

## **RICERCHE EFFETTUATE SANITA' ANIMALE**

Abel L, Havlicko P, Agren EO, Trogu° T, Lavazza° A, Lueert S, Smajs D, Knauf S  
**Investigating the genetic diversity of lagomorph infecting treponemes and their relatedness to human pathogenic *Treponema pallidum***

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 95 [Nr. Estr. 8005]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

The rabbit infecting *Treponema paraluisleporidarum* ecovar *Cuniculus* (TPeCuniculus) and the hare infecting orthologue TPeLepus belong to the group of uncultivable nonhuman pathogenic treponemes. Similar to human syphilis, rabbit treponemal disease is typically sexually transmitted and results in erythema and edema of the anogenital area, often followed by skin ulceration and crusting of the lesions. Infection of the nose, eyelids, lips, and paws can also occur. So far, limited information is available on the diversity, clinical relevance and occurrence of TPeCuniculus infection in wild rabbits and almost nothing is known about TPeLepus infections in hares. Based on serological results, infection of hares is widespread in the European area. However, infection seems to be mostly sub-clinical (no ulcerative skin lesions reported) compared to the frequency of seropositivity. Yet, the impact of infection on hare populations is currently unknown. Based on newly obtained genome data from treponemes infecting mountain hares (*Lepus timidus*), phylogeny suggests that the causative agent of hare syphilis is similar but distinct from the agent of rabbit syphilis. However, the genetic relatedness of strains infecting the two hare species *L. europaeus* and *L. timidus* is currently unsolved. We outline a study that aims to investigate whole genomes of uncultivable treponemes with a greater number of newly collected and characterized TPeCuniculus and TPeLepus strains from wild lagomorphs in Europe, Africa, and Australia. Trapping and sampling of live rabbits and hares is anticipated if samples cannot be obtained through collaborations or from dead animals. Sampling of dead animals and initial processing of samples will be followed by serology, DNA extraction and quantification of DNA via qPCR. Furthermore, a newly established and validated Loop-Mediated Isothermal Amplification (LAMP) will be performed under field conditions to rapidly test for treponemal DNA. Multi-locus strain typing analysis utilizing multiple different genes with reasonable heterogeneity will be used to select most suitable candidate strains for whole genome sequencing. In vivo cultivation of clinical isolates is anticipated to create new laboratory strains. The results will significantly contribute to basic research in treponematoses and to our understanding of *Treponema* evolution, pathogenicity, epidemiology, and ecology. Genome sequences of *Treponema* infection in rabbits and hares may help to clarify the evolution of syphilis and diseases caused by related subspecies (e.g., yaws).

Alborali° GL, Borella° L, D'Incau° M

### **Micoplasmosi di ieri e di oggi in veterinaria**

Leggiamo insieme : storie di sanità fra cronaca e scienza / Maurizio Zavanella. - Brescia : Fondazione Iniziative Zooprofilattiche e Zootecniche, 2018. - (Atti delle giornate di studio Fondazione Iniziative Zooprofilattiche e Zootecniche ; 107) p 208-219 [Nr. Estr. 8108]

Almugadam SH, Trentini A, Maritati M, Contini C, Rugna° G, Bellini T, Manfrinato MC, Dallochio F, Hanau S

### **Influence of 6-aminonicotinamide (6AN) on *Leishmania* promastigotes evaluated by metabolomics : beyond the pentose phosphate pathway**

Chem Biol Interact. - Vol. 294 ( 2018). - p 167-177. - 80 bib ref [Nr. Estr. ( ultimo accesso 26/02/2019 ) <https://doi.org/10.1016/j.cbi.2018.08.014> 8076]

6-Aminonicotinamide (6AN) is an antimetabolite used to inhibit the NADPH-producing pentose phosphate pathway (PPP) in many cellular systems, making them more susceptible to oxidative stress. It is converted by a NAD(P)<sup>+</sup> glycohydrolase to 6-aminoNAD and 6-aminoNADP, causing the accumulation of PPP intermediates, due to their inability to participate in redox reactions. Some parasites like *Plasmodium falciparum* and *Coccidia* are highly sensitive but not all cell types showed a strong responsiveness to 6AN, probably due to the different targeted pathway. For instance, in bacteria the main target is the Preiss-Handler salvage pathway for NAD<sup>+</sup> biosynthesis. We were interested in testing 6AN on the kinetoplastid protozoan *Leishmania* as another model to clarify the mechanisms of action of 6AN, by using metabolomics. *Leishmania* promastigotes, the life-cycle stage residing in the sandfly, demonstrated a three order of magnitude higher EC<sub>50</sub> (mM) compared to *P. falciparum* and mammalian cells (μM), although pre-treatment with 100 μM 6AN prior to sub-lethal oxidative challenge induced a supra-additive cell kill in *L. infantum*. By metabolomics, we did not detect 6ANAD/P suggesting that NAD<sup>+</sup> glycohydrolases in *Leishmania* may not be highly efficient in catalysing transglycosidation as happens in other microorganisms. Contrariwise to the reported effect on 6AN-treated cancer cells, we did not detect 6-phosphogluconate (6 PG) accumulation, indicating that 6ANADP cannot bind with high affinity to the PPP enzyme 6 PG dehydrogenase. By contrast, 6AN caused a profound phosphoribosylpyrophosphate (PRPP) decrease and nucleobases accumulation confirming that PPP is somehow affected. More importantly, we found a decrease in nicotinate production, evidencing the interference with the Preiss-Handler salvage pathway for NAD<sup>+</sup> biosynthesis, most probably by inhibiting the reaction catalysed by nicotinamidase. Therefore, our combined data from *Leishmania* strains, though confirming the interference with PPP, also showed that 6AN impairs the Preiss-Handler pathway, underlining the importance to develop compounds targeting this last route.

Amadori° M

**Control of bovine mastitis in the 21st century : immunize or tolerize?**

The 2018 International Bovine Mastitis Conference : Milano, June 11-13, 2018 / [s.l. : s.n., 2018]. - p 24-30. - 24 bib ref [Nr. Estr. 7999]

International Bovine Mastitis Conference (NMC) : Milano : June 11-13, 2018)

High production levels in animal husbandry can determine a difficulty of farm animals to adapt to the environment. This translates into increased replacement rates, reduction of life expectancy, more frequent occurrence of variegated and multifactorial diseases and increased drug usage. This is actually conflicting with major concerns of the public opinion about the very safety of the food production chains, let alone the ethical dimension of the latter. This is the current scenario of bovine mastitis, still underlying huge losses in dairy farms and still representing one of the main causes of early cull for dairy cows. There is in fact a correlation between prevalence of mastitis and high milk yield (Ingvarsen, 2003), despite the great advances in farm hygiene and management achieved over many years. Interestingly, whereas somatic cell counts have substantially decreased, this is not true of clinical mastitis cases (Ruegg, 2017), still giving rise to large drug usage on farm.

Amadori° M

**Control of bovine mastitis in the 21st century : immunize or tolerize?**

Res Vet Sci. - Vol. 121 ( 2018). - p 1-3. - 12 bib ref [Nr. Estr. ( ultimo accesso 26/02/2019 ) <https://doi.org/10.1016/j.rvsc.2018.09.010> 8123]

Amadori° M, Drigo M, Giacomini° E, Lazzaro° M, Pasotto D, Bilato° D, Ruggeri° J,

Boniotti° MB, Ferlazzo° G, Alborali° GL

**Un nuovo approccio alla valutazione del condizionamento delle scrofette per il controllo della PRRS** = A new approach to the evaluation of gilt acclimation for the control of PRRS

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 183-192. - 12 bib ref [Nr. Estr. 7827]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

La Sindrome Riproduttiva Respiratoria del suino (PRRS) è un modello complesso d'interazione virus/ospite. La protezione clinica del suino si basa essenzialmente (A) sulla ridotta recettività dei macrofagi al virus, (B) sul controllo della risposta infiammatoria indotta dal virus in associazione ad altri stressori ambientali, infettivi e non-infettivi. Il condizionamento efficace delle scrofette deve pertanto favorire tali azioni di controllo. Abbiamo paragonato le risposte immunitarie delle scrofette in condizionamento in 4 allevamenti stabili e 2 instabili per PRRS. Le scrofette sieronegative per virus PRRS (PRRSV) si infettavano regolarmente in tutti gli allevamenti oggetto di studio. Quattro aspetti vanno sottolineati: 1) La precocità della risposta anticorpale nei fluidi orali di gruppo era del tutto simile a quella determinata sul siero di sangue; la circolazione di PRRSV era presente in tutti i gruppi d'età solo negli allevamenti instabili; una risposta anticorpale precoce, bilanciata in IgG e IgA nei liquidi orali, era presente solo negli allevamenti stabili; 4) una risposta precoce in IFN-gamma a PRRSV si evidenziava solo negli allevamenti stabili. La viremia da PRRSV aveva un chiaro effetto inibente la risposta IFN-gamma. Negli allevamenti instabili si notava invece una diffusa risposta IFN-gamma nei suinetti sotto scrofa, di chiara origine materna. Vi era anche correlazione tra risposta IgA nei liquidi orali e cessazione dell'escrezione di PRRSV.

*The Porcine Reproductive and Respiratory Syndrome (PARS) is a complex model of host/virus relationship. Clinical protection of pigs is based: (A) on reduced susceptibility of macrophages to PRRSV infection and replication; (B) on the control over the inflammatory response caused by PRRSV and other environmental, infectious and non-infectious stressors. Therefore, successful acclimation of gilts should be conducive to such control actions. In order to establish correct parameters of evaluation, we compared the dynamics of the immune responses of gilts in four PRRS-stable and two unstable farms. PRRS-free gilts got regularly infected after entering PRRS-stable and unstable farms. Four main results should be highlighted: A) the precocity of the antibody response in oral fluids was generally similar to that recorded in sera; B) circulation of PRRSV was consistently detected in all age groups in unstable herds, only; C) an early, balanced, IgA and IgG response in oral fluids was only observed in PRRS-stable herds; D) an early IFN-gamma response after PRRSV infection was detected in PRRS-stable herds, only. This response was also observed in suckling piglets of unstable herds, likely due to transfer of maternal immunity. Furthermore, the mucosal IgA response was associated with cessation of virus excretion in oral fluid samples.*

Amadori° M, Soares-Felipe JF, Riva F, Vitali A, Ruggeri° J, Lacetera N

**Characterization of the blastogenic response to LPS of bovine peripheral blood mononuclear cells**

PLoS One. - Vol. 13 no 10 ( 2018). - p e0204827 (17 p). - 24 bib ref [Nr. Estr. ( ultimo accesso 05/12/2018 ) <https://doi.org/10.1371/journal.pone.0204827> 8042]

Mitogens are diverse compounds of plant and microbial origin, widely employed to test immunocompetence in animals. The blastogenic response of bovine Peripheral Blood Mononuclear Cells (PBMC) to lipopolysaccharides (LPS) has been investigated in our laboratories for a long time. In particular, a possible correlation between blastogenic response to LPS and disease resistance of periparturient dairy cows had been observed in previous studies. Most important, low responder cows presented a higher frequency of disease cases after calving, compared with high responder animals. Owing to the above, different aspects of the blastogenic response to LPS were investigated on PBMC of healthy Friesian cows, using a 72-hour Bromodeoxyuridin (BrDU) cell proliferation assay. Stimulation with LPS induced little if any replication of bovine PBMC over 72 hours despite consistent BrDU detection in all the PBMC samples under study. Poor replication of LPS-stimulated PBMC was confirmed by cell cycle and cell growth flow cytometry analyses. In particular, LPS

stimulation gave rise to very low percentages of S phase cells, sometimes lower than in control, unstimulated cells, as opposed to Concanavalin A-stimulated PBMC. Magnetic separation and analysis of BrDU-treated bovine PBMC after exposure to LPS showed that both B and CD4 T cells are involved in the blastogenic response to LPS, in contrast with current data based on human and murine models. Finally, LPS caused an early, specific up-regulation of TNF- $\alpha$  and TLR4 genes in bovine PBMC, and significant correlations were shown between the expression of inflammatory cytokine and Indoleamine-pyrrole 2,3-dioxygenase (IDO1) genes. On the whole, our data indicate that differences in the blastogenic response to LPS could be partly accounted for by heterogeneity of responding cells (B and T lymphocytes), which might also have an impact on induction and regulation of inflammatory responses and endotoxin tolerance.

Amato B, Di\_Marco Lo\_Presti V, Gerace E, Capucchio MT, Vitale M, Zanghi P, Pacciarini<sup>o</sup> ML, Marianelli C, Boniotti<sup>o</sup> MB

**Molecular epidemiology of Mycobacterium tuberculosis complex strains isolated from livestock and wild animals in Italy suggests the need for a different eradication strategy for bovine tuberculosis**

Transbound Emerg Dis. - Vol. 65 no 2 ( 2018). - p 416-424. - 76 bib ref [Nr. Estr. ( Ultimo accesso 18/06/2018 ) <https://doi.org/10.1111/tbed.12776> 7736]

Bovine tuberculosis (bTB) is an important zoonosis, which has been re-emerging in different ecological scenarios. In Sicily, Italy, from 2004 to 2014, an anatomopathological survey for tuberculosis-like lesions both in farmed and wild animals was performed. The isolates were genotyped using spoligotyping and Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats (MIRU-VNTR) techniques. High prevalence of lesions was observed for cattle (4%), pigs (4.9%) and wild boars (6.8%), and a total of 625 Mycobacterium bovis isolates were identified. Genotyping analysis showed the presence of 37 different spoligotypes including fifteen spoligotypes not present in other Italian regions and 266 MIRU-VNTR profiles. Spoligotype SB0120 exhibited the highest prevalence in cattle (50%) and pigs (56%) and the highest genetic variety with 126 different MIRU-VNTR profiles. The isolation of M. bovis in a farmer underlines the importance of M. bovis identification during the human TB diagnostic processes. This study supported the use of the genotyping analysis as a valuable tool for the evaluation of the epidemiological role of pigs and other domestic reservoirs such as goats and the role of wildlife in the maintenance of bTB infection.

Antongiovanni M, Buccioni A, Minieri S, Tosi<sup>o</sup> G

**Tannin from chestnut wood as an antimicrobial feed additive**

World Poultry Sci J. - Vol. 74 Suppl ( 2018). - p 72 (O9-07) [Nr. Estr. ( Ultimo accesso 05/02/2019 ) [http://www.wpsa.com/images/downloads/tables\\_of\\_contents/2018\\_MPS\\_BoA\\_online.pdf](http://www.wpsa.com/images/downloads/tables_of_contents/2018_MPS_BoA_online.pdf) 7883]

Mediterranean Poultry Summit (6th : Turin, Italy : June 18-20, 2018)

The problem of acquired antimicrobial bacterial resistance (AMR) is a global concern, not adequately considered so far. Since 2006, the use of antimicrobial as growth promoters in poultry production has been banned. For this reason, alternative additives could be adopted. Tannin from chestnut wood (CT) demonstrated to be efficient and reliable. CT exhibited a very strong antimicrobial action in vitro against Clostridium perfringens, Salmonella typhimurium, Escherichia coli and Campylobacter jejuni and in vivo with challenged birds. Moreover, its known antinutritional effect on the digestion of proteins was measured by means of a nitrogen balance trial with broilers. The overall effect of CT was surprisingly positive: the higher the level of CT in the diet, the better the body retention of nitrogen. Incidentally, the same effect was observed with pigs, in which faeces were separated from urines, thus highlighting that the advantage is exclusively metabolic. As a matter of fact, there are two limitations to the concentration of tannin in the feed: palatability and cost. According to our experience, 5 g/kg is the concentration not to be exceeded, but 3 g/kg is the

ideal level. In order to find out possible negative side effects, a live performance growth trial was carried out with broilers. The expected depressing effect of tannin on nitrogen digestibility was not observed, obviously because the negative effect on faeces was masked by the positive effect on urines, as observed in pigs. Performance data, oxidative status and meat colour of breasts were statistically comparable for the treated and untreated birds. In conclusion: CT is recommended as a reliable antimicrobial feed additive, with no negative side effects.

Arrigoni° N, Boldini° M, Galletti° G, Ruocco L, Brenzoni LG, Farioli M, Gradassi° M, Ventura° G, Giovannini° S, Paterlini° F, Rosignoli° F, Martinelli° C, Garbarino° C, Tamba° M

**Bovine paratuberculosis in Italy : results after four years of application of the national guidelines**

Bull Int Dairy Fed. - Vol. 493 ( 2018). - p 11-17. - 6 bib ref [Nr. Estr. 8088]

Paratuberculosis Forum (6th : Riviera Maya, Mexico : 4 June 2018)

Arrigoni° N, Boldini° M, Galletti° G, Ruocco L, Gemma Brenzoni L, Farioli M, Gradassi° M, Ventura° G, Giovannini° S, Paterlini° F, Rosignoli° C, Martinelli° C, Garbarino° C, Tamba° M

**Bovine paratuberculosis in Lombardy : results after four years of application of the national guidelines**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 85. - 2 bib ref [Nr. Estr. 7975]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

Introduction Paratuberculosis is widespread in Italy, where over 50% of bovine herds are infected (2). In order to improve the health status of dairy herds and to protect the dairy export market, the Italian Ministry of Health issued the "National guidelines for the control of bovine paratuberculosis and for assigning the health ranking of herds" (1). All of the regions have formally adopted the guidelines, but their application, being voluntary, is not uniform throughout the Italian territory. Methods Here we present the results of the first four years of application of the National guidelines in Lombardy, where 5,673 dairy herds and 1,000,000 total heads (including 500,000 dairy cows) are present and where nearly 40% of Italian milk is produced. Results In 2017, out of 5,673 herds, 4,157 officially adopted the guidelines. From 2014 to 2017 we observed: a rise in the number of herds joining the plan (from 56.0% to 73.3% of total dairy herds) a rise in the number of tested herds (from 7.2% to 20.9% of total dairy herds) a reduction of both infected herds (from 74.2% to 56.0% of tested herds) and positive cows (from 4.2% to 1.9% of tested cows) a reduction of "high within-prevalence herds" (>5%) (from 28.8% to 8.4%) a rise in the number of herds obtaining a health ranking (from 340 to 1,062), in particular PT1 (from 187 to 577) and PT2 (from 137 to 393). Conclusions In the Lombardy region, during the period 2014-2017, we recorded a general improvement in the situation of both prevalence of infected herds and infected animals, despite the absence of economical compensation for culled animals and financial support for the ELISA tests, which are paid for exclusively by the farmers. Among the critical points, there is the under-reporting of clinical cases, partially due to the fact that farmers tend to cull seropositive cows, in particular with high S/P values, anticipating the onset of clinical disease. The main strength of the improvements recorded is the synergic collaboration among health veterinary services, laboratories, practitioners and farmers through the specific creation of support groups, in order to sustain joining and participation in the program; for this purpose the information resources created by the National reference center for paratuberculosis were of help.

Bachanek-Bankowska K, Di\_Nardo A, Wadsworth J, Mioulet V, Pezzoni° G,

Grazioli<sup>o</sup> S, Brocchi<sup>o</sup> E, Kafe SC, Hettiarachchi R, Kumarawadu PL, Eldaghayes IM, Dayhum AS, Meenowa D, Sghaier S, Madani H, Abouchoaib N, Hoang BH, Vu PP, Dukpa K, Gurung RB, Tenzin S, Wernery U, Panthumart A, Seeyo KB, Linchongsubongkoch W, Relmy A, Bakkali-Kassimi L, Scherbakov A, King DP, Knowles NJ

**Reconstructing the evolutionary history of pandemic foot-and-mouth disease viruses : the impact of recombination within the emerging O/ME-SA/Ind-2001 lineage**

Sci Rep. - Vol. 8 ( 2018). - Article no. 14693 (11 p). - 44 bib ref [Nr. Estr. ( ultimo accesso 07/12/2018 ) <https://doi.org/10.1038/s41598-018-32693-8> 8037]

Foot-and-mouth disease (FMD) is a highly contagious disease of livestock affecting animal production and trade throughout Asia and Africa. Understanding FMD virus (FMDV) global movements and evolution can help to reconstruct the disease spread between endemic regions and predict the risks of incursion into FMD-free countries. Global expansion of a single FMDV lineage is rare but can result in severe economic consequences. Using extensive sequence data we have reconstructed the global space-time transmission history of the O/ME-SA/Ind-2001 lineage (which normally circulates in the Indian sub-continent) providing evidence of at least 15 independent escapes during 2013–2017 that have led to outbreaks in North Africa, the Middle East, Southeast Asia, the Far East and the FMD-free islands of Mauritius. We demonstrated that sequence heterogeneity of this emerging FMDV lineage is accommodated within two co-evolving divergent sublineages and that recombination by exchange.

Balboni A, Musto C, Kaehler E, Caniglia R, Fabbri E, Carra<sup>o</sup> E, Berzi D, Cotti C, Battilani M, Delogu M

**Is tongue an adequate sample for detection of canine adenovirus type 1 in free-ranging wolf (*Canis lupus*)?**

IX Convegno Nazionale della Ricerca nei Parchi "Conoscere per proteggere" (SIPI) : 4-7 Ottobre 2018 : book of abstracts / [s.l. : Settore Ricerca e Conservazione, Parco Natura Viva, 2018]. - p 31 [Nr. Estr. 8049]

Convegno Nazionale della Ricerca nei Parchi (9. : Bussolengo (VR) : 4-7 Ottobre 2018)

Collection of adequate tissue samples is essential to detect viral DNA in wild animals found dead. This study reports the detection of Canine Adenovirus Type 1 (CAV-1 DNA) by real-time PCR technique in the tongue tissue of a wolf. CAV-1 DNA detection was carried out using a real-time PCR able to simultaneously detect and differentiate the CAV-1 and CAV-2. Wolf age was estimated to be a second class (12-24 months). Genetic analyses confirmed the carcass belonged to a male pure Italian wolf. CAV DNA was detected in samples of the mesenteric lymph node and the tongue, characterised by a melting temperature specific for the type. Both the CAV-1 hexon and the fibre genes sequences differed from all the CAV-1 reference sequences at the nucleotide as well as at the amino acid levels. To the extent of our knowledge, this is the first detection of CAV-1 DNA in a tongue sample of wolf. Although lymphoid tissues are one of the main sites of viral replication, the higher amount of the target CAV-1 DNA detected in the tongue suggests that this organ may represent an important site for viral replication. Furthermore, a positive result in molecular biology could be explained by a contamination occurred on the tongue surface via saliva. To confirm the effective replication of CAV-1 in the tongue tissues, further immunohistochemical investigations are required. Genetic characterization of the identified CAV-1 revealed strict relationship with viruses detected both in dogs and red foxes in Italy suggesting that transmission of CAV-1 could occur among different animal species. This could have an impact on the populations of wolves included in species conservation programs. More studies are needed to confirm with a large case series the use of the tongue epithelium samples as alternative biological matrix for the routinely molecular detection of CAV-1 in deceased wild animals.

Balboni A, Musto C, Kaehler E, Fabbri E, Caniglia R, Carra° E, Battilani M, Delogu M

#### **Genetic characterisation of Canine adenovirus type 1 detected in the tongue of a wolf**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 396. - 3 bib ref [Nr. Estr. 7973]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

Evidence of exposure to Canine adenovirus type 1 (CA<sub>AdV</sub>-1), the aetiologic agent of the infectious canine hepatitis in dogs, has long been reported in wolves in several geographic areas. In this study, the genetic characterization of CA<sub>AdV</sub>-1 detected in the tongue sample of a male pure Italian wolf (*Canis lupus*) found dead in Tuscany, Italy, in December 2014, is described. A complete post mortem examination was carried out and samples of mesenteric lymph node and tongue were collected. CA<sub>AdV</sub>-1 DNA detection was carried out using a real-time PCR able to simultaneously detect and differentiate the CA<sub>AdV</sub>-1 and the CA<sub>AdV</sub> type 2 [1]. Amplification, nucleotide sequencing and assembling of hexon and fiber CA<sub>AdV</sub> genes were done as previously reported [2]. The obtained nucleotide sequences were aligned with reference sequences of canine adenoviruses from GenBank and translated into amino acid sequences using BioEdit 7.2.5. Phylogenetic relationships were evaluated for the concatenated hexon and fiber genes sequences using MEGA version 6.0.6. At necropsy, the wolf appeared to have been in good general health. The subject was killed by a motor vehicle collision. Wolf age was estimated to be a second class (12-24 months) according to tooth development. CA<sub>AdV</sub> DNA was detected in the lymph node (6.7x10<sup>4</sup> DNA copies/g) and in the tongue epithelium (1.6x10<sup>6</sup> DNA copies/g), with a melting temperature specific for the type 1. The CA<sub>AdV</sub>-1 hexon and fiber genes sequences differed from all the CA<sub>AdV</sub>-1 reference sequences both at the nucleotide and the amino acid levels. The position 388 of the predicted CA<sub>AdV</sub>-1 hexon protein differentiated the Italian sequences from the other reference strains by having serine instead of asparagine. Several nucleotide and amino acid mutations in the hexon and fiber genes allowed to distinguish all the Italian strains submitted to GenBank until now in two groups. Phylogenetic tree confirmed the clusterisation of the Italian CA<sub>AdV</sub>-1 sequences. This is the first detection of CA<sub>AdV</sub>-1 DNA in a tongue sample of wolf and a high amount of the target CA<sub>AdV</sub>-1 DNA was detected in this sample. Hence, it is possible to speculate that the tongue epithelium represents an important site for viral replication. This tissue could be used as a complementary sampling site for CA<sub>AdV</sub>-1 testing in wildlife, especially in deceased animals undergoing postmortem changes, as previously reported for parvovirus [3]. Sequence alignment and phylogeny confirmed that several genetic variants of CA<sub>AdV</sub>-1 circulate in Italy [2], and allowed to distinguish Italian CA<sub>AdV</sub>-1 in two subgroups, each of which infects various animal species, suggesting that the transmission of the virus from wild animals to the dog, and vice versa, can frequently occur.

Baldo° V, Scali° F, Giudici° F, Maisano° AM, Santucci° G, Giacomini° E, Lazzaro° M, Amicabile° A, Bontempi° G, Pongolini° S, Belluzzi G, Ianieri A, Colagiorgi A, Bruini I, Borrello S, Candela L, Vitali A, Begni E, Fadini M, Alborali° GL

#### **Antimicrobial consumption and extended-spectrum B-lactamase (ESBL)-producing Escherichia coli in Italian fattening units**

ESPHM 2018 : 10th European Symposium of Porcine Health Management : Barcelona, 9th - 11th May, 2018 : proceedings / [s.l. : s.n., 2018]. - p 179 (Poster BBD-001) [Nr. Estr. 7866]

European Symposium of Porcine Health Management (ESPHM) (10th : Barcelona : 9th - 11th May, 2018)

Introduction Extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* represents a risk for both human and pig health. Furthermore, antimicrobials misuses may increase ESBL spread. The aim of this study was to investigate, in latter stages of production, relationships between presence of ESBL-producing *E. coli* and antimicrobial consumption. Materials and Methods A convenience sample of forty-eight heavy pig fattening units was selected in Lombardy region of Italy. Ten samples of faeces were collect for each farm, five on farm and five at slaughterhouse, and ESBL presence was screened using a phenotypic diagnosis. Farms were classified as ESBL-positive if, at least, one sample collected on farm and one at slaughterhouse were positive. Antimicrobial consumption was estimated, as days of treatments per bred pig, using defined daily dose animal for Italy (DDDAit) and an average weight at treatment of 100 kg. Data were collected retrospectively on 2016. Differences between ESBL-positive and ESBL-negative farms were investigated using Mann-Whitney U test. Results Twenty out of forty-eight farms were found ESBL-positive. Number of bred pigs per year did not differ significantly ( $P = 0.3061$ ) between the two groups of farms. Medians of antimicrobials consumptions were 7.65 days/pig in ESBL-positive farms (range; 0.84-31.10) and 19.54 days/pig (range; 0.11-43.97) in ESBL-negative ones. Antimicrobials consumption were not significantly different ( $P = 0.1212$ ) between the two groups. Discussion & Conclusion The presence ESBL-producing *E. coli* is a source of relevant concern for public health. Lack of differences in antimicrobials consumption, between ESBL-positive and ESBL-negative farms, may be due to a limited sampling and difference in usage patterns of specific active ingredients (i.e.  $\beta$ -lactams). Moreover, identification ESBL-producing *E. coli* could be improved using molecular techniques. Further clarifications are required regarding ESBL, antimicrobials consumptions and active ingredients patterns during all production phases of Italian heavy pigs.

Barilli E, Bacci C, Villa ZS, Merialdi<sup>o</sup> G, D'Incau<sup>o</sup> M, Brindani F, Vismarra A

**Antimicrobial resistance, biofilm synthesis and virulence genes in Salmonella isolated from pigs bred on intensive farms**

Ital J Food Safety. - Vol. 7 no 2 ( 2018). - no 7223 (p 131-137). - 51 bib ref [Nr. Estr. ( ultimo accesso 31/08/2018 ) <https://doi.org/10.4081/ijfs.2018.7223> 7891]

*Salmonella* is the second cause of food-borne infection in humans in the USA and Europe. Pigs represent the second most important reservoir for the pathogen and the consumption of pork meat is a major risk factor for human salmonellosis. Here, we evaluated the virulence patterns of eleven *Salmonella* isolated from pigs (carcasses and faces) bred in intensive farms in the north of Italy. The two serotypes identified were *S. Typhimurium* and its monophasic variant 1,4,5,12:i:-. None of the isolates was an ESBL producer, as confirmed also by PCR. However, the presence of a multi-drug resistant pattern was evident, with all the isolates being resistant to at least to five antimicrobial agents belonging to various classes. Moreover, six out of eleven isolates showed important resistance profiles, such as resistance against colistin and ciprofloxacin, with nine to twelve recorded resistances. The isolates were negative for the biofilm synthesis test, while four different virulotypes were characterized. All the isolates showed the presence of *invA*, *hilA*, *stn*, *ssrA*, *sipC*. One sample also harbored *ssaR* and *spvC* genes. One strain was positive for all the virulence genes tested and was resistant to 12 antimicrobial agents. The present study contributes new data to the surveillance program for antibiotic resistance. Furthermore, the presence of eleven highly virulent isolates poses concern for human health in relation to their diffusion in the environment.

Barisani C, Merenda<sup>o</sup> M, Gibelli<sup>o</sup> LR, Fedrizzi<sup>o</sup> G, Rosignoli<sup>o</sup> C

**Episodio di intossicazione da gas in un gruppo di manzette da latte stabulate in vicinanza di foraggio di frumento appena insilato**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 154. - 2 bib ref [Nr. Estr. 7979]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)



Introduzione Viene descritto un episodio di intossicazione da gas, prodotto da frumento appena insilato, che ha coinvolto un gruppo di manzette di un allevamento da latte della provincia di Mantova. Il caso si è presentato nel mese di maggio del 2018 in un gruppo di 8 manzette di circa 10 mesi di età e del peso di 180-220 Kg di razza frisona. Le manzette erano stabulate in box adiacenti a elementi prefabbricati in cemento armato utilizzati come struttura di contenimento per l'insilamento orizzontale dei cereali. La forma clinica si è manifestata la mattina del giorno seguente all'ammassamento e alla copertura con film plastico di 20 ettari di trinciato di frumento. Tre manzette sono state trovate morte e altre 3 con gravi sintomi respiratori. Tutte presentavano una colorazione giallastra del pelo del dorso. È stato effettuato l'esame necroscopico su due soggetti deceduti e inviati i visceri di una manzetta al laboratorio per accertamenti diagnostici. Risultati Le manzette con forma clinica grave presentavano sintomi caratterizzati da tosse, dispnea intensa e fuoriuscita di schiuma dalla bocca. A questi soggetti è stato somministrato un cortisonico per via parenterale ma con scarsi risultati e nel corso della giornata sono poi venute a morte. Le altre manzette, quelle con sintomi più lievi, immediatamente spostate in un altro luogo sono invece guarite completamente. All'esame necroscopico dei soggetti deceduti veniva riscontrato edema polmonare emorragico accompagnato da intensa congestione della mucosa delle vie respiratorie e evidente ispessimento gelatinoso dei setti interlobulari. Si notavano anche grosse bolle enfisematose nei lobi diaframmatici. Il pH misurato a livello della mucosa dei grossi bronchi del polmone inviato al laboratorio si attestava su un valore oscillante tra 5,8 e 6,0. L'esame istologico rilevava una mucosa bronchiale disepitelizzata, edematosa con emorragie recenti multifocali e una broncopolmonite caratterizzata dalla presenza negli alveoli di essudato fibrino-emorragico e nei bronchioli di numerose cellule desquamate e macrofagi. Gli esami per la ricerca dei più comuni patogeni respiratori batterici e virali davano esito negativo. Conclusioni Le notizie anamnestiche, i segni clinici e le indagini di laboratorio portano ad attribuire l'episodio descritto ad una forma di intossicazione da gas, ed in particolare da biossido di azoto (NO<sub>2</sub>). Come riportato anche da altri Autori (1, 2), tale elemento si può sviluppare in quantità significativa nelle prime 24-48 ore dopo l'insilamento di cereali particolarmente ricchi in nitrati. Il biossido di azoto se inalato a livello della mucosa delle vie respiratorie reagisce con l'acqua e genera acido nitroso che, se prodotto in quantità significativa, può determinare bronchiolite obliterante e grave edema polmonare. Nel caso in esame, la concomitanza di più fattori (stabulazione degli animali adiacente ai silos, assenza di una adeguata ventilazione, probabile elevato tasso di nitrati nel foraggio ecc. ) ha generato il grave episodio di intossicazione descritto.

Bassi E, Betti<sup>o</sup> M, Menotta<sup>o</sup> S, Fedrizzi<sup>o</sup> G, Cappi<sup>o</sup> F, Ferloni M, Facchetti R, Bianchi<sup>o</sup> A

#### **Lead concentrations in vultures from 4 European countries**

2018 Scientific Meeting Italian Association for the study of Trace Elements in living organisms - AISETOV "The role of trace elements in health: from healthy environments to healthy living organisms" : Ozzano Emilia, Bologna, October 12, 2018 : abstract book / [s.l. : AISETOV], 2018. - p 32 (Poster P-08). - 2 bib ref [Nr. Estr. 7986]

Scientific Meeting Italian Association for the study of Trace Elements in living organisms (AISETOV) : Ozzano Emilia, Bologna : October 12, 2018)

Introduction: Very few data are available on lead levels in tissues of vultures; therefore the aim of the present work was to compare isotopic ratios of lead concentrations in the vultures carcasses with isotopic signature found in 19 types of hunting ammunition widely used in the Alps. Material and Methods: Lead concentrations were analysed by ICP-MS in liver, kidney and bones from 49 specimens of Eurasian Griffon (*Gyps fulvus*), from 6 specimens of Black Vulture (*Aegypius monachus*) and from 5 specimens of Bearded Vulture (*Gypaetus barbatus*) recovered from 4 European countries (Italy, France, Austria and Switzerland) by Stelvio National Park and Sondrio Province since 2008. Results and Discussion: In all the analyzed specimens lead concentrations reached the highest concentrations in bones. The maximum mean level was found in Black Vulture (13.3±25.9 mg/Kg) and the minimum in Bearded Vulture (2.7±1.5 mg/Kg). Liver and kidney mean lead concentrations were similar and lower than 1 mg/Kg, with exception of few specimens, which presented high lead concentrations; the maximum value (4.7 mg/Kg) was determined in a sample of kidney from Eurasian Griffon. Data are in the range of those reported by other authors. In the Alps,

most scavengers ingest lead from ammunition remains, by eating lived prey with embedded shots, carcasses and/or offal. Severe cases of saturnism, owing to the ingestion of high amounts of lead, caused acute poisoning in a few cases, leading to direct mortality. Sub-lethal lead quantities, however, are ingested throughout their lifetime. This intake may affect avian populations by altering cognition and behavior, diminishing reproductive success and favoring diseases, as well as starvation or traumatic events, or by increasing mortality through aggressive inter-specific interactions (Gugiatti et al. 2016). These evidences also demonstrated that similar recoveries are not exceptional events but more likely they represent the 'tip of the iceberg' of a substantial proportion of the Alpine populations affected by lead at sub-lethal levels demonstrating the need and urgency to replace lead bullets with other not-toxic metals (mostly made in copper).

Bassi° P, Procopio° A, Galletti° G, Gentile A, Famigli\_Bergamini P, Merialdi° G, Santi° A, Tamba° M, Taddei° R

**Besnoitiosi bovina : indagine sierologica in aziende linea vacca-vitello in Emilia Romagna, anni 2015-2018**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 160. - 3 bib ref [Nr. Estr. 7982]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

Introduzione La besnoitiosi bovina (*Besnoitia besnoiti*) è considerata malattia emergente in Europa e provoca perdite economiche dovute al calo di produzione latte, riduzione di incremento ponderale e deprezzamento delle pelli<sup>1</sup>. La diagnosi sierologica può essere effettuata in screening mediante test ELISA, con successiva conferma in immunofluorescenza (IFAT) o Western Blot (WB). Questo studio mira ad indagare la diffusione della besnoitiosi sul territorio dell'Emilia Romagna tra il 2015 e il 2018. Materiali e metodi In 89 aziende linea vacca-vitello (67 localizzate a Forlì-Cesena, 11 a Bologna, 10 a Rimini e 1 a Ravenna) sono stati prelevati i sieri di bovini di età superiore ai 24 mesi: 2273 animali nel 2015 e 2219 nel 2018. I sieri sono stati analizzati con Kit ELISA PrioCHECK®*Besnoitia* Ab 2.0, secondo le istruzioni fornite dal produttore. Ai fini delle analisi gli esiti dubbi sono stati considerati negativi. L'allevamento è stato classificato positivo se includeva almeno un capo positivo. Risultati I risultati dell'indagine evidenziano un calo delle percentuali di positività sia a livello di aziende, sia di capi. Nelle aziende la percentuale di positività si riduce da 52,8% (47/89) nel 2015 a 31,5% (28/89) nel 2018. A Forlì-Cesena, le aziende positive sono diminuite da 43 nel 2015 a 26 nel 2018, con un solo allevamento positivizzato nel 2018. A Bologna tutte le aziende positive nel 2015 (3/11) sono risultate negative nel 2018. A Rimini la situazione è rimasta invariata (1 positivo/10 aziende), mentre l'unica azienda campionata a Ravenna è risultata positiva solo nel 2018. Considerando i capi, le positività variano da 23,2% (528/2273) nel 2015 a 16,6% (370/2219) nel 2018. I capi esaminati in entrambi gli anni sono 1395. Di questi, 190 (13,6%) si riconfermano positivi e 1016 (72,8%) si riconfermano negativi dopo tre anni. Nel corso del triennio, 64 (4,5%) capi si sono positivizzati, mentre 125 (8,9%) si sono negativizzati al test ELISA. Conclusioni Pur considerando i limiti del test ELISA (bassa specificità), i dati esposti evidenziano una riduzione delle sieropositività nel 2018 rispetto al 2015. L'analisi dell'andamento della malattia, come riportato in due recenti studi longitudinali<sup>2,3</sup> effettuati in allevamenti con infezione endemica, ha evidenziato una tendenza alla riduzione della sieroprevalenza in ELISA<sup>2,3</sup> e dei titoli anticorpali in IFAT<sup>3</sup> nell'arco di 2-4 anni. I risultati del nostro studio, condotto su un numero più ampio di aziende, risultano in accordo con quanto riportato in letteratura per popolazioni più ridotte.

Bassi° S, Carpana E, Bergamini° F, Loglio G, Palminteri S, Galletti° G, Carra° E

**Identification of honeybee colonies infected by *Paenibacillus* larvae through the powdered sugar examination**

8th Congress of Apidology (EurBee 8) : 18-20 September 2018, Ghent, Belgium : program & abstract book / [s.l. : s.n., 2018]. - p 153 (Poster P010) [Nr. Estr. 7940]

European Conference of Apidology (EurBee) (8th : Ghent, Belgium : 18-20 September 2018)

We studied the possibility to identify *Paenibacillus* larvae infected honeybee colonies and determine their level of infection by using the powdered sugar examination. 105 colonies belonging to 10 apiaries were examined: Group A- 15 colonies with AFB symptoms; Group B -45 colonies without symptoms of disease but belonging to apiaries with AFB diseased colonies; Group C- 45 colonies of apiaries where no cases of AFB were reported in the last two years. Fifty grams of powdered sugar was dusted on the top bars of each brood combs and collected after 20 min on sheets of papers placed on the bottom of the hives. The sugar samples were examined by cultural method and with a new molecular method: a 16S rRNA gene based quantitative TaqMan real-time PCR. The results obtained by culture method are the following: Group A: all samples were positive with a spore load between  $1 \times 10^4$  and  $2,8 \times 10^7$  CFU/g. Group B: 10 samples were negative; 34 positives with a spore load between  $2 \times 10^1$  and  $6,2 \times 10^3$  CFU/g and 1 positive with a load of  $1,7 \times 10^4$  CFU/g. Group C: 37 samples were negative and 8 positive with a spore load between  $2 \times 10^1$  and  $1,4 \times 10^2$  CFU/g.

Bassi° S, Carpana E, Bergomi P, Galletti° G

#### **Detection and quantification of *Paenibacillus* larvae spores in samples of bees, honey and hive debris as a tool for American foulbrood risk assessment**

Bull Insectology. - Vol. 71 no 2 ( 2018). - p 235-241. - 37 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <http://www.bulletinofinsectology.org/pdfarticles/vol71-2018-235-241bassi.pdf> 8036]

American foulbrood (AFB) is a severe bacterial brood disease of honey bees (*Apis mellifera* L.) caused by the spore-forming bacterium *Paenibacillus* larvae. Low-levels of *P. larvae* in honey bee colonies are very widespread and can be considered endemic, whereas AFB occurs in clinical form only when a certain level of spores is reached in the colony. We studied for the first time the relationship between the level of the wintry contamination by *P. larvae* in materials taken from the hive and the onset of the disease in the following spring. In ten apiaries, from 125 colonies without signs of AFB, samples of adult bees, honey from the brood chamber and hive debris were collected in winter and cultured to detect *P. larvae* spore levels. The colonies were then checked in spring, until the end of May, for AFB symptoms. The performance of tests based on bees, honey and hive debris was evaluated, with respect to the results of clinical examination, by determination and comparison of sensitivity (Se), specificity (Sp) and positive predictive value (PPV) at different cut-off thresholds. Using the presence or absence of spores as a cut-off threshold, the examination of debris and bees showed a good Se (100%), while the Se of honey was limited (81.25%). At the same cut-off threshold, debris showed a greater Sp than that of bees (57.80% vs 7.34%). At increased cut-off thresholds, the Se was always higher for bee examination than for debris examination, whereas Sp and PPV were always higher for debris than for bees. The results showed that the levels of *P. larvae* spores in adult bees and, above all, in hive debris can be a useful tool for AFB risk assessment. The identification and proper management of the colonies with an increased risk of developing the disease is crucial for reducing the impact of AFB.

Bassi° S, Lavazza° A, Palamara\_Mesiano M, Perez\_Garcia F, Lupi D

#### **Can sublethal pesticides exposure in honeybee colonies with subclinical infections by *Paenibacillus* larvae favour the development of American foulbrood in clinical form?**

8th Congress of Apidology (EurBee 8) : 18-20 September 2018, Ghent, Belgium : program & abstract book / [s.l. : s.n., 2018]. - p 191 (Poster P084) [Nr. Estr. 7942]

European Conference of Apidology (EurBee) (8th : Ghent, Belgium : 18-20 September 2018)

Stress factors may interact with additive or synergic effects and a multi-stress condition is the most probable cause for honeybee decline. We evaluated how the combination of different stresses (sublethal dose of pesticides, and electromagnetism) affect survival and make bee susceptible to *Varroa* attack and viral diseases. Experimental hives, from the same origin and health status, were

equally and randomly placed on 5 April 2017 in 2 exposure sites (ES1-2) and in 1 control site (CS). The ESs were inside an experimental farm where chemical treatments are applied in orchards ([Si sublethal exposures to pesticides), and where there is also a high-voltage electric line (ES2 chemicals as in 1 plus electromagnetism). The CS was far from agricultural fields and from human settlements. Clinical inspections and debris collection from the hives for mite checkup were weekly performed. Monthly, sugar treatments have been also made to check the mite infestation level. During a routine check in mid-June, unexpectedly, one case of American foulbrood (AFB) caused by *P. larvae* genotype ERIC II was diagnosed in E51. In the following days AFB was diagnosed again in 1 colony in [Si and in 2 colonies in E52. No hive in CS resulted affected. After the diagnosis of AFB, sugar and debris previously harvested and stored have been examined for *P. larvae* detection (culture method). The sugar collected at the end of May in ES ranged from 174,000 to 5,000,000 CFU/g in diseased colonies and from 60 to 17,000 CFU/g in the asymptomatic ones. In CS it was <20 CFU/g (detection limit). The debris of three diseased colonies collected on 28 April showed already a high number of CFU. Since the bacteriological examination always underestimates the number of spores, we hypothesize that low contamination was present at the beginning of the trial in all the colonies. The presence of stressors in ES1-2 has probably favoured the development of the infection by sporadically giving rise to the AFB disease.

Bedendo G, Panzarin V, Fortin A, Zamperin G, Pretto T, Buratin A, Quartesan R, Sabbion M, Salogni C, Pascoli F, Toffan A

**Detection and characterization of a rhabdovirus causing mortality in black bullhead catfish, *Ameiurus melas***

J Fish Dis. - Vol. 41 ( 2018). - p 1063-1075. - 37 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1111/jfd.12797> 7893]

This study fully describes a severe disease outbreak occurred in 2016 in black bullhead catfish farmed in Italy. Affected fish showed nervous clinical signs as well as emaciations and haemorrhagic petechiae on the skin at the fin bases, abdomen and gills. Viral isolation in cell culture allowed the subsequent identification of a rhabdovirus, tentatively named ictalurid rhabdovirus (IcRV), through electron microscopy, immunofluorescence and whole genome sequencing (WGS). The newly isolated virus, together with 14 additional viral strains stored in our repository and detected during similar mortality episodes in the period 1993–2016, was phylogenetically analysed on the basis of the nucleoprotein and the glycoprotein nucleotide and amino acid sequences. The genetic distances among Italian IcRV strains were also estimated. Our results show that all the IcRV strains belong to the genus *Sprivirus* and are closely related to the tench rhabdovirus (TenRV). Italian catfish production is constantly decreasing, mainly due to viral infections, which include the newly characterized IcRV. Data presented in this work will assist to investigate the molecular epidemiology and the diffusive dynamics of this virus and to develop adequate surveillance activities.

Bernini A, Bolzoni L, Casagrandi R

**When details do matter : detecting superspreader farms in livestock diseases**

International School and Conference on Network Science (NetSci) : 11-15 June 2018, Paris, France : book of Abstracts, Posters / [s.l. : s.n., 2018]. - 1 p [Nr. Estr. 7910]

International School and Conference on Network Science (NetSci) : Paris, France : 11-15 June 2018)

Livestock infectious diseases spread between farms (network nodes) via both direct contacts, i.e. animal trade, and indirect contacts, due to visitors and vehicles that can act as pathogen vectors. The diffusion process can well be captured by temporal two-layer network models. While animal movements are registered in national databases in many EU-countries, little or no information about on-farm visits is available and rarely collected in a systematic manner. Here we evaluate how and to

what extent different levels of detail in describing indirect contact networks can result into qualitative and quantitative different outcomes of epidemic spread, in terms of both superspreaders identification and final epidemic size. We focus on a dairy farm system in Emilia Romagna (Northern Italy) as resulting from a 3-month comprehensive data collection campaign on itineraries of trucks for calves transportation. The indirect contact layer of the network is described at two different levels of detail. In the common contractors network (CCN), farms are connected with indirect links if visited by trucks of the same company. In the truck itineraries network (TIN), directed links connect each farm to those later visited by the same truck. Disease spread is simulated through a stochastic Susceptible-Infectious model initialized with one-at-a-time farm acting as epidemic seed. CCN, coarsely-described layering systematically brings to overestimations of final epidemic sizes compared to TIN, finely-described layering. Moreover, the identified sets of superspreaders (farms leading to the top 5% of final epidemic sizes), are very different using CCN or TIN. Most important, while epidemics starting from CCN-superspreaders typically infect the same farms, outbreaks seeded at TIN-superspreaders involve quite diversified sets of farms. We conclude that not only the detail of indirect contacts description deeply influences the identification of superspreaders, but also the set of infected farms, with non-trivial consequences in designing surveillance and control measures.

Bertocchi° L, Fusi° F, Angelucci° A, Bolzoni° L, Pongolini° S, Strano° RM, Ginestreti° J, Riuzzi° G, Moroni P, Lorenzi° V

**Characterization of hazards, welfare promoters and animal-based measures for the welfare assessment of dairy cows : elicitation of expert opinion**

Prev Vet Med. - Vol. 150 ( 2018). - p 8-18. - 51 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 )  
<https://doi.org/10.1016/j.prevetmed.2017.11.023> 7752]

An expert opinion elicitation, based on a modified Delphi technique, was organized to collect the opinion of 16 Italian veterinarians with the aim of conducting a hazard and a welfare promoter characterization for defining and weighing a list of management and housing factors potentially associated with negative or positive welfare outcomes in dairy cows kept in loose housing systems. In addition, the 16 experts judged a set of animal-based measures in order to rate them by appropriateness and by the level of animal pain and suffering due to the welfare consequences they measure. Veterinary experts were asked to score 52 hazards, 47 welfare promoters and 18 animal-based measures. Management and housing hazards, that were determined to be associated with a very high impact on the welfare of dairy cows, were mainly referred to lactating cows (absence of bedding material, presence of inadequate or slippery floor in walking areas, wrong design of the lying area), but also the use of harmful tools for animal handling and the lack of scheduled foot inspection, trimming and foot bathing reached very high impact scores. Management and housing welfare promoters dealing with optimal cow comfort around resting obtained the highest ratings, together with the presence of experienced and trained stockpersons, the implementation of an attentive animal grouping strategy and the control of temperature, humidity and ventilation. Considering animal-based measures, the highest ranking of importance was given to observation of lameness, records of mortality of adult cows and calves, observation of integument alterations and body condition scoring. This study has been the starting point for the development of the first Italian national protocol for the assessment of the welfare of dairy cows farmed in loose housing systems.

Bertocchi° L, Gaffuri° A, Angelucci° A, Fusi° F, Strano° RM, Ginestreti° J, Ferrara° G, Bolzoni° L, Zanardi° G, Lorenzi° V

**Expert knowledge elicitation for ranking hazards affecting dairy goat welfare in Italian farms**

The 30th World Buiatrics Congress : August 28 to September 1, 2018 : Sapporo, Japan : abstract book, keynote lectures, oral and poster presentations / [s.l. : s.n., 2018]. - p 345-346. - 1 bib ref [Nr. Estr. 8085]

World Buiatrics Congress (WBC) (30th : Sapporo, Japan : August 28 to September 1, 2018)

Bertocchi° L, Lorenzi° V, Strano° RM, Ginestreti° J, Fusi° F, Angelucci° A, Ferrara° G, Galletti° G, Bolzoni° G, Zanardi° G

**Preliminary results on the correlation between routinely collected bulk tank milk quality parameters and on-farm dairy cow welfare**

The 2018 International Bovine Mastitis Conference : June 11-13, 2018 : Milano / [s.l. : s.n., 2018]. - p 197. - 2 bib ref [Nr. Estr. 7916]

International Bovine Mastitis Conference (NMC) : Milano : June 11-13, 2018)

The on-farm assessment of dairy cow welfare could be time consuming and expensive, thus limiting its implementation in the field. To overcome this problem, some recent scientific works proposed to exploit national herd databases for continuously monitoring and estimating the level of dairy cow welfare. In particular, it has been suggested that data routinely collected on-farm could be an useful prescreening tool for identifying herds with welfare problems (de Vries et al., 2014). The present study aims at investigating the use of bulk tank milk quality parameters as possible indicators of cow welfare in Italian dairy farms. During the three-year period 2014-2016, the welfare assessment protocol of the Italian National Reference Centre for Animal Welfare (Bertocchi et al., 2018; CRenBA 2014) was applied by trained veterinarians in 287 dairy farms located in Northern Italy (mean lactating cows = 111; mean milk production = 28kg/cow/day) and an overall animal welfare score was obtained for each farm. In the same herds, bulk tank milk quality data, included in the monthly analysis for milk quality payment and referred to three-month period preceding the day of the welfare assessment, were also collected. Somatic cell count (SCC), total bacterial count (TBC), milk fat, milk protein, lactose and urea were the main bulk tank milk quality parameters considered for the analysis. Based on the available data, SCC geometric mean was calculated for 269 herds, TBC geometric mean was calculated for 253 farms, milk fat and milk protein arithmetic means were computed for 269 farms, while lactose and urea arithmetic means were determined for 234 and 257 herds, respectively. Kendall's Tau coefficient (r) was used to investigate the possible correlation between farms' milk quality parameters and farms' overall animal welfare scores. Data showed a mild negative correlation between SCC, TBC and farm overall animal welfare level (SCC:  $r = -0.118$ ,  $p\text{-value} < 0.01$ ; TBC:  $r = -0.197$ ,  $p\text{-value} < 0.01$ ), while lactose and urea resulted to be positive correlated with the overall welfare score (Lactose:  $T = 0.173$ ,  $p\text{-value} < 0.01$ ; Urea:  $T = 0.103$ ,  $p\text{-value} < 0.05$ ). Milk fat and milk protein did not show any significant association (Fat:  $T = 0.054$ ,  $p\text{-value} > 0.05$ ; Protein:  $T = 0.048$ ,  $p\text{-value} > 0.05$ ). Further in-depth analyses are necessary to confirm the possibility to exploit these parameters, in particular SCC, TBC and urea, as prescreening tools for dairy cow welfare.

Bonardi S, Longhi S, Pia F, Corradi M, Giliol S, Scaltriti° E

**Isolamento di Escherichia coil ESBL da linfonodi meseraici di cinghiale (Sus scrofa)**

Attualita nell'igiene degli alimenti: stato dell'arte e prospettive future : XXVIII Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) : Milano, 12-14 Settembre 2018 / [s.l. : s.n., 2018]. - p 7 (C013) [Nr. Estr. 7938]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (28. : Milano : 12-14 Settembre 2018)

Lo studio si prefigge lo scopo di valutare l'eventuale ruolo del cinghiale (Sus scrofa) quale "biosensore" di microorganismi resistenti agli antimicrobici (AMR) diffusi nell'ambiente nel quale vive. Il cinghiale è l'ungulato pi diffuso in Italia, sia in termini distributivi che di consistenza. Il suo areale si estende per circa 190.000 Km<sup>2</sup>, pari al 64% del territorio italiano. La crescita numerica della specie fa si che numerosi soggetti si trovino frequentemente nei pressi di aree antropiche e di allevamenti di animali da reddito. In questi territori, il cinghiale può facilmente ingerire microorganismi di derivazione umana, ambientale e animale. La colonizzazione intestinale da parte di batteri AMR, spesso derivati da reflui di allevamenti intensivi utilizzati in agricoltura, può comprendere stipti

resistenti ai beta-lattamici, tra i quali hanno grande importanza i microrganismi portatori di geni ESBL (Extended-Spectrum Beta-Lactamase) e di carbapenemasi. Per valutare la presenza di *Escherichia coli* portatori di geni ESBL e di carbapenemasi, 108 stipiti isolati da linfonodi meseraici di altrettanti cinghiali abbattuti nel 2017-2018 in un piano di controllo in provincia di Parma (Ente Parchi e Biodiversità Emilia-Occidentale) sono stati testati per la sensibilità a cefotaxime (CTX), ceftazidime (CAZ) e meropenem (MEM). È stato impiegato il test di Kirby-Bauer facendo riferimento al breakpoint proposto da CLSI (2017) per indicare la possibile produzione di enzimi ESBL e carbapenemasi: CTX <27 mm, CAZ <22 mm, MEM <24 mm. I ceppi resistenti sono stati saggiati mediante prove di sinergia con acido clavulanico e Polymerase Chain Reaction (PCR) per i geni blaSHV, blaCTX-M, blaTEM, blaAmpC, blaKPC, blaNDM, blaVIM, blaIMP, blaOXA-48, blaSPM, blaBIC, blaSIM, blaDIM, blaGIM, blaAIM. Nessun ceppo di *E. coli* è risultato resistente ai carbapenemi. Uno stipite di *E. coli* (WB-21L) - con aloni di inibizione CTX 9 mm e CAZ 14 mm - è stato sottoposto con esito positivo alle prove di sinergia con acido clavulanico. WB-21L è risultato positivo per il gene blaTEM e negativo per gli altri geni testati mediante PCR. Secondo le nostre conoscenze, questo rappresenta il primo isolamento di uno stipite di *E. coli* ESBL da cinghiali abbattuti in Italia. Il gene plasmidico blaTEM codifica per una beta-lattamasi di classe A. La presenza a livello linfonodale di *E. coli* produttore dell'enzima TEM conferma la diffusione al cinghiale di geni ESBL ed è coerente con il fenotipo osservato con il test di sensibilità agli antimicrobici. Il futuro sequenziamento dello stipite WB-21L fornirà indicazioni sulla variante dell'enzima TEM e su eventuali analogie con microrganismi isolati da pazienti umani o da animali da reddito nella stessa area geografica. Le implicazioni sulla salute umana sono evidenti, in quanto prodotti non sottoposti a cottura, quali le salsicce di cinghiale, o la manipolazione delle carni di cinghiale potrebbero diffondere all'uomo batteri commensali provvisti di geni di resistenza facilmente trasmissibili a microrganismi patogeni presenti nel tratto intestinale umano. Un'altra considerazione che si può trarre concerne il ruolo degli animali selvatici quali sensori della contaminazione ambientale da batteri resistenti agli antimicrobici e, in senso lato, da geni portatori di resistenze antimicrobiche.

Boniotti° MB, Papetti° A, Bertasio° C, Giacomini° E, Lazzaro° M, Cerioli° M, Faccini° S, Bonilauri° P, Vezzoli° F, Lavazza° A, Alborali° GL

#### **Porcine epidemic diarrhoea virus in Italy : disease spread and the role of transportation**

Transbound Emerg Dis. - Vol. 65 ( 2018). - p 1935-1942. - 46 bib ref [Nr. Estr. ( ultimo accesso 10/01/2019 ) <https://doi.org/10.1111/tbed.12974> 7919]

Porcine Epidemic Diarrhoea Virus (PEDV) causes watery diarrhoea, dehydration, and a high mortality rate among suckling pigs. Recently, PEDV had a large negative economic impact on the swine industries in Asia and North America. In 2014, PEDV reemerged in many European countries, but most countries only reported a few sporadic cases. Here, we report the epidemic wave that occurred in Italy from 2015 to 2017. During this time, PEDV was detected by real-time PCR in 438 farms located mainly in the high-density pig production area in Northern Italy. Most of the outbreaks were in farrow-to-finish, farrow-to-wean and finisher farms. Clinical signs were observed mainly in suckling and fattening animals, while mortality rates were higher in piglets, reaching 50%. A sequence analysis showed that a PEDV strain, similar to the OH851 S-INDEL strain isolated in the USA in January 2014, was responsible for the outbreaks in Italy in 2015 and 2016. However, from January 2017, a recombinant variant strain, containing a portion of the Swine Enteric Coronavirus in the S1 gene, spread and almost completely outcompeted the previous nonrecombinant strain. In total, 14.1% of the environmental swabs collected from trucks at slaughterhouses after animals were unloaded tested positive for PEDV before the trucks were cleaned and disinfected, and 46% remained positive after cleaning and disinfection processes were performed. Moreover, environmental swabs indicated that 17.3% of the empty trucks arriving at the farms to load animals were PEDV-positive. This study indicates that trucks can have an important role in the spread of PEDV in Italy.

Borella° L, Salogni° C, Vitale° N, Scali° F, Moretti VM, Pasquali P, Alborali° GL

**Antimicrobico-resistenza (AMR) di ceppi mobili di *Aeromonas* spp. isolati da specie ittiche dulciacquicole allevate e selvatiche**

Atti del XXIV Convegno Nazionale SIPI Società Italiana di Patologia Ittica : Torino, 11-13 Ottobre 2018 / [s.l. : s.n., 2018]. - p 48 (Poster P6) [Nr. Estr. 8091]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (24. : Torino : 11-13 Ottobre 2018)

La presenza di residui antibiotici nell'ambiente acquatico può avere serie implicazioni di salute pubblica. Le molecole antimicrobiche possono persistere a lungo nelle acque, sia pur a basse concentrazioni, favorendo in tal modo le condizioni per l'insorgenza di microrganismi antimicrobico-resistenti (AMR). Il problema dell'AMR è un fenomeno globale, complesso e sebbene sia stato ampiamente studiato nei batteri patogeni, meno si sa per quelli ambientali, fra cui gli *Aeromonas* spp. Nelle acque superficiali, la presenza di residui antibiotici è legata sia ai reflui degli allevamenti zootecnici (tra cui quelli ittici) che all'inquinamento urbano. Le specie mobili del genere *Aeromonas* comprendono microrganismi ambientali ubiquitari, patogeni opportunisti di varie specie acquatiche e terrestri, tra cui l'uomo. Nei pesci possono causare forme setticemiche emorragiche, che vengono generalmente tenute sotto controllo con la terapia antibiotica. Lo scopo di questo lavoro è stato quello di indagare l'AMR di *Aeromonas* mobili, isolati da specie ittiche d'acqua dolce, verso 30 molecole diverse di antimicrobici. Sono stati analizzati complessivamente 64 ceppi di *Aeromonas* mobili, isolati da 20 pesci allevati e 44 selvatici, conferiti al Laboratorio di Ittiopatologia della Sezione Diagnostica di Brescia (IZSLER). Tutti i campioni provenivano da allevamenti intensivi o corpi idrici del Nord-Italia, zona caratterizzata da un'elevata densità zootecnica ed urbana. I ceppi sono stati identificati biochimicamente a livello di complesso (macrometodo). La resistenza antimicrobica è stata determinata mediante la tecnica di Kirby-Bauer e il pannello testato comprendeva i seguenti antibiotici: amminosidina (Amn), amoxicillina (Am), amoxicillina/acido clavulanico (AMC), ampicillina (A), apramicina (Ap), cefaloridina (Cf), cefquinome (Cq), ceftiofur (Ct), cloxacillina (Cx), colistina (Cl), danofloxacin (Dn), doxiciclina (Dx), enrofloxacin (En), eritromicina (Er), florfenicol (Ffc), flumequina (Flu), gentamicina (Gn), kanamicina (K), marbofloxacin (Ma), acido nalidixico (AN), neomicina (N), penicillina (P), spiramicina (Sp), sulfadiazina (Su), tetraciclina (Te), tiamfenicol (Tf), tiamulina (Tm), tilmicosina (Tim), trimethoprim/sulfametossazolo (SXT), tilosina (Til). Gli isolati resistenti ad almeno una molecola appartenente a tre o più classi antimicrobiche sono stati considerati multi-resistenti (MDR). L'analisi statistica è stata eseguita mediante il test del chi-quadrato ( $\chi^2$ ) ed il test esatto di Fisher (GraphPad PRISM®, versione 6.05, San Diego, CA, USA). In base ai caratteri fenotipici, il 53.1% (34/64) degli isolati è stato identificato come *A. hydrophila* complex, il 37.5% (24/64) come *A. sobria* complex e il 9.4% (6/64) come *A. caviae* complex. Tutti gli isolati hanno mostrato resistenza verso A, Cx, Sp, e Tm. Alte frequenze di AMR (>80%) sono state osservate verso Am, P, Sf, Tm, e Til. Al contrario, la resistenza vs Amn, Cf, Cl, Dx, En, Ffc, Gn, Ma, Tf è stata osservata a bassi livelli (<10%). Nessun ceppo è risultato resistente a Cq. Tutti gli isolati hanno mostrato un pattern MDR ed *A. caviae* è risultato il complesso con il maggior numero di resistenze per ceppo. Nel campione analizzato non sono emerse differenze significative di AMR fra i complessi di *Aeromonas* mobili. Considerando invece la provenienza dei ceppi (allevata o selvatica), sono state osservate differenze significative di resistenza per 4 molecole ( $P < 0.05$ ): Apr, Sf, Dx, e Te. Eccetto la tetraciclina, nessuno fra i principi attivi registrati nella Comunità Europea per l'uso nei pesci ha mostrato percentuali di resistenza maggiori negli isolati da soggetti allevati, suggerendo un modesto impatto ambientale degli antibiotici autorizzati in acquacoltura. Tuttavia l'ampia diffusione di ceppi MDR verso alcune molecole critiche (macrolidi, chinoloni, fluorochinoloni, polimixine, cefalosporine di 3° e 4° generazione) costituisce un fattore di sicura importanza anche in campo medico umano.

