

## **RICERCHE EFFETTUATE SANITA' ANIMALE**

### **Alborali°G, Giacomini°E, Ferrari°N, Pasquali P, Pitozzi°A, Boniotti°B, Cordioli°P PRRSV and H1N1 detection in individual blood samples, nasal swabs and pen oral fluids in longitudinal study in post weaning pigs**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 177 (Poster P90) [Nr. Estr. 5720]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

Oral fluids have recently been used as a surveillance tool for Porcine reproductive and respiratory syndrome virus (PRRSv), Swine Influenza virus (SIV) and Porcine circovirus-2 (PCV-2) using reverse transcription polymerase chain reaction (RT-PCR). Methods to collect samples and analysis is most important to detect viruses. The sampling of oral fluids through cotton rope, where animals chew on the material and deposit oral fluid, represents an easy method to collect samples in pig pen. The aim of this study was to estimate and compare the detection of PRRSv and SIV with different sampling approaches through individual blood, nasal swab and oral fluid. The study was carried out in farrow to growing herd in North Italy with endemic PRRSv and H1 N1 infection in the post-weaning site. Pigs were sampled in a longitudinal study of 5 groups of 15 pigs from 25 to 85 days of age. Three sampling were included in the protocol: 25 days of life when/during? weaning (T1), at 55 days (T2) and at 85 days of life (T3). Samples were tested by RT-PCR. The analyses of PRRSv showed that the infection was detected earlier in blood samples compared to nasal swabs and oral fluid with a great variability among the three sampling times. The overall prevalence in blood samples was 66, 85 and 93% at T1, T2 and T3 respectively. In contrast, virus was detected later in oral fluid reaching comparable values to blood samples in T2 and T3 with overall prevalence of 20, 86 and 86%. Nasal swabs showed the lowest levels of infected animal with prevalence of 9, 22 and 25%. H1N1 was mostly detected in oral fluid with overall prevalence values of 13, 40 and 20% while in nasal swabs virus was detected in 0, 4 and 6% of the samples respectively. Surveillance of H1 N 1 and PRRSV in post-weaning pigs by oral fluids has allowed to know and monitor the health status of different batches of pigs for fattening before transfer. Oral fluid sampling is a promising approach for increasing the efficiency and cost effectiveness of virus surveillance in swine herds even if the infection status of the barn is considered variable. It is easy to performed, doesn't stress to animals, it is a rapid testing method and shows reliable diagnostic performance.

Alborali°GL, Boniotti°B, Lavazza°A

### **Surveillance and control of PED Coronavirus in pigs in Italy**

Swine Enteric Coronavirus Diseases (SECD) International Meeting : September 23 - 25, 2014 Chicago, Illinois, USA / [s.l. : s.n., 2014]. - p 7 [Nr. Estr. 5853]

Swine Enteric Coronavirus Diseases (SECD) International Meeting : Chicago, Illinois, USA : September 23 - 25, 2014)

In Italy, Porcine Epidemic Diarrhea (PED) is present since the early 90s. Its diffusion increased with the simultaneous decline of Transmissible Gastroenteritis (TGE), another coronavirus pig enteritis widespread in the 70-80s. A severe epidemic wave of PED outbreaks occurred in the early 90s and then it became endemic with sporadic outbreaks occurring between cyclic epidemic peaks; the last of which on 2005-2006 in pigs densely populated areas of North Italy. The clinical signs involved pigs of all ages, but mortality was registered only in piglets and lasted 3-4 weeks. On farms with only finisher pigs, diarrhea was acute, watery, without evidence of mucus and blood and on farrow-to-weaner herds, vomiting, diarrhea and death from dehydration were prominent in suckling pigs. MAb-based (anti-PEDV strain CV777) diagnostic tools were developed at IZSLER. They were set up for both antigen (immuno-electron-microscopy-IEM and double antibody sandwich-DAS-ELISA) and antibodies (antigen capture ELISA and immune-peroxidase monolayer

assay-IPMA) detection. Virological identifications were also confirmed by RT-PCR. From 2008 to 2014 only sporadic outbreaks were observed in growers and finishers herds: 71 PEDs, from 58 different farms, out of 1563 cases of enteritis (4.54%). The genetic variability of 26 strains identified during six-year period was analyzed by partial sequencing of 3 genes. During 2014, a serosurvey (antibodies were found in 11 out of 21 farms in 7% to 52% tested animals) confirmed the still active circulation of PEDV. IZSLER is carrying out a targeting surveillance on pig enteritis to detect old (as PEDV) and potentially new enteric pathogens.

Amadori°M, Fusi°F, Bilato°D, Archetti°IL, Bertoni°L

### **Lysozyme and interleukin-6 as disease-predicting parameters in transition dairy cows**

Proceedings of the XXVIII World Buiatrics Congress : Cairns, 2014 : oral communication and poster abstracts / editor, David S. Beggs. - Australia : Australian Cattle Veterinarians, 2014. - p 178 [Nr. Estr. 5783]

World Buiatrics Congress (WBC) (28th : Cairns : 2014)

Amadori°M, Fusi°F, Bilato°D, Archetti°IL, Bertoni°L

### **Lysozyme and interleukin-6 as disease-predicting parameters in transition dairy cows**

Proceedings of the First DairyCare Conference : Copenhagen, August 22nd and 23rd 2014 / editor, C.H. Knight. - [s.l. : DairyCOST action FA1308, 2014]. - p 34 [Nr. Estr. 5858]

DairyCare Conference (1st : Copenhagen : August 22nd and 23rd 2014)

Objectives. The lysozyme and interleukin(IL)-6 responses in the dry period have been shown to be useful predictive parameters of disease occurrence in the early lactation period of cows reared in an Experiment Station (Trevisi et al., 2012). Therefore, we set out to confirm these findings under field conditions in an ad hoc case-control study. Materials and methods. The study was carried out on 80 high-yielding dairy cows of 26 herds. Cows were submitted to clinical inspections in the dry and early lactation periods, and the number and type of disease cases and drug treatments after calving were thoroughly reported. With respect to the expected calving day, serum samples were collected between -23 and -33 days (T1) and between -2 and -6 days (T2), respectively. IL-6 and lysozyme reference values were < 160 pg/ml and 1-3 microg./ml, respectively. Values outside these physiological intervals (responder animals, IL-6R and LysR) were taken as disease risks. Results. LysR and (to a lesser extent) IL-6R subjects showed a greater prevalence of disease cases until the 60th day after calving compared with non-responder cows. In particular, by combining the results at T1 and T2, LysR and LysR + IL-6R cows showed a significantly higher number of serious disease cases (P< 0.01 for both, Odds Ratio 7.1 and 5.1, respectively). This was also confirmed by T1 results, alone. In fact, T1 LysR and T1 LysR + IL-6R cows showed a significantly higher number of serious disease cases (P< 0.01 and 0.05, respectively). Accordingly, the relevant Odds Ratio was equal to 4.9 and 3.5, respectively. Lysozyme and IL-6 were less informative at T2 (higher P values and lower Odds Ratios). The above data confirm that crucial events may affect the homeostatic response to inflammatory stimuli one month before calving (T1 in this study), and give rise to a negative imprinting of the innate immune response to non-infectious metabolic stressors after calving. Conclusions. Lysozyme and IL-6 can represent useful readout parameters of a poor regulation of the inflammatory response in dry cows, and as many risk factors for disease occurrence in the early lactation period.

Amadori°M, Razzuoli°E

### **Immune control of PRRS : lessons to be learned and possible ways forward**

Frontiers Vet Sci. - Vol. 1, Article 2 ( 2014). - p 1-14. - 128 bib ref [Nr. Estr. 5855]

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. The disease can be controlled by farm management programs, which can be supported by vaccination or conditioning of animals to circulating PRRS virus (PRRSV) strains. Yet, PRRS still represents a cause of heavy losses for the pig industry worldwide. Immunological control strategies are often compounded by poor and late development of adaptive immunity in both vaccinated and infected animals. Also, there is evidence that results of field trials can be worse than those of experimental studies in isolation facilities. Neutralizing antibody (NA) was shown to prevent PRRSV infection. Instead, the role of NA and adaptive immunity on the whole in virus clearance after established PRRSV infections is still contentious. Pigs eventually eliminate PRRSV infection, which may be correlated with an "educated," innate immune response, which may also develop following vaccination. In addition to vaccination, an immunomodulation strategy for PRRS can be reasonably advocated in pig "problem" farms, where a substantial control of disease prevalence and disease-related losses is badly needed. This is not at odds with vaccination, which should be preferably restricted to PRRSV-free animals bound for PRRSV-infected farm units. Oral, low-dose, interferon- $\alpha$  treatments proved effective on farm for the control of respiratory and reproductive disease outbreaks, whereas the results were less clear in isolation facilities. Having in mind the crucial interaction between PRRSV and bacterial lipopolysaccharides for occurrence of respiratory disease, the strong control actions of low-dose type I interferons on the inflammatory response observed *in vitro* and *in vivo* probably underlie the rapid clinical responses observed in field trials.

Amato B, Di\_Marco\_Lo\_Presti V, Gerace E, Mandanici F, Biasibetti E, Boniotti<sup>o</sup> MB, Souria L, Valenza F, Pacciarini<sup>o</sup> ML, Capucchio MT

#### **Bovine tuberculosis in pigs : a case of *Mycobacterium caprae* infection**

International Meeting on Emerging Diseases and Surveillance (IMED) : Vienna, Austria, October 31 - November 3, 2014 / [s.l. : s.n., 2014]. - p 144 [Nr. Estr. 6074]

International Meeting on Emerging Diseases and Surveillance (IMED) : Vienna, Austria : October 31 - November 3, 2014)

Purpose: Bovine tuberculosis is a worldwide zoonosis that has been reemerging in different ecological scenarios. The presence of wildlife reservoirs frequently hampers eradication. In Sicily cases of tuberculous lesions have been reported in a population of Sicilian Bleck pig, an autochthonous variety of pig that live in Natural Parks of Nebrodi and Madonie in free or semi-free roaming conditions, frequently sharing pastures with cattle. The authors report a description of *M. caprae* infection case in a Nebrodi Bleck pig with the characterization of pathological lesions and genotyping of isolated strains. Methods & Materials: In the first half of 2013, a Nebrodi Bleck pig carcass was subjected to a complete routine meat inspection at the abattoir. Macroscopical, histopathological and bacteriological investigations were carried out on suspected lesions. Molecular identification and genotyping of the isolate was also performed by Spoligotyping and MIRU-VNTR analysis (ETRA-E, VNTR 2163a, 2163b, 4052, 3155, 1895, 3232 and MIRU-26). Results: An adult sow, showed generalized granulomatous lesions involving lymph nodes of the head, thorax and abdomen, tonsils, udders and coxo-femoral joint. In the tonsils small translucent gray nodules with a pale yellow core were observed. Multiple granulomatous lesions of different size were identified in three udders. A voluminous neoplasm (10 cm) involving the right coxo-femoral joint was also detected. The X-ray examination revealed bone remodeling of the trochanter and ischial bones osteolysis. Histologically granulomatous lesions appeared as classical tuberculous granulomas with a necrotic center surrounded by epithelioid, multinucleated giant macrophages and lymphocytes with minimal or abundant calcifications. Mycobacterial colonies Ziehl-Neelsen positive were isolated from all the affected organs. By genotyping, all the isolates were *M. caprae*, spoligotype SB0866 and MIRU-VNTR 4.1.5,4,4,11. 4.2.4.3.8.7. Conclusion: In Sicily, the number of isolates of *M. caprae* compared to *M. bovis* in domestic and wild animals since 2004 till now is very low (0,01%). This is the first report of *M. caprae* isolation in pigs in Italy. The same genotype was found in two cattle herds from neighboring areas, suggesting an inter-Species transmission. At the moment, is known about the host susceptibility to *M. caprae* infection in swine.

Andreoli E, Radaelli E, Bertolotti I, Bianchi A, Scanziani E, Tagliabue S, Mattiello S

**Leptospira spp. infection in wild ruminants : a survey in Central Italian Alps**

Vet Ital. - Vol. 50 no 4 ( 2014). - p 285-291. - 40 bib ref [Nr. Estr. 5994]

La leptospirosi è un'importante zoonosi presente in tutto il mondo e gli animali selvatici sono considerati importanti vettori epidemiologici. Per indagare la presenza dell'infezione *Leptospira* spp. nei ruminanti allo stato brado delle Alpi Centrali Italiane, in provincia di Sondrio sono stati prelevati 441 campioni sierologici e 198 campioni renali da cervi, caprioli e camosci analizzati, rispettivamente, con il test di agglutinazione microscopica e l'esame istopatologico. Sono state riscontrate 19 reazioni sierologiche positive in 15 cervi. I sierotipi più frequenti sono risultati Bratislava e Grippotyphosa, seguiti da Pomona, Hardjo e Copenhageni. Nel 22% dei campioni renali appartenenti ai cervi risultati sieropositivi è stata riscontrata una nefrite tubulo-interstiziale multifocale cronica linfoplasmacitica e fibrotica da lieve a moderata. Nel presente studio, gli anticorpi per *Leptospira* spp. sono risultati infrequenti nei ruminanti selvatici e solo il cervo sembra essere sensibile all'infezione da *Leptospira* spp. Data la contenuta sieroprevalenza e il fatto che non sono state segnalate infezioni da *Leptospira* spp. in bovini, ovini, caprini e cacciatori nella area di studio durante il periodo di indagine, i ruminanti selvatici in ambiente alpino non possono essere considerati come reservoirs o importante fonte di infezione da *Leptospira* spp. per l'uomo o gli animali domestici.

*Leptospirosis is an important zoonotic disease diffused worldwide, and wildlife species are commonly considered to be important epidemiological carriers. Four-hundred and forty-one serological and 198 renal samples from red deer, roe deer and chamois collected in the Province of Sondrio were analysed using the microscopic agglutination test and histopathologic examination. Positive serological findings were found only in 15 red deer and 19 positive serologic reactions were recorded. The most frequent serovars were Bratislava and Grippotyphosa, followed by Pomona, Hardjo and Copenhageni. Twenty-two per cent of renal samples from seropositive red deer were affected by mild to moderate multifocal chronic lymphoplasmacytic and fibrosing tubulo-interstitial nephritis, mainly involving the cortical parenchyma. In this study, antibodies to Leptospira spp. were infrequent in wild ruminants, and only red deer seemed to be sensitive to the infection. Given the low presence and the fact that there was no record of Leptospira spp. infections in cattle, sheep, goats and also hunters in area during the study period, wild ruminants in Alpine environments cannot be considered as reservoirs or important sources of Leptospira spp. infection for humans or domestic animals.*

Angelucci A, Donati M, Fusi F, Lorenzi V, Bertolotti L

**Benessere : a che punto siamo nell'allevamento bovino a posta fissa**

Inf Zootec. - Vol. 60 no 2 ( 2014). - p 58-62. - 15 bib ref [Nr. Estr. 5639]

Ardigò P, D'Incau M, Pongolini S

**Abortion in cattle due to infection with *Staphylococcus lugdunensis***

J Vet Diagn Investig. - Vol. 26 no 6 ( 2014). - p 818-820. - 29 bib ref [Nr. Estr. 5930]

An aborted fetus of 7 months gestation, the associated placenta, and a single blood sample from the dam were submitted for diagnostic investigation to the diagnostic laboratory of the Lombardy and Emilia-Romagna Experimental Zooprophyllactic Institute in Parma, Italy. The serum was negative for *Neospora caninum*, *Coxiella burnetii*, *Chlamydophila abortus*, Bovine herpesvirus 1 (BHV-1), Bovine viral diarrhoea virus (BVDV), *Brucella abortus*, and *Brucella melitensis*. Fetal tissues and placental cotyledons were pooled and tested by polymerase chain reaction (PCR) for the presence of BHV-1, Bovine herpesvirus 4, BVDV, *N. caninum*, *C. burnetii*, *Chlamydophila* spp., Schmallenberg virus,

and *Leptospira interrogans*. All PCR assays were negative. Bacteriological examinations performed on the fetal organs revealed a pure growth of *Staphylococcus lugdunensis* in all organs cultured. In human beings, *S. lugdunensis* is responsible for community-acquired and nosocomial infections, in both immunocompetent and immunocompromised patients. In veterinary medicine, the pathogenic potential of *S. lugdunensis* has not been fully investigated. The incidence of *S. lugdunensis* is regarded as being underreported because it could be easily misidentified as *Staphylococcus aureus*. The current report documents the ability of *S. lugdunensis* to cause abortion in cattle, indicating the need for accurate diagnostic procedures to identify this emerging and zoonotic pathogen whose incidence is likely underestimated in both human and veterinary medicine.

Arioli E, Caleffi A, Leotti G, Ostanello F, Foni°E , Vila T, Joisel F

**Booster effect of swine influenza virus infection on serological response to Gripovac® vaccine in an Italian fattening unit**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014, Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 173 (Poster P82) [Nr. Estr. 5954]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

Arrigoni°N, Ostanello F, Ricchi°M, Bonilauri°P, Bonfante E, Giacometti F, Serraino A

**Screening for *Mycobacterium avium* subsp. *paratuberculosis* in Southern Italian dairy herds by bulk milk ELISA and in line milk filter PCR**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 185 (Poster P-06.2) [Nr. Estr. 5762]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

The knowledge of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection status of each herd is a key factor for the control of the disease, for informed decisions of risk managers and for creation of positive conditions for the conscious commerce of animals and their products. Few data are available about paratuberculosis prevalence in Southern Italian dairy herds; therefore an investigation to detect MAP infected herds by repetitive screening tests was performed. This screening (S-MAP) was based on analysis of Bulk Tank Milk (BTM) samples by a commercial ELISA test (ID VET, France) and of In Line Milk Filter (IMLF) samples by IS900-qPCR. BTM and ILMF were collected twice from 569 dairy herds in 3 Italian Regions. Additionally, a total of 12312 individual milk samples were collected and analysed by ELISA, 9509 from 102 herds which resulted positive to the initial screening (S-MAP positive) and 2803 from 24 herds which resulted negative to the initial screening (S-MAP negative). The S-MAP positive herds in these regions ranged between 18.8% and 23.9%; no significant differences were shown between regions in the prevalence of S-MAP positive herds. The within-herd Apparent Prevalence (AP) ranged between 0.00% and 22.73% and no significant differences were shown between Regions. The within-herd AP appears to be comparable to that reported in other Italian Regions. A highly significant correlation was shown between positivity to S-MAP and within-herd AP. In fact, S-MAP detected a minimum of 56.3% of low prevalence herds (within-herd AP < 2.00%), up to a maximum of 100% of high prevalence herds (within-herd AP > 8.00%). Overall, the S-MAP detected 85.6% of positive herds. Although it cannot be used for MAP-free herd certification, S-MAP could be a useful tool to prioritise appropriate control measures in the context of widespread control plans aimed at reducing the prevalence of infection in dairy herds and milk contamination in dairy production.

Arrigoni°N, Ruocco L, Paternoster°G, Tamba°M

**Bovine paratuberculosis in Italy : building infrastructures and defining the guidelines for control and for ranking of herds**

Proceedings of the 4th ParaTB Forum : 21 June 2014, Parma, Italy / [Parma : Efsa, 2014]. - p 53-59. - 9 bib ref [Nr. Estr. 5701]

ParaTB forum (4th : Parma, Italy : 21 June 2014)

Arrigoni°N, Ruocco L, Paternoster°G, Tamba°M

**Bovine paratuberculosis in Italy : building infrastructures and defining the guidelines for control and for ranking of herds**

Proceedings of the 4th ParaTB Forum : 21 June 2014, Parma, Italy / [Parma : Efsa, 2014]. - Bull Int Dairy Fed. - Vol. p 45-50. - 9 bib ref [Nr. Estr. 5701/A]

ParaTB forum (4th : Parma, Italy : 21 June 2014)

Bachanek-Bankowska K, Wadsworth J, Mioulet V, Valdazo-Gonzetlez B, Eldaghayes IM, Dayhum AS, Kammon AM, Sharif MA, Grazioli°S, Brocchi°E, Subramaniam S, Pattnaik B, King DP, Knowles NJ

**Outbreaks of foot-and-mouth disease virus in Libya and Saudi Arabia during 2013 due to an exotic O/ME-SA/IND-2001 lineage**

Where science and policy meet: FMD risk management in a world of changing disease landscapes : open session of the standing technical and research committees of the EuFMD : Cavtat, Croatia, 29-31 October 2014 : book of abstracts / [s.l. : FAO, 2014]. - p 154-155 [Nr. Estr. 5877]

Open Session of the Research Group of the Standing Technical and Research Committees of the EuFMD : Cavtat, Croatia : 29-31 October 2014)

Bardini R, Nigrelli°A, Benaglia P, Faccini°S, Ost anello F

**Confronto dell'efficacia di due vaccini commerciali anti PCV2 in condizioni normali di allevamento** = Comparative efficacy of two commercial PCV2 vaccines in conventionally pigs

Large Anim Rev. - Vol. 20 no 1 ( 2014). - p 25-31. - 22 bib ref [Nr. Estr. 5665]

L'efficacia di due vaccini anti Porcine Circovirus 2 (PCV2) è stata valutata, in suini convenzionali, in termini di risposta anticorpale, quantità di genoma virale presente nel sangue, sintomi clinici e performances di accrescimento dallo svezzamento fino a 220 giorni di vita. Allo scopo è stato realizzato uno studio di campo randomizzato cieco in una azienda a ciclo chiuso di 420 scrofe, utilizzando 1050 suinetti provenienti da 5 bande consecutive di produzione, distribuiti in 3 gruppi. All'inizio della sperimentazione (25±2 giorni di vita), 349 animali (gruppo A) sono stati vaccinati intramuscolo (i.m.) con una dose di 0,5 ml di Circovac®, 351 animali (gruppo B) hanno ricevuto una dose i.m. di 1 ml di CircoFLEX®. I 350 soggetti del gruppo C sono stati utilizzati come animali di controllo non vaccinati. Tutti i suini sono stati pesati a 25, 105 e 220 giorni di vita ed è stato calcolato l'incremento ponderale medio giornaliero (IPMG). Le valutazioni sierologiche e virologiche (quantità di genoma virale presente nel sangue, determinata mediante real time PCR), sono state condotte esaminando i campioni di sangue prelevati mensilmente (dallo svezzamento fino a 6 mesi di vita), da un gruppo di 36 soggetti selezionati in modo casuale e appartenenti alla terza banda. Gli animali vaccinati hanno presentato un IPMG significativamente più elevato rispetto ai soggetti del gruppo di controllo nei periodi 25-105, 105-220 e 25-220 giorni di vita, minori percentuali di morti, scarti e soggetti sottopeso e minori quantità di genoma virale nel sangue. Tra i due vaccini, non è stata osservata nessuna differenza statisticamente significativa relativamente ai parametri considerati.

*The efficacies of two commercial Porcine Circovirus 2 (PCV2) vaccines were compared in conventional pigs based on humoral response, viral load, clinical observation, and growth performance from weaning to 220 days of age. A double-blind, randomised, and controlled field trial was performed on an Italian 420-sow farrow-to-finish farm. One thousand and fifty piglets, from five consecutive batches, were included in this trial. The piglets were stratified by sex, weight, parity of*

*the sow and randomly allocated to 3 groups. At inclusion (weaning at 25±2 days of age), 349 animals (group A) received a 0.5 ml intramuscular dose of Circovac®, 351 animals (group B) received a 1 ml intramuscular dose of CircoFLEX®. Another group (group C) of 350 pigs was kept as unvaccinated control. Weights were recorded at inclusion, at 105 and 220 days of age, and the average daily weight gain (ADWG) was calculated. For serologic and virologic studies, blood samples were collected monthly, from weaning to 180 days of life, from a randomly selected sample of animals coming from batch no. 3. Compared to the control group, vaccinated animals showed a significant increase of ADWG from 25-105, 105-220 and 25-220 days of age. Both animals vaccinated with Circovac® and those vaccinated with Circoflex® showed ADWG significantly higher than those of the control group during the three considered periods. The difference between ADWG of group A and group B was not statistically significant for any of the three time periods. The percentage of 25-to-220-day dead pigs and runts were very similar; however, incidence of underweight pigs was statistically lower in the vaccinated groups. The vaccination seemed to reduce the viral pressure in the facilities. This observation is supported by the qPCR data, which showed, that vaccinated animals had a lower serum viral load. No statistically significant difference in the considered parameters was observed between the two vaccines.*

Bassi° S, Carpana° E

#### **Examination of winter debris as a tool for the control of American foulbrood**

Sixth European Conference of Apidology EurBee : Murcia (Spain), 9-11 September 2014 / [s.l. : s.n., 2014]. - p 164 [Nr. Estr. 5808]

European Conference of Apidology (EurBee) (6th : Murcia (Spain) : 9-11 September 2014)

American foulbrood (AFB), caused by the spore-forming bacterium *Paenibacillus larvae* (*P. larvae*), is the most serious and widespread brood disease in honey bees (*Apis mellifera*). The clinical examination is still considered the gold standard for the diagnosis of AFB but it does not enable the detection of colonies with asymptomatic infections. Debris collected on the hive bottom trays can be a good indicator of the presence of *P.larvae* infection in the colony. The aim of this work was to study the relationship at the colony level between the number of *P.larvae* spores in the winter debris and the probability of developing the disease in the following spring. Winter debris collected from 351 hives belonging to 17 apiaries in the Emilia Romagna Region (Italy) were examined. Each colony was then clinically monitored monthly from March to June in the following spring. During this period the disease evolved in only one out of the 154 colonies (0.6%) in which the presence of *P.larvae* spores in the winter debris had not been recorded. Instead the disease was observed in 19/25 (76%) colonies with a number of *P.larvae* spores >100,000 CFU/g and a total of 44% of colonies with a number of spores > 1,000 CF U/g developed symptoms of AFB. This study showed that there is a dose correlation between the load of *P.larvae* spores in the colony's winter debris and the probability of developing the disease in spring. Monitoring the winter debris could be a helpful tool for reducing the impact of AFB.

Bassi° S, Galletti° G, Pizzuto° A

#### **A new bacteriological method for the detection of *Paenibacillus larvae* from beehive debris**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 102 - 3 bib ref [Nr. Estr. 5917]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

*Paenibacillus larvae*, a gram-positive endospore-forming bacterium, is the causative agent of american foulbrood the most severe and widespread disease affecting the brood of *apis mellifera*. the search of *p. larvae* spores in samples taken from the hive (honey, bees or debris) is a useful tool to identify infected colonies. a method for the detection of *p. larvae* on debris collected on the bottom of the hive has been recently developed in the czech republic (titera and haklova 2003). in that

method is used toluene to extract the spores from the debris and this is a critical point, furthermore it has long run times. In 2007, it was introduced a variant of this method which make use of tween 80 (tm) instead of toluene and appears to be significantly more effective in detecting p. larvae spores (bzdil 2007). also this technique is laborious and this limits the possibilities of use, especially when many samples must be examined at the same time as often occurs in practice. we have developed a method based on distilled water extraction of spores (wm). the execution of this method is simpler and analysis time is shorter if compared to the tm. the aim of this work was to describe this method and to compare the results obtained with wm and tm on the same samples. materials and methods: we examined 30 samples of beehive debris collected in 2013 at the bottom of 30 hives belonging to 12 apiaries. each sample was analyzed for the detection of p. larvae spores with wm and tm. wm was carried out as follows: a) put 1 g of debris in a 15 ml falcon containing 9 ml of sterile distilled water; b) vigorously shake by hand for 30 seconds; c) heat in a water bath at 85° 90 °c for 15 minutes; d) filter immediately after the heat treatment through a sterile gauze; e) plate 500 microliters of the filtered sample onto 5 plates (100µl/plate) of mypgg agar supplemented with nalidixic acid and pipemidic acid; f) incubate the agar plates at 37 ° c in an atmosphere with 10% co<sub>2</sub>; g) examine the plates after 3 days for the first reading and after 8 days for the final reading. tm was performed as described by bzdil (bzdil, 2007). results were expressed in cfu/g for both methods. we used cohen's kappa coefficient (k) to compare the results between wm and tm. in this step, results were classified as presence/absence. then we used the wilcoxon signed-rank test (v) and spearman's rank correlation ( ) to compare colony counts. results: in 13 out of 30 samples colonies did not grow on plate neither with wm nor with tm. in 16 samples colonies grew with both methods, and in 1 sample they grew only with tm. the agreement between the methods was high (k=0.933). in the 17 samples where colonies grew we compared colony counts. analysis suggested that there was no difference between the two methods (v=112, p-value=0.098; =0.947). discussion and conclusions: the analysis performed shows no evidence of difference between the two methods, both from quantitative and qualitative viewpoint. whereas the results obtained with the wm and the tm method not show significant differences, the use of the wm compared to tm presents practical advantages: it is less expensive, it is easier to perform and the sample preparation time is shorter (about 20 minutes with wm, about 4 hours with tm). these characteristics are important, especially when you need to examine large numbers of samples.

Bello C, Reverberi M, Fanelli C, Fabbri A, Scarpari M, Dall'Asta C, Angelucci°A, Bertocchi°L

#### **Aflatoxin control in feed by *Trametes versicolor***

Proceedings of the First DairyCare Conference : Copenhagen, August 22nd and 23rd 2014 / editor, C.H. Knight. - [s. l. : DairyCOST action FA1308, 2014]. - p 36 [Nr. Estr. 5856]

DairyCare Conference (1st : Copenhagen : August 22nd and 23rd 2014)

*Aspergillus flavus* are well known widely diffused fungi able to contaminate, already in the field, food commodities like seeds. Once the crop is contaminated, these fungi can develop and produce aflatoxins, secondary metabolites which are carcinogenic, teratogenic and mutagenic for animals and humans. These mycotoxins can enter the human food chain by the direct ingestion of contaminated seeds or processed food and by the consumption of animal products coming from livestock fed with contaminated silages. The requirement of products with low impact on the environment and on human health, able to control aflatoxin production, has increased. Several papers report the use of extracts from fungi to inhibit fungal development and mycotoxin production [1]. In this work the effect of bioactive compounds produced by the basidiomycete fungus *T. versicolor* on the aflatoxin production by *A. flavus* both in vitro and in maize, was investigated. The goal was to propose an eco-friendly tool for a significant control of aflatoxin production, in order to obtain feedstuffs and feeds with a high standard of quality and safety to enhance the wellbeing of dairy cows. The presence of *T. versicolor*, grown on sugar beet pulp, was able to inhibit the production of aflatoxin B1 in maize by *A. flavus*. Furthermore, treatment with culture filtrates of *T. versicolor* containing ligninolytic enzymes, showed a significant reduction of the content of aflatoxin B1 in contaminated maize. Moreover, treated and control maize samples were also compared under in vitro ruminant digestive condition to simulate the possible releasing of aflatoxins upon cow's

digestion. Finally, the effect of the bioactive compounds has been verified in vivo. Feed, contaminated with aflatoxin B1 and treated with *T. versicolor* has been administered to dairy cows and the treatment effect assessed by examining carry-over of aflatoxin B1 to Aflatoxin M1 in milk.

Ben-Dov D, Hadani Y, Ben-Simchon A, Alborali°L, Po zzi PS

**Guidelines for pig welfare in Israel**

Isr J Vet Med. - Vol. 69 no 1 ( 2014). - p 4-15. - 24 bib ref [Nr. Estr. 5681]

Pig production in Israel is limited to about 20 farms, producing approximately 200,000 pigs per year. In January 2013 "Guidelines for Swine Keeping" in Israel became effective, regulating minimal standards for space requirements of pigs, use of individual crates for sows, correct implementation of castration, tail docking and teeth clipping. This article compares the "Guidelines" in place in Israel with the European Council Directive 2008/120/EC of 18 December 2008 (minimum standards for the protection of pigs) and legislations in place in other European Countries to further improve the Council Directive. Furthermore this article summarizes the findings of Veterinary Services inspections carried out in 2013 in Israel with the purpose of verifying the compliance to the Guidelines by local pig farmers. Corrective measures imposed for non-compliant farms are also indicated.

Bencetti F, Pedrini G, Gaffuri°A, Martinelli°N, L ombardi°G

**Risultati preliminari dell'uso di vaccini stabulogeni per il controllo della "malattia degli ascessi" in allevamenti di capre da latte del Nord Italia** = Preliminary results about autogenous vaccines used to control "abscess disease" in dairy goats farms in northern Italy

Large Anim Rev. - Vol. 20 no 4 (Suppl 1) ( 2014). - p 115. - 6 bib ref [Nr. Estr. 6067]

Congresso Nazionale Societa Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) (21. : Foggia : 9-12 Settembre 2014)

Benedetti°D, Pezzoni°G, Chiapponi°C, Grazioli°S , Pongolini°S, Brocchi°E

**Epidemiologia molecolare dei virus della malattia vescicolare del suino in Italia nel periodo 2002-2013**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 18 [Nr. Estr. 5734]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

La Malattia Vescicolare del Suino (MVS) è una malattia infettiva contagiosa dei maiali sostenuta da un virus a RNA con polarità positiva appartenente al genere Enterovirus. Il suo genoma è organizzato in un'unica Open Reading Frame (ORF) fiancheggiata alle estremità 5' e 3' da regioni non tradotte (UTRs), l'unica ORF codifica per 4 proteine strutturali nella regione P1 (VP1, VP2, VP3 and VP4) e sette non-strutturali nelle regioni P2-P3 (2A, 2B, 2C, 3A, 3B,3C e 3D). Negli ultimi 10 anni, la MVS è stata diagnosticata solo in Italia ad eccezione di sporadici focolai segnalati in Portogallo (2003, 2007). L'epidemiologia molecolare del virus MVS, condotta rispetto alla porzione del genoma codificante per la proteina strutturale VP1, ha evidenziato sin dal 2004 la contemporanea circolazione in Italia di due sotto-gruppi virali: uno comprendente virus evoluti in Italia dal 1992 (lineaggio italiano) e l'altro comprendente virus isolati inizialmente in Portogallo nel 2003 e successivamente nel Centro-sud Italia dal 2004 (lineaggio Portoghese). Questo quadro epidemiologico è stato confermato dall'analisi filogenetica basata su una porzione della regione genomica 3D di 73 virus isolati tra il 2002 ed il 2010, che ha mostrato una concordanza del 100% con la clusterizzazione ottenuta in VP1. Recentemente nell'ambito di uno studio di validazione di

metodiche molecolari per la ricerca del virus della MVS, l'analisi filogenetica comparativa condotta su 27 ceppi virali e basata su tre diverse regioni genomiche, VP1, 3D e 5'UTR, ha mostrato una differente clusterizzazione di sette ceppi, la maggior parte dei quali isolati tra il 2011 ed il 2013; questi risultavano classificati nel lineaggio portoghese in base all'analisi genomica delle porzioni VP1 e 5'UTR, e al lineaggio italiano rispetto alla regione 3D. Questi risultati hanno suggerito l'occorrenza di un evento di ricombinazione tra i ceppi appartenenti ai due lineaggi circolanti, ulteriormente indagato attraverso il sequenziamento genomico completo dei virus coinvolti. Nove isolati, comprendenti i tre sotto-gruppi virali evidenziati dagli studi precedenti (Italiano, Portoghese e possibile ricombinante), sono stati interamente sequenziati attraverso tecniche di sequenziamento di nuova generazione (piattaforma Illumina) e l'evento di ricombinazione è stato confermato da specifici software (Simplot, Recco, RDP); in particolare i ceppi ricombinanti presentano le regioni 5'UTR, P1 e parte della P2 del lineaggio Portoghese e la rimanente parte del genoma del lineaggio Italiano. Questi dati confermano come gli eventi di ricombinazioni intervengono nei meccanismi evolutivi degli Enterovirus e nel caso specifico hanno contribuito alla variabilità genetica del virus MVS..

Bergagna S, Barbero R, Dezzutto D, Vitale N, Possidente R, Rossi F, Soncin AR, Richelmi G, Varello K, Colussi S, Maniaci MG, Gorla M, Romano A, Arrigoni N, Gennero MS

**Estimate agreement between ELISA and culture results for Mycobacterium avium subspecies paratuberculosis in sera and fecal samples**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 107 (Poster P-03.7) [Nr. Estr. 5769]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

Diagnosis of paratuberculosis (*Mycobacterium avium* subspecies paratuberculosis - MAP) can be a challenge primarily in latent stages of the infection. In fact, the definite diagnosis of MAP is very difficult to perform, however the enzyme-linked immunosorbent assay (ELISA) is largely used. The aim of the study was to evaluate the predictive value of serological tests, in agreement with culture results, for the diagnosis of paratuberculosis. Blood and fecal samples were collected from 300 lactating dairy cows in two known infected Friesian dairy herds. The serological tests are performed with two commercial kits: one for screening (Pourquier® ELISA Para-tuberculosis Antibody Screening-Montpellier-France) and one for confirmation (Pourquier® ELISA Paratuberculosis Antibody Verification-Montpellier-France), according to the protocol provided by the manufacturer. Culture results were classified as: negative, high shedder, moderate shedder, low shedder, while ELISA results were expressed in terms of the relationship between optical density value S/P (sample to positive values) in order to quantify the degree of positivity. To evaluate the agreement between the two tests the intraclass Correlation coefficient (ICC) was calculated using GLM mixed model and the index Kappa was also estimated. Data showed fair agreement between ELISA and culture (ICC=0.46). Culture negative samples showed S/P average of 0.91 (CI95%: 0.58-1.23), low shedder showed a S/P average of 1.54 (CI95%: 1.24-1.84), moderate shedder showed a S/P average of 1.82 (CI95%: 1.29-2.50) and high shedder showed a S/P average of 2.34 (CI95%: 1.72-2.96). Kappa 0.66 (CI95%: 0.42-0.80). Data showed fair agreement between ELISA and culture with these premises, serological tests, expressed as S / P, can be considered a valuable predictive tool for the diagnosis of paratuberculosis when combined with excretion as infected head became ELISA 4 months prior then culture.

Bertasi B, Tilola M, Malanga M, De Medici D, Delibato E, Bilei S, De Santis, Lovari S, Acutis PL, Modesto P, Peletto S, Losio MN

**Metodiche di biologia molecolare per l'identificazione di specie in prodotti alimentari : applicazione di metodi basati su protocolli internazionali e verifica di approcci quantitativi**

Ital J Food Safety. - Vol. 3 suppl 1 ( 2014). - p 20 (C41) [Nr. Estr. 5798]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (24. : Bologna : 10-12 settembre 2014)

L'identificazione di specie nei prodotti alimentari è divenuta recentemente una problematica significativa, volta ad evitare frodi nei confronti del consumatore e prevenire il consumo di sostanze dannose legate a determinate specie. All'inizio del 2013 il RASFF ha notificato un'allerta relativa alla contaminazione di carne di cavallo in differenti prodotti, che ha coinvolto numerosi stati; di conseguenza la Comunità Europea ha emanato una raccomandazione europea relativa a un piano coordinato di controllo volto a stabilire la prevalenza di pratiche fraudolente nella commercializzazione di determinati prodotti alimentari, per verificare la presenza di carne di cavallo in differenti matrici alimentari. In tale attività sono stati coinvolti anche i laboratori italiani operanti nell'ambito del Controllo Ufficiale. Contestualmente alla Raccomandazione CE, il Laboratorio di Riferimento Europeo per le proteine animali nei mangimi (EURLAP) ha pubblicato il metodo analitico di Real-Time PCR da utilizzare per identificare il DNA di cavallo per la ricerca della carne di cavallo nei campioni prelevati nell'ambito del piano. Quest'ultimo metodo e differenti altri metodi di biologia molecolare (tutte determinazioni qualitative non in grado di distinguere fra aggiunta volontaria e contaminazione accidentale), la maggior parte validati e approvati dal Laboratorio di Riferimento e dal Ministero della Salute, sono stati applicati, in differenti laboratori ufficiali. In particolare l'IZSLER di Brescia ha analizzato 324 campioni, di cui 14 risultati non conformi per la presenza di DNA di cavallo sono stati confermati mediante analisi di revisione dall'ISS e IZSLT. Inoltre, le differenze riscontrate a livello delle varie metodiche e la condivisione dei dati fra differenti Istituti hanno consentito di confrontare i risultati e di identificare alcuni problemi relativi ai sistemi di quantificazione.

Bertocchi°L, Fusi°F

#### **Il sistema di valutazione CReNBA per il benessere e la biosicurezza nell'allevamento bovino**

Ital J Food Safety. - Vol. 3 suppl 1 ( 2014). - p 8 (T02) [Nr. Estr. 5806]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (24. : Bologna : 10-12 settembre 2014)

Il benessere degli animali e la biosicurezza negli allevamenti da reddito è un argomento che sta diventando sempre più pressante e stringente, a causa del vistoso interesse che suscita nell'opinione pubblica e per la grande attenzione che i media gli riservano. In particolare, a seguito delle grandi emergenze sanitarie degli ultimi anni (es. pollo alla diossina, BSE, influenza aviaria), l'attenzione dei consumatori si è focalizzata dapprima sulla qualità e salubrità dei prodotti di origine animale, e in seguito sulla sostenibilità ed eticità delle produzioni, soprattutto se di tipo intensivo. L'Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, attraverso il Centro di Riferenza Nazionale per il Benessere Animale (CReNBA), ha messo a punto un sistema di valutazione del benessere e della biosicurezza per l'allevamento dei bovini (bovina da latte a stabulazione libera e bovina da carne – sono in corso d'opera i sistemi per le altre tipologie d'allevamento), basato sulle più recenti acquisizioni in materia di valutazione del rischio prodotte dall'EFSA, sul progetto di ricerca Welfare Quality®, sulla bozza normativa per il benessere bovino in sede a Strasburgo e sulla normativa vigente in materia (D. L.vo 146/2001 e 126/2011). Il sistema è composto da una serie di quesiti a risposta multipla divisi in 5 aree di pertinenza: Area A (Management aziendale e personale); Area B (Strutture ed attrezzature); Area C [Animal Based Measures (ABMs)]; Area D (Controllo delle condizioni ambientali e dei sistemi di allarme); Area E (Biosicurezza). Al termine della valutazione, i dati sono inviati al CReNBA che li elabora per produrre un certificato da restituire al valutatore (un medico veterinario istruito tramite un apposito corso di formazione). Il certificato riporta una serie di indicazioni sulla situazione dell'allevamento ed esprime due numeri che danno un'indicazione del livello di benessere medio di tutti gli animali presenti e del livello di rischio in termini di biosicurezza. Il sistema è uno strumento valido, facilmente applicabile in campo e in grado di fornire al veterinario valutatore un quadro dettagliato dei maggiori rischi e pericoli per il benessere e per la biosicurezza presenti nell'allevamento. Queste indicazioni possono essere utilizzate sia dalla veterinaria pubblica per categorizzare gli allevamenti

in diverse classi di rischio, sia dai veterinari liberi professionisti come supporto nell'attività di consulenza all'allevatore. Il miglioramento del benessere e della biosicurezza negli allevamenti bovini sarà un passo indispensabile nelle filiere del latte e della carne, da un lato per accrescere la sostenibilità delle aziende zootecniche e dall'altro per garantire ai consumatori le qualità etica ed ambientale, sempre più richieste.

Bertocchi°L, Fusi°F

**Manuale per la valutazione del benessere e della biosicurezza nell'allevamento bovino da latte a stabulazione libera**

Manuale per la valutazione del benessere e della biosicurezza nell'allevamento bovino da latte a stabulazione libera / Luigi Bertocchi, Francesca Fusi. - Brescia : Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia (IZSLER), Centro di referenza Nazionale per il Benessere Animale (CRenBA), 2014. - p 1-176. - 40 bib ref [Nr. Estr. 6029]

Bertocchi°L, Fusi°F, Donati°M, Lorenzi°V, Angelucci°A

**Per la salute e per il mercato**

Inf Zootec. - Vol. 60 no 16 ( 2014). - p 20-26 [Nr. Estr. 6022]

Bertocchi°L, Fusi°F, Donati°M, Lorenzi°V, Angelucci°A

**Reddito : l'impatto economico**

Inf Zootec. - Vol. 60 no 16 ( 2014). - p 28-29 [Nr. Estr. 6023]

Bertocchi°L, Fusi°F, Donati°M, Lorenzi°V, Angelucci°A

**Valutazione/1 : con quali condizioni l'obiettivo è raggiunto**

Inf Zootec. - Vol. 60 no 16 ( 2014). - p 30-35 [Nr. Estr. 6024]

Bertocchi°L, Fusi°F, Donati°M, Lorenzi°V, Angelucci°A

**Valutazione/2 : il nuovo metodo del Crenba**

Inf Zootec. - Vol. 60 no 16 ( 2014). - p 36-40 [Nr. Estr. 6025]

Bertocchi°L, Fusi°F, Donati°M, Lorenzi°V, Angelucci°A

**In pratica : l'applicazione in campo del sistema**

Inf Zootec. - Vol. 60 no 16 ( 2014). - p 42-46 [Nr. Estr. 6026]

Bertocchi°L, Fusi°F, Donati°M, Lorenzi°V, Angelucci°A

**Biosicurezza : contro i rischi biologici e chimici a carico dei bovini**

Inf Zootec. - Vol. 60 no 16 ( 2014). - p 48-53 [Nr. Estr. 6027]

Bertocchi°L, Fusi°F, Donati°M, Lorenzi°V, Angelucci°A

**In conclusione : il benessere è una realtà**

Inf Zootec. - Vol. 60 no 16 ( 2014). - p 54-55 [Nr. Estr. 6028]

Bertocchi<sup>°</sup>L, Vitali A, Lacetera N, Nardone A, Vari sco<sup>°</sup>G, Bernabucci U

**Seasonal variations in the composition of Holstein cow's milk and temperature–humidity index relationship**

Animal. - Vol. 8 n 4 ( 2014). - p 667-674. - 35 bib ref [Nr. Estr. 5644]

A retrospective study on seasonal variations in the characteristics of cow's milk and temperature–humidity index (THI) relationship was conducted on bulk milk data collected from 2003 to 2009. The THI relationship study was carried out on 508 613 bulk milk data items recorded in 3328 dairy farms from the Lombardy region, Italy. Temperature and relative humidity data from 40 weather stations were used to calculate THI. Milk characteristics data referred to somatic cell count (SCC), total bacterial count (TBC), fat percentage (FA%) and protein percentage (PR%). Annual, seasonal and monthly variations in milk composition were evaluated on 656 064 data items recorded in 3727 dairy farms. The model highlighted a significant association between the year, season and month, and the parameters analysed (SCC, TBC, FA%, PR%). The summer season emerged as the most critical season. Of the summer months, July presented the most critical conditions for TBC, FA% and PR%, ( $52\ 054 \pm 183\ 655$ ,  $3.73\% \pm 0.35\%$  and  $3.30\% \pm 0.15\%$ , respectively), and August presented higher values of SCC ( $369\ 503 \pm 228\ 377$ ). Each milk record was linked to THI data calculated at the nearest weather station. The analysis demonstrated a positive correlation between THI and SCC and TBC, and indicated a significant change in the slope at 57.3 and 72.8 maximum THI, respectively. The model demonstrated a negative correlation between THI and FA% and PR% and provided breakpoints in the pattern at 50.2 and 65.2 maximum THI, respectively. The results of this study indicate the presence of critical climatic thresholds for bulk tank milk composition in dairy cows. Such indications could facilitate the adoption of heat management strategies, which may ensure the health and production of dairy cows and limit related economic losses.

Bianchini<sup>°</sup>V, Luini<sup>°</sup>M, Borella<sup>°</sup>L, Parisi A, Jonas R, Kittl S, Kuhnert P

**Genotypes and antibiotic resistances of Campylobacter jejuni isolates from cattle and pigeons in dairy farms**

Int J Environ Res Pub Health. - Vol. 11 ( 2014). - p 7154-7162. - 18 bib ref [Nr. Estr. 5792]

Campylobacter jejuni is the most common food-borne zoonotic pathogen causing human gastroenteritis worldwide and has assumed more importance in Italy following the increased consumption of raw milk. Our objectives were to get an overview of genotypes and antibiotic resistances in C. jejuni isolated from milk, cattle feces, and pigeons in dairy herds of Northern Italy. flaB-typing was applied to 78 C. jejuni isolates, previously characterized by Multi-Locus Sequence Typing, and genotypic resistances towards macrolides and quinolones based on point mutations in the 23S rRNA and gyrA genes, respectively, were determined. flaB-typing revealed 22 different types with one of them being novel and was useful to further differentiate strains with an identical Sequence Type (ST) and to identify a pigeon-specific clone. Macrolide resistance was not found, while quinolone resistance was detected in 23.3% of isolates. A relationship between specific genotypes and antibiotic resistance was observed, but was only significant for the Clonal Complex 206. Our data confirm that pigeons do not play a role in the spread of C. jejuni among cattle and they are not responsible for milk contamination. A relevant number of bulk milk samples were contaminated by C. jejuni resistant to quinolones, representing a possible source of human resistant strains.

Bianchini<sup>°</sup>V, Turi V, Romanò A, Maisano<sup>°</sup>A, Manfred ini<sup>°</sup>A, Vicari<sup>°</sup>N, Luini<sup>°</sup>M

**Monitoring of zoonotic pathogens in feral pigeons in a rural area of Northern Italy**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy),

12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 234 - 5 bib ref [Nr. Estr. 5948]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

Bianco C, Panarese S, Bacci ML, Dottori°M, Bonilauri°P, Lelli°D, Ostanello F, Leotti G, Vila T, Joisel F, Sarli G

**Vaccine efficacy against PCV2-related reproductive pathology in gilts**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - v. 2. - p 621. - 2 bib ref [Nr. Estr. 5748]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Biasi°G, Cavallari M, Bonilauri°P, Rosamilia°A, Maioli°G, Gherpelli°Y, Dottori°M, Luppi°A

**Impiego della vaccinazione nelle scrofe per il controllo della malattia di Glaesser** = The Glaesser disease control using the vaccination in sows

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 147-151. - 6 bib ref [Nr. Estr. 5676]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

Un focolaio di polisierosite ha colpito, nei mesi di gennaio e febbraio 2012, un allevamento multisito di 1400 scrofe, situato in nord Italia. Il 50% dei suinetti di 28 giorni d'età ha manifestato febbre alta (41,5 °C), tosse, respiro addominale, gonfiore arti colare con zoppia e sintomi nervosi (decubito laterale, pedalamento e tremori) con un tasso di mortalità del 20% circa. Cinque suinetti sottoposti a necropsia e ad esame anatomopatologico hanno evidenziato quadri riferibili a malattia di Glaesser (polisierosite, artrite fibrinosa, meningite). Da campioni patologici raccolti durante l'esame anatomopatologico è stato isolato un ceppo di *Haemophilus parasuis* successivamente sierotipizzato, tramite AGID, come sierotipo 13. Il ceppo isolato è stato utilizzato per la produzione di un vaccino stabulogeno. Le scrofe sono state vaccinate due volte ad intervallo di 28 giorni e le scrofette a 115 e 140 giorni di vita. Tutte le scrofe e le scrofette sono state rivaccinate a 77 giorni di gestazione. Dall'introduzione della vaccinazione non sono stati riportati casi di polisierosite in allevamento.

*During January and February 2012 an outbreak of polyserositis occurred in a 1400 sows multisite herd of northern Italy. A large number of piglets (50%) 28 days old showed high fever (41,5°C), coughing, abdominal breathing, swollen joints with lameness and central nervous signs (lateral decubitus, paddling and trembling) and the piglets mortality was approximately 20%. Necropsies performed on 5 piglets showed lesions compatible with a diagnosis of Glaesser disease (polyserositis, fibrinous arthritis and meningitis). A strain of *Haemophilus parasuis* identified as serotype 13 was isolated from samples collected during the necropsies. The strain isolated was used to produce an autovaccine and sows were firstly vaccinated twice with an interval of 28 days and gilts were vaccinated at 115 and 140 day of life. All sows and gilts were then revaccinated at 77 days of gestation. After the introduction of the vaccination no cases of Glaesser disease were recorded in the herd.*

Bilato°D, Drigo M, Pasotto D, Amadori°M

**Analisi comparativa di parametri immunologici sierici, mucosali e cellulo-mediati dopo infezione di campo da virus PRRS (PRRSV)** = Comparative evaluation of the immune response to a field porcine respiratory and reproductive syndrome virus (PRRSV) infection in terms of serum and mucosal antibody, and cell-mediated immunity

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 205-209. - 3 bib ref [Nr. Estr. 5673]

Scopo di questo lavoro è stato confrontare lo sviluppo temporale dell'immunità umorale e cellulo-mediata in 2 gruppi di scrofette PRRS-free introdotte in un allevamento da riproduzione infetto da PRRSV. In particolare, sono stati analizzati anticorpi IgG sierici, IgG ed IgA salivari, risposta cellulo-mediata (test di rilascio di interferon-gamma specifico per PRRSV). Tali parametri sono stati valutati al fine di evidenziare possibili differenze nello sviluppo e nella cinetica della risposta immunitaria nei confronti del virus della PRRS. Le scrofette hanno contratto l'infezione attorno alle 7-9 settimane dall'ingresso in allevamento. Sono emersi in particolare 4 risultati salienti: A) la precocità della risposta Ab nei liquidi orali di gruppo è analoga a quella evidenziata nei sieri; B) buone condizioni di sanità, benessere e conduzione aziendale si associano ad una precoce risposta immunitaria umorale e, soprattutto cellulo-mediata (test IFN-gamma), a differenza di quello che si osserva in allevamenti "problema" per PRRS; C) la risposta cellulo-mediata presenta differenze anche elevate tra soggetti dello stesso gruppo, ma i gruppi tendono a distinguersi chiaramente tra loro rispetto a tale parametro; D) la positività anticorpale nel liquido orale di gruppo viene assicurata anche solo da pochi suini sieropositivi sul totale degli 8-10 soggetti che depositano i liquidi orali. Sono state osservate infine alcune criticità riguardo all'uso del cordino di gruppo per il recupero dei liquidi orali in suini di età > 12 settimane, da affrontare con opportune modifiche del protocollo d'impiego.

*The aim of this work was to compare the time-course of humoral and cell-mediated immunity in 2 groups of PRRS - free gilts introduced into a PRRSV-infected breeding herd. In particular, we investigated serum IgG antibody, PRRSV-specific IgA and IgG in oral fluids and the cell-mediated response (PRRSV-specific release of interferon-gamma). These parameters were measured in order to identify possible discrepancies in the development and kinetics of the immune response against PRRS virus. Gilts got regularly infected by PRRSV around 7-9 weeks after entering the farm. 4 results must be highlighted: A) the precocity of the Ab response in oral fluids group was similar to that seen in sera; B) good conditions of animal health, welfare and farm management were associated with an early humoral immune response and cell-mediated immunity, as well (gamma-IFN test) , in contrast to what is observed in PRRS "problem" herds; C) the cell-mediated response may be considerably different among subjects of the same group, but each group tends to clearly distinguish itself with respect to this parameter; D) Ab-positive oral fluid samples can derive from a minority of seropositive pigs out of the 8-10 individuals that deposit the oral fluids. Lastly, some problems were reported regarding the use of the cotton rope for collecting oral fluids of pigs aged > 12 weeks. These problems should be dealt with by proper modifications of the applied protocol.*

Bolzoni<sup>o</sup>L, Tessonni V, Groppi M, De\_Leo GA

**React or wait : which optimal culling strategy to control infectious diseases in wildlife**

J Math Biol. - Vol. 69 ( 2014). - p 1001-1025. - 68 bib ref [Nr. Estr. 5872]

We applied optimal control theory to an SI epidemic model to identify optimal culling strategies for diseases management in wildlife. We focused on different forms of the objective function, including linear control, quadratic control, and control with limited amount of resources. Moreover, we identified optimal solutions under different assumptions on disease-free host dynamics, namely: self-regulating logistic growth, Malthusian growth, and the case of negligible demography. We showed that the correct characterization of the disease-free host growth is crucial for defining optimal disease control strategies. By analytical investigations of the model with negligible demography, we demonstrated that the optimal strategy for the linear control can be either to cull at the maximum rate at the very beginning of the epidemic (reactive culling) when the culling cost is low, or never to cull, when culling cost is high. On the other hand, in the cases of quadratic control or limited resources, we demonstrated that the optimal strategy is always reactive. Numerical analyses for hosts with logistic growth showed that, in the case of linear control, the optimal strategy is always reactive.

Bonfanti L, Monne I, Tamba°M, Santucci U, Massi°P , Patregnani T, Loli\_Piccolomini L, Natalini S, Ferri G, Cattoli G, Marangon S

**Highly pathogenic H7N7 avian influenza in Italy**

Vet Rec. - Vol. 174 no 15 ( 2014). - p 382. - 6 bib ref ( ultimo accesso <http://veterinaryrecord.bmj.com/content/174/15/382.2.full.pdf+html> ) [Nr. Estr. 5710]

Boni A, Vaccari G, Di\_Trani L, Zaccaria G, Alborali°GL, Lelli°D, Cordioli°P, Moreno°AM

**Genetic characterization and evolution of H1N1pdm09 after circulation in a swine farm**

BioMed Res Int. - Vol. Article ID 598732 ( 2014). - 9 p. - 28 bib ref (ultimo accesso 23/10/2014 <http://www.hindawi.com/journals/bmri/2014/598732/>) [Nr. Estr. 5863]

Following the emergence of the A(H1N1)pdm09 in humans, this novel influenza virus was reverse transmitted from infected people to swine population worldwide. In this study we investigated the molecular evolution of A(H1N1)pdm09 virus identified in pigs reared in a single herd. Nasal swabs taken from pigs showing respiratory distress were tested for influenza type A and A(H1N1)pdm09 by real-time RT-PCR assays. Virus isolation from positive samples was attempted by inoculation of nasal swabs samples into specific pathogen free embryonated chicken eggs (ECE) and complete genome sequencing was performed on virus strains after replication on ECE or from original swab sample. The molecular analysis of hemagglutinin (HA) showed, in four of the swine influenza viruses under study, a unique significant amino acid change, represented by a two-amino acid insertion at the HA receptor binding site. Phylogenetic analysis of HA, neuraminidase, and concatenated internal genes revealed a very similar topology, with viruses under study forming a separate cluster, branching outside the A(H1N1)pdm09 isolates recognized until 2014. The emergence of this new cluster of A(H1N1)pdm09 in swine raises further concerns about whether A(H1N1)pdm09 with new molecular characteristics will become established in pigs and potentially transmitted to humans..

Boni A, Zaccaria G, Di\_Trani L, Alborali°GL, Lelli °D, Cordioli°P, Vaccari G, Moreno°A

**Sequenziamento completo di virus influenzali mediante next generation sequencing e piattaforma ION Torrent PGM**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 19 [Nr. Estr. 5735]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

L'uomo è parte integrante del complesso ciclo epidemiologico dei virus influenzali di tipo A (INF A), che emersi da un ospite animale, possono causare nella popolazione umana infezioni sporadiche e autolimitanti. Ne sono esempi i casi di trasmissione all'uomo di H5N1 HPAIV, endemico in molti Paesi, anche del bacino mediterraneo, ed H7N9, LPAIV circolante in Cina dal 2013. Al contrario, il virus H1N1pdm 2009, quadruplo riassortante di virus influenzali aviari e suini, si è diffuso rapidamente dal serbatoio animale all'uomo, causando la prima pandemia del terzo millennio, con gravi ripercussioni socio-sanitarie ed economiche. La sorveglianza attiva globale e la caratterizzazione dei virus influenzali sono strumenti essenziali per la preparazione contro potenziali eventi pandemici. Il Next Generation Sequencing (NGS) offre la possibilità di ottenere in tempi brevi informazioni sul genoma completo di patogeni infettivi. In questo studio è stato sviluppato e validato un protocollo NGS utilizzando il sequenziatore Personal Genome Machine (PGM) Ion Torrent. Sono stati analizzati n°42 virus di influenza suina (SI Vs) isolati dal 1998 al 2013 in allevamenti di Lombardia ed Emilia-Romagna. Il genoma virale è stato amplificato utilizzando primers universali per INF A. Le librerie sono state costruite utilizzando dei barcode nucleotidici, in modo da analizzare

più SIVs contemporaneamente su uno stesso chip. L'analisi bioinformatica è stata realizzata utilizzando il Ion Torrent suite software o il software CLC bio per le analisi de novo e mapping. Il protocollo NGS ha consentito il sequenziamento full genome ed analisi di tutti i sottotipi (H3N2, H1N1, H1N2 ed H1N1pdm2009) di SIVs inclusi nello studio. Sequenze ottenute con metodo di sequenziamento Sanger, eseguito su alcuni campioni, e quelle ottenute con NGS hanno mostrato 100% di identità nucleotidica, confermando la correttezza dei dati NGS, ottenuti in tempi più brevi ed a costi ridotti. I risultati delle analisi filogenetiche hanno confermato i dati della iniziale tipizzazione antigenica, indicando anche fenomeni evolutivi di SIVs circolanti negli allevamenti di Lombardia ed Emilia-Romagna durante 15 anni dello studio. La tecnologia NGS messa a punto, consente quindi la sorveglianza dei flussi genici interspecie ed intraspecie di INF A, utile ai fini della prevenzione in sanità umana ed animale ed il controllo continuativo del passaggio diretto o indiretto all'uomo di virus influenzali aviari o suini con potenziale pandemico.