Brocchi° E, Abouchoaib N, El\_Mellouli F, Bugnetti° M, Rosso F, Ripani A, Pezzoni° G, Grazioli° S

**Field trial to estimate the effectiveness of the vaccination program implemented in the Maghreb region**

OS18 "Global vaccine security" : EuFMD Open session : October 2018, Puglia, Italy : online version,



Introduction Routine or emergency vaccination are strategic tools to control FMD. Preliminary estimates of vaccine effectiveness can be obtained by confined field studies, contributing to optimizing control programs. Field trials to evaluate effectiveness of FMD vaccines currently used in the Maghreb region have been designed; here we report the results obtained from the trial conducted in Morocco. Materials and Methods. A bivalent vaccine (A/Eritrea-98 6PD50 and 0-Manisa/0-3039 3PD50) was administered to 20 naïve calves and 20 previously vaccinated cattle. Sera were checked before and 30 days post vaccination (DPV), with an intermediate sampling 5 or 10 DPV for vaccinated and naïve cattle respectively. The level of virus neutralizing (VN) antibodies against the vaccine strains and the field viruses was determined; in addition, sera were titrated using IZSLER ELISA kits. Results In previously vaccinated cattle, vaccination elicited a strong and fast increase (up to 10X, 5 DPV) of neutralizing antibodies, suggestive of protective immunity, with overlapping titres against the two type 0 vaccine strains and the field virus 0-ALG/1/2014 (lineage 0-Ind2001); antibodies had further increased at 30 DPV, reaching average titres of 3 Log 10. Analogous trend was observed for type A, though titres to the vaccine strain were 3-fold higher than those against the field virus A/Algeria/1/2017 (despite both belong to the same lineage N Africa/G-IV). All naïve calves seroconverted from negative to positive after vaccination, but the level of VN antibodies remained lower than in the boost-vaccinated group. The best immune response was observed against the vaccine strain A/Eritrea-98, with 95% animals overcoming the presumed protective threshold, whilst only about 50% achieved sufficient immunity against the other FMDV strains tested. ELISA provided results consistent with VNT for boost-vaccinated cattle, whilst it was less sensitive to detect antibodies in prime-vaccinated calves. Discussion A booster vaccination is necessary to elicit a strong and fast increase of antibodies, cross-neutralizing field circulating viruses. Simple and feasible field trials enable producing relevant information for improving FMD control and preparedness against reoccurrence of outbreaks.

Burioli EAV, Varello K, Lavazza° A, Bozzetta E, Prearo M, Houssin M

**A novel divergent group of Ostreid herpesvirus 1  $\mu$ Var variants associated with a mortality event in Pacific oyster spat in Normandy (France) in 2016**

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<https://doi.org/10.1111/jfd.12883> 8147]

The acute course of disease in young oysters infected by OsHV-1 and the rapid tissue degradation often preclude histological examination of specimens collected during outbreaks in field. Herein, live spat originated from two geographical areas were sampled just at the onset of a mortality event that occurred in Normandy (France) in June 2016. The lesions, associated with high OsHV-1 DNA quantities, were characterized by severe and diffuse haemocytosis mainly involving blast-like cells, myocyte degeneration and large, irregularly shaped degenerate eosinophilic cells in the connective tissue. The herpesvirus was identified by negative staining TEM and real-time PCR. Sequencing of the C region and ORFs 42/43 confirmed that the variants met the definition of OsHV-1  $\mu$ Var. We sequenced 30 other ORFs in twenty OsHV-1-positive individuals and compared them to the  $\mu$ Var specimens isolated between 2009 and 2011. The ORFs encoding putative membrane proteins showed the highest number of variations. Seven different genotypes were identified, confirming the presence of relevant genetic diversity. Phylogenetic analysis provided evidence for a well-separated  $\mu$ Var new group, with an evolutionary divergence estimated at 0.0013 from the other  $\mu$ Var variants. The geographical distribution of these newly described variants and their effective virulence should be investigated in future.

Callegari° E, Bonilauri° P, Carra° E, Rugna° G, Dottori° M, Calzolari° M

**Co-circulation of Leishmania infantum and phleboviruses from sandflies in the Bologna**

## province, Italy

ECE 2018 : XI European Congress of Entomology : 2-6 July 2018, Napoli : book of abstracts / [s.l. : s.n., 2018]. - p 281 (Poster PO410) [Nr. Estr. 7991]

European Congress of Entomology (11. : Napoli : 2-6 July 2018)

Sandflies are tiny insects, often living close to domestic animals and humans. Females of these insects are hematophagous and their bites occur especially at night. Sandflies are poor fliers, flying silently for short distances, and with activity peaks during summertime. Sandflies are widely distributed in peri-Mediterranean countries and are vectors of many pathogens, particularly, protozoa of the genus *Leishmania* and some arthropod-borne viruses of the genus *Phlebovirus*. These pathogens often show an epidemiological link, sharing the same sandfly vectors: *Phlebotomus perniciosus* and *Phlebotomus perniciosus* are able to transmit *Leishmania infantum* and Toscana virus (TOSV) in Italy. The co-circulation of phleboviruses and *L. infantum* was detected by specific Real-Time PCRs in sandflies sampled in two sites localized in Valsamoggia municipality (BO), Emilia-Romagna region, Italy. Sandflies were sampled with attractive carbon dioxide traps from June 2017 to September 2017. A total of 28,283 sandflies were collected (site-1: 19,441, site-2: 8,842), about 5% of these were identified at species level of the genus *Phlebotomus* achieved by morphological identification of males: *P. perniciosus* (1,358, 98%) and *P. perniciosus* (30, 2%). One hundred ninety-four pools of 50 females were tested; *Leishmania* was detected in 60 pools (site-1: 38, site-2: 22), while, 63 pools tested positive for phleboviruses (site-1: 40, site-2: 23). Interestingly, 16 of these pools were positive for both protozoa and viruses. Amplicons obtained by the pan-phlebovirus PCR were sequenced and were ascribable to: Corfu virus (2), Fermo-like virus (40), Ponticelli virus (7) and TOSV (4). In conclusion, co-circulation of *Leishmania infantum* and phleboviruses was confirmed by their contemporary presence in the surveyed sites. Their coexistence was likely due to a common vector, *Phlebotomus perniciosus*, strongly incriminated as principal vector for its abundance in that area. Among phleboviruses only TOSV is known as human infectious agent, while the potential pathogenicity of other phleboviruses must be investigated.

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Calò° S, Vitale° N, Formenti° N, Chiari° M, Faccin° F, Tamba° M, Pannicià M, Morelli A, D'Alessio N, Guberti V, Zanoni° M, Moreno° MA

### **Epidemiological analysis of Aujeszky disease in wild boar in Italy**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 20 [Nr. Estr. 8007]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

Aujeszky's disease (AD) is a notifiable infection that causes substantial economic losses to the swine industry and has major economic impact due to trade implications and income losses for farmers. Pseudorabies virus (PRV), which belongs to the family of Herpesviridae, causes AD. Thus, the European general policy is to eradicate AD in order to support free intra-EU trade. In Italy, an AD national monitoring program was implemented in 1997. Extraordinary regional control plans were also applied in the Northern Italian regions with the highest concentrations of pig industry; these regions have recently been included in annex two of the EU Decision 2008/185/EC. PRV may infect a wide spectrum of mammals, although domestic and wild Suidae are the only hosts that can act as reservoir. Although PRV has been eliminated in domestic pigs in many European countries, AD is being continuously reported in wild boar populations; consequently, their possible impact on the application and success of AD eradication programs and the risk they pose to the PRV-free status should be taken into account. During 2016/2017 hunting season, a cross sectional study was conducted in order to evaluate the seroprevalence of AD in wild boar populations in different ecological areas in Italy. Overall 226 sera samples were collected from 245 hunted wild boars from four Italian ecological areas: 53 were collected from Alps, 25 from Northern-Apennine, 65 from Central-Apennine and 83 from Southern-Apennine. Sera samples were tested for anti-gE antibodies

using an ELISA kit. In addition, variables regarding wild boars (age; sex) and hunting area were recorded. An overall seroprevalence of 28.32% (64/226) emerged. The seroprevalence was respectively 13.21% in Alps (7/53 95% CI: 5.48% - 25.34%), 56.00% in Northern-Apennine (14/25 95% CI: 34.93% - 75.60%); 41.54% in Central-Apennine (27/65 95% CI: 29.44% - 54.44%), 19.28% (16/83 95% CI: 11.44% - 29.41%) in Southern-Apennine. The difference between seroprevalence in the four areas is statistically significant ( $\chi^2=24.338$ ,  $p$ -value < 0.001). Statistical analyses showed that seroprevalence was associated by gender ( $\chi^2=4.534$ ,  $p$ -value=0.033) and age ( $\chi^2=27.236$ ,  $p$ -value < 0.001); in particular seroprevalence was higher for females (31.96% 31/97) than males (18.07% 15/83); and it was higher for adult wild boars (43.48% 20/46) than young (0% 0/2) and sub-adult (14.71% 5/34). However, including all factors in a multivariate logistic regression models, only location resulted statistically significant (LRT = 24.079,  $p$ -value < 0.001), as age and gender dependent on ecological areas. Results of this study suggest that different ecological areas show different pattern of AD circulation, the structure of wildlife population may be influence the dynamics of AD transmission. The highest seroprevalence was recorded in areas with intense wild boar management. The epidemiology in wild boars should be continuously monitored and assessed. This study was funded by a National Research Project PRC2015001.

Calonzi D, Cremonesi P, Monistero V, Moroni P, Romano<sup>o</sup> A, Luini<sup>o</sup> MV, Castiglioni B

#### **Development of multiplex PCR assays for the molecular characterization of *Streptococcus uberis* strains isolated from bovine mastitis**

The 2018 International Bovine Mastitis Conference : Milano, June 11-13, 2018 / [s.l. : s.n., 2018]. - p 163-164. - 15 bib ref [Nr. Estr. 7998]

International Bovine Mastitis Conference (NMC) : Milano : June 11-13, 2018)

*Streptococcus uberis* is an important environmental udder pathogen in the modern dairy industry. It ranks among the most important causes of mastitis in countries around the world, including Australia, Brazil, Canada, Netherlands, New Zealand, United Kingdom and United States (Zadoks et al., 2011). *S. uberis* has a great ability to survive in cow habitat (soil, water, hay, faeces) and cattle are constantly exposed to risk of infections (Zadoks et al., 2005). This spreading ability could be due the capsulation of the bacteria which is coded by *hasA* (hyaluronate synthase A), *hasB* (UDP-glucose dehydrogenase), and *hasC* (UDP-glucose pyrophosphorylase) genes. (Reinoso et al., 2017). Indeed, *hasA* positive bacteria could be associated with clinical mastitis, while *hasA* negative strains were correlated with subclinical cases (Pullinger et al., 2006). Among virulence factors, *S. uberis* adhesion molecule (SUAM), encoded by *sua* gene, play a key role in the invasion of mammalian epithelial tissue, due to its high affinity for lactoferrin, a protein required for iron assimilation (Almeida et al., 2006). Further, the plasminogen activator A (*PauA*), responsible for degradation of milk proteins into small peptides and free amino acids useful for the growth (Loures et al., 2017), was considered from several authors as potential target for the development of new vaccine therapy due to its high prevalence in *S. uberis* population (Perrig et al., 2015; Leigh et al., 1999). The knowledge of the distribution of virulence genes is important to understand the infectivity and the pathogenesis of *S. uberis*. To date, molecular characterization has improved our knowledge of epidemiology of *S. uberis* identifying molecules that have roles in the establishment of the intra-mammary infection (Loures et al., 2017). Therefore, the aim of this study was to develop two multiplex PCR assays for the simultaneous detection of 10 virulence factors of *S. uberis*, useful both for a rapid characterization of *S. uberis* strains isolated from bovine milk and for epidemiological studies. The mPCR assays were called reaction 1 and reaction 2. The first one included: *tuf*, *cpn60* (Dmitriev et al., 2006), *pauA*, *sodA*, *sua*, *oppF* and *gapC* genes, while reaction 2 amplified the three genes responsible for the synthesis of capsule, i.e. *hasA*, *hasB* and *hasC*. All primer pairs, apart those for *cpn60* gene, were designed using PRIMER3 (<http://primer3.ut.ee/>) and their specificity was in silico and in vivo evaluated. *S. uberis* ATCC 9927 was used as reference strain for the development of mPCR assays and genomic DNA was extracted from pure cultures as described by Cremonesi et al. (2006). Once the specificity of the primer pairs has been determined, both the PCR conditions and the buffer and primer concentrations were optimised in order to combine the primers into the two multiplex PCR reactions, without affecting the ability of the primer pairs to generate

gene specific amplicons. Afterwards, the limit of detection (LoD) for each mPCR assay was determined using 2-fold serial dilution of the genomic DNA extracted from the reference strain, starting from 48 ng/pL of the DNA template. Finally, to check the reliability of the two mPCR assays, we screened 33 pure cultures of *S. uberis* strains isolated from bovine mastitis milk. All *S. uberis* strains have been previously identified by colony morphology, Gram stain, catalase reaction and esculin hydrolysis. Further, all streptococcal isolates were identified to the species level by the API 20 Strep. The mPCR assays were successfully optimized and the desired amplicons were obtained in both reactions. In reaction 1, it is possible distinguished 7 bands with size of 143 bp, 205 bp, 280 bp, 350 bp, 369 bp, 400 bp and 505 bp, corresponding to *tuf*, *pauA*, *soda*, *sua*, *cpn60*, *oppF* and *gapC* gene, respectively. While in reaction 2, three bands size of 193, 400, 599 bp were obtained, corresponding to *hasC*, *hasB* and *hasA*, respectively. All of the bands were well differentiated on gel. The sensitivity of the two mPCR reactions was comparable with other studies (Wang et al., 2015; Cremonesi et al., 2005): 12 pg and 6 pg of template DNA for reaction 1 and 2, corresponding approximately to  $12 \times 10^{(1)}$  CFU/ml e  $6 \times 10^{(1)}$  CFU/ml, respectively. Phuektes and co-workers (2001) reported 50 pg of DNA as threshold of detection of the multiplex PCR assay set up for the identification of *S. uberis*, while Shome and co-workers (2011) developed a really sensitive mPCR reaching 10 fg as LoD. We found that the most common pattern of virulence-associated genes was *hasA+* *hasB+* *hasC+* *tuf+* *cpn60+* *pauA+* *soda+* *sua+* *oppF+* *gapC+* (82%, 27 out of 33). Moreover our results showed the high presence of *pauA* (97%, 32 out of 33) and *sua* (91%, 30 out of 33) genes in the *S. uberis* strains analysed, confirming the data previously reported (Loures et al., 2017; Perrig et al., 2015). To our knowledge only few studies described mPCR assays developed for the molecular characterization of the *S. uberis* strains. Therefore, owing to specificity, rapidity and ease of use, the mPCR methods described in this study could be a powerful tool for studying the virulence and pathogenicity of *S. uberis* isolates. In conclusion, our mPCR assays will be of value in the dairy sector for *S. uberis* mastitis diagnosis and in epidemiological investigations.

Caltagirone M, Mattioni\_Marchetti V, Mercato A, Spalla M, Nucleo E, Scarsi° G, Prati° P, Fabbi° M, Migliavacca R

#### **Presenza di Escherichia coli MDR in campioni di origine animale destinati al consumo umano**

XLVII Congresso Nazionale AMCLI : Rimini, 10-13 Novembre 2018 / [s.l. : s.n., 2018]. - 1 p. (Poster P053) [Nr. Estr. 8134]

Congresso Nazionale AMCLI (47. : Rimini : 10-13 Novembre 2018)

**INTRODUZIONE** La resistenza agli antimicrobici rappresenta un problema di sanità pubblica e veterinaria. Recenti studi ipotizzano che alimenti di origine animale possano agire da vettore nel trasferimento di batteri multi-resistenti (MDR) all'uomo. Scopo dello studio è stato caratterizzare a livello fenotipico/molecolare ceppi di *E. coli* isolati da animali da allevamento destinati al consumo umano. **METODI** Nel periodo Dicembre 2017-Agosto 2018 sono stati raccolti, presso l'IZSLER di Pavia, 28 ceppi di *E. coli* con fenotipo MDR mediante Metodo Kirby-Bauer. Gli stipiti, inviati all'Unità di Microbiologia per la caratterizzazione molecolare, provenivano da 25 vitelli e 3 suini da allevamenti del Nord-Ovest Italia. Identificazione e sensibilità agli antibiotici sono stati ripetuti mediante Autoscan4 (Beckman Coulter) ed interpretati secondo breakpoint clinici e cut-off epidemiologici EUCAST 2018. Test fenotipico ES13L+AmpC Screen Kit (Rosco Diagnostica), microarray Check-MDR CT103XL (Check-Points) ed amplificazione genica hanno confermato la presenza di ESCA. I geni responsabili di resistenza a fluorochinoloni, aminoglicosidi e colistina (CO) (*QnrA*; *aac*-(6')-Ib-cr; *Arm-A*; *rmtB/C* e *mcr*) sono stati ricercati mediante PCR. La caratterizzazione plasmidica è stata ottenuta con PBRT Kit (Diatheva) e la tipizzazione condotta mediante PFGE e ricerca del gruppo filogenetico. **RISULTATI** I 28 ceppi di *E. coli* provenivano da differenti campioni biologici: 17/28 feci, 10/28 biopsie tessutali (7 da intestino; 1 da fegato; 1 da muscolo; 1 da polmone) ed 1/28 latte mastitico. Il profilo di antibiotico resistenza era il seguente: 78,6% isolati resistenti a trimetoprim/sulfametossazolo; 64,3% a fluorochinoloni e cloramfenicolo; 57,1% ad aminoglicosidi; 39,3% ad aztreonam e cefalosporine; 31% ad amoxicillina/clavulanato, 14,3% a CO e 100% sensibili a carbapenemi, fosfomicina e tigeciclina. La produzione di ESRL è stata confermata in 10/28 isolati positivi per la presenza dei geni: *bla*<sub>aux\_m</sub> in 8/10 (97 hla \_\_\_0-x-m-i e 12,5% *bla*<sub>crx\_m\_9</sub>), *bla*<sub>fiv-5</sub> in 1/10 e *bla*<sub>crx\_m\_i</sub>+*bla*<sub>crmy\_2</sub> in 1/10 isolati. 4/17 ceppi resistenti agli

aminoglicosidi sono risultati inoltre positivi per la presenza del gene *armA* ed 1/17 per *aac*-(6')-Ib-cr. L'analisi del profilo plasmidico ha evidenziato la presenza di diversi gruppi di incompatibilità. Gli isolati appartenevano a differenti cloni PFGE ed ai gruppi filogenetici A, B1 e D. **CONCLUSION!** I risultati ottenuti confermano come gli animali da allevamento rappresentino una possibile fonte di diffusione di microrganismi MDR. Da qui l'importanza di investigarne la prevalenza in ulteriori matrici animali, vegetali ed ambientali.

Calzolari<sup>o</sup> M, Callegari<sup>o</sup> E, Canziani<sup>o</sup> S, Corradi<sup>o</sup> M, Baisi<sup>o</sup> C, Lelli<sup>o</sup> D, Bonilauri<sup>o</sup> P  
**Anopheles mosquitoes of the Maculipennis complex in Emilia-Romagna and Lombardy (Northern Italy)**

ECE 2018 : XI European Congress of Entomology : 2-6 July 2018, Napoli : book of abstracts / [s.l. : s.n., 2018]. - p 91 (CO277) [Nr. Estr. 7985]

European Congress of Entomology (11. : Napoli : 2-6 July 2018)

After the Malaria eradication in Italy, the interest in characterization the Anopheline fauna has progressively decreased, and nowadays, the knowledge of the distribution of these mosquitoes is largely fragmentary. If mosquito vectors are present, autochthonous Malaria cases are still possible the arrival of infected people, as demonstrated by the recent outbreak in Greece or the autochthonous cases in Puglia during 2017. The definition of Anopheline fauna is hampered by the presence of complex of sibling species difficult to distinguish on a morphological basis. The Maculipennis complex is one of these groups, which includes primary Malaria vectors, and other species considered secondary or irrelevant vectors. Seven species of the complex are reported in Italy: *Anopheles labranchiae*, *An. sacharovi*, *An. atroparvus*, *An. maculipennis* ss, *An. melanoon*, *An. messeae*, *An. subalpinus*. The aim of this study is to define Maculipennis complex species present in studied area. *Anopheles* mosquitoes were directly collected (mainly by manual aspiration in resting sites) or retrieved from the entomological surveillance programs (particularly WNV surveillance plans). To preserve the body, DNA was extracted from two legs and submitted to ITS2 and COI PCRs and the obtained products were sequenced for molecular identification. Morphometry was applied to wings, in attempt to discern the different species preserving mosquitoes, which can thus be addressed to molecular analyses after species identification. More than 10,000 *Anopheles* mosquitoes were sampled, mainly in 2017. 420 specimens were identified by biomolecular analysis, of which 353 from Emilia-Romagna and 67 from Lombardy. The most abundant species was *An. messeae* (333 specimens), followed by *An. maculipennis* ss (78 specimens), more abundant in the western part of the surveyed area. Moreover 9 specimens of *An. atroparvus*, a good vector of Malaria, were identified. These preliminary results demonstrate that *Anopheles* mosquitoes, potentially able to transmit Malaria, are still present in Northern Italy.

Calzolari<sup>o</sup> M, Chiapponi<sup>o</sup> C, Bellini R, Bonilauri<sup>o</sup> P, Lelli<sup>o</sup> D, Moreno<sup>o</sup> A, Barbieri<sup>o</sup> I, Pongolini<sup>o</sup> S, Lavazza<sup>o</sup> A, Dottori<sup>o</sup> M

**Isolation of three novel reassortant phleboviruses, Ponticelli I, II, III, and of Toscana virus from field-collected sand flies in Italy**

Parasites & Vectors. - Vol. 11 ( 2018). - no 84 (11 p). - 40 bib ref [Nr. Estr. ( ultimo accesso 09/03/2018 ) <https://doi.org/10.1186/s13071-018-2668-0> 7806]

Background: Different phleboviruses are important pathogens for humans; most of these viruses are transmitted by sand flies. An increasing number of new phleboviruses have been reported over the past decade, especially in Mediterranean countries, mainly via their detection in sand flies. Results: At least five different phleboviruses co-circulated in sand flies that were collected in three sites in EmiliaRomagna (Italy) in the summer of 2013. The well-known Toscana virus (TOSV) was isolated; three new, closely related phleboviruses differing in their M segments and tentatively named Ponticelli I, Ponticelli II and Ponticelli III virus, respectively, were isolated; a fifth putative phlebovirus,

related to the sand fly fever Naples phlebovirus species, was also detected. The co-circulation, in a restricted area, of three viruses characterized by different M segments, likely resulted from reassortment events. According to the phylogenetic analysis of complete genome sequences, the TOSV belongs to clade A, together with other Italian isolates, while the Ponticelli viruses fall within the Salehabad phlebovirus species. Conclusions: Results highlight an unexpected diversity of phleboviruses that co-circulate in the same area, suggesting that interactions likely occur amongst them, that can present challenges for their correct identification. The co-circulation of different phleboviruses appears to be common, and the bionomics of sand fly populations seem to play a relevant role. Such a complex situation emphasizes the need for detailed investigations of the biology of these viruses to better characterize their pathogenic potential for mammals, including humans.

Calzolari° M, Rugna° G, Clementi E, Carra° E, Pinna° M, Bergamini° F, Fabbi° M, Dottori° M, Sacchi L, Votýpka J

**Isolation of a Trypanosome related to *Trypanosoma theileri* (Kinetoplastea: Trypanosomatidae) from *Phlebotomus perfiliewi* (Diptera: Psychodidae)**

BioMed Res Int. - Vol. 2018). - Article ID 2597074 (8 p). - 33 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1155/2018/2597074> 7888]

The *Trypanosoma theileri* group includes several trypanosome species hardly distinguishable due to the lack of discriminating morphological characters. Trypanosomes belonging to this group have been isolated from different bovine, ovine, and cervids in Europe, Africa, Asia, and Americas. The principal vectors of the *T. theileri* group are considered tabanid flies; however, *T. melophagium* is transmitted exclusively by sheep keds. In 2016, 128 sand flies out of 2,728 trapped in Valsamoggia municipality, Italy, were individually dissected and an unknown trypanosome strain, named TrPhp1, was isolated from a female of the sand fly *Phlebotomus perfiliewi*. Sequence analysis placed this trypanosome in the *T. theileri* group with very high homology to other trypanosomes detected in European cervids. This is the first report of the *T. theileri* group isolation from a sand fly, and the possible role of this insect group in the trypanosome transmission cycle is discussed. Within the *T. theileri* group, the phylogenetic analysis distinguished several lineages, which, unfortunately, do not correspond with their host specificity and their taxonomic status remains ambiguous.

Campanini G, Rigamonti° S, Paolucci S, Prati° P, Sarasini A, Vicari° N, Fratini A, Peli° S, Isernia P, Pezzoni° G, Antonini GC, Carughi P, Baldanti F, Fabbi° M, Marone P

**Circolazione di HEV nel parco lombardo della valle del Ticino : un approccio "one health"**

XLVII Congresso Nazionale AMCLI : Rimini, 10-13 Novembre 2018 / [s.l. : s.n., 2018]. - 1 p. (Poster 214) [Nr. Estr. 8133]

Congresso Nazionale AMCLI (47. : Rimini : 10-13 Novembre 2018)

INTRODUZIONE Nei paesi sviluppati, due genotipi del virus dell'epatite E (HEV), HEV-3 e HEV-4, infettano sia l'uomo che diverse specie animali dove suini e cinghiali rappresentano il maggiore reservoir. Il consumo della carne poco cotta o insaccati freschi rappresentano la via di trasmissione più frequente, benché alcune categorie di lavoratori esposte al contatto con carni o escrementi di animali infetti abbiano un rischio elevato di contrarre l'infezione. L'obiettivo dello studio è stato l'analisi epidemiologica nell'uomo e nel cinghiale nell'area geografica del Parco Lombardo della Valle del Ticino che si estende nelle provincie di Milano, Varese e Pavia. METODI Dal 2016 al 2017 sono stati testati 3008 sieri di cinghiali per la presenza di anticorpi IgG (HEV IgG ELISA, HM). 1156 fegati e contenuti biliari di cinghiali abbattuti nell'ambito del piano di monitoraggio regionale della fauna selvatica sono stati esaminati per la presenza di HEV-RNA mediante amplificazione della regione ORF3 mentre per la genotipizzazione è stato sequenziato un frammento della regione

ORF2. Sono stati inoltre testati per la presenza di IgG ed IgM anti-HEV (HEV IgM/IgG ELISA, DIA.PRO) i sieri di 40 dipendenti del Parco e di 120 donatori di sangue (gruppo di controllo).  
RISULTATI Dallo studio è emerso che: i) la prevalenza di anticorpi IgG anti-HEV nei cinghiali 6 del 8.7%; ii) 43/1156(3,7%) campioni di fegato/bile di cinghiali abbattuti sono risultati positivi per HEV-RNA e appartenenti tutti al genotipo 3.; iii) il 10% del sieri dei dipendenti del Parco 6 risultò positivo per IgG anti-HEV; iv) i dipendenti del Parco risultati positivi svolgevano mansioni (es. Guardia Parco) "a rischio" di contatto con escrementi o visceri di animali potenzialmente infetti; v) nel gruppo di controllo solo 11,7% dei donatori e risultato positivo per IgG anti HEV. CONCLUSIONI Il dato di sieroprevalenza (8.7%) di HEV nei cinghiali del Parco risulta lievemente superiore ai dati relativi al nord Italia del 2015 ma nettamente inferiore ai valori ottenuti nel centro Italia. Il riscontro (3,7%) di HEV-RNA nei cinghiali è in linea con quello del nord Italia e nettamente inferiore a quello di centro e sud Italia. Il dato di sieroprevalenza nella popolazione "a rischio" risulta in linea con quello italiano e del Nord Italia recentemente pubblicato, mentre risulta notevolmente più basso quello nella popolazione sana rispetto ai dati pubblicati della Lombardia e della Provincia di Pavia..

Canelli E, Catella A, Borghetti P, Ferrari L, Ogno G, De\_Angelis E, Bonilauri° P, Guazzetti S, Nardini R, Martelli P

**Efficacy of a modified-live virus vaccine in pigs experimentally infected with a highly pathogenic porcine reproductive and respiratory syndrome virus type 1 (HP-PRRSV-1)**

Vet Microbiol. - Vol. 226 ( 2018). - p 89-96. - 43 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.vetmic.2018.10.001> 8019]

PRRS is one of the main viral diseases in pig production, causing huge economic losses to the swine industry worldwide. The virus shows an intrinsic genomic instability and is able to change continuously, with the emergence of new strains, with different pathogenicity patterns. Commercially available vaccines only partially prevent or counteract the disease and the correlated losses. Moreover, the emergence of highly virulent and pathogenetic isolates represents a particular concern for PRRS control and diagnosis. The purpose of this study was to evaluate the efficacy of a modified-live virus (MLV) PRRSV-1 commercial vaccine in reducing the severity of the disease and minimizing losses upon challenge with a highly pathogenic PRRSV-1.1 Italian isolate (PRRSV1\_PR40/2014). Four different groups were compared: C (unvaccinated-uninfected), VAC-C (vaccinated-uninfected), PR40 (unvaccinated-infected) and VAC-PR40 (vaccinated-infected). The tested vaccine provided partial, but statistically significant clinical, virological and pathological protection after challenge under experimental conditions. In particular, vaccinated animals showed reduced viremia in terms of duration and magnitude, reduced respiratory signs and pathological lesions. Vaccination was able to trigger adaptive immunity able to respond efficiently also against the HP PR40 isolate. Vaccinated animals showed higher average daily weight gain, even during the viremic period, compared to non-vaccinated challenged pigs.

Cannistrà° M, Capelli° G, Garbarino° CA, Meriardi° G, Gandolfi P, Idropici E, Arrigoni° N

**On farm culture for bovine mastitis : a rapid tool to reduce the use of antimicrobials**

The 2018 International Bovine Mastitis Conference : Milano, June 11-13, 2018 / [s.l. : s.n., 2018]. - p 208 [Nr. Estr. 7997]

International Bovine Mastitis Conference (NMC) : Milano : June 11-13, 2018)

In the scientific community and in public opinion, there is strong concern about the development of antimicrobial resistance. In order to counteract the onset of this phenomenon in livestock, the "European guidelines for the prudent use of antimicrobials"(1) were published. These Guidelines recommend the use of antibiotic therapy in accordance with the indications of use, only for therapeutic purposes and following specific diagnosis. Moreover, the same Guidelines recommend

the use of rapid diagnostic tests to be used directly on the farm. Here we report the results of an on-farm culture (OFC) protocol, for rapid diagnosis of clinical mastitis which is the main cause of antibiotic use in dairy cattle breeding. The protocol based on the use of Tr-Plates with selective media for *Streptococcus* spp, *Staphylococcus* spp and Coliforms, was applied to 5 herds for a period of one year. Based on the result of OFC, antibiotic therapy was avoided in 61% of cases of clinical mastitis (ranging from 47% to 77% in the 5 herds), with a significant economic advantage for breeders, linked to lower milk loss and lower purchase of antimicrobials. 1-Commission notice (2015). Guidelines for the prudent use of antimicrobials in veterinary medicine; Official Journal of the European Union, 2015/C 299/04 Funded by "Rural development programme 2014-2020 — Operation 16.1.01 — Working group of European Innovation Partnership for "Agricultural productivity and Sustainability" — Focus Area 2A - Project "Integrated approach aimed at reducing the use of antimicrobial in milk production for regional PDO cheese, contributing to reduce the risk of antimicrobial-resistance occurrence".

Capelli° G, Cannistra° M, Scali° F, Merialdi° G, Garbarino° CA, Bertocchi° L, Alborali° GL, Trevisi P, Motta V, Salvarani C, Arrigoni° N

### **Risk assessment for animal welfare, biosecurity and antimicrobial use (AMU) in 63 Italian dairy herds**

The 2018 International Bovine Mastitis Conference : June 11-13, 2018 : Milano / [s.l. : s.n., 2018]. - p 209. - 3 bib ref [Nr. Estr. 7914]

International Bovine Mastitis Conference (NMC) : Milano : June 11-13, 2018)

In the Italian livestock farming system there is an increasing concern about antimicrobial resistance and the need to counteract its onset through the prudent use of antimicrobials. A rational approach to antimicrobial use (AMU), the improvement of biosecurity and animal welfare management are considered crucial key points. In order to evaluate AMU, the conditions of animal welfare and biosecurity, a survey was carried out on 63 dairy herds supplying milk to three dairies producing DOP hard cheese (Parmigiano Reggiano and Grana Padano). For each farm the following data related to 2016 were collected: milk quality, production data, pharmacological treatments. A risk assessment for animal welfare and biosecurity was carried out in each farm, according to the method developed by the National Reference Centre for Animal Welfare (CRenBA)(1) of Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER). The data on the pharmacological treatments were analyzed by a program developed by IZSLER("Classyfarm") for the calculation of the "Defined Daily Doses for Animals in Italy" (DDDAit) in each category of production (calves, heifers, adult cows), following similar principles regarding "Defined Daily Dose for animals" (DDDvet) set by EMA(2), but based on Italian summaries of product characteristics and available literature(3). Regarding the administration route, 40.27% of the antimicrobials used in cows were injectable, 32.51% intramammary for lactating cows, 21.48% intramammary for dry treatment, 5.70% intrauterine and 0.03% oral products. Moreover, this evaluation allowed to point out the consumption of Highest Priority Critical Antimicrobials (HP CIA)(4), for which a strong reduction of use is recommended. The incidence of HP CIA on total antimicrobial consumption ranged from 0% to 100% in each herd, both for cows and calves. The consumption rate of HP CIA in respect to the overall AMU in adult cows was 26.85% (cephalosporins of 3-4 generations 19.08%; fluoroquinolones 4.21%; macrolides 3.52%; colistin 0.04%) and 71.32% in calves (colistin 49.53%; macrolides 7.4%; fluoroquinolones 14.39%). Regarding animal welfare rate, 76% of loose housing herds and 81% of tie-stall herds reached the acceptable level (60% of the maximum score) defined by CRenBA, but none reached the optimal value (80%). The welfare values ranged from 50.10% to 79.29% in the 63 herds, with an average value of 66.65%. Regarding biosecurity rate, 67% of herds attained the acceptable level (33% of the maximum score) defined by CRenBA but only one herd reached the optimum level (66%). Biosecurity values ranged from 13.40%, to 72.62% in the 63 herds, with an average value of 37.32%. The collected data have been stratified for farm dimensions and for housing system in order to evaluate the statistical correlations between animal welfare, biosecurity and antimicrobial use. The results showed that for the loose housing herd, in the farms with a range between 20-119 cows the biosecurity level was positively correlated with the antimicrobial administration (DDD); in medium range farms ( n= 125-240) a negative correlation between welfare



and biosecurity levels with somatic cells counts and total bacteria count were observed. Moreover, in both medium and large farms (N= 264-665) the antimicrobial administration in calves was positively correlated with the total number of cows. No clear correlations were observed for tie-stall herds.

Capelli° G, Cannistrà° M, Scali° F, Meriardi° G, Garbarino° C, Bertocchi° L, Alborali° GL, Trevisi P, Motta V, Salvarani C, Diegoli G, Arrigoni° N

#### **A practical approach for reducing antimicrobial usage in dairy herds**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 94. - 2 bib ref [Nr. Estr. 7977]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

**Introduction** To counteract the phenomenon of antimicrobial resistance, the European Union recommended the adoption of measures aimed at reducing drug usage in the zootechnical sector (1). In Emilia Romagna, the project "Integrated approach aimed at reducing the use of antimicrobial in milk production for regional PDO cheese, contributing to reduce the risk of antimicrobial-resistance occurrence", has been developed using a "benchmarking approach", based on periodic data collection, ongoing interactive discussion and training of breeders and veterinarians. We report the preliminary results of the first year of the project below. **Methods** In 63 dairy herds of Emilia Romagna, conferring milk to three different cheese processors (2 of Parmigiano Reggiano and 1 of Grana Padano), data on antimicrobial consumption (in DDDAit, Defined Daily Dose Animal for Italy) of two consecutive years (2016 - 2017) were monitored. Data concerning risk assessment for animal welfare and biosecurity (CRENBA method) and zootechnical data were also collected, using an integrated monitoring system. The data on antimicrobial consumption (total consumption and HP CIAs, Highest Priority Critically Important Antimicrobials) were presented in individual reports to each farmer in periodic meetings, involving the bovine practitioners. In the same report, each farmer also received a list of risk factors regarding animal welfare and biosecurity, with suggestions for improving the situation of the herd (2). In 6 partner farms, rapid diagnostic tools (on farm culture) were also introduced, aimed at limiting the use of antimicrobials for mastitis therapy. **Results** The data on antimicrobials consumption showed a decrease in 2017 compared to 2016. In particular: in calves we observed a 21.3% decrease of total DDDAit and a 53.3% decrease of HPCIAAs. In cows we observed a slight decrease (0.6%) of total DDDAit but a larger HPCIAAs decrease (23.5%). In the 6 partner farms the reduction was more consistent. Regarding animal welfare rate, 75.6% of loose housing herds and 80.7% of tie-stall herds reached the level defined as acceptable by CRENBA method. Regarding biosecurity rate, 66.7% of herds attained the acceptable level. **Conclusions** Although there is room for improvement, the Project, still ongoing, is achieving its goal. The awareness actions and the training carried out in collaboration with farmers and practitioners, have led to a total reduction of antimicrobial consumption, in particular of HPCIAAs.

Caporali C, Albanese F, Vicari° N, Dawidowicz J, Mancianti F, Abramo F

#### **Pathology in practice**

J Am Vet Med Assoc. - Vol. 252 no 1 ( 2018). - p. 57-60. - 12 bib ref [Nr. Estr. 7753]

Capucchio MT, Salogni° C, Biasibetti E, Grossi C, Pazzaglia M, Chiappino L, Sereno A, Alborali° LG, Guarda F

#### **Endocardiosis of valvular complex in aging sturgeons (Acipenser spp.) : first report**

72° Convegno Nazionale della Società Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 228. - 6 bib ref [Nr. Estr. 7965]

Convegno Nazionale della Società Italiana delle Scienze Veterinarie (SISVET) : 72  
Convegno SICV : 18  
Convegno SIRA : 16  
Convegno AIPVet : 15  
Convegno ARNA : 10  
Convegno RNIV : 5  
Convegno ANIV : 2  
Convegno SICLIM-VET : 1  
Giornata studio AIVI  
Giornata studio SOFIVET :

Valvular endocardiosis is a degenerative process characterized by matrix proliferation and degeneration affecting cardiac valves. It is a common age-related lesion in animal and human species [1,2,3]. A limited documentation of fish valvular lesions is available [4] and to date no data have been reported on *Acipenser* species. The adult sturgeon heart is formed by sinus venosus, atrium, ventricle and outflow tract (conus arteriosus and bulbus arteriosus). Valve systems appear at the sinoatrial and atrioventricular junctions and in the conus arteriosus [5,6]. The present study represents the first description of morphological features of valvular endocardiosis in *Acipenser* spp. A total of 54 specimens of *Acipenser* spp. were collected in an Italian fish farm and divided into three groups according to age and body weight: group 1 (G-1), 2-5 years, 0.5-2 kg, male, *A. baerii* and *A. transmontanus* (13 animals); group 2 (G-2), 6-10 years, 6-9 kg, *A. baerii* (5 male), *A. transmontanus* (6 female); group 3 (G-3), 12-16 years, 27-60 kg, female, *A. gueldenstedtii* and *A. transmontanus*, 30 animals. Hearts were fixed in 10% buffered formalin solution and submitted to macroscopical evaluation. Samples of valve systems were processed for histological evaluation, embedded in paraffin and stained with Haematoxylin & Eosin, Weigert Van Gieson, Toluidine Blue and Alcian Blue stains. Valve lesions were scored on a 0 to 3 scale on the basis of the severity. Data were analyzed by Shapiro-Wilk normality test and Mann-Whitney U test ( $P < 0.05$ ) by means of GraphPad Prism® software. Valvular endocardiosis affected all the animals of the group 3. Particularly severe lesions characterized by verrucous proliferation with distortion of the valves and increase of myxomatoid Alcian positive matrix affected the valves at the bulboventricular junction. Fish of the group 1 and 2 generally showed no endocardiosis or low grade lesions; in particular atrioventricular valves were always less affected. All valves of group 3 showed a statistically significant increase of severity. The presence of most severe lesions in aged animals permits to consider endocardiosis an age-related lesion as observed in other species. A systematic study on a larger number of young fish is ongoing to confirm these preliminary results.

Capucchio MT, Salogni° C, Colombino E, Chiappino L, Sereno A, Grossi C, Pazzaglia M, Alborali° GL, Guarda F

#### **Patologia delle arterie coronarie dello storione allevato : ricerche preliminari**

Atti del XXIV Convegno Nazionale SIPI Società Italiana di Patologia Ittica : Torino, 11-13 Ottobre 2018 / [s.l. : s.n., 2018]. - p 41 (O26) [Nr. Estr. 8093]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (24. : Torino : 11-13 Ottobre 2018)

Le alterazioni delle arterie coronarie o coronaropatie costituiscono una tra le principali malattie cardiache nell'uomo e sono responsabili di alta mortalità. In medicina veterinaria, l'arteriosclerosi coronarica è stata descritta in un'ampia varietà di specie animali, tra cui mammiferi domestici ed uccelli, con un progressivo aumento della frequenza di lesioni stenotiche legate all'invecchiamento. Dal punto di vista istopatologico, è presente, in genere, un'iperplasia segmentale della tonaca intima associata a una frammentazione della membrana elastica interna e ad una focale degenerazione delle cellule muscolari lisce della tonaca media con successiva deposizione di tessuto fibroso. Molteplici fattori di natura ormonale, genetica e socio-comportamentale ne favoriscono l'insorgenza. Sebbene l'incidenza delle lesioni sia maggiore negli animali anziani, numerosi studi riportano lesioni anche in animali giovani, quali suini, polli e bovini. Sui pesci i dati sono pochi e mancano del tutto per gli storioni. Il presente lavoro riporta le alterazioni morfologiche osservate nelle arterie coronarie intra ed extramurali di diverse specie del genere *Acipenser* allevate in acque dolci prevalentemente per la produzione di caviale. 54 esemplari di *Acipenser* spp. sono stati campionati presso un'azienda del nord Italia e divisi in tre gruppi in base all'età ed al peso: gruppo 1 (G-1), 2-5 anni, 0.5-2 kg, maschi, *A. baerii* e *A. transmontanus* (13 animali); gruppo 2 (G-2), 6-10 anni, 6-9 kg, *A. baerii* (5 maschi), *A. transmontanus* (6 femmine); gruppo 3 (G-3), 12-16 anni, 27-60 kg, femmine, *A. gueldenstedtii* e *A. transmontanus*, 30 animali. I cuori sono stati fissati in formalina al 10% e sottoposti ad esame macroscopico. Campioni selezionati di atri e ventricoli sono stati inclusi in paraffina e le sezioni istologiche corrispondenti sono state colorate con ematossilina-eosina, Weigert

van Gieson, tricromica di Masson, Alcian-PAS e blu di Toluidina. Le arterie coronariche extramurali degli storioni di età più avanzata mostrano lesioni arteriosclerotiche di grado lieve consistenti in piccoli cuscinetti intimali fibroelastici modicamente protudenti nel lume. Tali arterie extramurali talvolta sono circondate da infiltrati linfo-emopoietici e mostrano melanomacrofagi nella tonaca media. Sporadicamente sono visibili mononucleati periazzentuziali senza alterazioni della struttura arteriosa. Nelle coronarie intramurali invece non è raro riscontrare delle vere e proprie lesioni fibrose di intima e media oppure fenomeni degenerativi-iperplastici della tonaca media con omogeneizzazione della struttura, rarefazione cellulare e sviluppo di cellule di Anitchkow con la loro caratteristica struttura ampiamente descritta nell'uomo ed in altre specie animali. In alcuni casi più gravi queste cellule possono invadere anche la tonaca intima; le cellule muscolari lisce restanti presentano talvolta fenomeni degenerativi dei nuclei con picnosi e cariolisi. Complessivamente l'arteriosclerosi extramurale osservata è stata minima di scarsa o nulla importanza fisiopatologica. Sono invece da considerarsi più importanti i fenomeni degenerativi della media delle arterie intramurali, potenzialmente imputabili alla senescenza o all'esposizione a fattori stressanti durante l'allevamento intensivo, come ipotizzato per alcuni mammiferi. Ulteriori studi si rendono comunque necessari per confermare questa ipotesi.

Caruso C, Vitale<sup>o</sup> N, Prato R, Radaelli MC, Zoppi S, Possidente R, Dondo A, Chiavacci L, Moreno<sup>o</sup> MA, Masoero L

**Pseudorabies virus in North-West Italian wild boar (*Sus scrofa*) populations : prevalence and risk factors to support a territorial risk-based surveillance**

Vet Ital. - Vol. 54 no 4 ( 2018). - p 337-341. - 19 bib ref [Nr. Estr. ( ultimo accesso 20/06/2019 )  
<https://doi.org/10.12834/VetIt.1006.6613.2.8204>]

Il virus della Pseudorabbia (PrV) rimane una minaccia per la popolazione di cinghiali non protetti nonostante sia in corso la sua eradicazione nei suini domestici. In questo studio sono riportati i dati sulla prevalenza e l'influenza di possibili fattori di rischio in 2 popolazioni (stato libero e in area protetta) di cinghiali italiani del Nord-Ovest, con l'obiettivo di supportare l'implementazione di un sistema di sorveglianza basato sul rischio, utile a determinare le aree in cui è più probabile la ricomparsa della malattia di Aujeszky. Dal 2011 al 2015 sono stati raccolti sieri di 1.425 cinghiali selvatici; la sieroprevalenza complessiva è stata del 30,39% (433/1.425, IC 95% 28,01-32,85%). Il tasso di prevalenza, invece, si è dimostrato significativamente differente tra la popolazione in condizione libera (90/902; IC 95%; 8,10-12,12%) e quella nel parco La Mandria in Piemonte (343/523 CI 95%; 61,51-69,65%). In entrambe le popolazioni è risultato positivo un numero significativamente maggiore di adulti e di femmine. Sull'AD dovrebbero essere acquisiti, tuttavia, dati territoriali specifici da altre regioni che possano indirizzare le misure basate sul rischio al fine di ridurre la minaccia della reinfezione da AD nella modalità economicamente più efficace.

*Although the eradication of Pseudorabies virus (PrV) in domestic pigs is ongoing, the circulation of this virus in wild boars remains a threat in the currently unprotected, low prevalence, pig population. In this study, we reported PrV prevalence data and the influence of possible risk factors in 2 North-West Italian wild boar populations (free and enclosed) with the goal of supporting the implementation of a risk-based AD surveillance system. Sera from 1,425 wild boars were collected between 2011 and 2015 and tested by ELISA for the presence of PrV antibodies; the overall raw seroprevalence was 30.39% (433/1,425; CI 95% 28.01-32.85%). A significant difference was however observed between the prevalence rates of the free range population (9.98%; 90/902; CI 95%; 8.10-12.12%) and the enclosed population of La Mandria park (Piedmont, Italy) (65.58%; 343/523; CI 95%; 61.51-69.65%). In both populations a significantly higher number of adults and females were found positive to PrV ELISA. Specific territorial data on PrV circulation in wild boars should be acquired from other regions for guiding risk-based measures in order to reduce the threat of AD re-infection in a more cost-effective manner.*

Cascone G, Bertocchi<sup>o</sup> L, Licitra F

**Correlazione tra benessere animale e prevalenza di clamidiosi e neosporosi in aziende di**

## **bovine da latte**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 87. - 4 bib ref [Nr. Estr. 7976]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

Introduzione L'obiettivo del lavoro è stato studiare la correlazione tra le principali malattie (diagnosticate attraverso esami sierologici), il grado di benessere e la biosicurezza riscontrate in ogni azienda di bovine da latte oggetto dello studio. Sono state scelte 35 aziende rappresentative del territorio siciliano in particolare della zona del ragusano. Delle quali 15 aziende con stabulazione libera e le restanti a stabulazione libera con pascolo. Metodi Nelle aziende è stata effettuata la valutazione del "benessere animale" compilando la check-list redatta dal Centro Di Referenza Nazionale Per Il Benessere Animale del IZS di Lombardia ed Emilia Romagna. Tale Check-list valuta i pericoli attraverso parametri collocati in 4 aree. In questo lavoro si è presa in considerazione l'Area di "Biosicurezza". Nelle 35 aziende sono stati effettuati prelievi sui capi presenti per la diagnosi sierologica di Clamidiosi e Neosporosi, tramite metodica ELISA . Risultati Nelle 35 aziende sono stati eseguiti prelievi su 3865 bovine da latte. Di seguito si riportano i valori minimo e massimo in percentuale dell'Area di Biosicurezza riscontrati nelle 35 aziende: (val. min.)18,12 e ( val. max) 82,58. La prevalenza della clamidia oscilla da 0 - 6 e la prevalenza di neospora da 0 a 41,3. Conclusioni Si è proceduto alla elaborazione del dato tramite la correlazione di Spearman. Si è riscontrata una correlazione inversa statisticamente significativa (con  $p=0$ ) ovvero all'aumentare del valore della biosicurezza vi è una diminuzione della prevalenza della malattia. Sulla base dei risultati riportati nella presente indagine che ha interessato 35 aziende si può affermare l'esistenza di una correlazione tra il livello di presenza di patologie aziendali e lo stato di benessere animale generale nelle aziende zootecniche.

Cavadini° P, Capucci° L, Lavazza° A

### **Identification in European hare of new RHDV2 recombinant virus**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 162 [Nr. Estr. 8009]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

Rabbit haemorrhagic disease (RHD) is a highly contagious and acute hepatitis of the European rabbit, caused by a lagovirus (RHDV). In 2010, a new phylogenetically and antigenically distinct virus, RHDV2, was identified in both rabbits and four hare species. Recently, the sequencing of the entire genomes of RHDV2 strains shown the occurrence of multiple recombinant events. To investigate the occurrence of recombination in Italy we amplified and sequenced a region over the RdRp-VP60 junction, of ~30 RHDV Italian RHDV2 strains between 2012-2015, including the first RHDV2 strain identified in 2012 in hare (RHDV2\_Bg12). Positive samples were originating from wild, domestic, rural and industrial farmed rabbits from whole Italy, including those detected from 2012 in Bergamo province, where the RHDV2-positive hare was found. This area is characterized by a high density of hares for hunting purposes, some industrial and many rural rabbits farms, and few confined close populations of wild rabbits. Overall, we identified four RHDV2 recombinant strains from rabbits, all detected on 2015 in Central Italy. These strains showed a recombination event with a RHDV-G1 strain and a very high nucleotide identity i.e. about 98%, for vp60 and p58 with the recombinant strain identified in Algarve on 2014. By analyzing the complete genome sequence of RHDV2\_Bg12, the sole Italian RHDV2 identified in brown hare till today, the vp60 gene sequence showed an 97.2% identity (98.4% at amino acid level) with UD11 strain the first Italian RHDV2 identified in farmed rabbits in 2011. Moreover, the nucleotide identity of the genomic region upstream the vp60 gene, showed an average of 86% to EBHSV, 70% to RHDV, RHDV2 and RCV and 81% to HaCV. Phylogenetic and SimPlot analysis performed with the lagovirus sequences present in GenBank, showed a recombination event between the non-structural proteins of an EBHSV-like virus and the structural protein of RHDV2 virus. In order to determine the nature of the "parental" lagovirus involved in the recombination event, by using RT-PCR with specific primers

designed on the p37 gene (NSP region) of RHDV2\_Bg12, we examined 19 EBHSV and 11 HaCV strains identified in hares in Italy from 2012 onwards, but we didn't get any positive amplification, making the nature of such virus apparently not referable to any known lagovirus. The occurrence of recombination among RHDV2 strains, as shown in other European countries, was confirmed, but we also identified a new lagovirus generated by recombination between RHDV2 and a still unknown hare lagovirus. The origin of RHDV2\_Bg12 remains indefinite but the lack of further detections in hares and rabbits suggests a likely origin outside Italy followed by an accidental introduction. In this case, albeit viable, the emerged new virus was not able to persist in the lagomorph populations, likely due to its low fitness more than to the absence of susceptible hosts. In conclusion, the ability to generate new genomes and viable lagoviruses throughout recombination mechanisms, even between pathogenic and non-pathogenic lagoviruses, could help to understand the genetic mechanism underpinning the emergence of new strains/variants/serotypes with different tropism, fitness and virulence, as assumed for other caliciviruses.

Cavirani S, Rosignoli<sup>o</sup> C, Cabassi CS, Taddei S, Gorrieri F, Valla G

**Studio di follow up su un approccio combinato, vaccinazione ed eliminazione degli animali immunotolleranti, per il controllo della Diarrea Virale Bovina (BVD)** = Follow up study on a combined approach of vaccination and test & cull of persistent infected animals for control of Bovine Viral Diarrhea (BVD)

Large Anim Rev. - Vol. 24 no 5 ( 2018). - p 179-182. - 13 bib ref [Nr. Estr. ( ultimo accesso 31-12-2018 ) <https://www.vetjournal.it/riviste/item/26589-s-cavirani-et-al.html> 7989]

Scopo dello studio e verificare, in condizioni di campo, l'efficacia di un protocollo combinato che associa l'identificazione e tempestiva rimozione dei soggetti immunotolleranti (PI) alla vaccinazione, eseguita con un vaccino vivo attenuato, che presenta l'indicazione della protezione fetale nei confronti dell'infezione transplacentare da virus della diarrea virale bovina (BVDV). Lo studio e stato condotto nel periodo novembre 2008-dicembre 2017 in tre allevamenti con una consistenza complessiva di 4.800 capi, nei quali, precedentemente all'inizio dello studio, era in atto un protocollo vaccinale che prevedeva l'utilizzo sia di un vaccino inattivato sia di un vaccino vivo attenuato per BVDV. All'inizio dello studio sono stati sottoposti a prelievo ematico tutti gli animali di eta superiore a 5 mesi per la ricerca di BVDV mediante RT-PCR eseguita su pool da 20 animali. In seguito, e stata effettuata, mediante RT-PCR, la ricerca di PI sui nuovi nati e di BVDV nel latte di massa su base quadrimestrale. Ai fini dello studio e stato introdotto un protocollo vaccinale per BVDV che prevedeva esclusivamente l'uso di un vaccino vivo attenuato (Mucosiffa®), utilizzato su base semestrale nelle manze e in somministrazione singola nel post-parto nelle bovine adulte. Nella fase di screening sono stati identificati 3 animali PI nati nel periodo compreso tra il 2008 e il 2010. Questi animali erano nati da bovine non PI, probabilmente infettate nei primi tre mesi di gravidanza e vaccinate con un vaccino inattivato utilizzato prima dell'inizio dello studio. Al contrario, dopo l'utilizzo del vaccino vivo attenuato, non sono stati identificati animali PI. I controlli eseguiti per la ricerca di BVD sul latte di massa sono risultati costantemente negativi durante tutto il corso dello studio. In conclusione, l'approccio combinato dell'applicazione di un piano vaccinale che prevede l'utilizzo del vaccino vivo attenuato, associato alla ricerca e rimozione di vitelli PI, ha consentito un ottimale controllo dell'infezione, evidenziato dall'assenza della nascita di nuovi vitelli PI e di circolazione virale attestata dalla negativita del latte di massa alla ricerca del virus.

*Introduction - The control of bovine viral diarrhea (BVD) infection is based on three main pillars: implementation of bio-safety measures, test and cull of persistently infected animals (PI) and adoption of a vaccine protocol to prevent trans-placental infection (fetal protection). Aim - The aim of this study is to verify, in field conditions, the efficacy of a combined approach that provides for detection and removal of PI animals and vaccination with a vaccine with the claim of fetal protection to BVDV. Materials and methods - The study lasted 9 years (November 2008-December 2017), was carried out in three farms located in the Province of Modena (Italy) and involved about 4,800 animals. Anamnestic data pointed out that in all the performed as well on a four-monthly basis. Regarding vaccination protocol, the animals were vaccinated with a modified-live vaccine (Mucosiffa0), namely heifers every six months and cows in the post-partum. Results and discussion - During the study, three PI animals were identified. These animals were born from non-PI cows*

*vaccinated with an inactivated vaccine. During the period when the animals were vaccinated with the modified-live vaccine, no PI animals were detected. All the tests on bulk milk were negative for BVD antigen. Conclusion - The combined approach of vaccination with a modified-live vaccine, associated with test and cull of PI calves, allowed the control of BVD infection, as demonstrated by absence of new PI calves and BVDV circulation assessed by RT-PCR in bulk milk.*

Chiapponi° C, Ebranati E, Pariani E, Faccini° S, Luppi° A, Baioni° L, Manfredi° R, Carta V, Merenda° M, Affanni P, Colucci ME, Veronesi L, Zehender G, Foni° E

### **Genetic analysis of human and swine influenza A viruses isolated in Northern Italy during 2010-2015**

Zoonoses Public Health. - Vol. 65 no 1 ( 2018). - p 114-123. - 46 bib ref [Nr. Estr. ( ultimo accesso 25/01/2019 ) <https://doi.org/10.1111/zph.12378> 7586]

Influenza A virus (IAV) infection in swine plays an important role in the ecology of influenza viruses. The emergence of new IAVs comes through different mechanisms, with the genetic reassortment of genes between influenza viruses, also originating from different species, being common. We performed a genetic analysis on 179 IAV isolates from humans (n. 75) and pigs (n. 104) collected in Northern Italy between 2010 and 2015, to monitor the genetic exchange between human and swine IAVs. No cases of human infection with swine strains were noticed, but direct infections of swine with H1N1pdm09 strains were detected. Moreover, we pointed out a continuous circulation of H1N1pdm09 strains in swine populations evidenced by the introduction of internal genes of this subtype. These events contribute to generating new viral variants—possibly endowed with pandemic potential—and emphasize the importance of continuous surveillance at both animal and human level.

Chiapponi° C, Faccini° S, Merenda° M, Amorico° A, Moreno° A, Rosignoli° C, Pariani E, Galli C, Affanni P, Colucci ME, Foni° E

### **Virological surveillance of influenza virus type A, B, C, D in Italy**

11th International Congress for Veterinary Virology, 12th Annual meeting of Epizone : August 27-30, 2018, Vienna : abstracts / [s.l. : s.n., 2018]. - p 66 (Poster 97) [Nr. Estr. 8116]

International Congress for Veterinary Virology : 11th Annual meeting Epizone : 12th : Vienna : August 27-30, 2018)

Background Influenza A, B, C and D viruses (IAV, IBV, ICV, IDV) are genus of Ortho-mixoviridae family and can cause influenza in humans and animals. IAVs infect humans, mammals and avian species. IBV is considered a common seasonal human pathogen but can occasionally infect pigs and seals, while ICV causes mild infection in humans. ICV has been isolated once from swine in China. IDV was first identified in 2011 in pigs with influenza-like illness but it was shown to be widespread and fairly common in cattle. IDV zoonotic role is currently under investigation. AIM: To investigate the circulation of IAV, IBV, ICV, and IDV in cattle, swine and humans in Italy. Methods From 2015 onwards we performed a virological screening by real-time RT-PCR for IVs on respiratory samples collected in Northern Italy from cattle (I BV, ICV, 10V) and swine (IAV, IBV, ICV, I DV). Full genomes of IAVs and IDVs were sequenced. Human samples collected from influenza-like illness cases in the framework of Influnet (annual active surveillance of IAV and IBV in humans) were examined for ICV and IDV by real-time RT-PCR. Results Swine: we examined 856 herds and found 34% positive for IAV and 1.7% for IDV. IBV and ICV were not detected. Cattle: we examined 888 herds and found 7.6% positive for IDV. IBV and ICV were not detected. Humans: IAV or IBV were detected in 46.9% of analysed samples. 1491 respiratory samples that resulted IAV/IV-negative were examined, and ICV was detected in 0.7% of cases. IDV was not detected. Genetic analysis of IDV from swine and cattle confirmed the circulation of viruses clustering with D/swine/Oklahoma/1334/2011 while the genetic analysis of IAVs from swine showed a more complex situation with the circulation of multiple reassortant genotypes (H1 N1, H1N2, and H3N2). Conclusion The study results showed that cattle are the most susceptible species to IDV infection

whilst it was confirmed that IAV circulates with high prevalence among pigs. The high genotypic variability of Italian swine Ms has undergone further progression. Circulation of ICV or IBV in cattle or swine was not demonstrated. The study did not prove IDV circulates among humans while ICV was detected only in 0.7% of re-spiratory illness. Despite the high circulation of IAV and IBV in humans, introduction of genes from human IVs to animal species was limited to H1N1pdm09 derived viruses detected in swine population.

Chitray M, Grazioli° S, Willems T, Tshabalala T, De\_Vleeschauwer A, Esterhuysen JJ, Brocchi° E, De\_Clercq K, Maree FF

**Development and validation of a foot-and-mouth disease virus SAT serotype-specific 3ABC assay to differentiate infected from vaccinated animals**

J Virol Methods. - Vol. 255 ( 2018). - p 44-51. - 38 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.jviromet.2018.02.006> 7811]

The effective control of foot-and-mouth disease (FMD) requires sensitive, specific and rapid diagnostic tools. However, the control and eradication of FMD in Africa is complicated by, among other factors, the existence of five of the seven FMD virus (FMDV) serotypes, including the SAT-serotypes 1, 2 and 3 that are genetically and antigenically the most variable FMDV serotypes. A key diagnostic assay to enable a country to re-gain its FMDfree status and for FMD surveillance, is the 3ABC or the non-structural protein (NSP) enzyme-linked immunosorbent assay (ELISA). Although many kits are available to detect 3ABC antibodies, none has been developed specifically for the variable SAT serotypes. This study designed a SAT-specific NSP ELISA and determined whether this assay could better detect NSP-specific antibodies from FMDV SAT-infected livestock. The assay's performance was compared to validated NSP assays (PrioCheck®-NSP and IZSLER-NSP), using panels of field and experimental sera, vaccinated and/or infected with FMDV SAT1, SAT2 or SAT3. The sensitivity ( ) of the SAT-NSP was estimated as 76% (70%, 81%) whereas the specificity was 96% (95%, 98%) at a 95% confidence interval. The sensitivity and specificity were comparable to the commercial NSP assays, PrioCheck®-NSP (82% and 99%, respectively) and IZSLER-NSP (78% and 98%, respectively). Good correlations were observed for all three assays.

Cirasella° L, Bergamini° F, Bassix [i.e. Bassi°] S, Antognetti V, Formato G, Cersini A, Carra° E

**Validation of three Real-Time PCR assays for the rapid differentiation of *Aethina tumida* from other Nitidulidae (Coleoptera) species**

8th Congress of Apidology (EurBee 8) : 18-20 September 2018, Ghent, Belgium : program & abstract book / [s.l. : s.n., 2018]. - p 153 (Poster P009) [Nr. Estr. 7941]

European Conference of Apidology (EurBee) (8th : Ghent, Belgium : 18-20 September 2018)

The small hive beetle (SBH), *Aethina tumida* (Coleoptera; Nitidulidae), a parasite of European honeybee colonies (*Apis mellifera*), has shown a significant range expansion from the native Southern Africa to other Continents. In 2014, it was detected in Southern Italy (Calabria and Sicilia), and its presence is so far confined in Calabria region. Some other Nitidulidae species could be associated with the honeybees colonies, but they could be confused with the SHB. Among these, *Cychramus luteus*, *Brachyepelus glaber* and *Meligethes aneus* could potentially be present in the territory under SHB surveillance, interfering with the analysis of identification of SBH. To assist the surveillance and control activities of SHB, three distinct TaqMan Real-Time PCR assays were developed and validated to ease the differentiation of SHB from these mentioned Nitidulidae species, above all when parts of insects are found in hive debris and in brood honeycombs. Three new sets of specific primers and MGB probes, targeting the COI sequences, were designed in silico. Distinct recombinant plasmids, containing one copy of the target sequences, were used as standard

reference DNA. The diagnostic specificity of the three real-time PCR was evaluated by testing DNA extracted from SHB larvae or adults, giving no positive results. The analytical sensitivity was determined by the limit of detection, generated by serial 10-fold dilutions of each standard DNA with a known copy number, either in water and in negative debris and brood honeycombs extracts, resulting to be 50 copies/ l in all matrices. For each PCR, the intra and inter-assay variability were determined using negative honeycombs samples artificially contaminated with different load of the standard DNA. All the replicates tested resulted positive. The Real-Time PCR assays we developed, could represent a reliable support to get a quick differential identification of SHB from *Cychramus luteus*, *Meligethes aeneus* and *Brachyepplus glaber*.

Cocchi M, Salogni° C, Agnetti F, Deotto S, De\_Zan G, Ustulin M, Toson M

#### **MIC distribution, MIC50 and MIC90 in motile *Aeromonas hydrophila* isolated from diseased fish**

Int J Health Anim Sci Food Safety. - Vol. 5 no 3s ( 2018). - p 67 [Nr. Estr. 8032]

International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM) (9th : Rome, Italy : October 16-19, 2018)

Motile *Aeromonads* cause atypical furunculosis in freshwater fish. The infection is characterized by skin ulceration, hemorrhages, fin rot, and septicemia. *Aeromonas* (*A.*) *hydrophila* has gained much attention as the most common fish pathogenic motile *Aeromonas* species. To study the antimicrobial susceptibility, interpretative criteria (epidemiological cut off values) are available only for *A. salmonicida*, regarding florfenicol, ormethoprim-sulfadimethoxine, oxytetracycline and oxolinic acid. Moreover, diverse susceptibility tests are performed for *A. hydrophila*. The minimum inhibitory concentration (MIC) test is conducted using broth dilution or agar dilution technique. Aim of this study was to investigate the MIC distribution, calculating MIC50 and MIC90 in *A. hydrophila* strains isolated from fishes affected by furunculosis. 53 strains of *A. hydrophila* were identified in diseased fish, sampled from farms located in the northern and central regions of Italy. A commercial broth microdilution plate (Micronaut S; Merlin Diagnostics; customized plate) was used, according to CLSI guidelines. *A. salmonicida* subsp. *salmonicida* ATCC 33568 was used as control. The molecules, belonging to seven antibiotic classes, were as follows (the test range, pg/ml, is reported in brackets): ampicillin (0.0625-32), apramycin (0.25-64), colistin (0.015625-8), trimethoprim-sulfamethoxazole (0.0625-1.1875-16/304), aminosidine (0.25-32) gentamicin (0.0625-32), flumequine (0.25-32), enrofloxacin (0.015625-32), florfenicol (1-64), tetracycline (0.03125-16). From the obtained distribution of MICs values, MIC50 and MIC90 were calculated. MIC values below the tested range were recovered for enrofloxacin, florfenicol, and flumequine. On the opposite, MIC values above the tested range were found for colistin. MIC's distribution showed two distinct peaks for colistin and aminosidine. In this study, the MIC50 for trimethoprim-sulfamethoxazole and for tetracycline of pathogenic *A. hydrophila* strains, is in agreement with that obtained by Baron et al. (2017) in strains collected from the aquatic environment, whereas it is lower than the value obtained in other reports. Moreover, gentamicin MIC50 and MIC90 values (0.5 and 1 pg/ml, respectively) were equivalent to those recorded in the literature for *Aeromonas* spp. and, as previously reported, intrinsic resistance to ampicillin was observed (MIC>32 pg/ml).