Bonilauri°P, Carra°E, Rugna°G, Luppi°A, Magistr ali CF, Nigrelli°AD, Alborali°G, Biasi°G, GherPELLI°Y, Bergamini°F, MeriALDI°G

#### **Genotype characterization and antibiotic resistance of *Brachyspira hyodysenteriae* isolates in Italy between 2003 and 2012**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 116 [Nr. Estr. 5719]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

Swine dysentery (SD) caused by *Brachyspira hyodysenteriae* is a major pig disease worldwide including Italy. The increased resistance to pleuromutilins recorded in many countries represents an important challenge. In this study the Multilocus Sequence Typing (MLST) scheme was applied to 108 *B. hyodysenteriae* strains, isolated from 86 different farms distributed in various Regions of Italy between 2003 and 2012. In order to identify possible associations between genotypes and antibiotic resistance patterns the MIC value for pleuromutilins was determined using VetMICTM Brachy SVA (ver. 2). For each isolates seven housekeeping genes were sequenced (*adh*, *alp*, *est*, *gdh*, *glpK*, *pgm*, *thi*). According to their allelic profiles isolates were assigned in 23 sequence types (STs) of which 21 were new and two (ST8 and ST52) were previously reported in PubMLST. Significant linkage disequilibrium was found ( $p < 0.000$ ) both considering number of isolates ( $1a = 3.0102$ ) and number of STs ( $1a = 0.5017$ ). Five Clonal complex (Cc) and three singletons were identified by BURST analysis. Based on MIC results strains were allocated in two groups: sensitive strains (MIC  $< 1$  mcg/ml for both antibiotics) and strains with reduced sensitivity (MIC  $> 1$  mcg/ml for one or both antibiotics). The proportion of sensible strains in respect to different independent variables (clonal complex, year and region of isolation) was evaluated by generalized linear model (GLM). No statistically significant association with farms geographical localization, was observed, while Cc and year of isolation resulted significantly associated to the proportion of sensible strains ( $p < 0.05$ ). In particular strains belonging to Cc 2 or 3 resulted about 11 times more likely to be sensitive to pleuromutilins than other Cc (Odds ratio equa) to 11.42; CI95% 2.98-43.71,  $p < 0.01$ ) and a significant trend in reduction of susceptibility to pleuromutilins during time from 2003 to 2012 (70% to 20% respectively) was observed (Pearson's correlation  $r = -0.37$ , extension of the Wilcoxon rank-sum test for the trend,  $p < 0.01$ ). This study confirms MLST as reliable tool to investigate the diversity of *B. hyodysenteriae* strains. The application of this technique could lead to better understanding of the epidemiology of the infection, including sources and patterns of introduction, potentially providing support to more effective control and eradication strategies. The association among Ccs and pattern of sensitivity have to be investigated in order to clarified the molecular bases of observed profiles.

Boniotti°B

#### **Genetic variability of Italian PEDV strains**

Swine Enteric Coronavirus Diseases (SECD) International Meeting : September 23 - 25, 2014  
Chicago, Illinois, USA / [s.l. : s.n., 2014]. - p 11 [Nr. Estr. 6073]

Swine Enteric Coronavirus Diseases (SECD) International Meeting : Chicago, Illinois, USA :  
September 23 - 25, 2014)

Boniotti°MB, Gaffuri°A, Gelmetti°D, Tagliabue°S , Chiari°M, Mangeli°A, Spisani  
M, Nassuato C, Gibelli°L, Sacchi°C, Zanoni°M, Pa cciarini°ML

**Detection and molecular characterization of Mycobacterium microti isolates in wild boar from Northern Italy**

J Clin Microbiol. - Vol. 52 no 8 ( 2014). - p 2834-2843. - 56 bib ref [Nr. Estr. 5909]

Approximately 23,000 hunter-harvested wild boars from the pre-Alpine area of northern Italy were examined for tuberculosis over a 9-year period (2003 to 2011). Retropharyngeal and mandibular lymph nodes from the wild boars were examined grossly, and 1,151 of the lymph nodes were analyzed in our laboratory by histology (728 samples) and culture isolation (819 samples). Mycobacterium tuberculosis complex (MTBC)-specific PCR (1,142 samples) was used for molecular-level detection in tissue samples, as was a gyrB restriction fragment length polymorphism (RFLP) assay (322 samples). Lesions compatible with tuberculosis and indistinguishable from those described in cases of Mycobacterium bovis infection had been observed since 2003. Mycobacterium microti was identified directly in 256 tissue samples by the adopted molecular approaches. However, only 26 M. microti strains were obtained by culture isolation due to the well-known difficulties in isolating this slow-growing mycobacterium. During 2006, a prevalence study was performed in two provinces of the area, and the diffusion of M. microti was calculated to be 5.8% (95% confidence intervals surrounding the estimated prevalences [CIP95%], 3.94 to 7.68%). Over the following years (2007 to 2011), the presence of M. microti appeared to be stable. All isolates were genotyped by spoligotyping and exact tandem repeat analysis (ETR types A to F). In addition to the typical vole type (SB0118), a new spoligotype lacking the 43 spacers was found. Spoligotyping was also applied directly to tissue samples, and a geographical cluster distribution of the two spoligotypes was observed. This is the first report studying the diffusion and genetic variability of M. microti in wild boar.

Boniotti°MB, Gaffuri°A, Gelmetti°D, Tagliabue°S , Chiari°M, Mangeli°A, Spisani  
M, Nassuato C, Gibelli°L, Sacchi°C, Zanoni°M, Pa cciarini°ML

**Detection and molecular characterisation of Mycobacterium microti in wild boar from Northern Italy**

VI International M. bovis conference : Cardiff, Wales, Great Britain, 16th - 19th June 2014 :  
Delegates Handbook / [s.l. : s.n., 2014]. - 2 p [Nr. Estr. 5914]

International M. bovis conference (6th : Cardiff, Wales : 16th - 19th June 2014)

Introduction. A monitoring activity for tuberculosis was conducted on about 23000 hunter-harvested wild boars from a Prealps area in Northern Italy over a 9 year period (2003-2011). Materials and methods Retropharyngeal and mandibular lymph nodes of wild boar were examined grossly. Histology and culture isolation were performed in 728 and 819 samples respectively. Molecular detection in tissue samples was done by a Mycobacterium tuberculosis Complex (MtbC) specific PCR (1142). Positive samples were submitted to a gyrB-RFLP assay (332) to identify MtbC species. Isolated strains were genotyped by Spoligotyping and Exact Tandem Repeat analysis (ETR A-F). Results Lesions compatible with tuberculosis and indistinguishable from those described in the case of Mycobacterium bovis infection had been observed since 2003. Mycobacterium microti was identified directly in tissue samples by the gyrB-RFLP assay. During 2006 a prevalence study was carried out in two provinces of the area: the diffusion of M. microti was calculated to be 8.5% (095%: 6.24 — 10.69). Over the following years (2007-2011) the presence of M. microti showed to be stable. Spoligotyping of the isolated strains (26) revealed the presence of the typical vole type (SB0118)

and of a new spoligotype lacking all the 43 spacers. Spoligotyping applied directly on tissue samples showed an evident geographical clustering distribution of the two spoligotypes. Discussion and Conclusion This study showed a considerable presence of *M. microti* in wild boar from Northern Italy and it highlighted the need of a constant monitoring to control possible interferences with the diagnosis of TB infections.

Boniotti<sup>o</sup>MB, Loda<sup>o</sup>D, Cozza D, Tagliabue<sup>o</sup>S, Galie ro G, Pacciarini<sup>o</sup>ML

**Genotyping of Mycobacterium bovis isolates from water buffalo (*Bubalus bubalis*)**

VI International *M. bovis* conference : Cardiff, Wales, Great Britain, 16th - 19th June 2014 : Delegates Handbook / [s.l. : s.n., 2014]. - 1 p [Nr. Estr. 5915]

International *M. bovis* conference (6th : Cardiff, Wales : 16th - 19th June 2014)

Introduction. *Mycobacterium bovis* can infect and cause bovine tuberculosis in all bovid animals including water buffalo. In Italy, mainly in Campania region, buffalo breeding is an important source of income and buffalo tuberculosis prevalence is 0.97%. In this study we genotyped *M. bovis*/*M. caprae* isolates using spoligotyping and variable number of tandem repeats (VNTR) typing to understand the molecular and epidemiological characteristics of the isolates from buffalo. Materials and methods More than 300 isolates coming from about two hundred outbreaks were genotyped by Spoligotyping and 12 variable number of tandem repeats (VNTR) loci: ETR A-E, 2163a, 2163b, 4052, 1895, 3155, 3232 and 2996. Introduction *Mycobacterium bovis* can infect and cause bovine tuberculosis in all bovid animals including water buffalo. In Italy, mainly in Campania region, buffalo breeding is an important source of income and buffalo tuberculosis prevalence is 0.97%. In this study we genotyped *M. bovis*/*M. caprae* isolates using spoligotyping and variable number of tandem repeats (VNTR) typing to understand the molecular and epidemiological characteristics of the isolates from buffalo. Materials and methods More than 300 isolates coming from about two hundred outbreaks were genotyped by Spoligotyping and 12 variable number of tandem repeats (VNTR) loci: ETR A-E, 2163a, 2163b, 4052, 1895, 3155, 3232 and 2996. Results By spoligotyping we differentiated 37 different profiles. As well as in cattle, the most widespread was the SB0120 (46% vs 53% in cattle) while the second one was SB0920 (13% vs 1%). We also found 3 *M. caprae* spoligotypes (SB0866, SB0415 and SB0418). Genotyping by spoligotyping and VNTR differentiated 118 genotypes: 85 unique and 31 clusters. The cluster more widespread was SB0920-5 3 5 3 3 10 4 4 4 3 6 5 (21 isolates) and SB0120-4 5 5 3 3 10 4 4 4 3 6 5 (19 isolates). Interestingly, related or different genotypes were frequently observed in the same herd. Discussion and Conclusion. This study showed a considerable genetic diversity among isolates coming from buffalo herds. Moreover, the coexistence of different strains in the same herd could likely originate from both different sources of contamination and reiterated infections within the farm.

Bontempo V, Jiang XR, Cheli F, Lo\_Verso L, Mantovani G, Alborali<sup>o</sup>L, Wu CC, Vitari F, Domeneghini C, Agazzi AP

**Administration of a novel plant extract product via drinking water to post-weaning piglets : improvement in performance and gut health despite *E. coli* challenge**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 231(Poster P191) [Nr. Estr. 5723]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

Increased susceptibility to enterotoxigenic *Escherichia coli* infections and acute diarrhea are common problems in newly weaned piglets in large commercial farms. With reduced emphasis on antibiotics to promote herd health, options to address bacterial infection include plant-based feed additives and restriction in access to feed in order to reduce proliferation of bacteria and incidence of scour. A novel plant extract (PE) derived from leaves of green tea and fruit of pomegranate and the

like, (Grazix solution, LiveLeaf Inc., USA) has been noted to reduce scour in small farms but its effect on animal performance and gut health has not been documented. The objective of this study was to assess performance and gut health after a challenge with a common pathogen in piglets provided with the PE or not. One hundred and forty-four piglets were weaned at 24 days and allocated to 8 groups according to a 2 X 2 X 2 factorial combination of (a) treatment [water without product (CT) vs. 8 pl/kg/d PE in drinking water (PE)], (b) feeding regimen [ad libitum (AD) vs. restricted (RE)], and (c) oral *E. coli* challenge [sham (-) vs. viable bacteria (+)], using 6 pens per group with 3 piglets per pen. Performance and characteristic of feces was measured every 7 days for a total of 5 weeks (35 days). On day 35, twelve piglets on restricted diets in both arms were slaughtered and their distal ileum examined. After 35 days, piglets given the PE had higher average daily gain ( $p = 0.03$ ) and higher gain to feed ratios ( $p = 0.10$ ) than piglets not given the PE. Irrespective of feeding regime, piglets provided with PE had lower fecal scores (meaning little to no scour) after administration of *E. coli* than did those in the CT groups. On a histologic level, the total area ( $p = 0.02$ ), medulla area ( $p = 0.06$ ) and cortex area ( $p = 0.01$ ) of follicles in PE piglets were smaller than those in the CT group. The *E. coli* challenge increased macrophage numbers in the ileum mucosa in CT pigs ( $p = 0.005$ ), while PE supplementation reduced the number of macrophages in challenged piglets ( $p = 0.003$ ). These results suggest that PE supplementation may improve gut health status of post-weaning piglets and counteract some negative effects when piglets are challenged with *E. coli*.

Bozzo G, Bonerba E, Di\_Pinto A, Bolzoni<sup>o</sup>G, Ceci E, Mottola A, Tantillo G, Terio V  
**Occurrence of Prototheca spp. in cow milk samples**

New microbiol. - Vol. 37 ( 2014). - p 459-464. - 31 bib ref [Nr. Estr. 5943]

Protothecosis is a potential zoonotic disease associated with bovine mastitis which can be transmitted to humans through contaminated milk. Considering the increasing prevalence of bovine mastitis due to *Prototheca* species, individual cow milk samples were analyzed using microbiological examination and biomolecular assay. Aspects related to health requirements for milk production, clinical and histological bovine mastitis were also described. The results showed 24/257 (9.3%) culture-positive samples and 42/257 (16.3%) PCR-positive samples. Moreover in 5 cows with somatic cell count over 106/mL presented histological features of mastitis. This study reveals that the presence of *Prototheca* species in dairy herds was related to the hygienic conditions of the milking equipment, showing an emerging public health issue.

Brocchi<sup>o</sup>E, Grazioli<sup>o</sup>S, Dho<sup>o</sup>G, Eldaghayes I, Dayh um A, Kammon A, Sharif M, Ferrari G

**Serological survey in Libya to assess FMD viruses circulation and vaccine immune response**

Where science and policy meet: FMD risk management in a world of changing disease landscapes : open session of the standing technical and research committees of the EuFMD : Cavtat, Croatia, 29-31 October 2014 : book of abstracts / [s.l. : FAO, 2014]. - p 162-163 [Nr. Estr. 5878]

Open Session of the Research Group of the Standing Technical and Research Committees of the EuFMD : Cavtat, Croazia : 29-31 October 2014)

Busi<sup>o</sup>C, Lavazza<sup>o</sup>A, Falcone E, Canelli<sup>o</sup>E, Monini M, Ruggeri FM, Boniotti<sup>o</sup>MB  
**Phylogenetic analysis of avian rotaviruses in Italy**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 263 - 1 bib ref [Nr. Estr. 5886]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa

(Italy) : October 12-15, 2014)

Camarda A, Pugliese N, Cavadini°P, Circella E, Cap ucci°L, Caroli A, Legretto M, Mallia E, Lavazza°A

**Detection of the new emerging rabbit haemorrhagic disease type 2 virus (RHDV2) in Sicily from rabbit (*Oryctolagus cuniculus*) and Italian hare (*Lepus corsicanus*)**

Res Vet Sci. - Vol. 97 n 3 ( 2014). - p 642-645. - 20 bib ref [Nr. Estr. 5900]

Rabbit haemorrhagic disease virus (RHDV), a member of the genus Lagovirus, causes rabbit haemorrhagic disease (RHD), a fatal hepatitis of rabbits, not previously reported in hares. Recently, a new RHDV-related virus emerged, called RHDV2. This lagovirus can cause RHD in rabbits and disease and mortality in *Lepus capensis* (Cape hare). Here we describe a case of RHDV2 infection in another hare species, *Lepus corsicanus*, during a concurrent RHD outbreak in a group of wild rabbits. The same RHDV2 strain infected rabbits and a hare, also causing a RHD-like syndrome in the latter. Our findings confirmed the capability of RHDV2 to infect hosts other than rabbits and improve the knowledge about the epidemiology and the host range of this new lagovirus.

Cano-Gómez C, Agüero M, Robles A, Villalba A , Elizalde M, Moreno°A, Fernandez-Pinero J

**Development of specific quantitative real-time RT-PCR assays for mediterranean bluetongue virus serotyping**

4th International Conference "Bluetongue and other Orbivirus diseases" : 5 - 7 November 2014, Rome / [s.l. : s.n., 2014]. - p 163-164 [Nr. Estr. 5964]

International Conference "Bluetongue and other Orbivirus diseases" (4th : Rome : 5 - 7 November 2014)

Capra E, Cremonesi P, Cortimiglia°C, Bignoli G, Ri cchi°M, Moroni P, Pesce A, Luini°M, Castiglioni B

**Simultaneous identification by multiplex PCR of major *Prototheca* spp. isolated from bovine and buffalo intramammary infection and bulk tank**

Lett Appl Microbiol. - Vol. 59 no 6 ( 2014). - p 642-647. - 32 bib ref [Nr. Estr. 5950]

Bovine mastitis caused by *Prototheca* spp. infection is increasing worldwide, therefore becoming more relevant to the dairy industry. Almost all *Prototheca* isolates from bovine mammary protothecosis came from *P. zopfii* genotype 2, with a lower prevalence of infection due to *P. blaschkeae* and rarely to *P. wickerhamii*. In this study, we report the development of two multiplex PCR assays able to discriminate among the three species responsible for bovine intramammary infection (IMI). Our assay is based on the specific amplification of new DNA target from mitochondria and chloroplasts partial sequences, of different *Prototheca* isolates. Both methods were set up using reference strains belonging to all *Prototheca* species and validated by the analysis of 93 isolates from bovine and buffalo IMI and bulk tank milk samples. The investigation involves 70 isolates from North, 13 from Central and 10 from South Italian regions. Isolates from bovine were most commonly identified as *P. zopfii* genotype 2, and only in one case as *P. blaschkeae*, whereas isolates from buffaloes belonged both to *P. zopfii* genotype 2 and *P. wickerhamii*. These findings proved the suitability of our multiplex PCRs as a rapid test to discriminate among pathogenic *Prototheca* strains.

Capucci°L, Cavadini°P, Botti°G, Brocchi°E, Lava zza°A

## **Diagnosi di laboratorio della Rabbit haemorrhagic disease (RDH) causata dall'RHDV2**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 24 [Nr. Estr. 5737]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

La Malattia emorragica del coniglio (RHD) è un'epatite acuta del coniglio adulto (*Oryctolagus cuniculus*) ad esito letale con mortalità e morbilità dell' 80-90%. Emersa negli anni '80, l'RHD si è rapidamente diffusa a più continenti. L'agente causale è un calicivirus del genere *Lagovirus*. Nel 2010 in focolai di RHD in Francia, è stata identificata una variante genetica dell'RHDV. Studi recenti ipotizzano che la variante sia emersa ex novo da un'origine non identificata e che costituisca quindi una nuova specie virale (RHDV2). La proteina capsidica (VP1 - 60 kDaltons), nella parte che contiene gli aa di superficie del virus (regione P2 aa 287-484), mostra una elevata divergenza, in media del 22% circa. Fra le diverse conseguenze della significativa diversità genetica fra i virus, quella che i metodi diagnostici sviluppati ed in uso per l'RHDV mostrano significativi limiti nella diagnosi dell'RHDV2. Per i metodi virologici, si è dimostrato che l'emo-agglutinazione (HA) mantiene la sua validità, fermo restando le sue limitazioni generali (possibili falsi negativi per ceppi non HA e strutturalmente degradati). Per le reazioni ELISA sandwich se basate su sieri policlonali prodotti verso l'RHDV, possono essere usate, ma considerando il significativo calo in sensibilità e quindi un concreto rischio di esiti falsi negativi. Presso il CRN di Brescia è stato prodotto un nuovo pannello di MAbs anti-RHDV2 con i quali è stata allestita un'ELISA specifica (differenziale) per il nuovo virus con performance diagnostiche simili a quella per l'RHDV. In aggiunta sono studiati nuovi primers (F: posizione nt sulla VP1 109-129; R: posizione nt VP1 567-590) che consentono di identificare in RT-PCR l'RHDV2, cross reagendo solo in alcuni casi e con bassa sensibilità con l'RHDV. Per la sierologia i diversi metodi usati per l'RHDV (ELISA competizione, ELISA IgG, IgA ed IgM) sono stati resi RHDV2 specifici semplicemente sostituendo i reagenti immunologici. Nel caso della cELISA il rapporto fra i titoli ottenuti con i due metodi (variabile da 1/4 a 1/64) permette di distinguere quale dei due virus ha infettato gli animali in esame (o quale vaccino è stato usato). Dati preliminari indicano che i titoli IgA consentono di identificare l'occorrenza di re-infezioni enteriche in animali immuno-protetti e il tipo di RHDV responsabile. In ogni caso va tenuto presente che la diagnosi dell'RHDV2 potrebbe risultare più difficoltosa di quella dell'RHDV, sia per una potenziale maggior presenza di casi cronici che per il concreto riscontro di una maggior variabilità antigenica.

Capucci°L, Cavadini°P, Botti°G, Brocchi°E, Lava zza°A

## **Epidemiologia della Rabbit haemorrhagic disease causata dall'RHDV tipo 2 ed evoluzione del virus**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 25 [Nr. Estr. 5738]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

La RHD è un'epatite acuta virale del coniglio adulto (*Oryctolagus cuniculi*) con indici di mortalità e morbilità superiori all'80%. Emersa in Cina negli anni '80, l'RHD ha devastato la conigliocoltura Europea sino all'introduzione del relativo vaccino. All'oggi è endemica in più continenti, in particolare la dove è più presente il coniglio selvatico. Nel 2010 in focolai occorsi in Francia è stata identificata una variante genetica dell'RHDV, un *Calicivirus*, con divergenza nucleotidica della proteina capsidica intorno al 20%. Sebbene apparentemente meno patogena, la malattia colpiva sia animali vaccinati che di poche settimane. Studi successivi hanno portato ad ipotizzare che la variante fosse in realtà l'emergenza di una nuova specie virale (RHDV2). I dati a disposizione sulla diffusione dell'RHDV2 sono desumibili quasi esclusivamente da pubblicazioni scientifiche recenti e per poco o nulla dai sistemi di sorveglianza nazionali. L'RHDV2 si è rapidamente diffuso in Italia e Spagna, con la Francia principali produttori di conigli, ove più focolai sono stati identificati nel 2011. In Spagna e Francia l'RHDV2 è stato ripetutamente identificato anche nella popolazione selvatica, ove sembrerebbe manifestare un maggior grado di patogenicità. È recente l'identificazione dell'RHDV2 anche in UK. Sebbene con capacità diffusive comparabili a quelle dell'RHDV, la percentuale di

mortalità indotta appare minore e variabile (fra il 10 e 60%). Analisi antigeniche effettuate con pannelli di MAb anti-RHDV e anti-RHDV2 portano a classificare i due virus al confine fra sottotipi distanti e sierotipi. Il dato è confermato anche dai dati sierologici comparati e dalla limitata protezione rilevata direttamente sul campo. Da qui la necessità di approntare un vaccino omologo per l'RHDV2. L'analisi antigenica degli isolati nazionali dal 2011 all'oggi, per quanto limitata dal basso numero, dimostra la rapida comparsa di più varianti RHDV2 con frequenza superiore a quella rilevata durante i primi anni di diffusione dell'RHDV. Ciò potrebbe derivare da almeno due fattori. Quale virus di recente emergenza, l'adattamento al nuovo ospite potrebbe essere ancora in corso, con selezione di genotipi a maggior fitness. La popolazione cunicola Europea, sia commerciale che selvatica, è in larga parte immunizzata e quindi anche protetta, dall'RHDV. Tale stato protegge invece in minima parte dall'RHDV2 che ha quindi relativa libertà di diffondersi. In aggiunta, quale virus ad RNA, potrebbe essere "spinto", attraverso successivi minimi cambi antigenici, verso una vero sierotipo. Per quanto sopra, risulta indubbia l'importanza di seguire l'evoluzione antigenica dell'RHDV2 anche in funzione della scelta del ceppo/i da utilizzare nella produzione dei vaccini.

Caruso C, Dondo A, Cerutti F, Masoero L, Rosamilia<sup>o</sup> A, Zoppi S, D'Errico V, Grattarola C, Acutis PL, Peletto S

**Aujeszky's disease in red fox (*Vulpes vulpes*) : phylogenetic analysis unravels an unexpected epidemiologic link**

J Wild Dis. - Vol. 50 no 3 ( 2014). - p 707-710. - bib ref [Nr. Estr. 6085]

We describe Aujeszky's disease in a female of red fox (*Vulpes vulpes*). Although wild boar (*Sus scrofa*) would be the expected source of infection, phylogenetic analysis suggested a domestic rather than a wild source of virus, underscoring the importance of biosecurity measures in pig farms to prevent contact with wild animals.

Casà G, Gallina L, Savini F, Morent M, Trentin B, Piquemal D, Lavazza<sup>o</sup>A, Puleio R, Cannella V, Purpari G, Di\_Marco P, Guercio A, Scagliarini A

**Lesioni cutanee proliferative nei ruminanti : evidenza di infezioni multiple da virus epiteliotropi**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 28 [Nr. Estr. 5739]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

Le famiglie Papillomaviridae and Poxviridae comprendono diverse specie virali in grado di infettare i mammiferi. Tra i Poxviridae, i generi Parapoxvirus ed Orthopoxvirus, includono virus che penetrano tramite soluzioni di continuo della pelle o delle mucose e provocano lesioni pustolose nei ruminanti e nell'uomo. La famiglia Papillomaviridae comprende numerosi generi nei quali sono compresi tipi virali in grado di infettare i ruminanti. Questo studio è stato effettuato allo scopo di identificare i virus epiteliotropici responsabili di forme cliniche definite comunemente "papillomatosi" nei bovini. A questo scopo, campioni cutanei prelevati da animali colpiti da lesioni proliferative, sono stati sottoposti ad analisi istopatologiche e virologiche. La Rolling circle amplifications (RCA) è stata effettuata allo scopo di identificare la presenza di genomi circolari, successivamente analizzati mediante enzimi di restrizione e sequenziamento. Tutti i campioni di DNA sono stati inoltre analizzati mediante mini- array, un test innovativo in grado di identificare nello stesso campione la presenza di acido nucleico virale di diversi generi di poxvirus zoonosici. I risultati ottenuti hanno permesso di dimostrare che, in molti casi, le lesioni proliferative erano il risultato di co-infezioni da parte di papillomavirus e poxvirus. In particolare Epsilon-papillomavirus e Delta-papillomavirus BPV-1 e 2, responsabili di fibropapillomatosi, sono stati identificati in un numero limitato di casi mentre la maggior parte degli animali è risultato infetto con Xi papillomavirus. Le analisi eseguite con miniarray

assay hanno permesso di rivelare che la maggior parte degli animali era co-infetto con Pseudocowpoxvirus o Bovine Papular Stomatitis virus e Cowpoxvirus. Le successive analisi tramite sequenziamento hanno confermato questi risultati. I dati ottenuti dimostrano che le cosiddette "papillomatosi" possono essere il risultato di infezioni multiple da parte di diverse specie di virus epiteliotropi e che le sole analisi istopatologiche e microscopiche non sono in grado di identificare tutti i virus presenti nelle lesioni alcuni dei quali trasmissibili all'uomo.

Casadio<sup>o</sup>M, Massi<sup>o</sup>P, Tosi<sup>o</sup>G, Fiorentini<sup>o</sup>L, Tadde i<sup>o</sup>R, Bolognesi PG, Lupini C, Cecchinato M, Catelli E

**Valutazione del livello di contaminazione batterica in uova ed embrioni di pollo presso un incubatoio industriale** = Evaluation of the level of bacterial contamination of eggs and dead-in-shell chicks in an industrial hatchery

Large Anim Rev. - Vol. 20 no 3 ( 2014). - p 119-123. - 16 bib ref [Nr. Estr. 6071]

Carenze igienico-sanitarie a livello di allevamento di riproduttori si amplificano in maniera esponenziale nell'incubatoio e la popolazione microbica presente in incubatoio ha un impatto rilevante sulla qualità dei pulcini, la loro possibilità di sopravvivenza nell'allevamento di destinazione e le future performances produttive. Scopo del presente lavoro è stato quello di valutare il grado d'inquinamento batterico di uova di pollo in un incubatoio industriale della Romagna nelle diverse fasi dell'incubazione. Sono state campionate e sottoposte ad esami batteriologici, dall'arrivo alla schiusa, 20 partite di uova. Nelle uova in arrivo la contaminazione batterica è risultata piuttosto scarsa sia a livello dei gusci che dei tuorli. Sono stati isolati E. coli, Pseudomonas aeruginosa e altri batteri minori quali Citrobacter freundii, Providencia alcalifaciens, Enterobacter spp., Enterobacter ag-glomerans, Pseudomonas cepacia e Sphingomonas paucimobilis. Alla schiusa la contaminazione batterica è risultata nettamente più consistente. Escherichia coli era presente nell'88,25% delle uova non schiuse "beccate" ed in gran parte delle uova non schiuse con guscio integro (22%). Anche alla schiusa sono stati isolati batteri minori appartenenti a varie specie (in associazione o meno ad E. coli o Salmonella Livingstone), in particolare: Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter spp., Escherichia fergusonii, Citrobacter koseri, Citrobacter amalonaticus, Citrobacter freundii, Acinetobacter calcoaceticus, Acinetobacter lwoffii, Serratia liquefaciens. Salmonella Livingstone è stata isolata da uova non schiuse di due partite. I risultati dell'indagine confermano il ruolo di E. coli quale principale contaminante dell'incubatoio ed importante causa di mortalità embrionale.

*Hygiene and biosecurity deficiencies in breeding farms are amplified exponentially in the hatchery. The microbial population present in the hatchery has a significant impact on the quality of the chicks, on their chances of survival and their productive performances. The purpose of the present study was to assess the degree of bacterial contamination of chicken eggs in an industrial poultry hatchery. Samples of twenty batches of eggs, collected at different stages of the incubation, from the arrival to the hatcher, were subjected to bacteriological tests. The bacterial contamination of the eggs at the arrival, both of the shells than of the yolks, was found to be rather low. Escherichia coli, Pseudomonas aeruginosa and other bacteria of minor importance such as Citrobacter freundii, Providencia alcalifaciens, Enterobacter spp., Enterobacter agglomerans, Pseudomonas cepacia, Sphingomonas paucimobilis were isolated. At the hatching, the bacterial contamination was significantly larger. Escherichia coli was present in the 88,25% of eggs pipped but chicks dead-in-shell, and in most of the chicks dead-in-shell without pipping (22%). Various other bacteria species were also isolated (in association or not with Escherichia coli or Salmonella Livingstone), in particular: Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter spp., Escherichia fergusonii, Citrobacter koseri, Citrobacter amalonaticus, Citrobacter freundii, Acinetobacter calcoaceticus, Acinetobacter lwoffii, Serratia liquefaciens. Salmonella Livingstone was isolated from dead-in-shell chicks of two batches of eggs. The results of the present survey confirm the role of Escherichia coli as one of the major bacterial contaminant of poultry hatcheries. Moreover, results of our study highlight how the hatchery may have an amplifier effect of the bacterial contamination. Pseudomonas aeruginosa and Escherichia coli, present respectively in the 5% and in the 10% of the batches of eggs at the arrival, were found during the hatching respectively in the 15% and 100% of dead-in-shell chicks.*

Castrucci MR, Facchini M, Di\_Mario G, Garulli B, Sciaraffia E, Meola M, Fabiani C, De\_Marco MA, Cordioli°P, Siccardi A, Kawaoka Y, Do natelli I

**Modified vaccinia virus Ankara expressing the hemagglutinin of pandemic (H1N1) 2009 virus induces cross-protective immunity against Eurasian 'avian-like' H1N1 swine viruses in mice**

Influenza Other Respir Viruses. - Vol. 8 no 3 ( 2014). - p 367-375. - 36 bib ref [Nr. Estr. 5833]

**Objectives** To examine cross-reactivity between hemagglutinin (HA) derived from A/California/7/09 (CA/09) virus and that derived from representative Eurasian "avian-like" (EA) H1N1 swine viruses isolated in Italy between 1999 and 2008 during virological surveillance in pigs. **Design** Modified vaccinia virus Ankara (MVA) expressing the HA gene of CA/09 virus (MVA-HA-CA/09) was used as a vaccine to investigate cross-protective immunity against H1N1 swine viruses in mice. **Sample Two** classical swine H1N1 (CS) viruses and four representative EA-like H1N1 swine viruses previously isolated during outbreaks of respiratory disease in pigs on farms in Northern Italy were used in this study. **Setting** Female C57BL/6 mice were vaccinated with MVA/HA/CA/09 and then challenged intranasally with H1N1 swine viruses. **Main outcome measures** Cross-reactive antibody responses were determined by hemagglutination-inhibition (HI) and virus microneutralizing (MN) assays of sera from MVA-vaccinated mice. The extent of protective immunity against infection with H1N1 swine viruses was determined by measuring lung viral load on days 2 and 4 post-challenge. **Results and Conclusions** Systemic immunization of mice with CA/09-derived HA, vectored by MVA, elicited cross-protective immunity against recent EA-like swine viruses. This immune protection was related to the levels of cross-reactive HI antibodies in the sera of the immunized mice and was dependent on the similarity of the antigenic site Sa of H1 HAs. Our findings suggest that the herd immunity elicited in humans by the pandemic (H1N1) 2009 virus could limit the transmission of recent EA-like swine HA genes into the influenza A virus gene pool in humans.