Colagiorgi A, Baldo° V, Scali° F, Varrà MO, Zanardi E, Ghidini S, Alborali° GL, Ianieri A

#### **Antibiotic resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) from Italian swine chain in planktonic and biofilm form**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 287. - 7 bib ref [Nr. Estr. 7968]



Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72  
Convegno SICV : 18  
Convegno SIRA : 16  
Convegno AIPVet : 15  
Convegno ARNA : 10  
Convegno RNIV : 5  
Convegno ANIV : 2  
Convegno SICLIM-VET : 1  
Giornata studio AIVI  
Giornata studio SOFIVET :

Pig herds are known to be important reservoir for methicillin resistant *Staphylococcus aureus* (MRSA), i.e. MRSA lineage ST398 [1]. MRSA is one of the most commonly identified antimicrobial-resistant pathogens worldwide [2]. Furthermore, it has been reported that some MRSA strains are able to form biofilm on different surfaces [3]. In this study, MRSA isolated from swine chain in Northern Italy were tested in their planktonic and biofilm form against the critically important antimicrobials gentamicin and enrofloxacin [4]. A total of 400 samples were collected from 50 pig finishing herds. In details: three environmental sites at each farm (150), and five carcasses per farm at slaughterhouse (250) were sampled. MRSA were identified by phenotypic analysis and a quadruplex-PCR. Biofilm-forming capacities were evaluated using a previously described method [5]. The Minimum Inhibitory Concentration (MIC) and Minimum Biofilm Eradication Concentration (MBEC) to gentamicin and enrofloxacin were evaluated using micro dilution assays [6]. In addition, the minimum concentration of antimicrobial that inhibits growth of the dispersed cells from the biofilm was also evaluated [7]. A total of 37 MRSA strains was isolated from 400 samples (9.25% prevalence). Six/37 (16.2%) isolates were moderate (1) and strong (5) biofilm producers. Among these, 2 strains (1 moderate and 1 strong) were isolated from carcasses, and 4 from environment. Resistance to gentamicin and enrofloxacin was assayed in planktonic and biofilm form. Results from MIC analysis showed that 4/6 and 2/6 planktonic MRSA strains were resistant to enrofloxacin and gentamicin, respectively. Conversely, MBEC assays on biofilm cultures showed 8- to 512-fold enhancement in resistance. In details, it was observed that sensitive planktonic isolates were able to acquire resistance, and that both antibiotics were unable to eradicate biofilms even at the highest tested concentrations. Furthermore, planktonic cells dispersed from biofilms were also found to be more resistant in 5/6 strains. In conclusion, it was observed that biofilm could act as a mechanism for the tested swine-isolated MRSA strains to get a better survival, and stress once more the importance of finding and developing new alternatives to common antibiotics to overcome the bacterial resistance issue. Further studies are needed to confirm these preliminary findings.

Colagiorgi A, Bruini I, Baldo° V, Scali° F, Giudici° F, Maisano° AM, Santucci° G, Giacomini° E, Lazzaro° M, Amicabile° A, Bontempi° G, Pongolini° S, Belluzzi G, Ianieri A, Borrello S, Candela L, Vitali A, Begni E, Fadini M, Alborali° GL

#### **Antibiotic resistance profiles of Methicillin-resistant *Staphylococcus aureus* (MRSA) from pig production chain in Northern Italy**

ESPHM 2018 : 10th European Symposium of Porcine Health Management : Barcelona, 9th - 11th May, 2018 : proceedings / [s.l. : s.n., 2018]. - p 413 (Poster VPH-001 ) [Nr. Estr. 7869]

European Symposium of Porcine Health Management (ESPHM) (10th : Barcelona : 9th - 11th May, 2018)

Pig herds are an important reservoir for Methicillin-Resistant *Staphylococcus aureus* (MRSA), one of the most commonly identified antimicrobial-resistant pathogens worldwide. The aim of this survey was to evaluate antimicrobial resistance (AMR) profiles of MRSA isolated from finisher pigs in relationship with antimicrobial usage (AMU). Materials and Methods A total of 400 samples were collected from 50 fattening herds, located in Lombardy (Italy). Three environmental samples were collected from each farm. Cutaneous swabs were also collected, from five pigs per farm, at slaughterhouse. MRSA identification was carried out by phenotypic and a quadruplex-PCR. AMR was evaluated by disk diffusion test, following the Clinical Laboratory Standard Institute recommendations. Fifteen prototype molecules, belonging to 12 different drug classes, were tested. AMU was estimated, as days of treatments per bred pig (days/pig), using defined daily dose animal for Italy (DDDAit) and a standard weight at treatment of 100 kg. DDDAit were established according to Italian summaries of product characteristics. Data were collected retrospectively regarding AMU and pig population of 2016. Results 37 MRSA strains were isolated from 21 out of 50 finishing pig herds. 37/37 (100%), 30/37 (81.1%) and 8/37 (21.6%) isolates were resistant to at least one, four and eight classes of antimicrobials, respectively. Medians of AMU were 22.8, 19.5, and 18.3

days/pig in farms where isolates were resistant to 8 or more, 4-7, and 3 or less classes of antimicrobials, respectively. AMU was not significantly different among the three different groups of farms. Discussion & Conclusion Multidrug resistance in MRSA was frequently observed in this survey. Nevertheless, AMR patterns seem not related to AMU, which may be due to a sampling limited in numbers and farms type (finishers only). Further studies are needed to confirm these preliminary findings, which should encompass a larger sample and MRSA molecular characterization.

Colella° EM, Lombardi° G, Amadori° M

#### **IL-1BETA : a potential indicator of the toxicity of autogenous vaccines**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 317. - 3 bib ref [Nr. Estr. 7970]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

Interleukin-1 $\beta$  (IL-1 $\beta$ ) is a pro-inflammatory cytokine mainly produced by activated macrophages and monocytes. The precursor (pro IL-1 $\beta$ ) is located at cytoplasmic level and must be cleaved by caspase-1 to generate the mature activated form [1]. Assessment of IL-1 $\beta$  production by macrophages in response to vaccine antigens could help evaluate the safety and efficacy of the vaccine-induced immune response. Macrophages were obtained after differentiation of pig monocytes from peripheral blood mononuclear cells (PBMC) frozen in liquid nitrogen. PBMC were thawed quickly at 38°C and cultured at the concentration of 6 to 10 million cells/ml in RPMI 1640 medium. The suspension was seeded in 48-well plates and incubated at 37° to promote monocyte adhesion. After 2-3 h, a medium change was made with RPMI 1640 + 10% fetal calf serum (FCS) + Macrophage-Colony Stimulating Factor (M-CSF) at a concentration of 10 ng/ml to stimulate macrophage differentiation [2]. After 4 days of differentiation, the macrophages were incubated for 24 hours at 37 °C with different dilutions of bacterial vaccine antigens. In the tests, a negative and a positive control were included. The negative control consisted of RPMI 1640 + 10% Fetal Calf Serum (FCS), only, and the positive one consisted of 50  $\mu$ L/well of LPS O:111 B4 at 10 micrograms/mL in complete medium, followed by 17  $\mu$ L/well of 150 mM ATP after 4 h at 37°C. At 24 h of incubation the cellular supernatant was collected and frozen at -80°C. Samples were analyzed for IL-1 $\beta$  by "Duo set ELISA for Porcine IL-1 $\beta$ /IL-1F2" (R&D System). Preliminary results reveal that each macrophage population shows different levels of basic activation profile, shown by the levels of IL-1 $\beta$  in the negative control. Most important, the sensitivity of macrophages to vaccine antigens was shown to vary depending on their own basic activation. Non-activated cells respond effectively to the antigen, whereas activated cells display a substantial tolerance, that could also be linked to the culture period before the assay. In fact, macrophages and monocytes, that are exposed to endotoxin are rendered "tolerant" and manifest a profoundly altered response when rechallenged with bacterial endotoxin [3]. It is therefore crucial for standardization to start from a batch of low-activation cells, to standardize the period of cell differentiation and to choose a batch of suitable FCS, in order to have representative and replicable data. The proposed work aims to present the potential of this methodology in the field of autogenous vaccine efficacy and safety control. This kind of evaluation may also pave the way to new studies on the effectiveness of the immune response and the risk of toxicity of autogenous vaccines.

Cooke BD, Duncan RP, McDonald I, Liu J, Capucci° L, Mutze GJ, Strive T

**Prior exposure to non-pathogenic calicivirus RCV-A1 reduces both infection rate and mortality from rabbit haemorrhagic disease in a population of wild rabbits in Australia**

Transbound Emerg Dis. - Vol. 65 no 2 ( 2018). - p e470-e477. - 38 bib ref [Nr. Estr. ( ultimo accesso 18/06/2019 ) <https://doi.org/10.1111/tbed.12786> 8203]

Mortality caused by rabbit haemorrhagic disease virus (RHDV) in wild rabbits is reduced in parts of Australia where the related, non-pathogenic calicivirus RCV-A1 is endemic. Laboratory experiments previously showed that prior infection with RCV-A1 enabled rabbits to better withstand subsequent infection with highly virulent RHDV, and this was assumed to explain higher survival. Here, we analyse serological data from the field suggesting that reduced mortality rates among wild rabbits may also result from rabbits previously infected with RCV-A1 having a reduced likelihood of RHDV infection. We discuss the possible mechanisms underlying this finding and its implications. The methods we describe for analysing field data gave far greater insights into epidemiological processes and virus interactions than gained from reporting basic seroprevalence rates alone.

Costassa EV, D'Angelo A, Mazza M, Meloni D, Baioni E, Maurella C, Colussi S, Martinelli° N, Lo\_Faro M, Berrone E, Favole A, Crociara P, Grifoni S, Gallo M, Lombardi° G, Iulini B, Casalone C, Corona C

#### **Clinical, pathological, and molecular features of classical and L-type atypical-BSE in goats**

PLoS One. - Vol. 13 no 5 ( 2018). - p e0198037 (22 p). - 46 bib ref [Nr. Estr. ( ultimo accesso 11/06/2018 ) <https://doi.org/10.1371/journal.pone.0198037> 7861]

Monitoring of small ruminants for transmissible spongiform encephalopathies (TSEs) has recently become more relevant after two natural scrapie suspected cases of goats were found to be positive for classical BSE (C-BSE). C-BSE probably established itself in this species unrecognized, undermining disease control measures. This opens the possibility that TSEs in goats may remain an animal source for human prion diseases. Currently, there are no data regarding the natural presence of the atypical BSE in caprines. Here we report that C-BSE and L-type atypical BSE (L-BSE) isolates from bovine species are intracerebrally transmissible to goats, with a 100% attack rate and a significantly shorter incubation period and survival time after C-BSE than after L-BSE experimental infection, suggesting a lower species barrier for classical agent in goat. All animals showed nearly the same clinical features of disease characterized by skin lesions, including broken hair and alopecia, and abnormal mental status. Histology and immunohistochemistry showed several differences between C-BSE and L-BSE infection, allowing discrimination between the two different strains. The lymphoreticular involvement we observed in the C-BSE positive goats argues in favour of a peripheral distribution of PrPSc similar to classical scrapie. Western blot and other currently approved screening tests detected both strains in the goats and were able to classify negative control animals. These data demonstrate that active surveillance of small ruminants, as applied to fallen stock and/or healthy slaughter populations in European countries, is able to correctly identify and classify classical and L-BSE and ultimately protect public health.

Cremonesi P, Ceccarani C, Curone G, Severgnini M, Pollera C, Bronzo V, Riva F, Addis MF, Filipe J, Amadori° M, Trevisi E, Vigo D, Moroni P, Castiglioni B

#### **Milk microbiome diversity and bacterial group prevalence in a comparison between healthy Holstein Friesian and Rendena cows**

PLoS One. - Vol. 13 no 10 ( 2018). - p e0205054 (17 p). - 47 bib ref [Nr. Estr. ( ultimo accesso 20/11/2018 ) <https://doi.org/10.1371/journal.pone.0205054> 8021]

Dry and early lactation periods represent the most critical phases for udder health in cattle, especially in highly productive breeds, such as the Holstein Friesian (HF). On the other hand, some autochthonous cattle breeds, such as the Rendena (REN), have a lower prevalence of mastitis and other transition-related diseases. In this study, milk microbiota of 6 HF and 3 REN cows, all raised on the same farm under the same conditions, was compared. A special focus was placed on the

transition period to define bacterial groups' prevalence with a plausible effect on mammary gland health. Four time points (dry-off, 1 d, 7–10 d and 30 d after calving) were considered. Through 16S rRNA sequencing, we characterized the microbiota composition for 117 out of the 144 milk samples initially collected, keeping only the healthy quarters, in order to focus on physiological microbiome changes and avoid shifts due to suspected diseases. Microbial populations were very different in the two breeds along all the time points, with REN milk showing a significantly lower microbial biodiversity. The taxonomic profiles of both cosmopolitan and local breeds were dominated by Firmicutes, mostly represented by the Streptococcus genus, although in very different proportions (HF 27.5%, REN 68.6%). Large differences in HF and REN cows were, also, evident from the metabolic predictive analysis from microbiome data. Finally, only HF milk displayed significant changes in the microbial composition along the transition period, while REN maintained a more stable microbiota. In conclusion, in addition to the influence on the final characteristics of dairy products obtained from milk of the two breeds, differences in the milk microbiome might, also, have an impact on their mammary gland health.

Curone G, Filipe J, Cremonesi P, Trevisi E, Amadori<sup>o</sup> M, Pollera C, Castiglioni B, Turin L, Tedde V, Vigo D, Moroni P, Minuti A, Bronzo V, Addis MF, Riva F

**What we have lost : mastitis resistance in Holstein Friesians and in a local cattle breed**

Res Vet Sci. - Vol. 116 ( 2018). - p 88-98. - 54 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 )  
<https://doi.org/10.1016/j.rvsc.2017.11.020> 7835]

In Holstein Friesian dairy cows, selective pressure for increased milk production has led to a higher propensity to disease, including mastitis, when compared to less selected and lower producing dairy breeds. The biology underpinning the higher resistance to disease of such "local breeds" is not fully understood. With the aim of investigating the factors associated to this phenomenon, we applied a multidisciplinary approach to compare innate immune response patterns, metabolic parameters, milk protein profiles and the milk microbiota in Holstein Friesian and Rendena cows reared in the same farm and under the same management conditions. Quarter milk samples and blood plasma were collected from all cows at dry-off, 1 day after calving, 7–10 days after calving and 30 days after calving. Quarter milk samples were subjected to bacteriological culture, characterization of the milk microbiota by 16S metagenomics, milk protein profiling by electrophoresis and densitometry, somatic cell counting, measurement of the inflammation marker cathelicidin and assessment of different innate immune-related mediators such as lysozyme, CD45, IL-1 $\beta$ , TNF- $\alpha$ , PTX3, IL-1R8. In parallel, the main inflammometabolic parameters were measured in blood plasma samples. Despite having relatively few animals (6 moderate-yielding Holstein Friesian and 4 low-yielding Rendena) some important differences were apparent. Holstein Friesian cows showed a more severe fat mobilization and systemic inflammatory response postpartum in comparison with Rendena cows, which had a greater postpartum muscle mass and an increased amino acid mobilization compared to Holstein Friesians. Upon bacteriological analysis, contagious bacteria such as *Staphylococcus aureus* and *Streptococcus agalactiae* were absent, but significant differences were seen in the general composition of the milk microbiota of the two breeds. Concerning the milk protein abundance profile, pronounced differences were seen in colostrum, with significantly higher amounts of immunoglobulins and other immune-related proteins in Rendena. Added to this, the expression of innate immune related genes such as PTX3, IL-1 $\beta$ , TNF- $\alpha$ , and KRT5 expression in milk epithelial and leukocyte cell components, respectively, was lower in Holstein Friesian colostrum compared with Rendena. In conclusion, several differences were observed in the two breeds, in spite of the same farming conditions. The observations reported in this work present numerous pointers to the factors that may provide autochthonous, more rustic breeds with a higher resistance to disease.

Curone G, Filipe J, Cremonesi P, Trevisi E, Amadori<sup>o</sup> M, Pollera C, Castiglioni B, Turin L, Tedde V, Vigo D, Moroni P, Minuti A, Bronzo V, Addis MF, Riva F

**Biodiversity as a tool to study the mastitis resistance**

In the last 60 years genetic selection in Holstein cattle has been mainly focused on the milk yield criterion, and this mono-aptitude selective criterion has led to their high propensity to develop critical physiological conditions in the pregnancy to lactation transition period with repercussions on reproductive efficiency (calving interval and conception rate), longevity in farm and resistance to stress and diseases (metabolic syndrome, ketosis, mastitis and lameness) [1][2]. A different scenario appears to happen with autochthonous Italian dairy breeds, which are typically characterized by much better fertility and higher resistance and resilience against disease [3]. Unfortunately, less is known about the biological mechanisms behind this rusticity. In this study, a multidisciplinary approach was applied to compare different physiological parameters related with the metabolism, innate immunity, milk protein profiles and the milk microbiota in Holstein (H) and Rendena (REN) breeds, reared in the same farm and under the same management conditions. The cows (6 H and 4 REN) were all among 2 and 4 lactations, with an average of 3.6 for H and 2.7 for REN. The average milk yield was significantly higher in H compared to REN (H=5,366 kg vs REN=3,769;  $p=0.0147$ ). The percentage of milk fat (H=3.52% vs REN=3.37%) and protein (H=3.02% vs REN=3.08%) content was comparable in the two breeds. Quarter milk samples and venous blood were collected from each animal at the following time points: dry-off (T1), 1 day after calving (T2), 7-10 days after calving (T3) and 30 days after calving (T4). Blood samples were used for the analysis of plasma metabolites such as: glucose, total cholesterol, urea, inorganic phosphorus, total protein, haptoglobin, globulin, albumin, total bilirubin, aspartate aminotransferase (GOT),  $\gamma$ -glutamyltransferase (GGT), creatinine, NEFA, 13-OH-butyric acid (BHBA), thiol groups (SHp) and ferric reducing antioxidant power (FRAP). On quarter milk samples different analysis were performed: bacteriological culture, somatic cell counting, protein profiles, and characterization of the milk microbiota. Milk samples were subjected also to measurement of the inflammation marker cathelicidin and assessment of different innate immune-related mediators such as lysozyme, CD45, IL-1b, TNF- $\alpha$ , PTX3, IL-1R8. H cows showed a more severe systemic inflammatory response at T2 and T3 in comparison with REN cows in terms of haptoglobin, total proteins, globulins and bilirubin.. We also observed a greater muscle mass, lower risk of oxidative stress and an increased amino acid mobilization in REN cows immediately after calving when compared to H. Upon bacteriological analysis, contagious bacteria such as *S. aureus* and *S. agalactiae* were not found, but significant differences were assessed in the general composition of the milk microbiota of the two breeds. The taxonomic profiles of cosmopolitan and local breeds were dominated by Firmicutes, mostly represented by *Streptococcus* genus, followed by Proteobacteria, Bacteroidetes, and Actinobacteria. Moreover, within the *Streptococcus* genus, the main species was *Str. thermophilus*, a lactic acid bacterium widely used in the fermentation of dairy products (fermented milks, yogurt, different cheeses), present in both REN and H breeds, although in different proportions: *Str. thermophilus* accounted for more than 48% of total *Streptococcus* abundance in REN cows, while H breed had less than 2%. Concerning the milk protein abundance profile, several bands associated to immunoglobulin components were present in consistently higher amounts in REN colostrum when compared to H cows. Moreover, at all time points H showed higher levels of the inflammation marker cathelicidin in milk. Added to this, the expression of innate immune related genes such as PTX-3, IL-1, TNF- $\alpha$ , and KRT5 expression in milk epithelial and leukocyte cell components, respectively, was lower in H colostrum compared with REN. Our results suggest that H cows develop a systemic and local mammary inflammatory response that could impair the capability of the animal to face the peripartum period and make them more susceptible to disease compared with REN cows. Furthermore, the relative abundance of caseins between the two breeds did also vary at all time points, prompting further investigations about its implications on cheesemaking properties. In conclusion, several differences were observed in the two breeds, in spite of the same farming.

D'Incau<sup>o</sup> M

#### **Surveillance of animal leptospirosis in Italy**

3rd ELS scientific meeting on Leptospirosis and other rodent borne haemorrhagic fevers : 24-26

May 2018, Alghero / [s.l. : s.n., 2018]. - p 17 [Nr. Estr. 7863]

ELS scientific meeting on Leptospirosis and other rodent borne haemorrhagic fevers (3rd : Alghero : 24-26 May 2018)

Leptospirosis is a worldwide zoonosis that infects a large spectrum of domestic and wild animals. Due to its prevalence on the Italian territory, and according to the national legislation, leptospirosis has always been included among the infections for which a mutual collaboration between human and veterinary medicine is required and, in particular, through sharing information of clinical cases and outbreaks in humans and animals. Currently, human infection has very low annual prevalence and incidence (rates of less than 1%), while infection in animal species is present in livestock (cattle, pigs), pet animals (dogs) and wild animals. Despite several seroprevalence studies have evidenced the continuous circulation of *Leptospira* in Italy, there are no systematic surveillance plans at national level; in the past, voluntary control plans were organized at regional level. However, in case of infection, it is mandatory to report to the Veterinary Authority with adoption of restriction measures. Even if official surveillance plans are not in place, the control of infection in animals is ensured by the network of Istituti Zooprofilattici Sperimentali (IIZZSS) coordinated by the National Reference Center. Monitoring is mainly guaranteed by serological checks, performed by agglutination microscopy (MAT), with the use of the same panel of antigens by all laboratories, and collection of the data in a national database. Multi locus sequence typing (MLST) is also performed to monitor genotypes circulation. During the period 2012 - 2017, IIZZSS laboratories examined, on average, over 18,000 sera per year; more than 80% of these sera came from cattle (over 40%), pigs (around 30%) and dogs (around 12%). Sera collected from wild animals (over 5%) and horses (over 2%) were also tested. On a yearly average, 5% of the examined cattle were positive: Sejroe serogroup recorded the highest prevalence, followed by Pomona and Icterohaemorrhagiae serogroups. In swine, more than 14% of the samples were positive: Australis serogroup was significantly the most prevalent serogroup followed by Pomona. MLST revealed two different Pomona sequence types referable to *L. interrogans* Pomona Pomona and *L. kirschneri* Pomona Mozdok. The Tarassovi serogroup has apparently disappeared (prevalence of less than 1%). The sera collected from dogs revealed a high percentage of positivity (over 30%), due to the presence of vaccine antibodies; in this case, if we consider only sera with a titre greater than 1:400, we observed a higher prevalence of the Icterohaemorrhagiae serogroup followed by the Australis serogroup. Among wild animals, mostly sera from wild boar were examined (over 80% of sera). In this species the Australis serogroup was the most prevalent, followed by Pomona and Grippotyphosa. The data obtained confirm the presence of *Leptospira* both in domestic and wild animals and the prevalence of Sejroe serogroup among cattle, of Australis serogroup between pigs and Icterohaemorrhagiae among dogs. In addition, the seroprevalence data in cattle and pigs, now well established over the years, suggest the possibility of implementing control or eradication programs focused on specific serogroups/ serovars.

Dai F, Tranquillo<sup>o</sup> M, Dalla\_Costa E, Barbieri S, Canali E, Minero M

#### **Outcomes of a web-survey to collect stakeholders' opinion on welfare requirements for horses**

Book of abstracts of the 69th Annual Meeting of the European Federation of Animal Science : Dubrovnik, Croatia, 27th -31st August, 2018 / Wageningen : Wageningen Academic Publisher, 2018. - no 24. - Book of abstracts) p 505 (Theatre 6) [Nr. Estr. 7950]

Annual Meeting of the European Federation of Animal Science (EAAP) (69th : Dubrovnik, Croatia : 27th - 31st August 2018)

The Animal Welfare Indicators (AWIN) project aimed at developing animal-based welfare assessment protocols for different species, including horses. To ensure a good acceptance of protocols, stakeholders were invited to participate in a multi-language web-survey. Participants answered 14 open questions about appropriate requirements to guarantee high levels of horse welfare on farm. Text mining was used to analyse answers. Participants properly completed 122 surveys. Most of them were women (85%), veterinarians (35%) and horse owners (34%), coming from Italy (30%) and United Kingdom (15%). To describe welfare requirements, the words 'water'

and 'feed' was the most frequently used (40 and 35 times, respectively): participants considered the welfare principle Good Feeding as the most relevant. As for the principle Good Housing, the shelter appears of primary importance (31); the presence of pasture is mentioned only 18 times, reflecting the habit of keeping horses in single boxes all over the year. It has been demonstrated that the possibility of free grazing prevents abnormal behaviours and enhances welfare, but owners frequently do not perceive it as feasible or safe for horses. The principle Good Health was linked to 'care' (24) and 'health' (20). To describe the principle Appropriate Behaviour, respondents used the words 'training' (23) and 'company', mentioned only 16 times. However, research demonstrated that social isolation is one of the main predisposing factors for behavioural problems development. The results showed that horse stakeholders consider welfare primary linked with appropriate feeding. At the same time, allowing the possibility to interact with conspecifics and spend time at pasture is paramount to guarantee horse welfare. Stakeholders' involvement is fundamental for any action intended to improve animal welfare; this work portrays the stakeholders' perception, highlighting the need of proper dissemination of scientific knowledge. Acknowledgements The Animal Welfare Indicators (AWIN) Project has been co-financed by the European Commission, within the VII Framework Program (FP7-KBBE-2010-4, Grant n. 266213).

Dayhum A, Sharif M, Eldaghayes I, Kammon A, Calistri P, Danzetta ML, Di\_Sabatino D, Petrini A, Ferrari G, Grazioli° S, Pezzoni° G, Brocchi° E

#### **Sero-prevalence and epidemiology of peste des petits ruminants in Libya**

Transbound Emerg Dis. - Vol. 65 ( 2018). - p e48-e54. - 35 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1111/tbed.12670> 7748]

We conducted a cross-sectional study during 2013 to quantify the serological prevalence of peste des petits ruminants (PPR) infection and to investigate host factors associated with PPR infection in small ruminants in Libya. A two-stage sampling design was carried out. A total number of 148 flocks owning at least 100 heads each were randomly selected. Sixteen to forty-eight samples were collected from each selected flock. A total number of 3,508 serum samples from unvaccinated animals were collected and analysed at IZSLER Brescia, Italy, by using competitive ELISA, IDvet innovative diagnostics (IDvet 310, France). The overall serological prevalence among SR was 33% (95% CI: 31.4–34.5). Significant differences between the prevalence in the geographical branches were observed. The lowest prevalence level was observed in Zawiyah branch (16.1%), whereas the highest value was obtained for the Sabha branch (56.8%). Considering the age, a serological prevalence of 24.7%, 31.5% and 42.1% was observed in SR < .001) in the sero-prevalence levels were also observed between the age groups. Our findings suggest that the southern part of Libya could be more exposed to the infections coming from the neighbouring countries and this should be better investigated to correctly identify wherever specific entry points can be considered at higher risk than others. The results also confirmed the endemic status of PPR in Libya, with a constant exposure to the infection of the animals during their life. In the framework of the global strategy for control and eradication of PPR, our results, even if obtained by a preliminary study, can contribute to the assessment of the epidemiological situation of PPR in Libya as required by the Stage 1 of the plan.

De\_Grossi L, Santori D, Barone A, Abbruzzese S, Ricchi° M, Marcario GA

#### **Detection of Mycobacterium avium subsp. paratuberculosis in faeces and tissue of small ruminants using a non - automated liquide culture system method**

14th International colloquium on paratuberculosis : 4-8 June 2018, Riviera Maya, Mexico : program & abstracts / [s.l. : s.n, 2018]. - p 70 (P-1.24) [Nr. Estr. 7902]

International colloquium on paratuberculosis (14th : Riviera Maya, Mexico : 4-8 June 2018)

In Italy the strains of Mycobacterium avium subsp. Paratuberculosis in bovine are frequently isolated

and studied while detection of MAP in sheep and goats is very rare and the circulating strains and their characteristics are unknown. The Paratuberculosis is very common in both species, it needs thereby to know more data about them for developing a control program. The aim of this study was to detect strains of MAP type 1 (S) in ovine and goats using a cultural liquid manual method. In this study, we investigated four flocks with Paratuberculosis and one flock considered to be free of Paratuberculosis in previous research. A total of 603 serum samples and 419 faeces samples were collected and serum samples were analysed by Enzyme- Linked Immunoabsorbent Assay (Paratuberculosis ELISA IDVET) Faeces samples were tested using Herrold's Egg Yolk Medium (HEYM), Middlebrook liquid medium (7H9+) and Real Time PCR (IS 900). During the experiment two positives animals died and tissues and faeces were investigate for MAP. All four flocks were positive to MAP and one confirmed negative. 86 serum samples were positive to MAP by Elisa test. 17 samples of faeces from the same subjects, almost with high value of % S/P (> 100), and 7 negatives were selected to be cultivated in liquid and solid medium and liquid culture analysed by RT PCR. Periodically, liquid cultural were tested by RT PCR to detect 15900 and positives were typed by F57. At last 28 samples, including faeces and tissues, were cultivated and 12 of these were positives at PCR IS 900 for MAP. 6 strains resulted type 1 (S) by PCR respectively from two flocks and four sheep. Results obtained are important because represent one of the first ovine strains of MAP isolated in Italy and cultural method used was non automated and not expensive.

De\_Grossi L, Santori D, Barone A, Abbruzzese S, Ricchi° M, Marcario GA

**Isolamento di Mycobacterium avium subsp. Paratuberculosis da feci e tessuti di piccoli ruminanti utilizzando un metodo colturale liquido non automatizzato** = Isolation of Mycobacterium avium subsp. Paratuberculosis from feces and tissues of small ruminants using a non-automated liquid culture method

XXIII Congresso Nazionale Societa' Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) : Napoli 12-14 settembre 2018 / edited by Antonio Bosco ... [et al.]. - [Naples : University of Naples Federico II, 2018]. - (Mappe parassitologiche ; 24) p 51. - 5 bib ref [Nr. Estr. 7945]

Congresso Nazionale Societa' Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) (23. : Napoli : 12-14 settembre 2018)

In Italia i ceppi di Mycobacterium avium subsp. Paratuberculosis nei bovini sono frequentemente isolati e studiati mentre l'isolamento di MAP nelle pecore e nelle capre e molto raro e i ceppi circolanti e le loro caratteristiche sono pressoché sconosciuti. La paratubercolosi e molto comune sia nei bovini che nei piccoli ruminanti, c'è quindi bisogno di approfondire questo aspetto per molteplici ragioni legate alle forme di malattia, contagio, sviluppo di eventuali vaccini ( ora vietati in Italia) e per migliorare i programmi di controllo della Paratubercolosi. Lo scopo di questo studio era coltivare ceppi di tipo MAP 1 (S) in ovini e caprini usando un metodo manuale liquido colturale.

De\_Lorenzi° G, Cannistrà° M, Capelli° G, Valentini C, Gherpelli° Y, Tamba° M, Arrigoni° N, Stefani E, Luppi° A

**Health management of a salmonellosis outbreak in a dairy farm**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 127. - 1 bib ref [Nr. Estr. 7978]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

Salmonellosis is an important disease for both animals and humans. The main risk factors in cattle breeding are the introduction of carrier animals, poor hygiene and overcrowding. Methods In July 2017 an outbreak of salmonellosis occurred in a dairy cow farm of 494 lactating cows. Salmonella enterica serovar Typhimurium monophasic variant 4,[5],12:i:- was isolated from 6 fecal samples collected from cows with diarrhea. Based on a farm risk analysis (using the evaluation system proposed by CReNBA of IZSLER), a health management plan was implemented in order to improve the levels of external and internal biosecurity and animal welfare. Vaccination based on the



autogenous vaccine made by IZSLER using the strain of S. 4,[5],12:i:- isolated in the farm was implemented. All cows, except for calf, have been vaccinated twice. The results of the plan were evaluated during two visits (October 2017 and February 2018), measuring biosecurity and welfare levels and through microbiological investigations. In every visit were collected 11 environmental samples from different stables of the farm and 60 feces of asymptomatic cows to verify the prevalence of Salmonella. Bacteriology was performed using ISO 6579:2002/Amd 1:2007 method and serotyping using ISO/TR 6579-3:2014-07 method. Results The second visit showed, compared to the first one, an improvement of welfare (73.1% and 58% respectively) and biosecurity (46.8% and 37% respectively). In the first inspection S. Agona and S. Typhimurium were isolated from 8 and 1 environmental samples, respectively, while in the second one S. Typhimurium was isolated from one sample collected in the lactation stable. Regarding microbiology investigations from the faeces, in the first inspection S. Typhimurium was isolated from 4 cows while in the second one the faeces resulted negative. S. Typhimurium monophasic variant was not isolated from samples collected in the 2 visits. Conclusions Biosecurity measures, hygiene and management play an important role in the epidemiology of salmonellosis. The frequent purchase of cows, the poor hygiene observed in the farm and the overcrowding were considered the most important outbreak's predisposing factors and could explain the variability of the isolated serotypes. The application of a health management plan, determined the disappearance of the clinical salmonellosis and the prevalence reduction in the farm. The plan coupled with the vaccination can represent the strategy to tackle salmonellosis outbreaks in dairy cow.

De\_Lorenzi° G, Kamphuisen K, Biscontinini G, Pacciarini° M, Zanoni° M, Luppi° A  
**Mycobacterium genavense infection in a domestic ferret (*Mustela putorius furo*)**

Top Companion Anim Med. - Vol. 33 ( 2018). - p 119-121. - 19 bib ref [Nr. Estr. ( ultimo accesso 14/01/2019 ) <https://doi.org/10.1053/j.tcam.2018.10.001.8080>]

Mycobacterium genavense infection was diagnosed in an adult ferret with ptosis of the left eye, a proliferative lesion of the conjunctiva of the nictitating membrane, conjunctival swelling, and tumefaction of the periorbital tissues with a watery ocular discharge and the presence of a retrobulbar mass. The diagnosis was based on characteristic cytology of the retrobulbar mass and left mandibular lymph node that revealed granulomatous inflammation. Ziehl-Neelsen staining showed the presence of positive acid-fast bacilli in the cytoplasm of the macrophages. The diagnosis was confirmed by sequence analysis of the 16S rRNA gene amplified by using a multiplex polymerase chain reaction from a fresh lymph node biopsy. Therapy with marbofloxacin, rifampicin, and clarithromycin was recommended for 6 months and after this period, the veterinarian who was treating the ferret reported the disappearance of clinical signs. Six months after the end of the antibiotic treatment, the symptoms described previously reoccurred. Confirmatory laboratory tests were not performed but a recurrence of M genavense infection was suspected and the veterinarian, in agreement with the owner, euthanized the ferret.

De\_Lorenzi° G, Pellacini M, Gherpelli° Y, Gibelli° L, Pangallo° G, Bonilauri° P, Dottori° M, Luppi° A

**Caso clinico: focolaio di clostridiosi da *Clostridium difficile* in suinetti neonati**

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 229-233. - 5 bib ref [Nr. Estr. 7828]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

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De\_Sabato L, Vaccari G, Lelli° D, Lavazza° A, Castrucci MR, Moreno° A

**Identification and full genome characterization of Alfa and Beta-coV viruses from bats in Italy**

2nd National Congress of the Italian Society for Virology "One Virology One Health" : Rome, November 28-30, 2018 / [s.l. : s.n., 2018]. - p 166 ( Poster no. P54 ) [Nr. Estr. 8156]

National Congress of the Italian Society for Virology (2nd : Rome : November 28-30, 2018)

Background: Bats are the natural reservoir of Coronaviruses (CoV). Human CoVs cause worldwide slight respiratory diseases but in the last decade, two Beta-CoVs (MERS-CoV and SARS), caused thousands of death and cases worldwide. Phylogenetic analysis suggested the evolutionary origin of mammalian CoVs in bats. In this study, we characterized three Alpha-CoVs and two Beta-CoVs to highlight bat strains circulating in Italy. Materials and methods: Isolates were sequenced using the Next Generation Sequencing (NGS) approach and genome reconstructed using the online tool Galaxy Aries. Phylogenetic analyses were conducted by MEGA7 and MrBayes software. Similarity plots were generated using SSE v1.2. Results: Bioinformatics analysis permitted the identification of three Alpha-CoVs complete genomes of 28 kb and 2 Beta-CoVs of 30 kb (named BatCoV-ITA1-5). BatCoV-ITA1 and 2 formed a monophyletic group with MERS-CoV sequences. The comparison of the concatenated domains within ORF 1 ab confirmed their classification into the MERS-CoV species. The 3D structure of RBD of Italian strains showed two aa deletions located in a region corresponding to the external subdomain of MERS-RBD. BatCoV-Ita3 and BatCoV-Ita4/5 were classified into two novel Alpha-CoVs species by comparison of concatenated domains within ORF1ab. Due to the high divergence with the Alpha human spike protein strains it was impossible to establish the protein structure and the potential affinity to human receptor. Conclusions: The Italian strains showed the typical organization of Alpha and Beta-CoVs. We reported two Beta-CoVs closely related to MERS-CoVs from bats belonging to common species (*P. kuhlii* and *H. savii*). The analysis of the RBD in the spike protein suggest the absence of human receptor-binding potential. The three Alpha-CoV strains, were classified into two novel species, confirming the high heterogeneity of CoV strains in bats. Overall our study enlarges the variability of CoV extending the number of CoV species circulating in bat.

Di\_Patrizi° G, Formenti° N, Scali° F, Chiari° M, Zanoni° M, Tironi° M, Vitale° N, Salogni° C, Giovannini° S, Alborali° GL, Pasquali P

**Toxoplasma gondii and Neospora caninum in wild boars : seroprevalence and potential biosecurity implications in areas with different levels of animal productions**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 173 [Nr. Estr. 8008]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

The protozoa *Toxoplasma gondii* and *Neospora caninum* can cause relevant economic losses on small domestic ruminants and cattle, respectively. Furthermore, *T. gondii* may represent a serious issue for human health through the consumption of raw/undercooked meat or manipulation and handling of infected carcasses. In addition to domestic animals, many wild species can have a role in those protozoa lifecycles and, among wildlife species, wild boars could have a relevant importance due to their scavenger behaviour and omnivorous diet. Moreover, the increasing density of wild boars populations and their distribution, even in close proximity to pastures and farms, should raise the importance for a more focused monitoring to assess the potential risk for these protozoan infections. A sero-epidemiological investigation was carried out on wild boars' populations from Northwest Italy (province of Brescia) in order to evaluate: (i) the spread, (ii) the spatio-temporal dynamics of those infections within populations from areas characterised by different levels of large animal productions. Overall, 1516 sera samples were collected during three hunting seasons

(n=number of samples), 2015-2016 (n=460), 2016-2017 (n=519), 2017-2018 (n=537), from wild boars of three macro-areas characterized by different levels of farms density: low (n=705), medium (n=193) and high (n=618). Sera were tested for *T. gondii* and *N. caninum* antibodies with an indirect ELISA (kit ID VET®, Grabels, France). Data were analysed through logistic regression. Average *Toxoplasma gondii* seroprevalence was 22.56%. Wild boars from the low farm density area were significantly less infected (13.90%) than individuals from both high (30.91%,  $p<0.001$ ) and medium (27.46%,  $p<0.001$ ) ones. Wild boars showed a significantly low seroprevalence in the hunting season 2016-2017 (19.08%) than in hunting season 2015-2016 (24.78%;  $p=0.008$ ) or in hunting season 2017-2018 (24.02%,  $p=0.010$ ). Overall, *N. caninum* antibodies were detected in five out of 1516 (0.46%) samples. Diffusion of *T. gondii* and *N. caninum* was very different within the studied wild boars sub-populations. The sporadic presence of *N. caninum* suggests that wild boars do not to play a relevant epidemiological role in the studied areas; conversely, *T. gondii* seems rather widespread. *Toxoplasma gondii* showed an increased seroprevalence in areas with high and medium density of farms. It is reasonable to hypothesize that this difference in seroprevalence is mostly attributable to the higher anthropization of those areas. Moreover, the large presence of pig smallholders (>60% of total pig farms) may further increase potential risks, which roaming wild boars can pose, in terms of biosafety. These findings highlight importance of biosecurity measures in farms (e.g. fencing, pest control) located in areas where wild boards are present. Differences in seroprevalence among hunting seasons should be interpreted cautiously. Although they could reflect yearly variations of *T. gondii* spread, this study timespan was limited. During the next hunting seasons, systematic monitoring should be implemented and annual sampling should be improved, in order to draw solid evaluations on the role of wild boards in *T. gondii* epidemiology and as a zoonotic risk source in human-domestic-wildlife interface.

Di\_Profio F, Melegari I, Sarchese V, Robetto S, Bermudez\_Sanchez S, Carella E, Orusa R, Cavadini° P, Lavazza° A, Marsilio F, Martella V, Di\_Martino B

**Potential role of wolf (*Canis lupus*) as passive carrier of European brown hare syndrome virus (EBHSV)**

Res Vet Sci. - Vol. 117 ( 2018). - p 81-84. - 25 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.rvsc.2017.11.016> 7725]

European brown hare syndrome virus (EBHSV) was detected in a faecal swab collected from a wolf carcass in Northern Italy. The full-length genome of the EBHSV WOLF/17/2016/ITA strain was determined. In the VP60 capsid gene, the wolf strain displayed the highest genetic identity (99.2-99.1% nucleotide and 99.6-99.7% amino acid) with two EBHSV strains recently found in the intestinal content of a red fox and in the spleen and liver of a hare in Northern Italy. This finding poses interrogatives on the potential role of carnivores as EBHSV passive carriers, favoring the introduction and spread of the virus among different hare populations.

Drigo M, Giacomini° E, Lazzaro° M, Pasotto D, Bilato° D, Ruggeri° J, Boniotti° MB, Alborali° GL, Amadori° M

**Comparative evaluation of immune responses of swine in PRRS-stable and unstable herds**

Vet Immunol Immunopathol. - Vol. 200 ( 2018). - p 32-39. - 27 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.vetimm.2018.04.007> 7849]

Porcine Reproductive and Respiratory Syndrome (PRRS) is an elusive model of host/virus relationship in which disease is determined by virus pathogenicity, pig breed susceptibility and phenotype, microbial infectious pressure and environmental conditions. Successful disease control in PRRS-endemic Countries corresponds to “stability”, i.e. a condition with no clinical signs of PRRS in the breeding-herd population and no viremia in weaning-age pigs. The aim of this work was to compare the profile and time-course of humoral and cell-mediated immunity in stable and unstable

herds, respectively. In particular, we investigated PRRS virus (PRRSV) in serum and group oral fluid samples by Real-time RT-PCR, PRRSV-specific IgA and IgG in oral fluids, serum IgG antibody and the cell-mediated response (PRRSV-specific release of interferon-gamma) in whole blood samples. These parameters were measured in order to identify possible discrepancies in the development and kinetics of the immune response against PRRSV. PRRS-free gilts got regularly infected after entering PRRS-stable and unstable farms. In an open cycle, unstable pig farm PRRSV infection could be demonstrated in all groups of pigs, including suckling piglets. Four main results should be highlighted: A) the precocity of the Ab response in group oral fluids was generally similar to that recorded in sera; B) circulation of PRRSV was consistently detected in all age groups in the unstable herds, as opposed to the stable ones; C) an early, balanced, IgA and IgG response in oral fluids was only observed in the stable herds; D) an early IFN-gamma response after PRRSV infection was often observed in stable herds, as opposed to the unstable ones. These were characterized by IFN-gamma responses in piglets, likely due to transfer of maternal immunity. Most important, the mucosal IgA response was associated with cessation of virus excretion in oral fluid samples of PRRS-unstable herds. The above findings indicate that a peculiar profile of immune response to PRRSV can be found in PRRS-stable herds. Therefore, the outlined immune parameters can represent a useful readout system to evaluate successful adaptation to PRRSV based on acclimatization of breeding animals and management of pig flow.

Drumo R, Chirullo B, Petrucci P, Pistoia C, Ruggeri° J, Alborali° L, Pasquali P

**Characterization of the invasiveness of monophasic Salmonella Typhimurium strains**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 44 (Poster 02.02) [Nr. Estr. 7955]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

Background: During the last decade, a monophasic variant of *S. Typhimurium* (*S. Typhimurium* 1,4,[5],12:i:-) has emerged as a public health threat. Here, we characterize the invasiveness of different strains of monophasic variant of *S. Typhimurium*. Material and methods: The wild-type strain *S. Typhimurium* ATCC 14028 (STM 14028) and the three monophasic variants of *Salmonella* (mSTM 18/11/14, 39/10/11 and SA412) were used throughout the study. The mouse macrophage RAW 264.7 and the human monocytic leukemia cell line (THPI-1) were employed for in vitro studies. Female CD1 and Balb/c mice, 6- 8 weeks old were used for in vivo experiments. Results and discussion: In THPI-1 cells, STM 14028 had a higher level of colonization than mSTM 39/10/11 or mSTM SA412 ( $P < 0.0001$ ). Conversely, in RAW 264.7 cells STM 14028 had a higher level of colonization only than mSTM 39/10/11 ( $P < 0.05$ ). In CD1 mice, STM 14028 and mSTM 18/11/14 showed a significant higher splenic colonization than mSTM 39/10/11 and mSTM SA412 ( $P < 0.05$ ), while in C57 mice all the monophasic variants are less virulent than STM 14028. Conclusion: Different isolates of monophasic variants of *S. Typhimurium* showed to have a different phenotype than *S. Typhimurium* 14028 either in vitro or in vivo experiments. These differences can account for the different epidemiological scenario observed in the last years.

Faccini° S, Merenda° M, Franzini° G, Rosignoli° C

**Bovine respiratory disease complex : rates of detection of main respiratory viruses in nasal swabs**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 156. - 9 bib ref [Nr. Estr. 7981]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

Introduction Bovine respiratory disease complex (BRDC) is a main health problem in cattle worldwide. Viral infections are crucial in predisposing bacterial colonization of the lower part of the

respiratory tract. A rapid and sensitive detection of respiratory viruses is essential to manage BRDC outbreaks and minimize economic loss. A retrospective study was conducted to investigate the rates of detection of the major viral pathogens in nasal swabs collected from cattle affected by BRDC in Italy. Methods Nasal swabs, submitted from January 2014 to June 2018 to IZSLER Diagnostic Laboratory of Mantova from BRDC affected cattle, were considered for this study. Only samples tested for all the viruses of the respiratory test panel - Bovine Viral Diarrhea virus (BVDV), Bovine Coronavirus (BoCV), Bovine Herpesvirus-1 (BoHV-1), Bovine Respiratory Syncytial virus (BRSV), and Bovine Parainfluenza virus-3 (BPI3) - were included. Moreover, Influenza D virus (IDV) assay results were considered, when available. All viruses were detected by Real-Time PCR as previously described (2-5; 8). Results A total of 373 bovine nasal swabs were included in the study; 274 had been tested also for IDV. Pools constituted 81%. The samples came from 232 farms, placed in 27 provinces of Italy. Collectively 185 samples (49.6%) were negative for all the targets included in the test panel. BoCV was the virus most frequently detected (34.9%), followed by BRSV (12.6%), BoHV-1 (9.7%), BPI3V (9.7%), BVDV (8.1%). Overall, 13.5% of samples tested for IDV were positive, and in 4.4% the virus was the only detected. Restricting data to 185 samples from young calves the percentage of positivity for BoCV increased significantly (45.91%;  $P < 0.0001$ ). Conclusions Data demonstrate the effectiveness of Real-Time PCR analysis of nasal swabs for rapid diagnosis of viral respiratory infections. Data are coherent with other studies from different countries (1; 6; 7; 9). BoCV was the most detected virus (34.9%), with a significant higher positivity ( $P < 0.0001$ ) in samples collected from calves (45.91%). The lack of a vaccine against BoCV in Italy, and also in Europe, probably contribute to the high diffusion of this virus. Moreover, the study confirms the circulation of IDV in Italian cattle.

Favole A, Mazza M, Vallino Costassa E, D'angelo A, Martinelli N, Lombardi G, Marconi P, Gallo M, Acutis P, Caramelli M, Casalone C, Corona C

**Rilevamento precoce e pre-clinico della proteina prionica patologica nel liquido cerebrospinale caprino mediante Real-Time Quaking-Induced Conversion assay** = Early and pre-clinical detection of pathological prion protein in cerebrospinal fluid of goats using Real-Time Quaking-Induced Conversion assay

XXIII Congresso Nazionale Societa' Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) : Napoli 12-14 settembre 2018 / edited by Antonio Bosco ... [et al.]. - [Naples : University of Naples Federico II, 2018]. - (Mappe parassitologiche ; 24) p 53-54. - 4 bib ref [Nr. Estr. 7946]

Congresso Nazionale Societa' Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) (23. : Napoli : 12-14 settembre 2018)

La diffusione dell'Encefalopatia Spongiforme Bovina (BSE) nei piccoli ruminanti e ad oggi uno dei punti di maggior interesse nella sorveglianza delle Encefalopatie Spongiformi Trasmissibili (EST) poiche non si pith escludere che, in passato, anche la popolazione ovicaprina si sia trovata esposta in condizioni naturali a questo tipo di infezione. Inoltre, il passaggio dell'agente infettivo in un nuovo ospite puo determinare una variazione delle proprieta del ceppo prionico e rendere difficile il riconoscimento del ceppo originario, aumentando il rischio della diffusione epidemica dell'agente infettivo. Ad oggi non sono stati riportati casi di BSE naturale negli ovini, mentre dal 2005, sono stati identificati due casi di BSE nella capra [1,3]. Inoltre, sono state recentemente riportate anche trasmissioni sperimentali della forma classica (C-BSE) e atipica di BSE (L-BSE) nella specie caprina [4]. Su queste basi, lo sviluppo di un nuovo approccio per la diagnosi ante mortem e la caratterizzazione dei ceppi delle EST dei ruminanti potrebbe aiutare a ridurre il rischio di diffusione epidemica delle EST animali. Il presente progetto di ricerca si e pertanto proposto di verificare l'applicabilita del metodo RT-QuIC nella diagnostica delle EST dei piccoli ruminanti. In particolare, e stata valutata la sensibilita e la specificita del test nel rilevare la presenza della PrP<sup>Sc</sup> in omogenati di cervello e nel liquido cerebrospinale (CSF), prelevato all'esordio clinico e durante il periodo asintomatico, di animali infettati naturalmente e sperimentalmente con Scrapie e con isolati di BSE classica ed atipica (CBSE e BASE).

Ferlazzo° G, Bilato° D, Amadori° M

**Bacterial contaminations of cell cultures can be detected by flow cytometry**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 316. - 2 bib ref [Nr. Estr. 7969]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

Cell culture control for bacterial contaminations is mandatory to enable correct cell growth. Contaminated cell cultures are often subvital, showing growth delay and unreliable responses to immunological tests. Nowadays, sterility control is carried out by microbiological methods, mainly in thioglycolate and trypticase soy broth liquid media incubated at 30 - 37°C over three days, which is hardly enough to detect low-grade microbial growth or slow-growing bacteria, let alone bacteria with complex growth requirements. Owing to the above, we decided to develop a method to detect bacterial contaminations in cell cultures on the basis of the scatter characteristics of *E. coli*. We used non-filtered and 0.22 micron-filtered cell culture samples. Samples were clarified and the supernatant was centrifuged at 10,000 g for 3 minutes; the pellet was resuspended in sterile saline and labeled with BacLight® live / dead kit containing Syto9 and Propidium iodide (PI) fluorochromes, to detect bacterial viability by flow cytometry [1,2]. The assays were carried out in a Guava EasyCyte HT flow cytometer (Merck Millipore), using Incyte software. By setting two gates i.e. R4 (low fluorescence) and R5 (high fluorescence) in a RED H-log x GRN H-log plot and analyzing a positive control containing only bacteria, we could discriminate between sterile and contaminated cell cultures. The morphological picture of a cell culture negative for bacterial contamination was characterized by events in R4 with limited presence in R5 and an upward oriented diagonal of events, the ratio R5/R4 being generally <1. Sample filtration profoundly alters the R5/R4 ratio which always drops to values by far <1. Also new events appear in R4, probably particles degraded as a result of the mechanical filtration stress, e.g. autofluorescent extracellular vesicles damaged after filtration, incorporating less fluorochrome and moving from R5 upper right to R4 lower left. On the other hand, in a contaminated sample =97% of events are concentrated in R5 as a cluster; in the contaminated, filtered sample the events cloud moves from R5 to R4 with only 1-2% of events remaining in R5. Experimental evidence indicates that contamination of cell culture flasks with bacteria in log phase growth diluted 1:2000 can be detected just after two hours of incubation. In conclusion, early detection of bacterial contaminations in cell cultures is badly needed. In this respect, flow cytometry was shown to detect bacterial growth very early, well before the three day-period of a successful microbiological assay. The cytometric approach is substantially cost-effective, also on the basis of a large prevalence of false-negative samples in bacteriological assays.

Ferlizza° E, Menotta° S, Rubini° S, Gigliotti° D, Andreani G, Bertocchi M, Fedrizzi° G, Isani G

**Trace metals and metal-binding proteins in seaweeds and cyanobacteria used as nutritional supplement**

2018 Scientific Meeting Italian Association for the study of Trace Elements in living organisms - AISETOV "The role of trace elements in health: from healthy environments to healthy living organisms" : Ozzano Emilia, Bologna, October 12, 2018 : abstract book / [s.l. : AISETOV], 2018. - p 12 (O-04). - 1 bib ref [Nr. Estr. 7988]

Scientific Meeting Italian Association for the study of Trace Elements in living organisms (AISETOV) : Ozzano Emilia, Bologna : October 12, 2018)

Introduction: For centuries, local populations around the world used seaweeds and cyanobacteria as

food. In fact, these aquatic organisms contain high concentrations of proteins, vitamins and minerals, in particular essential trace elements. The consumption of seaweeds in Western countries showed an increase in recent years, raising concern about their quality and safety. The purpose of this preliminary research was to analyze trace metal concentrations and to isolate metal-binding proteins in commercial samples of seaweeds and cyanobacteria used as human and animal nutritional supplementation. Material and Methods: Sixty samples of dry and fresh edible seaweeds and cyanobacteria were obtained from the market. Nineteen essential and non-essential trace elements (Mn, Fe, Co, Cu, Zn, Se, Mo, Cr, V, Pb, Cd, Hg, As, Al, Ag, Ni, Tl, U, Sb) were analyzed by ICP-MS after mineralization. Cytosolic proteins were separated by gel filtration chromatography. Results and Discussion: Trace element concentrations are in the range of those reported by other authors (Desideri et al., 2016) and vary depending on the species, the environmental conditions and the water content of the commercial products. In particular, Fe concentrations in fresh samples ranged from 6 to 95 µg/g. In cyanobacteria, a mean Fe concentration of 643 µg/g dw was measured, resulting higher than those generally found in food of animal and plant origin. Very low concentrations of Cd, Pb and Hg were determined in all the examined samples, while Al showed wide variations and raised concern due to high values found in dry samples of *Ulva* sp. Despite As concentrations were high in some dry samples, this toxic element should raise less concern, because it was present mainly as organic As. The amount of Fe and Zn bound to cytosolic proteins was one order of magnitude higher in cyanobacteria than in macroalgae, indicating a possible higher bioavailability of these trace elements in the former organisms.

Ferrara° G, Angelucci° A, Strano° RM, Lorenzi° V, Fusi° F, Ginestreti° J, Bertocchi° L

#### **Preliminary observations on the predictivity of bulk tank milk CFU count for the detection of *Staphylococcus aureus* infection at herd level**

The 2018 International Bovine Mastitis Conference : June 11-13, 2018 : Milano / [s.l. : s.n., 2018]. - p 240. - 3 bib ref [Nr. Estr. 7915]

International Bovine Mastitis Conference (NMC) : Milano : June 11-13, 2018)

Mastitis is one of the most important disease in dairy herd because reduces animal welfare and negatively influences farmer productivity and economy (EFSA, 2009). *Staphylococcus aureus* is the microorganism that mostly causes clinical and subclinical infections in the farm and more than others it is the main concern of veterinarians and farmers. This is due to the fact that this infection is difficult to eradicate and control, it is an occupational zoonosis and it could be a problem of food safety and public health. For these reasons it is very important to be able to diagnose this kind of infection quickly without spending too much money. The purpose of this work is to establish if the CFU (Colony Forming Units) count of *Staphylococcus aureus* in bulk tank milk is predictive of an herd infection, considering the possibility of using this kind of diagnostic exam as a starter screening of infection and an evaluating method of the efficacy of control/eradication programs. During 2016 — 2017, 18 Italian dairy herds of Lombardy region have been selected for the study based on an initial screening on the *Staphylococcus aureus* positivity in the bulk tank milk. It was decided to sampling twice each farm at a distance of 3-6 months from the first one, collecting both the bulk tank milk and milk samples of each cow. The average number of lactating cows was 74 and 81, respectively in the day of the first and second access in the farm. During the first access has been analyzed 1332 milk samples of each cow, while during the second one has been analyzed 1459 samples. On the udder milk samples has been done a qualitative bacteriological exam (presence/absence) on a selective terrain Baird Parker with Rabbit Plasma Fibrinogen supplement (BP+RFP); on bulk tank milk samples has been done a quantitative bacteriological exam for the CFU count on BP-RPF terrain. About the first access, 356 udder milk samples were positive and 976 were negative to the diagnostic exam. *Staphylococcus aureus* infection prevalences were between 2,13% and 72,97%. The bulk tank milk CFU count has resulted variable from <10 (no detected) to 1200 CFU/ml. During the second farms access, positive samples were 314, negative samples were 1137 and 7 were polluted, on a total of 1459 udder milk samples. In this second case, *Staphylococcus aureus* infection prevalences were between 0% and 62% and the bulk tank milk CFU count has resulted variable from <10 (no detected) to 610 CFU/ml. A preliminary statistical analysis has been done with

the collected data, correlating the infection prevalence's data carried out from udder milk analysis and CFU count's data from bulk tank milk of the 18 daily herds. For this statistical analysis has been used the Pearson correlation coefficient, for the evaluation of the linearity of the data, in association with Student's T-Test, to know how the correlation obtained was significant. In the first case, the data analysis shows a moderate and statistically significant correlation ( $r$  value=0,60;  $p$  value <0,03). Also for the data of the second access to the farms, the correlation results moderate and statistically significant with a  $r$  value of 0,70 and a  $p$  value <0,01. From the results obtained comes out that the CFU count on bulk tank milk samples can be considered a good screening method to evaluate an initial situation in a herd and to monitor the outcomes of *Staphylococcus aureus* control/eradication programs, as previously demonstrate in other studies (Bertocchi et al., 2010; Varisco et al., 2008). The use of this method is surely an useful tool for the farmer, in particular for the money saving, and for the veterinarian is useful to undertake subsequent investigations on infection situation of the dairy herd. However it is important to do further studies to better understand the degree of the herd infection's predictivity using the bulk tank milk CFU count.

Ferrari L, Canelli E, De\_Angelis E, Catella A, Ferrarini° G, Ogno G, Bonati L, Nardini R, Borghetti P, Martelli P

**A highly pathogenic porcine reproductive and respiratory syndrome virus type 1 (PRRSV-1) strongly modulates cellular innate and adaptive immune subsets upon experimental infection**

Vet Microbiol. - Vol. 216 ( 2018). - p 85-92. - 41 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.vetmic.2018.02.001> 7817]

Highly pathogenic (HP) PRRSV isolates have been discovered within both PRRSV-1 and PRRSV-2 genotypes and investigated in recent years especially for their ability to cause extremely severe disease in conventional pig herds. The exacerbation of general and respiratory clinical signs has been attributed not only to an efficient replication (virulence) but also to the ability to dysregulate viral recognition and induce mechanisms of immune evasion or immune enhancement of humoral and cellular anti-viral responses differently from non-HP PRRSV isolates in terms of intensity and temporal onset. Thus, the understanding of the immunopathogenesis of HP PRRSV is a major concern for the study of virus biology and development of efficacious vaccines. The present study aims at addressing the modulation of relevant immune cell subsets by flow cytometry in the blood of 4- week-old pigs experimentally infected with the recently discovered PR40/2014 HP PRRSV-1.1 strain phenotypically characterized in Canelli et al. (2017) compared to pigs infected with a non-HP PRRSV isolate (PR11/ 2014) and uninfected controls. PR40 infected animals showed an early and marked reduction of pro-inflammatory CD172a+ CD14+CD16+ and CD14+CD163+ monocytes and TCR d+CD8a+/CD8a- lymphocytes when pigs were most infected, possibly due to a recruitment sustaining an acute inflammatory response in target tissues. The prolonged increased CD3+CD16+ NKT cell levels may sustain peripheral inflammation and/ or the anti-viral response. The late reduction (potential depletion) of  $\alpha$ /d T lymphocytes and CD3+CD4+CD8a- naïve Th lymphocytes paralleled with the delayed increase of CD3+CD4+CD8a+ memory and CD3+CD4- CD8a/ $\beta$  + cytotoxic T lymphocytes. In addition, PR40 infection showed an early depletion of activated CD4+CD25+ T lymphocytes and Tregs together with an intense and lasting depletion of CD21+ B lymphocytes. Overall, these features demonstrate that the more severe clinical signs observed upon infection with the HP PR40 strain are sustained by remarkable changes in the peripheral blood distribution of immune cells and provide further insights into the immune regulation/immunopathogenesis induced by PRRSV-1 subtype 1 European isolates.

Filipe J, Curone G, Cremonesi P, Trevisi E, Amadori° M, Pollera C, Castiglioni B, Turin L, Tedde V, Vigo D, Moroni P, Minuti A, Bronzo V, Addis MF, Riva F

**Mastitis resistance in Holstein and in Rendena cattle breeds**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII



Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 114. - 3 bib ref [Nr. Estr. 7964]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

The selective pressure for increased milk production in Holstein Friesian dairy cows has led to their higher propensity to develop diseases in the transition period, including mastitis, when compared to less selected and lesser producing dairy breeds which are typically characterized by a higher resistance to diseases. With the aim of investigating the factors associated to this phenomenon, this study applied a multidisciplinary approach to compare innate immune response patterns, metabolic parameters, milk protein profiles and the milk microbiota in 6 Holstein and 4 Rendena cows reared in the same farm and under the same management conditions. Quarter milk samples and blood plasma were collected from all cows at dry-off (T1), and 1 (T2), 7-10 (T3) and 30 days after calving (T4). Quarter milk samples were subjected to bacteriological culture, characterization of the milk microbiota by 16S metagenomics, milk protein profiling by electrophoresis and densitometry, somatic cell counting, measurement of the inflammation marker cathelicidin and assessment of different innate immune-related mediators such as lysozyme, CD45, IL-1 $\beta$ , TNF- $\alpha$ , PTX3, IL-1R8. In parallel, the main inflammometabolic parameters were measured in blood plasma samples. Holstein cows showed a more severe fat mobilization and systemic inflammatory response at T2 and T3 in comparison with Rendena cows. Rendena cows showed a greater muscle mass (i.e. higher creatinine) and an increased amino acid mobilization immediately after calving compared to Holstein. Upon bacteriological analysis, contagious bacteria such as *Staphylococcus aureus* and *Streptococcus agalactiae* were absent, but significant differences were seen in the general composition of the milk microbiota of the two breeds. The taxonomic profiles of both breeds were dominated by Firmicutes (mostly *Streptococcus* (average HF=27.5%, REN=68.6%)), followed by Proteobacteria, Bacteroidetes, and Actinobacteria. However, Rendena cows showed a lower microbial diversity and a more stable microbiota along the transition period. Concerning the milk protein abundance profile, pronounced differences were seen in colostrum (T2), with significantly higher amounts of immunoglobulins and other immune-related proteins in Rendena. Adding to this, the expression of innate immune related genes such as PTX-3, IL-1 $\beta$ , TNF- $\alpha$ , as well as the CD45/KRT5 expression ratio in milk cells, indicating the epithelial and leukocyte components, respectively, was lower in Holstein Friesian compared with Rendena at T2. In conclusion, several differences were observed among breeds, in spite of the same farming conditions. The observations reported in this work present numerous hints on the factors that may provide autochthonous, more rustic breeds with a higher resistance to metabolic diseases and mastitis.

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#### **Development of a new TaqMan based rRT-PCR for encephalomyocarditis virus (EMCV) detection**

11th International Congress for Veterinary Virology, 12th Annual meeting of Epizone : August 27-30, 2018, Vienna : abstracts / [s.l. : s.n., 2018]. - p 63 (Poster 164) [Nr. Estr. 8144]

International Congress for Veterinary Virology : 11th Annual meeting Epizone : 12th : Vienna : August 27-30, 2018)

Background The encephalomyocarditis virus (EMCV) is a member of genus *Cardiovirus*, family *Picornaviridae*, and is a causative agent of myocarditis, encephalitis, and reproductive disorders, depending on host species and aging. It infects various mammals, including domestic, captive and wild animals, with zoonotic potential for humans; swine is the most receptive between farm species. The infection is widespread in many countries, both industrialized and not industrialized.