Cecchinato M, Lupini C, Bolognesi PG, Marzadori°F, Raffini°E, Frasnelli°M, Poppi L, Shivaprasad HL, Catelli E

**An outbreak of blindness due to retinopathy in nine flocks of Guinea fowl**

Avian Dis. - Vol. 58 ( 2014). - p 337-339. - 7 bib ref [Nr. Estr. 5965]

Blindness was observed in 10- to 14-day-old guinea fowl. The incidence ranged from 25% to 80% in nine flocks within a total population of 110,000 guinea fowls. Clinical signs of blindness in birds included aimless wandering, failure to find feed and water, lateral recumbency, loss of weight, and increased mortality. The birds lacked papillary reflexes to light, and there were no gross lesions in the eyes. Histologically there was degeneration and disorganization of photoreceptors in the retina. The guinea fowl came from three different breeder sources but all of the birds were given the same feed. The condition was not observed in the subsequent flocks that came from the same breeder sources but that were given different feed. Based on these observations, toxicity of an unknown ingredient in the feed is suspected as the cause of blindness in the guinea fowl.

Chiapponi°C, Baioni°L, Luppi°A, Moreno°A, Caste llan A, Foni°E

**Temporal insight into the natural generation of a new reassortant porcine influenza virus in a swine holding**

Vet Microbiol. - Vol. 174 ( 2014). - p 9-15. - 38 bib ref [Nr. Estr. 5901]

The influenza A virus (IAV) subtypes H1N1, H3N2 and H1N2 are the most prevalent subtypes in swine in Italy. Reassortant influenza A viruses subtypes in swine appeared in European pig population. In particular reassortant viruses carrying genome segment from the pandemic H1N1

(H1N1pdm) are reported in many European countries, including Italy. In a 1000 sows farrow-to-feeder farm, in Northern Italy, we isolated 10 IAVs from recurrent episodes of respiratory disease in 45–70 days-old pigs from September 2012 to June 2013. The antigenic and genetic characterization of the swine IAV isolates showed the contemporary circulation of H1N1 avian-like and H1N1pdm strains in the first outbreak. The analysis of the viruses isolated subsequently showed the circulation of H1N1pdm IAV and then the establishment of a new previously undescribed H1N1 reassortant strain with a pandemic derived hemagglutinin gene and all the other seven segments of swine H1N1 avian-like lineage.

Chiapponi°Ch, Moreno°A, Baioni°L, Luppi°A, Faccini°S, Foni°E

**Genetic characterization of Italian swine influenza viruses : 2011-2013**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - v. 2. - p 315. - 4 bib ref [Nr. Estr. 5756]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Chiari°M, Ferrari°N, Giardiello°D, Avisani°D, Pacciarini°ML, Boniotti°B, Alborali°L, Zanoni°M

**Wild boar (*Sus scrofa*) and MTB complex, something new : spatiotemporal and biological patterns of *M. microti* infection in wild boar**

Hystrix Ital J Mammalogy. - Vol. 25 (supplement) ( 2014). - p 52 [Nr. Estr. 5696]

Congresso Italiano di Teriologia (9. : Civitella Alfedena (AQ) : 7-10 Maggio 2014)

Tuberculosis is a chronic disease caused by mycobacteria belonging to MTB complex that include, *M. microti*, the causative agent of the vole tuberculosis. Due to the slow growth in vitro of the bacteria on primary isolation, *M. microti* prevalence, geographical distribution and host range has been probably underestimated. This study aims to firstly describe the epidemiological trend of the infection of *M. microti* in free-living wild boar and to identify the host risk factors linked with its occurrence. Tuberculosis like lesions of 3041 hunted wild boar coming from 5 different hunting areas were analysed through IS6110 PCR and the positive ones were subsequently analysed by gyrB-RFLP assay in order to identify the presence of *M. microti*. From 2008 to 2012, 190 examined wild boar showed TBL only at submandibular lymph nodes. PCR gyrB assay identified the presence of *M. microti* in 99 free-ranging wild boar (P=3.26%). The age class distribution of the 99 *M. microti* positive wild boars was: n=11 (11.11%) "young", n=36 (36.36%) "sub adult" and n=46 (46.47%) "adult". The prevalence changed spatiotemporally with a generalized increase over the years of 1.26. Age class and index of abundance influenced the prevalence of *M. microti* infection while sex was not significant. In particular, the probability of being infected was higher in older individuals and in animals coming from area and year with higher wild boars abundances. The obtained data will stimulate further indications on TB surveillance in wildlife and management of this expanding species regarding tuberculosis infection.

Chiari°M, Ferrari°N, Giardiello°D, Avisani°D, Pacciarini°ML, Boniotti°B, Alborali°L, Zanoni°MG

**Wild boar (*Sus scrofa* [i.e. *scrofa*]) and MTB complex, something new : spatiotemporal and biological patterns of *M. microti* infection in wild boar**

11th European Wildlife Disease Association Conference (EWDA) : Edinburgh, 25-29 August 2014 / [s.l. : s.n., 2014]. - 1 p. [Nr. Estr. 5850]

European Wildlife Disease Association Conference (EWDA) (11th : Edinburgh : 25-29 August

2014)

Tuberculosis is a chronic disease caused by mycobacteria belonging to MTB complex that include, *M. microti*, the causative agent of the vole tuberculosis. Due to the slow growth in vitro of the bacteria on primary isolation, *M. microti* prevalence, geographical distribution and host range has been probably underestimated. This study aims to firstly describe the epidemiological trend of the infection of *M. microti* in free-living wild boar and to identify the host risk factors linked with its occurrence. Tuberculosis like lesions of 3041 hunted wild boar were analysed through IS6110 PCR and the positive ones were subsequently analysed by gyrB-RFLP assay in order to identify the presence of *M. microti*. From 2008 to 2012, 190 examined wild boar showed TBL only at submandibular lymph nodes. PCR gyrB assay identified the presence of *M. microti* in 99 free-ranging wild boar (P=3.26%). A widespread presence of *M. microti* infection in wild boar with a marked spatial variability and an overall temporal increase of the prevalence were registered. Moreover a positive effect of abundance and age on the prevalence was detected, proving how old individuals coming from high density are the most affected one. The obtained data will provide further advice on TB surveillance in wildlife and management of this expanding species regarding specifically tuberculosis infection.

Chiari°M, Ferrari°N, Giardiello°D, Avisani°D, Zanoni°M, Alborali°GL, Lanfranchi P, Guberti V, Capucci°L, Lavazza°A

**Temporal dynamics of European brown hare syndrome infection in Northern Italian brown hares (*Lepus europaeus*)**

Eur J Wild Res. - Vol. 60 ( 2014). - p 891-896. - 22 bib ref [Nr. Estr. 5874]

The progressive decline in the hare population across Europe has been associated with the occurrence of European brown hare syndrome (EBHS), a highly contagious disease considered endemic in all European countries. This study aimed to evaluate the in-field temporal dynamics of European brown hare syndrome virus (EBHSV) infection in wild European brown hares (*Lepus europaeus*) and to test the influence of population density on EBHS seroprevalence. A total of 512 blood samples were collected from free ranging hares captured for restocking in seven different areas of the province of Brescia (Northern Italy) during seven consecutive years (2006–2013) and tested using a competitive ELISA. A generalized linear mixed model estimated the yearly effects of population density on EBHS prevalence. Of the 512 tested, 344 (67.2 %) tested positive for EBHSV antibodies, with the annual seroprevalence ranging from 94.3 to 35.8 %. The prevalence was 3.303 times higher in areas with a density of over 15 hares/km<sup>2</sup> and declined over the years. The results indicate the ongoing transmission of the virus in the tested brown hare population. Since the eradication of EBHS in a wild population is not feasible, a strategy aimed at promoting the endemic stability of the virus through density-dependent mechanisms could be applied; however, this seems more difficult in practice than in theory and would most likely require a very high density of brown hares.

Chiari°M, Ferrari N, Giardiello°D, Lanfranchi P, Zanoni°MG, Lavazza°A, Alborali°LG

**Isolation and identification of *Salmonella* spp. from red foxes (*Vulpes vulpes*) and badgers (*Meles meles*) in Northern Italy**

Acta Vet Scand. - Vol. 56 no 86 ( 2014). - 4 p. - 21 bib ref [Nr. Estr. 5906]

Background *Salmonella* spp. have been isolated from a wide range of wild animals. Opportunistic wild carnivores such as red foxes (*Vulpes vulpes*) and badgers (*Meles meles*) may act as environmental indicators or as potential sources of salmonellosis in humans. The present study characterizes *Salmonella* spp. isolated from the intestinal contents of hunted or dead red foxes (n =

509) and badgers (n = 17) in northern Italy. Findings Thirty-one strains of Salmonella belonging to 3 Salmonella enterica subspecies were isolated. Fourteen different serovars of S. enterica subsp. enterica were identified, among which were serovars often associated with human illness. Conclusions Wild opportunistic predators can influence the probability of infection of both domestic animals and humans through active shedding of the pathogen to the environment. The epidemiological role of wild carnivores in the spread of salmonellosis needs to be further studied.

Chiari°M, Ferrari°N, Zanoni°M, Alborali°L

### **Mycoplasma hyopneumoniae temporal trends of infection and pathological effects in wild boar populations**

Eur J Wild Res. - Vol. 60 ( 2014). - p 187-192. - 25 bib ref [Nr. Estr. 5534]

Mycoplasma hyopneumoniae (Mhyo) is the principal etiological agent of enzootic pneumonia (EP), one of the most economically important diseases in the pig production industry worldwide. Although swine and wild boars (*Sus scrofa*) share susceptibility to Mhyo infection, information regarding the dynamics of Mhyo infections and pathology in wild boars is currently limited. Therefore, the aim of this study was to determine the spatiotemporal dynamics of Mhyo infections in free-living wild boars through serological ELISA testing. Additionally, the presence of EP-like gross lesions and their association with the presence of Mhyo, based on a PCR assay, were assessed. Over a period of 5 years, antibodies against Mhyo were detected in 655 (30.0 %) of the 2,177 analyzed wild boars. A generalized temporal increase of seroprevalence coupled with an increase of mean antibody titers of seropositive individuals was found. Moreover, a similar seroprevalence between age classes associated with higher antibody titers of younger individuals indicated the wild boars were infected during the early stages of their life. Out of 99 lungs tested, 43 showed EP-like lesions and 45 were PCR positive for the presence of Mhyo DNA. The lung lesion scores were related to the sex and age of the wild boars, with young individuals having higher lung scores than others, and there was a positive association with the Mhyo PCR-positive status. The temporal increase of the Mhyo seroprevalence and infection association with EP-like lesions, when coupled with the spreading of wild boar populations, raises concerns on the epidemiological role of this species.

Chiari°M, Lelli°D, Moreno°A, Sozzi°E, Tironi°M , Avisani°D, Zanoni°M, Farioli M, Dottori°M, Lavazza°A

### **West Nile Virus surveillance in Lombardy, North Italy**

8th Annual Epizone Meeting "Primed for tomorrow" : 23 - 25 September 2014 Copenhagen, Denmark : Posters "Intervention strategies and legislation" / [s.l. : s.n., 2014]. - 1 p (Poster CONTR01) [Nr. Estr. 5952]

Annual meeting Epizone (8th : Copenhagen, Denmark : 23 - 25 September 2014)

West Nile virus (WNV) is one of the most serious public health threats that Europe and the Mediterranean countries are currently facing. Following the first outbreak of West Nile disease (Tuscany, 1998), a national surveillance program supported by the Ministry of Health was established since 2002 in order to early identify WNV circulation. This multispecies surveillance planned to screen wild birds, sentinel-chickens, sentinel horses, equine neurological cases, mosquitoes and humans and blood donors. To tackle WNV continuous incursions, more comprehensive Regional surveillance programs are also carried out in WNV affected areas. After the first WNV outbreak (lineage 1), WND apparently disappeared for almost ten years and it was newly detected in 2008. In the subsequent years, the number of cases increased in the Po river Valley. Until 2013, the Lombardia Region was only marginally involved in 2008 epidemic (2 human cases in Mantova Province). In fact, no other reports were described before the identification of WNV lineage 2 in a pool of *Cx. pipiens* sampled for experimental purposes in Cremona Province (July 5, 2013). Since just Mantova Province was at that time enclosed in the national plan, a regional program was

designed to define prevention and control strategies for human health. Considering the role given to entomological and wild birds surveillance in WNV early detection, this plan aimed to improve these keys activities. By placing 25 CO2-CDC traps in 5 different Provinces of the southern part of the Region in the Po valley, a total of 147 capture sessions were done (July-October 2013). In addition, 632 wild birds of different species (*Pica pica*, *Corvus corone cornix*, *Streptopelia decaocto*, *Garrulus glandarius*) were sampled. PCR carried out on wild birds, led to identify 2 infected birds out of 632 tested (1 European magpies and 1 carrion crows). Within the entomological surveillance 7 pools of *Cx. pipiens* resulted PCR positive. In addition, passive and active surveillance on horses allowed to identifying 8 cases in 7 different farms. In the same period 18 cases of human WNV infection were diagnosed in the Lombardia Region. These results support the hypothesis that WNV is becoming endemic in Po valley (northern Italy). Therefore, the regional 2014 monitoring plan is implemented with a more comprehensive geographical distribution of the traps and a greater number of wild birds sampled. In particular, the plan territory of the region is split into 30 square areas of 20Km<sup>2</sup> and one trap is placed in each square, taking into account risk areas. Night trap sessions are programmed every 15 days from mid May to the end of September. The monitoring on wild birds is spatially and temporally improved in order to ensure a total of 155 wild birds sampled every month from April to September. The 2014 surveillance program supported by the Lombardy Public Veterinarian Service represents an integrated surveillance aiming to early identification of WNV circulation. Having such a rapid health alarm system in place is of extreme value as it enables regional infrastructures for optimal management of acute human cases and adoption of prevention strategies.

Chiari<sup>°</sup>M, Lelli<sup>°</sup>D, Sozzi<sup>°</sup>E, Moreno<sup>°</sup>A, Alborali<sup>°</sup> L, Zanoni<sup>°</sup>MG, Cordioli<sup>°</sup>P, Lavazza<sup>°</sup>A

#### **Serosurveillance for Schmallenberg virus (SBV) in wild boar (*Sus scrofa* [i.e scrofa]) in Northern Italy**

11th European Wildlife Disease Association Conference (EWDA) : Edinburgh, 25-29 August 2014 / [s.l. : s.n., 2014]. - 1 p. [Nr. Estr. 5851]

European Wildlife Disease Association Conference (EWDA) (11th : Edinburgh : 25-29 August 2014)

Schmallenberg Virus (SBV), a novel Orthobunyavirus, was first detected in cattle in northwestern Europe in 2011. Since then, SBV infection has been cited as the cause of congenital malformations and stillbirths in cattle and sheep in several European countries. The virus appears to be transmitted by *Culicoides* spp. and specific SBV antibodies have been detected in wild ruminants (red deer, roe deer and chamois). This study aimed to assess whether SBV antibodies were present in the sera of free ranging wild boar (*Sus scrofa*) in North Italy in order to verified the susceptibility of this species to SBV. The tested sera (924) were collected from hunted wild boars during different hunting season from 2009 to 2013. All the sera were tested using a serological "in-house" SBV MAbs based competitive ELISA. Neutralizing SBV antibody titres were determined by the virus neutralization test, using Vero cells and SBV strain BH80/11-4 (RBV 1099-FLI) for most of the positive ELISA samples. A total of 43 wild boar tested positive for the presence of SBV antibodies, in particular 3 during the hunting season 2009/10 (P=0.67%), 15 on 2011/12 (P=4.02%) and 25 on 2012/13 (23.36%). Because this serosurvey is suggestive of an active SBV circulation in wild boars, targeted surveillance should be performed on this wild species to monitor the spread of the virus and to assess the epidemiological role of wildlife at the interface with domestic ruminants.

Chiari<sup>°</sup>M, Sozzi<sup>°</sup>E, Zanoni<sup>°</sup>M, Alborali<sup>°</sup>LG, Lavazza<sup>°</sup>A, Cordioli<sup>°</sup>P

#### **Serosurvey for Schmallenberg virus in alpine wild ungulates**

Transbound Emerg Dis. - Vol. 61 ( 2014). - p 1-3. - 10 bib ref [Nr. Estr. 5533]

Because Schmallenberg virus (SBV) was first reported in domestic ruminants in Northern Italy in

February 2012, we conducted a serosurvey to assess the presence of SBV-specific antibodies in free-ranging alpine ruminants. The tested serum samples were from chamois (23) and red deer (352) hunted from 2007 to 2013. All of the serum samples collected through September, 2012, tested negative, whereas a single chamois serum and 21 red deer sera taken during the 2012–2013 hunting season tested positive for the presence of SBV antibodies. Because this serosurvey is suggestive of an active SBV circulation in Alpine wildlife, targeted surveillance should be performed on wild ruminants to monitor the spread of the virus and to assess the epidemiological role of wildlife at the interface with domestic animals.

Chiari°M, Zanoni°M, Alborali°LG, Zanardi°G, Aviani°D, Tagliabue°S, Gaffuri°A, Pacciarini°ML, Boniotti°MB

**Isolation of *Mycobacterium caprae* (Lechtal genotype) from red deer (*Cervus elaphus*) in Italy**

J Wild Dis. - Vol. 50 no 2 ( 2014). - p 330-333. - 15 bib ref [Nr. Estr. 5752]

During tuberculosis (TB) surveillance, 53 hunted red deer (*Cervus elaphus*) were collected to determine whether TB was present in free-ranging animals from an Italian alpine area. Samples (lungs, liver, intestine, and lymph nodes) were cultured and analyzed by real-time PCR assay carried out directly on tissue. *Mycobacterium caprae* was isolated from small granulomatous, tuberculosis-like lesions in the liver of a 12-yr-old female. Identification of suspect colonies was done by PCR restriction fragment length polymorphism analysis of the *gyrb* gene, and genotyping was performed by spoligotyping and mycobacterial interspersed repetitive unit variable number tandem repeat analysis. The isolated strain was genetically identical to strains isolated in the study area in 2001 from dairy cows imported from Austria and in 2010 from an indigenous cow. The genotype, called "Lechtal," is the most frequently detected in the TB outbreaks in Austria and Germany. The possibility that red deer act as a maintenance host of *M. caprae* between TB outbreaks could be not excluded. Despite the high red deer population density, the detection of only one infected red deer could suggest that the wildlife management measures applied in the study area (prohibition of artificial feeding and secure removal of offal from hunted animals) may reduce the risk of TB spreading.

Circella E, Legretto M, Pugliese N, Caroli A, Bozzo G, Accogli G, Lavazza°A, Camarda A

**Psittacine beak and feather disease–like illness in Gouldian finches (*Chloebia gouldiae*)**

Avian Dis. - Vol. 58 n 3 ( 2014). - p 482-487. - 37 bib ref [Nr. Estr. 5829]

Beak and feather disease virus (BFDV) is a member of the genus *Circovirus* and causes psittacine beak and feather disease (PBFD) in Psittaciformes. PBFD is a severe disease generally characterized by immunodeficiency and beak and feather disorders. Although *Circovirus* spp. have been detected in several nonpsittacine species, little is known about the symptoms and the disease associated with this infection in birds other than Psittaciformes. In this study, we report the identification of *Circovirus* infection in a flock of Gouldian finches showing beak and feather disorders. Sequence analyses on the *rep* gene of the virus highlighted a strong similarity at nucleotide and amino acid levels with the corresponding regions of BFDV from psittacine species. By contrast, it was more distant to circoviruses identified in finch and canary.

Colussi S, Peletto S, Modesto P, Riina MV, Meistro S, Varello K, Giorgi I, Soncin AR, Gennero MS, Dezzutto D, Irico L, Vitale N, Romano A, Gorla M, Arrigoni°N,

Acutis PL

**Identification of single nucleotide polymorphisms in SLC11A1 and card15 genes and their association with infection by Mycobacterium avium subspecies paratuberculosis**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 136 (Poster P-04.7) [Nr. Estr. 5766]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

Using the approach of the candidate gene, a case-control study on Friesian cattle, naturally infected by Mycobacterium avium subspecies paratuberculosis (MAP), was carried out. Solute carrier family 11 member 1 (SLC11A1) and Caspase recruitment domain 15 (CARD15) genes were analysed. Cases and controls were defined considering blood serum ELISA, faecal culture, and PCR tests using a Bayesian probability weighting function. Twelve polymorphisms were reported in CARD15 and two were statistically associated ( $p < 0.05$ ) with susceptibility to MAP infection. Presence of polymorphism c.2886-14 A>G, at intron 10 was found in infected animals 2-fold higher than in healthy ones (OR=2.35; CI 95%: 1.08-5.10;  $X^2=4.87$   $p=0.03$ ); it was formerly reported associated to health and productive traits in Canadian Holstein. Analysis based on TFSearch software showed, when mutation was present, the alteration of the site for GATA3 transcription factor, involved in phagocytosis and apoptosis. Interestingly GATA3 and Interleukin 5 expression was reported altered in MAP-infected cattle. We confirmed the role of mutation 1908C>T, at 3'UTR, (OR=2.04; CI 95%: 1.03-4.99;  $X^2=4.45$   $p=0.03$ ), previously associated with susceptibility; this could be attributed to changes in a regulatory motif comprising the mutation site. Seven polymorphisms were reported in SLC11A1; one associated with susceptibility, found in the promoter region, c.-90 C>A (OR=2.04; CI 95%: 1.03-4.04;  $X^2=6.02$   $p=0.01$ ); and the second, already described, c.1157-91 A>T, at intron 11, related to susceptibility when the wild-type allele A is present (OR=3.46; CI 95%: 1.37-8.74;  $X^2=6.86$   $p=0.01$ ). The association with infection, of polymorphisms c.-90 C>A and c.2886-14 A>G is described here for the first time. These data could be considered for future application in programs to control paratuberculosis in cattle, in which highly susceptible animal could be negatively selected according to the genotype.

Corpus<sup>o</sup>F, Carra<sup>o</sup>E, Baldelli R, Codeluppi M, Genna ri W, Lombardini A, Salvatore D, Tamba<sup>o</sup>M, Vitale F, Meriardi<sup>o</sup>G, Rugna<sup>o</sup>G

**Characterization of Leishmania infantum strains circulating in dogs and humans in Emilia-Romagna Region (Northern Italy) by using the Multi Locus Microsatellite Typing (MLMT)**

8th Annual Epizone Meeting "Primed for tomorrow" : 23 - 25 September 2014 Copenhagen, Denmark : Posters "Vector borne diseases" / [s.l. : s.n., 2014]. - 1 p. (Poster VECT08) [Nr. Estr. 5932]

Annual meeting Epizone (8th : Copenhagen, Denmark : 23 - 25 September 2014)

Abst.

Costarelli S, Giammarioli M, Iscaro C, Caporali A, Canonico C, Ciullo M, Crotti S, Vicari<sup>o</sup>N, Gavaudan S

**Surveillance of tick borne encephalitis and other tick borne diseases in Umbria Region**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 149 - 7 bib ref [Nr. Estr. 5946]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

De\_Marco MA, Delogu M, Di\_Trani L, Boni A, Frasnelli°M, Venturi L, Marzadori°F, Donatelli I, Raffini°E

**Serologic evidence of occupational risk for avian influenza virus transmission at wildfowl-poultry-human interface**

4th International Influenza meeting : September 21-23, 2014 Germany / [s.l. : s.n., 2014]. - p 63 (poster 16) [Nr. Estr. 5955]

International Influenza meeting (4th : Germany : September 21-23, 2014)

Low pathogenic (LP) avian influenza viruses (AIVs) can cause human infections, as the result of direct transmission from infected birds. Human cases are related to mild or subclinical disease, although severe outcomes have been recently reported in subjects infected by H7N9 LPAIVs. Some farming practises are modelled to house birds outdoor, thus exposing them to AIVs harboured in wild avian reservoir. These relatively low bio-security conditions enable the emergence of AIVs in captive-reared birds, increasing the potential zoonotic risk to occupationally exposed workers. Main purpose of this study is to provide serological evidence of LPAIV infection in workers operating in farms located in the Emilia-Romagna Region (Italy). In April 2005-July 2006 period, sera were collected from 57 bird-exposed workers and 7 non bird-exposed controls, planning three blood sample collections from each individual to assess seroconversion against AIVs. Study population included: 46 poultry workers (PWs) and 4 veterinarians operating in 14 farms housing birds under outdoor conditions; 2 veterinarians and 4 technicians involved in AIV laboratory diagnostics; a wildlife professional exposed to wild waterfowl. Haemagglutination inhibition (HI) assay and microneutralization-ELISA (MN) were used as screening and confirmatory tests, respectively. Sera were tested for specific antibodies against twenty LPAIVs belonging to antigenic subtypes from H1 to H14 and including twelve reference strains and eight avian isolates from farms under study. MN results showed serologic evidence of exposures to AIV in 3/46 PWs, showing antibodies against H3, H6, H8 and H9 AIVs. In addition, the wildlife professional was seropositive against H11 AIV subtype.

De\_Marco MA, Valentini A, Foni°E, Savarese MC, Cot ti C, Chiapponi°C, Raffini°E, Donatelli I, Delogu M

**Is there a relation between genetic or social groups of mallard ducks and the circulation of low pathogenic avian influenza viruses?**

Vet Microbiol. - Vol. 170 ( 2014). - p 418-424. - 46 bib ref [Nr. Estr. 5685]

We investigated the circulation dynamics of low pathogenic avian influenza viruses (LPAIVs) in the mallard (*Anas platyrhynchos*) reservoir in Italy. In particular, we evaluated the temporal distribution of virologic findings by combining virus isolation data with a new population genetic-based study approach. Thus, during 11 consecutive sampling periods (wintering periods between 1993/94 and 2003/04), categorised into 40 sampling sub-periods, cloacal swab samples were collected from 996 wild and 16 captive-reared mallards, to be screened by RT-PCR before attempting influenza A virus isolation in embryonated eggs. Forty-eight LPAIVs were isolated from wild mallards and antigenically characterised by haemagglutination-inhibition and neuraminidase-inhibition assays. When considering LPAIV antigenic subtypes in which more than one mallard tested virus isolation positive (H1N1, n. 22; H2N3, n. 2; H5N3, n. 2; H6N5, n. 3; H6N8, n. 2; H7N3, n. 3; H11N6, n. 5), at least two birds infected with a specific HN subtype clustered within one same sampling sub-period. In the context of the novel population genetic approach, total DNA was extracted from a subset of 16 captive-reared and 65 wild ducks (2000/01 and 2001/02 sampling periods) to assess genetic diversity by amplified fragment length polymorphisms (AFLP) markers. Analyses of AFLP results showed that captive-reared mallards clustered together, whereas two main independent clusters characterised the distribution pattern of most wild mallards. Within this subset of samples, nearly identical H7N3 LPAIV strains were isolated from two wild mallards belonging to the same genetic cluster. Blood sera were also collected from the above subset of mallards and examined for antibodies to the homologous H7N3 virus strain. Four out of six wild mallards testing

H7N3-seropositive by haemagglutination-inhibition assay (2001/02 period) belonged to the genetic cluster including H7N3 virus shedding ducks. Overall, our data raise the possibility of an enhanced transmission and circulation of LPAIVs in genetic or social groups of wild mallards, gathered in flocks possibly related by parentage and/or geographic origin.

Del\_Amo J, Llorente F, Figuerola J, Soriguer RC, Moreno<sup>o</sup>AM, Cordioli<sup>o</sup>P, Weissenboeck H, Jiménez\_Clavero MA

**Experimental infection of house sparrows (*Passer domesticus*) with West Nile virus isolates of Euro-Mediterranean and North American origins**

Vet Res. - Vol. 45 ( 2014). - no 33 (9 p). - 32 bib ref (ultimo accesso 12/02/2015 : <http://www.veterinaryresearch.org/content/45/1/33/abstract> ) [Nr. Estr. 5987]

West Nile virus (WNV) is a zoonotic arboviral pathogen transmitted by mosquitoes in a cycle involving wild birds as reservoir hosts. The virus has recently emerged in North America and re-emerged in Europe. North American WNV outbreaks are often accompanied by high mortality in wild birds, a feature that is uncommon in Europe. The reason for this difference is unknown, but the intrinsic virulence of the viruses circulating in each continent and/or the susceptibility to the disease of Palearctic as opposed to Nearctic wild bird species could play a role. To assess this question, experimental inoculations with four lineage 1 WNV strains, three from southern Europe (Italy/2008, Italy/2009 and Spain/2007) and one from North America (NY99) were performed on house sparrows (*Passer domesticus*), a wild passerine common in both continents. Non-significant differences which ranged from 0% to 25% were observed in mortality for the different WNV strains. Viremias lasted from 1 to 5–6 days post-inoculation (dpi) in all cases; individuals inoculated with NY99 had significantly higher titres than those inoculated with any of the Euro-Mediterranean strains. Remarkably, host competence was found to be higher for NY99 than for the other strains. Consequently, albeit being pathogenic for house sparrows, some Euro-Mediterranean strains had reduced capacity for replication in -and transmission from- this host, as compared to the NY99 strain. If applicable also to other wild bird host species, this relatively reduced transmission capacity of the Euro-Mediterranean strains could explain the lower incidence of this disease in wild birds in the Euro-Mediterranean area.

Della\_Noce I, Stevenson K, Parkhill J, Ricchi<sup>o</sup>M, Lazzari B, Del\_Corvo M, Zanetti E, Messina V, Arrigoni<sup>o</sup>N, Williams JL, Minozzi G

**Insights in the genome of *Mycobacterium avium* subsp. paratuberculosis by next generation sequencing approaches**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 166 (Poster P-05.3) [Nr. Estr. 5765]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

*Mycobacterium avium* subsp. paratuberculosis (MAP) is the causative agent of paratuberculosis in farmed and wild animals. In the present study the genomic variability of field strains of MAP isolated from different hosts and from several regions, in Italy was analyzed by whole genome sequence comparison using next generation sequencing approaches. The preliminary results on 15 strains are presented. All the MAP strains were isolated from single animals from cattle herds located in 6 provinces in the north of Italy, in collaboration with National Reference Centre for Paratuberculosis (Piacenza, Italy). Nine samples were paired end sequenced at 150bp end and six samples at 75bp per end on the Illumina Myseq. The K-10 (NC\_002944.2) strain was used as the reference, and bioinformatic analyses were performed using the BWA-mem, FreeBayes, GATK, and SNPEff software. The reads were mapped to the K-10 reference sequence with 99.96% reads finding a match, with a mean coverage of 138.26 X. In total 844 variants were identified, of which 698 SNP, 23 MNP, 45 INS, and 78 DEL. Each strain has 25.90% private SNP on average. The variants

identified are 1.40% missense, 1.14% nonsense, and 37.46% silent. About 43% of variants were located in coding regions, while 25% were in the -60 upstream regions. Fewer variants were identified than expected. This could be functional, due to the position of the variants, which mainly fall in coding and regulatory sequences. In the future, phenotypic information linked to MAP strains and their hosts may help to disentangle the genetic variability linked to virulence and MAP population substructure.