Phylogenetic analyses show that viral strains detected worldwide are grouped into five distinguishable lineages, though the majority that includes all those from domestic species cluster in the two lineages A and B. In Italy, the disease is frequently confirmed in pig farms of highly-dense populated regions, where it causes fatal myocarditis. Molecular assays are the preferred diagnostic

tools, therefore this work aimed to develop a one-step TaqMan-based real-time RT-PCR (rRT-PCR) able to detect EMC viruses belonging to lineages A and B, by targeting a portion of the well-conserved 3D-coding region. Method The optimal primers and probe, expected to detect any EMCV strain belonging to lineages A and B, were designed by the alignment of 3D-coding sequences obtained from GenBank and from 50 Italian isolates collected between 2013 and 2015. Analytical sensitivity was evaluated on ten-fold serial dilutions of virus grown on cell cultures with known infectious titre, while analytical specificity was verified by testing different swine viral pathogens, including other picornaviruses. A total of 266 heart homogenates from swine, previously evaluated with a routinely used conventional RT-PCR, were analysed to assess the diagnostic performances. Each sample originated from a different herd, including Italian farms with clinical suspect (n.100) or healthy (n. 131), in addition to 35 positive samples from Belgium and Greece. Results The reaction efficiency resulted of 100% on average, with a detection limit of 100 TCID<sub>50</sub>/nnl. No cross reaction was observed with SVDV, FMDV, enteroviruses, PCV2, PRRSV and ADV. The concordance between the rRT-PCR and the conventional assay was 100%, with 131 samples that scored negative and 135 positive in both assays. Moreover, 83 out of 100 Italian positive samples were submitted to virus isolation in BHK-21 cells and all the relevant isolates were confirmed EMCV-positive by ELISA and rRT-PCR, substantiating test effectiveness. Conclusion These data indicate that the newly developed real-time RT-PCR assay shows satisfying diagnostic specificity and sensitivity and is suitable for surveillance and diagnosis of EMCV-infections. Acknowledgments This study was funded by the National grant PRC2013001.

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#### **Replication dynamics of mixed FMD viruses in vitro**

OS18 "Global vaccine security" : EuFMD Open session : October 2018, Puglia, Italy : online version, book of abstracts / [s.l. : s.n., 2018]. - Day 1. - p 29-30 [Nr. Estr. 8018]

EuFMD Open session : Borgo Egnazia (Brindisi), Puglia, Italy : 29-31 October 2018)

Introduction Foot-and-mouth disease (FMD) is one of the most infectious viral diseases of livestock worldwide. The etiological agent (Aphthovirus, Picornaviridae) is present as seven serotypes with multiple variants. In endemic countries different serotypes and variants of virus often co-circulate with the possibility that animals become infected with multiple viruses. Therefore, simultaneous presence of two viruses in the same sample can occur, making virus isolation (VI) tricky and sometimes misleading. The aim of this work was to gain insight into the dynamics of replication of two serotypes of FMDV co-infecting various cell lines in vitro. Materials and Methods Three cell lines, BHK-21, IBRS-2 and LFBK.vp6, were co-infected with two FMDV serotypes (0 and A) at different ratios; samples were collected sequentially up to 48 hours after infection and analysed by ELISA and a serotype-specific rRT-PCR, that enabled identification and quantification of the grown viruses. To investigate the possible impact of virus strains, experiments were repeated using two different topotypes per each serotype, namely 0/ME-SA/Ind-2001d with A/ASIA/Iran-05 and 0/EA-3 with A/AFRICA/G-IV. Results The results of both serotype-specific Ag-ELISA and rRT-PCR showed that FMD viruses of serotype A have a better fitness than type 0 viruses when cultured in BHK-21 and IBRS-2 cell lines, while LFBK.p6 cells allowed replication of the various co-infecting viruses without promoting one specific serotype. In this cell line the selection was only oriented versus one virus when its concentration was 100X compared to the other virus, suggesting that LFBK.vp6 cells supports the growth of both serotypes with similar efficiency. Discussion Our results corroborate previous observations that LFBK.vp6 are the preferable cell line for VI from field suspect samples, thanks to the speed of viral replication and to a wider susceptibility to various FMD viruses, with no predilection for a specific serotype. Conversely, BHK-21 and IBRS-2 cells are more susceptible to FMDV serotype A compared to 0.

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## **Development and evaluation of two multi-antigen serological assays for the diagnosis of bovine tuberculosis in cattle**

J Microbiol Methods. - Vol. 153 ( 2018). - p 118-126. - 56 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.mimet.2018.09.013> 8100]

There is currently an increased interest in the use of serological approaches in combination with traditional cell-mediated immunity-based techniques to improve the detection of tuberculosis (TB)-infected animals. In the present study, we developed and validated two different serological TB-detection assays using four antigens, MPB70, MPB83, ESAT6 and CFP10, and the tuberculin PPD<sub>b</sub>. A conventional multi-antigen TB-ELISA method and a novel TB multiplex test, based on Luminex technology, were developed to detect antibodies to multiple antigen targets. The performance levels of the two tests were evaluated and compared using selected panels of samples having known TB states. The TB-ELISA test (containing five antigens, including PPD<sub>b</sub>) had a sensitivity (Se) of 74.2% and a specificity (Sp) of 94.9%, while the TB-Luminex test had higher Se (79.0%) and Sp (99.1%) rates even when only one reactive antigen was used to classify the test as positive. If a more restrictive criterion, requiring two positive antigens to classify the test as positive, was used, then the TB-ELISA's Sp rate increased to 99.8% but the Se decreased to 61.3%, while the TB-Luminex test's Sp rate increased to 100% but the Se decreased to 51.2%. TB-ELISA and TB-Luminex were applied to a panel of 257 sera collected from bTB-positive herds, as determined by a post-mortem inspection. They showed good performance levels, identifying 49 (80.3%) and 48 (78.7%), respectively, of 61 samples that had tested positive by the intradermal tuberculin (IDT) test and/or interferon-gamma assay. In addition, TB-ELISA and TB-Luminex were able to identify 60 and 42 samples as positive, respectively, out of the 196 samples that tested negative to IDT and interferon-gamma at the time of serum collection. Subsequent IDT tests performed after 1–2 months, confirmed the positivity of 18 samples, indicating the strategic value of having two serological assays to detect TB-infected herds that were not reactive to initial IDT testing, thereby allowing for the rapid control of outbreaks and eradication of the disease.

Formenti° N, Calò° S, Chiari° M, Vitale° N, Eriksson H, Dalgaard T, Soederlund R, Albiñ A, Giovannini° S, Salogni° C, D'Incau° M, Pacciarini° ML, Zanoni° M, Alborali° LG

### **Indagine epidemiologica di *Erysipelothrix* spp. nei suidi domestici e selvatici : diffusione, isolamento e potenziali rischi zoonosici e zoo-economici**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 141. - 6 bib ref [Nr. Estr. 8053]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Within the genus *Erysipelothrix*, *E. rhusiopathiae* (ER) may pose zoonotic and economic concerns while *E. tonsillarum* (ET) is thought to be avirulent for swine. In addition to pigs, wild boar is considered a potential reservoir of *Erysipelothrix* spp.. In the presented project, the analysis of 582 swine sera revealed an overall ER seroprevalence of 41.58%; however all of 58 tonsils investigated were negative by culture. Wild boars showed an ER seroprevalence of 59.63% (579/971) with the highest value (71.4% (298/417);  $p < 0.0001$ ) registered in subjects from an area with high density of pig farms. Of the 91 wild boar isolates (ntot=223 tonsils), 88 were ER and 3 were ET. The supposed relation between wild boar seroprevalence and high density of pig farms should be studied more deeply including both species density in statistical analyses. Healthy boars carrying ER in their tonsils may serve as a source of infection for pigs and pose zoonotic and economic risks.*

Formenti° N, Chiari° M, Calò° S, Vitale° N, Eriksson H, Dalgaard T, Soederlund R, Albiñ A, Giovannini° S, Salogni° C, D'Incau° M, Zanoni° M, Alborali° GL

### **Epidemiological investigation on erysipelas in wild boars : spread, isolation and potential**

### **impacts at the wildlife-domestic-human interface**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 178 [Nr. Estr. 8002]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

The genus *Erysipelothrix* was originally associated with the species *Erysipelothrix rhusiopathiae* (ER) which may pose zoonotic and economic concerns due to its wide range of domestic and wild hosts. Furthermore, *E. tonsillarum* (ET) was described and differentiated from ER by fermentation of saccharose, serological and molecular techniques, and the lack of pathogenicity for pigs. Despite erysipelas outbreaks occur regularly among domestic animals, the source of infection is seldom investigated. The increasing popularity of animal friendly production systems leads to concerns of outbreaks becoming more prevalent in the future. Although domestic pigs are considered the main reservoir of *Erysipelothrix*s, many wildlife species could have a role in the spread of the infection and wild boar (*Sus scrofa*) have been suggested as a healthy host. Moreover, the increase of wild boar densities, the expansion of its ranges even in proximity to farms and the little information available about the distribution of the infection in this species should be considered. The need is therefore to investigate if wild boars could represent a risk of erysipelas at the wildlife-domestic-human interface. Wild boars from Northwest Italy were analysed to evaluate (i) the seroprevalence of ER and (ii) the prevalence of *Erysipelothrix* spp. isolates to disclose (iii) the epidemiological factors associated with the presence and the transmission of the infection. During two hunting seasons (2015/2016 and 2016/2017) 971 sera samples were collected from 8 macro-areas with different density of pig farms. Moreover, during 2016/2017 overall 223 wild boar tonsils were collected from the high and low density pig farms macro-areas. ER seroprevalence was evaluated through a commercial ELISA kit while *Erysipelothrix* spp. were isolated and confirmed by Gram stain, cell morphology, catalase activity, and H<sub>2</sub>S production on triple sugar iron agar medium. Results from serology and microbiology were analysed by multivariate logistic regression. An overall ER seroprevalence of 59.63% (579/971) emerged. Wild boars from high density pig farms macro-area showed the highest seroprevalence (71.4% (298/417),  $p < 0.0001$ ): in this macro-area the probability to be seropositive was 11.4 (6.05-24.5) times higher than in others. *Erysipelothrix* spp. was isolated from 58 wild boars (26.01%). No significant association were found between isolates and study factors. Partial 16S sequencing was performed on 10 of 58 isolates: 7 were ER while 3 were identified as ET. The increased seroprevalence in the high density pig farms macro-area could support the hypothesis of a relation between the seroprevalence of wild boars and the presence of farms, although the role of wild boar density cannot be ruled out. Further statistical analyses would be performed to deepen this aspect. However, as 60.41% of farms in this macro-area belongs to smallholders, wild boars could represent a biosecurity risk. Wild boars may pose zoonotic risks through ER infected carcasses while the importance of ET in erysipelas diagnosis emerged considering its lack of pathogenicity for pigs. Comparative whole-genome sequence analysis of isolates originating from wild boars and domestic pigs is ongoing and will provide further clues to better evaluate the potential economic and zoonotic risks related to wild boars.

Formenti° N, Chiari° M, Calo° S, Vitale° N, Eriksson H, Dalgaard T, Soederlund R, Albiñ A, Giovannini° S, Salogni° C, D'Incau° M, Zanoni° M, Alborali° LG

### **Epidemiological investigation on *Erysipelothrix* spp. in wild boars and potential risks at the wildlife-domestic-human interface**

5th Congress of the European Association of Veterinary Laboratory Diagnosticians (EAVLD) : 14-17 October, 2018, Brussels, Belgium : abstract book / [s.l. : s.n., 2018]. - p 133 (Poster 63) [Nr. Estr. 8046]

Congress of the European Association of Veterinary Laboratory Diagnosticians (EAVLD) (5th : Brussels, Belgium : 14-17 October, 2018)

Introduction: *Erysipelothrix rhusiopathiae* (ER) and *E. tonsillarum* (ET) are the two main species of the genus *Erysipelothrix*. As ER may pose zoonotic and economic concerns while ET is thought to be avirulent for swine, their different epidemiological role emerges. Wild boar (*Sus scrofa*) have been suggested as potential reservoir of *Erysipelothrix* spp. and the little information available warrants investigation of a possible risk of infection at the wildlife-domestic-human interface. The ER seroprevalence and the prevalence of *Erysipelothrix* spp. isolates were evaluated in wild boars from Northwest Italy to disclose factors associated with the epidemiology of the infection. Materials and Methods: During the 2015/2016 and 2016/2017 hunting seasons 971 sera were collected from 8 macro-areas with different density of pig farms. Additionally, 223 tonsils were gathered during 2016/2017. Results were analysed by multivariate logistic regression. Results: An overall ER seroprevalence of 59.63 % emerged. Subjects from high density pig farms macro-area showed the highest seroprevalence ( $p < 0.0001$ ). *Erysipelothrix* spp. was isolated from 67 boars. Until now, partial 16S sequencing was performed on 10 isolates: BLAST analysis identified 7 as ER and 3 as ET. Discussion and Conclusion: The increased seroprevalence in the high density pig farms macro-area could support a relation between seroprevalence and the presence of farms, although in this mechanism an effect of wild boar density cannot be ruled out. Healthy wild boars carrying ER in their tonsils may serve as a source of infection for domestic pigs and pose zoonotic and economic risks. Further sequencing will provide clues to better evaluate the potential risks related to wild boars.

Formenti° N, Chiari° M, Trogu T, Gaffuri° A, Garbarino° C, Boniotti° MB, Corradini, P. Lanfranchi, N. Ferrari

**Molecular identification of cryptic cysticercosis : *Taenia ovis* *krabbei* in wild intermediate and domestic definitive hosts**

J Helminthol. - Vol. 92 ( 2018). - p 203-209. - 41 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 )  
<https://doi.org/10.1017/S0022149X17000177> 7759]

The complex life cycle of taeniids represents an ideal model of a multi-host system. The complexity of these parasites can therefore cover the epidemiological issues of the interface between wild and domestic animals, especially once spatial overlap between wild and domestic definitive and intermediate hosts occurs. Here we use the occurrence of *Taenia ovis* *krabbei* in two model areas as an example of this epidemiological complexity. In two contiguous areas in the Italian northern Apennines, two hunted roe deer (*Capreolus capreolus*) showed numerous cysticerci in the muscles of their whole body and an adult tapeworm was recorded in a semi-stray dog (*Canis lupus familiaris*). Through molecular typing of the mitochondrial cytochrome c oxidase I (cox1) gene, cysticerci and the adult tapeworm of *T. krabbei* were identified. *Taenia krabbei* cysticercosis was recorded for the first time in Italy. Although the role of dogs in the parasite's life cycle emerges, the overlap between wild and domestic definitive hosts and the increase of wild population densities raise concerns about the temporal (old or new) introduction and the spread of this parasite by one of these canid species (wolf (*Canis lupus*) or dog). Although *T. krabbei* is not a public health issue, economic concerns emerged for hunters and meat producers, related to the damage of carcasses by cysticerci. Therefore, there is a need to evaluate the spread of *T. krabbei* in the intermediate and definitive host populations, and to ensure the relevant sanitary education for hunters in order to avoid practices that could favour the spread and maintenance of its life cycle.

Formenti° N, Gaffuri° A, Zanardi° G, Braga° A, Ricchi° M, D'Incau° M

**Fatal co-occurrence of *Mycoplasma ovipneumoniae*, *Mycoplasma agalactiae* and Pasteurellaceae in an alpine chamois**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 109 [Nr. Estr. 8004]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

Co-infections, or alternatively called mixed infections, are the rule in natural conditions. Furthermore, wildlife has to face the pathological effects known to be related to multiple and concomitant infections. Focusing on wild ungulates, the co-occurrence of multiple strain infection can indeed lead to severe disease outbreaks and the diagnosis of all etiological agents involved is necessary for the control strategies of the disease. Among strains of main interest, infections with multiple *Mycoplasma* species and/or in association with Pasteurellaceae can occur severely. Although for the latter a precise microbiological diagnosis can be obtained, for *Mycoplasmas* the small number of biochemical and physiological properties available together with the serological cross-reactions between subspecies or types complicate the identification and the detection of co-infections. Conversely, molecular approaches can overcome these limitations providing useful tools for rapid and reliable identification allowing the detection of multiple infections. The need is to apply a diagnostic process that combines techniques that allow the typing of all the etiological agents involved in the disease. Here we propose the case of the co-occurrence of two *Mycoplasma* species and Pasteurellaceae detected in an old (>6 years old) female chamois (*Rupicapra r. rupicapra*) culled during the 2017 hunting season. The animal was culled since its poor body conditions and the hypertrophic udder. Macroscopically, the udder was enlarged and lungs showed bronco-pneumonia, multiple abscesses were recorded in both organs. Liver was congest and kidneys had an altered parenchyma. All the organs were cultured on blood agar and Gassner media; PPLO agar and broth were used for bacteriological exams of lungs and udder. DNA was extracted from both organs and the cultured *Mycoplasma* spp. colony; four real-time PCR protocols were designed to differentiate *Mycoplasma* species that likely infect chamois: *M. ovipneumoniae*, *M. agalactiae*, *M. bovis* and *Mycoplasma mycoides* cluster. Bacteriological analyses showed *Bibersteinia trehalosi* in lung, *Mannheimia haemolytica* in udder and *Mycoplasma* spp. in both organs. PCRs detected *M. ovipneumoniae* and *M. agalactiae* in the lung while the latter was recorded even in the udder. Our diagnostic process showed the fatal co-occurrence of bacterial and multiple *Mycoplasma* infections in chamois giving evidence of the severity of the disease. The use of these series of PCRs allowed the identification and differentiation of two *Mycoplasma* species and lead to exclude other *Mycoplasmas* that can affect chamois. *M. ovipneumoniae* and *M. agalactiae* are widespread in wild and domestic ruminants and are responsible of both subclinical infections and severe disease. Particularly, *M. agalactiae* infection can affect individuals causing agalactia in lactating females, conjunctivitis, pneumonia and arthritis. Moreover, the pathogenic role of Pasteurellaceae on these animal species is documented. As during summer pastures chamois can have a spatial overlap with flocks, sheep and goats may have a role in the spread of these infections crossing the wild-domestic interface. Further analyses should be performed to compare strains of *Mycoplasmas* and Pasteurellaceae from wild and domestic ruminants to assess if an interspecies transmission really occurs and to study the capacity of these changing microorganisms to adapt to different species.

Formenti<sup>o</sup> N, Trogu<sup>o</sup> T, Bellometti S, Gugliatti A, Pedrotti L, Gaffuri<sup>o</sup> A, Lanfranchi P, Ferrari N

**Toxoplasma gondii in naturally infected red deer (*Cervus elaphus*) : spread, infection dynamics and effects on host behaviour**

XXX Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) "Mutamenti ambientali e parassiti" : 26-29 Giugno 2018, Milano / [s.l. : s.n., 2018]. - p 116 [Nr. Estr. 8118]

Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) (30. : Milano : 26-29 Giugno 2018)

INTRODUCTION. *Toxoplasma gondii*, beyond its zoonotic, economic, and conservation values, has been associated with behavioural changes in several hosts, including humans. Recent studies constitute indeed a convincing body of evidence that parasite's activity can manipulate intermediate host behaviour through neurological symptoms/alterations. In humans, loss of psychomotor performance and concentration or mental disorders were related to Toxoplasmosis, while in animals *T. gondii* may promote "risky behaviours" that may favour the parasite transmission from

intermediate to other/s or to definitive hosts. As the occurrence of *T. gondii* behavioural manipulation has not been investigated in natural conditions, we focused our attention on wild red deer (*Cervus elaphus*). Therefore, we investigated (1) the spread and dynamics of *T. gondii* and (2) if the parasite may induce more risky behaviours, assuming as parameter an increase in the probability for the infected host to be culled. **MATERIALS AND METHODS.** For epidemiological analysis 464 sera were collected during 2014-2017 culling plans from three areas with different level of anthropization (low, moderate, high) in Stelvio National park, while the behavioural hypothesis was tested on 81 adult females. Samples were analysed by a commercial ELISA kit. The epidemiology of the infection was analysed through a Binomial and a Gaussian Generalized Linear Models (GLM). While the behavioural hypothesis was tested by a Gaussian GLM modelling the "days from the beginning of culling" to evaluate the potential increase in the culling probability induced by *T. gondii*. **RESULTS AND CONCLUSIONS.** An overall prevalence of 26.9% emerged. Adults were significantly more infected than yearlings and calves. Subjects of low anthropised area were significantly less infected than those of other anthropised ones. Deer of 2014 were significantly less infected than those of other years and seropositive subjects of this study year showed the lowest serological titres. The effect of age class and anthropization on the spread of *T. gondii* supports horizontal transmission as the main route. Seropositive adult females (49/81) had a significant higher culling probability, being culled sooner, than the seronegative ones. *T. gondii* appears to induce behavioural alterations in red deer making them more "at risk" to be culled. The supposed emerged mechanism leads to a *T. gondii* manipulation in this species that could increase its spread and transmission even to humans. Further analyses should be carried out to extend this analysis to other age classes and to preyed/found dead/roadkill animals for a wide assessment of the potential alterations induced by *T. gondii*.

Formenti<sup>o</sup> N, Vigano R, Fraquelli C, Trogu T, Bonfanti M, Lanfranchi P, Palme R, Ferrari N

**Increased hormonal stress response of Apennine chamois induced by interspecific interactions and anthropogenic disturbance**

Eur J Wildl Res. - Vol. 64 ( 2018). - no 68 (8 p). - 59 bib ref [Nr. Estr. ( ultimo accesso 10/12/2018 ) <https://doi.org/10.1007/s10344-018-1228-4> 8020]

Responses of animals to environmental changes and their interactions with other species play an important role in conservation. Sharing a common habitat may lead to interspecific competition for resources, but field assessment of these biological events is not always easily accomplished. By using a non-invasive method, we evaluated the physiological stress responses of Apennine chamois (*Rupicapra pyrenaica ornata*) to the presence of cattle, sheep and goat, red deer (*Cervus elaphus*), people (hikers), and predators to identify which factors may affect this endangered species. During September 2012, November 2012, and July 2013, a total of 318 faecal samples were collected in representative sites and analysed for faecal cortisol metabolites (FCM). FCM concentration was analysed through linear mixed-effect models. A significant increase in FCM values in Apennine chamois sharing their habitat with domestic animals was recorded during all study periods. On the contrary, stress responses to red deer and people were limited in time and emerged only during summer months, when hikers are more frequent and red deer extend their altitudinal range reaching chamois' habitat. The observed effects of domestic animals, red deer, and hikers should be considered in future Apennine chamois management plans, which should include the regulation of pastured domestic livestock, anthropogenic disturbances, and possible interferences with other wild species within parks.

Francesco<sup>o</sup> D, Annalisa<sup>o</sup> G, Giorgia<sup>o</sup> DL, Lucia G, Andrea D, Michele<sup>o</sup> D, Paolo<sup>o</sup> B [i.e. Defilippo<sup>o</sup> F, Grisendi<sup>o</sup> A, De\_Lorenzi<sup>o</sup> G, Gibelli<sup>o</sup> L, Dottori<sup>o</sup> M, Bonilauri<sup>o</sup> P]

**Laboratory trial for to evaluate the vector competence of Oriental Cockroach (*Blatta orientalis*) for ETEC, Salmonella typhimurium and Brachyspira hyodysenteriae**

ESPHM 2018 : 10th European Symposium of Porcine Health Management : Barcelona, 9th - 11th May, 2018 : proceedings / [s.l. : s.n., 2018]. - p 234 (Poster BBD-056 ) [Nr. Estr. 7871]

European Symposium of Porcine Health Management (ESPHM) (10th : Barcelona : 9th - 11th May, 2018)

The Oriental Cockroaches (*Blattella germanica*) are important pest in swine production in recent years, have long been investigated to assess the carry capacity for different swine pathogenic bacteria. They are known to carry about 57 species of pathogenic bacteria. Though various studies indicated that cockroaches play an important role as mechanical as well as biological vectors for foodborne bacterial pathogens, but cockroaches individual contribution lack. The objective of this study was to evaluate the vector competence of Oriental cockroach for three important porcine pathogens isolated from Italian swine farms: ETEC (F18 STa, STb), *Salmonella typhimurium*, and *Bacteroides hyodysenteriae*. About one hundred adult cockroaches were reared under controlled conditions (RH 60%, photoperiod 14:10 h (L:D) and temperature 28°C). For each pathogen tested, 16 adult cockroaches were randomly picked, divided into 2 groups of 8 and transferred individually in sterile plastic containers. The first group was infected by contaminated food (potatoes) supplied for 5 days, which was then removed and replaced to sterile one. Individual faeces were collected daily and the presence of each pathogen was verified (ETEC only numbered). The second group was maintained as negative control. The oral infection was carried out with bacterial quantity of 10<sup>8</sup>-10<sup>9</sup> CFU/g. Cockroach faeces started to be positive for *S. typhimurium* (8/8) the day after contamination and for ETEC (6/8) two days after contamination. No positivity was observed for *B. hyodysenteriae*. Faeces remained positive until contaminated food was removed: 4 days after removal no more positivity was observed for both *S. typhimurium* and ETEC (0/8). Faeces of the control group remained negative for each pathogen. In this study the vector competence of cockroaches for swine bacterial pathogens was confirmed for ETEC and *S. typhimurium*, but only as mechanical vector (not infected). The possible role in the diffusion of *B. hyodysenteriae* was not evidenced.

Franzo G, Cecchinato M, Tosi° G, Fiorentini° L, Faccin° F, Tucciarone CM, Trogu° T, Barbieri° I, Massi° P, Moreno° A

### **GI-16 lineage (624/I or Q1), there and back again : the history of one of the major threats for poultry farming of our era**

PLoS One. - Vol. 13 no 12 ( 2018). - p e0203513 (15 p). - 46 bib ref [Nr. Estr. ( ultimo accesso 11/01/2019 ) <https://doi.org/10.1371/journal.pone.0203513> 8079]

The genetic variability of Infectious bronchitis virus (IBV) is one of the main challenges for its control, hindering not only the development of effective vaccination strategies but also its classification and, consequently, epidemiology understanding. The 624/I and Q1 genotypes, now recognized to be part of the GI-16 lineage, represent an excellent example of the practical consequences of IBV molecular epidemiology limited knowledge. In fact, being their common origin unrecognized for a long time, independent epidemiological pictures were drawn for the two genotypes. To fix this misinterpretation, the present study reconstructs the history, population dynamics and spreading patterns of GI-16 lineage as a whole using a phylodynamic approach. A collection of worldwide available hypervariable region 1 and 2 (HVR12) and 3 (HVR3) sequences of the S1 protein was analysed together with 258 HVR3 sequences obtained from samples collected in Italy (the country where this genotype was initially identified) since 1963. The results demonstrate that after its emergence at the beginning of the XX century, GI-16 was able to persist until present days in Italy. Approximately in the late 1980s, it migrated to Asia, which became the main nucleus for further spreading to Middle East, Europe and especially South America, likely through multiple introduction events. A remarkable among-country diffusion was also demonstrated in Asia and South America. Interestingly, although most of the recent Italian GI-16 strains originated from ancestral viruses detected in the same country, a couple were closely related to Chinese ones, supporting a backward viral flow from China to Italy. Besides to the specific case-study results, this work highlights the misconceptions that originate from the lack of a unified nomenclature and poor molecular epidemiology data generation and sharing. This shortcoming appears particularly relevant since the described scenario could likely be shared by many other IBV genotypes and pathogens in general.



Franzoni G, dei\_Giudici S, Razzuoli E, Sanna G, Galleri G, Fiori M, Pilo G, Modesto P, Amadori° M, Graham SP, Oggiano A

**Characterization of the interaction of African swine fever virus strains of diverse virulence with macrophage subsets**

4th GARA Scientific Workshop : April 11-13 2018, Cagliari / [s.l. : s.n., 2018]. - 2 p. - 2 bib ref [Nr. Estr. 7761]

GARA (Global African Swine Fever Research Alliance) Scientific Workshop (4th : Cagliari : April 11-13, 2018)

African swine fever (ASF) is a devastating viral disease for which there is no vaccine available. The interactions between the ASF virus (ASFV) and the macrophages they infect are thought to be crucial for disease pathogenesis. This study aimed to conduct a detailed in vitro characterization of the responses of porcine unactivated (moM0), classically (moM1) and alternatively (moM2) activated monocyte-derived macrophages to infection with a virulent Sardinian isolate (22653/14) and a tissue culture attenuated strain (BA71V) of ASFV. Using an MOI of 1, both isolates were able to infect macrophage subsets, but 22653/14 presented a greater ability to infect moM1 compared to BA71V. Higher expression of early (p30) compared to late (p72) proteins was observed in BA71V-infected moM1. Using an MOI of 0.01, only 22653/14 was able to replicate in all the macrophage subsets, with initial lower expression of ASFV proteins in moM1 and moM2 compared to moM0. Higher levels of IL-18, IL-13 and IL-1 $\beta$  were released from moM1 after infection with BA71V compared to 22653/14 or the mock-infected control. ASFV down-regulated CD16 expression on macrophages, whereas the virus did not modulate MHC class II or CD 163 levels on these cells. Interestingly, BA71V-infected but not 22653/14 infected moM1 and moM2 presented lower percentages of MHC class II cells compared to the mock-infected controls. We tested another attenuated ASFV strain (NH/P68) and we observed a comparable down-regulation of MHC class I on moM1. Preliminary data showed that infection with NH/P68 resulted in an increased expression of IFN- $\beta$  gene compared to the virulent 22653/14. Differences between IFN- $\beta$  subtypes are under investigation. These significant differences in the response of macrophages to virulent and attenuated ASFV may help our understanding of immunity and pathogenesis of ASF.

Franzoni G, Dei\_Giudici S, Razzuoli E, Sanna G, Galleri G, Fiori MS, Ledda M, Madrau MP, Carta T, Amadori° M, Graham SP, Oggiano A

**Characterization of the interaction of African swine fever virus strains of diverse virulence with dendritic cells and macrophage subsets**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 53 (Poster 02.20) [Nr. Estr. 7957]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

African swine fever (ASF) is a devastating disease for which there is no vaccine available. ASF virus (ASFV) has a tropism for cells of the myeloid lineage, including macrophages and dendritic cells (DC). Despite the important role of DC in induction of immunity, few studies analysed their response to ASFV infection. There were also no studies of the interaction of ASFV with activated macrophages. We therefore conducted an in vitro characterization of the interactions of porcine monocyte-derived DC (moDC), unactivated, classically (M1) and alternatively (M2) activated monocyte-derived macrophages with a virulent (22653/14) and a low virulence (NH/P68) strain of ASFV. Using a multiplicity-of-infection (MOI) of 1 both strains infected moDC, but maturation with IFN- $\alpha$  and TNF- $\alpha$  increased susceptibility to infection with 22653/14. On the contrary, NH/P68

presented a reduced ability to infect IFN- $\alpha$  but not TNF- $\alpha$  treated moDC. Using an MOI of 0.01, both strains efficiently replicated in moDC. Infection with NH/P68 resulted in lower expression of MHC I on nnoDC, whereas all the strains up-regulated MHC II DR and decreased CD16 expression. ASFV infection resulted in CD80/86 down-regulation on mature nnoDC. ASFV did not induce a strong cytokine response from both immature and mature moDC. Using an MOI of 1, both isolates infected all the macrophage subsets, however NH/P68, but not 22653/14, down-regulated MHC class I and induced IFN- $\gamma$  gene expression. These results revealed differences between ASFV strains, suggesting that virulent isolates are better able to evade host immune response, promoting their survival in infected pigs.

Franzoni G, Graham SP, Sanna G, Angioi P, Fiori MS, Anfossi A, Amadori<sup>o</sup> M, Dei\_Giudici S, Oggiano A

### **Interaction of porcine monocyte-derived dendritic cells with African swine fever viruses of diverse virulence**

Vet Microbiol. - Vol. 216 ( 2018). - p 190-197. - 35 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.vetmic.2018.02.021> 7798]

African swine fever (ASF) is a devastating disease for which there is no vaccine. The ASF virus (ASFV) can infect dendritic cell (DC), but despite the critical role these cells play in induction of adaptive immunity, few studies investigated their response to ASFV infection. We characterized the in vitro interactions of porcine monocytederived DCs (moDC) with a virulent (22653/14), a low virulent (NH/P68) and an avirulent (BA71V) ASFV strain. At a high multiplicity of infection (MOI = 1), all three strains infected immature moDC. Maturation of moDC, with IFN- $\alpha$ /TNF- $\alpha$ , increased susceptibility to infection with 22653/14 and other virulent strains, but reduced susceptibility to NH/P68 and BA71V. The reduced moDC susceptibility to BA71V/NH/P68 was IFN- $\alpha$  mediated, whereas increased susceptibility to 22653/14 was induced by TNF- $\alpha$ . Using an MOI of 0.01, we observed that BA71V replicated less efficiently in moDC compared to the other isolates and we detected increased replication of NH/P68 compared to 22653/14. We observed that BA71V and NH/P68, but not 22653/14, downregulated expression of MHC class I on infected cells. All three strains decreased CD16 expression on moDC, whereas ASFV infection resulted in CD80/86 down-regulation and MHC class II DR up-regulation on mature moDC. None of the tested strains induced a strong cytokine response to ASFV and only modest IL-1 $\alpha$  was released after BA71V infection. Overall our results revealed differences between strains and suggest that ASFV has evolved mechanisms to replicate covertly in inflammatory DC, which likely impairs the induction of an effective immune response.

Franzoni G, Razzuoli E, Sanna G, Zinellu S, Angioi P, Sanna ML, Galleri G, Modesto P, Amadori<sup>o</sup> M, Dei\_Giudici S, Oggiano A

### **Characterization of the interaction of diverse virulence African swine fever virus strains with macrophage subsets**

72<sup>o</sup> Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 318. - 2 bib ref [Nr. Estr. 7971]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

African swine fever (ASF) is a devastating disease for which there is no vaccine available [1]. ASF virus (ASFV) has a tropism for cells of the myeloid lineage, including macrophages and dendritic cells (DC) [2]. Despite the central importance of macrophages for ASFV pathogenesis and the

polarising effects of classical and alternative activation on macrophage phenotype/function, there are very few studies of the interaction of ASFV with activated macrophages. We therefore conducted an in vitro characterization of the interactions of porcine monocyte-derived unactivated (moM), classically (moM1) and alternatively (moM2) activated monocyte-derived macrophages with ASFV strains of diverse virulence. Monocytes were differentiated using 50 ng of hM-CSF and were then left untreated or activated with IFN-gamma and LPS (moM1) or IL-4 (moM2). Cells were infected with 1 multiplicity-of-infection (MOI) of a virulent (22653/14) or a low virulence (NH/P68) ASFV strains, along-side mock infected control. Twenty-one hours post-infection (pi) the expression of ASFV proteins and surface markers were assessed with flow cytometry. At different time pi (3, 6, 9, 12, 21 hours) total RNA was extracted and retrotranscribed, then gene expression of IFN-gamma and 17 different IFN-gamma subtypes was determined by q-PCR. We observed that both isolates infected all the macrophage subsets, however NH/P68, but not 22653/14, down-regulated MHC class I and induced IFN-gamma gene expression. These results revealed differences between ASFV strains, suggesting that virulent isolates are able to evade host immune response and promote their survival in infected pigs.

Fusaro A, Zecchin B, Mulatti P, Zamperin G, Schivo A, Ormelli S, Marciano S, Bonfanti L, Azzolini A, Cunial G, Massi° P, Moreno° A, Mandola ML, Marangon S, Terregino C, Monne I

**Transmission dynamics of highly pathogenic avian influenza virus A (H5N8) in Italy, 2016-2017**

2nd National Congress of the Italian Society For Virology "One Virology One Health" : Rome, November 28-30, 2018 / [s.l. : s.n., 2018]. - p 68 ( OC33 ) [Nr. Estr. 8155]

National Congress of the Italian Society for Virology (2nd : Rome : November 28-30, 2018)

Between December 2016 and December 2017, Italy was affected by Highly Pathogenic Avian Influenza virus (HPAIV) H5N8 outbreaks both in domestic and in wild birds. The first epidemic wave lasted for six months, from December 2016 to May 2017, with 16 outbreaks in poultry farms and 7 in migratory birds. The second epidemic wave began on the third week of July 2017 and continued until mid. December of the same year. Sixty-seven outbreaks were observed in poultry farms, while only seven cases were reported in wild birds. To shed light on the source of the Italian H5N8 outbreaks and to investigate the inter- and intra-farm genetic diversity of the circulating viruses, we used a next generation sequencing approach to characterize the complete genome of 115 samples collected from multiple hosts both from each infected farm and from viruses identified in wild species. Maximum-likelihood phylogenetic trees of the eight gene segments indicated four distinct introductions of AIV genotypes at the beginning of the epidemic (December 2016-February 2017). Since March 2017 one single genotype (H5N8-A/wild duck/Poland/82A/2016-like) had been identified, with the exception of a single virus detected in a turkey farm in October, which turned out to be a reassortant virus for the NP and PA genes, likely acquired from low pathogenic H9 viruses circulating in resident wild birds. Evolutionary and phylogeographic analyses performed using the BEAST v1.8.4 package suggested that genotype H5N8-A/wild duck/Poland/82A/2016-like had further evolved into two distinct clusters, namely Italy-A and Italy-B. Italy-A had probably emerged between February-April 2017 and circulated in the north-east of Italy; differently, Italy-B seems to have appeared between March-July 2017 and mainly spread in the north-western regions. During the second epidemic wave, epidemiological investigations identified four clusters of secondary cases, involving 36 infected farms. Viruses isolated from each of these clusters grouped together in the phylogenetic trees of all the eight AIV genes and in our median-joining (MJ) network of the eight concatenated gene segments, thus supporting the findings of the epidemiological analysis. In addition, we demonstrated a high intra-farm genetic diversity (0-9 nucleotide substitutions/genome). This highlighted the importance of genetically characterizing viruses from multiple hosts within a single farm, so as to correctly reconstruct the evolution and the transmission dynamics of an AI epidemic. Overall these results proved to be instrumental to help epidemiological investigations to discriminate between new introductions and lateral spreads. This type of analyses could be performed in almost a real-time fashion, providing quickly accessible information to generate and/or corroborate hypotheses on the likely epidemiologic pattern of contacts between cases, in order to

adjust AI control and prevention policies.

Fusi° F, Ferrara° G, Lorenzi° V, Angelucci° A, Bolzoni° L, Bertocchi° L

**Indagine dell'Iszler : le cause di morte delle bovine da latte**

Inf Zootec. - Vol. 65 no 18 ( 2018). - p 62-70. - 14 bib ref [Nr. Estr. 7990]

Fusi° F, Ginestreti° J, Lorenzi° V, Strano° RM, Ferrara° G, Angelucci° A, Galletti° G, Bergagna S, Bolzoni° G, Zanardi° G, Buffoli° E, Marcolini° A, Bertocchi° L

**Qualità del latte di massa : indicatore a distanza del livello di benessere delle bovine da latte**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Società Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 284-285. - 4 bib ref [Nr. Estr. 8065]

Congresso Nazionale Società Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Since on-farm assessment of dairy cow welfare could be too-time consuming, previous studies suggested the potential of routinely collected herd data for estimating welfare conditions at herd-level (3). A retrospective study (2014-2016) on 287 Italian dairy farms revealed significant negative correlations between monthly bulk tank somatic cell count (SCC) and total bacterial count (TBC) and the overall animal welfare score (AWS) (2), gained by the CReNBA protocol (i.e. Italian Reference Centre for Animal Welfare). The present work aimed to study, in 42 dairy farms, the association between monthly (Oct 2016-Dec 2017) bulk tank milk parameters and the overall AWS gained in two different periods (Oct 2016-Mar 2017; May 2017-Nov 2017). Preliminary results showed a significant negative correlation ( $t = -0.273$ ,  $p = 0.011$ ) between SCC and overall AWS, proposing bulk tank SCC as a pre-screening tool for herd in poor welfare conditions.*

Gabai G, Amadori° M, Knight CH, Werling D

**The immune system is part of a whole-organism regulatory network**

Res Vet Sci. - Vol. 116 ( 2018). - p 1-3. - 14 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.rvsc.2017.09.018> 8122]

Gaibani P, Campoli C, Lewis RE, Volpe SL, Scaltriti° E, Giannella M, Pongolini° S, Berlingeri A, Cristini F, Bartoletti M, Tedeschi S, Ambretti S

**In vivo evolution of resistant subpopulations of KPC-producing Klebsiella pneumoniae during ceftazidime/avibactam treatment**

J Antimicrob Chemother. - Vol. 73 ( 2018). - p 1525-1529.- 20 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1093/jac/dky082> 7854]

Objectives: KPC-producing *Klebsiella pneumoniae* (KPC-Kp) represent a serious problem worldwide. Herein, we describe the evolution of ceftazidime/avibactam resistance by sequencing longitudinal clinical isolates from a patient with KPC-Kp bloodstream infection undergoing ceftazidime/avibactam treatment. Methods: WGS was performed on one ceftazidime/avibactam-susceptible KPC-Kp (BOT-CA-S) and two phenotypically different ceftazidime/avibactam-resistant KPC-Kp with low (BOT-CA-R) and high (BOT-EMO) carbapenem MICs. The population diversity was assessed by the frequency of allele mutations and population analysis profiles (PAPs). Results: Phylogenetic analysis demonstrated clonal relatedness of the KPC-Kp isolates, all belonging to the clone ST1519. The D179Y mutation in blaKPC-3 was detected in both of the ceftazidime/avibactam-resistant KPC-Kp, whereas it was absent in the ceftazidime/avibactam-susceptible isolate. The mutation emerged independently in the two

ceftazidime/avibactam-resistant isolates and was associated with a significant reduction in carbapenem MICs in BOT-CA-R, but not in BOT-EMO. WGS analysis revealed that the frequency of the D179Y mutation was 96.32% and 51.05% in BOT-CA-R and BOT-EMO, respectively. PAP results demonstrated that carbapenem resistance in BOT-EMO was due to the coexistence of mixed subpopulations harbouring WT and mutated blaKPC-3. A bacterial subpopulation with high ceftazidime/avibactam resistance for BOT-EMO KPC-Kp showed low carbapenem MICs, whereas a subpopulation with high meropenem resistance had a low MIC of ceftazidime/avibactam. Conclusions: Our analysis indicates that mixed subpopulations of ceftazidime/avibactam-resistant KPC-Kp emerge after ceftazidime/avibactam treatment. The evolution of different subpopulations that are highly resistant to ceftazidime/avibactam likely contributes to treatment failure, thereby highlighting the need for combination treatment strategies to limit selection of ceftazidime/avibactam-resistant KPC-Kp subpopulations.

Galiero° A, Leo° S, Garbarino° C, Arrigoni° N, Russo° S, Giacomelli° S, Bianchi° A, Trevisiol K, Idrizi I, Daka G, Fratini F, Turchi B, Cerri D, Ricchi° M

**Mycobacterium avium subsp. [i.e. avium subsp.] paratuberculosis isolated from wild red deer (*Cervus elaphus*) in Northern Italy**

Vet Microbiol. - Vol. 217 ( 2018). - p 167-172. - 37 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.vetmic.2018.03.015> 7834]

Paratuberculosis (or Johne's disease) is an infectious disease which affects mainly ruminants and it is caused by *Mycobacterium avium* subsp. paratuberculosis (MAP). During a culling program (years 2011–2015) aimed at controlling the red deer (*Cervus elaphus*) population in Stelvio National Park (Italian Alps), where paratuberculosis was already described in this species, 382 tissue samples from the Lombardy Region and 102 fecal specimens from the Autonomous Province of Bolzano were analyzed by PCR. Of these, 77 samples (20.16%) from the Lombardy area and 19 specimens (18.63%) from the Bolzano area resulted PCR positive. The cultural test was carried out on PCR positive samples (n = 96), enabling the isolation of 19 MAP field strains which were genotyped using MIRU-VNTR typing and Short Sequence repeats (SSRs). Our results suggest that all isolates share an identical VNTR profile corresponding to the INMV1 genotype. The only variation was on the locus SSR2, but the utility of this last locus has already been questioned because of its instability. Overall, these data suggest a common clonal origin and host adaptation during the diffusion of paratuberculosis in this population. Finally, this profile is the same as that which has already been described in the cattle population in Northern Italy, suggesting a possible inter-species disease transmission pattern from wildlife to domestic ruminants and vice versa.

Galiero° A, Leo° S, Garbarino° C, Arrigoni° N, Russo° S, Ricchi° M

**Genotype profile of *Mycobacterium avium* paratuberculosis isolated from red deer (*Cervus elaphus*) in Northern Italy**

14th International colloquium on paratuberculosis : 4-8 June 2018, Riviera Maya, Mexico : program & abstracts / [s.l. : s.n, 2018]. - p 132 (O-2.73) [Nr. Estr. 8135]

International colloquium on paratuberculosis (14th : Riviera Maya, Mexico : 4-8 June 2018)

Paratuberculosis (or Johne's disease) affects ruminants and it is caused by *Mycobacterium avium* subsp. paratuberculosis (MAP). In deer, signs of illness include weight loss, poor body condition and diarrhea with fecal staining around the perineum and hindquarters. From an epidemiological point of view, the detection of MAP in the wild red deer population points to a possible inter-species disease transmission from wildlife to domestic ruminants and vice versa. The objective of our study was to measure the prevalence of the disease in red deer population in the Lombardy Region and the Autonomous Province of Bolzano (Italy). In addition, MAP field strains isolated were sub-typed by Micro and Mini satellites loci in order to investigate the role of wildlife in the transmission of the

disease between domestic and wild animals. Three hundred and twenty eight tissue samples from the Lombardy Region and 102 fecal specimens from the Autonomous Province of Bolzano were analyzed by PCR. Then, cultural test was carried out on PCR positive samples and subsequently isolated strains were typed by DMC PCR and sub-genotyped by VNTR typing and Short Sequence repeats (SSRs). Out of the sample analyzed, 77 samples (20.16%) from the Lombardy area and 19 samples (18.63%) from the Bolzano area resulted PCR positive. The cultural test carried out on PCR positive samples (n=96), enabled the isolation of 19 MAP field strains which were genotyped. All isolates resulted as type C and share an identical VNTR profile (already known as INMV1 genotype) and SSR profile. All isolates showed the same VNTR pattern profile, which has previously been encountered in Red Deer in other European countries. Notably, the same VNTR pattern and the same SSR profile were detected in Germany in a farm cattle suggesting the possibility of interspecies transmission for this genotype. More studies should be carried out to understand the epidemiological role of wildlife in the transmission of paratuberculosis, including also nonruminant wildlife, so that control strategies could be adopted to avoid interspecific transmission.

Galletti° G, Santi° A, Guberti V, Paternoster° G, Licata E, Loli Piccolomini L, Procopio° A, Tamba° M

**A method to identify the areas at risk for the introduction of avian influenza virus into poultry flocks through direct contact with wild ducks**

Transbound Emerg Dis. - Vol. 65 ( 2018). - p 1033-1038. - 28 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1111/tbed.12838> 7897]

Wild dabbling ducks are the main reservoir for avian influenza (AI) viruses and pose an ongoing threat to commercial poultry flocks. Combining the (i) size of that population, (ii) their flight distances and (iii) their AI prevalence, the density of AI-infected dabbling ducks (DID) was calculated as a risk factor for the introduction of AI viruses into poultry holdings of Emilia-Romagna region, Northern Italy. Data on 747 poultry holdings and on 39 AI primary outbreaks notified in Emilia-Romagna between 2000 and 2017 were used to validate that risk factor. A multivariable Bayesian logistic regression was performed to assess whether DID could be associated with the occurrence of AI primary outbreaks. DID value, being an outdoor flock, hobby poultry trading, species reared, length of cycle and flock size were used as explanatory variables. Being an outdoor poultry flock was significantly associated with a higher risk of AI outbreak occurrence. The probability of DID to be a risk factor for AI virus introduction was estimated to be 90%. A DID cut-off of 0.23 was identified to define high-risk areas for AI virus introduction. Using this value, the high-risk area covers 43% of the region. Seventy-four per cent of the primary AI outbreaks have occurred in that area, containing 39% of the regional poultry holdings. Poultry holdings located in areas with a high DID value should be included in a risk-based surveillance programme aimed at AI early detection.

Ghidini S, Scalfi° F, Borrello S, Colagiorgi A, Ianieri A, Di\_Ciccio PA, Varrà MO, Zanardi E, Maisano° AM, Giacomini° E, Alborali° GL

**Applicazione del sistema CLASSYFARM su allevamenti suinicoli da ingrasso della Lombardia : relazioni tra consumo di antibiotici, benessere e lesioni al macello**

Attualità nell'igiene degli alimenti: stato dell'arte e prospettive future : XXVIII Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) : Milano, 12-14 Settembre 2018 / [s.l. : s.n., 2018]. - p 30 (P014) [Nr. Estr. 7931]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (28. : Milano : 12-14 Settembre 2018)

Il presente lavoro, svolto nell'ambito di un progetto di ricerca "Classyfarm" e finanziato dal Ministero della Salute, ha avuto come obiettivo quello analizzare la presenza o meno di relazioni tra consumo di antimicrobici, lesioni al macello e benessere animale in allevamento, nell'ottica di un approccio

integrato alla razionalizzazione dell'uso di antimicrobici. Lo studio è stato svolto su 13 allevamenti, situati in Regione Lombardia. Le partite di suini incluse nelle valutazioni al macello sono state macellate nella stessa struttura, a carattere industriale (400 suini/ora). I dati di consumo degli antimicrobici sono stati raccolti retrospettivamente per l'anno 2016 mediante consultazione dei documenti cartacei (registri, bolle e ricette). Il consumo è stato stimato come giorni di trattamento per animale allevato, abbreviato in giorni/suino, utilizzando le "defined daily dose animal for Italy" (DDDAit) ed un peso standard al trattamento pari a 100 Kg. L'impiego di antimicrobici in categorie diverse dai suini grassi, anche se presenti in azienda, non è stato riportato nel presente lavoro. Le rilevazioni sul benessere animale dei suini all'ingrasso sono state effettuate utilizzando la check-list elaborata dal Centro di Referenza Nazionale per il Benessere Animale. Al macello, durante l'ispezione post-mortem, sono state valutate 53 tipologie di lesioni. Dieci tra le lesioni più frequenti e rilevanti sono state selezionate per il confronto con il consumo del farmaco. Le lesioni particolarmente pertinenti alla valutazione del benessere, inoltre, sono state raggruppate nelle quattro categorie (polmonite, pleurite, pericardite, white spot). Le informazioni riguardanti benessere animale, consumo di antimicrobici e lesioni al macello sono state inserite e processate attraverso il sistema di monitoraggio integrato Classyfarm. Le correlazioni tra i consumi di farmaco, benessere e lesioni al macello sono state indagate secondo il coefficiente di correlazione per ranghi di Spearman. I consumi medi di antimicrobici sono risultati, per il 2016, 21 giorni/suino (range 0,1-87,8). Le ABMs rilevate in allevamento (2336 suini, media per azienda 172±14) hanno mostrato un'ampia variabilità. Tale varianza si è riscontrata sia per quanto riguarda ABMs tendenzialmente più legate allo stato sanitario, come sindromatologie enteriche lievi (range 0,0-39,3%) e respiratorie (range 0,0-9,1%), che per ABMs fattori di rischio per lo sviluppo di patologie, come l'imbrattamento fecale del corpo di grado 1 (range 2,6-34,6%) e 2 (range 0,0-85,4%). Le osservazioni al macello (70000 carcasse) hanno riportato una notevole variabilità, con netta prevalenza delle lesioni polmonari tra cui polmonite enzootica (9,3±3,5%) e non ..(5,1±1,2%) sono state le più rappresentate. Non vi è stata alcuna correlazione significativa tra consumi di antimicrobici, benessere e lesioni al macello. I risultati preliminari di questo lavoro dovranno essere confermati su un numero più ampio di aziende, tuttavia, l'assenza di correlazione tra i parametri registrati al macello, gli ABMs ed il consumo di antimicrobici, suggerisce la possibilità che i trattamenti effettuati sugli animali possano essere stati non sempre razionali.

Giacomelli° S, Bianchi° A, Rota\_Nodari° S, Gibelli° LR, Vicari° N, Archetti° I, Bertoletti° I

#### **Messa a punto di metodiche per la determinazione di presenza ed intensità di infestazione da *Sarcocystis* spp. in ungulati selvatici**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Società Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 290-294. - 7 bib ref [Nr. Estr. 8066]

Congresso Nazionale Società Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Sarcocystosis is one of the most widespread tissue parasitosis of domestic and wild herbivores. Wild ungulates have generally high prevalence of infestation (4; 7) and often burden unknown species (1; 5). A toxin, isolated from Sarcocystis has been recently reported to be a causative agent of acute food poisoning, inducing nausea, vomiting and diarrhea (6). Data for prevalence and intensity of infection are rarely comparable, due mainly to the different methodologies applied in studies (7). With the increase of consumption of raw or poorly cooked game meat, it is necessary to have a simple and reliable method of detection and quantification of this parasite. In our study we compared four different methods of Sarcocystis detection in samples of wild boars, deer, roe deer and chamois.*

Giacomini° E, Gasparri° S, Lazzaro° M, Scali° F, Boniotti° MB, Corradi A, Pasquali P, Alborali° GL

## The role of transportation in the spread of *Brachyspira hyodysenteriae* in fattening farms

BMC Vet Res. - Vol. 14 ( 2018). - Article no 10 (6 p). - 22 bib ref [Nr. Estr. ( ultimo accesso 09/02/2018 ) <https://doi.org/10.1186/s12917-017-1328-5> 7778]

Background: Direct and indirect contact among animals and holdings are important in the spread of *Brachyspira hyodysenteriae*. The objective of this study was to investigate the role of slaughterhouse vehicles in spreading *B. hyodysenteriae* between unconnected farms. Results: Multilocus sequence typing (MLST) and Multiple Locus Variable number tandem repeat Analysis (MLVA) were used to characterize *B. hyodysenteriae* strains isolated from trucks. Before cleaning, 976 batches of finishing pigs transported by 174 trucks from 540 herds were sampled. After cleaning, 763 of the 976 batches were also sampled. Sixty-one of 976 and 4 of 763 environmental swabs collected from trucks before and after cleaning and disinfection operations, respectively, were positive for *B. hyodysenteriae*. The 65 isolates in this study originated from 48 farms. Trucks were classified into five categories based on the number of visited farms as follows: category 1: 1–5 farms, category 2: 6–10 farms, category 3: 11–15 farms, category 4: 16–20 farms, category 5: >21 farms. Although the largest number of vehicles examined belonged to category 1, the highest percentage of vehicles positive for *B. hyodysenteriae* was observed in categories 3, 4 and 5. Specifically, 90.9% of trucks belonging to category 5 were positive for *B. hyodysenteriae*, followed by categories 4 and 3 with 85.7% and 83.3%, respectively. The results of MLST and MLVA suggest that trucks transporting pigs from a high number of farms also play a critical role in spreading different *B. hyodysenteriae* genetic profiles. STVT 83–3, which seems to be the current dominant type in Italy, was identified in 56.25% of genotyped isolates. The genetic diversity of isolated strains from trucks was high, particularly, in truck categories 3, 4 and 5. This result confirmed that MLST and MLVA can support the study of epidemiological links between different *B. hyodysenteriae* farm strains. Conclusions: This study highlights the potential role of shipments in *B. hyodysenteriae* spread. Moreover, it emphasizes the importance of strict vehicle hygiene practices for biosecurity programmes.

Ginestreti° J, Lorenzi° V, Fusi° F, Angelucci° A, Strano° RM, Ferrara° G, Scali° F, Alborali° GL, Bertocchi° L

### A preliminary study on antibiotic usage in Italian dairy herds: the DDDAit method

Proceedings of the fifth DairyCare Conference 2018 : Thessaloniki, March 19th and 20th 2018 / editor, C.H. Knight. - [s.l.] : DairyCare COST Action FA1308, 2018. - p 50 (Poster 14). - 4 bib ref [Nr. Estr. 7819]

DairyCare Conference (5th : Thessaloniki : March 19th and 20th 2018)

It has been estimated that in the European Union the 50% of all antimicrobials, consumed annually, are used in livestock animals<sup>1</sup>. Even if the transmission of antimicrobial resistance from animals to humans is not fully explored, it is recognized that an unreasonable antimicrobial usage (AMU) in food producing animals relates to the selection of resistant bacteria or genes<sup>2</sup>. Promotion of antibiotic correct use can thus preserve the efficacy of molecules that are “critically important” for human medicine. In 2017, Italy was confirmed as the third country in Europe with the highest veterinary antimicrobial consumption based on wholesale data<sup>3</sup>. Currently, there is no an internationally accepted standard to quantify and report veterinary AMU in Europe, but the use of the Defined Daily Dose method<sup>4</sup> is given high priority also in veterinary medicine. Since there are still no data about AMU in Italian dairy farms, based on DDD metrics, the aim of this preliminary study was to quantify AMU in 20 Italian herds. Farm records of antibiotic treatments used during 2016 (whole year) were collected and analysed. Animal category, treatment indication and administration route were also registered. The AMU index, expressed as number of days per cow per year, was calculated using Defined Daily Dose Animal for Italy (DDDAit), based on Italian summaries of product characteristics. Considering the studied farms, mean AMU for adult cows was 11.18 days/year (range 3.47-21.17). In adult cows, antibiotics were mostly used for udder problems (40.67%), dry cow therapy (33.66%), locomotion problems (14.24%) and urogenital diseases (6.81%). Intramammary (51.51%) and injectable routes (46.42%) were the most common routes of administration. Most used antimicrobials in adult cows are reported in Figure 1. A significant



correlation was found between AMU for adult cows and herd size (Pearson's  $r$ : 0.48;  $P$ value= 0.03). Despite the few sampled herds, the preliminary results confirmed the primary role of udder problems and dry cow therapy in boosting antibiotic consumption in dairy farms.

Girault G, Wattiau P, Saqib M, Martin B, Vorimore F, Singha H, Engelsma M, Roest HJ, Spicic S, Grunow R, Vicari° N, De\_Keersmaecker SCJ, Roosens NHC, Fabbi° M, Tripathi BN, Zientara S, Madani N, Laroucau K

#### **High-resolution melting PCR analysis for rapid genotyping of *Burkholderia mallei***

Infect Genet Evol. - Vol. 63 ( 2018). - p 1-4. - 8 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 )  
<https://doi.org/10.1016/j.meegid.2018.05.004> 7852]

*Burkholderia* (*B.*) *mallei* is the causative agent of glanders. A previous work conducted on single-nucleotide polymorphisms (SNP) extracted from the whole genome sequences of 45 *B. mallei* isolates identified 3 lineages for this species. In this study, we designed a high-resolution melting (HRM) method for the screening of 15 phylogenetically informative SNPs within the genome of *B. mallei* that subtype the species into 3 lineages and 12 branches/sub-branches/groups. The present results demonstrate that SNP-based genotyping represent an interesting approach for the molecular epidemiology analysis of *B. mallei*.

Gibellini M, Rossi R, Ferro P, Pitozzi° A, Trogu E, Fusco L, Foresti F, Turco G, Boniotti° B, Alborali° GL

#### **Dinamica dell'infezione da *Mycoplasma hyopneumoniae* in scrofette da rimonta in 3 allevamenti di tipo commerciale** = Infection dynamics of *Mycoplasma hyopneumoniae* in replacement gilts in 3 commercial herds

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 135-140. - 11 bib ref [Nr. Estr. 7826]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. :  
Montichiari (BS) : 15-16 Marzo 2018)

La trasmissione di *Mycoplasma hyopneumoniae*, agente eziologico della polmonite enzootica, avviene prevalentemente per contatto diretto. Nonostante il problema sia maggiormente apprezzabile nei suini in fase di accrescimento-ingrasso, il contatto tra scrofa escrettrice e suinetto gioca un ruolo chiave nel mantenimento dell'infezione in allevamento. In particolare le scrofette e le scrofe giovani rappresentano un fattore di criticita per la trasmissione del *Mycoplasma hyopneumoniae* in sala parto. Scopo di questo studio è stato quello di valutare la presenza, la dinamica e la persistenza dell'infezione da *Mycoplasma hyopneumoniae* nelle scrofette da rimonta. 168 scrofette provenienti da 3 allevamenti positivi per *Mycoplasma hyopneumoniae* sono state selezionate e testate nel corso dello studio. Tamponi tracheo-bronchiali e prelievi ematici sono stati effettuati a 110, 150, 180, 210, 270, 330 e 360 giorni di vita. 52 tamponi su 164 sono risultati positivi mentre la sierologia ha evidenziato una sieroprevalenza variabile nel corso degli intervalli di tempo valutati. Le scrofette oggetto dello studio sono risultate naturalmente infettate con *Mycoplasma hyopneumoniae* per un minimo di 2 mesi ad oltre 160 giorni. Inoltre è stata osservata l'estensione dell' infezione tardiva fino alla sala parto, fattore di rischio importante per il protrarsi della patologia in allevamento.

*Mycoplasma hyopneumoniae* is prevalent transmitted by direct contact; despite pathology is mainly impactful in fattening unit, the direct contact between piglets and their dams play a crucial role for the persistence of the microorganism in the herd. Gilts and young sows represent a critical factor for *Mycoplasma* transmission in farrowing unit. The aim of the study was to assess detection, dynamic and persistence of *Mycoplasma hyopneumoniae* infection in replacement gilts. 168 gilts from three commercial herds positive for *Mycoplasma hyopneumoniae* were selected and tested during the study. Tracheobronchial swabs and blood were collected at 110, 150, 180, 210, 270, 330 and 360

*days of age. Positive swabs were founded in 52 out of 164 evaluated; seroprevalence was variable during the observed time. The study has shown that naturally infected gilts are positive for a minimum of 2 months for more than 160 days post infection. If infection occurs late during acclimation the possibility to have shedders in farrowing unit is high and this can compromise the herd health status.*

Grassi A, Baldo° V, Maniscalco L, Facchetti L, Fracassetti P, Salogni° C, Alborali° GL

**Prevalence of mcr-1 and ESBL-producing uropathogenic Escherichia coli isolated from companion animals in Northern Italy in 2017**

28th European Congress of Clinical Microbiology and Infectious diseases (ECCMID) : Madrid, Spain, 21-24 April 2018 / [s.l. : s.n., 2018]. - 1 p. - 4 bib ref [Nr. Estr. 7855]

European Congress of Clinical Microbiology and Infectious diseases (ECCMID) (28th : Madrid, Spain : 21-24 April 2018)

Graziani C, Bellino S, Busani L, Pasquali P, Pacciarini° ML, Boniotti° MB, Zanoni° MG, Bollo E, De\_Carlo E, Vendetti S, Pezzotti P, Amadori° M, Gibelli° L, D'Incau° M, Zanardi° G, Borrello S, Ruocco L, Lomolino R, Primavera A

**Micobatteri tubercolari negli animali e implicazioni di sanità pubblica in Italia** = Mycobacterial infections in animals and implications for public health in Italy

Micobatteri tubercolari negli animali e implicazioni di sanità pubblica in Italia / Caterina Graziani ... [et al.]. - Roma : Istituto Superiore di Sanità, 2018. - (Rapporti Istisan ; 18 / 3) 93 p [Nr. Estr. 7853]

Questo rapporto, aggiorna e raccoglie in modo organico ed esaustivo tutte le informazioni disponibili sulla circolazione negli animali e nell'uomo di Mycobacterium bovis e di altri micobatteri appartenenti al Mycobacterium tuberculosis complex dal 2001 al 2016 in Italia. In particolare, oltre agli aspetti generali della malattia, sono stati raccolti e analizzati i dati ufficiali disponibili sulla diffusione della malattia nell'uomo e negli animali, consentendo una lettura integrata del fenomeno. Viene presentata l'importanza di un approccio integrato, con attenzione ai settori di sanità animale, sicurezza alimentare e salute pubblica per individuare tempestivamente i punti critici e le possibilità di intervento per la prevenzione e il controllo della malattia in Italia. Il rapporto è uno strumento di conoscenza e di pianificazione a disposizione sia dei ricercatori sia delle autorità, e fornisce un quadro delle azioni svolte e delle informazioni disponibili sulla malattia.

*This report presents a comprehensive picture of all available information on the circulation in animals and humans of Mycobacterium bovis and other mycobacteria belonging to Mycobacterium tuberculosis complex from 2001 to 2016 in Italy. The disease has been described analyzing the official data available in Italy from the surveillance in animals and in humans. This document presents the integrated approach, under the "One Health, One Medicine" concept that in Italy has been followed for the control of the disease, emphasizing the importance of such approach and the need to extensive cooperation between public health and animal health professionals. This report is intended as a tool for both scientists and authorities, providing them with the available knowledge of the disease and focussing on critical points and conditions that still affect the capacity of control of the disease.*

Grippi F, Barreca S, Sciacca C, Arnone M, D'Incau° M, Galluzzo P, Guercio A  
**Outbreaks of bovine leptospirosis infection in two Sicilian livestock (Southern Italy)**

3rd ELS scientific meeting on Leptospirosis and other rodent borne haemorrhagic fevers : 24-26 May 2018, Alghero / [s.l. : s.n., 2018]. - p 56 [Nr. Estr. 7864]

ELS scientific meeting on Leptospirosis and other rodent borne haemorrhagic fevers (3rd : Alghero : 24-26 May 2018)

Until now, there are no data about Leptospirosis cases in bovines in Sicily. In this work, we first report leptospirosis infection outbreaks in bovines farmed in two different livestock located in mountainous area in the heart of Madonie Natural Park (Sicily). After some cases of abortion and fertility problems, the freelance and public veterinary, supposed a probable leptospirosis infection. The bovines object of our study belonged to two different livestock. The first one was composed of only bovine of Charalaise race. The second was composed of Limousine bulls and Cinisare and Modicane cows. The outbreaks emerged in October 2017. Number 23 samples of bovine sera were taken in the first livestock and n. 75 in the other one. Sampling was carried out before and after the antibiotic treatment with oxytetracycline (10mg/kg of body weight with two injections at four days interval). Two urine samples were taken from positive bovines (2/2) belonging to the first livestock and four (4/29) in the second one; in addition, a sample of brain and placenta were taken from a single abortion. The diagnosis of leptospirosis infection was based on the antibodies detection through serological Microagglutination Test (MAT) conducted as prescribed in the O.I.E. Manual (Chapt. 2.1.12. Par. B2.1. 2014). The analysis concerned eight common serogroups circulating in Italy (L. interrogans serogroup Australis serovar Bratislava, L. interrogans serogroup Ballum serovar Ballum, L. interrogans serogroup Canicola serovar Canicola, L. kirschneri serogroup Grippotyphosa serovar Grippotyphosa, L. interrogans serogroup Icterohaemorrhagiae serovar Copenhageni, L. interrogans serogroup Pomona serovar Pomona, L. borgpetersenii serogroup Sejroe serovar Hardjo, L. borgpetersenii serogroup Tarassovi serovar Tarassovi). "Real-Time PCR method" was performed for the gene lipL32 detection of pathogenic *Leptospira* spp. in the brain, placenta and urine samples. The "isolation method" was performed only urine sample. Afterwards a differential diagnosis, no presence of bacterial or viral abortion agents was detected. In the first livestock, 2 serological positive bovines (2/23) to antibodies against L. borgpetersenii serogroup Sejroe serovar Hardjo were found. While in the other livestock, there were 29 serological positive bovines (29/75), with different antibody titres against L. borgpetersenii serogroup Sejroe serovar Hardjo (1:100 — 1:3200). The Real Time PCR test detected *Leptospira* DNA in one of the two samples of urine of the first livestock. But the same Real Time PCR test on both placenta and brain was negative. The isolation is still in progress. After 40 days from antibiotic treatment, the antibody titres to L. borgpetersenii serogroup Sejroe serovar Hardjo decreased in the first livestock. Instead, in the second livestock, the titres of 6 bovines increased, the titres of 4 diminished and 2 new positives emerged. The titres of the other positive 19 remained at the same level as before. In both livestock the symptoms disappeared. The good results obtained with antibiotic treatment in the bovines of the first livestock, let us to believe that outbreak will be over soon. Instead, the situation of the second livestock seems to be more complicated and makes the leptospirosis eradication more difficult. Infact, a second antibiotic treatment is still on going. In order to control the progression of the *Leptospira* spp. infection, it is our intent to check the bovine antibody titres in the next future.

Guarda F, Salogni° C, Chiappino L, Sereno A, Colombino E, Capucchio MT

**Appunti di anatomia del cuore e delle sue arterie nei pesci Osteitti con spunti di patologia** = Notes of anatomy of the heart and of its arteries in fishes with hints of pathology

Ittiopatologia. - Vol. 15 no 1-2 ( 2018). - p 3-28. - 37 bib ref [Nr. Estr. 8202]

L'anatomia del cuore dei pesci è stata ampiamente studiata, ma la patologia cardiaca è stata sinora piuttosto trascurata, se si escludono le alterazioni conseguenti a malattie infettive da batteri o virus. Gli autori offrono una panoramica basilare delle strutture cardiovascolari degli Osteitti sulla base della letteratura e delle esperienze personali. A completamento presentano alcune delle piu comuni alterazioni del cuore e dei vasi cardiaci osservate durante il loro lavoro in alcune specie di pesci allevati e selvatici.

*The anatomy of the fish's heart has been extensively studied, but the cardiac pathology has so far been rather neglected, if the alterations resulting from bacterial or viral infectious diseases are excluded. The authors provide a basic overview of the cardiovascular structures of Osteichthyes based on literature and personal experiences. To complete, they present some of the most common alterations of the heart and of the cardiac vessels observed during their work in some species of farmed and wild fish.*

Guerrini A, Fedrizzi° G, Andreotti A, Iannone L, Roncada P

**Evaluation of Pb and Hg levels in wild migratory water-birds nesting in different areas of Po Delta**

2018 Scientific Meeting Italian Association for the study of Trace Elements in living organisms - AISETOV "The role of trace elements in health: from healthy environments to healthy living organisms" : Ozzano Emilia, Bologna, October 12, 2018 : abstract book / [s.l. : AISETOV], 2018. - p 14 (O-06) [Nr. Estr. 7993]

Scientific Meeting Italian Association for the study of Trace Elements in living organisms (AISETOV) : Ozzano Emilia, Bologna : October 12, 2018)

The use of fossil fuels as main energy resource led to metal emissions that spread all over the environment and contaminate ecosystems and food. This has resulted in even serious toxic effects, both in humans and animals. A characteristic feature of some metals is their pronounced biological accumulation and biomagnification in food chains. For these reasons, their monitoring in the environment should be constant over time. This study shows data on Pb and Hg levels in tissues of various species of wild migratory water-birds nesting in different areas of Po Delta. The sampling was carried out from May to July 2016. Analyses were performed according to the internal test method of the IZSLER by inductively coupled plasma mass spectrometry (ICP/MS). The two metals were found in most of the analyzed samples. The maximum levels of Pb were found in the natal down of nestlings (3.5515 mg/kg), and in feathers, femur and humerus of adult birds (5.2363, 15.469 and 12.9760 mg/kg, respectively). In adults the highest concentrations of Hg were found in feathers and liver (6.0144 and 6.1939 mg/kg, respectively) while in nestlings the highest level was found in down (4.9411 mg/kg). The differences between the average concentrations detected in adults and those measured in nestlings can be explained by chronic exposure to metals. This exposure is usually highest at the top of food chains in predatory species. Thus, the birds of prey may be suited for monitoring purposes, but their sampling may be difficult due to their low population densities and high mobility. Moreover, in the present study only birds dead of natural causes were used. In the future, the analysis of a higher number of samples will provide a more complete picture.

Hamdy ME, Del\_Carlo M, Hussein HA, Salah TA, El-Deeb AH, Emara MM, Pezzoni° G, Compagnone D

**Development of gold nanoparticles biosensor for ultrasensitive diagnosis of foot and mouth disease virus**

J Nanobiotechnology. - Vol. 16 ( 2018). - no 48 (12 p). - 49 bib ref [Nr. Estr. ( Ultimo accesso 04/09/2018 ) <https://doi.org/10.1186/s12951-018-0374-x> 7885]

Background Nano-PCR is a recent tool that is used in viral diseases diagnosis. The technique depends on the fundamental effects of gold nanoparticles (AuNPs) and is considered a very effective and sensitive tool in the diagnosis of different diseases including viral diseases. Although several techniques are currently available to diagnose foot and mouth disease virus (FMDV), a highly sensitive, highly specific technique is needed for specific diagnosis of the disease. In the present work, a novel AuNPs biosensor has been designed using thiol-linked oligonucleotides that recognize the conserved 3D gene of FMDV. Results The AuNPs-FMDV biosensor specifically recognizes RNA standards of FMDV, but not that of swine vesicular disease virus (SVDV) isolates. The analytical sensitivity of the AuNPs-FMDV biosensor was 10 copy number RNA standards in RT-PCR and 1 copy number RNA standard in real-time rRT-PCR with a 94.5% efficiency, 0.989 R<sup>2</sup>, a - 3.544 slope and 100% specificity (no cross-reactivity with SVDV). These findings were confirmed by the specific and sensitive recognition of 31 Egyptian FMDV clinical isolates that represents the three FMDV serotypes (O, A, and SAT2). Conclusions The AuNPs-FMDV biosensor presents in this study demonstrates a superior analytical and clinical performance for FMDV diagnosis. In addition, this biosensor has a simple workflow and accelerates epidemiological surveillance, hence, it is qualified as an efficient FMDV diagnosis tool for quarantine stations and farms particularly in FMDV endemic areas.

Jucker C, Loni A, Calzolari<sup>o</sup> M, Belokobylskij S, Lupi D

**Accidental introduction in Italy of the parasitoid *Spathius vulnificus* Wilkinson (Hymenoptera braconidae Doryctinae)**

Redia. - Vol. 101 ( 2018). - p 189-192. - 32 bib ref [Nr. Estr. ( ultimo accesso 14/01/2019 )  
<http://dx.doi.org/10.19263/REDIA-101.18.25.8081>]

This paper reports the finding of the Hymenoptera Doryctinae *Spathius vulnificus* Wilkinson for the first time in Europe. This is an idiobiont ectoparasitoid attacking woodborer larvae of some Bostrichidae (Coleoptera) species. Such exotic parasitoid, mainly spread in the Oriental and southeastern part of the Palaearctic Region, has been intercepted in association with the lesser auger beetle *Heterobostrychus aequalis* (Waterhouse) (Coleoptera, Bostrichidae), imported from the Asian Far East in wood packaging material. This report represents an updating on the worldwide distribution of the parasitoid.

Jurado C, Martínez-Avilés M, De\_La\_Torre A, Štukelj M, de\_Carvalho\_Ferreira HC, Cerioli<sup>o</sup> M, Sánchez\_Vizcaíno JM, Bellini<sup>o</sup> S

**Relevant measures to prevent the spread of African swine fever in the European Union domestic pig sector**

Front Vet Sci. - Vol. 5 ( 2018). - Article no. 77 (16 p). - 103 bib ref [Nr. Estr. ( ultimo accesso 04/03/2019 ) <https://doi.org/10.3389/fvets.2018.00077.8126>]

During the past decade, African swine fever (ASF) has spread from the Caucasus region to eastern European Union countries affecting domestic pig and wild boar populations. In order to avert ASF spread, mitigation measures targeting both populations have been established. However, despite these efforts, ASF has been reported in thirteen different countries (Georgia, Azerbaijan, Armenia, the Russian Federation, Ukraine, Belarus, Estonia, Latvia, Lithuania, Poland, Moldova, Czech Republic, and Romania). In the absence of an effective vaccine or treatment to ASF, introduction and spread of ASF onto domestic pig farms can only be prevented by strict compliance to control measures. This study systematically reviewed available measures to prevent the spread of ASF in the EU domestic pig sector distinguishing between commercial, non-commercial, and outdoor farms. The search was performed in PubMed and using a common browser. A total of 52 documents were selected for the final review process, which included scientific articles, reports, EU documents and official recommendations, among others. From this literature review, 37 measures were identified as preventive measures for the introduction and spread of ASF. Subsequently, these measures were assessed by ASF experts for their relevance in the mitigation of ASF spread on the three mentioned types of farms. All experts agreed that some of the important preventive measures for all three types of farms were: the identification of animals and farm records; strict enforcement of the ban on swill feeding; and containment of pigs, so as to not allow direct or indirect pig–pig and/or pig–wild boar contacts. Other important preventive measures for all farms were education of farmers, workers, and operators; no contact between farmers and farm staff and external pigs; appropriate removal of carcasses, slaughter residues, and food waste; proper disposal of manure and dead animals, and abstaining from hunting activities during the previous 48 h (allowing a 48 h interval between hunting and being in contact with domestic pigs). Finally, all experts identified that the important preventive measures for non-commercial and outdoor farms is to improve access of those farms to veterinarians and health services.

Jurado C, Sanchez\_Vizcaino JM, Martínez-Avilés M, de\_la\_Torre A, Bellini<sup>o</sup> S

### **Systematic review and assessment of measures to prevent the spread of African swine fever in the domestic pig sector**

4th GARA Scientific Workshop : April 11-13 2018, Cagliari / [s.l. : s.n., 2018]. - 2 p. - 3 bib ref [Nr. Estr. 8125]

GARA (Global African Swine Fever Research Alliance) Scientific Workshop (4th : Cagliari : April 11-13, 2018)

During the last decade, African swine fever (ASF) has spread from the Caucasus region to eastern European Union (EU) countries affecting domestic pig and wild boar. Since its introduction in 2007, numerous mitigation actions targeting both populations have been established. However, ASF spread was not blocked and it reached up to thirteen different countries (Georgia, Azerbaijan, Armenia, the Russian Federation, Ukraine, Belarus, Estonia, Latvia, Lithuania, Poland, Moldova, Czech Republic and Romania). Due to the lack of vaccine or treatment, prevention is key to avoid the entrance and spread of ASF onto domestic pig farms. This work systematically reviewed available measures to prevent the spread of ASF in the domestic pig sector distinguishing between commercial, non-commercial and outdoor farms. The search was performed by using PubMed and a common browser. A total of 52 documents were selected for the final review process. Such pieces of literature pointed out 37 measures to prevent ASF spread. Topics such as the source of new animals, farming practices, animal facilities and farm perimeter, among others, were covered. Moreover, identified preventive measures were assessed through expert opinion elicitation. All experts agreed that the identification of animals and farm records; prohibit swill feeding; and containment of pigs to not allow contact with pigs from other farms, feral pigs or wild boar or their products, were important preventive measures for the three types of farms. Other important preventive measures for all operators; no contact between farmers and farm staff and external pigs; appropriate removal of carcasses, slaughter residues and food waste; proper disposal of manure and dead animals, and no hunting activities during the last 48 hours. Finally, all experts identified as important preventive measures for non-commercial and outdoor farms, to improve access of those farms to veterinarians and health services.

Lanave G, Dowgier G, Decaro N, Albanese F, Brogi E, Parisi A, Losurdo M, Lavazza<sup>o</sup> A, Martella V, Buonavoglia C, Elia G

#### **Novel orthopoxvirus and lethal disease in cat, Italy**

2nd National Congress of the Italian Society for Virology "One Virology One Health" : Rome, November 28-30, 2018 / [s.l. : s.n., 2018]. - p 74 ( OC39 ) [Nr. Estr. 8157]

National Congress of the Italian Society for Virology (2nd : Rome : November 28-30, 2018)

Orthopoxviruses (OPXVs) are complex, double-stranded DNA viruses that belong to the family Poxviridae, subfamily Chordopoxvirinae, genus Orthopoxvirus. Some OPXVs, such as monkeypox virus, camelpox virus, buffalopox virus, and cowpox virus (CPXV), have zoonotic potential and reports of OPXV infections in animals and humans have largely increased during recent decades, leading to the perception of an increasing risk for humans. The study reports the detection and full-genome characterization of a novel OPXV responsible for fatal infection in a cat. The virus induced skin lesions histologically characterized by leukocyte infiltration and eosinophilic cytoplasmic inclusions. Different panchordopoxvirus PCRs specific for the subfamily Chordopoxvirinae and other unclassified chordopoxviruses were unable to assign the virus to a defined OPXV species. Large amounts of typical brick-shaped virions, morphologically related to OPXV, were observed by electron microscopy. The OPXV strain named as Italy\_09/17 was isolated on cell cultures and embryonated eggs. Virus stocks were extracted and subjected to Next Generation Sequencing protocols by using the Nextera DNA Sample Prep Kit (Illumina, San Diego, CA, USA) and a MiSeq instrument. Sequences from the genome assembled were aligned with cognate OPXVs representative of North American and Old World (African and Eurasian) viruses by using MAFFT algorithm implemented in Geneious version 10.1.3 (Biomatters LTD, Auckland, 1010, New Zealand). Phylogenetic analyses were performed using ModelTest to identify the most appropriate model of evolution and Mr Bayes using general time reversible model. The virus was not

a classical CPXV, already identified in felids. Upon analysis of 9 concatenated genes, the feline isolate was only distantly related to all CPXV lineages currently known and formed a separate cluster with respect to ectromelia virus (ECTV), with which it was strictly related. The feline strain grouped with an OPXV strain isolated from captive macaques in Italy (strain Abatino) (Fig.1). The genetic distance between these 2 viruses (strains Italy\_09/17 and Abatino) and ECTVs was higher than the distance observed between variola minor virus and variola major virus. Therefore, the ECTV-like strains Italy\_09/17 and Abatino likely represent a novel OPXV species. Moreover, in the HA gene, strain Italy\_09/17 appeared more closely related to strain Abatino than to feline-derived human OPXV strains identified in Italy in 2009. Extensive epidemiologic surveillance in cats and rodents will assess whether cats are incidental hosts and rodents are the main reservoir of the virus. The zoonotic potential of the feline ECTV-like OPXV also deserves further investigation. Consequently, this feline poxvirus could represent a threat to human health. Thus, veterinarians and cat breeders and owners should be aware of this additional risk associated with handling of cats with skin lesions.

Lanave G, Dowgier G, Decaro N, Albanese F, Brogi E, Parisi A, Losurdo M, Lavazza° A, Martella V, Buonavoglia C, Elia G

#### **Novel orthopoxvirus and lethal disease in cat, Italy**

Emerg Infect Dis. - Vol. 24 no 9 ( 2018). - p 1665-1673. - 49 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://dx.doi.org/10.3201/eid2409.1712837925>]

We report detection and full-genome characterization of a novel orthopoxvirus (OPXV) responsible for a fatal infection in a cat. The virus induced skin lesions histologically characterized by leukocyte infiltration and eosinophilic cytoplasmic inclusions. Different PCR approaches were unable to assign the virus to a defined OPXV species. Large amounts of typical brick-shaped virions, morphologically related to OPXV, were observed by electron microscopy. This OPXV strain (Italy\_09/17) was isolated on cell cultures and embryonated eggs. Phylogenetic analysis of 9 concatenated genes showed that this virus was distantly related to cowpox virus, more closely related to ectromelia virus, and belonged to the same cluster of an OPXV recently isolated from captive macaques in Italy. Extensive epidemiologic surveillance in cats and rodents will assess whether cats are incidental hosts and rodents are the main reservoir of the virus. The zoonotic potential of this novel virus also deserves further investigation.

Lauzi S, Trogu° T, Bellometti S, Koni A, Pedrotti L, Gugiatti A, Corlatti L, Piccinini R, Lanfranchi P, Luzzago C

#### **Free-ranging red deer contribution to environmental contamination of Shiga toxin-producing Escherichia Coli in Italian alps**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 182 [Nr. Estr. 8000]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

Wild ungulates are increasing their density and distribution across Europe. At the same time, habitat fragmentation caused by human activities and infrastructures favours proximity with humans, livestock and other animal species. This critical issue for public health and for conservation, highlights the need for ecological studies of multi-host infections within an eco-evolutionary framework. Red deer (*Cervus elaphus*) is among the most representative species of such patterns: in the Stelvio National Park (SNP, central Italian Alps), for example, very high population density is associated with intense browsing impacts and cross transmission of pathogens with domestic

ruminants. In turn, a culling plan has been initiated in the Lombardy sector of the Park, with the aim to reduce red deer density: this led to the introduction of some 37.3 tons of meat into the food chain over just 5 years, between 2011 and 2016, with potential consequences on human health. Among food-borne pathogens, herbivorous are the main reservoir of Shiga toxin-producing *Escherichia coli* (STEC). The microorganism is shed in feces and can persist in the environment, rising the risk of ingestion, intestinal colonization and fecal shedding in increased numbers of grass-feeding animals. This study aims to evaluate the prevalence, antimicrobial susceptibility and spatial distribution of STEC in red deer in the Lombardy sector of the SNP in January and February 2017. The analysis was based on the collection of feces from 65 red deer from three culling areas with different anthropization levels (low, moderate, high), for which radio-tracking showed low or absence of deer movements among areas. Feces were enriched into modified Tryptone Soya Broth, and DNA was extracted for stx genes PCR. Positive samples were plated on Levine-eosin methylene blue agar and 20 single *E. coli* colonies were tested by PCR to confirm STEC identification and evaluate the presence of eaeA gene. The isolates were tested for their susceptibility to 12 antimicrobial agents by disc diffusion. Twelve of 65 feces were positive for STEC, showing a prevalence in red deer of 18.4% (95% CI:10.8-29.5), with strains harboring stx2 (n=7) or stx1 (n=5). No strain carried both stx genes and eae gene was never detected. STEC positive calves were only detected in high anthropised area. The STEC isolates were resistant to at least one of the antimicrobial agents tested, mostly ampicillin. The present study showed that red deer shed STEC in environment during winter, with prevalence similar to cattle. Additional samples and characterization of STEC serotypes are needed to highlight the effects of environmental and human-mediated factors influencing the infection dynamics.

Lavazza° A, Cooke BD

#### **Diseases of lagomorphs**

Lagomorphs : pikas, rabbits, and hares of the world / edited by Andrew T. Smith ... [et al.]. - Baltimore : Johns Hopkins University Press, 2018. - p 18-21 [Nr. Estr. 7857]

Lazzaro° M, Giacomini° E, Santucci° G, Maisano° A.M, Scali° F, Boniotti° MB, Corradi A, Gasparrini° S, Pasquali P, Alborali° GL

**Il ruolo del trasporto al macello nella diffusione della dissenteria suina** = The role of transportation to slaughterhouse in the swine dysentery spreading

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 77-83. - 15 bib ref [Nr. Estr. 7825]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

*Brachyspira hyodysenteriae*, principale agente della dissenteria suina (SD), può essere introdotta in azienda attraverso diverse vie. L'obiettivo dello studio è stato indagare la presenza dell'agente nei camion che trasportano suini al macello, così da definirne il potenziale ruolo nella diffusione. Sono stati selezionati casualmente 212 camion presso due macelli industriali ed i campioni ambientali prelevati tra lo scarico dei suini e le procedure di sanificazione. Lo screening per la ricerca di *B. hyodysenteriae* è stato effettuato con PCR-Real Time. Sono risultati positivi 84 dei 1322 (6,26%) campioni raccolti che provenivano da 49 dei 212 (23,11%) camion controllati. La probabilità d'identificare un campione positivo da un camion aumentava col numero di controlli fino al 94,44% (>20 campioni/veicolo). L'odds ratio dei veicoli con almeno 10 campioni è risultato 37,94 (IC 95% 14,03-102,61, P<0,0001) rispetto agli altri. I veicoli possono rappresentare una fonte di rischio per la diffusione di *B. hyodysenteriae* e le procedure di sanificazione degli automezzi al macello sono un punto critico. Un monitoraggio regolare, su campioni pre- e post-sanificazione, può fornire informazioni rilevanti su fonti di diffusione e prevenzione della SD. Per limitare i rischi di propagazione di *B. hyodysenteriae* attraverso il trasporto, pulizia e disinfezione degli automezzi al macello sono fondamentali così come le misure di biosicurezza in azienda, soprattutto legate al carico dei suini.



*Brachyspira hyodysenteriae*, the main cause of swine dysentery (SD), can be introduced in a herd through different ways. The aim of this study was to investigate the presence of *B. hyodysenteriae* in trucks transporting pigs to slaughterhouses, hence, to define their potential role in spreading the agent. Trucks were randomly selected at two large slaughterhouses; environmental samples were collected between the unload of pigs and cleaning procedures. A multiplex real time-PCR was used to screen the presence of *B. hyodysenteriae*. 84 out of 1332 (6.26%) collected samples were PCR positive, those samples were collected from 49 out of 212 (23.11%) selected trucks. Likelihood of identifying, at least, one positive sample from a truck increased according to number of samples per trucks, up to 94.44% (<20 samples/truck). Odds ratio of trucks sampled at least 10 times was 37.94 (95% CI 14.03-102.61;  $P < 0,0001$ ) when compared with others. Trucks may represent a potential source of *B. hyodysenteriae* spreading while cleaning and disinfection procedures at the slaughterhouse are a critical point which should be controlled. Regular screening, on samples collected before and after cleaning, should provide relevant information regarding spread and prevention of SD. Risks of spreading *B. hyodysenteriae*, via trucks, can be greatly decreased by proper cleaning and disinfection at the slaughterhouse. Biosecurity measures at farm, particularly when loading pigs, can further reduce those risks.

Lelli° D, Luini° M, Gazzola° A, Boccardo A, Sozzi° E, Sala° G, Trogu° T, Canziani° S, Moreno° A, Lavazza° A

#### **Pseudo-lumpy skin disease (BoHV-2) : a case study**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 152. - 3 bib ref [Nr. Estr. 8054]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Pseudo-lumpy skin disease (PLSD), caused by Bovine herpesvirus 2 (BoHV-2), is a viral cutaneous diseases of cattle which can be clinically indistinguishable from LSD making necessary a specific approach by using both serological and virological methods. BoHV-2 is also the agent of bovine herpes mammillitis (BHM) and may transiently cross-react with BoHV-1, thus creating problems for veterinary diagnostic laboratories in areas submitted to control programs against BoHV-1. In addition BoHV-2 may cause economical losses in livestock but its presence is under-investigated and likely underestimated. This study describes the clinical and laboratory findings of a PLSD outbreak occurred in a small piemontese cattle breeding in Northern Italy where animals were affected by a multifocal papular lesions evolving in a persistent alopecia. BoHV-2 was demonstrated by RFLP-PCR, isolation and electron microscopy and specific sero-conversion were evidenced in affected animals.*

Lelli° D, Moreno° A, Prosperi° A, Chiapponi° C, Faccin° F, Decaro N, Baioni° L, Sozzi° E, Lavazza° A

#### **Bats as reservoir hosts of novel and emerging viruses in Italy**

11th International Congress for Veterinary Virology, 12th Annual meeting of Epizone : August 27-30, 2018, Vienna : abstracts / [s.l. : s.n., 2018]. - p 13 (Oral 56) [Nr. Estr. 8113]

International Congress for Veterinary Virology : 11th Annual meeting Epizone : 12th : Vienna : August 27-30, 2018)

Background The diversity of viral agents found in bats worldwide is still largely unknown, and it is thought that a great number of viruses are yet to be discovered and characterized. Herein we report the most recent virus detections obtained within a survey for emerging viruses associated with bats implemented in Italy. Methods The survey involved the analyses of dead animals collected from bat rehabilitation centers or from known roost sites between 2010 and 2017. Fecal samples collected from hospitalized individuals were also sampled. Fresh carcasses were fully necropsied, and tissue specimens from different organs were analyzed. Samples were examined through a diagnostic protocol broadly targeting viral agents, which was set up using both "classical" virologic methods

(virus isolation and negative staining electron microscopy (nsEM)), and molecular techniques (PCRs and NGS sequencing) in order to enhance the possibility to detect newly-emerging viruses with a potential zoonotic importance or proving dangerous for bats. Results Almost 700 tissue and fecal samples from 12 different bat species (mostly pipistrelle bats) were collected and analyzed. The survey firstly provided evidence that insectivorous bats carry a wide variety of Mammalian orthoreoviruses (including new reassortant strains) and coronaviruses (COVs), both AlphaCoVs and BetaCoVs (MERS and SARS-like CoVs). Then, a novel and previously unknown Rhabdovirus, named Vaprio virus (VAPV), belonging to the genus Ledantevirus was isolated from organs of a *Pipistrellus kuhlii* spontaneously dead in a wild animal recovery centre. Preliminary screening for VAPV performed on 76 bats sampled in the same area revealed three more positivity with a prevalence of 5.2%. Moreover, an adenovirus type2, strongly related to a virus previously detected in bats from Germany [Th252129] in 2007, re-emerged in a new area in Northern Italy. More recently, a bat-borne poxvirus was also identified for the first time in bats in Europe. This virus was isolated from a pool of viscera of an adult male of *Hypsugo savii* with humerus fracture and osteo-malacia. The preliminary genome sequence analysis performed on almost the whole-genome sequence (80%) revealed a significant aa similarity (84%) with the Eptesipoxvirus strain Washington [KY747497] isolated from the elbow joint of a big brown bat in US. Conclusion A quite large variety, including new and emerging viruses have been identified in bats in Italy. This provides new targets for the development of specific diagnostic and surveillance assays and opens new prospective for viral research in bats.

Lelli° D, Prosperi° A, Chiapponi° C, Faccin° F, Papetti° A, Boniotti° B, Vaccari G, Leopardi S, Decaro N, De\_Benedictis, Bonilauri° P, Sozzi° E, Moreno° A, Lavazza° A

#### **A passive surveillance for emerging viruses in bats in Italy implications for public health and biological conservation**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 32 [Nr. Estr. 8006]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

Bats are recognized as the reservoir hosts of emerging viruses with zoonotic potential in tropical regions; however, little is known on viruses which may be present in bats in European countries. Considering the poor eco-epidemiological data on the circulation of viruses in bats in Italy, a passive surveillance on bat populations for emerging viruses has been implemented since 2010. The survey involved the analyses of dead animals collected from bat rehabilitation centers or from known roost sites. Fresh carcasses were fully necropsied, and tissue specimens from different organs were analyzed. Fecal samples collected from injured and hospitalized individuals were also sampled and tested. Organs and fecal samples were examined through a broadly reactive PCR-based protocol for the presence of viral agents. Next generation sequencing (NGS), virus isolation techniques and negative staining electron microscopy were also used in order to detect newly-emerging viruses with a potential zoonotic importance or proving dangerous for bats. A total of 635 fecal and tissue samples from 12 different bat species (mostly pipistrelle bats) were collected and analysed between 2010-2017. We demonstrated that coronaviruses (COVs), both AlphaCoVs and BetaCoVs, are widespread among Italian bat populations. The whole-genome sequences of representative viruses belonging to the clade 2c of the genus BetaCoVs (MERS-like CoVs), clade 2b (SARS-like COVs) and to the genus AlphaCoVs were obtained and characterized. The survey also provided evidence that insectivorous bats carry a wide variety of Mammalian orthoreoviruses (MRVs), with members of the type 3 mostly represented and new reassortant strains belonging to serotype 1 and 2. A novel and previously unknown Rhabdovirus named Vaprio virus belonging to the genus Ledantevirus was isolated in cell culture from organs of an adult female of *Pipistrellus kuhlii* spontaneously dead in a wild animal recovery center. Positivity for astroviruses and adenoviruses were also detected by PCR respectively in faecal and organ samples. An adenovirus 2 strongly related to a virus previously

detected from *P. pipistrellus* bats in Germany [JN252129] in 2007 was also isolated from the intestine of a young female of *P. kuhlii*. Molecular tests for rabies and related lyssaviruses performed on brain samples resulted constantly negative and no arboviruses have been so far detected in insectivorous bats, despite the endemic presence of Usutu virus and West Nile virus in the sampled areas since 2008. Results offer a preliminary dataset on the distribution of major viral infections in bats in Italy, an achievement so far not yet obtained, which improves our understanding on their spread and evolution and enhanced the methods for detecting the viruses that may emerge from bats. The knowledge of the high biodiversity of bats, the broad geographical distribution and the genetic diversity of bat-associated viruses is crucial for a comprehensive study from which viral discovery studies, viral disease prevention and biological conservation issues can benefit. This study was partially funded by the Italian Ministry of Health under the Research projects: WFR GR-2011-02350591 PGR201100; ISS PE-2011-02351681 PRF201130.

Lelli° D, Prosperi° A, Moreno° A, Chiapponi° C, Gibellini AM, De\_Benedictis P, Leopardi S, Sozzi° E, Lavazza° A

#### **Isolation of a novel Rhabdovirus from an insectivorous bat (*Pipistrellus kuhlii*) in Italy**

Virology - Vol. 15 ( 2018). - no 37 (7 p). - 30 bib ref [Nr. Estr. ( Ultimo accesso 09/03/2018 )  
<https://doi.org/10.1186/s12985-018-0949-z> 7794]

**Background** Rhabdoviridae is one of the most ecologically diverse families of RNA viruses which can infect a wide range of vertebrates and invertebrates. Bats, among mammals, are pointed to harbor a significantly higher proportion of unknown or emerging viruses with zoonotic potential. Herein, we report the isolation of a novel rhabdovirus, detected in the framework of a virological survey on bats implemented in North Italy. **Methods** Virus isolation and identification were performed on samples of 635 bats by using cell cultures, negative staining electron microscopy and PCRs for different viruses. NGS was commonly performed on cell culture supernatants showing cytopathic effect or in case of samples resulted positive by at least one of the PCRs included in the diagnostic protocol. **Results** A rhabdovirus was isolated from different organs of a *Pipistrellus kuhlii*. Virus identification was obtained by electron microscopy and NGS sequencing. The complete genome size was 11,774 nt comprised 5 genes, encoding the canonical rhabdovirus structural proteins, and an additional transcriptional unit (U1) encoding a hypothetical small protein (157aa) (3'-N-P-M-G-U1-L-5'). The genome organization and phylogenetic analysis suggest that the new virus, named Vaprio virus (VAPV), belongs to the recently established genus *Ledantavirus* (subgroup B) and it is highly divergent to its closest known relative, Le Dantec virus (LDV) (human, 1965 Senegal). A specific RT-PCR amplifying a 350 bp fragment of the ORF 6 gene, encoding for L protein, was developed and used to test retrospectively a subset of 76 bats coming from the same area and period, revealing two more VAPV positive bats. **Conclusions** VAPV is a novel isolate of chiropteran rhabdovirus. Genome organization and phylogenetic analyses demonstrated that VAPV should be considered a novel species within the genus *Ledantavirus* for which viral ecology and disease associations should be investigated.

Leo S, Calamari L, Arrigoni° N, Tamba° M, Amadori° M

#### **Effect of paratuberculosis infection on welfare parameters of dairy cows**

14th International colloquium on paratuberculosis : 4-8 June 2018, Riviera Maya, Mexico : program & abstracts / [s.l. : s.n., 2018]. - p 87 (P-1.41) [Nr. Estr. 7905]

International colloquium on paratuberculosis (14th : Riviera Maya, Mexico : 4-8 June 2018)

Paratuberculosis causes predisposition of cattle to metabolic disorders, mainly close to calving, but data on these aspects are often lacking. The aim of this study was to evaluate the impact of paratuberculosis on metabolic conditions of infected cows compared with healthy cows reared in the same herd, around calving period. A case-control study was performed: cases were positive to

serum ELISA (IDEXX Paratuberculosis Ab test®) and/or fecal culture, controls were repeatedly test-negative and asymptomatic animals. Blood samples of 30 positive and 30 negative cows were collected 4 times, at days -30, +3, +10, +30, with respect to calving. The metabolic profile was measured in each sample, including positive and negative acute phase proteins (APP) (i.e. haptoglobin, bilirubin), as well as markers of oxidative status (i.e. Reactive Oxygen Metabolites -ROMt- and Ferric Reducing Ability of Plasma). Some APP values were used to calculate the Post-calving Inflammatory Response Index (PIRO), and the Liver Functionality Index (LFI) which define the inflammatory response during the first week and the first month of lactation, respectively. At each sampling point, BCS and hygienic score were also measured. Nine of the 30 positive cows were culled before 30 days post partum due the onset of symptoms. No differences were found for LFI, BCS and hygienic score. Significant differences were found in Ca and albumin (lower in the positive group) and ROMt (higher in the positive group). Selecting cows positive to both tests (ELISA and fecal culture), ceruloplasmin became significant too (higher in positive group). PIRI, calculated at 3 days post-calving, was significantly different for the entire case-control group, and for the serological only positive subjects. The lower PIRI values in the case group highlighted their altered metabolic profile. LFI did not differ significantly between groups, probably because the early culling of 9 cows decreased too much the cases number. A greater number of observations are needed though to corroborate the initial hypothesis. Funded by Italian Ministry of Health, PRC2013/016.

Leopardi S, Priori P, Zecchin B, Poglayen G, Trevisiol K, Lelli° D, Zoppi S, Scicluna MT, D'Avino N, Schiavon E, Bourhy H, Serra-Cobo J, Mutinelli F, Scaravelli D, De\_Benedictis P

**Active and passive surveillance for bat lyssaviruses in Italy revealed serological evidence for their circulation in three bat species**

Epidemiol Infect. - Vol. 147 ( 2018). - Article no. e63 (6 p). - 16 bib ref [Nr. Estr. ( ultimo accesso 29/01/2019 ) <https://doi.org/10.1017/S0950268818003072> 8103]

The wide geographical distribution and genetic diversity of bat-associated lyssaviruses (LYSVs) across Europe suggest that similar viruses may also be harboured in Italian insectivorous bats. Indeed, bats were first included within the passive national surveillance programme for rabies in wildlife in the 1980s, while active surveillance has been performed since 2008. The active surveillance strategies implemented allowed us to detect neutralizing antibodies directed towards European bat 1 lyssavirus in six out of the nine maternity colonies object of the study across the whole country. Seropositive bats were *Myotis myotis*, *M. blythii* and *Tadarida teniotis*. On the contrary, the virus was neither detected through passive nor active surveillance, suggesting that fatal neurological infection is rare also in seropositive colonies. Although the number of tested samples has steadily increased in recent years, submission turned out to be rather sporadic and did not include carcasses from bat species that account for the majority of LYSVs cases in Europe, such as *Eptesicus serotinus*, *M. daubentonii*, *M. dasycneme* and *M. nattereri*. A closer collaboration with bat handlers is therefore mandatory to improve passive surveillance and decrypt the significance of serological data obtained up to now.

Leotti G, Polloni A, Candotti° P, Merdy O

**Assessment of atrophic turbinate lesions in Italian farms**

ESPHM 2018 : 10th European Symposium of Porcine Health Management : Barcelona, 9th - 11th May, 2018 : proceedings / [s.l. : s.n., 2018]. - p 216 (Poster BBD-038) [Nr. Estr. 7867]

European Symposium of Porcine Health Management (ESPHM) (10th : Barcelona : 9th - 11th May, 2018)

Atrophic Rhinitis (AR) is a disease impacting the upper part of the respiratory tract characterized by hypoplasia of nasal turbinates associated with decreased performance and increased respiratory

problems. Routine diagnosis is uncommon in Italy. This absence of regular diagnosis can lead to underestimate the prevalence and the impact of the disease. The aim of the present study was to evaluate the presence of AR lesions in Italian pig farms at risk. Material & Methods Veterinarians from Northern Italy were asked to select for this survey farms with no to low AR vaccination plan (e.g. gilt vaccination only, recent vaccination plan discontinuation). On these farms, cross sections of the snout of the 3 -5 dead pigs considered at risk and weighing 20-180 kg were scored (0-22 scale). Pictures of turbinates were taken and were submitted to a blinded expert for scoring and procedure controls. Vets answered to a questionnaire about farm practices as well. Results Seventeen vets have submitted pictures and questionnaires from 25 farms. In total 97 noses were scored. From nasal pictures, 70% of the sections were correctly performed. Among these, complete absence of lesions was found in only 15%; 53% showed low score (1-7), 24% medium score (8-14) and 9% high score (15-22). At the farm level, 19/25 farms provided correct sections for scoring, among which 11 farms provided sections showing moderate to severe lesions. Discussion & Conclusion This study possibly revealed that the impact of AR in Italian farms could be underestimated since 35% of interpretable snout sections collected in farms with limited prevention program showed non negligible medium to severe AR lesions. Nevertheless no bacteriological exam was performed to confirm the etiology of these cases. This observation advocates reinforcement of disease surveillance routines on farm by encouraging regular diagnosis and according to best practices..

Lupi D, Palamara\_Mesiano M, Tremolada P, Benocci R, Parenti P, Giacchini R, Marino G, Lavazza° A, Bassi° S, Mazzoni E, Panini M, Colombo° M

#### **A multifactorial approach of stress factors influencing bee health**

ECE 2018 : XI European Congress of Entomology : 2-6 July 2018, Napoli : book of abstracts / [s.l. : s.n., 2018]. - p 316 (Poster PO525a) [Nr. Estr. 8047]

European Congress of Entomology (11. : Napoli : 2-6 July 2018)

Since the late '90s, a complex pathology known as Colony Collapse Disorder (CCD) brought to widespread events of honeybee disappearance. Many adversities may be responsible of honeybee decline: recrudescence of old and new pathologies, contamination from pesticides and emerging contaminants (e.g. nanoparticles) and environmental stresses, including climate change. Stress factors may interact among them with additive or synergic effects and, currently, a multi-stress condition is accepted as the most probable and diffuse decline cause for honeybees. This work is an in-field evaluation of combined stress factors (pesticide mixtures, electromagnetic fields, pest and diseases) on honeybees. Experimental hives were placed in April 2017 in a "control site" far from agricultural field and from significant human settlements and in an "exposure site" located inside an experimental farm where both a high-voltage electric line is present and multiple chemicals treatments for orchards crops are applied. Honeybee families were sampled weekly throughout the year to evaluate health status (parasites, virus, and bacteria) and population parameters (queen presence, brood status, mortality in underbaskets and honey and pollen reserves) and monthly for biomarker analyses (acetylcholinesterase, catalase, glutathione S-transferase, alkaline phosphatase, reactive oxygen species (RO8), lipid peroxidation, and DNA fragmentation). Preliminary data analysis revealed that population parameters and biomarkers were both affected by stress sources. Direct effects on bees were registered in relation to pesticide applications. Multi-stress position showed the most severe effects, leading to colony death. Colony losses were directly related to the development of different pathologies. Reduced immunity defences and social disorders by frequent queen replacement seem to be the main effects related to the multi-stress condition.

5.

Luppi° A, Lelli° D

#### **Case report : Multiple cutaneous nodules in a dairy cow (LSD differential diagnosis)**

11th International Congress for Veterinary Virology, 12th Annual meeting of Epizone : August 27-30,

2018, Vienna : abstracts / [s.l. : s.n., 2018]. - p 110 (Poster 200) [Nr. Estr. 8151]

International Congress for Veterinary Virology : 11th Annual meeting Epizone : 12th : Vienna : August 27-30, 2018)

**Background** The nodular cutaneous lesions in dairy cow recognize several causes for which, the differentiation, requires diagnostic investigations. The recent spread of Lumpy skin disease (LSD) in several European countries, such as Albania, Greece and Macedonia, has led greater attention to cases not easily differentiable from a clinical point of view from the aforementioned disease. Severe LSD is highly characteristic, but milder forms can be confused with: bovine herpes mammillitis, bovine papular stomatitis, papillomavirus, pseudocowpox, vaccinia virus and cowpox virus infections, dermatophilosis, demodicosis, insect or tick bites, besnoitiosis, rinderpest, hypoderma bovis, photosensitisation, urticaria, cutaneous tuberculosis, onchocercosis. Milder forms of these diseases/syndromes can be distinguished from LSD by histopathology and other laboratory tests. The present case report describes the clinical presentation and the investigation performed in a dairy cow with multiple diffuse nodular skin lesions. **Methods** In February 2018, multiple diffuse nodular skin lesions were found in 1 out of 60 cattle in a dairy farm in Reggio Emilia, northern Italy. The cow was 5-year-old. The lesions were distributed mostly around the neck, shoulders, back, forelimbs, hindlimbs, and bilateral of midline portion. Lesions were characterized by firm, hypotrichous to alopecic nodules of 0.5-3 cm of diameter. The enlargement of the right precrural lymph node was observed. Clinical examination of the affected cow revealed slight emaciation and rectal temperature of 39°C. A representative cutaneous nodule was sampled by biopsy. The pathological material was submitted to cytological and histopathological evaluation, to virological investigation (cell culture) and tested for capripox virus (real time PCR) and for bovine papillomavirus (PCR). The precrural lymph node was sampled by fine needle aspiration for cytology. Blood sample was collected and tested for bovine leukaemia virus (BLV) antibodies using the agar gel immune diffusion method (AGID). **Results** The real time PCR for capripox virus resulted negative, as well as the virological examination on cell culture. PCR for bovine papillomavirus (BPV) resulted positive (99% of homology with BPV-1). Cytology revealed neoplastic lymphocytes both in cutaneous and lymph nodal samples. Histologically, epidermal and dermal infiltrations of neoplastic lymphoid cells were observed. No histological lesions compatible with BPV infection were observed. AGID result excluded the BLV infection. **Conclusion** The present case report describes a cutaneous lymphoma in a dairy cow, occurring independently of BLV infection and affecting only a single cow in the herd. The case described underlines the importance of a correct diagnostic approach in case of nodular lesions in cattle.

Luppi° A, Taddei° R, Gherpelli° Y, De\_Lorenzi° G, Fontana° MC, Bassi P, Pangallo° G, Merialdi° G

**Antimicrobial resistance patterns of Escherichia coli isolated from canine urinary samples submitted to IZSLER diagnostic laboratories in 2016-2017**

Int J Health Anim Sci Food Safety. - Vol. 5 no 3s ( 2018). - p 94 [Nr. Estr. 8027]

International Conference on Antimicrobial Agents In Veterinary Medicine (AAVM) (9th : Rome, Italy : October 16-19, 2018)

Bacterial urinary tract infections (UTI) represent a common reason requiring veterinary care and Escherichia coli is the most frequently isolated pathogen. Early diagnosis and prompt antimicrobial therapy are recommended when clinical signs of a UTI are present. Submission of urine samples for culture and sensitivity testing is the best practice approach to diagnosis. Two hundred-thirty strains of E. coli isolated from January 2016 to December 2017 from urine samples belonging to as many dogs suffering of UTI were tested for the antimicrobial susceptibility using the Kirby-Bauer disc diffusion method, following the procedures of the Clinical and Laboratory Standards Institute (CLSI). A standard panel of antimicrobials including amikacin (30 µg), amoxicillin + clavulanic acid (20/10 µg), ampicillin (10 µg), cefpodoxime (10 µg), cephazolin (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), nitrofurantoin (300 µg), tetracyclin (30 µg), trimethoprim+sulphamethoxazole (1.25/23.75mg) was tested against E.coli isolates. The strains were classified as resistant, susceptible or intermediate to the tested

antimicrobials by interpreting the zones of growth inhibition according to the CLSI. Intermediate strains were grouped with the resistant ones. The E.coli strains included in this study showed the following percentages of resistance: tilmicosin (87.2%), tetracyclin (73.6%), ampicillin (70.7%), cephalosporin (57.74 amoxicillin + clavulanic acid (43%), enrofloxacin (34.8%), trimethoprim+sulphamethoxazole (29.9%), cefpodoxime (28.7%), kanamycin (23%), gentamicin (13%), chloramphenicol (10.64 nitrofurantoin (6.3%), amikacin (2.9%). Empirical antimicrobial therapy is often instituted while awaiting for the results of culture and sensitivity testing. The sensitivity testing results obtained in this study showed that the antibiotics classified as highest priority critically important antimicrobials (3rd and 4th generation cephalosporins, quinolones, macrolides and polynyxins) by WHO (3) do not necessarily represent the best choice in terms of in vitro efficacy and some therapeutic alternatives, based on less critical antimicrobials are to be preferred. Periodic monitoring of pathogens isolated from UTI and their susceptibility patterns are helpful in guiding the first line empirical therapy and can also be effective for monitoring the eventual spread of resistant bacteria.

Magistrali CF, Curcio L, Luppi° A, Pezzotti G, Orsini S, Tofani S, Feudi C, Carattoli A, Villa L

#### **Mobile colistin resistance genes in Escherichia coli from pigs affected by colibacillosis**

Int J Antimicrob Agents. - Vol. 52 no 5 ( 2018). - p 744-746. - 7 bib ref [Nr. Estr. ( ultimo accesso 11/01/2019 ) <https://doi.org/10.1016/j.ijantimicag.2018.08.008> 7923]

Maisano° AM, Luini° M, Vitale° N, Scali° F, Alborali° GL, Rota\_Nodari° S, Vezzoli° F

#### **Animal-based measures on Italian heavy pigs at slaughterhouse and relations with animal welfare on farm**

ESPHM 2018 : 10th European Symposium of Porcine Health Management : Barcelona, 9th - 11th May, 2018 : proceedings / [s.l. : s.n., 2018]. - p 173 (Poster AWN-057) [Nr. Estr. 7865]

European Symposium of Porcine Health Management (ESPHM) (10th : Barcelona : 9th - 11th May, 2018)

Introduction Animal-based measures (ABMs) can be used effectively in the evaluation of animal welfare (AW) on farm. Some ABMs might be also efficiently used during slaughterhouse inspections. The aim of this work was to investigate the possibility of exploiting ABMs, collected at slaughterhouse on Italian heavy pigs (165Kg), to obtain information about AW on farm. Material & Methods Twenty-eight ABMs were tested at slaughterhouse on 62 batches of finishing pigs belonging to 54 different pig farms of Northern Italy. The observations were carried out during ante-mortem (n=10,085 pigs) and post-mortem inspections (n=7,952 pigs). Fifteen selected ABMs were evaluated both at slaughterhouse (118.5} 45 pigs per farm) and on farm (26.3} 4.8 pigs per farm) for 16 different farms chosen randomly. Results Four ABMs were not observed at slaughterhouse (tail biting, scouring, rectal prolapse and twisted snouts).The two ABMs with a higher prevalence at slaughterhouse were manure on the body grade 1 (28.36%) and dermatitis (28.03%). The prevalence of ABMs showing AW problems was higher at slaughter compared to farm. In particular, significant differences between ABMs were founded for non-uniformity of size, 3.0} 0.6 and 1.8} 0.9 (P<0.05), and dermatitis, 34.3} 4.8 and 1.8} 0.7 (P<0.001) for slaughterhouse and on farm observations respectively. Discussion & Conclusion The comparison between ABMs observed at slaughterhouse and at farm-level showed a good correlation and slaughterhouse proved to be a better detection site for some indicators. Besides, observations at slaughterhouse are easier to perform compared to observations on farm and allow to monitor and record constantly AW in batches all year round. According to the results of this study, slaughterhouse seems an excellent and feasible detection point to evaluate ABMs in pigs and it could be used as a tool to complete the evaluation of AW at farm-level.

Maisano° AM, Romano° A, Maraschi F, Morandi S, Orlandelli C, Spelta° C, Vezzoli° F, Bertocchi° L, Luini° M

**Indagini epidemiologiche, biomolecolari e di antibiotico-resistenza in aziende di bovine da latte con infezione da *Streptococcus agalactiae*** = Epidemiological, molecular and antimicrobial-resistance investigations in dairy farms infected with *Streptococcus agalactiae*

Large Anim Rev. - Vol. 24 no 1 ( 2018). - p 11-18. - 18 bib ref [Nr. Estr. 7833]

Lo scopo di questo lavoro è stato quello di approfondire l'epidemiologia di *Streptococcus agalactiae* in aziende con recenti positività, in particolare a riguardo: delle condizioni d'allevamento, della prevalenza di bovine infette, della suscettibilità agli antibiotici e delle caratteristiche genotipiche dei ceppi. I risultati hanno dimostrato la presenza del patogeno in 13 allevamenti su 15 (1794 campioni di latte individuale), con prevalenza di bovine infette molto variabile (10,4-54,7%). Le condizioni d'allevamento (check-list) correlate con la prevalenza d'infezione sono risultate significative per: la preparazione igienica della mammella, l'esecuzione del post-dipping, la routine di mungitura, le procedure di lavaggio e disinfezione dell'impianto/tank, la somministrazione di latte infetto ai vitelli, l'igiene delle mammelle e la conta sia delle cellule somatiche che delle UFC/mL di *S. agalactiae* nel latte di massa. I risultati della minima concentrazione inibente su un ceppo per stalla (n. 13), hanno evidenziato resistenze alla Tetraciclina (n. 5), Eritromicina (n. 1) e Pirlimicina (n. 1); tutti i ceppi sono risultati sensibili ai (3-lattamici. La RAPD-PCR su almeno 4 ceppi per stalla, ha evidenziato una certa variabilità genetica (6 profili diversi su 55 ceppi), ed in 11 stalle la circolazione dei ceppi ha mostrato un profilo clonale, mentre in 2 80.11e Si Sono evidenziati ceppi differenti. 18 ceppi rappresentativi di tutte le stalle sono risultati tutti positivi per il gene specie specifico e per i fattori di virulenza, *neuC* e *cpsL* (PCR-RT). I nostri risultati confermano il carattere contagioso di questa infezione e sottolineano la necessità di un controllo costante delle corrette procedure-igiene di mungitura, del management degli animali e del latte di massa.

*The aim of our study was to investigate the infection epidemiology in dairy farms with recent history of *Streptococcus agalactiae* intramammary-infection with special reference to: farming conditions, prevalence of infected cows, susceptibility to antimicrobials and molecular characteristics of the involved strains. In 15 dairy farms of the Lodi's Province, resulted positive for *S. agalactiae* at the official tank bulk milk test in the last two years, 1794 individual composite milk samples were obtained (range 24-217) and selective bacteriological examination were performed. The results showed the presence of *S. agalactiae* in 13 herds, with a prevalence of infected cows ranging from 10.4 to 54.7%. The analysis of the milking and biosecurity checklists on farms highlight some critical factors correlating with the prevalence of infection, such as: insufficient udder preparation ( $R = 0.66$ ), failure to perform post-dipping ( $R = 0.77$ ), incorrect milking routine ( $R = 0.58$ ), inappropriate washing and disinfection procedures of the milking machine ( $R = 0.61$ ), the administration of the infected milk to the calves ( $R = 0.85$ ), poor udder hygiene ( $R = 0.64$ ), high counts of both somatic cells and UFC/mL of *S. agalactiae* in bulk tank milk (respectively  $R = 0.63$  and  $R = 0.64$ ). Results of the minimum inhibitory concentration on one strain for farm (n. 13) showed strains resistant to tetracycline (n. 5), erythromycin (n. 1) and pirlimycin (n. 1); all strains were sensitive to  $\beta$ -lactams tested. Results of RAPD-PCR on 55 strains (4 for each farm) showed a genetic variability between strains (6 different profiles), but also evidenced that in 11 herds out of 13, strains with the same genetic profile were spread (clonal diffusion), while in 2 herds, different strains were simultaneously present. Eighteen strains representative of the strains circulating in the investigated farms were all positive at PCR-RT for a specific species gene and for *neuC* and *cpsL* virulence genes factors. Our findings confirm the severity of infection by *S. agalactiae* and underline the need of constant monitoring of the correct and hygienic procedures of milking, the animal management and bulk tank milk analysis.*

Maisano° AM, Rota\_Nodari° S, Alborali° GL, Begni E, Fadini M, Giacomini° E, Invernizzi° A, Lazzaro° M, Lombardi° G, Polloni° A, Prati° P, Rosignoli° C, Santucci° G, Scali° F, Vitali A, Vezzoli° F

**Osservazioni preliminari sull'uso delle animal based measures negli allevamenti intensivi suini** = Preliminary observations on the use of animal based measures in intensive pigs farm



Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 67-76. - 17 bib ref [Nr. Estr. 7824]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

Il benessere animale è una tematica con un alto impatto sia sull'opinione pubblica che sull'allevamento suinicolo soprattutto nell'ottica della filiera etica. Lo scopo del lavoro stato quello di verificare su diverse categorie produttive una selezione di Animal Based Measures (ABMs) e valutarne l'applicabilita su larga scala. Lo studio si è svolto su 125 allevamenti della pianura Padana. Le categorie produttive campionate sono state: verri, scrofe (gestazione e sala parto), sottoscrofa, svezzati, magroni e grassi. Le ABMs pid rilevanti (per frequenza e/o impatto sul benessere) in questo studio sono risultate analoghe a quelle raccomandate nella short-list dell'EFSA. Inoltre, altre ABMs, riflesso dell'adeguatezza delle strutture, sono state registrate frequentemente, ad esempio bursiti e imbrattamento fecale >30% nei grassi. La frequenza media di alcune ABMs 6 stata influenzata da poche aziende caratterizzate da un'elevata prevalenza; nella fattispecie: diarree nei sottoscrofa, lesioni alle code nei grassi e zoppie nelle scrofe in box gestazione. Concludendo, la valutazione del benessere non dovrebbe limitarsi ai soli parametri legislativi ma essere integrata anche da osservazioni dirette sull'animale, quali le ABMs, poiche esse rispecchiano il grado di adattamento dell'animale all' ambiente in cui vive. Le ABMs possono essere applicate su larga scala solo se in numero limitato e accuratamente selezionate secondo la loro frequenza attesa, impatto e riproducibilita.

*Animal welfare is an important topic with a relevant impact on both public opinion and pig production, particularly, in the so-called ethical production chain. The aims of this study were to test, on different pig categories, a selection of Animal Based Measures (ABMs) and evaluate their feasibility for monitoring welfare in large animal populations. Assessments were performed on 125 farms, located in Po valley. Productive categories sampled were: boars, sows (farrowing and pregnancy), sucking piglets, weaners, pre-finishers, and finishers. The most relevant ABMs (frequency and/or impact on animal welfare) observed in this study were similar to the ones recommended by EFSA ABMs short list. Furthermore, other ABMs, which reflect structures adequacy, has been observed frequently, such as "presence of bursitis" and "manure on the body >30%" (fattening pigs only). Average frequency of some ABMs was strongly influenced by a small number of farms with high prevalences. These ABMs were: piglets' diarrhoea, fattening's tail biting, and pregnant sow's lameness. In conclusion, animal welfare should not be evaluated only with legislative parameters but also with ABMs which reflect the adaptation of animals to the environment where they live. A feasible approach, on a large animal population, should contemplate a short list of ABMs based on expected frequency, impact, and reproducibility.*

Maisano° AM, Santucci° G, Ghidini S, Scali° F, Costa A, Ballista C, Zaghini L, Attanasio G, Belluzzi G, Borrello S, Candela L, Colagiorgi A, Giacomini° E, Giudici° F, Ianieri A, Lazzaro° M, Frazzi P, Vitali A, Vezzoli° F, Zanardi E, Alborali° GL

**Consumo antimicrobico, lesioni al macello e benessere animale : relazioni negli allevamenti in intensivi da ingrasso** = Antimicrobial consumption, lesions at slaughterhouse and animal welfare : relationships in intensive fattening pig's farm

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 57-66. - 11 bib ref [Nr. Estr. 7823]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

L'uso di antimicrobici nel suino rappresenta un rischio sanitario per la diffusione dell'antibiotico-resistenza. Parametri importanti, quali benessere animale e lesioni al macello, potrebbero fornire dati utili ai fini di razionalizzare i trattamenti. Lo scopo di questo studio e stato esaminare le relazioni tra uso di antimicrobici, lesioni al macello e benessere. Sono stati analizzati consumo di antimicrobici, benessere animale ed ispezioni al macello di 13 aziende da ingrasso. L'uso di antimicrobici è stato misurato nel 2016, come giorni di trattamento per suino allevato (giomi/suino). Il benessere è stato rilevato, tramite una selezione di animal based measures (ABMs), su 2336 suini (media 172±14). Le lesioni al macello su 67721 soggetti (media 5074±1701). La mediana di consumo degli antimicrobici è stata 21 giorni/suino (range 0,1-87,8); le ABMs piü

frequenti sono state imbrattamento fecale di grado 2 (media 23,3±32,0%) e 1 (media 15,0±10,7%); le lesioni al macello: pleuriti (media 16,3±6,1%) e polmoniti (media 14,4±3,5%). Non vi sono correlazioni tra consumo di antimicrobici, ABMs e le lesioni al macello. Sebbene i risultati di questo studio siano preliminari, l'assenza di correlazioni tra i parametri analizzati potrebbe implicare trattamenti non sempre razionali. L'ottimizzazione dei protocolli terapeutici dovrebbe prevedere un approccio integrato con i principali aspetti dell'allevamento (diagnostica, biosicurezza, consumo antimicrobici, benessere, ispezioni al macello).

*Antimicrobial usage (AMU) in pig farms pose a threat to public health due to the negative consequences on spread of antimicrobial resistance. Important factors, such as animal welfare and slaughterhouse records, may provide useful data regarding rational use of antimicrobials. AMU, animal welfare, and slaughterhouse inspection of 13 fattening units were investigated. AMU was collected retrospectively for 2016 and calculated as mean days of treatment per reared pig (days/pig). Welfare was analysed, using a selection of animal based measures (ABMs), on 2336 pigs (mean 172±14). Slaughterhouse records (FSIS/FSAJWQ standards) of 67721 pigs (mean 5074±1701) were also collected. Median AMU was 21 days/pig (range 0.1-87.8); the two most frequent AMBs were manure on the body score 2 (mean 23.3±32.0%) and 1 (mean 15.0±10.7%); the two most frequent slaughterhouse records were pleurisy (mean 16.3±6.1%) and pneumonia (mean 14.4±3.5%). No correlations were observed among AMU, animal welfare, and slaughterhouse records. Although results of this study are still preliminary, lack of correlations among AMU and the other factors may suggest that treatments were not always rational. In order to optimise AMU an integrated approach should be encompassed, which includes monitoring and reviewing: diagnostic protocols, biosecurity, animal welfare, and slaughterhouse protocols.*

Mancin M, Barco L, Losasso C, Belluco S, Cibin V, Mazzucato M, Bilei S, Carullo MR, Decastelli L, Di\_Giannatale E, D'Incau° M, Goffredo E, Lollai S, Piraino C, Scuota S, Staffolani M, Tagliabue° S, Ricci A

#### **Salmonella serovar distribution from non-human sources in Italy; results from the IT-Enter-Vet network**

Vet Rec. - Vol. 183 no 2 ( 2018). - 8 p. - 22 bib ref [Nr. Estr. ( ultimo accesso 24/07/2018 ) <https://doi.org/10.1136/vr.104907.7898>]

The study summarises the results obtained over the period 2002–2013 by the Italian IT-Enter-Vet network, aimed at collecting data on Salmonella isolates from non-human sources. A total of 42,491 Salmonella isolates were reported with a progressive decrease over the years. S. Typhimurium was the most frequent serovar up to 2011, but then, it was overtaken by S. 4,[5],12,i:-, S. Derby, S. Livingstone and S. Enteritidis alternated as the third most commonly isolated serovars. With regard to the sources of isolation, S. Typhimurium was distributed ubiquitously among the animal species. On the contrary, S. 4,[5],12,i:- and S. Derby were strictly associated with pigs, whereas S. Livingstone, S. Enteritidis and S. Infantis were clearly related to poultry. Intriguingly, when the frequency of serovar distribution along the food chain was considered, it was evident that S. Typhimurium and S. Derby tended to persist along the chain, as they were isolated even more frequently from foods than from animals. A similar distribution was found for S. Enteritidis and S. Hadar. Despite limitations related to nonmandatory participation of laboratories in the network, the data presented are valuable to obtain a picture of the evolution of Salmonella from non-human sources over time in Italy.

Manev I, Genova K, Lavazza° A, Capucci° L

#### **Humoral immune response to different routes of myxomatosis vaccine application**

World Rabbit Sci. - Vol. 26 no 2 ( 2018). - p 149-154. - 30 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.4995/wrs.2018.7021.7920>]

The aim of our study was to monitor the dynamics of the serological response to different application

routes of live attenuated myxomatosis vaccine. The study included 42 Californian breed rabbits, aged 3 mo, of both sexes. They were separated into 7 groups: 6 experimental and 1 control. All experimental groups were vaccinated on day 0 with a single dose of myxomatosis vaccine (min 10<sup>3.3</sup> tissue culture infective dose 50 [TCID<sub>50</sub>], max 10<sup>5.8</sup> TCID<sub>50</sub>). Three of the groups were injected with monovalent attenuated myxomatosis vaccine using different types of application: intradermal (i.d.), intramuscular (i.m.) and subcutaneous (s.c.). The other 3 groups were injected with bivalent attenuated vaccine against myxomatosis and rabbit haemorrhagic disease; again the routes of administration were i.d., i.m. and s.c.. There were no clinical signs or serious side effects after vaccination. The serological response was evaluated on days 7, 15 and 30 with a monoclonal antibody based-competition enzyme-linked immunosorbent assay (cELISA). More rapid and potent humoral response was detected in groups with i.d. inoculation in comparison to i.m. and s.c. routes. Vaccination with monovalent vaccine against myxomatosis induced higher antibody titre in comparison to bivalent vaccine. Our study showed that the vaccine application route and the type of vaccine used influence the speed and intensity of antibody response.

Martinelli° C, Giovannini° S, Alborali° GL

#### **Indagini sierologiche su latte di massa in provincia di Brescia**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 80. - 1 bib ref [Nr. Estr. 7974]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

Il latte di massa in un allevamento bovino può essere un semplice ed economico strumento per la valutazione sanitaria non solo della singola realtà ma dell'intero territorio provinciale. Tramite esso si può infatti monitorare la presenza di diverse malattie infettive e valutare le migliori strategie di controllo Methods Nel corso del primo semestre 2017, nell'ambito del monitoraggio sul latte di massa per la brucellosi previsto dal D.D.U.O. n. 97/2011 e successive modifiche, sono stati raccolti 1570 campioni di latte di massa di allevamenti bovini presenti sul territorio della provincia di Brescia; di questi, 1236 fanno parte dell'ATS di Brescia e 334 dell'ATS della Montagna (Val Camonica). Ciascun campione, oltre che per Brucellosi, è stato analizzato tramite tecnica ELISA per la ricerca anticorpale nei confronti di IBRgE, IBRtot, Neospora caninum e BVD anti-ns2-3. Results Gli esami di laboratorio evidenziano una positività per IBR gE del 23% (356/1570 aziende) tra gli allevamenti della provincia di Brescia; di questi, solo uno si trova nell'ATS della Val Camonica, mentre tutti gli altri sono nell'ATS di Brescia. Per quanto riguarda la prova IBR tot è stata rilevata una positività complessiva del 64,45%, di cui il 41,8% si riferisce ad aziende che, molto verosimilmente, applicano piani vaccinali secondo quanto previsto dal Piano Regionale. Per quanto riguarda Neospora caninum, il 21% (331/1570) delle aziende è risultata positiva, anche in questo caso con una prevalenza maggiore nell'ATS di Brescia (25%) rispetto all'ATS della Montagna (8%). La prova ELISA anti-ns2-3 ha evidenziato una elevata prevalenza nei confronti del virus BVD (81,8%). Quelli con titoli anticorpali maggiori o uguali a 1,0 s/p sono l'11,5% (180/1570), mentre i negativi rappresentano il 18% (286/1570). Nel caso dell'IBR le prove sierologiche su latte di massa vengono già utilizzate per l'attribuzione delle qualifiche previste dal Piano Regionale. La prova ELISA per Neospora su latte di massa permette di rilevare solo sieroprevalenze relativamente elevate (superiori al 15%) (Bartles et al., 2005). La prova ELISA anti-ns2-3 su latte ha messo in evidenza una prevalenza piuttosto elevata sul territorio che dovrebbe essere approfondita al fine di stabilire la reale diffusione del virus. Conclusions Il latte di massa può quindi costituire un'ottima matrice per il monitoraggio sanitario del territorio nel tempo. Le positività delle singole aziende potranno poi essere approfondite tramite ulteriori indagini sui singoli animali.