Di\_Ciccio P, Ossiprandi MC, Ghidini S, Zanardi E, Belluzzi G, Pongolini S, Ianieri A

### **Detection of Salmonella on carcasses and environment in pig slaughterhouses in Northern Italy**

LXVIII Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : Convegno SICV : XI Convegno AIPVet : XII Convegno SIRA16-18 Giugno 2014 Pisa / [s.l. : s.n., 2014]. - p 161. - 3 bib ref [Nr. Estr. 5991]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 68 Convegno SICV Convegno AIPVet : 11 Convegno SIRA : 12 : Pisa : 16-18 Giugno 2014)

The aim of the present study was to evaluate the presence of Salmonella on carcasses and environment in pig slaughterhouses. Three slaughterhouses (A-B-C) with high slaughter capacity (~400 pigs/h) were selected for the survey and visited 6 times with 4-week interval. On each visit, samples were collected from n.5 carcasses by using sponges (10x10 cm), caecal contents (225g pools of the 5 pigs) and the slaughterhouse environments: a surface 10x10 cm per site was sampled using sponges in three different places: floor after the bleeding stage, gut container, runoff pit. The samples were pre-enriched in buffered peptone water and analyzed according to the Salmonella Precis method (OXOID - Milan). Presumptive colonies were submitted to phenotypic identification (API ID 32E - bioMérieux, France); serotyped by agglutination tests with specific O and H antisera and then genotyped by pulsed field gel electrophoresis (PFGE) using the Xba enzyme. From a total 216 samples, Salmonella was recovered from 11 (5%) samples in two slaughterhouses: A-B. From a total 90 pre-chill carcasses sampled in 18 batches, Salmonella was isolated from 4 (4.44%) in only one slaughterhouse (B). Two out of the 18 batches (each batch: ~140 animals), originating from different farms, were positive for Salmonella (11.1%). From the 108 environmental samples (54 collected before and 54 during slaughter activities), Salmonella was detected only in the samples collected during the slaughter activities (7/54 - 12.9%). None of the pooled faeces were positive. Four different serotypes were detected: S.Rissen (n=5), S.Tiphymurium (n.1) and the monophasic-variant of the serotype Tiphymurium (n.3), S. Derby (n.2). The serotypes from the carcasses were S.Rissen and S.Derby whereas those from the environmental samples were S.Rissen, S.Tiphymurium and the monophasic-variant. In serotype S.Rissen isolates, two different PFGE restriction profiles could be distinguished; among S.Tiphymurium monophasicvariant isolates two PFGE patterns were identified; the S.Derby strains showed the same pulsotype. The European Union (EU) is currently discussing Salmonella control in pigs and deliberating on setting targets for such control programs. In this study, differences in Salmonella contamination were observed in relation both to the sampling day and to the slaughterhouses. Salmonella was recovered in two of the three abattoirs tested. These findings are in accordance with the results of Botteldoorn et al.(1), indicating that sampling results depend on the slaughterhouse, the sampling day and the origin of the pigs. The detected percentage of carcass contamination (4.44%) is in accordance with other studies (2). Regarding serotype, our results are consistent with the Baseline EU survey (3). As for as the PFGE analysis is concerned, a diversity of strains has been identified. However, it was not possible to isolate the same pulsotype within the same slaughterhouse in different sampling days. S.Rissen isolates recovered from carcasses had the same PFGE profile and the same isolate was also detected from the floor after the bleeding stage during the same sampling visit in slaughterhouse B. The slaughterhouse environment may be a source of carcass contamination of the slaughtered pigs passing along the slaughter line.

Dilda F, Molinari°S, Capucci°L, Williams J, Lelli °D, Potenza G, Luini°M

**Caratterizzazione immuno-fenotipica della risposta anticorpale all'inoculazione di un vaccino vivo attenuato contro la BVD in bovini da latte**

Simposio Immunologia Veterinaria / [s.l. : s.n., 2014]. - 1 p. [Nr. Estr. 6080]

Simposio Immunologia Veterinaria : Firenze : 28 Maggio 2014)

L'infezione da BVDV (Bovine Viral Diarrhea Virus) causa importanti danni economici all'allevamento bovino in tutto il mondo, in particolare per problemi riproduttivi nelle bovine da latte. Una completa protezione contro la malattia è difficile da ottenere per la complessità della risposta immunitaria a questa infezione e la eterogeneità dei ceppi BVDV. La presente indagine è stata effettuata con l'obiettivo di differenziare gli animali in funzione della risposta immunitaria alla vaccinazione e di caratterizzare i bersagli immunologici del vaccino. **METODI** — In 4 aziende, sono state vaccinate con un vaccino vivo attenuato (Mucosiffa-Merial) 120 manze sieronegative, indenni da BVDV. La risposta anticorpale è stata misurata dopo 40 gg.(+5) mediante ELISA NS2/3 e siero-neutralizzazione (SN). Un sotto-campione di sieri è stato analizzato mediante Western Blot (WB) utilizzando il siero degli animali vaccinati come anticorpo primario e le proteine virali separate su gel denaturante e trasferite su substrato di PVDF. **RISULTATI** — Le analisi hanno evidenziato differenze quali-quantitative nella risposta anticorpale individuale e fra i gruppi di diversa provenienza e una scarsa correlazione tra valori ELISA e SN. In particolare, alcuni animali con bassi valori S/P in ELISA hanno mostrato un elevato livello di anticorpi neutralizzanti e viceversa. L'analisi in WB ha messo in evidenza proteine di 125, 118 e 55 kDa, identificabili in tutti gli animali dopo la vaccinazione. **DISCUSSIONE E CONCLUSIONI** - Il lavoro sta proseguendo per approfondire l'origine delle differenze nella risposta anticorpale evidenziata e la correlazione tra i titoli anticorpali, in particolare quelli SN e gli epitopi riconosciuti in WB. Questi dati contribuiranno alla comprensione della risposta immunitaria a BVDV ed alla identificazione degli epitopi più immunogeni per lo sviluppo di vaccini sempre più efficaci. Il lavoro è stato sostenuto da un finanziamento MIPAFF, progetto " Inovagen ".

Elizalde M, Agüero M, Buitrago D, Yuste M, Arias ML, Munoz MJ, Lelli°D, Pérez-Ramírez E, Moreno\_Martin°AM, Fernández-Pinero J

**Rapid molecular haemagglutinin subtyping of avian influenza isolates by specific real-time RT-PCR tests**

J Virol Methods. - Vol. 196 ( 2014). - p 71-81. - 62 bib ref [Nr. Estr. 5623]

Sixteen haemagglutinin (HA) subtypes of avian influenza viruses (AIV) have been described to date. Rapid subtype identification of any AIV is of major interest because of the possible serious consequences for the poultry industry and even public health. Molecular techniques currently allow immediate accurate subtype characterisation prior to virus isolation. In this study, a set of fourteen specific real-time RT-PCR methods were developed and evaluated for AIV HA subtyping (H1–H4, H6–H8, H10–H16), H5 and H9 being excluded on the basis of the current validity of the European Union (EU) recommended specific assays. Specific primers and probes sets for each HA-subtype were designed to hybridise the largest iso-lates range within each single subtype, considering the Eurasian lineage as a major target. The robustness and general application of the 14 HA-subtype methods were verified by the analysis of 110 AIV isolates belonging to all 16 HA-subtypes, performed in different laboratories. The developed real-time RT-PCR assays proved to be highly specific and revealed suitable sensitivity, allowing direct HA-subtyping of clinical material. In summary, this study provides for the first time a panel of molecular tests using specific hydrolysis probes for rapid and complete AIV HA-subtype identification.

Faccini°S, Barbieri°I, Franzini°G, Rosignoli°C, Moreno°A, Morganti P, Nigrelli°

AD

### **First occurrence of an emergent PCV2B variant in Italy**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 76 - 5 bib ref [Nr. Estr. 5928]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

Porcine circovirus type 2 is highly prevalent in pig population worldwide and is associated with several clinical manifestations collectively named PCV disease (PCVD) or PCV acquired disease (PCVAD). Three different genotypes are recognized for this virus: PCV2a, PCV2b, and PCV2c. In 2010 a PCV2b variant (vPCV2b), initially classified as PCV2d, has been described in China (1). Highly similar strains were subsequently identified in the United States, Serbia, and Brazil (3-5). This study reports the occurrence of this emergent PCV2b variant in Italy. **MATERIALS AND METHODS:** In March 2014, lymph nodes from two pigs of 50Kg with gastric ulcers were submitted to the IZSLER laboratories for diagnostic investigation. The farm was a farrow to finish with about 150 productive sows. The herd was routinely vaccinated against PCV2. A PRRSV break had been active in the farm since the end of February. Mortality was about 5%: 3.5% linked to respiratory symptoms and 1.4% to gastric ulcers. PCV2 detection and quantification was performed as previously described (2). PRRSV was detected by TaqMan NA and EU PRRSV kit (Ambion, USA). Full-length genome of PCV2 was amplified by a previously described method (4). Sequencing was performed on both strands by BigDye Terminator Cycle Sequencing kit v1.1 on 3500xl genetic analyzer (Life Technologies). Sequences were analyzed using Lasergene software (DNASTar, USA). Phylogenetic analysis was performed using Neighbor-Joining method with K2P model (MEGA 6). **RESULTS:** A PCV2 load of about 9 Log(10) genome copies/g was estimated in both lymph nodes. Both samples were also positive for PRRSV Type 1. The full genome of the two strains was composed of 1,767 nucleotides. ORF1 was 945nt long and encoded a protein of 314 amino acids (aa); ORF2 was 705nt long and encoded a protein of 234aa instead of 233aa as in classic PCV2. Sequence analysis showed that IZSLER PCV2 strains shared 100% nucleotide identity and had a high level of identity (>99.5%) with vPCV2b strains previously isolated in other countries (1,3-5). Phylogenetic analysis confirmed that IZSLER strains were closely related to the emerging vPCV2b (fig.1). Further sequence analysis revealed one nucleotide substitution (T1471C) leading to aa change in ORF1 (I84V) not previously reported and the presence of an additional K residue, at position 234 of ORF2 (K234), already detected in other vPCV2b. **DISCUSSION AND CONCLUSIONS:** In recent years the emergence of a new variant of PCV2b has been described in many countries. The strains here reported represent the first demonstration of the occurrence of this emergent vPCV2b in Italy. Differently from what described for previously isolated vPCV2b strains (1,3,5), clinical signs in the herd were not attributable to a typical severe PCVAD outbreak so there are not enough elements to affirm that the present isolation occurred in a case of vaccine failure. This report confirms the increasing diffusion of vPCV2b worldwide.

Faccini<sup>o</sup>S, Podgórska K, Tomasz S, Boniotti<sup>o</sup>MB, Le Ili<sup>o</sup>D, Dottori<sup>o</sup>M, Nigrelli<sup>o</sup>AD, Bonilauri<sup>o</sup>P

### **Development and validation of a real time PCR for the detection of all subtypes of PRRSV type 1 (European genotype)**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 177 - 2 bib ref [Nr. Estr. 5926]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major threat to swine production worldwide and causes huge economic losses in the pig industry. PRRS European (EU, type 1) viruses are currently divided in 3 subtypes: subtype 1 (Lelystad viruslike), 2 and 3 and tentative

evidence was found for potential additional subtypes [1]. Divergent subtypes of Type 1 PRRSV have caused high rates of false-negative RT-PCR results in diagnostic tests [2]. Aim of this study was to develop and validate a new Real Time PCR able to detect all known Type 1 PRRSV strains. MATERIALS AND METHODS: A library constituted by a 495bp region, covering entire PRRSV ORF7 and 5' and 3' bordering regions, of 306 Italian subtype 1 field isolates and subtypes 1, 2, 3 and 4 strains available on GenBank was analyzed to check candidate conserved sequences. The detection limit of the method was assessed by testing serial dilutions of two plasmids, containing the artificially synthesized (MWG, Eurofins Genomics) target region of reference sequences PRRSV Lelystad strain (M96262.2, subtype1, pLely) and Lena strain (JF802085.1, subtype3, pLena). Moreover two cultures of PRRSV type 1 field isolates, with known viral titer, were serially diluted in PRRSV negative sera and tested. A total of 292 sera coming from 8 seronegative farms, 25 sera and tissues coming from 12 artificially infected animals, 16 reference positive Type 1 PRRSV cDNA coming from EPIZONE ring trial, 4 cDNA of Eastern Europe strains (Bor and LT3 subtype 2; Sza subtype 3; Okt subtype and 5 cultures of Italian Type 1 field strains were analyzed in order to assess diagnostic sensitivity (DSe) and specificity (DSp). For testing the field application of the method 97 sera samples collected in two conventional farms during the probable viremic stage of the animals (between 7 and 8 weeks of age) were examined. RESULTS: One forward primer, 2 reverse primers and a probe (sequence available on request) were selected as virtually able to detect all subtypes of Type 1 PRRSV strains included in our library. Analytical sensitivity (ASe): the viral LOD of the method, estimated by viral culture dilution, was equal to 3.9 Log(10) TCID<sub>50</sub>/50µl. Linearity was found (R<sup>2</sup>=0.99) across seven 10 fold dilutions of the 2 viruses in negative porcine serum. The LOD estimated with plasmid dilution was equal to 10 copies/reaction for pLena and 100 copies/reaction for pLely. Diagnostic sensitivity (DSe) and specificity (DSp): none of the 292 sera coming from 8 seronegative farms gave positive amplification. This confers 100% (CI95% lower limit 98.38%) of DSp. All known positive samples were found positive before Cq38 by the method, conferring 100% (CI95% lower limit 92.87%). Field application: 92 over 97 sera from probable viremic animals were tested positive by the method. DISCUSSION AND CONCLUSIONS: This new method can be considered fit for the purpose of Type 1 PRRSV strains detection. The application of this methods in field samples from European countries, will improve the validations of this new Real Time PCR developed. Acknowledgements: The work was funded by Italian Ministry of Health research project PRC201001..

Faccini°S, Rosignoli°C, Franzini°G, Nigrelli°AD

**Resistenza agli antibiotici e fattori di virulenza in ceppi di Escherichia coli isolati da suini sottoscrofa e svezzamento** = Antimicrobial resistance and virulence factors in E. coli strains isolated from piglets

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 245-249. - 6 bib ref [Nr. Estr. 5679]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

366 ceppi di E.coli sono stati isolati da campioni di suini, in lattazione e svezzamento, con patologie enteriche, conferiti presso sezione IZSLER di Mantova dal 2010 al 2013. I ceppi sono stati analizzati per la presenza di geni per alcuni fattori di patogenicità (PFs): F4, F5, F6, F41, e F18, LT, STa e STb, mediante PCR multiplex. Inoltre, è stato eseguito un test di sensibilità agli antibiotici, mediante agar-diffusione, per un pannello di 19 agenti antimicrobici, appartenenti a 9 classi differenti. Il numero medio di resistenze è stato di 10.2 ± 3.5 riferito a singoli farmaci e 6.9 ± 1.6 per le classi di appartenenza. I fenotipi di resistenza più comuni sono stati: Tetraciclina (95.24%), Tiamfenicolo (94.16%), e Amoxicillina (93.14%). Gli isolati multi-resistenti (resistenti a più di 3 classi di antibiotici) rappresentavano il 96%. In particolare: il 32% era resistente a 4-6 classi di agenti antimicrobici, il 49 % a 7-8 classi e il 15% a 9-10 classi. Il 51.4% dei ceppi possedeva almeno un gene per PFs. I più frequenti sono stati: STb (38.25%), LT (28.14%), STa (26.23%). La resistenza ad Apramicia è risultata più frequente nei ceppi con almeno un gene per PF. Al contrario, nei ceppi senza PFs, i resistenti ai chinoloni erano significativamente maggiori. Questi risultati mostrano la necessità d'indagini più approfondite sulle relazioni tra fattori di virulenza e antibiotico-resistenza, per far luce sui meccanismi di diffusione e indirizzare gli approcci terapeutici.

*A total of 366 E.coli strains were isolated from samples from diseased piglets submitted to the IZSLER diagnostic section of Mantova between 2010 and 2013. A multiplex PCR was performed for detection of some pathogenic factors: F4, F5, F6, F41, F18, LT, STa, and STb. A panel of 19 antimicrobial agents, belonging to 10 different classes, was considered for testing antibiotic sensitivity by Kirby—Bauer disk diffusion method. The mean number of resistances was  $10.2 \pm 3.5$  in reference to single drugs, and  $6.9 \pm 1.6$  for antimicrobial classes. Most common resistance phenotypes were against Tetracycline (95.24%), Thiamphenicol (94.16%), and Amoxicillin (93.14%). Multi-drug resistant isolates (resistant against more than 3 antimicrobial classes) were 96%. In particular 32% had resistances against 4-6 classes of antimicrobial agents, 49% against 7-8 antimicrobial classes and 15% against 9-10 classes. At least one gene for pathogenic factor was detected in 188 strains (51.4%). Most frequent were: STb (38.25%), LT (28.14%), STa (26.23%). Interestingly resistance against Apramycin was more frequent among E. coli strains with at least one gene for PFs. On the contrary, in nPFs strains, the proportion of those resistant to quinolones was significantly higher. These results highlight the importance of deeper investigations on relations between antimicrobial resistance and virulence factors to better understand their mechanism of diffusion and therapeutic approaches.*

Faccini°S, Rosignoli°C, Luppi°A, Franzini°G, Ni grelli°AD

### **Antimicrobial resistance and virulence factors in E. coli strains isolated from pig in Italy**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - v. 2. - p 467. - 2 bib ref [Nr. Estr. 5927]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Introduction Escherichia coli is part of the commensal flora of the lower intestine of humans and animals. On the other hand pathogenic strains of this species represent a major cause of morbidity and mortality worldwide. Both antimicrobial resistance and virulence genes can be horizontally transferred among E.coli strains and other bacterial species through transmissible genetic elements (2). This study evaluated antimicrobial resistances and pathogenic factors among E.coli strains, isolated from pig with enteric disorders, in order to describe possible relationships. Materials and Methods A total of 549 E.coli strains were isolated from pig specimens submitted to the IZSLER diagnostic laboratory of Mantova between 2010 and 2013. All isolates were tested for antibiotic sensitivity by Kirby—Bauer disk diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of 19 different antimicrobial agents, belonging to 10 different classes, was considered: Spectinomycin (Aminocyclitols), Aminosidine, Apramycin, Gentamicin, Neomicyn (Aminoglycosides), Cefalexin and Ceftiofur (Cephalosporins), Tilmicosin (Macrolides), Amoxicillin, Amoxicillin+Clavulanic acid (Penicillins), Tiamphenicol and Florfenicol (Phenicol), Colistin (Polipeptides), Flumequine, Danofloxacin, Enrofloxacin and Marbofloxacin (Quinolones), Tetracycline (Tetracyclines) and Trimetoprim+Sulfonamides (Sulfonamides). E. coli strains, found resistant to more than 3 or different classes of antibiotics, were considered as multi-drug resistant (MDR). A multiplex PCR was performed for detection of 5 different adhesins (F4, F5, F6, F41, and F18) and 3 enterotoxins (LT, STa, and STb) (1). The Chi-square test was used evaluate the difference between the proportions of resistant isolates in strains with pathogenic factors (PFs) and those without (nPFs). A p-value <0.05 was considered as significant. Results All the isolates showed resistance against at least 2 antimicrobial agents. The mean number of resistances was  $10.04 \pm 3.5$ , in reference to single drugs, and  $6.86 \pm 1.6$  for antimicrobial classes. The most common resistance phenotypes were detected against Thiamphenicol (94.16%), Tetracycline (93.71%), Amoxicillin (93.14%) and Tilmicosin (88.97%). Genes for PFs were detected in 274 strains (49.9%). The most frequent were: STb (37.2%), LT (27.7%), STa (26.6%), F18 (21.7%), F4 (19.7%). The analysis of proportions of resistant strains demonstrated, for some antimicrobial agents, significant differences between the group with genes for PFs and that without (Table1). MDR isolates Conclusions and Discussion The study confirms the high prevalence of MDR E.coli in swine population. Interestingly resistance against Aminosidine and Apramycin was more frequent among E.coli strains with at least one gene for PFs. On the contrary, in nPFs strains, the proportion of those resistant to Quinolones was significantly higher. No relevant differences were demonstrated for the other antimicrobial agents. The obtained results highlight the importance of deeper investigations on relations between

antimicrobial resistance and virulence factors to better understand their mechanism of diffusion.

Falcone E, Boniotti<sup>°</sup>MB, Canelli<sup>°</sup>E, Monini M, Busi <sup>°</sup>C, Vignolo<sup>°</sup>E, Lavazza<sup>°</sup>A, Ruggeri FR

**Monitoraggio di Rotavirus aviari di gruppo A e D in diverse specie aviarie da allevamenti del Nord Italia**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 46 [Nr. Estr. 5740]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

I Rotavirus (RVs), tra i principali agenti eziologici di malattie enteriche in diverse specie di mammiferi e uccelli, sono classificati in sette gruppi (A-G), sulla base della mobilità elettroforetica degli 11 segmenti di RNA a doppio filamento e delle caratteristiche antigeniche della proteina capsidica VP6. Nelle specie aviarie sono stati riscontrati con maggiore frequenza i rotavirus appartenenti ai gruppi A e D, mentre solo occasionalmente sono stati identificati rotavirus aviari appartenenti ai gruppi F e G. Le infezioni causate da rotavirus aviari (AvRVs) sono più frequenti nei tacchini, ma sono diffuse anche tra polli, fagiani, faraone, pernici, quaglie e, più sporadicamente, piccioni e anatre. La sindrome enterica nei giovani uccelli è una delle principali preoccupazioni per l'industria del pollame, in grado di causare gravi perdite economiche. Il monitoraggio della distribuzione dei AvRVs in diverse specie aviarie è uno strumento utile per l'acquisizione di dati epidemiologici e per comprendere meglio l'ecologia dei rotavirus aviari in natura. Lo scopo di questo studio retrospettivo è quello di fornire informazioni sulla prevalenza e l'epidemiologia dei rotavirus aviari circolanti in Italia durante il periodo 2006-2012, per monitorare la diversità e la variabilità nel tempo dei due gruppi antigenici (A e D) maggiormente diffusi tra queste specie. Sono stati analizzati 115 campioni di feci e/o contenuti intestinali, positivi per la presenza di RVs alla microscopia elettronica, provenienti da allevamenti di diverse specie aviarie del Nord e del centro Italia in presenza di focolai di enterite. I risultati del nostro studio indicano che il 14,8% e il 50,4% dei campioni analizzati appartengono rispettivamente ai gruppi A e D. Sono stati inoltre individuati nel 34,8% dei campioni co-infezioni dei due gruppi AvRV-A e AvRV-D. È interessante notare, come la co-infezione con entrambi i gruppi è stata evidenziata soprattutto tra i polli (48%) e tra i tacchini (20%). L'elettroferotipizzazione eseguita su campioni selezionati ha confermato i risultati ottenuti mediante RT-PCR. I risultati di questo studio forniscono nuovi dati sulla prevalenza dei gruppi AvRV-A e AvRV-D nelle specie aviarie in Italia. L'analisi dettagliata delle sequenze, permetterà di definire eventuali correlazioni con gli stipti isolati nei mammiferi e individuare l'eventuale presenza di ceppi appartenenti a gruppi meno diffusi tra la popolazione aviaria.

Ferreri L, Giacobini M, Bajardi P, Bertolotti L, Bolzoni<sup>°</sup>L, Tagliapietra V, Rizzoli A, Rosà R

**Pattern of tick aggregation on mice : larger than expected distribution tail enhances the spread of tick-borne pathogens**

PLoS Comput Biol. - Vol. 10 no 11 ( 2014). - p e1003931 (12 p). - 57 bib ref [Nr. Estr. 5882]

The spread of tick-borne pathogens represents an important threat to human and animal health in many parts of Eurasia. Here, we analysed a 9-year time series of *Ixodes ricinus* ticks feeding on *Apodemus flavicollis* mice (main reservoir-competent host for tick-borne encephalitis, TBE) sampled in Trentino (Northern Italy). The tail of the distribution of the number of ticks per host was fitted by three theoretical distributions: Negative Binomial (NB), Poisson-LogNormal (PoiLN), and Power-Law (PL). The fit with theoretical distributions indicated that the tail of the tick infestation pattern on mice is better described by the PL distribution. Moreover, we found that the tail of the distribution significantly changes with seasonal variations in host abundance. In order to investigate the effect of

different tails of tick distribution on the invasion of a non-systemically transmitted pathogen, we simulated the transmission of a TBE-like virus between susceptible and infective ticks using a stochastic model. Model simulations indicated different outcomes of disease spreading when considering different distribution laws of ticks among hosts. Specifically, we found that the epidemic threshold and the prevalence equilibria obtained in epidemiological simulations with PL distribution are a good approximation of those observed in simulations feed by the empirical distribution. Moreover, we also found that the epidemic threshold for disease invasion was lower when considering the seasonal variation of tick aggregation.

Ferri M, Dal\_Zotto M, Sala L, Todaro A, Baranceková M, Fontana R, Lanzi A, Armaroli E, Musarò C, Andina L, Allegri M, Adorni PL, Peloso F, Gelmini°L, Levrini M, De\_Pietri A

**Three sika deer *Cervus nippon* recently hunted in the Emilia-Romagna's area of <A.C.A.T.E.R. West> question the management of Italian *Cervus elaphus* population**

Hystrix Ital J Mammalogy. - Vol. 25 (supplement) ( 2014). - p 104 [Nr. Estr. 5842]

Congresso Italiano di Teriologia (9. : Civitella Alfedena (AQ) : 7-10 Maggio 2014)

The European Red deer (*Cervus elaphus*) population of ACATER West management unit (northern Appennines, Emilia-Romagna) is the target of censuses since 2009, and under hunting plan since 2012. In February 2011, a deer was tentatively identified as <Sika deer> (*Cervus nippon*) and after several attempts of trapping it was finally shot by the Provincia officers in March 2012, in the same locality where it was observed one year before. The details of the event, involving an adult male of 72 kg weight, have been reported in a poster presented at the VIII National Congress of Teriology (ATIt, Piacenza, 9-11 May 2012), where the case was described as the first in Italy. Only after the meeting a previous case was brought to our knowledge, having occurred in October 2010 in the province of Bolzano also concerning an adult male of 73 kg. A second Sika deer was hunted in Modena, in October 2012, at the same site of the previous killing; also this time it was an adult male weighting 113 kg. After these cases an investigation has started in order to identify farms or detention sites of Sika deer from which the specimens could have escaped. The survey, particularly challenging and often frustrating, has excluded recent escapes from Sika farms of the Emilia-Romagna, Marche, Lombardia and Liguria regions. However 3-4 specimens escaped in 1999 from a farm in the mountains of southern Emilia-Romagna region. A variable degree of hybridization between Scottish Red deer (*Cervus elaphus*) and Sika deer has been reported in several European areas, therefore, additional concern for the <ACATER West> Red deer population derives from the existence of potential hybrids of *Cervus elaphus* x *Cervus nippon* purchased in Scotland and bred since 40 years in central Italy and Emilia Romagna, some of which have escaped from captivity and settled near the site of the Sika deer shot. Morphological variability due to potential hybridization and degree of introgression may make difficult to distinguish between Sika and Red deer. Consequently, it was proposed to the Emilia-Romagna Region and Modena's Province the implementation of an information sheet (made with the coordination of ISPRA) concerning the morphological characters of *Cervus nippon* and its similarities and differences with *Cervus elaphus* and *Dama dama* (Fallow deer), with the aim of raising awareness in the volunteer staff in charge of biometric monitoring at the checking stations. The circulation of information has in fact contributed to alert several hunters and volunteers of <ACATER West>, with interesting feedbacks and rising questions regarding cases deemed suspect or doubtful. Among these, could be the case of a Sika deer hunted in January 2014 in the province of Parma, about 30 miles far from Modena's site, but still included in the same <ACATER West> management unit. As in the past, the animal was an adult male, weighing 123 kg. The collaborative attitude of the hunters allowed to start collecting samples for genetic tests aimed at determining if the three Sika deer so far shot were pure species or hybrids. Additional tests will determine whether some apparently pure European deer shot may in fact derive from introgression from Sika or not. The genetic surveys based on microsatellites analysis, in cooperation between Czech and Italian laboratories, are in progress.

Ferri M, Ghirardelli R, Corsini C, Gelmini°L, Rugna°GL

**A case of death by starvation of a group of wild boar *Sus scrofa* in the high Apennines of Modena during a long snow period, in February 2012**

Hystrix Ital J Mammalogy. - Vol. 25 (supplement) ( 2014). - p 105 [Nr. Estr. 5841]

Congresso Italiano di Teriologia (9. : Civitella Alfedena (AQ) : 7-10 Maggio 2014)

In February 2013 near a village of the municipality of Frassinoro, in the high Apennines of Modena province, have been reported some wild boar *Sus scrofa* in trouble in the high snow that lingered the local mountains since several weeks; after some attempts to rescue them only some carcasses were found scattered in the thick blanket of snow. Over a period of a week were recovered the carcasses of three sub-adult and finally were found also an adult. Other carcasses less precisely reported in the same area were not found. The wildboars were as crouched in the snow without fresh paths around them; after recovery were transported to the Zoo-prophylactic Institute of Modena where they were subjected to autopsy. For all the four subjects were found: absence of signs of infectious diseases, absence of wounds and poisoning, extreme weight loss, absence of subcutaneous fat, absence of visceral fat, empty stomach and intestines. The stomach of the adult actually showed a small amount of a dark mush that apparently looked like a mixture of potting soil and rotted wood. Also it was registered a strong pediculosis. In addition to the effects of long snow, animals may have also been badly affected by a scarce availability of beechnuts, acorns and chestnuts caused by a severe drought in summer and autumn. Few other episodes were reported in the woodlands of nearby municipalities but without available information to retrieve the carcasses.