Mattioni\_Marchetti V, Caltagirone M, Mercato A, Novazzi F, Nucleo E, Spalla M, Prati° P, Scarsi° G, Migliavacca R, Pagani L, Fabbì° M

**Deadly puppy infection caused by an MDR ST58 E. coli O141 blaCTX-M-15, CMY-II, DHA-1 and aac(6')-Ib-cr-positive in a breeding of Central Italy**

46° Congresso Nazionale della Società Italiana di Microbiologia : 26-29 Settembre 2018, Palermo : abstract book / [s.l. : s.n., 2018]. - p 134 (Poster P058) [Nr. Estr. 8132]

Congresso Nazionale della Società Italiana di Microbiologia (SIM) (46. : Palermo : 26-29 Settembre 2018)

**Introduction.** Companion animals are considered as emerging reservoirs of antibiotic-resistant bacteria, but few epidemiological data on Beta-Lactamases (BLs)-producing *Escherichia coli* in pet dogs are available in Italy. The most prevalent acquired BLs in both companion and breeding animals are CTX-M-1 and CMY-II, with CTX-M-15 and DHA-1 being rarely identified in veterinary medicine in Europe. The aim of this study was to assess the presence of Extended-Spectrum BL, AmpC and fluoroquinolone/aminoglycoside resistance genes among *E. coli* from a Bulldog puppy in a dog breeding located in Pesaro area, Central Italy. **Materials and Methods.** A total of five *E. coli* were collected in September 8th 2017, from biopsy specimens of the same two-weeks old dog. Species identification and serotyping were performed by API-20E system (bioMérieux) and hot tube agglutination with specific sera. Antibiotic susceptibilities were obtained by AutoScan4 System (Beckman Coulter); the results were interpreted according to EUCAST 2017 guidelines: epidemiological cut-offs or clinical breakpoints for those drugs for which epidemiological cut-offs have not been made available. Check-MDR CT103XL (Checkpoint) microarray and/or PCR and sequencing were used for resistance genes investigation. Conjugation experiments were performed using *E. coli* J53 as a recipient. Molecular characterization of plasmids and clones was accomplished using PBRT Kit (Diateva), Pulsed-Field Gel Electrophoresis (PFGE), Phylogroup identification and Sequence Typing (ST). **Results.** All the n=2 (from liver) and n=3 (from intestine) dog specimens resulted *E. coli* O141 positive. The isolates revealed resistance to ampicillin, piperacillin, third generation cephalosporins, aztreonam, ciprofloxacin, gentamycin and moxifloxacin. The *bla*<sub>TEM</sub>, *bla*<sub>CMY-II</sub> and *bla*<sub>CTX-M-15</sub> genes were detected by microarray, while *aac*(61)-Ib-cr were always identified by PCR. Sequencing of *b/aDHA*\_type genes showed the presence of *b/aDHA*\_i variant. Conjugation experiments confirmed the transferability of *b/aCTX-M-15*, *b/aDHA*, and *aac*(6)-Ib-cr genes; plasmid characterization detected *IncHI2*, *IncX3*, *IncX1*, *IncFII*. The strains showed an identical PFGE profile, of phylogenetic group B1 and ST58. **Discussion and Conclusions.** This is the first Italian report on a two-weeks old dog invasive infection caused by a MDR ST58 *E. coli* O141 *b/aCTX-M-15*, *bla*<sub>CMY-II</sub>, *b/aDHA*\_i and *aac*(6)-Ib-cr positive. MDR *E. coli* clone here characterized was probably the cause of death of all the eight dogs belonging to the same litter. The ST58 reservoir could be the water tank-stored used to prepare the milk-based litter meal. As One health approach, the dissemination of MDR bacteria in companion animals' environment should be regularly monitored to avoid cross-contamination.

Mazzariol S, Centelleghè C, Cozzi B, Povinelli M, Marcer F, Ferri N, Di\_Francesco G, Badagliacca P, Profeta F, Olivieri V, Guccione S, Cocumelli C, Terracciano G, Troiano P, Beverelli M, Garibaldi F, Podestà M, Marsili L, Fossi MC, Mattiucci S, Cipriani P, De\_Nurra D, Zaccaroni A, Rubini° S, Berto D, Beraldo\_de\_Quiros Y, Fernandez A, Morell M, Giorda F, Pautasso A, Modesto P, Casalone C, Di\_Guardo G

**Multidisciplinary studies on a sick-leader syndrome-associated mass stranding of sperm whales (*Physeter macrocephalus*) along the Adriatic coast of Italy**

Sci Rep. - Vol. 8 ( 2018). - Article no. 11557 (18 p). - 102 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1038/s41598-018-29966-7> 7911]

Mass strandings of sperm whales (*Physeter macrocephalus*) are rare in the Mediterranean Sea. Nevertheless, in 2014 a pod of 7 specimens stranded alive along the Italian coast of the Central Adriatic Sea: 3 individuals died on the beach after a few hours due to internal damages induced by prolonged recumbency; the remaining 4 whales were refloated after great efforts. All the dead animals were genetically related females; one was pregnant. All the animals were infected by dolphin morbillivirus (DMV) and the pregnant whale was also affected by a severe nephropathy due to a large kidney stone. Other analyses ruled out other possible relevant factors related to weather

conditions or human activities. The results of multidisciplinary post-mortem analyses revealed that the 7 sperm whales entered the Adriatic Sea encountering adverse weather conditions and then kept heading northward following the pregnant but sick leader of the pod, thereby reaching the stranding site. DMV infection most likely played a crucial role in impairing the health condition and orientation abilities of the whales. They did not steer back towards deeper waters, but eventually stranded along the Central Adriatic Sea coastline, a real trap for sperm whales.

Mazzariol S, Centelleghè C, Povinelli M, Bonsembiante F, Cozzi B, Marcer F, Ferri N, Di\_Francesco G, Di\_Provvido A, Di\_Renzo L, Badagliacca P, Di\_Guardo G, Di\_Francesco C, Cocumelli C, Terracciano G, Cersini A, Cardeti G, Petrella A, Troiano P, Beverelli M, Garibaldi F, Podesta M, Marsili L, Fossi C, Capanni F, Mattiucci S, Cipriani P, De\_Nurra D, Rossi R, Zaccaroni A, Andreini R, Rubini° S, Berto D, De\_Beraldo Quiros Y, Fernandez A, , Morell M, Giorda F, Pautasso A, Modesto P, Biolatti C, Mignone W, Casalone C

#### **Multidisciplinary studies on a sperm whales' mass stranding**

"Marine conservation forging effective strategic partnerships" : the 32nd Conference of the European Cetacean Society : 6th April to 10th April 2018, La Spezia, Italy / [s.l. : s.n., 2018]. - p 66-67 [Nr. Estr. 7846]

Conference of the European Cetacean Society (32nd : La Spezia, Italy : 6th April to 10th April 2018)

Sperm whales' (*Physeter macrocephalus*) mass strandings remain peculiar and rather unexplained events. In September 2014, 7 whales were found stranded along the Italian coastline of the central Adriatic and, while 4 animals were extraordinarily refloated, 3 animals died on the shore. During necropsies of these 3 individuals, a complete set of tissues were collected and preserved for microbiology, genetics, virology, parasitology, stomach content and stable isotopes analysis, toxicology and for microscopic examination. A nested RT-PCR was used to investigate evidences of dolphin morbillivirus (DMV) along with immunohistochemistry (IHC) using an antibody targeting canine distemper virus nucleoprotein antigen. Finally, gas and fat embolic syndrome was assessed. The 3 dead individuals were all females belonging to the same Mediterranean population and social unit; the wind and marine currents pushed them towards the beach where they died. Postmortem analyses revealed that the older female, likely the stranded pod's leader, was pregnant and exhibited an hydronephrosis secondary to a large kidney stone. All the animals were infected by DMV with an immunopositive reaction confirming the infection. Scant and highly digested food remains and the parasitic burden support the hypothesis of a non recent feeding as confirmed also by stable isotope analyses. Gas and fat embolic syndrome was excluded as well as ongoing military exercises and seismic surveys. The results of postmortem analyses revealed that the 7 sperm whales entered the Adriatic Sea encountering adverse conditions and followed northward the ill and pregnant leader of the pod toward the stranding site. DMV infection could have played a crucial role in impairing their health condition and in recognizing the way towards the high seas. In fact, molecular and IHC analyses support the hypothesis of an infection at a very initial phase associated to a "general discomfort condition" secondary to the viral circulation.

Medici F, Bertocchi M, Ferlizza° E, Fedrizzi° G, Andreani G, Isani G

**Caratterizzazione dell'alga bruna *Ascophyllum nodosum* e valutazione della sua attività sulla salute del cavo orale nel cane** = Characterization of the brown seaweed *Ascophyllum nodosum* and evaluation of its activity on canine oral cavity health

XXVI Congresso Nazionale di Fitoterapia : Fuggi, 25 - 27 maggio 2018 / [s.l. : s.n., 2018]. - 2 p. - 3 bib ref [Nr. Estr. 8141]

Congresso Nazionale di Fitoterapia (26. : Fuggi : 25 - 27 maggio 2018)

*Ascophyllum nodosum* (L.) Le Jolis is a brown alga belonging to the family of Fucaceae. The interest on this seaweed is related to the presence of bio-active molecules, including alginates, chlorophyll, carotenoids, fucoxanthin, as well as other antioxidant molecules. The great variability of these natural products suggested us to fix the first aim of this preliminary study in the biochemical characterization of commercial samples of *A. nodosum*. The second aim was the *in vivo* evaluation of its activity on canine oral cavity health. On these samples, 19 trace elements were determined by ICP-MS; cytosolic proteins were extracted and subjected to chromatographic fractionation on Sephadex G-75. For the *in vivo* study, six dogs received *A. nodosum* powder (15mg/kg) for 33 days. Salivary proteins were separated by SDS polyacrylamide gel electrophoresis. High concentration of Fe, As, cytosolic proteins and free amino acids were determined in samples of *Ascophyllum*. Of particular interest are the mycosporine-like amino acids (MAAs), secondary metabolites produced by aquatic organisms, included seaweeds, for protection against UV exposure. The administration of *A. nodosum* to dogs affected by oral cavity diseases resulted in the improvement of gingivitis and halitosis in all the subjects. As regards salivary proteins, the treatment determined a reduction in the number and in the intensity of most protein bands. In conclusion, the results obtained from the *in vivo* study suggest a positive effect on canine oral cavity health, however, more attention should be paid to the characterization of *A. nodosum* commercial preparations, particularly for what concerns the concentration and the bio-availability of toxic trace elements.

Meroni G, Martino PA, Garbarino<sup>o</sup> CA, Bertocchi<sup>o</sup> L, Bronzo V

**Assessment of *Staphylococcus aureus* biofilm-producing genes, enterotoxins and antibiotic resistance in field strains isolated in a Italian dairy farm**

The 2018 International Bovine Mastitis Conference : Milano, June 11-13, 2018 / [s.l. : s.n., 2018]. - p 285. - 4 bib ref [Nr. Estr. 7995]

International Bovine Mastitis Conference (NMC) : Milano : June 11-13, 2018)

*Staphylococcus aureus* is one of the most important pathogen causing clinical, subclinical and chronic infections in both humans and animals, and one of the most relevant aetiological agent of clinical and subclinical bovine mastitis. The evolution of intramammary infection is often determined by factors both related to host immunity and bacterium (Mazzilli et al., 2015,2013; Piccinini et al., 2012). Several virulence factors (e.g enterotoxins, adhesins, haemolysins) act during the initial phase of colonization and other are required for internalization in epithelial cells. Among these, the ability to produce biofilm significantly increases the possibility to adhere and penetrate cells, overcoming the host immunity response and altering the antimicrobial-based therapies. Biofilm, indeed, promotes horizontal transfer of plasmids-borne antibiotic resistance (Marques et al., 2017). The aims of this study were the evaluation of biofilm-forming ability, assessment of beta-lactamic resistance and production of enterotoxins in *S. aureus* strains isolated from milk of cows affected from clinical mastitis. Quarter milk of 15 cows coming from a farm with a high prevalence (>30%) of *S. aureus* were collected resulting in 15 *S. aureus* isolates. The strains were characterized by phenotypic and genotypic assays, including Gram-staining, catalase and coagulase test, growth on Mannitol Salt Agar and/or Baird Parker Agar and ITS-PCR (Internal Transcribed Spacers). Furthermore, the phenotypic ability to produce biofilm was determined comparing two different methods commonly used: Congo Red Agar (CRA) and microtiter assay. The genetic determination of biofilm formation ability was assessed by the amplification of two specific genes (*icaA* and *icaD*). Moreover, genes such as *mecA* and *blaZ*, which are related to beta-lactamic resistance, were tested. The dissemination of enterotoxins genes such as *sea*, *sec*, *sed*, *seg*, *seh*, *sei*, *sej* was also analysed. All the strains belong to genotype B (GTB) and show the same ITS profile underlying the genetic relatedness of these contagious strains. CRA method showed objective difficulties during data interpretation, which were easier to understand using microtiter assay. In fact all the strains were classified as strong (26% = 4/15) and moderate (73% = 11/15) biofilm producers, none weak producer was observed. Additionally, these data were confirmed by *icaA* and *icaD* detection; all the strains were surprisingly positive for both genes. As already reported in other studies (2018; Artursson et al., 2016; Marques et al., 2016), the amplification of *mecA* gene does not occur in any isolate but on the other hand, *blaZ* was detected in all the strains. Moreover, only three (*sea*, *sed*, *sej*) out seven enterotoxins genes analysed were detected in all the strains. In conclusion, strains able to produce biofilm could be considered more dangerous and clinically relevant than

non-producers because the exopolysaccharide matrix acts like a physical barrier that alters the diffusion of antibiotics, resulting in non-successful elimination of this pathogen from mammary gland. These results are concordant with other studies and can help in understanding the relationship between biofilm, antibiotic resistance and mastitis caused by *S. aureus*.

Mescolini G, Silveira F, Lupini C, Felice V, Fiorentini° L, Tosi° G, Massi° P, Cecchinato M, Catelli E

**Caratterizzazione di un ceppo del virus della malattia di Marek rilevato in tacchini da carne con forma viscerale**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) 2018 : LVII Convegno annuale, III Simposio scientifico : Marmirolo (MN), 10-11 Maggio 2018 - Parma (PR), 14 Settembre 2018 / [s.l. : s.n., 2018]. - p 147-151. - 13 bib ref [Nr. Estr. 7935]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (57. : Marmirolo (MN) Parma (PR) : 10-11 Maggio 2018 14 Settembre 2018)

*Marek's disease (MD) is a lymphoproliferative disease caused by Gallid alphaherpesvirus 2 (GaHV-2), which affects primarily the chicken. The virus is able to induce tumours also in turkeys, even if this finding is relatively unusual when compared to the frequency of occurrence in chickens. This study reports the detection and the molecular characterization of a GaHV-2 strain from a flock of Italian meat-type turkeys showing visceral lymphomas. The meq gene was sequenced, aligned and compared with reference strains and with other Italian GaHV-2 detected in the last few years. Results showed that the turkey MD virus has molecular features of high virulence and is closely related to MD strains detected in Italian commercial chickens.*

Minuti A, Amadori° M, Mezzetti M, Lovotti G, Piccioli\_Cappelli F, Trevisi E

**The interferon gamma response to Mycobacterium avium in vitro can be correlated with a higher risk of clinical ketosis in dairy cows**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 30 (Poster 01.01) [Nr. Estr. 7951]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

Background: Ketosis is a metabolic disease associated with reduction of immune competence. The aim of this study was to evaluate the association between clinical ketosis after calving and adaptive immune responses. Material and methods: Thirteen pluriparous Friesian dairy cows were monitored from 21 days before till 28 days after calving. Blood was collected from the jugular vein at different days from calving (DFC) from -21 till 28 DFC. Plasma samples were analyzed for energy parameters (glucose, NEFA and BHBA). Moreover, at -21 and 28 DFC an IFN-gamma (IFNG) release assay for *Mycobacterium avium* was carried out on heparinized whole blood. Results were evaluated in terms of Delta OD (difference between avian PPD-stimulated and control wells). Cows were retrospectively grouped according to their plasma BHBA concentrations after calving in Control (CTR, BHBA < 1.4 mmol/L; 7 cows) and Ketosis (KET, BHBA > 1.4 mmol/L; 6 cows). Data were analysed as a repeated measures study using the MIXED procedure of SAS considering the group (CTR or KET) as fixed effect. Results: The IFNG response showed differences, both before (-21 DFC) and after calving (28 DFC), delta OD being higher in KET compared to CTR cows (P < 0.05). In practice, a higher IFNG response to avian PPD was associated with a higher risk of ketosis after calving. Conclusions: Considering that IFNG is related to metabolic pathways of energy use, a vigorous IFNG response to environmental microbial stressors like *M. avium* may represent a risk after calving, when homeostatic control circuits are less effective.

Minuti A, Amadori<sup>o</sup> M, Mezzetti M, Lovotti G, Piccioli\_Cappelli F, Trevisi E

**The Interferon gamma response to Mycobacterium avium in vitro can be correlated with a higher risk of clinical ketosis in dairy cows**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 108. - 2 bib ref [Nr. Estr. 7960]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

Ketosis is a common metabolic disease often associated with reduction of immune competence and linked to other pathologies (mainly mastitis and metritis) of the transition dairy cow. These may be preceded by innate immune responses well before parturition [1,2]. The aim of this study was to evaluate the association between clinical ketosis after calving and adaptive immune responses during the transition period. Thirteen pluriparous Italian Friesian dairy cows were monitored from 21 days before till 28 days after calving. Cows were housed in a tie-stall barn with ad libitum feed and water. Diets were formulated to cover requirements according to the National Research Council Recommendations. During the trial, the animals' health status was checked every day and daily feed intake and rumination were recorded. After calving the cows were milked twice a day, and milk yield was recorded. Blood was collected from the jugular vein before the morning feeding, at different days from calving (DFC) from -21 till 28 DFC. Plasma samples were analyzed by a clinical analyzer (ILAB 650, Instrumentation Laboratory, USA) for energy parameters (glucose, NEFA and BHBA). Moreover, at -21 and 28 DFC a blood tube was collected and used in an IFN-gamma (IFNG) release assay for Mycobacterium avium subsp. avium on heparinized whole blood (internal method IZSLER, MP 13/011). Results were evaluated in terms of Delta OD (difference for IFNG between avian PPD-stimulated and control wells). Cows were retrospectively grouped according to their plasma BHBA concentrations after calving in Control (CTR, BHBA<1.4 mmol/L; 7 cows ) and Ketosis (KET, BHBA>1.4 mmol/L; 6 cows). Data were analysed as a repeated measures study using the MIXED procedure of SAS with group (CTR or KET) as fixed effect. KET cows showed a lower feed intake during the 1st month of lactation with difference at 3 and 4 weeks after calving (P<0.05). Moreover, KET cows showed a lower milk production at 4th week (37.6 vs 40.3 kg/d of CTR, respectively; P<0.1) and, a lower rumination time (453 vs. 500 min/day of CTR; P<0.1). The markers of energetic metabolism confirmed the worse negative energy balance after calving in KET cows. In particular, BHBA concentrations were higher from -7 to 14 DFC (P<0.05), NEFA levels were higher from 0 to 28 DFC (P<0.05) and glucose concentrations were lower at 3 DFC (P<0.05). The IFNG response during challenge showed differences, both before (-21 DFC) and after calving (28 DFC), delta OD being higher in KET compared to CTR cows (P<0.05). In practice, cows mounting a higher IFNG response to Mycobacterium avium subsp. avium showed a higher risk to develop ketosis after calving. Considering that IFNG is related to metabolic pathways of energy use, a vigorous IFNG response to environmental microbial stressors may represent a risk after calving, when homeostatic control circuits are less effective. Our study confirms that peculiar features.

Montarsi F, Martini S, Arnoldi D, Calzolari<sup>o</sup> M, Ballardini M, Capelli G

**Current distribution of the invasive Mosquito Aedes koreicus (Diptera; culicidae) in Italy**

ECE 2018 : XI European Congress of Entomology : 2-6 July 2018, Napoli : book of abstracts / [s.l. : s.n., 2018]. - p 92 (CO281) [Nr. Estr. 7992]

European Congress of Entomology (11. : Napoli : 2-6 July 2018)

Mosquitoes belonging to genus Aedes are often recorded out of their native places as invasive species. In addition, these species are proven or potential vectors of important arboviruses and are a threat for human and animal health. Several invasive Aedes species are now established in Europe and Italy is one of the most infested European countries. At present, three invasive species



occur in Italy: *Aedes albopictus*, *Ae. japonicus* and *Ae. koreicus*. The latter, was first found in North-Eastern Italy in 2011. The constant monitoring of this species shows an increasing spreading trend. *Aedes koreicus* develops in artificial containers, sometimes sharing the breeding sites with the other invasive mosquito species and is adapted to tolerate the cold winter temperature. For this reason, it is present in mountainous and hilly areas where *Ae. albopictus* is present in low density or absent. Starting from the area of the first report, it has now spread over five Italian Regions and 123 municipalities, from West to East colonizing wide areas of Northern Italy. According to these records, northern Italy has a high probability to be invaded by *Ae. koreicus* in the next decade. The North-East Italy in particular, confirms to be one of the areas with the most frequent experience of invasive mosquito introduction in Europe. This is likely a consequence of the intensive trade of goods. The establishment of invasive mosquito species complicates the current surveillance system and requires well trained personnel for identification. A new competent vectors of pathogens may represent a challenge for the Health System.

Moreno<sup>o</sup> A, Chiapponi<sup>o</sup> C, Canziani<sup>o</sup> S, Baioni<sup>o</sup> L, Faccini<sup>o</sup> S, Luppi<sup>o</sup> A, Vaccari G, Foni<sup>o</sup> E, Alborali<sup>o</sup> GL, Lavazza<sup>o</sup> A

#### **Swine influenza A viruses : extremely high genomic heterogeneity in Italy in the last two decades**

2nd National Congress of the Italian Society for Virology "One Virology One Health" : Rome, November 28-30, 2018 / [s.l. : s.n., 2018]. - p 162 ( Poster no. P50 ) [Nr. Estr. 8154]

National Congress of the Italian Society for Virology (2nd : Rome : November 28-30, 2018)

Influenza A viruses (IAVs) have a wide range of hosts including avian species and mammals. In the European swine population, three subtypes (H1N1, H1N2, and H3N2) are currently spread: Eurasian avian-like H1avN1 (EU-Avianlike), A/sw/Gent/1/84-like H3 N2 (Gent/84), A/sw/Scotland/410440/94- like H1huN2 (scot/94) (3) and, since 2009, the novel pandemic virus pdm09H1N1. Phylogenetic analysis of European swIAVs showed high genome diversity and several different reassortment events involving swine, human and avian IAVs. Swine are indeed one of the main reservoir species for IAVs and play a key role in the transmission of IAVs between species. Additionally, the pdm09H1N1 IAVs highlighted the role of pigs in the emergence of IAVs with pandemic potential. Influenza surveillance between 1998 and 2018 was based on investigation of pigs showing respiratory disease and included genome detection, virus isolation, sequencing and genomic characterization. In this study, we investigated the complete genomic characterization of 444 SwIAV strains isolated in Italy with particular attention to the detection of reassortant strains and genotype diversity. Whole genome sequencing of Italian swIAVs was performed using Illumina MiSeq and Ion Torrent NGS platforms (1). Phylogenetic analysis was performed using MEGA6 including swine, avian and human IAVs retrieved from public databases. Phylogenetic analysis showed high genome diversity with several different reassortment events that involved swine and human IAVs but no avian IAVs. A total of 24 different genotypes were identified but with differences between subtypes (Fig1). Extremely high heterogeneity was observed within the H1N2 genotype, where only 5.3% of strains showed the characteristics of scot/94. The other strains (94.7%) were divided in 11 different genotypes; of these, 44.3% belonged to genotype A/Sw/Italy/4675/2003- like H1huN2 (Gen 3) first detected in 2003 and originated from reassortment between scot/94 and human seasonal H3N2 of 1997-98 (2). The H1N1(pdm09H1N1excluded) and H3N2 subtypes showed lower genetic diversity with 92.3% and 98.8% of strains exhibiting the characteristics of the EU-Avianlike and Gent/84 respectively. Almost all the reassortant strains in these subtypes contained different constellations involving pdm09H1N1 genes. Reassortment events involving swine and human influenza viruses were frequent in Italian pigs but with different patterns. Some of these patterns were highly frequent, suggesting the presence of new gene constellations and well balanced HA-NA combinations, able to contribute to efficient replication and successful transmission among pigs. The frequent reassortment events between human and swine IAVs in pigs provide the conditions to investigate adaptive evolution of IAVs in different hosts and to acquire more information on their capability to cross host barriers and become a pandemic strain.

Moreno<sup>o</sup> A, Chiapponi<sup>o</sup> C, Lelli<sup>o</sup> D, Morelli A, Silenti V, Gobbi M, Lavazza<sup>o</sup> A, Paniccia M

**Detection of a gE-deleted PRV strain in an Italian red fox**

11th International Congress for Veterinary Virology, 12th Annual meeting of Epizone : August 27-30, 2018, Vienna : abstracts / [s.l. : s.n., 2018]. - p 30 (Oral 223) [Nr. Estr. 8115]

International Congress for Veterinary Virology : 11th Annual meeting Epizone : 12th : Vienna : August 27-30, 2018)

Aujeszky disease (AD) is a notifiable disease that results from infection by Pseudorabies virus (PRV) that causes substantial economic losses to the swine industry. Suids are the natural reservoir of PRV whereas the disease is self-limiting in other species. AD in pigs is controlled by using live and inactivated gE-deleted vaccines. Although PRV has been eliminated from domestic pigs in many European countries, AD is continuously being reported in wild boars and in related hunting dogs. Clinical cases have rarely been detected in other wildlife such as foxes, Iberian lynxes, panthers and skunks. This case reports AD caused by a gE deleted PRV strain in a red fox in Italy. Methods A fox displaying atypical behaviour was found in an urban area in Central Italy and submitted to a Veterinary Hospital. Death occurred within 48 hours. The presence of PRV DNA in the brain was determined by specific gE/gB genes real-time PCRs. Rabies, canine distemper, Parvovirus, Hepatitis E and Leptospirosis were excluded by direct immunofluorescence and PCRs. Partial sequencing of the gC gene was also performed on clinical specimens. Virus isolation was attempted through inoculation in PK15 cells. The virus isolate was tested for PRV gE and gB antigen detection by two virological ELISAs using MAbs specific to gB and gE proteins. Full sequencing on the virus isolate was attempted by NGS approach. Results Clinical signs included ataxia, poor coordination, unsteady walk, tendency to stumble and diarrhoea without skin lesions. Only PRV gB was detected by real time and traditional PCRs. Virological ELISAs performed on the virus isolate resulted positive for gB antigen detection and negative for gE. Phylogenetic analysis of gC gene showed that the fox PRV sequence belonged to a different clade from those circulating in Italy but was closely related to the Nia strain. Full genome sequencing is in progress. Conclusion This case reported the isolation of a PRV strain in a fox suffering neurological signs found in an urban area. These results support the idea that the virus is a gE deleted mutant closely related to a vaccine strain and implicates domestic pigs as the primary source of infection for the fox. However, the epidemiologic link between a PRV vaccine strain and the infection in a fox remains unclear. These findings underscore the importance of biosecurity measures on pig farms and the relevance of virological surveillance.

Moreno<sup>o</sup> A, Dalloli C, Chiapponi<sup>o</sup> C, Bregoli<sup>o</sup> A, Vinco<sup>o</sup> LJ, Lelli<sup>o</sup> D, Sozzi<sup>o</sup> E, Lavazza<sup>o</sup> A, Testi S, Ardigò P, Giorgi M

**One health challenge : detection of a swine H1N2 influenza virus in commercial turkeys**

11th International Congress for Veterinary Virology, 12th Annual meeting of Epizone : August 27-30, 2018, Vienna : abstracts / [s.l. : s.n., 2018]. - p 19 (Oral 24) [Nr. Estr. 8114]

International Congress for Veterinary Virology : 11th Annual meeting Epizone : 12th : Vienna : August 27-30, 2018)

Background Three subtypes of influenza A viruses (IAVs), H1N1, H1N2 and H3N2 are commonly found in pigs in Italy. These were found to derive from mammalian or avian viruses or their reassortants. Since January 2017, 83 outbreaks of highly pathogenic (HP)IAV(H5N8) were reported in North Italy, mainly in industrial farms. Of these, 39 were reported in meat turkey farms in the same area, which is also characterized by the highest concentration of the national poultry and pig production. This study reports a natural infection of swine(sw)IAV H1N2 in a turkey farm located in North Italy in an area subjected to movement restrictions due to a previous H5N8HPAIV outbreak near the farm. Methods IAV and H5, H7 and other HAs genome detection was performed on pools

of 10 swabs each by real time RT-PCRs. Full genome was performed using NGS approach. Serological analyses were performed by competitive ELISA and HI test using different antigens belonging to H5, H7, and other subtypes of avian IAVs and H1 N1, H1 N2 and H3N2 swIAVs. Results A flock of 15280 10-wk-old meat turkeys experienced anorexia, depression and respiratory signs in a farm located in Lombardia Region. 60 tracheal swabs (TS) were taken following clinical signs and 105 TS and 45 sera before slaughter. PCR for IAV on one pool, collected after clinical signs, showed positivity near to cutoff, whereas six pools collected before slaughter resulted strongly positive. PCR for H5, H7 and other avian IAVs resulted negative. Full genome sequencing on positive samples taken at slaughter showed the presence of a 5wIAVH1N2. Genomic analysis evidenced an interesting gene constellation resulting from reassortment between H1 human-like (A/Sw/Italy/4675/2003-like) and human seasonal H3N2 of 1997-98. Twenty-five % of the samples resulted serologically positive towards swIAVH1N2 (HI titres from 16 to 32). Conclusion Interspecies transmission of SwIAVs to turkeys has been reported in several cases although mainly caused by H1N1 or H3N2 strains. This report provides evidence of a slow circulation of a 5wIAVH1N2 in a turkey farm and strengthens the arguments that IAVs can cross species complicating diagnosis and characterization of new isolates. The presence of both avian and human-type receptors on turkey tissues supports the finding that turkeys can be infected with IAVs containing avian or mammalian HA genes and can act as a potential intermediate host for interspecies transmission and spread of reassortment viruses between birds and humans.

Nardoni S, Pisseri F, Pistelli L, Najari B, Luini M, Mancianti F

**In vitro activity of 30 essential oils against bovine clinical isolates of *Prototheca zopfii* and *Prototheca blaschkeae***

Vet Sci. - Vol. 5 no 2 ( 2018). - no 45 (13 p). - 39 bib ref [Nr. Estr. ( ultimo accesso 01/06/2018 ) <https://doi.org/10.3390/vetsci50200457841>]

Protothecal mastitis poses an emergent animal health problem in dairy herds, with a high impact on dairy industries, causing heavy economic losses. Current methods of treating protothecal infections are ineffective, and no drug is licensed for use in cattle. The aim of the present study was to check the anti-algal activity of 30 chemically defined essential oils (EOs) against *Prototheca zopfii* and *Prototheca blaschkeae* isolated from the milk of dairy cows with mastitis. A microdilution test was carried out to estimate the anti-algal effectiveness of the selected chemically defined EOs. The microdilution test showed different degrees of inhibition among the examined *Prototheca* species. The activity of some of the examined EOs seem interesting. In particular, *Citrus paradisi* yielded the lowest minimal inhibitory concentration values (0.75%) for both algal species. *P. zopfii* appeared to be more sensitive to EOs in comparison to *P. blaschkeae*. The present study investigated the in vitro susceptibility of *P. zopfii* and *P. blaschkeae* to a wide range of EOs, obtained from different botanical families. Further investigations are necessary to evaluate the efficacy of EO-based formulations intended for the disinfection of both udder and milking products.

Neimanis AS, Ahola H, Zohari S, Larsson\_Pettersson U, Brojer C, Capucci L, Gavier-Widn D

**Arrival of rabbit haemorrhagic disease virus 2 to northern Europe : emergence and outbreaks in wild and domestic rabbits (*Oryctolagus cuniculus*) in Sweden**

Transbound Emerg Dis. - Vol. 65 ( 2018). - p 213-220. - 37 bib ref [Nr. Estr. ( ultimo accesso 09/03/2018 ) <https://doi.org/10.1111/tbed.126507804>]

Incursion of rabbit haemorrhagic disease virus (RHDV) into Sweden was documented in 1990 and it is now considered endemic in wild rabbit (*Oryctolagus cuniculus*) populations. Rabbit haemorrhagic disease virus 2 (RHDV2), a new, related lagovirus was first detected in France in 2010, and has spread rapidly throughout Europe and beyond. However, knowledge of RHDV2 in northern Europe

is sporadic and incomplete, and in Sweden, routinely available diagnostic methods to detect rabbit haemorrhagic disease (RHD) do not distinguish between types of virus causing disease. Using RHDV2-specific RT-qPCR, sequencing of the VP60 gene and immunological virus typing of archived and prospective case material from the National Veterinary Institute's (SVA) wildlife disease surveillance programme and diagnostic pathology service, we describe the emergence of RHDV2 in Sweden in both wild and domestic rabbits. The earliest documented outbreak occurred on 22 May 2013, and from May 2013 to May 2016, 10 separate incidents of RHDV2 were documented from six different municipalities in the southern half of Sweden. Phylogenetic analysis of the VP60 gene shows clear clustering of Swedish isolates into three separate clusters within two different clades according to geographic location and time, suggesting viral evolution, multiple introduction events or both. Almost all cases of RHD examined by SVA from May 2013 to May 2016 were caused by RHDV2, suggesting that RHDV2 may be replacing RHDV as the predominant cause of RHD in Sweden.

Neretti G, Morandi B, Taglioli M, Poglayen G, Galuppi R, Tosi° G, Borghi CA

**Inactivation of Eimeria oocysts in aqueous solution by a dielectric barrier discharge plasma in contact with liquid**

Plasma Med. - Vol. 8 no 2 ( 2018). - p 155-162. - 21 bib ref [Nr. Estr. 7884]

This study presents a novel technique to inactivate coccidian oocysts in an aqueous solution. The technique consists of treating the contaminated liquid by using an atmospheric-pressure air dielectric barrier discharge (DBD) plasma in contact with it. Many experiments in several operating conditions were performed. The discharge was supplied by sinusoidal and nanosecond-pulsed voltages with a constant average power of —7 W in both cases. Biological effects due to the plasma were investigated by performing tests with increasing treatment time. A sudden —40% drop in the number of survived oocysts was reached in 4 min and a two-fold reduction was detected after 12 min of exposure. No significant differences in the biocidal efficacy were detected between the AC-driven and the nanosecond-pulsed discharge. Chi-squared statistical analysis on the treated samples showed significant statistical difference (with a statistical significance P value parameter less than 0.01) and nonrandomness warranty of the results, opening interesting scenarios for future developments.

Ouagal M, Brocchi° E, Grazioli° S, Adel BY, Keith S, Kiram D, Oussiguéré A, Hendriks P, Berkvens D, Saegerman C

**Study on seroprevalence and serotyping of foot and mouth disease in Chad**

Rev Sci Tech OIE. - Vol. 37 no 3 ( 2018). - p 937-947. - 35 bib ref [Nr. Estr. ( ultimo accesso 05/03/2019 )

[http://www.oie.int/fileadmin/Home/eng/Publications\\_%26\\_Documentation/docs/pdf/revue\\_plurithematique/2018/23112018-00139-EN\\_Ouagal-Saegerman.pdf](http://www.oie.int/fileadmin/Home/eng/Publications_%26_Documentation/docs/pdf/revue_plurithematique/2018/23112018-00139-EN_Ouagal-Saegerman.pdf) 8143]

Foot and mouth disease (FMD) is a highly contagious viral disease that affects all Artio'dactyla. Seven immunologically distinct serotypes of FMD virus (FMDV) exist. In Chad, although FMD is included in the list of diseases monitored by the Chadian Animal Disease Surveillance Network (REPIMAT), the epidemiological situation remains unclear. A serological survey was conducted in the cattle population in eight of the nine administrative regions of the country (those regions with the highest cattle densities), to evaluate the prevalence and serotypes of circulating FMDV. A total of 796 sera from randomly selected cattle were analysed at the World Organisation for Animal Health/Food and Agriculture Organization of the United Nations FMD Reference Laboratory at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), in Brescia (Italy). An enzyme-linked immunosorbent assay (ELISA), called 3ABC ELISA, was used to detect antibodies against non-structural proteins (NSPs), as well as a series of six competitive ELISAs to

detect and serotype antibodies against the structural proteins of FMDV serotypes 0, A, SAT 1, SAT 2, Asia 1 and C. Based on the detection of anti-NSP antibodies, the animal-level seroprevalence was 35.6% (95% confidence interval [CI]: 32.2-38.9) and the herd-level seroprevalence was 62.3% (95% CI: 53.0-71.5). FMD was present in all livestock administrative divisions surveyed, with a higher prevalence in southern regions, which are characterised by higher rainfall and humidity and more important transboundary animal movements. Cattle aged more than four years had a higher seroprevalence, which may be due to repeated exposure. Semi-sedentary farming and transhumance were also risk factors. Antibodies against serotypes A, 0, SAT 1 and SAT 2 were detected.

Pajoro M, Pistone D, Varotto\_Boccazzi I, Mereghetti V, Bandi C, Fabbi° M, Scattorin F, Sasseria D, Montagna M

**Molecular screening for bacterial pathogens in ticks (*Ixodes ricinus*) collected on migratory birds captured in northern Italy**

Folia Parasitol. - Vol. 65 ( 2018). - no 8 (6 p). - 42 bib ref [Nr. Estr. ( ultimo accesso 12/07/2018 ) <https://doi.org/10.1007/s12550-017-0305-y> 7874]

*Migratory birds have an important role in transporting ticks and associated tick-borne pathogens over long distances. In this study, 2,793 migratory birds were captured by nets in a ringing station, located in northern Italy, and checked for the presence of ticks. Two-hundred and fifty-one ticks were identified as nymphs and larvae of Ixodes ricinus (Linnaeus, 1758) and they were PCR-screened for the presence of bacteria belonging to Borrelia burgdorferi sensu lato, Rickettsia spp., Francisella tularensis and Coxiella burnetii. Four species of Borrelia (B. garinii, B. afzelii, B. valaisiana and B. lusitanae) and three species of Rickettsia (R. monacensis, R. helvetica and Candidatus Rickettsia mendelii) were detected in 74 (30%) and 25 (10%) respectively out of 251 ticks examined. Co-infection with Borrelia spp. and Rickettsia spp. in the same tick sample was encountered in 7 (7%) out of the 99 infected ticks. We report for the first time the presence of Candidatus Rickettsia mendelii in I. ricinus collected on birds in Italy. This study, besides confirming the role of birds in dispersal of I. ricinus, highlights an important route by which tick-borne pathogens might spread across different countries and from natural environments towards urbanised areas.*

Pajoro M, Pistone D, Vicari° N, Peli° S, Rigamonti° S, Viganò R, Colombo L, Riccardi F

**Survey on ticks and bacterial tick-borne pathogens : Ossola Valley, North-West Italy (2010-2017)**

XXX Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) "Mutamenti ambientali e parassiti" : 26-29 Giugno 2018, Milano / [s.l. : s.n., 2018]. - p 205 (Poster P17) [Nr. Estr. 8130]

Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) (30. : Milano : 26-29 Giugno 2018)

**INTRODUCTION.** In Italy, the availability of eco-epidemiological data on the distribution of different tick species and associated tick-borne pathogens (TBPs) is highly variable. Such information constitutes a useful guideline for the medical healthcare system, which in general has a proper awareness on the epidemiology of these vector borne diseases. However, the incidence of human tick-borne diseases in non-endemic Italian areas is probably underestimated, perhaps due to limited surveillance, asymptomatic cases, improper diagnosis (e.g. false negative in serological tests) and lack of follow up studies. Despite a recent study has provided data on *Borrelia burgdorferi sensu lato* prevalence in *Ixodes ricinus* ticks collected in Piemonte (Pintore et al., 2015, Zoonoses Public Health 62:365-374), corresponding to the increase in the number of reported Lyme disease cases in the area, this region remains one of those territories rarely investigated for the presence of ticks and TBPs. **MATERIALS AND METHODS.** In our survey, a molecular screening for four important agents of zoonoses (*Rickettsia* spp., *B. burgdorferi sensu lato* complex, *Francisella tularensis* and *Coxiella burnetii*) was performed on both questing ticks collected by dragging on vegetation in different

forested areas in Ossola valley and engorged ticks collected on domestic and wild animals during the hunting seasons 2010 (Pistone et. al., 2017, Exp. Appl. Acarol. 73: 477) and 2017 (data not published). RESULTS AND CONCLUSIONS. Two different species of Rickettsia (*R. helvetica* and *R. monacensis*), known to cause human illnesses and five different Borrelia species, proved (*B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii*) or suspected (*B. valaisiana* and *B. lusitanae*) to cause clinical manifestations of Lyme disease in humans, were found in both questing and engorged *I. ricinus*. Moreover, PCR positivity for *F. tularensis* was obtained in engorged ticks collected on two different wild ungulate species (*Capreolus capreolus* and *Cervus elaphus*). This work provided further data and broadened our knowledge on bacterial pathogens present in ticks in North-Western Italy.

Pangallo° G, Amorico A, De\_Lorenzi° G, Gherpelli° Y, Bonilauri° P, Dottori° M, Gibelli° L, Luppi° A

**Pleuropolmonite fibrino-necrotico-emorragica in suinetti sottoscrofa**

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 239-243. - 6 bib ref [Nr. Estr. 7830]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

Scopo del presente lavoro 6 la descrizione di un caso di pleuropolmonite fibrino-necrotico emorragica da *Actinobacillus pleuropneumoniae* in suinetti sottoscrofa appartenenti ad un allevamento da riproduzione. L'esame anatomico-patologico eseguito su 2 suinetti deceduti improvvisamente senza segni clinici apparenti, ha permesso di evidenziare le lesioni tipiche a carico del parenchima polmonare, nel dettaglio si osservava un quadro di pleuropolmonite fibrino-necrotico-emorragica monolaterale. La diagnosi è stata confermata dall'esame batteriologico che ha permesso l'isolamento dai pohnoni di un ceppo di *Actinobacillus pleuropneumoniae* biotipo 1 sierotipo 9 da tutti i suinetti conferiti. *Actinobacillus pleuropneumoniae* (APP) e un batterio cocco bacillare, Gram negativo e anaerobio facoltativo. Gli isolati di APP sono suddivisi in biotipo 1 e biotipo 2, in base alla loro dipendenza dal nicotinamide adenine dinucleotide (NAD) per la crescita (rispettivamente NAD-dipendente e NAD- non dipendente) e in 15 sierotipi diversi (1-12,15 per APP biotipo 1; 13, 14 per APP biotipo 2). La specificita sierologica è conferita da polisaccaridi capsulari (CPS) e da lipopolisaccaridi di membrana (LPS) (Blackall et al., 2002; Kamp et al., 1987; Nielsen 1985a, b, 1986b; Nielsen et al., 1997; Nielsen and O'Connor, 1984; Rosendal and Boyd, 1982). I ceppi di APP sono produttori di 4 tossine differenti chiamate "APX". APX I 6 fortemente emolitica e citotossica, APX II 6 debolmente emolitica e citotossica e APX III non 6 emolitica ma fortemente citotossica. Per quel che riguarda APX IV non 6 ancora chiaro il ruolo nella patogenesi della malattia, tuttavia sembrerebbe necessaria a completare l'espressione della virulenza del patogeno (Frey et al. 1994; Jansen et al., 1995). La trasmissione di APP pu6 avvenire sia per contatto diretto sia tramite aerosol per brevi distanze. Negli allevamenti dove APP è endemico la trasmissione avviene generalmente tra scrofe infette e suinetti. Tale quadro influenzato sia dalla quantita di batteri eliminati dalla scrofa che dal livello di immunita passiva nei suinetti (durata da 2 settimane a 2 mesi) acquisito tramite colostro. Determinanti per la perpetuazione e l'introduzione della patologia, infine, risultano essere i portatori sub-clinici. Nel suino, APP è causa di pleuropolmonite fibrino-necrotico-emorragica, anche se la gravita della malattia è determinata sia da fattori legati all'ospite e all'ambiente, sia dalla virulenza del ceppo coinvolto. Si possono osservare forme acute, sub-acute o croniche della malattia caratterizzate da morbilita e mortalita differenti. E' importante sottolineare come l'infezione da APP possa essere caratterizzata da forme sub-cliniche della malattia che si caratterizzano da bassa o assente mortalita, con frequente localizzazione tonsillare del patogeno o da forme clinicamente lievi con lesioni polmonari che frequentemente cronicizzano. In quest'ultimo caso si osservano, con elevata prevalenza in animali regolarmente macellati, pleuriti fibrose o croniche a localizzazione prevalentemente dorso-caudale.

Pangallo° G, Amorico A, De\_Lorenzi° G, Gherpelli° Y, Bonilauri° P, Dottori° M,

Luppi° A

### **Hemorrhagic fibrino-necrotic pleuropneumonia in suckling piglets**

ESPHM 2018 : 10th European Symposium of Porcine Health Management : Barcelona, 9th - 11th May, 2018 : proceedings / [s.l. : s.n., 2018]. - p 220 (Poster BBD-042) [Nr. Estr. 7868]

European Symposium of Porcine Health Management (ESPHM) (10th : Barcelona : 9th - 11th May, 2018)

**Introduction** In May 2017 two 14-day-old suckling piglets belonging to an Italian farrow to wean farm (987 sows), with a clinical history of sudden death without clinical signs, were sent to the Laboratory of Reggio Emilia (IZSLER) for diagnostic investigations. They belonged to a litter of 16 piglets born from a first parity sow in a unstable PRRS farm. After the first case other 5 litters, belonging to 5 first parity sows were affected. The total morbidity and lethality was 76.2% and 45.8% respectively. **Material and methods** Necropsies were performed following standardized procedures as well as bacteriology (including biochemical confirmatory tests) and histological examinations, that were performed on lungs, kidneys and spleen collected during the anatomopathological evaluation. Sensitivity to antibiotics was determined using the disc diffusion method (Kirby-Bauer). **Results** Anatomopathological examination showed a diffuse monolateral hemorrhagic fibrinonecrotic pleuropneumonia. *Actinobacillus pleuropneumoniae* (APP) biotype 1 and serotype 9 was isolated from lungs. Microscopic examination confirmed the presence of fibrinous pleuropneumonia with bacterial aggregates in the alveoli and a depletion of the splenic lymphoid follicles in both piglets. In agreement with the results of the antibiogram the piglets were treated twice by injection with florfenicol (15 mg/kg BW). The antibiotic treatment was effective to tackle the problem. **Discussion and conclusion** This is a rare case of pleuropneumonia in 14-day-old suckling piglets as most of the outbreaks are described in fattening pigs. The early onset of pleuropneumonia described could be due to the introduction of a new APP strain; to the lack of vaccination and immunity for APP in gilts; to the lack of passive immunity to protect piglets belonging to first parity sows and to the presence of PRRSV-viremic and immunodepressed piglets. No other outbreaks of pleuropneumonia have been described in the herd in the following months.

Parigi° M, Ardolino F, Falletta M, Petrini D, Rota S, Rinaldi L, Dipineto L, Massi° P

### **Detection of *Giardia duodenalis* in pets, Italy**

XXX Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) "Mutamenti ambientali e parassiti" : 26-29 Giugno 2018, Milano / [s.l. : s.n., 2018]. - p 219 (Poster P30) [Nr. Estr. 8120]

Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) (30. : Milano : 26-29 Giugno 2018)

**INTRODUCTION.** Rabbits, reptiles and birds are becoming popular companion animals in several countries, including Italy (Assalco, 2016). However, little is still known on their role as potential zoonotic reservoir of various pathogens. The aim of the present study was to investigate the presence of *Giardia duodenalis*, the only species within the *Giardia* genus responsible for infection of humans and other mammals, in exotic animals kept as pets in different Italian regions. **MATERIALS AND METHODS.** In 2017, 99 exotic pets belonging to different species have been investigated for the coprological detection of DNA of *G. duodenalis*. Of the sampled pets, 52.5% (52/99) were small mammals (36/52 rabbits and 16/52 rodents), 27.3% (27/99) reptiles (23/27 Chelonian and 4/27 Squannata) and 20.2% (20/99) pet birds (16/20 Passeriformes, 2/20 Psittaciformes and 2/20 Columbiformes). All the animals were kept as pets and sampled individually with the exception of 15 pooled faecal samples of pet birds collected from the cages' floor. From each sample, the extracted DNA was tested with a real-time PCR targeting a portion of the *gdh* locus common to the A-H assemblages of the parasites (Yang et al., 2014, Exp Parasitol 137:46-52). **RESULTS AND CONCLUSIONS.** Fifteen faecal samples tested positive for the presence of *G. duodenalis* DNA (15.2%; 15/99). The highest prevalence was found in rabbits (53.3%; 8/15), followed by chelonian (39.6%; 6/15) and birds (6.6%; 1/15). Only one rabbit was referred to show gastrointestinal symptoms at the moment of the visit. The prevalence of infection found in rabbits (22.2%; 8/36) is higher than those reported in studies conducted in Europe and

China (7.6% and 9.9%, in Pantchev et al., 2014, Vet Rec 175(1): and in Jiang et al., 2018 In Press, respectively). The most interesting result of this study is the positivity found in chelonian (5 tortoise and 1 turtle). To date, studies on the presence of Giardia in reptiles did not show evidence of the parasite in these hosts (Rinaldi et al., 2012, Parasite 19(4): 437:440), with the exception of free-living lizards sampled in Spain that tested positive for different assemblages (Reboredo-Fernandez et al., 2017, Rev Bras Parasitol Vet 26(3): 395-399). The role of both reptiles and birds in the cycle of G. duodenalis is still unclear and further studies are needed to distinguish between actual infection and simply mechanical dissemination of cysts. Thus, the sequencing of our positive samples and the analysis of a larger number of animals are of importance in order to define the role of these animal species in the transmission and the maintenance of this zoonotic parasite the environment.

Parini M, Paoli A, Buccioni A, Antongiovanni M, Massi° P, Tosi° G, Fiorentini° L

**Efficacy of specific compositions of 1-Monoglycerides of Short- and Medium Chain Fatty Acids in controlling Salmonella typhimurium and other serotypes of Salmonella spp. in broiler chickens and in vitro conditions**

XVth European Poultry Conference : 17th to 21st September 2018, Dubrovnik, Croatia : conference information and proceedings / [s.l. : s.n., 2018]. - p 179 (Abstract ID 165) [Nr. Estr. 8033]

European Poultry Conference (15th : Dubrovnik, Croatia : 17th to 21st September 2018)

Specific compositions of 1-iVMonoglycerides of Short- and Medium Chain fatty acids showed antibacterial efficacy in vitro against S. typhimurium, S. enteritidis, S. jawa, S. heidelberg and S. dublin, S. infantis, S. alachua and other serotypes at pH 6-7, corresponding to the pH values of the gut of chickens. The MIC of the 1-Monoglycerides compositions against above Salmonella serotypes resulted to be comprised between 0.01% and 0.1%, while Lactobacillus plan tarum and Lactobacillus acidophilus were not inhibited. A trial with broiler chickens was carried out to assess the efficacy of a specific 1-Monoglycerides composition in reducing Salmonella typhimurium counts in the caeca after experimental infection. Sixty female one-day old Ross 308 broiler chicks were randomly housed in isolators and allotted to two treatments. The control group received a standard commercial feed, while the 1-Monoglycerides group received the same feed supplemented with 0.3% of the composition during the whole experimental period. At 7 days of age all the birds were challenged via endoesophageal inoculation with 1 mL of saline solution containing  $1 \times 10^7$  CFU/bird of S. typhimurium. At day 14, 24 and 34 ten chickens from each group were sacrificed; samples from caeca were analyzed for Salmonella spp. counting. Results showed that in the 1-Monoglycerides group the Salmonella CFU counts was reduced by 6 logs compared to the control group ( $p = 0.02$ . Two-way ANOVA). In the control group a mortality of 30% was recorded, while no mortality was observed in the 1-Monoglycerides group. The 1-Monoglycerides composition prevented Salmonella typhimurium colonization in the caeca and the pathogenic effects of the bacterium which caused high mortality in the control group. The in vitro trial showed a selective antibacterial effect of the i-Monoglycerides compositions against Salmonella spp. without inhibiting beneficial Lactobacillus. Specific compositions of Short- and Medium-Chain 1-Monoglycerides may represent an alternative to enteric antibiotics in broiler feed.

Parisi E, Vencia W, Amadori° M, Ferrari A, Razzuoli E

**Yersinia enterocolitica interaction with jejunal epithelial cells**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 113. - 1 bib ref [Nr. Estr. 7963]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5



*Yersinia enterocolitica* are zoonotic bacteria able to infect humans and animals, recognized as the third cause of foodborne disease in Europe (EU) in terms of prevalence (1). Important studies highlighted the molecular basis of pathogenesis of *Y. enterocolitica* infection, while scanty data are available about other environmental 1A biotypes, often isolated in cases of foodborne disease but not included in pathogenicity studies. Owing to the above, the aim of our work was to verify the modulation of intestinal innate immunity by different *Y. enterocolitica* strains. In our study, overnight cultures of 5 different *Y. enterocolitica* strains: 1B (O:8, ail+, ystA+, inv+, myfA+, ymoA+); 1A (O:9, ystB+, inv+, ymoA+); 1A (O:5, ail+, ystB+, inv+, myfA+, ymoA+); 1A (O:8, ystB+, inv+, myfA+, ymoA+); 1A (O:5, ystA+, ystB+, inv+, myfA+, ymoA+) isolated from wild boar livers were sub-cultured for 1 h at 37°C in BHI medium. Each bacterial strain was re-suspended at 100,000,000 CFU/ml in DMEM/F12 medium (2) and used to infect pig intestinal IPEC-J2 cells; untreated cells were employed as negative control. Innate immune responses were evaluated by real time PCR as previously described (2,3). Differences between data sets were checked for significant differences by ANOVA, and the significance threshold was set at  $P < 0.05$ . Our results showed different abilities to modulate gene expression by the strains under study with respect to controls. In particular, *Y. enterocolitica* 1B determined a pro-inflammatory effect characterized by up-regulation of IL-8 ( $P < 0.0001$ ) and TNF- $\alpha$  ( $P = 0.0024$ ), and decrease of antimicrobial peptide gene expression: bD3 ( $P = 0.049$ ), bD4 ( $P = 0.049$ ). At the same time we observed down-regulation of CD14 ( $P = 0.011$ ), MD2 ( $P = 0.004$ ), TLR1 ( $P = 0.0415$ ), TLR4 ( $P = 0.0038$ ) and TLR5 ( $P = 0.0360$ ). *Y. enterocolitica* 1A strain 1 caused a pro-inflammatory response with increased expression of IL-8 ( $P = 0.0002$ ), TNF- $\alpha$  ( $P = 0.0045$ ), bD1 ( $P = 0.049$ ), bD2 ( $P < 0.0001$ ) and down-regulation of NF-K $\beta$ 1 ( $P = 0.0130$ ), bD4 ( $P = 0.0164$ ), MD2 ( $P = 0.0397$ ), and TLR4 ( $P = 0.0242$ ). *Y. enterocolitica* 1A strain 2 caused pro-inflammatory response with increased expression of IL-1 $\beta$  ( $P = 0.0168$ ), IL-8 ( $P < 0.0001$ ), TNF- $\alpha$  ( $P = 0.0076$ ), bD1 ( $P = 0.0010$ ), bD3 ( $P = 0.0086$ ) and down regulation of NF-K $\beta$ 1 ( $P = 0.0392$ ), MYD88 ( $P = 0.0425$ ), MD2 ( $P = 0.0429$ ), TLR1 ( $P = 0.0256$ ) and TLR4 ( $P = 0.0212$ ). *Y. enterocolitica* 1A strain 3 determined a pro-inflammatory effect characterized by up-regulation of IL-1 $\beta$  ( $P = 0.0309$ ), IL-8 ( $P = 0.0493$ ), IL-18 ( $P = 0.0138$ ), and decrease of antimicrobial peptide gene expression: bD3 ( $P = 0.0010$ ), bD4 ( $P = 0.08t$ ). At the same time we observed down-regulation of CD14 ( $P = 0.011$ ), MD2 ( $P = 0.0132$ ), TLR1 ( $P = 0.0029$ ), TLR4 ( $P = 0.048$ ) and TLR5 ( $P = 0.0123$ ). *Y. enterocolitica* 1A strain 4 determined a pro-inflammatory effect characterized by up-regulation of IL-8 ( $P < 0.0001$ ), TNF- $\alpha$  ( $P = 0.0071$ ), and decrease of antimicrobial peptide gene expression: bD1 ( $P = 0.0078$ ), bD3 ( $P = 0.0163$ ). The adopted cell line had been shown to give valuable information about pathogenicity of bacteria (4). Our data suggest a potential pathogenic role of 2 out of 4 *Y. enterocolitica* 1A strains under study and different interactions with the host.

Passeri B, Martinelli° N, Alborali° LG, Cantoni AM, Di\_Lecce R, Bertani V, Corradi A  
**Immunohistochemical evaluation of TLR-4 response in the mediastinal adipose tissue of pigs infected naturally with *Mycoplasma hyopneumoniae***

J Comp Pathol. - Vol. 158 ( 2018). - p 138 [Nr. Estr. ( ultimo accesso 11/07/2019 )  
<https://doi.org/10.1016/j.jcpa.2017.10.136> 8201]

Joint European Congress of the European Society of Veterinary Pathology, European Society of Toxicologic Pathology and European College of Veterinary Pathologists : 3rd Meeting of the European Society of Veterinary Pathology : 35th Meeting of the European Society of Toxicologic

Introduction: *Mycoplasma hyopneumoniae* (MH) is the agent of enzootic pneumonia of pigs. Toll-like receptor-4 (TLR4) binds lipopolysaccharides (LPS) of gram-negative bacteria. In adipose tissue TLR4 appears to be involved in activation and support of inflammation. Materials and Methods: Mediastinal adipose tissue and lungs were sampled from 49 pigs at slaughter. Each lung lesion was evaluated by MH RT-PCR. Frozen sections (5  $\mu$ m) of lungs were evaluated histologically, with scores ranging from 0 to 4, for BALT, bronchial lumen, bronchiolar epithelial loss and subacute inflammation. Immunohistochemistry (IHC) was performed by the ABC technique using commercial anti-TLR4 antibodies. The average histological lung score ranged from 0.14 to 1.98. Thirty-two samples were RT-PCR positive and 17 were negative. Ten showed subacute inflammation and 22

showed MH lesions. Twenty samples, 14 MH positive and six MH negative, were evaluated for TLR4, using a semiquantitative classification for extent and intensity of labelling at four levels: absent, mild, medium or intense. Four fields were evaluated. Results: Three samples (two positive), with severe pulmonary inflammation, showed intense expression of TLR4 in the adipose tissue. Six samples (three positive), with mild/severe inflammation, showed medium TLR4 labelling. Eight adipose tissue samples, with mild alveolar—bronchial inflammation (six positive), showed TLR4 0 to I. Three adipose tissue samples were negative with a lung score 0. Conclusions: Semiquantitative analysis of the presence of TLR4 in mediastinal adipose tissue might confirm that more severe pulmonary inflammation shows a higher concentration of TLR4 than mild inflammation.

Pezzoni° G, Bregoli° A, Grazioli° S, Barbieri° I, Foglia° EA, Madani H. Omani A, Wadsworth J, Bachanek-Bankowska K, Knowles NJ, King DP, Brocchi° E

#### **FMD outbreaks due to an exotic virus serotype A lineage (A/Africa/G-IV) in Algeria in 2017**

OS18 "Global vaccine security" : EuFMD Open session : October 2018, Puglia, Italy : online version, book of abstracts / [s.l. : s.n., 2018]. - Day 3. - p 52-53 [Nr. Estr. 8014]

EuFMD Open session : Borgo Egnazia (Brindisi), Puglia, Italy : 29-31 October 2018)

In the spring of 2017, a new introduction of FMDV serotype A occurred in the Maghreb region (Algeria and Tunisia). These were the first reports of field outbreaks of FMD due to serotype A in these countries after >25 years. Here, we report the whole genome sequence of viruses recovered during the outbreaks in Algeria and phylogenetic analysis, based on the VP1 coding region, highlighting connections with countries in sub-Saharan Africa. Methods and Results A total of six samples originated from three different outbreaks in Algeria were analyzed, FMDV serotype A was detected by Ag-ELISA. Virus isolates from all three outbreaks were obtained after the first passage in LFBK-EIV6 cells, which showed a better performance for virus isolation compared to BHK-21 and IB-RS2 cells. The phylogenetic analysis of the VP1 coding sequences grouped them within the A/AFRICA/G-IV lineage, most closely related to sequences originating from Nigeria in 2015, sharing more than 98% nt identity; older FMDV sequences (2009-2013) from Nigeria had lower nt identities (86.3-94.2%). FMDV sequences from countries in West Africa (Cameroon, Togo, Mali) and North-East Africa (Egypt, Eritrea, Sudan) were found to be more distantly related with nt identities ranging between 84.2- 90.2% and 82.8-87.8%, respectively. One complete full genome sequence (8119 nt) and two near-complete sequences (7616 and 7627 nt) were obtained from the three isolates by Miseq Illumina platform. Sequences differed at only 27 nt sites, 19/24 located within the polyprotein-coding region were synonymous and five non-synonymous. Discussion This study provides evidence for the transmission of the A/AFRICA/G-IV lineage outside its endemic areas in West Africa into the Maghreb region. Together with FMD cases due to serotype 0 that have also been previously reported in the Maghreb, these serotype A outbreaks represent the second independent introduction of FMD into region since 2013. These unpredictable dynamic FMDV movements may lead to new viral lineages becoming endemic in the region, which will inevitably heighten the risk of FMD introduction to Europe.

Piredda I, Palmas B, Noworol M, Rebechesu L, Pedditzi A, Pintore P, Boniotti° MB, Ruiu A, Ponti MN

#### **Isolamento e caratterizzazione di Leptospira spp. da matrici ambientali in Sardegna**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 257-258. - 5 bib ref [Nr. Estr. 8062]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Leptospirosis is an important environmental disease and represents a major threat to human health causing not less than 1 million clinical infections every year. Twenty-four Leptospira spp. strains were isolated from water and soil samples from various areas of Sardinia. The isolates showed the typical motility and morphology of the genus Leptospira under dark field microscopy, developing in liquid EMJH medium and EMJH STAFF after ten days of incubation at 29°C. All isolates were negative by the Multilocus Sequence typing (MLST). Serological characterization and Molecular identification by rpoB gene sequencing identified all strains as nonpathogenic leptospires. Seven isolates showed a genetic profile identical to that of the reference strain Leptospira biexa serovar Patoc, and fourteen isolates revealed sequence similarities within the 97/98% range, closely related to Leptospira vanthielii/biflexa.*

Polloni A, Candotti° P, Ostanello F, Leotti G

**Indagine sulle alterazioni anatomo-patologiche delle cavita' nasali negli allevamenti suinicoli italiani** = Survey on gross pathology lesions of the nasal cavity in the Italian swine farms

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 193-200. - 15 bib ref [Nr. Estr. 7832]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

La rinite atrofica è una patologia multifattoriale diffusa nell'allevamento intensivo del suino. Scopo del lavoro è valutare, tramite un appropriato percorso diagnostico, la gravità delle lesioni dei turbinati e del setto nasale in modo da poterle classificare in: 1) anatomicamente normali; 2) di lieve entità.; 3) di media entità o 4) di grave entità, secondo uno schema di score (punteggio) predefinito. Per ogni suino identificato in ciascuna delle 20 aziende prese in esame è stato effettuato e fotografato il taglio trasversale del naso in corrispondenza del primo o del secondo premolare superiore. La fotografia è stata scattata utilizzando uno smartphone, strumento comunemente utilizzato dal professionista e in grado di trasmettere l'immagine a distanza. L'osservazione delle fotografie scattate ha permesso l'attribuzione di un valore di score da parte di un unico esperto, in modo da ridurre al minimo la variabilità del giudizio. Inoltre, per ogni azienda e per ogni suino, sono stati raccolti dei dati e osservazioni sui singoli gruppi di animali secondo una scheda anamnestica fornita ai veterinari. Il 14,7% dei nasi valutati non presentava alterazioni (score 0), il 52,9% presentava alterazioni di lieve entità. (score tra 1 e 7), il 23,5% presentava alterazioni di media entità (score tra 8 e 14) e l'8,8% alterazioni gravi (score >15). È stata osservata una differenza statisticamente significativa ( $p < 0,05$ ) tra la media dei punteggi dei turbinati superiori ed inferiori con questi ultimi che presentano un più alto valore medio degli score.

*Atrophic rhinitis is a multifactorial disease widespread through pig farms. The aim of this field study is to assess the injuries of the meatus and the nasal septum according to a score pattern. For every pig identified in each of the 20 farms was performed a cross cut of the snout at the level of the first or the second upper premolar and photographs were taken. The photograph was taken using a smartphone, a tool commonly used by the professional and able to send image. The photographs were scored by a single expert in order to minimize variability. In addition, for each farm and for each pig data and observations were collected according to the data sheet provided to the veterinarians. The 14.7% of the snout were pointed as 0 because they does not show alterations, the 52.9% has light alterations therefore with a score included between 1 and 7, 23.5% alterations of average entity therefore included between 8 and 14 points and 8.8% serious changes between 15 and 22 points. The statistical analysis shows that there is a statistically significant difference ( $p < 0.05$ ) between the mean of the upper and lower meatus scores with the latter having a greater degree of injury.*

Pozzi P, Arraf M, Boniotti° MB, Barbieri° I, Hadani Y, Etinger M, Alborali° GL

**First outbreak of Porcine Reproductive and Respiratory virus (PRRSV) in swine farms in Israel**

Isr J Vet Med. - Vol. 73 no 1 ( 2018). - p 15-22. - 15 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) [http://www.ijvm.org.il/sites/default/files/pozzi\\_2.pdf](http://www.ijvm.org.il/sites/default/files/pozzi_2.pdf) 7836]

This article summarizes the clinical findings and confirmation laboratory procedures occurring during

the first outbreak the American strain of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), in swine farms in Israel. So far, (2017), Israel has been considered free from PRRS. At least 7 pig farms were involved in the PRRSV outbreak, in the North Region of Israel, involving about 10,000 breeders. Main clinical signs were premature farrowings, increase of stillbirth and pre-weaning mortality. Abortion waves, typical of PRRSV outbreaks, were not observed. PRRSV involvement in the outbreak was confirmed through ELISA-antibody test on blood samples from sows and piglets and RT-PCR on blood and organs. Sequencing and genetic analysis confirmed the involvement of the American PRRSV strain. The outbreak was contained through vaccination of breeders before insemination and at mid-pregnancy, and of piglets before weaning. The source of introduction of PRRSV into Israel remained unknown.

Prati° P, Spalla M, Scarsi° GS, Mattioni\_Marchetti V, Caltagirone M, Mercato A, Novazzi F, Nucleo E, Migliavacca R, Pagani L, Fabbi° M

**Infezione fatale in cuccioli di bulldog causata da Escherichia coli ST58, O141, multi resistente agli antibiotici, CTX-M-15, CMY-2, DHA-1 E AAC(6')-ib-cr produttore, in un allevamento del Centro Italia**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 191-192. - 5 bib ref [Nr. Estr. 8057]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Companion animals are considered as emerging reservoirs of antibiotic-resistant bacteria. One of the most important mechanisms of antimicrobial resistance in Enterobacteriaceae is the enzymatic inactivation of penicillins and cephalosporins by means of plasmid-mediated Extended-Spectrum  $\beta$ -lactamases (ESBLs), such as the TEM-, SHV-, or CTX-M-group enzymes (2). Few epidemiological data on  $\beta$ -Lactamases ( $\beta$ Ls)-producing Escherichia coli in pet dogs are available in Italy. A total of five E. coli were collected from biopsy specimens of the same two-weeks old dog. Species identification, serotyping, antibiotic susceptibilities, resistance genes investigation, conjugation experiments and molecular characterization of plasmids were performed. All the dog specimens resulted E. coli O141 positive. The isolates were multi drug resistant. The molecular typing has identified the strains as ST58 E. coli blaCTX-M-15, blaCMY-2, blaDHA-1 and aac(6')-Ib-cr positive.*

Procopio° A, Paternoster G, Leo° S, Mammi L, Fustini M, Galletti° G, Santi° A, Formigoni A, Tamba° M, Arrigoni° N

**Evaluation of economic losses due to paratuberculosis in a bovine dairy herd in Northern Italy, 2012-2016**

The 15th International Symposium on Veterinary Epidemiology and Economics : 12-16 November 2018, Chiang Mai, Thailand : abstract book / [s.l. : s.n., 2018]. - p 414 [Nr. Estr. 8129]

International Symposium of International Society for Veterinary Epidemiology and Economics (ISVEE) (15th : Chiang Mai, Thailand : 12-16 November 2018)

Objective: Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of Johne's disease (JD), a chronic gastrointestinal inflammation affecting especially ruminant species worldwide. JD causes substantial economic losses in dairy sector. Moreover, due to the concern over the zoonotic potential of MAP, specific trading warranties have been required by some extra-UE countries. This work aimed at estimating the economic impact of JD in an infected dairy herd located in Northern Italy. Materials and methods: In an infected farm, between 2012 and 2016, we selected 120 cows older than 36 months using serological (every six months) and fecal culture (once a year) tests. We considered an animal which resulted positive to at least one test as MAP-positive, while animals tested at least twice and resulted always negative as MAP-negative. The study population included 45 MAP-positive and 75 MAP-negative. Data on animal characteristics and production were

obtained from different databases. Results: We estimated an average unrealized income of 3340.06 EUR per infected animal due to the lower mean lifetime milk production (-3207.16 EUR) and to the lower average slaughter weight (-132.90 EUR). The relative risk of death on the farm for MAP-positive animals was 8.33 (95% CI, 1.01-69.08) compared to their negative herdmates ( $p < 0.05$ ). We estimated an additional mean loss of 892.80 EUR per year, due to direct mortality-related costs in infected cows. Due to the shorter production life in infected cows, we estimated the need to purchase four additional replacement heifers per year, thus increasing the mean replacement cost of 7200 EUR per year. Conclusions: This is the first study of JD economic impact in Southern Europe. Our results indicate that MAP infection causes considerable economic losses to dairy producers and suggest the need to carry out farm control programs.

Prosperi° A, Fabbi° M, Prati° P, Brollo V, Casadei° G, Scarsi° GS, D'Incau° M, Barbieri° I, Cambieri P, Pongolini° S

#### **Paracoccus yeei in feline acute cystitis : a case report**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 193. - 6 bib ref [Nr. Estr. 8058]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Paracoccus yeei* was firstly identified in 2003 in the US as an unusual human pathogen in immunocompromised patients (1), and subsequently detected in other countries (2-5). A urine sample belonging to an eight-year-old neutered cat with cystitis, was conferred for bacteriological analysis to the IZSLER Diagnostic Laboratory. After 48 h the growth of mucoid whitish-greyish colonies was detected on sheep blood agar. The Gram staining revealed O-shaped Gram negative bacteria, which were catalase and oxidase positive. The API 20 NE (BioMérieux) was not conclusive, but the MALDI-TOF Bruker Biotyper® (Bruker Daltonics), VITEK® (BioMérieux) and sequencing of a 16S rRNA fragment identified the bacterium as *Paracoccus yeei*. To the authors' knowledge this is the first report of *P. yeei* in a pet, additionally linked to a pathological condition. Further investigations are certainly needed to clarify the pathological role of *P. yeei* in mammals and its relevance to public health.

Purpari G, Giudice E, Antoci F, Di\_Pietro S, Barreca S, Macaluso G, Di\_Bella S, Mira F, Gucciardi F, Cannella V, Dimarco P, Lavazza° A, Crino C, Guercio A

#### **Outbreak of "Winter Dysentery" (Bovine Coronavirus, BCoV) infection in dairy cows housed in a farm in Sicily**

Large Anim Rev. - Vol. 24 no 2 ( 2018). - p 59-62. - 20 bib ref [Nr. Estr. ( ultimo accesso 31-12-2018 ) <https://www.vetjournal.it/riviste/item/26574-purpari-g-et-al.html> 7881]

Introduction - Bovine Coronavirus (BCoV) is an important pathogen of livestock that causes an enteric and respiratory disease in cattle (Winter Dysentery) and calves (Neonatal Calf Diarrhea). The BCoV infection rarely causes death but shows high morbidity (up to 100%) that induces economic losses in farms. Aim - This paper describes a case of BCoV infection occurred on December 2016 in a high milk production farm, placed in Ragusa (Sicily, Italy). Materials and methods - All bovines farmed (nr. 150 subjects) showed hyperthermia (41°C), watery dysentery mixed to blood and fibrin, buccal and gingival erosions, high reduction of milk production. Four days before the appearance of these symptoms, the involved animals had been vaccinated with a delete vaccine against Bovine Viral Diarrhea Virus (BVDV). Whole blood, sera, feces and nasal swabs were collected from 15 animals that showed more severe symptoms. Serological (ELISA) and Virological tests (RT-PCR, Real Time RT-PCR, ELISA) were carried out to detect BCoV, Rotavirus, BVDV, Bluetongue Virus (BTV), Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis Virus (IBR/IPV), Herpes Bovine Virus type 4 (BHV4), Bovine Respiratory Syncytial Virus (BRSV) and Schmallenberg Virus (SBV). Feces were also analyzed for BCoV detection through

Immunoelectron Microscopy (IEM). Results and discussion - Blood and swabs samples gave negative results for all virological tests. All feces samples resulted positive for BCoV by Semi Nested RT-PCR but they resulted negative by ELISA. Viral particles, referable to BCoV, were also observed at IEM. Serological tests gave positive results for Rotavirus, BVDV, IBR/IPV, VRSB, BHV4. In this study, BCoV was the only etiological agent detected in feces collected from cows affected by severe clinical signs. RT-PCR and IEM were found to be two reliable methods for the diagnosis of BCoV, showing a greater sensitivity than ELISA. Serological positivity detected for IBR/IPV, BTV, BVDV, BHV4 and VRSB were connected to previous vaccinations, while, the presence of antibodies against Rotavirus showed previous circulation of this virus in farm. Conclusions - BCoV infection, usually referred as "Winter Dysentery" (WD) is also reported as "Conditioned Disease" because it is induced by various stressors. Vaccination against BVDV could have been one of the putative stressor, responsible for the disease. Although BCoV infection has had a benign evolution, without mortality in affected animals, it has caused significant economic losses. Therefore, a control of stressors and a correct management of animal welfare represent the only valid prevention tool.

Rasmussen TB, Boniotti<sup>o</sup> MB, Papetti<sup>o</sup> A, Grasland B, Frossard JP, Dastjerdi A, Hulst M, Hanke D, Pohlmann A, Blome S, Van\_der\_Poel WHM, Steinbach F, Blanchard Y, Lavazza<sup>o</sup> A, Bøtner A, Belsham GJ

#### **Full-length genome sequences of porcine epidemic diarrhoea virus strain CV777; use of NGS to analyse genomic and sub-genomic RNAs**

PLoS One. - Vol. 13 no 3 ( 2018). - p e0193682 (17 p). - 40 bib ref [Nr. Estr. ( ultimo accesso 08/03/2018 ) <https://doi.org/10.1371/journal.pone.0193682> 7813]

Porcine epidemic diarrhoea virus, strain CV777, was initially characterized in 1978 as the causative agent of a disease first identified in the UK in 1971. This coronavirus has been widely distributed among laboratories and has been passaged both within pigs and in cell culture. To determine the variability between different stocks of the PEDV strain CV777, sequencing of the full-length genome (ca. 28kb) has been performed in 6 different laboratories, using different protocols. Not surprisingly, each of the different full genome sequences were distinct from each other and from the reference sequence (Accession number AF353511) but they are >99% identical. Unique and shared differences between sequences were identified. The coding region for the surface-exposed spike protein showed the highest proportion of variability including both point mutations and small deletions. The predicted expression of the ORF3 gene product was more dramatically affected in three different variants of this virus through either loss of the initiation codon or gain of a premature termination codon. The genome of one isolate had a substantially rearranged 5'-terminal sequence. This rearrangement was validated through the analysis of sub-genomic mRNAs from infected cells. It is clearly important to know the features of the specific sample of CV777 being used for experimental studies.

Razzuoli E, Lazzara F, Cosma V, Ratto A, Vivaldi B, Zoppi S, Gorla M, Vencia W, Ferrari A, Amadori<sup>o</sup> M

#### **Porcine Circovirus-associated disease in wild boars following cadmium poisoning**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 43 (Poster 02.01) [Nr. Estr. 7954]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

Background. PCV2, in combination with other infectious and non-infectious stressors, has been implicated as the necessary cause of Porcine Multisystemic Wasting Syndrome (PMWS), part of the

Porcine Circovirus-associated disease (PCVAD). In this work we report 3 cases of PMWS in wild boars. Material and methods. In November 2015 three female wild boars were found moribund. Necropsy was performed, and serum and tissue samples from lung, stomach, spleen, liver, kidney and lymph nodes (mesenteric, superficial inguinal) were collected. Over 70 analyses were conducted on each animal: microbiological, toxicological, chemical, parasitological, virological, serological and histological. All samples were stored at -80 °C except for the portions fixed in 10% formalin for histopathology assays. Results. Animals showed poor body conditions with evident cachexia, smaller body size with respect to the estimated age, rarefaction of mantle with large alopecic areas, and sensory obtundation. Histologic examination showed various degrees of lymphocyte depletion and macrophage infiltration in lymphoid tissues. PMWS was recognized on the basis of characteristic lesions (lymphadenonegaly, lymphocyte depletion with or without histiocytic infiltration), associated with detection of PCV2 in tissues by PCR for viral DNA. All animals shared high levels of cadmium (Cd) with mean values of  $1.3 \pm 0.5$  mg/kg in liver and  $15.9 \pm 3.2$  mg/kg in kidney. Conclusions. Cd poisoning can be associated with PCVAD occurrence in wild boars. The induction of oxidative stress by Cd and the related amplification of the inflammatory response probably underlie the synergism between chronic exposure to Cd and PCV2 infection for PMWS occurrence.

Razzuoli E, Mignone G, Lazzara F, Vencia W, Ferraris M, Masiello L, Vivaldi B, Ferrari A, Bozzetta E, Amadori° M

#### **Impact of cadmium exposure on swine enterocytes**

Toxicol Lett. - Vol. 287 ( 2018). - p 92-99. - 49 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 )  
<https://doi.org/10.1016/j.toxlet.2018.02.005> 7805]

We tested cadmium (Cd<sup>2+</sup>) effects on porcine IPEC-J2 cells, which represent an in vitro model of the interaction between intestinal cells and both infectious and non-infectious stressors. Accordingly, we investigated the effects of low (2 µM) to moderate (20 µM) concentrations of Cd<sup>2+</sup>, in terms of pro-inflammatory gene expression and protein release, as well as of infectivity in a Salmonella typhimurium penetration model. Our data showed a significant ( $P < .001$ ) increase of intracellular Cd<sup>2+</sup> after 3, 6 and 24 h of exposure with respect to levels at 1 h. These data showed the ability of IPEC-J2 to absorb Cd<sup>2+</sup> as a function of both time and concentration. Also, the absorption of this heavy metal was related to a significant modulation of important pro-inflammatory messengers. In particular, down-regulation of IL-8 was associated with a significant decrease of Salmonella typhimurium ability to penetrate into IPEC-J2 cells, in agreement with a previous study in which an anti-IL 8 antibody could significantly inhibit Salmonella penetration into the same cells (Razzuoli et al., 2017). This finding demonstrates the ability of Cd<sup>2+</sup> to affect the outcome of an important host-pathogen relationship. In conclusion, our study highlighted the ability of an environmental pollutant like Cd<sup>2+</sup> to modulate innate immune responses in terms of chemokine release and gene expression, and susceptibility to microbial infections..

Razzuoli E, Mignone G, Vencia W, Capellini MF, Parisi E, Amadori° M, Ferrari A

#### **Cadmium exposure causes modulation of innate immunity in kidney epithelial cells**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 30 (Poster 01.02) [Nr. Estr. 7952]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

Background. Cadmium (Cd) induces a stress response. In previous studies, Cd<sup>2+</sup> was shown to modulate the activity of cellular enzymes, to initiate oxidative stress, to suppress mitochondrial functions and disrupt calcium homeostasis. Chronic exposure to low concentrations can evolve to

the development of a variety of pathologies including tumors and renal dysfunctions. No data are available on innate immune responses in kidneys after Cd<sup>2+</sup> exposure. Material and methods. Madin-Darby Canine Kidney (MDCK) cells were treated with 20 pM Cd<sup>2+</sup> in DMEM culture medium. After 3 and 24 h of treatment at 37°C, mRNA was extracted to investigate the expression of innate immunity genes: IL-8, IL-6, IL-113, TLR1, TLR3, TLR5, TLR9, iNOS, CD14, MYD88, NF-kappaB p65, TLR4, MD2, IL-18. Experiments were performed in quintuplicate; cells treated with medium only were used as negative control. Each test was repeated twice. Results. After 3 h of Cd<sup>2+</sup> treatment we observed up-regulation (P< 0.05) of important pro-inflammatory cytokine genes (IL-8, IL-6, IL-1[3], iNOS, TLRs (TLR1, 9, 3, 5) and down-regulation of CD14. After 24 h we observed up-regulation of MYD88, NF-kappaB p65, IL-18 and down-regulation of iNOS, IL-13, MD2 and TLRs (5, 9). Conclusions. Our results show the ability of Cd<sup>2+</sup> to modulate innate immune responses in terms of gene expression as a function of time, and suggest a possible alteration of host-pathogen interactions. This is in agreement with the results obtained in previous studies [1;2]. References. Razzuoli et al., 2018. Toxicol Lett. 287:92-99. 2 Checconi et al., 2013. Int J Mol Sci. 19:4148-62.

Ricchi° M, Galletti° G, Russo° S, Gamberale F, DeCarlo E, Arrigoni° N

**Evaluation of serological tests for the detection of paratuberculosis in Italian buffalos (Bubalus bubalis) : a class latent approach**

14th International colloquium on paratuberculosis : 4-8 June 2018, Riviera Maya, Mexico : program & abstracts / [s.l. : s.n., 2018]. - p 51 (P-1.5) [Nr. Estr. 7901]

International colloquium on paratuberculosis (14th : Riviera Maya, Mexico : 4-8 June 2018)

The ELISA test is the most used assay for paratuberculosis control and it is utilised in the Paratuberculosis Italian Guidelines for assigning the health ranking to cow and buffalo herds. Currently, available commercial ELISA kits for the diagnosis of paratuberculosis in buffaloes are not supported by robust validation data. The gold standard recommended by the OIE Terrestrial Manual for the in vivo diagnosis of paratuberculosis is the cultural assay. However, the sensitivity of these tests is low, not suitable to be used as standard in indirect test validation. Some researchers have proposed PCR tests as gold-standard because of its sensitivity and rapidity. Aim of this work was the validation of commercial ELISA tests aimed at detecting paratuberculosis infected buffaloes. The project was designed to collect data from herds located in various areas with different prevalences. So far, the sampling was carried out in Frosinone and Rome provinces from two different herds, one with a low and another with a higher prevalence. The blood and faeces of 449 buffaloes were analysed in parallel by Id-Vet ELISA test and 15900-qPCR. In order to evaluate the accuracy/performance of both tests, these preliminary data were analysed by a Bayesian two latent class model, combining different cut off for both tests. The model included strong prior only for performance of qPCR (Se 50%, 90% sure is between 40% and 60%; Sp 70%, 95% sure is higher than 50%). Results showed that at each combination of cut off considered (ELISA 0.6 vs PCR 38, 36, 34 Cq; ELISA 0.7 vs PCR 38 Cq) Sp of both tests was very high, with Posterior Median always higher than 95% and narrow high density intervals (HDI). Conversely, the model failed in evaluating Se: for ELISA, the HDI are too wide, for PCR, the strong prior was not modified and, finally, the prevalences estimated by the model are similar, in contrast with what requested by the model. We believe further samplings will improve the model..

Ricchi° M, Galletti° G, Russo° S, Gamberale F, Martucciello A, Pietrella G, De\_Carlo E, Tamba° M, Arrigoni° N

**Valutazione delle performance di kit sierologici per la diagnosi di paratubercolosi nel bufalo (Bubalus bubalis) : un approccio a classi latenti : dati preliminari**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 194-195. - 6 bib ref [Nr. Estr. 8059]



Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*The ELISA methods are the most used assays for paratuberculosis control and they are the tests required by the Paratuberculosis Italian Guidelines for assigning the health ranking to cow and buffalo herds. Currently, available commercial ELISA kits in buffaloes are not supported by robust validation data. Aim of this work was the validation of these tests. Blood and faeces of 554 buffaloes were analysed in parallel by Id-Vet ELISA test and IS900-qPCR combining different cut off for both tests. These preliminary data were analysed by a Bayesian two latent class model. The samples were from five herds from two different areas, one with a low and another with a higher apparent prevalence. Specificity of both tests was very high, while the model failed to evaluate Sensitivity: for ELISA, the high-density intervals are too wide, for PCR, the strong prior was not modified and, finally, the prevalences estimated by the model are similar. Further samplings are needed to improve the model.*

Ricchi° M, Russo° S, Scaltriti° E, Leo° S, Bolzoni° L, Arrigoni° N

**On the use of whole genome sequencing to unveil local spread of a paratuberculosis clone within a single herd**

14th International colloquium on paratuberculosis : 4-8 June 2018, Riviera Maya, Mexico : program & abstracts / [s.l. : s.n., 2018]. - p 131 (O-3.1) [Nr. Estr. 7903]

International colloquium on paratuberculosis (14th : Riviera Maya, Mexico : 4-8 June 2018)

*Mycobacterium avium subsp. paratuberculosis (MAP) is characterized by a low genomic rate of mutation. Current subtyping tools, such as Mini-Micro-satellite analyses, have not sufficient discriminatory power to follow MAP's evolution on a small spatial and temporal scale. Aim of the study was to investigate the evolution and spread of MAP within a single dairy herd through whole genome sequencing (WGS). For this purpose, the DNAs of 48 field isolates (with identical satellite subtype), collected in the 2012-2016 period from faeces of cows of the same herd, were sequenced by Miseq system (Illumina). The herd rarely bought new animals and in one case, it was possible to identify an isolate collected from a cow born outside the herd (bread Ottonese). The comparison of the genomes found no indels and 44 polymorphic sites, seven of which were shared between two or more isolates. Bayesian evolutionary analysis showed a mutation rate compatible with that previously reported. The MAP population evolves according to a strict clock model, confirming the clonal origin of the infection. Moreover, according to herd prevalence data, the coalescent model assuming constant population provided a better fit of the clone evolution than that assuming a growing population. Four separate MAP sub-lineages were identified within the herd, suggesting a sufficient phylogenetic signal to investigate transmission patterns. The observed patterns are consistent with the life history data since the ancestral sub-lineage displays larger genetic variability and it was found in the older cows. The other lineages emerged concurrently in more recent years indicating heterogeneity in within-herd transmission. The isolate collected from the Ottonese cow clustered with the others suggesting the infection occurred after the introduction in the herd. These findings show that WGS, coupled with appropriate epidemiological information, represents a valuable tool to provide new insight on MAP's spread and evolution at herd level.*

Rigamonti° S, Campanini G, Paolucci S, Prati° P, Sarasini A, Vicari° N, Fratini A, Peli° S, Isernia P, Pezzoni° G, Antonini G, Carughi P, Baldanti F, Marone P, Fabbio° M

**Circolazione del virus dell'epatite E (HEV) nel Parco Lombardo della Valle del Ticino : un approccio "one health"**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 261. - 4 bib ref [Nr. Estr. 8063]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*In industrialised countries, HEV-3 and HEV-4 are two genotypes of the hepatitis E virus (HEV) which infect both humans and animals, with pigs and wild boars representing the main reservoirs. The consumption of undercooked pork or wild boar meat and sausages is the most common route of transmission to humans. Some categories of workers exposed to contact with meat or faeces of infected animals have a high risk to become infected. The objective of our study was to investigate the prevalence of HEV in wild boars and humans in a wide area of the Parco Lombardo della Valle del Ticino, a regional park in Lombardy, Italy. Altogether, 3008 sera and 1156 livers of wild boars and 40 sera from park rangers were examined. The prevalence of anti-HEV IgG antibodies was 8.7% in wild boars and 10% in park rangers, while 3.7% of samples were PCR-positive for HEV, all belonging to genotype 3.*

Rigamonti° S, Floriano AM, Longbottom D, Scaltriti° E, Comandatore F, Casadei° G, Capucci° L, Donati M, Fabbi° M, Vicari° N, Magnino° S

**Analisi genomica delle PMPS e della plasticity zone di due isolati di Chlamydia pecorum da camoscio e bufalo**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 196-197. - 7 bib ref [Nr. Estr. 8060]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Few whole genome sequences of chlamydial strains and isolates of veterinary relevance have been published so far worldwide. We sequenced the whole genome of two Chlamydia pecorum field isolates grown and stored at the Italian National Reference Laboratory for Chlamydioses. Isolate PV7855 was recovered from a chamois affected with pneumonia and isolate PV6959 was recovered from the brain of a water buffalo calf with encephalomyelitis. The two isolates have few differences in two polymorphic regions of chlamydiae, the gene cluster of polymorphic membrane proteins (pmgs) and the plasticity zone (PZ).*

Rigamonti° S, Floriano AM, Longbottom D, Scaltriti° E, Comandatore F, Casadei° G, Capucci° L, Donati M, Vicari° N, Magnino° S

**Genomic analysis of pmgs and plasticity zone of two C. pecorum isolates from a chamois and a water buffalo**

5th European Meeting on Animal Chlamydioses and Zoonotic Implications (EMAC-5) : October 3-5, 2018, Odesa, Ukraine : final program and book of abstracts / [s.l. : s.n., 2018]. - p 46-47 [Nr. Estr. 8030]

European meeting on animal chlamydioses and zoonotic implications (5th : Odesa, Ukraine : October 3-5, 2018)

Two C. pecorum isolates stored at the Italian NRL for Animal Chlamydioses, PV7855 from the lung of a chamois affected with pneumonia and PV6959 from the brain of a water buffalo calf with encephalomyelitis, were grown respectively in LLC-MK2 and McCoy cells, their elementary bodies were purified according to the sucrose gradient separation protocol and their whole genome sequences were obtained with MiSeq System (Illumina). Analyses focused on two polymorphic regions of chlamydiae, the gene cluster of polymorphic membrane proteins (pmgs) and the plasticity zone (PZ). We compared the two isolates against the reference strains E58 and PV3056/3 and against each other with the Artemis Comparison Tool (ACT). The E58 strain was isolated from the brain of a calf with encephalomyelitis in the US and PV3056/3 was isolated from a dairy cow with endometritis in Italy. The genomes of PV3056/3, PV6959 and PV7855 strains were aligned to the reference genome E58 using Mauve. Then single nucleotide polymorphisms (SNPs) were called and annotated (at the nucleotide and amino acid level) using an in-house Pen i script. Comparisons of

coding sequences between genomes were made using the alignment tool ClustalW. PV7855 showed a much higher homology with E58 (192 SNPs in total, of which 125 are non-synonymous substitutions) than with PV3056/3 (15,198 SNPs in total, of which 6,639 are non-synonymous). In particular, the amino acid alignment of all pmps showed a homology between 99 and 100% when compared to E58, and a homology between 85.49 and 96% when compared to PV3056/3. As to the PZ of PV7855, we focused our analyses on the phospholipase genes (pld1, pld2, pld3 and pld4) and the toxin B genes (1 and 2). Also in this case PV7855 is more similar to E58 than to PV3056/3: indeed pld1, pld2, pld3 and pld4 show a homology of 100% in the amino acid sequences, plc/.1 being the most divergent compared to PV3056/3, with a homology of 56.81%. Also the toxin B genes of PV7855 are more similar to the ones in E58 (99% both) than in PV3056/3 (87 and 86%, respectively). The comparison between PV6959 and E58 showed a difference of 23,943 SNPs with 8,631 non-synonymous substitutions, while the comparison with PV3056/3 resulted in 25,799 SNPs with 9,425 non-synonymous SNPs. There are no significant differences in pmps region, the homology being between 78 and 98% when PV6959 is compared to E58 and between 77 and 98% when compared to PV3056/3. As to the PZ, the amino acid sequence of pld1 has a high degree of similarity (95.93%) with the one in E58, while pld2 and pld3 have a much lower similarity (54.45 and 59.15%). The two toxin B genes are highly homologous (between 83 and 94%) to both reference strains. In the PZ of PV6959 we also detected a hypothetical protein which seems to be a unique gene. The comparison between our two isolates showed that they differ by 23,985 SNPs, of which 8,664 are non-synonymous substitutions. Overall, the region of pmps is highly homologous and pmp6 is the least similar pmp with 78.22% similarity in the amino acid sequence. The analysis of PZ is under way. These preliminary results show that the PV7855 and PV6959 isolates have few differences in some highly variable regions of their genomes. In addition, the genomes of PV6959 and E58 (which were both isolated from animals affected with encephalomyelitis) are slightly different both as a whole and in the variable regions studied. These data suggest that the genomic regions considered in this study may not be related to tissue tropism. The investigation of further regions is ongoing.

Riva F, Filipe J, Curone G, Vigo D, Addis MF, Castiglioni B, Cremonesi P, Moroni P, Bronzo V, Trevisi E, Amadori M

#### **Mastitis resistance in Holstein and autochthonous dairy cattle**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 40 (Poster 01.22) [Nr. Estr. 7953]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

**Background.** Genetic selection in Holstein cattle has been mainly focused on the milk yield criterion, leading to high propensity to develop critical physio-pathological conditions in the transition period [1]. On the contrary, autochthonous dairy breeds are characterized by higher disease resistance and resilience [2]. A multidisciplinary approach was applied to compare different physiological parameters in Holstein (H) and Rendena (REN) breeds, reared in the same farm under the same conditions. **Materials and methods.** Quarter milk samples and venous blood were collected from 6 H and 4 REN cows at: dry-off, 1, 7-10 and 30 days after calving. Blood samples were used for the analysis of plasma metabolites. Quarter milk samples were submitted to bacteriological culture, cell counting, protein profiles and characterization of the microbiota, cathelicidin and innate immune mediators. **Results.** H cows showed more severe fat mobilization and systemic inflammatory response at T2 and T3 compared with REN cows. There were significant differences between the two milk microbiota. Immunoglobulins were present in consistently higher amounts in REN colostrum. H always showed higher levels of cathelicidin. The expression of innate immunity genes at T2 were also different. **Conclusions.** Our results suggest that H cows develop a systemic and local mammary inflammatory response underlying higher susceptibility to disease compared with REN cows. This is associated to critical differences in innate immunity in the colostrum phase..

Rizzo F, Braghin S, David M, Rigamonti° S, Vicari° N, Magnino° S, Mandola ML

**Chlamydia spp. detected in poultry in Piedmont, Italy**

World Poultry Sci J. - Vol. 74 Suppl ( 2018). - p 51 (Oral O7-03) [Nr. Estr. ( Ultimo accesso 05/03/2019 )

[http://www.wpsa.com/images/downloads/tables\\_of\\_contents/2018\\_MPS\\_BoA\\_online.pdf](http://www.wpsa.com/images/downloads/tables_of_contents/2018_MPS_BoA_online.pdf) 8131]

Mediterranean Poultry Summit (6th : Turin, Italy : June 18-20, 2018)

Chlamydia spp. are agents of re-emerging infections in poultry worldwide and deserve attention from a public health perspective due to the well-known zoonotic nature of *C. psittaci*, the agent of psittacosis-ornithosis. In 2014, the family Chlamydiaceae has been expanded with two new species, *C. avium* and *C. gallinacea*, which have been found so far in pigeons, parrots and poultry in Europe, China, Argentina and North America. Zoonotic potential of these new chlamydial species is under investigation. We undertook a study to investigate the prevalence of *C. psittaci* and other Chlamydia spp. in the northwestern Italian region of Piedmont, an area highly devoted to the poultry industry. In the context of the 2015 National monitoring plan for Avian Influenza, pharyngeal and cloacal swabs from poultry farms rearing ducks, chickens, turkeys and geese were collected and analyzed with a PCR screening test for the Chlamydiaceae family. Upon an automated nucleic acid extraction with magnetic beads, a real-time PCR targeting the 23S ribosomal gene was performed. Subsequently, all positive samples were characterized by four different real-time PCR assays specific for the species under investigation: *C. psittaci*, *C. abortus*, *C. avium* and *C. gallinacea*. The raw prevalence of Chlamydia spp. infections was found to be 23% with 20 out of 86 farms testing positive. The highest prevalences of infection with Chlamydia spp. were found in geese (29.4%; 5/17) and chicken farms (32.3%; 11/34). A lower prevalence was detected in duck farms (15.4%; 4/26) and interestingly, all turkey farms tested negative at the screening. *C. gallinacea* was the most commonly detected chlamydial species, with the highest prevalences in chicken (26.5%; 9/34) and duck farms (11.6%; 3/26), followed by *C. psittaci* in geese (23.5%; 4/17). Neither *C. avium* nor *C. abortus* were detected in any farm. Based on the results of our study, this year we started a Research Project on Chlamydia spp. in poultry, supported by the Italian Ministry of Health, to further investigate chlamydial prevalences and associated professional health risks in our region.

Roccaro M, Piva S, Scagliarini A, Giacometti F, Serraino A, Meriardi° G, Frasnelli° M, Romano A, Bellio A, Decastelli L, Peli A

**Case report of a pustular dermatitis outbreak in sheep : clinical and food safety considerations**

Ital J Food Safety. - Vol. 7 ( 2018). - no 6980 (p 24-27). - 22 bib ref [Nr. Estr. 7896]

The objective of this report is to describe an outbreak of pustular dermatitis in a flock of about 200 sheep, its clinical evolution and food safety implications. The onset of the symptoms was sudden and the lesions spread very quickly from ewe to ewe, so that in about 3 days almost all of the lactating sheep were stricken. Pustules from 5 different animals, six milk samples, two cheese samples, teat cup samples from the milking machine and farmer's hands were analysed. A pure culture of *Staphylococcus aureus*, producing staphylococcal enterotoxin (SE) C, was isolated from pustules. Milk and cheese showed a contamination by coagulase positive staphylococci <15 and 30 colony forming units respectively and the absence of SE. Farmer's hands and teat cups samples resulted negative for coagulase positive staphylococci. Therapy with daily topical medicaments was prescribed and a prophylactic intervention was suggested by the administration of an auto-vaccine. The low level of milk and cheese contamination and the absence of SE in cheese supported the decision to not advise the farmer to recall cheese produced with milk from affected animals.

Romano° C, Colella° EM, Stoppani° E, Lucchini F, Dotti°S, Villa° R

**SiRNA : new strategy for the influenza A virus control**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 273. - 3 bib ref [Nr. Estr. 7966]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

The viral recombination of influenza A virus is an impending problem, since animals act as a reservoir for the transmission of the pathology to humans. In particular, the animal species have a relevant role in the potential recombination between different influenza A virus strains. Hence, there is the need to create new molecules that can be an alternative to vaccination plans. RNA interference (RNAi) may play a key role because several studies highlight how short interfering RNAs (siRNAs) act as antiviral agents [1]. In this study, siRNA cellular clones [2] were obtained on MDCK cell line, permissive against influenza A virus. The genomic region coding for the viral nucleoprotein (NP), a well-conserved sequence among the different viral strains, was examined. The NP sequences were aligned and the strains containing siRNA target were selected. Three sequences (NP6, NP7, NP8) showed the greatest effectiveness. The siRNA clones were infected and the ability of siRNAs to inhibit the viral replication were evaluated on five avian (1067/1999 H7N1, 1082/1999 H7N1, 22A/1998 H5N9, 251/2003 H7N3, 8000/2002 H7N3) and five human (9/2014 H1N1, 79/2014 H1N1, 56/2014 H1N1, 53/2013 H1N1, 224/2015 H1N1) influenza A virus strains. To evaluate the viral titre of the different strains, the MDCK cell line was prepared in 96-well plates, infected with serial dilutions of the viruses (from 10<sup>-1</sup> to 10<sup>-7</sup>) and incubated at 37°C + 5% CO<sub>2</sub> for 5 days. The viral titre was determined on the basis of the cytopathic effect observed. At the end of the incubation period, the cultures were frozen at -80°C and thawed to obtain cell cryolase. The clones showed a different sensitivity to the viral strains analyzed. Inhibition results were confirmed by hemagglutination (HA) and Real Time PCR (RT-PCR) methods. In the experiments performed, the siRNA antiviral potency appears relevant but different for each strain examined. Moreover, preliminary data related to siRNA production in E. coli strains (data not shown), gives encouraging results to proceed for a large-scale production [3]. However, in vivo studies are necessary to confirm the antiviral effect of siRNAs as an alternative "ready to use" therapy.

Rosignoli° C, Merenda° M, Franzini° G, Faccini° S

**Sensibilità agli antimicrobici in ceppi di Streptococcus uberis isolati da casi di mastiti bovine cliniche e subcliniche dal 2007 al 2016**

Atti Soc Ital Buiatria. - Vol. 50 ( 2018). - p 155. - 1 bib ref [Nr. Estr. 7980]

Congresso Nazionale della Società Italiana di Buiatria (50. : Bologna : October 10th - 13th 2018)

Introduzione Streptococcus uberis è uno tra i più comuni patogeni responsabili di mastite nella bovina da latte (1). Per avere a disposizione dei dati oggettivi su cui basare la scelta terapeutica, il veterinario buiatra, in caso di isolamento di Strep. uberis da campioni di secreto mammario, richiede spesso al laboratorio il test di sensibilità agli antimicrobici. Lo scopo del presente studio retrospettivo è stato quello di elaborare i dati relativi alla sensibilità agli antimicrobici di ceppi di Strep. uberis isolati da secreti mammari di bovine con mastiti cliniche o subcliniche e di valutarne il trend in un periodo di 10 anni. Metodi Sono stati analizzati i dati relativi a 5.335 ceppi di Strep. uberis isolati da campioni di secreto mammario conferiti presso la Sezione di Mantova dell'IZSLER nel periodo 2007-2016. La sensibilità agli antimicrobici in vitro è stata valutata mediante il test di diffusione in agar secondo Kirby-Bauer in accordo con gli standard del CLSI. Gli isolati di Strep. uberis sono stati classificati come sensibili, intermedi o resistenti rispetto a 9 antimicrobici: penicillina G, amoxicillina, amoxicillina/acido clavulanico, cefazolina, cefoperazone, cefquinome, tetraciclina, eritromicina e trimethoprim/sulfametossazolo. Il tasso di sensibilità agli antimicrobici di Strep. uberis è stato determinato, per ogni anno e per ogni molecola o associazione antimicrobica, mediante il calcolo del

rapporto tra il numero dei ceppi risultati sensibili e il totale dei ceppi esaminati. L'andamento del tasso di sensibilità di *Strep. uberis* nel periodo in esame è stato valutato mediante il test del  $\chi^2$  per il trend. La soglia di significatività è stata definita con  $P = 0,05$ . Risultati Dall'analisi complessiva dei dati, la proporzione dei ceppi sensibili è risultata pari al 94,2% per amoxicillina/acido clavulanico, 91,9% per cefquinome, 89,3% per cefazolina, 85,5% per cefoperazone, 70,0% per penicillina G, 68,4% per amoxicillina, 67,3% per trimethoprim/sulfametossazolo, 44,1% per eritromicina e 22,4% per tetraciclina. Il trend del tasso di sensibilità, nei 10 anni presi in esame, si è dimostrato in crescita per 6 antimicrobici su 9 esaminati. Solo nei confronti della penicillina G e dell'amoxicillina la proporzione di ceppi sensibili ha dimostrato un trend in decrescita mentre per la tetraciclina si è mantenuto stabile. Conclusioni I risultati del presente studio evidenziano un buon tasso di sensibilità in vitro di *Strep. uberis* nei confronti delle cefalosporine e dell'amoxicillina associata con acido clavulanico. Inoltre, l'analisi del trend del tasso annuale di sensibilità depone per il fatto che *Strep. uberis*, nei 10 anni presi in esame, non abbia sviluppato specifici meccanismi di resistenza nei confronti di tali antimicrobici. E' auspicabile mantenere anche in futuro una continua sorveglianza dell'andamento della sensibilità di *Strep. uberis* nei confronti delle molecole, o associazioni di molecole, maggiormente impiegate in terapia.

Rubini° S, Faggionato° E, Merialdi° G, Merenda° M, Massi° P, Frasnelli° M, Gelmini° L, Garbarino° CA, Luppi° A, Biancardi° A, Frisoni P, Talarico A, Gaudio RM, Cotti C, Musto C, Delogu M, Strano\_Rossi S, Locatelli CA, Mazzariol S

#### **Monitoring of wildlife poisoning in Emilia Romagna (Italy)**

2nd World Conference and Exhibition on Forensic Science : 11-12 June 2018 Rome / [s.l. : s.n., 2018]. - 1 p [Nr. Estr. 7892]

World Conference and Exhibition on Forensic Science (2nd : Rome : 11-12 June 2018)

Rubini° S, Locatelli CA, Strano\_Rossi S, Faggionato° E, Bertocchi S, Govoni G, Merialdi° G, Fontana° MC, Barbieri S, Talarico A, Frisoni P, Gaudio RM

#### **Non-accidental poisoning of domestic and wild animals in Ferrara Province, Italy**

Clin Toxicol. - Vol. 56 no 6 ( 2018). - p 476-477 [Nr. Estr. ( ultimo accesso 11/03/2019 )  
<https://doi.org/10.1080/15563650.2018.1457818> 7856]

International Congress of the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) (38th : Bucharest, Romania : 22-25 May 2018)

Objective: The purpose of this paper is to describe our experience of animal poisoning after the Italian Legislative Decree of 18 December 2008 prohibiting the use of poisoned baits. Methods: Since 2008, the Ferrara section of the Lombardy and Emilia Romagna Experimental Zootechnic Institute (IZSLER) had received 465 samples for testing for toxic or harmful substances. These were 256 suspected baits, 152 carcasses and gastric contents of pets, and 55 carcasses of wild animals. Baits had been subjected to an inspection and then, if necessary, directed to the chemical laboratory for toxicological analysis. A necropsy was carried out on the carcasses and, on the basis of the findings, organs were taken for chemical analysis. Results: In total, 166 samples (35.7%) were positive for the presence of toxic/harmful substances. The most frequent toxins were rodenticides (36.1%), followed by carbamate/organophosphate pesticides (16.9%), carbamates (11.4%), and chlorinated pesticides (9.0%). In 7.2% of the baits, foreign bodies (spikes or needles) were found and 3.0% of the positive samples contained strychnine. The carcasses or the gastric contents of pets were mainly dogs (53.3%) and cats (46.7°M. Between the pets, there were 31 (38.2%), poisoned dogs and 26 (36.6%) poisoned cats. The toxic agents responsible for these poisonings were anticoagulant rodenticides (31.4%) in the dogs, and anticoagulant rodenticides (19.10/0) and carbamates (19.1%) in the cats. Of the wild animals 41.8% tested positive for poison; pesticides were the most commonly encountered toxic substance, while in synanthropic animals, the most common toxins found were anticoagulants. Conclusion: Many toxic substances are easy to find in the market, but in five cases, strychnine was also detected and it has been banned for years. The

Decree requires the involvement of the Official Veterinary Service and of the official laboratory in suspected cases but this is often neglected, so the number of cases reported to the Judicial Authority is certainly lower than actual cases. Although a good diagnostic rate was achieved by the laboratories, the improvement margins remain wide and it is therefore very important to collaborate among the different organisations, in our case, the Forensic Toxicology Laboratory at the University of Ferrara, the Pavia Poison Control Centre, and the Institute of Legal Medicine, Catholic University of Sacred Heart, Rome, to increase the number and categories of toxic substances that can be identified.

Ruggeri° J, Chirullo B, Martinelli° N, Drumo R, Scaglione F, Preget P, Ammendola S, Battistoni A, Corradi A, Ossiprandi M, Bollo E, Pasquali P, Alborali° L

**Salmonella Typhimurium monophasic variant attenuated vaccine is effective in controlling faecal shedding and organ colonization of piglets experimentally challenged**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 66 (Poster 06.01) [Nr. Estr. 7958]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

Background: Salmonella Typhimurium and its monophasic variant (S. Typhimurium 1, 4, [5], 12:i-) are increasingly responsible of food borne infections in humans and pork represents the principal source of infection. The aim of the study was to test the efficacy and safety of an attenuated vaccine of S. Typhimurium 1, 4, [5], 12:i- (S. Typhimurium Monophasic variant AznuABC). Material and methods: Twenty weaned piglets were divided in 4 groups. Group A (5 piglets) and B (5 piglets) were vaccinated with an oral administration of S. Typhimurium Monophasic variant AznuABC at the final dose of 5 x 10<sup>7</sup> CFU. At day 35 after vaccination, all piglets were challenged by an oral gavage with 5 x 10<sup>8</sup> CFU of S. Typhimurium Monophasic variant (group A and D) or S. Typhimurium (group B and C). Results and discussion: Vaccine strains were not isolated in faeces after three weeks from vaccination and they did not affect growth of animals, proving a good level of safety. In addition, both attenuated vaccines reduced the colonization of both virulent strains in target organs, in comparison to unvaccinated groups.

Ruggeri° J, Ferlazzo° G, Boniotti° MB, Capucci° L, Amadori° M

**Role of IgA antibodies in pig oral fluids for the control of PRRS virus infection**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 50 (Poster 02.14) [Nr. Estr. 7956]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

Background: Porcine Reproductive and Respiratory Syndrome (PRRS) is a complex model of host/virus relationship. Disease control is often pursued by "acclimatization" of gilts, i.e. the exposure of PRRS-naïve gilts to PRRSV-infected pigs before the breeding period. In this respect, we had repeatedly observed an association between IgA responses in oral fluids (OF) and block of PRRSV spread through OF. Material and methods: We investigated the neutralization of PRRSV by OF samples with different, PRRSV-specific, IgA/IgG ratios using Real-time RT PCR. Results: PRRSV yield reduction in macrophages was dependent on the Ig isotypes in OF samples, the IgG-rich samples being sometimes associated with Antibody-dependent Enhancement (ADE) of replication. These effects varied as a function of the susceptibility to PRRSV replication of macrophage batches, and also of the PRRSV strains under study. We could discriminate between ADE-positive and ADE-negative PRRSV strains. Next, we separated IgG and IgA in OF samples of PRRSV-infected

pigs by means of protein A and size exclusion chromatography. The above results were confirmed by using separated Ig isotypes. In general, the combination of dimeric and monomeric IgA was associated with the strongest reduction of PRRSV replication. Also, the treatment of pig macrophages with separated OF antibodies before PRRSV infection was associated with PRRSV yield reduction, along with a down-regulation of both CD163 and CD169 surface expression. Conclusions: OF IgA could control PRRSV excretion by extra or intracellular interaction with PRRSV, as well as by induction of signals leading to a reduced susceptibility of macrophages to PRRSV infection.

Ruggeri° J, Ferlazzo° G, Boniotti° MB, Capucci° L, Amadori° M

### **Role of IGA antibodies in pig oral fluids for the control of PRRS virus infection**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 110. - 1 bib ref [Nr. Estr. 7961]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

The Porcine Reproductive and Respiratory Syndrome (PRRS) is a complex model of host/virus relationship, in which the role of adaptive immunity after infection is ill-defined [1]. More convincingly, clinical protection of pigs can be accounted for by (A) a reduced susceptibility of macrophages to PRRSV infection and replication; (B) an effective control over the inflammatory response caused by PRRSV and other environmental, infectious and non-infectious stressors. This kind of adaptation is likely to take place during the so-called "acclimatization" of gilts, i.e. the controlled exposure of PRRS-naïve gilts to PRRSV-infected pigs before the breeding period. In this respect, we had repeatedly observed on farm a clear correlation between a balanced IgA to IgG anti-PRRSV Ab ratio in oral fluids (OF) and block of environmental virus spread through OF samples of PRRSV-infected gilts. Vice versa, OF samples with peak IgG titers were correlated with peak PRRSV titers in OF, in agreement with major antibody-dependent enhancement (ADE) of virus replication (Amadori M., submitted). Owing to the above, we decided to investigate the neutralization of PRRSV by OF samples with widely different IgA/IgG Ab titers to PRRSV in terms of s/p ratios in ELISA. IgA and IgG Ab to PRRSV in OF were measured by ELISA (kit IDEXX P3) with anti-Ig isotype conjugates. After overnight antibiotic treatment, OF samples were tested for yield reduction of different PRRSV strains in swine pulmonary alveolar macrophages and monocyte-derived macrophages. Yield reduction of PRRSV was measured by Real-time RT-PCR. In agreement with our field studies, PRRSV yield reduction in pig macrophage cultures was shown to be critically dependent on the Ig antibody isotypes in OF samples, ADE being associated with IgG-rich samples. Most important, the extent of these effects varied as a function of the susceptibility to PRRSV replication of different macrophage batches, and also of the PRRSV strains under study. In particular, we could discriminate between ADE-positive and ADE-negative PRRSV strains. Next, we took to separating IgG and IgA in OF samples of PRRSV-infected pigs by means of protein A and size exclusion chromatography. The above results were confirmed by using separated Ig isotypes. In general, the combination of dimeric and monomeric IgA was correlated with the strongest reduction of PRRSV replication. Finally, we decided to check yield reduction in pig macrophages, pre-treated with separated Ig isotypes, washed, and then infected with different PRRSV strains. This treatment was also correlated with a substantial PRRSV yield reduction, which was Ab isotype and PRRSV strain-dependent, and went along with a down-regulation of CD163 and CD169 surface expression. On the whole, our data point at major role of IgA in the control of PRRSV excretion. This might be accomplished by extra or intracellular interaction of IgA Ab with PRRSV, as well as by signals leading to a reduced susceptibility of macrophages to PRRSV infection. Accordingly, major epigenetic regulations in pig macrophages exposed to PRRSV and/or OF antibodies should be investigated in the near future.



Ruggeri° J, Foresti F, Pavesi R, Terrini° A, Giudici° F, Padoan D, Corradi A, Ossiprandi MC, Pasquali P, Alborali° GL

**The synergistic effect of organic acids, phytochemicals and a permeabilizing complex reduces *Salmonella Typhimurium* 1,4,[5],12:i-shedding in pigs**

Vet Res Commun. - Vol. 42 ( 2018). - p 209-217, 219. - 27 bib ref [Nr. Estr. ( ultimo accesso 24/08/2018 ) <https://doi.org/10.1007/s11259-018-9723-3> 7921]

*Salmonella Typhimurium* (including *S. Typhimurium* 1,4,[5],12:i-) and other enteric pathogens cause acute infection in pigs during the weaning stage, often evolving into chronic infections responsible for the introduction of zoonotic bacteria into the slaughterhouse and thus determining carcass contamination. In addition to being zoonotic hazards, these pathogens are responsible for economic losses in affected farms. Traditionally, antibiotic treatments have been largely administered in order to reduce the infection burden but it favored, as a direct consequence, an increase in the number of multidrug resistance strains. In order to overcome antibiotic-resistance concerns, new alternative control strategies should be developed. In this context, a blend of organic acids, phytochemicals and a permeabilizing complex, administered in feed (Group A - 459 piglets) or water (Group B - 458 piglets), was tested in field conditions for its capability of reducing *Salmonella*-infection in weaned piglets of an endemic farm. Data recorded were compared to results of a control group (Group C - 456 piglets). Zootecnical parameters were recorded in all animals, while microbiological, serological and PCR analyses were conducted in 15 piglets for each group. Results demonstrated that additive administered in feed improved animal weight gain (better average daily gain [A.D.G.] and increment), and rapidly reduced *Salmonella*-shedding in feces. Administration of additive in feed gave better results than in water.

Rugna° G, Carra° E., Bergamini° F, Calzolari° M, Salvatore D, Corpus° F, Gennari W, Baldelli R, Fabbi° M, Natalini S, Vitale F, Varani S, Meriardi° G

**Multilocus microsatellite typing (MLMT) reveals host-related population structure in *Leishmania infantum* from northeastern Italy**

PLoS Neglect Trop Dis. - Vol. 12 no 7 ( 2018). - p e0006595 (18 p). - 47 bib ref [Nr. Estr. ( ultimo accesso 27/08/2018 ) <https://doi.org/10.1371/journal.pntd.0006595> 7918]

Background Visceral leishmaniasis (VL) caused by *Leishmania infantum* is an ongoing health problem in southern Europe, where dogs are considered the main reservoirs of the disease. Current data point to a northward spread of VL and canine leishmaniasis (CanL) in Italy, with new foci in northern regions previously regarded as non-endemic. Methodology/Principal findings Multilocus microsatellite typing (MLMT) was performed to investigate genetic diversity and population structure of *L. infantum* on 55 samples from infected humans, dogs and sand flies of the E-R region between 2013 and 2017. E-R samples were compared with 10 *L. infantum* samples from VL cases in other Italian regions (extra E-R) and with 52 strains within the *L. donovani* complex. Data displayed significant microsatellite polymorphisms with low allelic heterozygosity. Forty-one unique and eight repeated MLMT profiles were recognized among the *L. infantum* samples from E-R, and ten unique MLMT profiles were assigned to the extra E-R samples. Bayesian analysis assigned E-R samples to two distinct populations, with further sub-structuring within each of them; all CanL samples belonged to one population, genetically related to Mediterranean MON-1 strains, while all but one VL cases as well as the isolate from the sand fly *Phlebotomus perfiliewi* fell under the second population. Conversely, VL samples from other Italian regions proved to be genetically similar to strains circulating in dogs. Conclusions/Significance A peculiar epidemiological situation was observed in northeastern Italy, with the co-circulation of two distinct populations of *L. infantum*; one population mainly detected in dogs and the other population detected in humans and in a sand fly. While the classical cycle of CanL in Italy fits well into the data obtained for the first population, the population found in infected humans exhibits a different cycle, probably not involving a canine reservoir. This study can contribute to a better understanding of the population structure of *L. infantum* circulating in northeastern Italy, thus providing useful epidemiologic information for public health authorities.

Salogni° C, Colzani A, Giovannini° S, Boniotti° B, Alborali° GL

**Caso clinico: focolaio di enterite da rotavirus in suinetti neonati**

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 235-238. - 5 bib ref [Nr. Estr. 7829]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

Salogni° C, Corti I, Malandra R, Capucchio MT, Chiappino L, Sereno A, Alborali° GL, Guarda F

**Riscontro delle Escrescenze di LambI nelle valvole di pesci teleostei d'acqua dolce**

Atti del XXIV Convegno Nazionale SIPI Società Italiana di Patologia Ittica : Torino, 11-13 Ottobre 2018 / [s.l. : s.n., 2018]. - p 39 (O24) [Nr. Estr. 8095]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (24. : Torino : 11-13 Ottobre 2018)

Le lesioni delle valvole cardiache conosciute come Escrescenze di LambI dapprima descritte nelle valvole semilunari aortiche del cuore umano sono poi state successivamente riscontrate anche in altre sedi (valvola mitrale) ed in alcuni altri mammiferi tra cui il cavallo, il cane ed il suino, in modo particolare negli esemplari più vecchi. Un reperto eccezionale è stato descritto da Guarda et al. (2017) in un tonno rosso (*Thunnus thynnus*) pescato nel Mediterraneo. Tali strutture appaiono morfologicamente come linguette o lamine, singole o multiple ai margini delle valvole cardiache. Strutturalmente tali proliferazioni sono rivestite da cellule endoteliali ed il tessuto interno è del tutto simile a quello valvolare. Dal momento che la discussione su frequenza, morfologia e istogenesi di queste formazioni tuttora un caso aperto, lo scopo di questo lavoro è stato quello di indagarne la presenza e la possibile istogenesi nei pesci d'acqua dolce. Nell'ambito di una collaborazione tra l'ATS di Milano Città metropolitana, presso il mercato ittico di Milano, l'IZSLER e il Dipartimento di Scienze Veterinarie dell'Università di Torino sono stati prelevati 104 cuori provenienti da pesci adulti e appartenenti a 12 specie ittiche regolarmente pescate in acque dolci d'Europa in assenza di lesioni macroscopiche. I complessi cardiaci prelevati sono stati fissati in formalina tamponata al 10% e processati per ottenere sezioni istologiche coinvolgenti seno venoso, parete atriale inclusi valvola e seno atriale, parete del ventricolo inclusa la valvola atrio-ventricolare, parete del bulbo inclusa la valvola bulbo-ventricolare ed eventuali valvole succenturiate. Le sezioni istologiche sono state colorate con i comuni metodi istologici quali Ematossilina-Eosina e con tecniche selettive in funzione delle lesioni osservate (PAS-Alcian, blu di toluidina, Weigert van Gieson). Di tutti i campioni esaminati sono state evidenziate le classiche morfologie delle escrescenze di LambI in 6 soggetti appartenenti a specie diverse (*Ameiurus melas*-2, *Silurus glanis*-1, *Cyprinus carpio*-1, *Tinca tinca*-1 e *Rutilus rutilus*-1). In tutti i casi il riscontro è avvenuto a livello di valvole atrio-ventricolari, in un caso a livello di valvola seno-atriale e in un altro nella valvola bulbo-ventricolare. Interessante è notare che le valvole sede di escrescenze erano sempre colpite da endocardiosi. Dal punto di vista istologico l'aspetto delle escrescenze è del tutto simile a quello riscontrato nell'uomo e nel cavallo anche se, a seconda del soggetto, possono variare di forma e volume, talvolta singole oppure raggruppate sul bordo valvolare per lo più dritte, altre volte piegate dalla direzione del sangue. Tali proliferazioni sono sempre rivestite da cellule endoteliali in fase di replicazione, a volte sono disposte a ciuffi. Il tessuto interno è morfologicamente analogo a quello valvolare. La diagnosi differenziale si è posta con le deformazioni valvolari quali figure ovoidi oppure disordinate che promanano dalla valvola colpita da endocardiosi per aumento di volume del tessuto valvolare ricco di glicosoamminoglicani. Sebbene la frequenza delle lesioni riscontrate sia stata bassa (3,8%) dal punto di vista della patologia comparata i risultati ottenuti dal presente lavoro sono interessanti. Le moltissime analogie strutturali, morfologiche e di localizzazione rilevabili tra specie ittiche diverse tra loro ed i mammiferi suggerisce che probabilmente, sotto l'azione di "stressors", il tessuto valvolare reagisca similmente in tutte le specie. Fattori quali Peta, l'ambiente in cui vive l'animale, il diverso metabolismo, stress meccanici emodinamici, patologie concomitanti ecc. possono influenzare, almeno in parte, l'insorgenza della lesione, ma non la natura della stessa. Risulta tuttavia difficile individuare quali agenti stressogeni abbiano giocato un ruolo prioritario in pesci che vivono in ambienti naturali, ma

che tuttavia possono essere anche fortemente alterati dall'impatto antropico. Futuri approfondimenti da effettuarsi sia su pesci selvatici che d'allevamento potrebbero aiutare a capire meglio tali dinamiche. Rimane aperto il problema istogenico della derivazione delle Escrescenze di Lambl dallo sviluppo di fibrina e formazione di trombi che poi si organizzano in tessuto connettivo oppure se si formano l'endocardiosi, con la trasformazione in glicosamminoglicani della sostanza fondamentale della valvola a favorirne lo sviluppo.

Salogni<sup>o</sup> C, Corti I, Malandra R, Capucchio MT, Chiappino L, Sereno A, Alborali<sup>o</sup> GL, Guarda F

#### **Lesioni cardiache osservate in pesci selvaggi d'acqua dolce**

Atti del XXIV Convegno Nazionale SIPI Società Italiana di Patologia Ittica : Torino, 11-13 Ottobre 2018 / [s.l. : s.n., 2018]. - p 65 (Poster P23) [Nr. Estr. 8096]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (24. : Torino : 11-13 Ottobre 2018)

Se dal punto di vista dell'anatomia del cuore normale dei teleostei sono state ben studiate le caratteristiche macroscopiche ed istologiche, meno invece si sa dal punto di vista patologico nonostante si affermi che tale organo sia coinvolto in svariate malattie. Nell'ambito di una collaborazione tra l'ATS di Milano Città metropolitana, presso il mercato ittico di Milano, l'IZSLER e il Dipartimento di Scienze Veterinarie dell'Università di Torino sono stati prelevati 104 cuori provenienti da pesci adulti e appartenenti a 12 specie ittiche regolarmente pescate in acque dolci d'Europa in assenza di lesioni macroscopiche. I cuori prelevati sono stati fissati in formalina tamponata al 10% e processati per ottenere sezioni istologiche coinvolgenti seno venoso, parete atriale inclusa valvola e seno atriale, parete del ventricolo inclusa la valvola atrio-ventricolare, parete del bulbo inclusa la valvola bulbo-ventricolare ed eventuali valvole succenturiate. Le sezioni istologiche sono state colorate con le colorazioni standard per l'istologia quale Ematossilina-Eosina e con tecniche selettive in funzione delle lesioni osservate (PAS-Alcian, blu di toluidina, Weigert van Gieson). Le specie indagate sono state in proporzioni variabili: il pesce persico (*Perca fluviatilis* -25), il lucio (*Sander lucioperca* -10), il luccio (*Esox lucius* - 2), il salmone atlantico (*Salmo salar* -1) il coregone (*Coregonus lavaretus* -19), la carpa (*Cyprinus carpio* -11), la tinca (*Tinca tinca* -13), il carassio (*Carassius carassius* -9), il gardon (*Rutilus rutilus* -5), la scardola (*Scardinius erythrophthalmus* -3), il pesce gatto (*Ameiurus melas* -4) ed il siluro (*Silurus glanis* -1). Sebbene nessun soggetto campionato evidenziasse lesioni macroscopicamente evidenti, il 62,5% di essi presentava lesioni istologiche cardiache (singole o multiple), in alcuni casi anche nel 100% dei soggetti per una determinata specie. Tra queste lesioni sono state rilevate: epicarditi (14 casi), miocarditi (11), flogosi del bulbo arterioso (9) ed endocarditi (2) perlopiù non purulente tra i processi infiammatori; endocardiosi (14), arteriosclerosi coronarica (10), escrescenze di Lambl (5) tra quelli degenerativi. Tali riscontri dimostrano la diffusione e la complessità dei quadri istopatologici cardiaci che, pur compatibili con la vita in condizioni naturali, sono apparsi simili a quanto già riscontrati nei mammiferi ed uccelli. Non si è invece potuta osservare alcuna correlazione tra un determinato quadro istopatologico e una determinata specie ittica o provenienza. Se tra le lesioni più comuni si sono osservate le epicarditi (13,5%) e le endocardiosi (13,5%) segnaliamo anche quadri più particolari per i quali è difficile definirne il grado patologico quali la persistenza d'infiltrazioni di tessuto embrionale timico nel grasso cardiaco (12 casi) o la presenza di residui di urati (3). I dati preliminari emersi da questo lavoro indicano l'importanza della patologia cardiaca in ambito ittico sia nel senso della sua varietà e complessità che prevalenza. Necessita un ulteriore approfondimento in termini di numerosità campionaria e specie ittiche da osservare, considerando anche l'età e l'habitat di provenienza onde poter individuare eventuali processi eziopatogenetici alla base delle lesioni riscontrate.

Salvetti M, Marangi M, Bianchi<sup>o</sup> A, Bertoletti<sup>o</sup> I, Roy L, Giangaspero A

#### **Microscopy and molecular investigation on *Lipoptena* (Diptera: Hippoboscidae) circulating in wild animal species in Italy**

XXX Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) "Mutamenti ambientali e parassiti" : 26-29 Giugno 2018, Milano / [s.l. : s.n., 2018]. - p 118 [Nr. Estr. 7900]

Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) (30. : Milano : 26-29 Giugno 2018)

**INTRODUCTION.** *Lipoptena* Nitzsch, 1818, is a blood-sucking ectoparasite of domestic and wild animals as well as, accidentally, of humans. Although unclear, they cause distress and alopecia and are suspected vectors of *Bartonella*, *Rickettsia* spp., *Anaplasma ovis* (Hornok et al., 2011, *Vector Borne Zoon. Dis*, 10:1319-1321). In Europe, four species of *Lipoptena* have been identified, i.e. *Lipoptena cervi*, *Lipoptena capreoli*, *Lipoptena couturieri* and *Lipoptena fortisetosa*. The aim of the study is to microscopically and molecularly investigate *Lipoptena* species circulating in Northern Italian areas in wild ungulates host species. **MATERIALS AND METHODS.** A total of 140 specimens from *Rupicapra rupicapra* (n. 23), *Capreolus capreolus* (n.74) and *Cervus elaphus* (n.43), living in seven different areas of the Sondrio province, were collected and microscopically identified. For molecular confirmation, specimens were individually subjected to DNA extraction and PCRs amplification by using generic primers for COI gene (Folmer et al., 1994, *Mol. Mar. Biol. Biotech.* 3:294-299). PCRs positive samples were then sequenced, aligned each other and phylogenetically analyzed. **RESULTS AND CONCLUSIONS.** All specimens were identified as *Lipoptena cervi*, males (40%) and females (60%). Out of 140 collected specimens, nine, so far molecularly examined, were positive to PCRs. The percentage of identity of the obtained sequences was 98.5% with *L. cervi*, 90.8% with *L. fortisetosa*, 90.1% with *Lipoptena* sp., 87.1% with *L. depressa* and 86.6% with *L. mazamae*. Phylogenetic analysis showed that sequences cluster with *L. cervi* group with homology of 100%. The results so far obtained highlight the presence of *L. cervi* in all investigated areas and in all investigated species, including chamois, considered by literature as an uncommon host species. Once completed, the molecular investigation may help in: i) overcoming potentially wrong identification; ii) identifying new unrecorded species in Italy, such as *L. fortisetosa* (recorded in Switzerland, Germany, Poland, Czech Republic and Slovakia), *L. capreoli* (spread in the Mediterranean countries, and apparently recorded (attested?) also in Italy), and the still unconfirmed *L. couturieri* identified in Spain and France; iii) understanding their distribution on the Alpine area close to the borders. This is the first epidemiological study in Italy, providing a noteworthy picture of the *Lipoptena* species distribution in Italy, also in the light of their possible zoonotic vector role.