Ferroglio E, Chiari°M, Zanet S, Triscioglio A, La vazza°A

***Leishmania infantum* infection in European brown hare and cotton-tail rabbit from North Italy**

11th European Wildlife Disease Association Conference (EWDA) : Edinburgh, 25-29 August 2014 / [s.l. : s.n., 2014]. - 1 p. [Nr. Estr. 5852]

European Wildlife Disease Association Conference (EWDA) (11th : Edinburgh : 25-29 August 2014)

Iberian hares (*Lepus granatensis*) have been recently deemed to be responsible of a major outbreak of human leishmaniasis that affected the metropolitan area of Madrid (Spain). However, the reservoir potential of lagomorphs in Europe is poorly known. A lagomorph introduced for hunting purposes from North America to Europe where, in certain areas like Northern Italy, its population has reached a conspicuous size. We report a cross-sectional survey on the *Leishmania infantum* —the causal agent of zoonotic endemic leishmaniasis in the Mediterranean basin — infection status of European hares (*Lepus europaeus*) and cotton-tail rabbit (*Sylvilagus floridanus*) in North Italy. A PCR protocol targeting a fragment present in *L. infantum* genome was performed over spleen samples from 24 hares and 76 cotton-tail rabbit. DNA prevalence in hare and cotton tail spleen samples was 25.93% (95%CI 11.87-46.59%) and 31.58% (95%CI 21.66-43.78%) respectively. The recent evidence of the ability of naturally infected hares to transmit *L. infantum* to *Phlebotomus perniciosus* — its main vector in Italy — and the widespread presence of the pathogen suggests lagomorphs may have an unexpectedly relevant role in the epidemiology of *L. infantum*.

Fiorentini°L, Tosi°G, Milandri C, Usberti R, Rosetti M, Ricci G, Casadio°M, Massi°P

**Prevalenza di *Campylobacter jejuni*, *Campylobacter coli* e *Campylobacter lari* in broiler regolarmente macellati; profilo di antibiotico resistenza**

Atti della Società Italiana di Patologia Aviare (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio

2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 145-154. - 16 bib ref [Nr. Estr. 5975]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*Thermophilic Campylobacter spp. have been recognized as major cause of foodborne infections in many countries throughout the world. Poultry meat is the most common source for foodborne cases of human campylobacteriosis. In order to study the prevalence of Campylobacter in broiler chickens a study was carried out in a slaughterhouse during 2012. The study was performed on 50 flocks. The overall prevalence of the Campylobacter spp. infection was 76% of the tested flocks. The prevalence of the C. jejuni infection was 36%, C. coli 32%, association C. jejuni and C.coli 6% and C. lari infection 2% of the tested flocks. In the second part of the study twenty Campylobacter strains (ten strains of C. jejuni and ten strains of C. coli from skin and ceca), were tested by Minimum Inhibitory Concentration (MIC) for their susceptibility to Penicillin, Betalactams, Macrolides, Tetracyclines, and Fluoroquinolones. All isolated strains of Campylobacter were fully susceptible to Penicillin. Low resistance were detected for Amoxicillin ranging from 10% and 20%. Very high resistance rates were detected for Macrolides (ranging from 10% and 60%), Tetracyclines (60%), and Fluoroquinolones (ranging from 40% and 50%). No isolates showed resistance to Tiamulina. Campylobacter is a leading foodborne bacterial pathogen, which causes gastroenteritis in humans. This pathogenic organism is increasingly resistant to antibiotics, especially Fluoroquinolones and Macrolides, which are the most frequently used antimicrobials for the treatment of campylobacteriosis when clinical therapy is warranted. For this reason, the study's antibiotic resistance is a major public health problem and current.*

Fiorentini°L, Tosi°G, Taddei°R, Barbieri°I, Casadio°M, Massi°P

#### **Approfondimenti diagnostici su casi di proventricolite in polli da carne**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 138-144. - 10 bib ref [Nr. Estr. 5969]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*A proventriculitis syndrome (associated to a malabsorption syndrome) in a multi age organic broiler farm was observed. Clinical signs and gross lesions were detected between 19 and 38 days of age. The same symptoms and lesions were also observed in the previous production cycle. In younger groups (19 and 24 days of age), the enteric lesions were most prominent and Reovirus and Rotavirus were observed using Electron Microscopy. At 31 and 38 days of age proventriculitis lesions were more evident. At 31 days of age the PCR and the RT-PCR revealed an infection caused by fowl adenovirus serotype 2 (in liver and intestine) and Enterovirus-like particles were observed by Electron Microscopy. At 38 days of age a mixed infection caused by Fowl Adenovirus serotype 2 (FAV2), in the proventriculus, intestine and liver and Reovirus, in the intestine, was detected using PCR and RT-PCR. Other viruses (such as Birnavirus, Infectious Bronchitis Virus, Chicken Infectious Anemia Virus and Infectious Bursal.*

Floris I, Carpana E, Bassi°S, Formato G, Ceresini A, Lodesani M

#### **Malattie batteriche**

Patologia e aversità dell'alveare / a cura di Emanuele Carpana, Marco Lodesani. - Milano : Springer-Verlag Italia 2014, 2014. - p 49-121. - 187 bib ref [Nr. Estr. 5903]

Foni°E, Arioli E, Bongiovanni E, Bresola M, Codato A, Donna R, Faccenda M, Fruttero F, Gamba F, Giorgiutti M, Salvini F, Zizioli B, Leotti G, Merdy O, Vila T, Joisel F, Ostanello F

**Influenza suina : profili sierologici in animali vaccinati di allevamenti del Nord Italia** = Swine influenza : serological responses in vaccinated pigs in Italian herds

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 251-260. - 9 bib ref [Nr. Estr. 5671]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

Nel corso del 2013 è stato eseguito uno studio sull'andamento temporale degli anticorpi nei confronti dei sottotipi H1N1, H3N2 e H1N2 del virus dell'influenza suina (SIV) in soggetti allevati in 11 aziende e immunizzati nei confronti di SIV con un vaccino trivalente inattivato. In ciascuna azienda sono stati selezionati tre gruppi di animali: un primo gruppo è stato vaccinato a 70-90 giorni di vita, con richiamo a distanza di quattro settimane e a circa 180 giorni di vita, con un vaccino trivalente nei confronti di SIV e con un vaccino nei confronti della malattia di Aujeszky (ADV), mediante inoculazione i.m. in due punti diversi. Un secondo gruppo è stato vaccinato, con la stessa tempistica, utilizzando una miscela dei due vaccini somministrata i.m. nello stesso punto di inoculo. Il terzo gruppo di animali è stato vaccinato solo nei confronti di ADV come tutti gli altri suini presenti nell'allevamento (gruppo di controllo). È stato effettuato un prelievo ematico da tutti gli animali appartenenti ai tre gruppi prima della vaccinazione, quattro settimane dopo il richiamo, in concomitanza con il terzo intervento vaccinale a 180 giorni di vita e prima dell'invio alla macellazione. I sieri sono stati analizzati per la ricerca di anticorpi nei confronti dei sottotipi H1N1, H3N2 e H1N2 mediante inibizione dell'emoagglutinazione. La vaccinazione nei confronti di SIV ha determinato un incremento significativo dei titoli anticorpali nei soggetti vaccinati rispetto a quelli di controllo. Tale differenza è stata riscontrata anche nelle aziende nelle quali è stato possibile, su base sierologica, ipotizzare la circolazione di SIV. La conferma virologica si è avuta soltanto in una azienda nella quale sono stati riscontrati evidenti sintomi respiratori. Le diverse modalità di somministrazione della vaccinazione non hanno determinato differenze significative nella risposta anticorpale.

*Serological responses of pigs vaccinated or not vaccinated with a commercial inactivated influenza vaccine containing H1N1, H3N2 and H1N2 strains, were compared in 11 farms in Northern Italy during 2013, to evaluate vaccine serological efficacy under current field conditions. In each farm two groups of 12-14 pigs were vaccinated against swine influenza virus (SIV) concomitantly to vaccination against Aujeszky Disease virus (ADV): twice before turn-to-fattening (70-90 days of age and 4 weeks later) and at 180 days of age, during fattening. One group received separated injections of the two vaccines and one group was vaccinated following dilution of the ADV vaccine dry pellet in the SIV vaccine. A third group of 12-14 pigs (controls) was also included in the study and were vaccinated only with ADV vaccine at the same dates as the other pigs of the flow. Blood samples were roughly collected at around 70-90, 130-150, 180-210 and 250 days of age (just before slaughter) for antibody titration against recent Italian SIV subtypes H1N1, H3N2, and H1N2 by inhibition of hemagglutination test. The vaccination appeared capable of inducing a significant antibody response against the SIV strains circulating in Italy. No clear difference was found in SIV antibody titers whatever the vaccination protocol.*

Foni°E, Garbarino°C, Chiapponi°C, Baioni°L, Zan ni°I, Cordioli°P

### **Epidemiological survey of swine influenza A virus in the wild boar population of two Italian provinces**

World Biomedical Frontiers. - Vol. 2014). - Ultimo accesso 10/04/2014 - <http://biomedfrontiers.org/> [Nr. Estr. 5565/a]

**Objectives** An epidemiological survey was carried out in order to obtain a better understanding of the role of wild boars in the epidemiology of the influenza virus. **Design** The samples were submitted to Real-Time PCR testing for gene M of the swine influenza virus (SIV), and virus isolation was performed from the positive PCR samples. Genome sequence analysis was performed on the isolates. Additionally, 1,977 boar sera samples were analyzed using ELISA and hemoagglutination inhibition. **Setting** Over recent years, the wild boar population has greatly increased in Italy, including in areas of high-density industrial pig farming, where the influenza virus is widespread. From July to December 2012, wild boar lung samples were collected in the Parma and Piacenza area, in the Emilia Romagna region. **Sample** 354 wild boar lung samples were collected. **Main outcome measures** Wild-boar influenza A virus infection should be studied more broadly in order to obtain a better understanding of the epidemiological role played by this species. **Results** Three SIV strains were isolated out of 12 samples that resulted positive using PCR analysis and they were identified

as avian-like SIV subtype H1N1. Phylogenetic analysis of the sequences obtained from isolate A/wild boar/291320/2012 showed that it clustered with recent Italian avian-like H1N1 SIVs isolated from domestic pigs. Sixty-eight sera samples showed a positive titer to the isolate A/wild boar/291320/2012. Conclusions This study suggests that SIV actively circulates in the wild boar population in the investigated area.

Foresti F, Alborali°G, Giacomini°E, Gianazza S, Klein U

**Clinical efficacy and performance improvement of Econor® (valnemulin) vs lincomycin for the control of Brachyspira hyodysenteriae infection in a pig fattening unit in Italy**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 232 (Poster P194) [Nr. Estr. 5724]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

Swine dysentery (SD) infections are of growing concern in Italian pig sector where it is estimated that 40% of fattening units are affected. Large farm units and the long fattening cycle typical of Italian production up to 160 kg be are two of the main reasons of SD spreading. A trend of increasing MIC values of antimicrobials against *Brachyspira hyodysenteriae* (*B.hyo*) is observed. Lincomycin is currently intensively used, while Econor® (valnemulin - Novartis AH) is considered to be the "Last resort choice" in case of heavy outbreaks of SD. A pig fattening unit with a recent history of a heavy outbreak of SD was chosen to run a comparative trial with valnemulin and with lincomycin. Two groups of 480 fattening pigs each were treated via medicated feed with valnemulin (4 mg/kg bw, group A) or with lincomycin (10 mg/kg bw, group B) for 15 days starting at 90 days of age when entering the fattening unit. Thirty pigs in each group were ear-marked and underwent periodic weighing and blood and faeces sampling from the beginning of the trial up to slaughter for isolation of *B. hyo* and *Lawsonia intracellularis* from faeces and *L. intracellularis* titers in the serum. Clinical observation and possible necropsy were carried out weekly. ADG and FCR were 0,670 and 0,640 and 3,55 and 3,70 respectively in group A (valnemulin) and group B (lincomycin). Mortality rate was 1,47% (7 pigs) in group A and 3,31 % (16 pigs) in group B. At the end of the trial no outbreaks of SD were determined and all faecal samples collected were negative. *B. hyo* strains were isolated from colon contents in 3 out of 16 animals necropsied (group B) and in 0 out of 7 animals in group A. At the slaughterhouse *B. hyo* strains were isolated in 2 out of 30 colon contents in each treatment groups. These positive samples confirmed active circulation of *B. hyo* within the pigs. *Lawsonia intracellularis* was not isolated from faeces but a serological titer increase was demonstrated within the population. The valnemulin treated group demonstrated a significantly lower mortality and a significantly better performance with a higher ADG, a lower FCR and a better ROI.

Foresti F, Pussi A, Giacomini°E, Lazzaro°M, Migliori M, Ruggeri°J, Zanoni°MG, Parini E, Coceva G, Pasquali P, Alborali°GL

**Effect of specific mix of monoglycerides and diglycerides of short and medium chain fatty acids in fattening pigs diets to control swine dysentery**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - v. 1. - p 320. - 4 bib ref [Nr. Estr. 5730]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Forlenza J, Rugna°G, Bonilauri°P, Carra°E, Luppi °A, Martelli P, Merialdi°G

**L'utilizzo della tipizzazione molecolare (MLST) e della sierologia per lo studio dell'epidemiologia di *Brachyspira hyodysenteriae* in un allevamento suino multisito = Use of**

molecular typing (MLST) and serology to study the epidemiology of *Brachyspira hyodysenteriae* infection in a multisite pig farm

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 161-169. - 20 bib ref [Nr. Estr. 5677]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

Un programma di controllo nei confronti della dissenteria suina (DS), specialmente in allevamenti multisito, per essere efficace deve basarsi sulla conoscenza dell'epidemiologia intra-allevamento di *Brachyspira hyodysenteriae*. Lo scopo di questo studio è stato l'identificazione delle fonti di infezione e delle vie di trasmissione dell'agente patogeno in un allevamento con DS endemica, utilizzando la sierologia e la tipizzazione molecolare (MLST) dei ceppi circolanti. L'allevamento è composto da 5 siti (A, B, C, D, E) tra loro strettamente correlati funzionalmente; è autosufficiente e solo occasionalmente vengono comprati lattoni da un allevamento non di proprietà, considerato indenne da DS. Nell'allevamento si verificano episodi ricorrenti di dissenteria emorragica in tutte i siti eccetto A (scrofe grand-parent) e D, dove la malattia non si presenta clinicamente da almeno due anni. Il protocollo di studio ha previsto il prelievo di campioni di sangue da tutte le unità e campioni fecali e/o visceri da soggetti con sintomi di DS in un periodo di 18 mesi. Gli isolati di *B. hyodysenteriae* sono stati sottoposti a determinazione della MIC ed MLST. I campioni di sangue sono stati testati sierologicamente con un prototipo di kit ELISA in fase di sperimentazione. La MLST ha mostrato la circolazione di isolati appartenenti tutti allo stesso sequence type (ST 77). La sierologia ha rilevato positività in tutte i siti ad eccezione di E in cui erano presenti suini introdotti da fonte esterna indenne. Sulla base della storia clinica e dei risultati di laboratorio è possibile ipotizzare che le scrofe granparentali del sito A, sebbene non affette da DS, siano la probabile fonte di diffusione di *B. hyodysenteriae*. L'uso combinato di MLST e sierologia si è dimostrato un potenziale strumento per una eventuale futura implementazione di strategie di controllo aziendale nei confronti dell'infezione da *B. hyodysenteriae*.

*A successful control program for Swine dysentery (DS), especially in multisite pig herds, relies on the accurate understanding of the intra-herd epidemiology. The aim of the study was to attempt the identification of the infection's sources and the transmission patterns of B. hyodysenteriae in an Italian pig farm suffering from DS by using a molecular typing method (MLST) and serology. The study was conducted in a herd based on 5 sites (A, B, C, D, E). Recurrent episodes of DS were observed, but 2 sites (A and D) have no history of DS in the last 2 years. Fecal samples were collected from pigs suffering from clinical signs of DS. Blood samples from at least 40 pigs were collected from each site. Fecal samples were cultivated for B. hyodysenteriae and the isolates were submitted to MLST. Two hundred blood samples were tested with a prototype of ELISA kit. The MLST results showed the circulation of isolates belonging to the same sequence type (ST 77). The ELISA test showed that B. hyodysenteriae antibodies were present in all groups including those without a recent history of clinical DS but in site E where pigs introduced from an DS free herd were allocated. Based on the case history and the laboratory results it is possible to hypothesize that grandparent sows site A), even though not affected by clinical DS, are the probable single source of B. hyodysenteriae spreading. The combined use of molecular typing and serology could be of help for the correct implementation of control strategies.*

Formenti N, Ferrari N, Pedrotti L, Gaffuri°A, Trogu T, Lanfranchi P

#### **Epidemiological investigation of *Toxoplasma gondii* in Alpine red deer (*Cervus elaphus*) : spread and effects on pregnancy**

Hystrix Ital J Mammalogy. - Vol. 25 (supplement) ( 2014). - p 61 [Nr. Estr. 5843]

Congresso Italiano di Teriologia (9. : Civitella Alfedena (AQ) : 7-10 Maggio 2014)

Several animal species can be infected by the widespread proto-zoan *Toxoplasma gondii* contributing to maintain both domestic and sylvatic parasite lifecycle and favouring the raise of public health issues related to its zoonotic value. As wild ungulates can be source of *T. gondii* for humans through consumption, manipulation and evisceration of carcasses, risk of infection should be evaluated in relation to the amount of game meat available from harvest plans every year. Therefore in Italy the attention should be focused on red deer (*Cervus elaphus*) because of the intense hunting activity in many Alpine areas and since this species is the most frequently consumed raw or

undercooked. In particular, despite the well documented zoo-economic losses in livestock, little is known about the epidemiology of *T gondii* infection in red deer particularly regarding any impact on populations' dynamics. Besides no assessments are reported about *T gondii* associated-reproductive pathologies in this species although vertical transmission recorded in white tailed deer (*Odocoileus virginianus*) and natural transplacental toxoplasmosis documented in a stillborn reindeer (*Rangifer tarandus*) foetus point out the hypothesis that also red deer could be affected. In addition just few studies have investigated the effect of parasitism on fecundity of ungulates in natural conditions. Here we performed a sero-epidemiological investigation of *T gondii* in red deer from two areas in Stelvio National park (Italian Central Alps), and three Generalized Linear Models were set up to evaluate: (1) the epidemiological factors influencing the probability to get infected; (2) if the infection is acquired before the breeding season or in early pregnancy and (i) could cause early abortion and drive hinds to lose reproduction, (ii) may influence hinds' fertility through a delay in the physiological development of foetus. During two consecutive weeks between the end of November and the beginning of December 2012, 81 red deer sera were collected during the culling management plan scheduled by the park, for each subject age, sex, location and morpho-biometric measures were recorded. In females, lactation and pregnancy were also registered together with foetus body weight and length. Sera were tested for the presence of anti-*T gondii* IgG using a commercial ELISA kit (IDVET, Montpellier, France). An overall seroprevalence of 39.5% emerged, giving evidence to the circulation of the pathogen in the study area. In particular, a significant effect of age class (calves, 1-year-old and >2-year-old deer) was recorded: the probability to contract infection is significant lesser in calves than in the two others. No significant difference emerged between 1-year-old and >2-year-old deer pointing out an equal infection in these age classes supporting the hypothesis of a high level of environmental contamination. Considering the sporadic presence of lynx (*Lynx lynx*) in Italian Alps, feral and semi-domestic cats are the only definitive hosts responsible for *T gondii* spreading. Calves did not contract the infection, apart just one female, and this fact suggests an almost total lack of vertical transmission in the studied population. *T gondii* infection seems not to prevent hinds to become pregnant or to cause early abortion, leaving females apparently barren although a negative effect of the pathogen on foetuses development of 2-3 year-old hinds and of hinds from area 1 was recorded. These results highlight that the pathogen could anyway affect pregnancy supporting the hypothesis that these hinds had acquired the infection before the breeding season or in early pregnancy. In particular *T gondii* seems to have influenced their fertility through a delay in the physiological development of foetus or to have affected hinds' breeding season provoking a delay in mating or in pregnancy. Data arisen give evidence to a high level of *T gondii* environmental contamination with horizontal transmission as the only route of infection in the study area. In this sense a widespread exposure to infection is supposed and should be taken into account in relation to the parasite zoonotic potential. The recorded negative impact of *T gondii* on foetus development of both 2-3 year-old hinds and females from area 1 points out that under specific conditions the pathogen could give an impact on population dynamics of this intermediate host. Further analysis are needed to evaluate the distribution, densities and *T gondii*-cero prevalente of semi-domestic and feral cats in order to define their role in environmental contamination and thus their effect in red deer infection.

Formenti N, Gaffuri<sup>o</sup>A, Trogu T, Ferrari N, Pedrotti L, Viganò R, Lanfranchi P

**Comparison between serological and molecular diagnostic approach to toxoplasma gondii infection in Alpine red deer (*Cervus elaphus*)**

XXVIII Congresso Nazionale Societa' Italiana di Parassitologia SOIPA : Roma, 24-27 giugno 2014 / [s.l. : s.n., 2014]. - p 229 [Nr. Estr. 5825]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA (28. : Roma : 24-27 giugno 2014)

**INTRODUCTION:** The widespread *Toxoplasma gondii* affects several domestic and wild animal species raising public health issues related to its zoonotic value. As wild ungulates can be source of human infection, risk should be evaluated in relation to the amount of game meat available from harvest plans and besides a reliable diagnostic trial to monitoring *T. gondii* in free-ranging populations should be defined. Focusing on red deer (*Cervus elaphus*), because of intense hunting

activity in many Alpine areas and since this species is consumed even raw or undercooked, we performed a serological and molecular investigations of *Toxoplasma gondii* to evaluate (i) the reliability of cardiac tissue fluids as an alternative to sera in ELISA test and (ii) the applicability of three PCR protocols. **MATERIALS AND METHODS:** Overall 78 sera and cardiac tissue fluids and 159 brain tissue samples were collected for respectively serological and molecular investigations from Lepontine Alps (VB) during the hunting season 2011 and during the culling management plan scheduled by the Stelvio National Park in 2012. A commercial ELISA kit (IDVET, Montpellier, France), validates for ruminants' sera and tissue fluids, was used and the agreement between analytical approach was assessed calculating the Kappa (K) value. DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Italy) and the whole samples were assayed by targeting a 529 bp noncoding region (Homan et al., 2000, *Int. J. Parasitol.*, 30:69-75) (protocol 1). Positive and doubtful results were then submitted to a single tube nested PCR (Hurtado et al., 2001, *Vet. Parasitol.*, 102:17-27) (protocol 2) and to a PCR-RFLP using primers that identify also *Neospora caninum* and *Sarcocystis* spp. (Magnino et al., 1998, *Proc. IX ICOPA:1269-1272*) (protocol 3). **RESULTS:** A *T. gondii* prevalence of 29% and 19% was recorded respectively in sera and cardiac fluids showing a "fair agreement" (K value = 0.38) between the two matrices and a loss in sensitivity and specificity using cardiac fluids. The whole analysed samples resulted negative for *T. gondii* DNA. In 15 samples doubtful PCR products with no-specific bands resulted from the protocol 1 and thus they were submitted to the second and the third PCRs. The other two protocols cleaned PCR amplified products confirming the *T. gondii* negativity but *Sarcocystis* spp. DNA was detected by protocol 3 in a 6-month-old male and in a 1-year-old female. Sequencing analysis identified *Sarcocystis hjerti* DNA from the calf brain tissue. **CONCLUSIONS:** The study highlights a few diagnostic difficulties in both *T. gondii* serological and molecular investigation in red deer. Although working with wildlife it could be easier sampling an heart than a serum sample, cardiac tissue fluids cannot be considered as an alternative of serum in this species using this ELISA test. The doubtful results emerged using PCR protocol 1 suggest that this molecular technique can have a lower specificity than two others with red deer samples and besides protocol 3 was useful to detect the cross reaction with *Sarcocystis* spp.. As application of diagnostic tests developed from livestock or human to wildlife could compromise their performances, the need is to set up diagnostic methods specific for wild species mainly for pathogens of public health significance.

Fusi°F, Zanardi°G, Bolzoni°G, Angelucci°A, Lore nzi°V, Donati°M, Bertocchi°L

#### **Could bulk tank somatic cell count be an indicator of overall welfare status for dairy cows?**

Proceedings of the First DairyCare Conference : Copenhagen, August 22nd and 23rd 2014 / editor, C.H. Knight. - [s. l. : DairyCOST action FA1308, 2014]. - p 41 [Nr. Estr. 5857]

DairyCare Conference (1st : Copenhagen : August 22nd and 23rd 2014)

Public opinion is increasingly sensitive to animal welfare. The percentage of European citizens worry about farm animal welfare has increased from 60% in 2005 to 64% in 2010 (Eurobarometer 354) and many of them would be willing to pay a higher price for food sourced from an animal Welfare friendly production system (Eurobarometer 229). In this contest, the Italian National Reference Centre for Animal Animal (CReNBA - IZSLER Brescia) has proposed a system to assess the overall dairy cow welfare on farm, using both animal-based measures (ABMs) and non-animal based measures (NABMs). This system comprises 78 different assessment items and is divided into 4 areas: Area A - management and personnel; Area B - structures and equipments; Area C - ABMs; Area D - exposure to environmental hazards. The amount of all scores gives the overall level of animal welfare ori farm, with a range from 43 (min) to 185 (max) points. The animal assessment was carried out in 265 loose housing system dairy farms in Northern Italy, characterized by an average number of 217 animals, 99 lactating cows and 27 kg/per cow as daily milk production. The average total welfare score (WS) was 139 and the average value of Bulk Tank Somatic Cell Count (BTSCC) was 288,000 cells/mi, calculated by 5 samples analyzed during 3 months before the welfare assessment. Farms were divided into 2 groups, according to the media of the total WS, and compared with average BTSCC, herd size and individual milk production. The strongest correlations were found between WS and both BTSCC and individual milk production. Farms were then divided into 4 groups, according to their BTSCC (Table 1). No correlation was found between WS and

BTSCC < 300,000 cells/ml; conversely, farms with BTSCC > 300,000 and even more > 400,000 cells/ml had the worst WS. Since mastitis is a multi-factorial disease, very susceptible to management and structural factors (which are also very important for the animal status of an animal), in the near future we would like to better understand the relationship between milk quality and the overall animal welfare status in a given farm.

Gaia S, Alfonsi V, Bruni R, Busani L, Cappelletti B, Ciccaglione AR, De\_Medici D, Di\_Pasquale S, Escher M, Guizzardi S, Lena R, Losio°MN, Martini V, Massaro M, Menghi A, Montano\_Remacha M del Carmen, Monteleone D, Pavoni°E, Pompa MG, Ricotta L, Rizzo C, Taffon S, Tosti ME, Vellucci L, Borrello S

### **Epidemia transnazionale di epatite A connessa al consumo di frutti di bosco congelati : l'indagine epidemiologica in Italia**

PROgramma di Formazione in Epidemiologia Applicata (PROFEA) : "L'epidemiologia applicata al servizio della costruzione della Salute" : V Conferenza PROFEA, Orvieto, 22 ottobre 2014 : riassunti / a cura di Paola Scardetta, Valerio Occhiodoro e Luana Penna. - Roma : Istituto Superiore di Sanità, c2014. - (ISTISAN congressi ; 14/C4) p 40-41 [Nr. Estr. 5894]

Conferenza Profea (5. : Orvieto : 22 ottobre 2014)

Introduzione. L'infezione da virus Epatite A (HAV) è causa di malattia a decorso generalmente benigno. Il consumo di alimenti contaminati e il contatto persona-persona (via oro-fecale) sono le principali vie di trasmissione del virus. In Italia la malattia è considerata a bassa/media endemicità, con picchi elevati d'incidenza nelle regioni ove è consolidata l'abitudine a consumare frutti di mare crudi. Obiettivi. Dal gennaio 2013 il nostro Paese è stato interessato da una vasta epidemia transnazionale di infezione da HAV genotipo IA che ha coinvolto 12 Paesi. Il presente lavoro descrive i risultati delle indagini svolte in Italia. Materiali e metodi. Le indagini sono state coordinate da una task-force multidisciplinare, istituita presso il Ministero della Salute, con l'obiettivo di armonizzare le attività di indagine dei casi epidemici, i criteri di campionamento e analisi microbiologica degli alimenti. Le attività si sono articolate in stretto raccordo con le autorità del SSN competenti sul territorio. Il case finding si è avvalso di protocolli diagnostici basati sul sequenziamento del genoma virale (regioni VP1/VP2A), capaci di discriminare i casi associati al ceppo epidemico e di consentire il confronto con le sequenze ottenute dagli alimenti. L'associazione tra consumi alimentari e casi epidemici è stata indagata attraverso uno studio caso-controllo (119 casi e 419 controlli). La possibile fonte di contaminazione virale è stata indagata attraverso uno studio di tracciabilità degli alimenti. Risultati. Tra gennaio 2013 e giugno 2014 sono stati identificati 1.300 casi di epatite A, con un incremento 4 volte superiore al 2012. In 228 casi è stata rilevata identità nucleotidica con il ceppo epidemico di riferimento (GenBank KF182323). Le indagini microbiologiche ed epidemiologiche hanno entrambe fornito una 'forte' evidenza che frutti di bosco congelati fossero la fonte epidemica. 15 degli oltre 1.300 campioni di frutti di bosco testati per HAV, sono risultati positivi. Per uno di essi è stato rilevato il 100% di similarità con il ceppo epidemico. I frutti di bosco congelati risultavano associati ai casi epidemici anche nello studio analitico (ORacii, 4,2; 95% IC, 2,54-7,02). L'analisi delle oltre 1.200 transazioni da 400 fornitori in 19 Paesi non ha consentito di individuare fornitori comuni a tutti i lotti tracciati, tuttavia il 100% di essi conteneva ribes provenienti dalla Polonia. Conclusioni. Eventi epidemici di grande complessità come quello descritto richiedono una forte capacità di coordinamento e un approccio multidisciplinare che il nostro SSN ha saputo mettere a disposizione confermano alimento ad elevato profilo di rischio. L'assenza di punti critici di controllo nella filiera produttiva, la lunga shelf-life e la modalità di consumo a crudo impediscono l'abbattimento di eventuali contaminazioni accidentali. Lo studio di tracciabilità sembra escludere una contaminazione per effetto di un singolo ingrediente, rimandando a scenari alternativi più complessi quali la contaminazione di intere zone aree produttive ovvero meccanismi di cross-contaminazione nella filiera distributiva.

Galletti°G, Paternoster°G, Santi°A, Renzi°M, Caminiti°A, Rugna°G, Magnolini C,

Stefini G, Tamba°M

**Web-based information system for the surveillance of canine Leishmaniasis in Emilia-Romagna public kennels**

8th Annual Epizone Meeting "Primed for tomorrow" : 23 - 25 September 2014 Copenhagen, Denmark : posters "Vector borne disease" / [s.l. : s.n., 2014]. - 1 p [Nr. Estr. 5922]

Annual meeting Epizone (8th : Copenhagen, Denmark : 23 - 25 September 2014)

In 2007 a regional surveillance program for canine Leishmaniasis (CanL) has been implemented in all public kennels of Emilia-Romagna region (ER, Northern Italy). Serological and entomological monitoring activities - IFAT and sticky traps, respectively - have been performed annually to rank kennels in four risk classes, in order to apply specific risk-based control interventions. According to this approach, all stray dogs are sampled for CanL at the moment of admittance to the kennel. Seropositive (IFAT titre 1/160) or inconclusive dogs (IFAT titre of 1/40 or 1/80) (1) are re-tested to follow up the health status and decide for appropriate therapy. Clinical suspects are sampled to confirm CanL diagnosis, and in kennels where the presence of the vector has already been proved, a sample of sentinel housed dogs, tested negative at least once, is checked yearly to monitor the spread of Can infection. Being monitoring activities and control measures carried out by the official veterinary service well-established, in 2014 we have developed a web-based information system (WIS) for the surveillance of CanL in public kennels, with the aim of enhancing quality and data collection. The system, available online at <http://seer.izsler.it>, currently collects data on more than 25,000 univocally identified dogs, tested in the 73 kennels of ER. The results of serological testing, which are updated daily, can be consulted online by the official veterinary service, accessing the WIS by signing in with a user name and password. The system displays the microchip number or tattoo of positive and inconclusive dogs to facilitate the planning of (i) further serological testing, (ii) an appropriate therapy and (iii) the adoption of preventive measures. Moreover, the WIS permits the integration of anamnestic information over time (symptoms, therapy, etc.), as well as the registration of the date of adoption or death. Finally, possible inconsistencies of the dog identification or mistakes while filling in the sampling forms are automatically highlighted, providing timely and consistent information which are a key for the success of a surveillance program. The online informative system, released in May 2014, could be further developed by adding a risk map on the spread of CanL infection in ER. This would provide physicians and veterinary practitioners with updated information on the epidemiology of CanL in their territory of competence.