Santi° A, Galletti° G, Procopio° A, Di\_Nardo° S, Romanelli° C, Loli Piccolomini L, Tamba° M

#### **Impatto dell'applicazione di una mappa di rischio introduzione di influenza aviaria sull'allevamento avicolo all'aperto dell'Emilia-Romagna**

Atti della Societa' Italiana di Patologia Aviare (SIPA) 2018 : LVII Convegno annuale, III Simposio scientifico : Marmirolo (MN), 10-11 Maggio 2018 - Parma (PR), 14 Settembre 2018 / [s.l. : s.n., 2018]. - p 207-210. - 7 bib ref [Nr. Estr. 7983]

Convegno annuale Societa' Italiana Patologia Aviare (SIPA) (57. : Marmirolo (MN) Parma (PR) : 10-11 Maggio 2018 14 Settembre 2018)

Gli anatidi selvatici di superficie sono il principale reservoir dei virus di Influenza Aviaria (AI) (Comm et al., 2011) e costituiscono una costante minaccia per gli allevamenti avicoli. Gli allevamenti all'aperto hanno un rischio di infettarsi maggiore rispetto a quelli al chiuso: ad esempio, per le galline ovaiole all'aperto il rischio è 6 volte superiore (Bouwstra et al., 2017). In Emilia-Romagna (E-R) tra il 2013 e il 2017 sono occorsi diversi focolai di influenza aviaria ad alta e bassa patogenicità (HPAI e LPAI). I focolai primari delle epidemie di HPAI del 2013 e del 2016 si sono verificati in aziende di galline ovaiole allevate all'aperto in un'area ricca di avifauna selvatica, ed è stato dimostrato l'ingresso del virus in allevamento tramite contatto diretto o indiretto con uccelli selvatici (Bonfanti et al., 2014; Mulatti et al., 2017). A seguito di queste epidemie, nel 2016, per ridurre il rischio di introduzione di influenza aviaria, con disposizioni regionali è stato vietato l'allevamento di pollame all'aperto in una larga porzione del territorio dell'E-R. PER meglio definire i territori interessati dal divieto, nel 2017 è stato sviluppato un indice di rischio di introduzione dei virus influenzali legato al

possibile contatto con avifauna selvatica infetta (Density of AI-Infected Dabbling Ducks, DID) (Galletti et al., 2018) e disegnata una mappa di rischio. Obiettivo dello studio 6 valutare l'impatto dell'applicazione di questa mappa di rischio sugli allevamenti avicoli regionali, in particolare per quelli di galline ovaiole e polli da carne all'aperto, considerando anche i costi delle misure di polizia veterinaria da sostenere in caso di focolai in queste strutture o in allevamenti ad essi collegati.

*Wild dabbling ducks are the main reservoir for avian influenza (AI) viruses, and pose an ongoing threat to commercial poultry flocks. Outdoor farms have a significantly higher risk for AI introduction than indoor farms. In 2013 and 2016 epidemics broke out in Northern Italy, included Emilia-Romagna region (E-R). Outbreak investigations and virus phylogenetic analysis showed that in E-R, AI viruses were first introduced into outdoor layer flocks, due to contact between free-ranging hens and wild waterfowl. After the above-mentioned AI outbreaks, in 2017 an index to quantify the risk of introduction of AI viruses in poultry farms was developed in E-R; this index represents the theoretical Density of AI Infected dabbling Ducks per sq.km (DID). A DID cut-off of 0.23 was identified to define high risk areas for introduction of AI viruses. In Emilia-Romagna, the high risk area covers 43% of the territory and involves 40% of regional poultry farms. In this area only indoor farms are allowed. This biosecurity measure has a relevant economic impact on free-range and organic poultry flocks, that should be moved to low risk areas or converted to conventional production. Considering the huge cost of outbreaks eradication and the need of preventing them, the restriction of outdoor farming to low risk areas is certainly the priority for Public Health.*

Savini° F, Gallina L, Mazza F, Mariella J, Castagnetti C, Scagliarini C

#### **Molecular detection of Bovine papillomavirus in the placenta and blood of healthy mares and respective foals**

72° Convegno Nazionale della Società Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 280. - 6 bib ref [Nr. Estr. 7967]

Convegno Nazionale della Società Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

Sarcoids are cutaneous fibroblastic tumours representing up to 90% of all skin neoplasms in horses worldwide [1]. Even if non metastasizing, sarcoids can become very aggressive locally, in addition no effective therapy is available [2]. Their etiology is considered multifactorial, but infection with Bovine Papillomaviruses type 1 and 2 (BPV-1,-2) has been implicated as major factor in the pathogenesis. The mode of BPV transmission within and between animals is still unclear: the transmission of the virus can occur horizontally between animals in close contact [3], by stable management practices [4] and flies [5], while scarce information is available about vertical transmission. For this reason, we investigated blood and placenta of mares and respective foals for the presence of the virus through DNA detection. Twelve pregnant mares, admitted to the Equine Perinatology Unit of the Department of Veterinary Medical Sciences of the University of Bologna, were involved in the study based on the absence of clinical signs or anamnestic history of sarcoid. K3EDTA blood, from mothers and foals before suckling, as well as a portion of placenta were collected after birth. DNA was extracted after digestion and separation of PBMCs from placenta and whole blood respectively. Qualitative PCR targeting a fragment of the L1 gene was performed on every sample. Our results give support to the hypothesis that the infection can be asymptomatic, with virus remaining latent in PBMCs of healthy subjects [6] as showed by 7/12 L1 positive mothers that gave birth to positive foals, in addition, the placenta of the subjects also tested positive. Sequencing of obtained amplicons demonstrated the presence of BPV-1, BPV-2 and BPV-1 EqSarc, where nucleotide substitutions were identified after alignment with reference sequences. Interestingly, the viral type was the same between mare and foal only in three cases, positive for BPV-1. Our data contribute to further knowledge on epidemiology and tropism of the BPVs and provide a starting point to better understand the transmission routes of the virus.

Savoca S, Capparucci F, Guarnera S, Salvo A, Borella° L, Salogni° C, Marino F

**Vibrio alginolyticus quale causa di mortalità in avannotteria**

Atti del XXIV Convegno Nazionale SIPI Società Italiana di Patologia Ittica : Torino, 11-13 Ottobre 2018 / [s.l. : s.n., 2018]. - p 47 (Poster P5) [Nr. Estr. 8092]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (24. : Torino : 11-13 Ottobre 2018)

L'orata (*Sparus aurata* Linnaeus, 1758), rappresenta una delle più importanti specie allevate lungo le coste italiane. Nonostante le patologie alle quali la specie risulta più suscettibile siano ben note, ancora relativamente poco esplorato risulta il campo delle patologie larvali e poche informazioni vi sono circa gli eventi di mortalità di massa che possono verificarsi nelle avannotterie degli impianti di allevamento. In questo studio è stato esaminato un fenomeno di alta mortalità, verificatosi in un'azienda italiana nei primi mesi del 2018. Il caso di studio manifestava una moria del 40 % delle larve di orata tra il diciannovesimo e il ventiseiesimo giorno di vita. Al fine di rilevare la causa di un così elevato tasso di mortalità sono state effettuate numerose analisi preliminari a partire da febbraio 2018, sia delle acque che dei campioni di pesce. In particolare, esami di qualità delle acque, chimici e tossicologici (IPA, PCB, Pesticidi Organoclorurati e metalli pesanti) sono stati effettuati sui pozzi che forniscono acqua all'impianto, nonché sulle acque in entrata e in uscita dalle vasche. I campioni larvali (circa 100) sono stati sottoposti a esami istologici e batteriologici e virologici. I risultati delle analisi delle acque, le analisi chimiche e tossicologiche, hanno mostrato tutti valori entro la norma. L'esame virologico per ricerca del VER/VNNv tramite PCR end-point ha dato esito negativo. L'esame batteriologico, eseguito su terreni agarizzati di primo isolamento (Agar Sangue, TSA 1,5% NaCl e TCBS) ha evidenziato la presenza di *Vibrio alginolyticus*, importante patogeno frequentemente coinvolto in fenomeni di mortalità di massa e forte perdita economica nell'acquacoltura Mediterranea (Babelona et al., 1988). Tra i sintomi caratteristici dell'infezione da *V. alginolyticus* negli esemplari adulti si registrano setticemia, emorragie, ulcere, pigmentazione scura del dorso. Nelle larve, invece uno dei sintomi più frequenti è il gonfiore addominale (Sedano et al., 1996). L'esame istologico degli esemplari larvali prelevati ha infatti mostrato una peculiare distensione addominale in circa il 60 % dei campioni, probabilmente riconducibile ad un'enteropatia larvale, precedentemente descritta come "Distended gut syndrome" (DGS) (Beraldo et al., 1999; Galeotti et al., 2001). Generalmente le larve affette da questa patologia mostrano una marcata distensione addominale associata a dilatazione intestinale e presenza di cibo non del tutto digerito; il patogeno *V. alginolyticus* sembrerebbe il principale responsabile di questa condizione. Tuttavia, rimane alquanto difficile attribuire gli eventi di mortalità di massa ad una singola causa o ad un singolo agente eziologico. Ulteriori e approfondite analisi, nonché, un piano di monitoraggio accurato si rendono necessari.

Scali° F, Bontempi° G, Sardo S, Lazzaro° M, Giacomini° E, Amicabile° A, Giudici° F, Santucci° G, Maisano° AM, Candela L, Borrello S, Alborali° GL

**Monitoring antimicrobial usage with a business intelligence approach : the Classyfarm system for Italian pig farms**

Quantification, benchmarking and stewardship of veterinary antimicrobial usage : first International Conference, 27-28 February 2018 : abstracts book : oral presentations / [s.l. : s.n., 2018]. - 1 p [Nr. Estr. 8031]

International Conference "Quantification, benchmarking and stewardship of veterinary antimicrobial usage" (1st : Ghent, Belgium : 27-28 February 2018)

Veterinary antimicrobials are extensively used in Italy and monitoring antimicrobial usage (AMU) in pig farms represents a priority for Italian Veterinary Services. Nonetheless, processing AMU data at farm level could be difficult not only due to issues on quality of data sources but also due to a potentially high data density. Indeed, information on usage of a single veterinary medicinal product (VMP) may encompass data on: route of administration, active ingredients (AIs) present, species and/or age group exposed, farm characteristics, target of treatment, etc. Processing and depicting

all this information require relevant resources and proper tools. The Classyfarm system, formerly BioFaBenMa, started in 2014 analysing data on AMU in pig farms. Additionally, information on biosecurity and animal welfare is also collected. All collected data are processed using a business intelligence tool (iDashboards). Currently, a full digitalisation of antimicrobial prescriptions is not available in Italy; hence, monitoring AMU is feasible only on a limited number of farms. Data are collected on a convenience sample of 100-150 pig farms per year. AMU is measured using a dose-based metric; namely, Defined Daily Dose Animal for Italy (DDDAit). DDDAit were established using, as primary data source, Italian summaries of product characteristics (SPCs). Scientific papers were also encompassed as additional sources of information. A specific DDDAit was assigned to each AT of every VMP which contains one or more antimicrobials. AMU is collected separately per age groups (sows/boars, sucking piglets, weaners, finishers) and processed accordingly. Finally, AMU is expressed as days per animal per year which represents the potential days of exposure to antimicrobials of each age group present in a farm. All the calculations are automatically performed by iDashboards and results are displayed via interactive dashboards. AMU, at farm-level, is showed per each reared age group and various stratifications of data are available, such as AIs, administration routes, and targets of treatment. Moreover, AMU can be aggregated at different geographical levels using a specific dashboard. Conclusion Business intelligence tools can provide a useful instrument for data analysis of AMU and cross-referencing information from different aspects of animal husbandry (e.g. population data, animal welfare, biosecurity status). Furthermore, a business intelligence approach can offer analyses on data from different sources, flexibility on calculations, and a widely customisable interface to illustrate results. Finally, the Classyfarm system could be a useful example of how AMU data can be processed and depicted even in larger dataset.

Scaltriti° E, Bolzoni° L, Russo° S, Pongolini° S, Casadei° G, Arrigoni° N, Ricchi° M

**Studio dell'evoluzione di un clone di *Mycobacterium avium* subsp. *paratuberculosis* all'interno di un singolo allevamento utilizzando whole genome sequencing**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 64-65. - 5 bib ref [Nr. Estr. 8051]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Mycobacterium avium* subsp. *paratuberculosis* (MAP), the agent of paratuberculosis, is characterized by a low genomic rate of mutation. Current subtyping tools, such as Mini-Micro-satellite analyses, have not sufficient discriminatory power to follow MAP's evolution on a small spatial and temporal scale. We investigate the evolution and spread of MAP within a single dairy herd through whole genome sequencing (WGS) of 45 field strains, recovered in a single herd from 2102 to 2017. The comparison of the genomes found no indels and 43 polymorphic sites, seven of which were shared between two or more isolates. Bayesian evolutionary analysis showed a mutation rate compatible with that previously reported. The high genomic similarity among isolates confirms the clonal origin of the infection. Moreover, according to herd prevalence data, the coalescent model assuming constant population provided a better fit of the clone evolution than that assuming a growing population.

Serra V, Cafiso A, Formenti° N, Verheyden H, Plantard O, Bazzocchi C, Sasser D

**Molecular and serological evidence of the presence of *Midichloria mitochondrii* in roe deer (*Capreolus capreolus*) in France**

J Wildl Dis. - Vol. 54 no 3 ( 2018). - p 597-600. - 24 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://www.jwildlifedis.org/doi/pdf/10.7589/2017-09-241> 7899]

*Midichloria mitochondrii* is a tickborne intracellular bacterium of the order Rickettsiales, found with high prevalence in the sheep tick (*Ixodes ricinus*). *Midichloria mitochondrii* is capable of vertical transmission in the tick, but recently evidence of potential horizontal transmission to the tick hosts

through the blood meal has been reported. We investigated the presence of the bacterium in the blood of roe deer (*Capreolus capreolus*) collected from an area known to be highly infested with *I. ricinus* ticks. We collected blood and sera samples for 3 yr in Gardouch (Haute Garonne, France) and subjected them to molecular screening through PCR and to serological investigation using enzyme-linked immunosorbent assay and western blot. Bacterial DNA was detected in the blood of four of seven animals, but only at one or two points in time, whereas all sera were positive for *M. mitochondrii* antigens at all times. Our results indicated that the presence of the bacterium in the blood is transient, but the antibody response appeared to be long-lasting, possibly due to constant exposure to tick bites, and thus to repeated injection of bacteria. The role of *M. mitochondrii* in the mammalian host, and its interaction with other tick-borne bacteria, remains unknown.

Severgnini M, Lazzari B, Capra E, Chessa S, Luini<sup>o</sup> M, Bordoni R, Castiglioni B, Ricchi<sup>o</sup> M, Cremonesi P

### **Genome sequencing of *Prototheca zopfii* genotypes 1 and 2 provides evidence of a severe reduction in organellar genomes**

Sci Rep. - Vol. 8 ( 2018). - Article no. 14637 (9 p). - 52 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1038/s41598-018-32992-0> 8048]

*Prototheca zopfii* (*P. zopfii*, class Trebouxiophyceae, order Chlorellales, family Chlorellaceae), a non-photosynthetic predominantly free-living unicellular alga, is one of the few pathogens belonging to the plant kingdom. This alga can affect many vertebrate hosts, sustaining systemic infections and diseases such as mastitis in cows. The aim of our work was to sequence and assemble the *P. zopfii* genotype 1 and genotype 2 mitochondrial and plastid genomes. Remarkably, the *P. zopfii* mitochondrial (38 Kb) and plastid (28 Kb) genomes are models of compaction and the smallest known in the Trebouxiophyceae. As expected, the *P. zopfii* genotype 1 and 2 plastid genomes lack all the genes involved in photosynthesis, but, surprisingly, they also lack those coding for RNA polymerases. Our results showed that plastid genes are actively transcribed in *P. zopfii*, which suggests that the missing RNA polymerases are substituted by nuclear-encoded paralogs. The simplified architecture and highly-reduced gene complement of the *P. zopfii* mitochondrial and plastid genomes are closer to those of *P. stagnora* and the achlorophyllous obligate parasite *Helicosporidium* than to those of *P. wickerhamii* or *P. cutis*. This similarity is also supported by maximum likelihood phylogenetic analyses inferences. Overall, the *P. zopfii* sequences reported here, which include nuclear genome drafts for both genotypes, will help provide both a deeper understanding of the evolution of *Prototheca* spp. and insights into the corresponding host/pathogen interactions..

Shimmon G, Kotecha A, Ren J, Asfor AS, Newman J, Berryman S, Cottam EM, Gold S, Tuthill TJ, King DP, Brocchi<sup>o</sup> E, King AMQ, Owens R, Fry EE, Stuart DI, Burman A, Jackson T

### **Generation and characterisation of recombinant FMDV antibodies : applications for advancing diagnostic and laboratory assays**

PLoS One. - Vol. 13 no 8 ( 2018). - p e0201853 (23 p). - 95 bib ref [Nr. Estr. ( ultimo accesso 23/08/2018 ) <https://doi.org/10.1371/journal.pone.0201853> 7924]

Foot-and-mouth disease (FMD) affects economically important livestock and is one of the most contagious viral diseases. The most commonly used FMD diagnostic assay is a sandwich ELISA. However, the main disadvantage of this ELISA is that it requires anti-FMD virus (FMDV) serotype-specific antibodies raised in small animals. This problem can be, in part, overcome by using anti-FMDV monoclonal antibodies (MAbs) as detecting reagents. However, the long-term use of MAbs may be problematic and they may need to be replaced. Here we have constructed chimeric antibodies (mouse/rabbit D9) and Fabs (fragment antigen-binding) (mouse/cattle D9) using the Fv



(fragment variable) regions of a mouse MAb, D9 (MAb D9), which recognises type O FMDV. The mouse/rabbit D9 chimeric antibody retained the FMDV serotype-specificity of MAb D9 and performed well in a FMDV detection ELISA as well as in routine laboratory assays. Cryo-electron microscopy analysis confirmed engagement with antigenic site 1 and peptide competition studies identified the aspartic acid at residue VP1 147 as a novel component of the D9 epitope. This chimeric expression approach is a simple but effective way to preserve valuable FMDV antibodies, and has the potential for unlimited generation of antibodies and antibody fragments in recombinant systems with the concomitant positive impacts on the 3Rs (Replacement, Reduction and Refinement) principles.

Sozzi° E, Lavazza° A, Gaffuri° A, Bencetti FC, Prosperi° A, Lelli° D, Chiapponi° C, Baioni° L, Moreno° A

#### **Nuovo pestivirus isolato da un feto ovino in Italia**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 58-59. - 5 bib ref [Nr. Estr. 8148]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*This study reports the isolation and genetic characterisation of a pestivirus, designated as Ovine/IT/1756/17, detected in an aborted lamb foetus in North Italy. The molecular analysis provided enough evidence for genomic differences from known atypical pestiviruses to consider it as the first member of a putative novel pestivirus genotype.*

Sozzi° E, Lavazza° A, Gaffuri° A, Bencetti FC, Prosperi° A, Lelli° D, Chiapponi° C, Baioni° L, Moreno° A

#### **Novel pestivirus isolated from an aborted lamb foetus in Italy**

14th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID) : 6-9 November 2018, Sitges, Spain / [s.l. : s.n., 2018]. - 1 p (SYM3.02.05) [Nr. Estr. 8098]

International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID) (14th : Sitges, Spain : 6-9 November 2018)

Introduction: This study reports the isolation and genetic characterisation of a pestivirus detected in an aborted lamb foetus in North Italy. Methods: In January 2017, an abortion was reported in a sheep in the last third of pregnancy. Foetus showed only hairy fleece without internal macroscopic lesions. Bacteriological and parasitological examinations were performed to detect abortogenic microorganisms. Virological analysis was conducted by RT-PCRs for pan-pestivirus and Schmallenberg virus (SBV). Virus isolation was attempted through inoculation to MDCK cells. Partial (5'UTR region) and the complete genome sequencing was carried out on both tissue homogenate (TH) and cell culture (CC) isolate using Sanger sequencing and MiSeq platform (Illumina, USA) respectively. Phylogenetic analysis was performed using MEGA6. Putative recombination events were verified using the RDP4 software with five different methods. Results: All the bacteriological, parasitological analyses and PCR for SBV resulted negative. Analysis of the complete sequences (TH and CC) showed that this pestivirus, named Ovine/IT1756/17, formed a well-supported single clade distinct from the known pestiviruses (Fig-1a); it showed a higher sequence identity to CSFV across the whole genome respect to other ovine pestivirus sequences (Fig-1 b); the % of similarity to CSFV sequences was 78-77 (89.9% in the 5'UTR region) and revealed that it is more closely related to CSFV than BDV or BVDV or any other pestiviruses. Recombination analysis did not detect any recombination event. Discussion: The molecular analysis provided enough evidence for genomic differences from known atypical pestiviruses to consider it as the first member of a putative novel pestivirus genotype. Although Italian pestivirus showed 10,1% of divergence within the UTR region

and 23-22% within the complete genome to CSF, we could consider that differences in host origin and disease meet the criteria of the International Committee on Taxonomy of Viruses for the definition of a novel pestivirus. Antigenic characterization is still in progress.

Sozzi° E, Moreno° A, Lelli° D, Perulli° S, Prosperi° A, Brocchi° E, Capucci° L, Papetti° A, Giacomini° E, Alborali° GL, Lavazza° A

**Development and validation of a monoclonal antibody-based competitive ELISA for detection of antibodies against porcine epidemic diarrhoea virus (PEDV)**

Res Vet Sci. - Vol. 121 ( 2018). - p 106-110. - 41 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 ) <https://doi.org/10.1016/j.rvsc.2018.10.011> 8022]

Porcine epidemic diarrhoea virus (PEDV), belongs to the genus Alphacoronavirus in the family Coronaviridae and causes severe diarrhoea, vomiting, dehydration and high mortality in seronegative newborn piglets. Thus, a precise and rapid diagnosis of PEDV infection is important for the application of control measures to limit viral dissemination. In this investigation, a monoclonal antibodies (MAbs)-based competitive enzyme-linked immunosorbent assay (ELISA) for detecting antibodies against PEDV was developed and validated. The diagnostic performance of the test was evaluated by receiver operating characteristic (ROC) analysis using a panel of 829 known sera collected from different commercial pig farms, with or without a history of PED presence and from experimentally infected pigs. The competitive ELISA showed excellent diagnostic performance and discriminatory power with high sensitivity (Se) and specificity (Sp) values (Se = 96.5%, 95% IC 94.1–98.1; Sp = 98.2%, 95% IC 96.3–99.3). Moreover, this competitive ELISA method has other properties, such as high feasibility of testing sera without pre-treatment and automatic and instrument-mediated revealing, that makes it appropriate for large-scale screenings of affected pig farms in endemic regions or for monitoring plans in PEDV-free areas.

Sozzi° E, Parisio° G, Faccin° F, Lelli° D, Alborali° GL, Giacomini° E, Barbieri° I, Lavazza° A, Moreno° A

**Caratterizzazione genetica di BVDV isolati da bovini del Nord Italia nel periodo 2010-2018**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 179-180. - 8 bib ref [Nr. Estr. 8149]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*The genetic heterogeneity of 148 North Italian BVDV isolates was investigated by phylogenetic analysis of the partial 5'-UTR and Npro regions. At the subgroup level, pairwise similarity provided a clear-cut assignment to 9 distinct genotypes of 143 isolates typed as BVDV-1 namely respectively as BVDV-1a (n=5), BVDV-1b (n=71), BVDV-1d (n=17), BVDV-1e (n=33), BVDV-1f (n=8), BVDV-1g (n=1), BVDV-1h (n=4), BVDV-1k (n=1) and BVDV-1r (n=3). Five isolates were typed as BVDV-2c. This results provided evidence of a high BVDV genetic heterogeneity in North Italy with higher prevalence of groups 1b and 1e.*

Sozzi° E, Parisio° G, Faccin° F, Lelli° D, Salogni° C, Barbieri° I, Alborali° GL, Moreno° A, Lavazza° A

**Presenza di atypical porcine pestivirus (APPV) in un feto suino in Italia**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 208-209. - 13 bib ref [Nr. Estr. 8150]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*Atypical porcine pestivirus (APPV) is a newly recognized member of the Flaviviridae family. This novel porcine pestivirus was first described in the U.S. in 2015 and subsequent reports have shown that this virus is widely distributed in domestic pigs in Europe and Asia. APPV has been shown to be associated with congenital tremor (CT) type A-II in new-born piglets. In December 2017, samples from an Italian swine herd with three aborted piglets were submitted to the laboratory. The pool of tissue samples tested positive for APPV by PCR and nucleotide sequencing of a part of the NS3 coding region showed high similarity (95%) to other APPV strains identified in Europe on 2017.*

Stacchiotti A, Favero G, Lavazza° A, Monsalve M, Rodella LF, Rezzani R

**Taurine supplementation alleviates puromycin aminonucleoside damage by modulating endoplasmic reticulum stress and mitochondrial-related apoptosis in rat kidney**

Nutrients. - Vol. 10 no 6 ( 2018). - no 689 (17 p). - 79 bib ref [Nr. Estr. ( Ultimo accesso 04/09/2018 ) <https://doi.org/10.3390/nu10060689> 7886]

Taurine (TAU) is a sulfur-containing beta amino acid that is not involved in protein composition and anabolism, conditionally essential in mammals provided through diet. Growing evidence supports a protective role of TAU supply in osmoregulation, calcium flux, and reduction of inflammation and oxidant damage in renal diseases like diabetes. Endoplasmic reticulum (ER) stress, due to abnormal proteostasis, is a contributor to nephrotic syndrome and related renal damage. Here, we investigated the effect of dietary TAU (1.5% in drinking water for 15 days) in an established rat model that mimics human minimal change nephrosis, consisting of a single puromycin aminonucleoside (PAN) injection (intraperitoneally 15 mg/100 g body weight), with sacrifice after eight days. TAU limited proteinuria and podocytes foot processes effacement, and balanced slit diaphragm nephrin and glomerular claudin 1 expressions. In cortical proximal tubules, TAU improved lysosomal density, ER perimeter, restored proper ER-mitochondria tethering and mitochondrial cristae, and decreased inflammation. Remarkably, TAU downregulated glomerular ER stress markers (GRP78, GRP94), pro-apoptotic C/EBP homologous protein, activated caspase 3, tubular caspase1, and mitochondrial chaperone GRP75, but maintained anti-apoptotic HSP25. In conclusion, TAU, by targeting upstream ER stress separate from mitochondria dysfunctions at crucial renal sites, might be a promising dietary supplement in the treatment of the drug-resistant nephrotic syndrome.

Strano° RM, Ginestreti° J, Lorenzi° V, Fusi° F, Angelucci° A, Ferrara° G, Bertocchi° L

**Main causes of culling in dairy cows: preliminary study in Northern Italy herds**

Proceedings of the fifth DairyCare Conference 2018 : Thessaloniki, March 19th and 20th 2018 / editor, C.H. Knight. - [s.l.] : DairyCare COST Action FA1308, 2018. - p 63 (Poster 35) [Nr. Estr. 7822] DairyCare Conference (5th : Thessaloniki : March 19th and 20th 2018)

Farmer's financial return is the most important reason for culling dairy cows. Animals addressed to slaughterhouse have often problems that could affect their welfare status. The aim of this study was to investigate the major causes that lead to dairy cows culling in Northern Italy and the animal welfare conditions of the culled animals. Three loose housing dairy farms (average number of lactating cows=360), were involved in the study. From April to December 2017, 41 cows were culled. At the day of culling for each cow, the following data were collected: culling reason and indicators of poor animal welfare. These latter were assessed using animal-based measures (i.e. cleanliness, presence of lameness, skin lesions) recorded both before the loading on the truck at the farm and then after the unloading at the slaughterhouse. In addition, it was verified if there were any signs of poor welfare detectable on the carcass during the post mortem inspection. Culling reasons are

shown in Figure 1. There were some changes in animal conditions before and after the journey from herd to slaughterhouse: regarding cleanliness before the loading 35 animals were clean while after the journey only 12 cows were still clean; regarding lameness only one cow showed lameness after the journey; finally skin lesions were the same both before and after the journey. No signs of poor welfare were detected on the carcass during the post mortem inspection. Based on the collected data the situation in the assessed herds reflects what it is reported in literature confirming that reproductive problems and udder health are the most important reasons for culling<sup>1</sup>. Besides it is also interesting to underline that the transport has a big influence on the animal welfare as it was shown by the cleanliness of animals and the presence of lameness before and after the journey.

Tavernier P, Capucci° L, Lavazza° A

### **Searching for lagoviruses in Flemish hares**

13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 160 [Nr. Estr. 8003]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

Different lagoviruses are known in rabbits and hares in Europe. Next to EBHSV, also RHDV2 and non pathogenic HaCV have been found in European brown hares (*Lepus europeus*). In Flanders, information about the prevalence of lagoviruses in hares is marginal. We screened livers from healthy hares, hunted in 2004 and 2017. Liver tissue samples from hares shot at random locations in Flanders were collected *secundum artem* in a game processing plant in 2004 (n=22) and 2017 (n=16), and frozen at -20°C until analysis. A sandwich typing ELISA based on the use of specific MAb and capable of distinguishing between EBHSV and RHDV2 with a specificity >98%, was run. One single sample (1/16: 6%) from the 2004 series was EBHSV positive whereas all hares sampled in 2017 tested virologically negative. No other lagoviruses were detected. Antibodies against lagoviruses were searched on liver homogenates, using IgG-ELISA (cross reactive lagovirus test) and cELISA specific tests respectively for EBHSV and for RHDV2. For the antibody search, the use of liver homogenate instead of serum (when not available) decreases the sensitivity (factor 8-16). This means that samples negative at the first dilution of 1/40 could correspond to a serum titer range between neg-1/320. The IgG-ELISA on liver homogenates resulted in 50% with titers equal or below the dilution 1/40 for the 2004 series. Conversely, the 2017 series showed 100% positives with medium titers (from 1/80 to 1/1280). By using the more specific cELISA, the 2004 samples showed 32% EBHSV antibody positives with titres from 1/10 to 1/80 whereas in 2017 only 12% of sera resulted positive with very low titre (1/10) close to the threshold value. In hunter shot healthy hares, the absence of ELISA detectable virus was as expected. Nevertheless, the 2004 sample showing a weak positive signal for EBHSV, could reveal a hare having survived acute EBHSV at least 4-8 days before shooting. Antibody detection showed a clear difference between the 2004 and the 2017 results: IgG ELISA indicates a general high circulation of lagovirus, possibly a non-pathogenic one such as HaCV, in Flemish hare populations in 2017, but cELISA EBHSV suggests a higher circulation of EBHSV in 2004. Taken together with the EBHSV positive liver, a circulation of EBHSV with a high population susceptibility is probable for the period 2002-2004. These findings, together with the apparent phylogenetic evolution of lagoviruses in Europe (emergence of RHDV2) underline the need for continued surveillance in order to monitor the population health and immunity of hares and rabbits.

Tonni° M, Guarneri° F, Giacomini° E, Pitozzi° A, Lazzaro° M, Scali° F, Boniotti° B, Alborali° GL

**Studio longitudinale e variabilita' genotipica di *Mycoplasma hyopneumoniae* in episodi di malattia respiratoria in suini all'ingrasso** = Longitudinal study and genetic variability of

Mycoplasma hyopneumoniae in respiratory outbreaks in fattening pigs

Atti Convegno SIPAS. - Vol. 44 ( 2018). - p 127-133. - 11 bib ref [Nr. Estr. 7831]

Meeting Annuale della Societa Italiana di Patologia ed Allevamento dei Suini (SIPAS) (44. : Montichiari (BS) : 15-16 Marzo 2018)

L'obiettivo dello studio è quello di valutare la dinamica dell'infezione da Mycoplasma hyopneumoniae (M. hyo) in episodi di malattia respiratoria in suini all'ingrasso e caratterizzare la variabilità genetica dei ceppi circolanti. Sono stati esaminati in totale 750 suini provenienti da episodi di malattia respiratoria riscontrati in 5 allevamenti da ingrasso. In ogni episodio sono stati testati 30 suini per la ricerca di M. hyo dapprima al momento della comparsa della sintomatologia respiratoria, successivamente in fase di convalescenza dopo 4 settimane mediante prelievo di tamponi tracheobronchiali e in fine al momento della macellazione prelevando i polmoni. I campioni sono stati testati mediante RealTime PCR per rilevare la presenza di M. hyo. Un totale di 314 campioni positivi sono stati caratterizzati mediante Multi Locus Variable-number tandem-repeat Analyses (MLVA). L'analisi MLVA stata eseguita tramite amplificazione delle regioni genomiche Locus 1, Locus 2, p 97-1 e p 97-2. Un'elevata prevalenza di M. hyo in suini all'ingrasso è stata riscontrata al momento della sintomatologia (88%) che è ulteriormente aumentata in fase convalescente (91%). Al momento della macellazione il 64% dei polmoni presentava lesioni e l'83% dei lobi con lesione è risultato positivo per RealTime PCR. La caratterizzazione dei campioni positivi con MLVA ha identificato un totale di 22 ceppi diversi e la presenza di più di un ceppo per allevamento. È stata osservata anche la presenza di più ceppi nello stesso animale. I risultati di questo studio evidenziano che la circolazione di M. hyo in episodi di malattia respiratoria in suini all'ingrasso è elevata pur variando in funzione dell'allevamento. Inoltre, la variabilità genetica di M. hyo evidenziata nei diversi allevamenti con focolai di malattia respiratoria suggerisce che la diversità può essere specifica per ogni allevamento.

*The aim of this study is to assess the infection dynamic of Mycoplasma hyopneumoniae (M. hyo) in outbreaks of respiratory disease in fattening pigs and to characterize the genetic diversity of the strains. A total of 750 fattening pigs were selected from respiratory outbreaks from 5 farms. In each outbreak 30 pigs were tested for M. hyo at the time of the appearance of respiratory clinical signs, in the convalescent phase after 4 weeks by tracheobronchial swabs (TBS) and at slaughterhouse by lung sampling. Samples were tested by RealTime PCR to detect the presence of M. hyo. A total of 314 samples were further characterized by Multiple Locus Variable-number tandem repeats Analysis (MLVA) using amplification of Locus 1, Locus 2, p 97-1, p 97-2. High prevalence of M. hyo in fattening pigs was detected at the beginning of outbreak (88%) which increased after 4 weeks (91%). At slaughterhouse, 64% of lungs showed lesions and the 83% of lobes with lesions was RealTime PCR positive. The characterization of M. hyo showed a total of 22 genetic variants and the circulation of more than one strain in each farm. Moreover, different strains have been observed even in the same animal. The results of this study showed that circulation of M. hyo in fattening pigs in outbreaks of respiratory disease is high and varied among farms. In addition, the molecular variability of M. hyo detected within farms with respiratory outbreaks suggests that genetic diversity could be farm-specific.*

Tosi° G, Fiorentini° L, Parigi° M, Fregnani° G, Benedetti V, Leotti G, Ostanello F, Massi° P

**Controllo dell'infezione da Eimeria tenella, Eimeria maxima ed Eimeria acervulina in polli da carne mediante l'impiego di un'associazione trimetoprim+sulfadimetossina (rapporto 1:5)**

Atti della Societa' Italiana di Patologia Aviare (SIPA) 2018 : LVII Convegno annuale, III Simposio scientifico : Marmirolo (MN), 10-11 Maggio 2018 - Parma (PR), 14 Settembre 2018 / [s.l. : s.n., 2018]. - p 179-184. - 6 bib ref [Nr. Estr. 7933]

Convegno annuale Societa' Italiana Patologia Aviare (SIPA) (57. : Marmirolo (MN) Parma (PR) : 10-11 Maggio 2018 14 Settembre 2018)

*A clinical study was conducted to determine the efficacy of an association of trimethoprim and sulfadimethoxine (ratio 1:5) in controlling coccidiosis in broiler chickens. Five groups of 20 broilers (raised in poultry isolators) were orally infected, at 21 days of age, with 1mL of a suspension*

containing 5000 sporulated oocysts of *Eimeria tenella*, 5000 sporulated oocysts of *Eimeria maxima* and 10000 sporulated oocysts of *Eimeria a6ervulina*. The treatment (in the drinking water) started at the beginning of the mortality. Different therapeutic schemes were tested: 0.5 mL/L for 5 consecutive days (group "B"); 1 mL/L for the first day followed by 0.5 mL/L over the next 4 days (group "C"); 1 mL/L for 5 consecutive days (group "D"); 2 mL/L for the first day followed by 1 mL/L over the next 4 days (group "E"); infected and not treated group (group "A"). Clinical signs appeared 6 days post-infection and mortality due to coccidiosis beginning 14 days post-infection in all groups and continued in the following two days-in the control group only. Mortality and intestinal lesion score were significantly less ( $p < 0,001$ ) in all treated groups compared to the control group. No significant differences in terms of efficacy were noted between group "B" and group "C" and between groups with constant dosage (groups B and D) and groups with variable dosage (groups C and E). The proportion of animals without intestinal lesions (score "0") showing "moderate" (score 1 and 2) and "severe" lesions (score 3 and 4) was significantly different ( $p < 0.001$ ) in the different groups with a greater prevalence of "moderate" and "severe" lesions in the control group.

Tosi° G, Massi° P

#### **Casi clinici 2017 ed esperienze di campo, casi clinici 2018 IZSLER Forlì**

Atti della Società Italiana di Patologia Aviaria (SIPA) 2018 : LVII Convegno annuale, III Simposio scientifico : Marmirolo (MN), 10-11 Maggio 2018 - Parma (PR), 14 Settembre 2018 / [s.l. : s.n., 2018]. - p 33-35 [Nr. Estr. 7932]

Convegno annuale Società Italiana Patologia Aviaria (SIPA) : Marmirolo (MN) Parma (PR) : 10-11 Maggio 2018 14 Settembre 2018)

Tremolada P, Palamara\_Mesiano M, Parolini M, Giacchini R, Benocci R, Marino G, Parenti P, Lavazza° A, Bassi° S, Colombo M, Lupi D

#### **Multi-stress approach for the assessment of decline causes for honeybee**

8th Congress of Apidology (EurBee 8) : 18-20 September 2018, Ghent, Belgium : program & abstract book / [s.l. : s.n., 2018]. - p 227 (Poster P155) [Nr. Estr. 7943]

European Conference of Apidology (EurBee) (8th : Ghent, Belgium : 18-20 September 2018)

Honeybee decline is a problem of high concern since a complex pathology (Colony Collapse Disorder) brought to worldwide events of colony losses. Many adversities may be responsible of this decline: recrudescence of old and new pathologies, contamination from pesticides and emerging contaminants (e.g. nanoparticles) and environmental stressors. Stress factors may interact among them with additive or synergic effects and, currently, a multi-stress condition is accepted as the most probable decline cause for honeybees. Experimental hives were placed in two experimental sites in Northern Italy: an exposure site ES and a control site CS (14 km far from ES, agricultural field and significant human settlements). ES is located inside an experimental farm where a high-voltage electric line is present together with a complex and controlled pesticide application schedule for orchards. In the ES, two experimental areas were set up (one just below the electric line, with the combined presence of electromagnetic fields and sublethal pesticide exposure SPE, and one exposed only to SPE). Honeybees were sampled from April to October 2017 weekly for health status (mites, virus, bacteria spores and fungi), and population parameters (queen and brood status and food stores, together with daily mortality), and monthly for biomarker analyses (acetylcholinesterase, catalase, glutathione S-transferase and alkaline phosphatase activity, amount of reactive oxygen species, lipid peroxidation, and DNA fragmentation). Preliminary analysis revealed that population parameters and biomarkers were both affected by stresses. Effects on biomarkers were registered in relation to pesticide applications. Multi-stress position showed the most severe effects, leading to colonies death. Colony losses were related to the development of different pathologies. Reduced immunity defences and social disorders by frequent queen replacement seem to be the main effects related to the multi-stress condition.

Trevisi E, Riva F, Filipe JFS, Massara M, Minuti A, Bani P, Amadori° M

**Innate immune responses to metabolic stress can be detected in rumen fluids**

Res Vet Sci. - Vol. 117 ( 2018). - p 65-73. - 60 bib ref [Nr. Estr. ( ultimo accesso 11/03/2019 )  
<https://doi.org/10.1016/j.rvsc.2017.11.008> 7707]

Many production diseases of dairy cows are related to digestive troubles. The rumen subacute acidosis is the most relevant one, albeit not easily recognized. Recent studies suggest that forestomachs can perform regulatory actions at both regional and systemic levels, since forestomach walls express immune receptors and cytokines, and the rumen liquor is infiltrated by leukocytes. Therefore, the rumen fluid could be conveniently collected for investigating metabolic production diseases. Thus, we investigated the origin of the leukocytes of the rumen fluid and demonstrated that they partly derive from saliva. Next, we carried out a field survey of innate immunity in rumen fluids of 128 cows from 12 dairy farms, along with clinical inspections, assessment of milk yield, rumen pH, volatile fatty acids (VFA) and major inflammo-metabolic parameters. Significant statistical correlations were found between immune markers in rumen fluids and biochemical parameters. A significant negative correlation was found in rumen between CD45 gene expression (leukocyte infiltration) and pH level. B cells were the most frequent mononuclear leukocyte population in the rumen liquor and their infiltration was negatively affected by low ruminal pH and high concentrations of VFA. Moreover, total Ig and IgM in rumen fluids were negatively correlated with ruminal pH and positively correlated with uremia. Our data suggest that forestomach immune responses could be directed to “dangers” arising within the forestomach environment. The immune markers could integrate consolidated diagnostic parameters (e.g. rumen pH) and contribute to robust, early diagnosis of tricky digestive troubles of cattle.

Trogu° T, Faccin° F, Canziani° S, Lelli° D, Sozzi° E, Lavazza° A, Moreno° A

**Genotipi del virus della bronchite infettiva aviare circolanti negli allevamenti aviari nel 2018**

Atti della Societa' Italiana di Patologia Aviare (SIPA) 2018 : LVII Convegno annuale, III Simposio scientifico : Marmirolo (MN), 10-11 Maggio 2018 - Parma (PR), 14 Settembre 2018 / [s.l. : s.n., 2018]. - p 185-190. - 6 bib ref [Nr. Estr. 7984]

Convegno annuale Societa' Italiana Patologia Aviare (SIPA) (57. : Marmirolo (MN) Parma (PR) : 10-11 Maggio 2018 14 Settembre 2018)

La bronchite infettiva aviare è una patologia del polio a diffusione mondiale, caratterizzata da lesioni a carico degli apparati respiratorio, riproduttivo e dei reni. Questa patologia è soprattutto presente nei paesi in cui l'industria avicola è altamente sviluppata e determina notevoli perdite economiche, legate fundamentalmente alla scarsa crescita, al peggioramento dell'indice di conversione, all'aumento del numero di scarti e di mortalità nei broilers, al cab o dell'ovodeposizione e alla minore qualità dell'uovo nelle ovaiole e nei riproduttori. Il virus della Bronchite Infettiva (IBV) appartiene alla famiglia Coronaviridae e, insieme a quell° del tacchino e del fagiano, viene inquadrato nel genere Gammacoronavirus. E' caratterizzato da un'elevata variabilità antigenica riconducibile fundamentalmente alle modificazioni che si verificano a carico di una sola proteina strutturale, la proteina S degli spikes, e in particolare della Si, una delle due sub-unità che la compongono. A partire del 1956 (3), Si sono progressivamente identificati nuovi sierotipi o varianti di IBV nei van i continenti e a oggi sono stati riportati oltre 60 sierotipi, anche se si pensa che solo una piccola parte di quelli esistenti sia stata individuata. La continua comparsa di nuove varianti IBV avviene malgrado l'uso diffuso negli allevamenti intensivi di presidi immunizzanti contenenti singole varianti (M41, 793B, QX) o associazioni tra di esse. La mancanza di una protezione vaccinale completa determina in molti casi la comparsa di forme cliniche sempre più variabili che vanno dalle respiratorie o renali, più frequenti nei broilers, a quelle caratterizzate da cab o di ovodeposizione e alterazione della qualità del guscio, tipiche di ovaiole e riproduttori. Il frequente riscontro di nuove varianti antigeniche pu6 quindi rendere problematica la realizzazione di adeguate profilassi immunizzanti e, per questo motivo, le indagini epidemiologiche indirizzate alla caratterizzazione dei ceppi IBV, isolati in ogni territorio, risultano di notevole importanza nella scelta di programmi vaccinali in grado di conferire una buona protezione. Lo scopo di questo lavoro è indagare i genotipi IBV circolanti in animali

vaccinati senza sintomatologia respiratoria appartenenti a diverse tipologie produttive, allo scopo di meglio comprendere la diffusione del virus IBV negli allevamenti vaccinati.

*Avian infectious bronchitis (IB) is a highly contagious viral disease caused by a Coronavirus (IBV). It is considered an important cause of economic losses in the poultry industry affecting primarily the respiratory tract but potentially spreading to other organs. The high mutation and recombination rate of IBV has led to the emergence of several new variants that contribute to incomplete protection by current vaccines. In this study we investigate different genotypes in poultry farms with different types of productions characterized by absence of IB clinical symptoms. In January and February 2018, in North Italy, 190 pool of 10 tracheal swabs each one have been analyzed by RT-PCR and successively sequenced. Genotypes 793B, QX and M41 are the most observed, with a total prevalence of 62%. Genotypes recognized are in agreement with current vaccines used, but only a part of samples has a complete identity with the vaccine sequence. This partial variance and the presence of 22% of mixed sequences suggest a potential modification of the virus that could led to clinical symptoms and economic losses.*

Trogu° T, Formenti° N, Ferrari N, Bellometti S, Pedrotti L, Corlatti L, Lanfranchi P  
**Parasitological community of red deer (*Cervus elaphus*) : effects on population and reproduction**

XXX Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) "Mutamenti ambientali e parassiti" : 26-29 Giugno 2018, Milano / [s.l. : s.n., 2018]. - p 115 [Nr. Estr. 8119]

Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) (30. : Milano : 26-29 Giugno 2018)

INTRODUCTION. Parasites are important indicators of biodiversity and biological parameter in wildlife as they can provide information about population health status, and about the potential interactions with other animal species and humans. Here we analyse parasite community in red deer to evaluate (i) its effects on the species' health status and, by focusing on adult females, (ii) its potential impacts on reproduction. Indeed, to our knowledge no studies are available on helminths-dependent abortion or reproductive disorders in wild ungulates. MATERIALS AND METHODS. A culling management plan within three macro-areas with different level of anthropization was conducted in Stelvio National Park in 2015, and faecal samples and abomasa were collected from 92 deer (47 adults including 32 females; 17 yearling and 28 calves). Parasitological investigations were performed in order to evaluate quantitatively parasite emission stages. Moreover, abomasal inspection and helminth morphological identification were carried out. Generalized Linear Models fitting kidney fat index (KFI), pregnancy and fetus weight as response variables and presence of pulmonar and abomasal helminths and emission of coccidia and helminthic eggs as predictors. RESULTS AND CONCLUSIONS. An overall prevalence of 59.8% and of 40.2% emerged for *Eimeria* spp. and helminthic eggs, respectively. Lung larvae had a prevalence of 80.4%. Abomasal inspection highlighted a prevalence of 29.3% showing *Spiculoptera* spiculoptera, *Tricostrongylus axei* and *Ostertagia leptospicularis* (dominant species) and *Rinadia mathevossiani* (co-dominant species). Statistical analyses highlighted a significant negative effect of lung larvae on KFI. None of the variables influenced the pregnancy probability, but fetus weight was significantly affected by abomasal abundance. Weight of fetus had average values of 438 g in negative females, 314 g with abomasal charge up to 40 helminths and 98 g when helminths was >40. Pulmonar parasites showed an impact on red deer health although larvae species require species identification to discriminate their patogenicity. Conversely the recorded gastro-intestinal helrnints did not affect animals. However, although parasites had no impact on pregnancy, abomasal intensity showed an indirect negative effect on fetus development. This result would suggest that helminths may influence the reproductive potential of red deer.

Trogu° T, Formenti° N, Ferrari N, Bellometti S, Viganò R, Pedrotti L, Citterio C, Lanfranchi P

**Cryptosporidium spp. and Giardia duodenalis in wild ungulates : zoonotic risk from the Alps?**



13th European Wildlife Disease Association Conference (EWDA) : Larissa, Thessaly, Greece 27-31 August, 2018 "Wildlife health and conservation : expectations in a challenging era" : abstracts book / edited by Ioannis Chloptsios, Panagiota Argyraki, Charalambos Billinis. - [s.l. : s.n., 2018]. - p 181 [Nr. Estr. 8001]

European Wildlife Disease Association Conference (EWDA) (13th : Larissa, Thessaly, Greece : 27-31 August, 2018)

*Cryptosporidium* spp. and *Giardia duodenalis* are ubiquitous parasites able to infect a wide host range, including humans, wild and farming animals, with severe zoonotic, economic and ecological concerns. The aim of this study was to investigate on presence of these protozoa in wild alpine ungulates as a potential source of transmission for humans and livestock. The investigation has been carried out in both hunting grounds and protected areas from three non contiguous territories: the first two areas lie in South-Eastern Alps in (i) Lombardy (Lecco and Sondrio provinces) and in (ii) Veneto (Belluno province); the last is located in North-Western Alps in (iii) Piedmont (Verbano-Cusio- Ossola province). Faecal samples were collected from the rectum of animals culled during the regular hunting activity and a specific population control plan of red deer in Sondrio province. In protected areas samples were collected from the ground after localization of animal groups. From 2013 to 2015 faecal samples were collected from 348 chamois, 437 red deer (only from Piedmont and Lombardy) and 119 roe deer (sampled only in Piedmont). Samples were analysed by an immune enzymatic test for the detection of copro-antigen of *Cryptosporidium* spp. and *G. duodenalis* (RIDASCREEN® *Cryptosporidium* – RIDASCREEN® *Giardia*). Positive faecal samples with a sufficient amount of material, have been subsequently analysed through PCR in order to identify *Cryptosporidium* species and *Giardia* assemblages. In Veneto chamois showed a *G. duodenalis* prevalence of 4.4%, while in Lombardy a prevalence of 0.8% and 7.0% in red deer and chamois respectively was detected. In these areas all animals tested were negative to *Cryptosporidium* infection. In Piedmont *G. duodenalis* had a prevalence of 4.5%, 1.5% and 8.4% in chamois, red deer and roe deer respectively. *Cryptosporidium* showed a prevalence of 1.2%, 0.5% and 3.4 % in chamois, red deer and roe deer respectively. No animal was infected simultaneously by *Cryptosporidium* spp. and *G. duodenalis*. Concerning species and assemblages, isolates from chamois and roe deer clustered within the zoonotic genotype C. ubiquitous. As regard to *G. duodenalis*, the zoonotic assemblage A was detected in chamois and red deer. Both the protozoa detected are characterized by strong environmental survival, and the higher prevalence of *G. duodenalis* could be explained through the longer emission by infectious animals. Although the low prevalence recorded for both protozoa suggests a low risk of infection, considering the zoonotic species and assemblage detected, a potential role of wild ungulates as source of infections for humans and livestock cannot be excluded. In this sense, focused studies are desirable mainly in areas showing high densities of wild ungulates together with positive trend of human outdoor activity and livestock breeding.

Tura G, Pellegrino V, Avallone G, Barone F, Bacci ML, Villa° R, Spadari A, Ventrella D, Dondi F, Corradetti V, La\_Manna G, Sarli G

#### **Post-transplant lymphoproliferative disorder (PTLD) in a gilt**

72° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 384. - 4 bib ref [Nr. Estr. 7972]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

One of the 18 pigs enrolled in a study aimed to identify early biomarkers of renal rejection (Ministerial Authorization n.279/2013-B) developed a Post-Transplant Lymphoproliferative Disorder (PTLD) and the anatomopathological features are here presented. To develop a model of renal transplantation a 4-month old commercial pig underwent monolateral nephrectomy and a

subsequent renal transplantation, receiving tacrolimus as immunosuppressive therapy. Thirty days after transplantation euthanasia and necropsy followed. Samples of tissues were formalin fixed paraffin embedded, stained with haematoxylin-eosin and by immunohistochemistry to CD79 and CD3 markers. Paraffin embedded tissues from lymph nodes was used to assess the presence of porcine lymphotropic herpesvirus-1 (PLHV-1) and porcine endogenous retroviruses (PERVs) genomes by PCR (1,2). Blood sampled before euthanasia showed cell counts within normal range. During necropsy several lymph nodes (superficial inguinal, splenic, gastric, mesenteric, aortic, tracheobronchial and mediastinal) appeared markedly enlarged (up to 5 cm), edematous and pale. The cut surfaces were white, homogeneous and firm. The spleen was moderately enlarged and on cut surface white pulp appeared as multiple round prominent and bulging nodules, 0.5 to 1.2 cm in diameter. Histologically, in the lymph node, the architecture was effaced by neoplastic tissue, composed of densely cellular sheets of round cells, supported by scant fibrovascular stroma. Neoplastic cells were 40-50 µm, with distinct cell borders, high N/C ratio and scant eosinophilic cytoplasm. Nuclei were round and paracentral with finely stippled chromatin and 2-3 basophilic nucleoli. Anisokaryosis and anisocytosis were severe. Mitosis were 10 per HPF. Intermixed there were numerous, 10 µm in diameter, round cells with high N/C ratio, consistent with small lymphocytes. Splenic architecture was replaced by neoplastic lymphocytes and remnants of red pulp were visible at the periphery. Neoplastic cells in both lymph nodes and spleen were CD79a positive and CD3 negative. PCR allowed the identification of the genome of PERVs and failed to identify the genome of PLHV-1. PTLD is an abnormal lymphoid proliferation that affects both humans and animals (3,4). In humans PTLD is strictly linked with Epstein-Barr virus. PTLD in swine is mainly described in miniature pigs and PLHV-1 seems to be involved as causative agent (3, 4) but few PTLD cases are negative for PHLV-1 genome and some non-PTLD cases are positive (3,4). Both PHLV and PERVs are common infections in conventional pig herds and their precise role on the pathogenesis of tumours in the specie has still to be elucidated. The present case of PTLD is, to the best of our knowledge, the first reported in a non-miniature breed of swine, and shares the following features with the model known in the pig: time of appearance after the transplantation (30 days in this case, from 21 to 45 days from literature); a prevalent involvement of lymphoid organs rather than invasion in non lymphoid tissues; a B cell phenotype. PTLD should be take into account in the management of swine during transplantation and its management can be a useful model for the human counterpart of the disease.

Van\_Diemen PM, Byrne A, Ramsay A, Watson S, Nunez A, Moreno\_Martin° AMV, Chiapponi° C, Foni° E, Brown IH, Brookes SM, Everett HE

**Assessment of zoonotic transmission of swine influenza A viruses from pigs to naïve or vaccinated ferrets**

6th European veterinary immunology workshop (EVIW) : Sept 5-7, 2018 : abstract book / co-editors, Victor Rutten, Christine Jansen, Femke Broere. - [s.l. : s.n., 2018]. - p 67 (Poster 06.03) [Nr. Estr. 7959]

European veterinary immunology workshop (EVIW) (6th : Utrecht, The Netherlands : Sept 5-7, 2018)

Background: An in vivo study was conducted to assess the infection dynamics of two swine-origin H1N1 influenza A viruses - a pandemic 2009 (Sw-pdm09) strain and human isolate (A/Pavia/65/16) which is phylogenetically indistinguishable from avian-like Eurasian swine influenza A viruses (H1 avN1). The efficacy of the human 2016-17 seasonal influenza vaccine was gauged using ferrets as human model. The vaccine incorporates a human pdm09 virus strain. Methods and Results: Five donor pigs were infected with Swpdm09 or Pavia strains and co-housed with five naïve and five vaccinated ferrets held in separate cages. Both viruses readily infected pigs producing similar, mild, disease profiles, and reached peak shedding at 2-4dpi, which ceased by 7dpi. The vaccinated ferret group exposed via bio-aerosol to Sw-pdm09 virus showed a significant reduction in viral shedding and did not develop an influenza-specific antibody titre, indicating (partial) protection from productive infection by the vaccine. The remaining ferret groups (1 vaccinated, 2 naïve) had a virus shedding profile characteristic of infection, and seroconverted. Their antibodies recognised the cognate infection antigen. All infected ferrets exhibited mild clinical signs and controlled the infection.

Discussion and Conclusion: This study confirms that vaccine strains must be highly matched to the challenge strain in order to afford protection. Pre-existing immunity to Human-origin pdm09 strains may not provide protection to all circulating swine influenza A virus HI N1 strains. There was no evidence of increased risk to human health - using animal models - from the Pavia strain compared to the swine-origin pdnn09 strain assessed in parallel.

Vangroenweghe F, Luppi° A

**Prevalence of virulence factors of Escherichia coli isolated from piglets with post-weaning diarrhoea in Belgium and The Netherlands**

5th Congress of the European Association of Veterinary Laboratory Diagnosticians (EAVLD) : 14-17 October, 2018, Brussels, Belgium : abstract book / [s.l. : s.n., 2018]. - p 107 (Poster 40) [Nr. Estr. 8043]

Congress of the European Association of Veterinary Laboratory Diagnosticians (EAVLD) (5th : Brussels, Belgium : 14-17 october, 2018)

Introduction: Post-weaning Escherichia coli diarrhea (PWD) remains a major cause of economic losses for the pig industry. PWD typically causes mild to severe watery diarrhea and is caused primarily by enterotoxigenic E. coli (ETEC). The most common fimbriae in PWD are F4 and F18, while the predominant enterotoxins are LT, STa and STb. The objective of the study was to determine the prevalence ETEC subtypes causing PWD in Belgium and The Netherlands. Materials and Methods: A total of 504 pig herds distributed in the Benelux showing clinical signs of PWD were sampled between January 2014 and December 2016. Rectal swab samples (n=5) from diarrheic pigs were collected and submitted to IZSLER (Brescia, Italy) to analyze the presence of virulence factors — adhesins (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb, Stx2e). Results: - In total, 526 non-hemolytic and 784 hemolytic E. coli strains were isolated and subsequently tested by PCR. The prevalence of the different ETEC subtypes was as follows: F4-ETEC (24.4 %) and F18-ETEC (19.2 %). On a herd level, the prevalence of the different ETEC subtypes was as follows: F4-ETEC (45.8 %) and F18-ETEC (37.5 %). Besides ETEC, 22 isolates (1.7 %) were classified as Shiga toxin-producing E. coli (STEC). Discussion and Conclusion: This study confirms that fimbriae type F4 was slightly more prevalent than F18 among E. coli isolates from PWD cases in Belgium and The Netherlands. Laboratory diagnostics, including characterization of virulence factors, are essential to understand the role of E. coli in PWD outbreaks and initiate appropriate preventive and control measures such as live oral vaccination.

Vencia W, Lazzara F, Cosma V, Ratto A, Vivaldi B, Zoppi S, Gorla M, Ferrari A, Amadori° M, Ercolini C, Razzuoli E

**Infezione da Porcine circovirus in cinghiali selvatici esposti a cadmio**

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 210-211. - 6 bib ref [Nr. Estr. 8061]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (18. : Perugia (PG) : 7-8-9 Novembre 2018)

*PCV2, in combination with other infectious and noninfectious stressors, has been implicated as the necessary cause of Porcine Multisystemic Wasting Syndrome (PMWS), as part of the Porcine Circovirus-associated disease (PCVAD). In this work, we report 3 cases of PMWS in wild boars. Over 70 analyses were conducted on each animal under study. Animals showed poor body conditions with evident cachexia. Histologic examination showed various degrees of lymphocyte depletion and macrophage infiltration. PMWS was recognized based on typical lesions associated with detection of PCV2 in tissues by PCR for viral DNA. All animals shared high level of cadmium (Cd) with mean values of 1.3±0.5 mg/kg in liver and 15.9±3.2 mg/kg in kidney. Accordingly, Cd*

*poisoning can be associated with PCVAD occurrence in wild boars. The induction of oxidative stress by Cd and the related amplification of the inflammatory response probably underlie the synergism between chronic exposure to Cd and PCV2 infection.*

Vencia W, Mignone G, Capellini MF, Parisi E, Amadori<sup>o</sup> M, Ercolini C, Ferrari A, Razzuoli E

### **Modulation of innate immunity in kidney epithelial cells by infective and non-infective stressor**

72<sup>o</sup> Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVIII Convegno S.I.C.V, XVI Convegno S.I.R.A, XV Convegno AIPVet, X Convegno ARNA, V Convegno RNIV, II Convegno ANIV, I Convegno SICLIM-VET, Giornata studio AIVI, Giornata studio SOFIVET / [s.l. : s.n., 2018]. - p 112. - 3 bib ref [Nr. Estr. 7962]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 72 Convegno SICV : 18 Convegno SIRA : 16 Convegno AIPVet : 15 Convegno ARNA : 10 Convegno RNIV : 5 Convegno ANIV : 2 Convegno SICLIM-VET : 1 Giornata studio AIVI Giornata studio SOFIVET :

Madin-Darby Canine Kidney (MDCK) are cell lines commonly used as models to study the characteristics of epithelial cells [1], they are involved to study viral infection of cells and in vaccine production [2]. Previous studies suggest the sensibility of MDCK to invasion and penetration of bacterial strain and the constitutive expression in MDCK of genes involved in the innate immunity response and cell cycle regulation [3]. However, there are no data about the ability of these cell line to respond to infective and non-infective stressor. The aim of this study was to evaluate the MDCK cells innate immunity response to non-infectious stressor (Cd<sup>2+</sup>) and infectious stressor (LPS). To this purpose we performed two experiments; in the first step we evaluated the ability of MDCK to respond to Cadmium (Cd<sup>2+</sup>) a non-infectious stressor, that at molecular level induces a cellular stress Cells were treated with 20 µM of Cd<sup>2+</sup> dissolved in DMEM culture medium. After 3 h and 24 h of treatment at 37°C in 5% CO<sub>2</sub>, mRNA was extracted to test gene expression. The second experiment provided the treatment of cells with 1 µg/mL of lipopolysaccharide (LPS) from Escherichia coli O111:B4 recognized an infectious stressor. After 3 h and 24 h of incubation t 37°C in 5% CO<sub>2</sub> mRNA was extracted to study gene expression. In both experiments we tested the modulation of followed parameters involved in innate immune response: IL-8, IL-6, IL-1β, TLR1, TLR3, TLR5, TLR9, INOS, CD14, MYD88, P65, TLR4, MD2, IL-18. Experiments were carried out in quintuplicate; cells treated with medium only were used as negative control. Each test was repeated twice. After 3 h of Cd<sup>2+</sup> treatment we observed up regulation (P<0.05) of important pro-inflammatory cytokines (IL-8, IL-6, IL1β), INOS, TLRs (TLR1, 9, 3, 5) and down regulation of CD14. Cd<sup>2+</sup> treatment at 24 h determined up regulation of MYD88, p65, IL-18 and down regulation of INOS, IL-1β, MD2 and TLRs. After 3 h of LPS treatment we detected up regulation (P<0.05) of MYD88 and down regulation of INOS, CD14, IL1β, TLRs (TLR 5, 4). No significant differences were reported after 24 h of treatment. Our results show the ability of MDCK to respond to infectious and non-infectious stressor; in particular, Cd<sup>2+</sup> and LPS to modulate innate immune response in terms of gene expression as a function of time. These data suggest a possible alteration of host-pathogen interactions due to inflammatory response and modulation of TLRs expression.

Veo C, Moreno<sup>o</sup> A, Rovida F, Percivalle E, Galli M, Baldanti F, Zehender G

### **Phylogeographical reconstruction of the origin and dispersion of WNV-2 in Italy**

2nd National Congress of the Italian Society for Virology "One Virology One Health" : Rome, November 28-30, 2018 / [s.l. : s.n., 2018]. - p 37 ( OC1 ) [Nr. Estr. 8158]

National Congress of the Italian Society for Virology (2nd : Rome : November 28-30, 2018)

West Nile virus (WNV) lineage 2 is an emerging virus whose circulation was documented until 2004 only in Sub-Saharan Africa, but recently it caused extensive epidemics in Central Europe spreading also to Italy. An increasing number of cases has been reported by the Italian National surveillance of WNV, established by law in 2008. The purpose of this study is the reconstruction of the origin and

dispersion of WNV-2 in Italy on the basis of the evolutionary (phylogenetic) study of whole viral genomes obtained by Whole Genome Sequencing (WGS). Material and methods: A total of 39 viral strains circulating in Italy in the 2015-2017 seasons were characterized by next generation sequencing (NGS) technology and analyzed through advanced phylogenetic analysis techniques (phylodynamics and phylogeography). Results: The sequences obtained were aligned with complete genomes isolated in various regions of the world. From the phylogenetic and phylogeographic analysis performed on the Italian sequences, it emerged that WNV-2 entered Italy around 2008 in an area between the Adriatic sea and the central Po Valley. The phylogeographic analysis of the Italian clade suggested that the epidemic spread simultaneously eastward and westward. According to our reconstruction, the eastern strain spread to the north reaching Emilia Romagna and Veneto Regions. The western strain dispersed rapidly in an area between Cremona and Piacenza reaching Lombardy Region. From 2014 to present, the Western strain has simultaneously expanded in South Eastern direction, reaching again Modena, Bologna and Ravenna. Discussion and conclusions: It might be speculated that the virus entered Italy around 2008 and remained confined in autochthonous reservoir birds, spreading locally along the Po Valley following the main river and its tributaries. These data underline the importance of a careful monitoring of the circulation of WNV in our territory, specially through the use of innovative molecular surveillance tools such as NGS and molecular evolution.