Gamberale F, Barlozzari G, Scaramella P, Pietrella G, De\_Santis G, Turriziani G, Ricchi°M, Arrigoni°N, Macrì G

**First isolation and genotyping of *Mycobacterium avium* subsp. *paratuberculosis* in Italian mediterranean buffalo (*Bubalus bubalis*) in Italy**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 167 (Poster P-05.5) [Nr. Estr. 5764]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

The current study aimed at reporting the first isolation and -typing of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in the Italian Mediterranean Buffalo, in Italy. In the international scientific literature, few data are available about MAP in buffalo. In Italy, *Mycobacterium avium* subsp. *paratuberculosis* in buffalo was isolated in 1999 even if at that time it was not genotyped. In this study, we tested two adult subjects bred in two different farms. The first animal showed clinical signs (weight loss, diarrhea) but ELISA tests resulted repeatedly negative. The culture tests on Herrold's Egg Yolk Medium (HEYM) yielded a positive result for mesenteric lymph node, ileum, ileocecal valve and stool samples. Kinyoun stain confirmed the presence of acid-fast bacilli. The second animal was asymptomatic but showed positive results in ELISA. Culture and microscopic tests on stool samples resulted positive and both animals were high shedders. These results were confirmed by IS900 commercial end-point PCR. The isolates were sent to the National Reference Centre for Paratuberculosis (IZSLER- Piacenza, Italy) to be confirmed by F57-PCR and typed by IS1311 REA-PCR and by PCR-DMC. The isolates were confirmed as MAP and identified as type C

(ll or cow type).

Garbarino°C, Bianchi°A, Gaffuri°A, Ricchi°M, Sa vi°R, Cammi°G, Leo°S, Bertoletti°I, Arrigoni°N

**Mycobacterium avium subsp. paratuberculosis in wild ruminants of Italian Central Alps**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 189 (Poster P-06.9) [Nr. Estr. 5761]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

Investigation on Mycobacterium avium subsp. paratuberculosis (MAP) diffusion in wildlife encounters many difficulties: collection of suitable samples, limits of serological tests (validated only for domestic ruminants) and difficult growth of some MAP strains. We examined samples of free ranging wild ruminants (red deer, roe-deer, chamois and mouflon from Italian Central Alps. During 2012-2013 hunting season, we collected 760 samples for direct diagnosis (290 tissues and 470 feces) and 1656 sera. Nine animals showed gross pathology: in 7 young hinds/stags and one adult stag we observed loss of body conditions, rough coat, enteritis and lymph nodes extremely enlarged; in a roe-deer enlarged lymph nodes with micro-calcifications. Tissues and feces were examined by IS900-9PCR and the positive samples were cultured, both in solid (HEYM) and liquid media (Versatrek™ instrument). Sixty-nine samples (9%) resulted positive to PCR and 10 strains of MAP, all from deer, were isolated. All animals with gross lesions resulted positive to PCR and to histopathology. All deers positive to PCR came from an area of Stelvio National Park where MAP is endemic in this species and deer population density is high (winter density: 8.5 animals per 100 ha). The positive roe deers, chamoises and mouflons came from Bergamo Alps. For serology, we used a commercial ELISA kit (IDVET) and AGID. Less than 1% of samples resulted positive for AGAQ and ELISA. On 37 selected sera (all from PCR positive animals), we tested different ELI-SA kits in parallel. Out of these 37 sera, 5 were positive (13.5%) for all the kits. The majority of positive sera (60%) came from animals with macroscopic lesions, suggesting that it is necessary to improve serological sensitivity. In conclusion, PCR resulted the most sensitive method for the surveillance of MAP infection in wild ruminants; on the contrary, serological and cultural methods didn't provide sufficient sensitivity.

Garbarino°C, Gaffuri°A, Bianchi°A, Ricchi°M, Sa vi°R, Leo°S, Bertoletti°I, Giacomelli°S, Formenti N, Arrigoni°N

**Diagnostic tools for paratuberculosis in free ranging wild ruminants in endemic and sporadic area**

11th European Wildlife Disease Association Conference (EWDA) : Edinburgh, 25-29 August 2014 / [s.l. : s.n., 2014]. - 1 p [Nr. Estr. 5924]

European Wildlife Disease Association Conference (EWDA) (11th : Edinburgh : 25-29 August 2014)

During 2011-2013 hunting seasons we tested samples of red deer, roe deer, chamois and mouflon from three Northern Italian areas: 1)Stelvio National Park, where paratuberculosis is endemic in red deer; and 2)Orobic Alps and 3)Appennini mountain chain, where it is sporadic. Tissues and feces were tested for Mycobacterium avium subsp. paratuberculosis (MAP) by IS900-qPCR; positive samples were cultured on HEYM and liquid media in a Versatrek instrument. Sera were analyzed by IDVET ELISA and AGID tests. In the first area, 47/218 deer tissue samples were positive by PCR, 10 of which were positive by culture. Seven young animals and one stag showed poor body condition, rough coat, enteritis and enlarged lymph nodes. Out of 231 sera, 11 were positive by AGID and 15 by ELISA, with moderate agreement ( $K=0.424$ ) between serological and PCR. In the second area, out of 489 samples of different species, 8 were positive by PCR, but none by culture; 5/1939 sera were ELISA positive and 1/1646 AGID positive, with no agreement ( $K=-0.014$ ) between

serological and PCR. In the third area, 5/95 tissue samples, all from animals found dead, were positive by PCR and 1/88 sera was positive by both ELISA and AGID. PCR was the most sensitive method for the surveillance of MAP infection in wild ruminants, but it is recommended to do associated serology before translocation. Passive surveillance, particularly in areas of unknown paratuberculosis status, is essential. Granted by Ministry of Health RC2011/012.

Gazzonis AL, Alvarez Garcia G, Zanzani SA, Maggioni A, Garippa G, Rossi L, Maggiora M, Invernizzi<sup>o</sup>A, Luini<sup>o</sup>MV, Dini V, Tranquillo<sup>o</sup>V, Ortega Mora L, Manfredi MT

**Epidemiological study of *Besnoitia besnoiti* infection among cattle in insular and North-Western Italy**

XXVIII Congresso Nazionale Societa' Italiana di Parassitologia SOIPA : Roma, 24-27 giugno 2014 / [s.l. : s.n., 2014]. - p 188 [Nr. Estr. 5823]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA (28. : Roma : 24-27 giugno 2014)

Bovine Besnoitiosis is a disease in expansion in Europe both in prevalence and in distribution. Up to now, in Italy only few autochthonous outbreaks were recorded in the Northern Apennine Mountains (Manuali et al., 2011, *Transbound Emerg Dis*, 58:464- 467; Mutinelli et al., 2011, *Vet Parasitol*, 178:198-198; Gentile et al., 2012, *Vet Parasitol*, 184:108-115). Further, a serological survey was recently carried out in Southern Italy (Rinaldi et al., 2013, *Parasitol Res*, 112:1805-1807) revealing a high seroprevalence (44%). According to recent indications of ESFA (2010), epidemiological surveys are necessary to monitor the presence of *Besnoitia besnoiti* and to increase the knowledge on its biology and associated risk factors. The aims of the study were: i) to determine the frequency of *Besnoitia* infection in Northern Italy and Sardinia; ii) to investigate a besnoitiosis outbreak in a beef farm. MATERIALS AND METHODS: The serosurvey was carried out in the areas in Northern Italy more suitable for cattle breeding (Lombardy, Piedmont and Emilia Romagna) and in Liguria and Sardinia regions. The investigated farms were representative of a high variety of management systems and of differences in landscape and climate. On the whole, 3141 bovine blood samples from 149 dairy and beef farms were collected between October 2012 and August 2013. Individual and farm data were registered. Additional 404 sera were collected in a positive farm in Lombardy (approximately 700 Limousine cattle bred), where suspected clinical cases were detected and investigated. Sera were analyzed using a standardized in house ELISA; for confirmation, 200 ELISA-positive samples were randomly selected and retested by a tachyzoite based Western Blot performed under non-reducing conditions (Garcia-Lunar et al., 2013, *Transbound Emerg Dis*, 60:59-68). RESULTS: ELISA seroreactors were 709 (22.5%), but only 10 samples (0.3%) from 5 farms were also positive at the confirmation test. All positive animals were found in Lombardy and Piedmont, in both dairy and beef farms (two and three, respectively). In the selected positive farm, a high prevalence (22.2%) was confirmed by Western Blot analysis. Cutaneous scarification and biopsies were taken from four seroreactors with suspected cutaneous clinical signs; cytology was not conclusive but the typical cysts were visualized in all bioptic samples. When two of these animals were slaughtered, histology and immunohistochemistry highlighted several tissue cysts of *B. besnoiti* in all tested organs. Testicular atrophy was also remarkably severe. CONCLUSIONS: Concluding, in the study area bovine besnoitiosis seems so far limited to spot areas and isolated outbreaks. In seropositive farms, however, the disease may involve several animals potentially causing economic losses. In the case of breeding bulls, besnoitiosis can lead to transient or definitive infertility. Besides obvious epidemiological interest, serological screening with appropriate methods can be useful to detect the disease in the early phases, and eventually implement control programs.

Gazzonis AL, Garcia GA, Zanzani SA, Garippa<sup>o</sup>G, Rossi L, Maggiora M, Dini V, Invernizzi<sup>o</sup>A, Luini<sup>o</sup>M, Tranquillo<sup>o</sup>VM, Mora LO, Manfredi MT

## **Besnoitia besnoiti among cattle in insular and northwestern Italy : endemic infection or isolated outbreaks?**

Parasites & Vectors. - Vol. 7 ( 2014). - no 585 (8 p). - 38 bib ref (ultimo accesso 27/02/2015: <http://www.parasitesandvectors.com/content/7/1/585> ) [Nr. Estr. 5983]

Background Bovine besnoitiosis, caused by the apicomplexan *Besnoitia besnoiti*, is a chronic and debilitating disease considered as emerging in Europe. In Spain, Portugal and France it is endemic and foci of infection were recorded in Germany, Switzerland, Hungary, Greece and Italy. In Italy, cases of bovine besnoitiosis were registered both in imported and autochthonous cattle, and mostly in central regions; high seroprevalence was also revealed by an epidemiological survey performed in the southern part of the country. Aiming to update information on the disease in northwestern and insular areas of Italy, where data on bovine besnoitiosis were missing, a serosurvey was designed for the present study. Method Three thousand one hundred and forty bovine blood samples from both dairy and beef farms (n = 126) were collected in northwestern regions (Lombardy, Piedmont and Liguria) and in the island of Sardinia. Samples were analyzed by a standardized in-house ELISA and those resulted positive were re-tested by Western Blot (WB) for confirmation. On results obtained by both ELISA and WB, apparent (AP) and true prevalence (TP) were calculated at individual and herd levels. Further, a panel of sera resulted positive to ELISA was analyzed by IFAT. Results A total of 712 animals (AP = 22.7%; TP = 18.8%) and 109 farms (AP = 86.5%; TP = 88.2%) showed a positive reaction in ELISA. Only ten (AP = 0.3%; TP = 0%) specimens proceeding from five farms (AP = 3.9%; TP = 1.7%) from Lombardy were confirmed positive to the WB, corresponding to two Holstein Friesian cows and eight beef cattle. IFAT showed a low sensitivity (44.4%) scoring positive in only four samples out of 9 positive to WB. Conclusions The survey demonstrated that bovine besnoitiosis cannot still be considered endemic in whole Italy. In fact, independent foci of infection were registered only in Lombardy region. Therefore, a sanitary strategy aimed to increase control measures and to organize monitoring plans, by adequate diagnostic tools is necessary to avoid overestimation of *B. besnoiti* in Italy.

Gazzonis AL, Veronesi F, Zanzani SA, Molineri G, Moretta I, Moretti A, Piergili Fioretti D, Invernizzi<sup>o</sup> A, Manfredi MT

### **Seroprevalence and risk factors for *Toxoplasma gondii* in small ruminants in Northern Italy**

XXVIII Congresso Nazionale Societa' Italiana di Parassitologia SOIPA : Roma, 24-27 giugno 2014 / [s.l. : s.n., 2014]. - p 194 [Nr. Estr. 5824]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA (28. : Roma : 24-27 giugno 2014)

INTRODUCTION: Small ruminants are considered an important source of *Toxoplasma gondii* human infection (Kijlstra and Jongert, 2008, *Int J Parasitol*, 38:1359-1370); moreover, in these species *T. gondii* represents a major cause of infectious reproductive failure (Dubey 2009, *Vet Parasitol*, 163:1-14). Despite in Europe a wide range of seroprevalence was reported, only few data are available for Northern Italy. Therefore, to fill up this gap a seroepidemiological survey was conducted in goats and sheep reared in Lombardy. MATERIALS AND METHODS: From May 2011 to May 2013, sera from 474 goats and 502 sheep from 42 farms were collected. An audit form on farm management was filled. Sera were tested for IgG antibodies to *T. gondii* by indirect Immunofluorescence Antibody Assay (IFAT) using commercial antigens (MEGACOR Laboratories, Horbranz, Austria) and fluorescein isothiocyanate-labelled rabbit anti-sheep IgG and anti-goats IgG (Sigma-Aldrich, USA) as conjugate diluted 1:100 and 1:200, respectively. Sera were screened at 1:64 dilution (cut-off) and those testing positive were serially diluted. To identify risk factors, a univariate binary logistic regression analysis of the variables was performed with SPSS 19.0. RESULTS: Antibodies to *T. gondii* were found in 96.5% (28/29) goat farms and in 87.5% (21/24) sheep farms. At individual level, 198 out of 474 goats (41.7%) and 298 out of 502 sheep (59.3%) resulted positive. Seroprevalence was significantly higher in sheep than in goats (Pearson's chi-square, P=0.000). The highest percentage of infected goats was found in 4-year-old animals, whereas in animals over six years of age seroprevalence decreased. Goats sampled in eastern and western areas showed similar seroprevalence values, whereas goats from southern area had a

lower probability to be infected (OR=0.41). Considering breed, Saanen goats presented the lowest seroprevalence (30.7%), whereas cross-breed exhibited the highest rate (48.7%). The risk factor "number of animals on farm" resulted highly significant (P=0.000). Animals from farms where both sheep and goats were reared had a risk to be infected 1.396 times higher than goats from farms without sheep. Goats bred in intensive farms showed lower prevalence (22.1%, OR=0.18) in comparison to those bred in extensive (45.6%, OR=0.55) or semi-intensive ones (60%, OR=1). Sampling area was by far one of the stronger predictor of *T. gondii* infection in sheep flock: the risk of a sheep from south-eastern area being diagnosed as seropositive to *T. gondii* was 3.25 times higher than a sheep from the western area. Finally, transhumant herds in comparison to semi-intensive ones appeared more at risk of *T. gondii* infection (66.8% vs 38.4%). **CONCLUSIONS:** The survey recorded a high *T. gondii* seroprevalence values. The highest prevalence was registered in sheep transhumant herds and in small family-run goat farms, speculating that these rearing systems might support the spreading and maintenance of the infection. Representing these traditional activities important resources for the conservation of the territory and its economy, it could be useful to improve management practices for a better control of the disease.

Gelmetti<sup>o</sup>D, Gibelli<sup>o</sup>L, Gelmini<sup>o</sup>L, Sironi G

#### **Malakoplakia with digestive tract involvement in a pig**

Vet Pathol. - Vol. 51 n 4 ( 2014). - p 809-811. - 10 bib ref [Nr. Estr. 5717]

Malakoplakia is a rare, granulomatous, inflammatory disease that mimics malignant tumors and can affect any organ. Herein is described a case of malakoplakia in a 10-month-old slaughter pig. Diffuse, pleomorphic, round cell infiltrates, mainly histiocytes, with a tumor-like growth pattern at gross examination, infiltrated the stomach, pancreas, omentum, and mesenteric lymph nodes. The histiocytes and multinucleated giant cells had concentric, target-like inclusions known as Michaelis Gutmann bodies. Microorganisms were not detected by the periodic acid–Schiff reaction, Ziehl Neelsen, Gram, and Warthin-Starry staining or by electron microscopic and bacteriologic investigations. Porcine circovirus type 2 and porcine reproductive and respiratory syndrome viruses were not detected by immunohistochemistry in the sections examined.

Gelmini<sup>o</sup>L, Bianchi<sup>o</sup>A, Giacomelli<sup>o</sup>S, Ferloni M, Milani F, Redaelli K, Milani P, Bertoletti<sup>o</sup>I

#### **Analysis of mortality in the roe deer (*Capreolus capreolus*) aid in structures of wild animals recovery in Northern Italy**

11th European Wildlife Disease Association Conference (EWDA) : Edinburgh, 25-29 August 2014 / [s.l. : s.n., 2014]. - 1 p [Nr. Estr. 5847]

European Wildlife Disease Association Conference (EWDA) (11th : Edinburgh : 25-29 August 2014)

The wildlife rehabilitation centers can provide a wealth of information on the health status of animal populations living in a given territory. In particular, provide important elements on the demographics and allow passive monitoring of infectious diseases. For years, the peripheral sections of Istituto Zooprofilattico of Lombardia ed Emilia-Romagna collaborate with the private and public rehabilitation facilities in order to provide a diagnostic service and consulence. In this work we report the results of 118 case of the diagnostic activities (mainly necropsies and bacteriological examinations) on recovered roe-deers in the last two years in two wildlife rehabilitation centers. The main causes of mortality are due to traumas (e.g. car crash), some cases concern secondary predation by stray dogs. The occurrence of infectious diseases concerns about 50% of the subjects, while the mortality from massive infestation concerns about 20%. Among the most commonly encountered infections are colibacillosis (36= 30%), clostridial diseases (10 = 8% ), pyogenic infections (8 =7% ) and pasteurellosis (5 = 4%), other recurrent infections in roe deer are caused by respiratory viruses. The

demographic data collected confirm an increased incidence of infectious and pathological phenomena in young and elderly individuals; while the trauma affecting most adult individuals. The detection of potentially zoonotic diseases in animals hospitalized in rehabilitation centers is important for health monitoring.

Genchi°M, Prati°P, Manfredini°A, Vicari°N, Bragioni°R, Sacchi L, Epis S, Fabbi°M

**Can Ixodes ricinus and Dermacentor reticulatus be a reservoir for Francisella tularensis?**

XXVIII Congresso Nazionale Societa' Italiana di Parassitologia SOIPA : Roma, 24-27 giugno 2014 / [s.l. : s.n., 2014]. - p 119 [Nr. Estr. 5822]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA (28. : Roma : 24-27 giugno 2014)

**INTRODUCTION:** *Ixodes ricinus* and *Dermacentor reticulatus* (Acari: Ixodidae) are small hard ticks able to transmit a large variety of pathogens to humans and animals. These ticks have been described as potential vectors of *Francisella tularensis*, a highly contagious zoonosis for a wide number of mammals, reptiles and birds. Transtadial transmission of this bacterium from larva to adult has been demonstrated under laboratory conditions. However, transovarial transmission is still debated. The aim of this study was to evaluate the possible transovarial transmission of *F. tularensis* in these two ticks. **MATERIALS AND METHODS:** four guinea pigs were infected with 0.5 ml of *F. tularensis* type B (1000 cfu/ml). After 2 days, 25 females and 35 males of *I. ricinus* and 25 female and 35 males of *D. reticulatus* were placed on 4 guinea pigs. The experiment was done in six replicates for a total of 150 *I. ricinus* females and 150 females of *D. reticulatus*. After 6 days from the infection, 24 engorged females of both species were dissected and salivary glands, midgut and ovary were collected and analyzed by real-time PCR target 23 kDa, transmission electron microscopy (TEM) and fluorescence in situ hybridization (FISH). The remaining females were maintained in incubator at 26°C and 90 RU to allow oviposition. Eggs were recovered and the ticks analyzed by PCR, TEM and FISH. Pool of 50 eggs were analyzed by PCR, culture and bioassay. Approximately 600 eggs were homogenized, suspended in saline solution and injected sc in 2 mice. Other groups of eggs were used to obtain larvae and nymphs. Three hundred larvae and 300 nymphs were used to infect 4 mice. A similar quantity of larvae and nymphs was tested by culture and PCR. **RESULTS:** All ticks were positive by PCR (midgut), TEM (ovary), FISH (ovary, salivary glands) and culture (midgut, ovary). The PCR carried out on eggs was positive but the culture and bioassay were negative. The same result was obtained on larvae: PCR positive and culture and bioassay negative. The nymphs after blood meal on mice were negative by PCR and culture. **CONCLUSIONS:** TEM and FISH showed the successful infection of the ovary by *F. tularensis* and PCR showed the presence of bacterial DNA in eggs larvae. However, the negative results obtained by culture of eggs, larvae and nymphs and especially the negativity of bioassay, indicate the passage of non-viable DNA from ticks to eggs, larvae and nymphs. In conclusion, the data suggest the failure of transovarial transmission of *F. tularensis* in eggs of *I. ricinus* and *D. reticulatus*.

Genchi°M, Prati°P, Manfredini°A, Vicari°N, Bragioni°R, Sacchi L, Epis S, Fabbi°M

**Are dermatocentor reticulatus and ixodes ricinus the real reservoir of Francisella tularensis?**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 93 - 2 bib ref [Nr. Estr. 5895]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

*Ixodes ricinus* and *Dermacentor reticulatus* (Acari: Ixodidae) are a small hard ticks able to transmit a large variety of pathogens to humans and animals. These ticks have been described as potential vectors of *Francisella tularensis*, a highly contagious zoonosis for a wide number of mammals, reptiles and birds (1). Transtadial transmission of this bacterium from larva to adult has been demonstrated under laboratory conditions. However, transovarial transmission is still debated (2). The aim of this study was to evaluate the possible transovarial transmission of *F. tularensis* in these two ticks. **MATERIALS AND METHODS:** Four guinea pigs were infected with 0.5 ml of *F. tularensis* type B (1000 cfu/ml). After 2 days, 25 females and 35 males of *I. ricinus* and 25 female and 35 males of *D. reticulatus* were placed on 4 guinea pigs. The experiment was done in six replicates for a total of 150 *I. ricinus* females and 150 females of *D. reticulatus*. After 6 days from the infestation, 24 engorged females of both species were dissected and salivary glands, midgut and ovary were collected and analyzed by realtime PCR target 23 kDa, transmission electron microscopy (TEM) and fluorescent in situ hybridization (FISH). The remaining females were maintained in incubator at 26°C and 90 RH to allow oviposition. Eggs were recovered and the ticks analyzed by PCR, TEM and FISH. Pool of 50 eggs were analyzed by PCR, culture and bioassay. Approximately 600 eggs were homogenized, suspended in saline solution and injected sc in 2 mice. Other groups of eggs were used to obtain larvae and nymphs. Three hundred larvae and 300 nymphs were used to infect 4 mice. A similar quantity of larvae and nymphs was tested by culture and PCR. **RESULTS:** All engorged ticks were positive by PCR (midgut), TEM (ovary) (Fig. 1), FISH (ovary and salivary glands) and culture (midgut and ovary). The PCR carried out on eggs was positive but the culture and bioassay in mice were negative. The same result was obtained on larvae: PCR positive and culture and bioassay negative. The nymphs after blood meal on mice were negative by PCR and culture. **DISCUSSION AND CONCLUSIONS:** TEM and FISH showed the successful infection of the ovary by *F. tularensis* and PCR showed the presence of bacterial DNA in eggs larvae. However, the negative results obtained by culture of eggs, larvae and nymphs and especially the negativity of bioassay, indicate the passage of non-viable bacteria from ticks to eggs, larvae and nymphs. In conclusion, the data suggest the failure of transovarial transmission of *F. tularensis* in eggs of *I. ricinus* and *D. reticulatus*.

Giacomelli°S, Bianchi°A, Nodari°S, Polloni°A, Milani P, Gelmini°L, Archetti°LI, Bertolotti°I

#### **Prognostic value of complete blood count parameters applied to Roe deer (*Capreolus capreolus*) recovery : preliminary results**

11th European Wildlife Disease Association Conference (EWDA) : Edinburgh, 25-29 August 2014 / [s.l. : s.n., 2014]. - 1 p. [Nr. Estr. 5849]

European Wildlife Disease Association Conference (EWDA) (11th : Edinburgh : 25-29 August 2014)

Roe deer (*Capreolus capreolus*) is one of the most represented species in Italian wildlife rehabilitation centers. Despite great prognostic value demonstrated for domestic species, hematological analysis are not routinely performed in these facilities mainly because of handling difficulties and the lacking of reference values. In this work (part of a project funded by the Italian Ministry of Health, Department of Veterinary Public Health, Food Safety and Collegial Bodies for Health Protection) we have collected blood sample from 52 roe deer in 3 different rehabilitation center in north and central Italy and performed complete blood count (CBC) through automated hematology analyzer. We have compared results between subjects died in a five days period after sampling (DS, n=7) and fully recovered animals (FR, n=45). Extreme values can identify poor prognosis especially if combined together. In particular the association of two or more extremely high or low values of Red Cell Distribution Width, Mean Corpuscular Hemoglobin, Mean Corpuscular Volume, Hemoglobin, Neutrophilic Granulocyte, White Blood Cells and Red Blood Cells is present only in DS, while only one DS and four FR had a single extreme value. As we expected CBC can provide some good information from prognostic and therapeutic point of view. Nevertheless a correlation with basic biochemical analysis, and a good anamnesis would complete the single clinical case.

Giacomelli°S, Bianchi°A, Polloni°A, Archetti°LI , Bertoletti°I, Rota\_Nodari°S

**Alpha-naphthyl acetate esterase activity in white blood cells of wild ruminants : preliminary results**

Proceedings International conference on diseases of zoo and wild animals 2014 : May 28-31, Warsaw, Poland / [s.l. : s.n., 2014]. - p 139-141. - 9 bib ref [Nr. Estr. 5778]

International conference on diseases of zoo and wild animals : Warsaw, Poland : May 28-31, 2014)

Alpha-naphthyl acetate esterase (ANAE) staining was performed on 22 peripheral blood smears obtained from roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*) and alpine ibex (*Capra ibex*), in order to study the enzyme activity in different leukocyte subtypes. The obtained leucogram was compared with the results of the classical Hemacolor staining. The analysis of the results indicated that ANAE staining provides additional information compared to Hemacolor through a better differentiation of mononuclear blood cells. In particular the presence of this esterase in T-lymphocytes seems to better characterise the health status of the individuals.

Giacomelli°S, Bianchi°A, Polloni°A, Rota\_Nodari° S, Bertoletti°I

**Lice (Phthiraptera: Trichodectidae) infestation on roe deer (*Capreolus capreolus*) from Northern Italy**

Hystrix Ital J Mammalogy. - Vol. 25 (supplement) ( 2014). - p 107 [Nr. Estr. 5697]

Congresso Italiano di Teriologia (9. : Civitella Alfedena (AQ) : 7-10 Maggio 2014)

The study was carried out on 70 roe deer (*Capreolus capreolus*) carcasses sent to our laboratory for diagnostic purposes between November 2012 and November 2013. The animals were mostly collected after car accident or found dead (n=33) in the province of Sondrio or died at a local rescue center (n=37). During routine necropsy facial and inguinal areas were examined for lice detection. An "infestation score" was calculated (mild—medium—severe) according to the number of lice collected. Some (1350) lice collected from the rostral part of the face area (nose and chin) were stored in 70% ethanol for species, sex and age characterization through dichotomical keys. 22 out of 70 (31%) roe deer were found visually infested by lice. *Damalinea (Cervicola) meyeri*, the typical louse of roe deer, is the only species of louse identified and, despite the severe dermatitis and massive infestation observed, we couldn't find any exotic and more pathogenic species as reported for cervids in North America. The nymphs/adult females ratio of *Damalinea (Cervicola) meyeri* varied from 0.13 to 4.92. Only 3 male lice were collected, from 3 different roe deer, supporting the hypothesis of parthenogenesis, as already observed in other Trichodectidae species. According to the data analysis, a seasonal pattern both of age ratio and lice prevalence can be described. In particular a statistically significant increase of nymphs/adult female ratio can be described, in spring and autumn (1.92 and 0.82 respectively) compared to summer and winter (0.38 and 0.31 respectively) (ANOVA  $p < 0.05$ ) while an opposite prevalence trend can be seen in spring (25%) compared to autumn (60%), winter (50%) and summer (56%). The severity of dermatitis was positively correlated (ANOVA  $p < 0.01$ ) with the nymphs/adult females ratio. Roe deer infested also by other arthropods (*Lipoptena* spp., *Cephenemyia* spp., *Ixodes* spp.) demonstrated a younger population of lice (ANOVA  $p < 0.05$ ). The positive correlation between infestation score and age and gender (massive infestation in females more than ten years old, Fisher's exact test  $p < 0.05$ ) suggest a hidden biotic factor that drive parasite population to rise. Furthermore, peculiar pathological states are able to interfere with the normal time budget of ungulates (i.e. resting and self grooming) and also on their home range and their intraspecific interaction, giving lice more chance to grow. However we couldn't find any statistically significant correlation between pathological findings and infestation indices. Based on these results we can assume that abiotic factors can influence the prevalence of lice in roe deer but more studies such as analysis on immune response or hematological parameters could help understanding the interaction between non blood sucking ectoparasites and host welfare

status.

Giacomini°E, Boniotti°MB, Ferrari°N, Salogni°C, Pasquali P, Alborali°GL

**PRRSV and SIV detection in individual blood samples, nasal swabs and pen oral fluids in a field longitudinal study in post weaning piglets**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - v. 2. - p 557. - 2 bib ref [Nr. Estr. 5731]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Giacomini°E, Lazzaro°M, Giovannini°S, Ferrari°N, Razzini P, Costa A, Pozio E, Pasquali P, Alborali°L

**Anti-T. Gondii antibodies IgG and IgM in heavy pigs reared in Northern Italy**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - v. 2. - p 404. - 4 bib ref [Nr. Estr. 5732]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Gibelli°L, Bertoletti°I, Bianchi°A, Mondani°H, Genchi°M, Lavazza°A, Gelmetti°D

**Sarcocystis in wild ruminant of Central Italian Alps a four years survey**

11th European Wildlife Disease Association Conference (EWDA) : Edinburgh, 25-29 August 2014 / [s.l. : s.n., 2014]. - 1 p. [Nr. Estr. 5848]

European Wildlife Disease Association Conference (EWDA) (11th : Edinburgh : 25-29 August 2014)

Monitoring the health of the wildlife population helps to control possible disease transmission between wildlife and human. Parasites whose developmental stages reside within game muscle are often not noticed by standard veterinary examination of hunted cloven hoofed game. A survey on wild ruminants found dead mainly in central Italian Alps comprising Stelvio National Park is ongoing since 2002; in the last 4 years we histologically found an increasing number of positive muscles for Sarcocystis. Sarcocystis have two host life cycle with herbivores and omnivores as intermediate hosts and mainly carnivores as definitive hosts. Sarcocystis have been detected in many wild and farm animals and cervids may act as intermediate host for many Sarcocystis species. In our study we included 35 wild ruminants: 24 roe deer, 5 red deer, 5 chamois, 1 ibex, that didn't show any macroscopic evidence of sarcosporidiosis. The aim is to make a preliminary study on the Sarcocystis species that parasitize the wild species of the central Italian Alps by studying the cyst wall structure by light (LM) and transmission electron microscopy (TEM).

Giorda F, Pautasso A, Iulini B, Pintore MD, Petrella A, Pintore A, Toffan A, Sholl F, Terracciano G, Di\_Nocera F, Latini M, Ferri N, Caracappa S, Rubini°S, Mazzariol S, Di\_Guardo G, Ballardini M, Mignone W, Casalone C

**Cetacei spiaggiati lungo le coste italiane : indagini diagnostiche post mortem**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 52-53 [Nr. Estr. 5726]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

Giorgi I, Varello K, Meistro S, Romano A, Gorla M, Chiavacci L, Vitale N, Gennero MS, Bergagna S, Modesto P, Biolatti C, Arrigoni°N, Dondo A

**Evaluation of agreement between culture, PCR, histopathology for post mortem diagnosis of paratuberculosis from faeces and tissues**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 66 (Poster P-02.8) [Nr. Estr. 5773]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

The purpose of this study was to acquire a more detailed knowledge in the direct diagnosis of Paratuberculosis (PTB) by post mortem tests. Portions of intestine, gut-associated lymph nodes and faeces from 49 dairy cattle belonging to 2 PTB infected herds were collected at slaughter and processed for *Mycobacterium avium* subsp. *para-tuberculosis* (MAP) detection (culture, PCR, histopathology). Strain isolation from faeces and tissues were carried out according to the NRC. PCR was performed by amplifying a portion of the IS900 specific for MAP. For histopathology, samples were fixed in formalin, embedded in paraffin, stained with hematoxylin-eosin and Ziehl-Neelsen stain. Kappa index of Coehn and exact CI95% was calculated to evaluate the agreement between the tests at animal level and for each sampled organ. A head was classified "positive" if at least one organ resulted positive. 21 cows resulted negative and 28 positive. At animal level the highest agreement resulted between culture and histopathology (K=0.71, CI95%: 0.52-0.90). Considering only organs the highest agreement was found for lymph node between PCR and histopathology (K=0.67, CI95%: 0.46-0.89), for rectum between culture and histopathology (K=0.50, CI95%: 0.23-0.77), for small intestine between PCR and histopathology (K=0.49, CI95%: 0.21-0.76), for cecum between culture and PCR (K=0.48, CI95%: 0.20-0.75), and for ileocecal valve PCR and histopathology (K=0.43, CI95%: 0.14-0.71). The agreement for faeces between isolation and PCR was found to be excellent (k=0.73, CI95%: 0.50-0.93). Lymph node showed the highest sensitivity (Se=69.2; CI95%: 0.48-0.90), while rectum the lowest (Se=48.0; CI95%: 0.18-0.72). Results confirmed good agreement between culture and histopathology at animal level and between PCR and histopathology for lymph node. Based on results, to improve the post mortem protocol it is advisable the sampling and analysis of all tissues considered, associated with faeces.

Gladue DP, O'Donnell V, Baker-Bransetter R, Pacheco JM, Holinka LG, Arzt J, Pauszek S, Fernandez-Sainz I, Fletcher P, Brocchi° E, Lu Z, Rodriguez LL, Borca MV

**Interaction of foot-and-mouth disease virus nonstructural protein 3A with host protein DCTN3 is important for viral virulence in cattle**

J Virol. - Vol. 88 no 5 ( 2014). - p 2737-2747. - 28 bib ref [Nr. Estr. 5668]

Nonstructural protein 3A of foot-and-mouth disease virus (FMDV) is a partially conserved protein of 153 amino acids in most FMDVs examined to date. The role of 3A in virus growth and virulence within the natural host is not well understood. Using a yeast two-hybrid approach, we identified cellular protein DCTN3 as a specific host binding partner for 3A. DCTN3 is a subunit of the dynactin complex, a cofactor for dynein, a motor protein. The dynactin-dynein duplex has been implicated in several subcellular functions involving intracellular organelle transport. The 3A DCTN3 interaction identified by the yeast two-hybrid approach was further confirmed in mammalian cells. Overexpression of DCTN3 or proteins known to disrupt dynein, p150/Glued and 50/dynamitin, resulted in decreased FMDV replication in infected cells. We mapped the critical amino acid residues in the 3A protein that mediate the protein interaction with DCTN3 by mutational analysis and, based on that information, we developed a mutant harboring the same mutations in O1 Campos FMDV (O1C3A-PLDGv). Although O1C3A-PLDGv FMDV and its parental virus (O1Cv) grew equally well in LFBK-\_v\_6, O1C3APLDGv virus exhibited a decreased ability to replicate in primary bovine cell cultures. Importantly, O1C3A-PLDGv virus exhibited a delayed disease in cattle compared to the virulent parental O1Campus (O1Cv). Virus isolated from lesions of animals inoculated with

O1C3A-PLDGv virus contained amino acid substitutions in the area of 3A mediating binding to DCTN3. Importantly, 3A protein harboring similar amino acid substitutions regained interaction with DCTN3, supporting the hypothesis that DCTN3 interaction likely contributes to virulence in cattle.

Gómez-Morales MA, Ludovisi A, Amati M, Bandino E, Capelli G, Corrias F, Gelmini L, Nardi A, Sacchi C, Cherchi S, Lalle M, Pozio E

**Indirect versus direct detection methods of *Trichinella* spp. infection in wild boar (*Sus scrofa*)**

Parasites & Vectors. - Vol. 7 no 171 ( 2014). - 8 p. - 35 bib ref [Nr. Estr. 5907]

Background *Trichinella* spp. infections in wild boar (*Sus scrofa*), one of the main sources of human trichinellosis, continue to represent a public health problem. The detection of *Trichinella* spp. larvae in muscles of wild boar by digestion can prevent the occurrence of clinical trichinellosis in humans. However, the analytical sensitivity of digestion in the detection process is dependent on the quantity of tested muscle. Consequently, large quantities of muscle have to be digested to warrant surveillance programs, or more sensitive tests need to be employed. The use of indirect detection methods, such as the ELISA to detect *Trichinella* spp. infections in wild boar has limitations due to its low specificity. The aim of the study was to implement serological detection of anti-*Trichinella* spp. antibodies in meat juices from hunted wild boar for the surveillance of *Trichinella* spp. infections. Methods Two tests were used, ELISA for the initial screening test, and a specific and sensitive Western blot (Wb) as a confirmatory test. The circulation of anti-*Trichinella* IgG was determined in hunted wild boar muscle juice samples in 9 provinces of 5 Italian regions. Results From 1,462 muscle fluid samples, 315 (21.5%, 95% C.I. 19.51-23.73) were tested positive by ELISA. The 315 ELISA-positive muscle fluid samples were further tested by Wb and 32 (10.1%, 95% C.I. 7.29-13.99) of these were positive with a final seroprevalence of 2.2% (95% C.I. 1.55-3.07; 32/1,462). *Trichinella britovi* larvae were detected by artificial digestion in muscle tissues of one (0.07%, 95% C.I. 0.01-0.39) out of the 1,462 hunted wild boars. No *Trichinella* spp. larvae were detected in Wb-negative wild boar. From 2006 to 2012, a prevalence of 0.017% was detected by muscle digestion in wild boar hunted in the whole Italian territory. Conclusions The combined use of both serological methods had a sensitivity 31.4 times higher than that of the digestion (32/1,462 versus 1/1,462), suggesting their potential use for the surveillance of the *Trichinella* spp. infection in wild boar populations.

Grisendi A, Defilippo F, Gatti F, Dottori M, Boni lauri P

**Preliminary observation of ADD value of six landmarks within the pupal stage of the forensically important fly *Lucilia sericata* (Diptera, Calliphoridae)**

11th Meeting of the European Association for Forensic Entomology (EAFE) : 9th to 11th April 2014, Lille, France : abstract book / [s.l. : s.n., 2014]. - p 41 [Nr. Estr. 5918]

European Association for Forensic Entomology (EAFE) (11th : Lille, France : 9th to 11th April 2014)

The pupal stage needs about 50% of the total immature development time of a blow fly species. Establishing the age of this big window of time is difficult and needs alive specimens which are reared up to the adult stage or expensive molecular techniques. However, estimating the age of a pupae can be a crucial task for determining the minimum postmortem interval. This study reports on a series of laboratory experiments that explore the effects of constant temperature on the development of the species *Lucilia sericata* with particular reference to Accumulated Degree Days (ADD) estimation and the observation of six landmarks to fix different pupal phases. The constant temperatures were 20, 22, 24, 26, and 28°C ( $\pm 1^\circ\text{C}$ ). Lower developmental threshold temperature (12,5°C) for the development were extrapolated from the linear regression of the developmental rates on each temperature. Following the removal of pupal case, five pupae every 24h were examined with stereo-microscope using 15x magnification, and six landmarks are showed as

described below. Cryptocephalic pupa [S1]: the head is still invaginated. Phanerocephalic pupa [S2]: complete eversion of the head. Pharate adult [S3]: visible segmentation in the thorax, legs and abdomen. Yellow eyed [S4]: beginning of eye pigmentation and ocelli are visible. Tanned chaetae [S5]: all the bristles, both macro- and microchaetae, are fully tanned, except a few at the distal margin of the leg and abdominal segments, which are still brownish. Tanned legs [S6]: all the bristles, legs and wings are fully tanned. Emergence [S7]. For each of the landmarks, the ADD value was calculated for every rearing temperature involved. Data processing showed that the mean ADD value at 20°C was 67.5 for S1, 75 for S2, 85.5 for S3, 112.5 for S4, 127.5 for S5, 142.5 for S6 and 157.5 for S7. In this study the development times from oviposition to adult eclosion were different from those reported in other studies. This discrepancy could be attributed to a variation in the geographic adaptation (intrinsic factors) or to the experimental method (extrinsic factors). Therefore it would be necessary to create a suitable protocol for the observation of pupal characters.

Lange-Consiglio A, Spelta C, Garlappi R, Luini M, Cremonesi F

### **Intramammary administration of platelet concentrate as an unconventional therapy in bovine mastitis : first clinical application**

J Dairy Sci. - Vol. 97 ( 2014). - p 6223-6230. - 32 bib ref [Nr. Estr. 5967]

Bovine udder infections induce a variety of changes in gene expression of different growth factors that may suggest their possible role in glandular tissue protection or repair processes. Growth factors and also chemokines and cytokines may act synergistically to increase the infiltration of neutrophils and macrophages to promote angiogenesis, fibroplasia, matrix deposition, and, ultimately, re-epithelialization. Considering the vast applications, typically in human medicine, of platelet concentrate (PC) and its ease of preparation, the aim of our study was to evaluate an alternative therapy to stimulate the regeneration of glandular tissue, administering a concentration in excess of the growth factors contained in the PC. In each one of the 3 farms examined in the trial, PC was prepared from donor cows in good health, free from infections, and with no records of medications administered during the previous 2 mo. The platelet produced in one farm was used only for treating the cows of the same farm in a heterologous way. A total of 229 mastitic quarters were divided in 3 groups: antibiotic group (treated with intramammary antibiotic), antibiotic and PC group (treated intramammary with antibiotics in association with PC), and PC group (treated with intramammary PC alone). The diagnosis of mastitis was based on somatic cell count and bacteriological evaluation of the milk from the affected quarter. Platelet concentrate, alone or in association with antibiotic, was used for 3 consecutive days as an unconventional therapy in bovine acute and chronic mastitis. Our data show that the associated action of antibiotic and PC performed significantly better than the antibiotic alone, either for the recovery of the affected mammary quarters or for somatic cell count reduction. In the same way, the association antibiotic plus PC showed significantly fewer relapses compared with the antibiotic alone, either for acute or chronic mastitis. The treatment with only PC did not show statistically significant differences compared with both antibiotic alone or associated treatment for acute mastitis, and it was better than the use of only antibiotic for chronic mastitis. Our results show that PC alone may be useful for a quick resolution of the inflammatory response, playing a role in limiting the tissue damage to the mammary gland parenchyma and reducing the recurrence rates.

Lavazza A, Dall'Olio R

### **Virosi**

Patologia e avversità dell'alveare / a cura di Emanuele Carpana, Marco Lodisani. - Milano : Springer-Verlag Italia 2014, 2014. - p 123-162. - 165 bib ref [Nr. Estr. 5904]

Lelli D, Moreno A, Prospero A, Lavazza A, Bonio M, Raffini E, Cordioli P

## **Mammalian orthoreovirus : caratterizzazione genomica di isolati da cane, gatto e pipistrello**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 59 [Nr. Estr. 5741]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

Gli orthoreovirus dei mammiferi (MRVs) sono RNA virus della famiglia Reoviridae in grado di infettare potenzialmente tutte le specie di mammiferi. Originariamente descritti come virus "orfani" (REO = Respiratory Enteric Orphans), in quanto non associati ad alcuna patologia, sono oggi considerati possibile causa di forme respiratorie, enteriche e neurologiche. Recentemente sono stati identificati nuovi MRVs in pipistrelli in Italia e Germania altamente correlati con un virus isolato nel cane in Italia nel 2004. Successivamente è stato descritto un caso di gastroenterite acuta in un bambino in Slovenia causato da un virus che presenta il più alto valore di similarità nucleotidica per i sopraccitati virus da pipistrello. Allo scopo di approfondire le possibili relazioni tra MRVs da differenti specie di mammiferi, viene riportato l'isolamento di MRVs da cane, gatto e pipistrello e la relativa caratterizzazione genomica del gene S1 completo. Sessantacinque campioni di feci di cane e 31 di gatto sono stati sottoposti ad esame virologico in coltura cellulare (linea LLCMK2). La caratterizzazione genomica è stata eseguita su 6 ceppi virali isolati da cane, 3 da gatto e 16 da pipistrello tramite sequenziamento completo del gene S1 (1416 bp). I ceppi sono stati precedentemente tipizzati come MRV tipo 3 tramite Multiplex RT-PCR. Le sequenze sono state confrontate con quelle di riferimento ottenute da GenBank mediante allineamento con il programma clustal W. L'albero filogenetico è stato costruito con il programma MEGA 5 utilizzando il metodo neighbour-joining, modello Tamura-Nei. Le topologie evidenziate sono state confermate con i metodi maximum likelihood e maximum parsimony. L'analisi filogenetica ha evidenziato che i virus analizzati si dividono in 4 cluster ben differenziati. Un primo cluster è formato da tutti gli isolati da cane e gatto che formano insieme ad alcuni ceppi da pipistrello un gruppo omogeneo con percentuale di identità >99%. Un secondo cluster è costituito dall'isolato umano identificato in Slovenia e dai due isolati da pipistrello in Italia e Germania. I restanti due cluster sono costituiti dai soli isolati da pipistrello in Italia. Lo studio ha evidenziato una scarsa variabilità tra i ceppi isolati da cane e gatto mentre risultano maggiormente differenziati gli isolati da pipistrello. Da sottolineare l'elevata correlazione con la più alta percentuale di identità (99,2%) tra il ceppo umano sloveno ed un ceppo italiano isolato da pipistrello. I dati ottenuti ampliano le conoscenze sull'epidemiologia dei reovirus in considerazione del limitato numero di sequenze MRVs disponibili in banca dati.

Lelli°D, Moreno°A, Prosperi°A, Lavazza°A, Bonio tti°MB, Raffini°E, Cordioli°P

### **Molecular characterization of Orthoreovirus isolated from bats, dogs and cats**

8th Annual Epizone Meeting "Primed for tomorrow" : 23 - 25 September 2014 Copenhagen, Denmark : Posters "Domestic-wildlife animal interactions" / [s.l. : s.n., 2014]. - 5939]

Annual meeting Epizone (8th : Copenhagen, Denmark : 23 - 25 September 2014)

Orthoreoviruses, with mammalian orthoreovirus (MRV) as the type species, have been recognized as respiratory and enteric orphan viruses since 1950s. MRVs have been reported to date in various hosts, including human and animal species. In the last few years, MRVs have been often described as the sole pathogen in various hosts presenting severe clinical manifestations, such as hemorrhagic enteritis, acute respiratory infections, central nervous system implications, and others. Novel MRVs, closely related to a virus isolated in dogs in Italy in 2004, have recently been identified in bats in Italy and Germany (1,2). In addition, a novel orthoreovirus with high similarity to MRVs found in European bats was detected in Slovenia from a child with acute gastroenteritis requiring hospitalization (3). In order to obtain a clearer overview of MRVs molecular epidemiology and to verify any possibility of zoonotic transmissions, we report the molecular characterization of MRVs isolated from bats, dogs and cats in Italy. One-hundred-ninety samples collected from bats (134 carcasses and 56 feces), 65 faecal samples collected from dogs and 31 from cats were analyzed by in vitro isolation using LLCMK2 cell line. A total of 25 viral strains were isolated, respectively from bats (16), dogs (6) and cats (3). They were firstly typed as MRV 3 by Multiplex RT-PCR and then genomically characterized by complete sequencing of the S1 gene (1416 bp). The sequences were

compared with those of reference obtained from GenBank under alignment with the program Clustal W. The phylogenetic tree was generated by Neighbour-joining method using the Kimura 2-parameter model. Phylogenetic analysis showed that the 25 isolates were divided into 4 different clusters. The first cluster was formed by all the viruses isolated from dogs (6) and cats (3) and by 7 bat strains which showed >99% of similarity between them. The second cluster consisted of the human-MRV identified in Slovenia and two bat-MRVs detected respectively in Germany and in Italy, during this survey. The other two clusters included respectively 2 and 6 remaining Italian strains isolated from bats. The results showed low variability among MRVs identified in dogs and cats while bat-MRVs appeared genetically more differentiated. To remark the high correlation (99.2% similarity) between the human-MRV identified in Slovenia and an Italian strain isolated from a bat (*Pipistrellus kuhlii*). In conclusion, the obtained data extend our knowledge on the epidemiology of MRVs since a limited number of nucleotide sequences are available in public database. The real epidemiological situation regarding MRVs in bats and their transmission to other animal species, including humans, should be further investigated in order to understand their full pathogenesis and zoonotic potential.

Leotti G, Foni°E, Arioli E, Bongiovanni E, Bresola M, Codato A, Donna R, Faccenda M, Fruttero F, Gamba F, Giorgiutti M, Salvini F, Zizioli B, Ostanello F, Merdy O, Vila T, Joisel F

**Serological responses in 11 Italian herds after vaccination with a combined vaccine against H1N1, H3N2 and H1N2 swine influenza virus subtypes**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - p 318. - 1 bib ref [Nr. Estr. 5956]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Lombardo°T, Villa°R, Dotti°S, Renzi°S, Chiapponi°C, Pongolini°S, Ferrari°M

**Variazioni biologiche e genomiche di biotipi dell'influenza aviaria propagati in differenti substrati biologici**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 60 [Nr. Estr. 5742]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

Il sistema biologico d'elezione impiegato per l'isolamento e la replicazione dei virus dell'Influenza è rappresentato dall'embrione di pollo. Tale sistema biologico permette la crescita di un'ampia gamma di virus influenzali. È tuttavia da rilevare che taluni isolati di campo non presentano una crescita immediata e la propagazione seriale può causare mutazioni, con comparsa di varianti, differenti dal punto di vista antigenico ai virus originali. Di notevole interesse è la possibilità di sostituire l'embrione di pollo con le colture cellulari, sistema più disponibile in laboratorio. Obiettivo dello studio è stata la valutazione delle caratteristiche biologiche e genomiche di differenti biotipi del virus dell'Influenza aviaria, amplificati serialmente utilizzando quale substrato sia embrione di pollo SPF (Specific Pathogen Free) che tre differenti linee cellulari, note per essere recettive ai virus influenzali: NSK (Newborn Swine Kidney), MDCK (Madin-Darby Canine Kidney), UMNSAH/DF1 (Chicken embryo fibroblasts). I biotipi di virus selezionati sono stati: H5N1 LPAI A/mallard/Italy/3401/05, H5N2 HPAI A/chicken/Italy/8/A98, H7N1 HPAI A/turkey/Italy/4580/99, H7N3 LPAI A/turkey/Italy/2962/V03, H9N2 LPAI A/turkey/Wisconsin/66, forniti dall'Istituto Zooprofilattico Sperimentale delle Venezie (Padova). La variazione di patogenicità, a seguito della propagazione seriale dei virus in esame, è stata valutata mediante prove in vivo condotte su polli SPF di razza White Leghorn di 6 settimane di età, inoculati per via endovenosa al fine di determinare l'indice di patogenicità intravenosa (IVPI). I biotipi virali propagati al primo e decimo passaggio nei sistemi biologici utilizzati, sono stati inoltre sottoposti a sequenziamento al fine di rilevare possibili mutazioni acquisite durante l'amplificazione seriale. La maggior parte dei biotipi virali è risultata in grado di replicare già dal primo passaggio sui tre tipi cellulari con induzione di effetto citopatico, tranne il

biotipo H9N2 LPAI A/turkey/Wisconsin/66 che non ha replicato nella linea cellulare UMNSAH/DF1. I valori dell'indice di patogenicità intravenosa, invece, sono risultati inferiori a seguito della propagazione virale nelle linee cellulari rispetto all'embrione di pollo, ad eccezione di H7N1 HPAI A/Turkey/Italy/4580/99. Le indagini perseguite hanno confermato come le colture cellulari possano rappresentare un valido sistema alternativo all'embrione di pollo per l'amplificazione del virus dell'Influenza aviare. Il sequenziamento genomico completo ha evidenziato che l'adattamento del virus dell'Influenza A ai differenti sistemi biologici può essere responsabile di mutazioni non descritte in precedenza in letteratura, con conseguente sostituzione amminoacidica il cui ruolo, sotto il profilo antigenico, deve essere accuratamente valutato.

Lopes AM, Capucci°L, Gavier\_Widén D, Le\_Gall\_Recul é G, Brocchi°E, Barbieri°I, Quéméner A, Le\_Pendu J, Geoghegan JL, Holmes EC, Esteves PJ, Abrantes J  
**Molecular evolution and antigenic variation of European brown hare syndrome virus (EBHSV)**  
Virology. - Vol. 468-470 ( 2014). - p 104-112. - 50 bib ref [Nr. Estr. 5966]

European brown hare syndrome virus (EBHSV) is the aetiological agent of European brown hare syndrome (EBHS), a disease affecting *Lepus europaeus* and *Lepus timidus* first diagnosed in Sweden in 1980. To characterize EBHSV evolution we studied hare samples collected in Sweden between 1982 and 2008. Our molecular clock dating is compatible with EBHSV emergence in the 1970s. Phylogenetic analysis revealed two lineages: Group A persisted until 1989 when it apparently suffered extinction; Group B emerged in the mid-1980s and contains the most recent strains. Antigenic differences exist between groups, with loss of reactivity of some MAbs over time, which are associated with amino acid substitutions in recognized epitopes. A role for immune selection is also supported by the presence of positively selected codons in exposed regions of the capsid. Hence, EBHSV evolution is characterized by replacement of Group A by Group B viruses, suggesting that the latter possess a selective advantage.

Luzzago C, Lauzi S, Ebranati E, Giammarioli M, Moreno°A, Cannella V, Masoero L, Canelli°E, Guercio A, Caruso C, Ciccozzi M, De\_Mia GM, Acutis PL, Zehender G, Peletto S

**Extended genetic diversity of bovine viral diarrhea virus and frequency of genotypes and subtypes in cattle in Italy between 1995 and 2013**

BioMed Res Int. - Vol. 2014). - ID 147145 (8 p). - 41 bib ref (ultimo accesso 02/09/2014  
<http://www.hindawi.com/journals/bmri/2014/147145/>) [Nr. Estr. 5793]

Genetic typing of bovine viral diarrhea virus (BVDV) has distinguished BVDV-1 and BVDV-2 species and an emerging putative third species (HoBi-like virus), recently detected in southern Italy, signaling the occurrence of natural infection in Europe. Recognizing the need to update the data on BVDV genetic variability in Italy for mounting local and European alerts, a wide collection of 5' UTR sequences (n = 371) was selected to identify the frequency of genotypes and subtypes at the herd level. BVDV-1 had the highest frequency, followed by sporadic BVDV-2. No novel HoBi-like viruses were identified. Four distribution patterns of BVDV-1 subtypes were observed: highly prevalent subtypes with a wide temporal-spatial distribution (1b and 1e), low prevalent subtypes with a widespread geographic distribution (1a, 1d, 1g, 1h, and 1k) or a restricted geographic distribution (1f), and sporadic subtypes detected only in single herds (1c, 1j, and 1l). BVDV-1c, k, and l are reported for the first time in Italy. A unique genetic variant was detected in the majority of herds, but cocirculation of genetic variants was also observed. Northern Italy ranked first for BVDV introduction, prevalence, and dispersion. Nevertheless, the presence of sporadic variants in other restricted areas suggests the risk of different routes of BVDV introduction.

Luzzago C, Lauzi S, Ebranati E, Giammarioli M, Moreno<sup>o</sup>A, Cannella V, Masoero L, Canelli<sup>o</sup>E, Guercio A, Caruso C, Ciccozzi M, De\_Mia GM, Acutis PL, Zehender G, Peletto S

### **Variabilità genetica di BVDV e frequenza di genotipi e sottotipi in Italia nel periodo 1995-2013**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 62 [Nr. Estr. 5743]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

Il virus della diarrea virale bovina (BVDV) è un pestivirus con un impatto rilevante sulla salute e sulla produttività dei bovini. La tipizzazione molecolare di BVDV ha permesso la differenziazione del virus in due genotipi (BVDV-1 e BVDV-2) e l'identificazione di una nuova specie putativa, denominata HoBi-like, la cui circolazione in bovini naturalmente infetti è stata di recente segnalata, per la prima volta in Europa, in Italia meridionale. Con l'intento di aggiornare le informazioni sulla variabilità genetica di BVDV in Italia, sia per monitorare la situazione nazionale che per porre le basi per studi di epidemiologia molecolare, una vasta collezione di sequenze (n=371) della regione 5'UTR del virus è stata selezionata per identificare genotipi e sottotipi circolanti sul territorio nazionale con una risoluzione a livello di singolo allevamento. I risultati hanno confermato che BVDV-1 è il genotipo con più alta frequenza, mentre BVDV-2 è stato rilevato sporadicamente e la segnalazione più recente risale al 2004. Nessun BVDV della nuova specie HoBi-like è stato identificato. Tra i sottotipi di BVDV-1, sono stati osservati quattro pattern di distribuzione: sottotipi ad alta prevalenza con un'ampia distribuzione spaziotemporale (BVDV-1b, 1e); sottotipi a bassa prevalenza con una vasta diffusione geografica (BVDV-1a, 1d, 1g, 1h, 1k), sottotipi a bassa prevalenza presenti in aree geografiche ristrette (BVDV-1f) e sottotipi sporadici rilevati solo in singoli allevamenti (BVDV-1c, 1j, 1l). BVDV-1c, k, l sono peraltro segnalati per la prima volta in Italia. Nella maggioranza degli allevamenti risulta circolare un'unica variante genetica, ma i risultati dimostrano la possibilità di co-infezione con varianti genetiche diverse, sia in allevamenti da latte che da carne. L'Italia settentrionale conferma il ruolo preponderante per l'introduzione e la dispersione di BVDV sul territorio nazionale. Tuttavia l'identificazione di varianti sporadiche in aree più ristrette suggerisce anche il rischio di introduzione attraverso differenti flussi commerciali o prodotti biologici contaminati.

Magistrali CF, Cucco L, Sebastiani C, Scoccia E, Ruggeri<sup>o</sup>J, Pitozzi<sup>o</sup>A, Pasquali P, Alborali<sup>o</sup>G

### **B. hyodysenteriae loads in faeces of experimentally infected pigs are associated with diarrhoea and faecal consistency**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 226 (Poster P183) [Nr. Estr. 5722]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

In many European countries, *B. hyodysenteriae*, *B. pilosicoli* and *L. intracellularis*, are considered the major causes of intestinal diseases of growers and fatteners. The symptoms of these diseases are often similar and mixed infections are frequent in field conditions, making a differential diagnosis awkward. Multiplex real time PCR, targeting these three pathogens simultaneously, could be useful when a mixed infection is suspected, to reduce the time and cost of a differential diagnosis. Since this test can also quantify the bacteria, it has been suggested that it can be used to interpret the test outcomes. An association between faecal loads and low growth rate has been shown for *L. intracellularis*. For *B. hyodysenteriae*, an inverse relationship between faecal consistency and faecal loads was suspected in cases occurring in the field. The aim of this work was to confirm the hypothesis of an inverse relationship between the presence of diarrhoea, the faecal consistency and

the amount of *B. hyodysenteriae* excreted by artificially challenged pigs. 151 faecal samples were taken from pigs artificially challenged by *B. hyodysenteriae* 21 to 48 days before. The pigs were from Salmonella- and *B. hyodysenteriae*- free herds and tested negative for these pathogens. The samples were categorized as non diarrhoeic or diarrhoeic by the same veterinarian, cultured for *Brachyspira* spp, and examined by RT-PCR targeting *B. hyodysenteriae*, *L. intracellularis* and *B. pilosicoli*. The dry matter content of 64 faeces samples was evaluated by a microwave procedure; then the faeces were divided in different categories, 1 to 4, with decreasing dry matter content. All samples tested negative for *L. intracellularis* and *B. pilosicoli*. Non diarrhoeic faeces were grouped in category 1 only. The bacterial load, expressed as log<sub>10</sub>, increased when diarrhoea was present ( $p=0,7011$ ;  $p$ -values<sub>0,05</sub>) and it was on average 4,3 log in normal faeces, and 7,1 log in diarrhoeic samples. An inverse relationship between the dry matter content and the *B. hyodysenteriae* excretion, was shown ( $p=-0,4983$ ;  $p$ -value<sub>50,05</sub>). This relationship was stronger in diarrhoeic samples ( $p=-0,8047$ ). The bacterial load in faeces increased progressively with the dry matter content category ( $p=0,6734$ ,  $p$ -value<sub>50,05</sub>), as it was greater in faeces belonging to category 3 and 4. In conclusion, this work confirms the correlation between *B. hyodysenteriae* loads excreted by faeces and the presence and the severity of the diarrhoea, suggesting a possible use of DNA quantification in interpreting test results.

Magnani D, Cafazzo S, Calà P, Razzuoli°E, Amadori° M, Bernardini D, Gerardi G, Nanni\_Costa L

**Effect of long transport and environmental conditions on behaviour and blood parameters of postweaned piglets with different reactivity to backtest**

Livestock Sci. - Vol. 162 ( 2014). - p 201-208. - 31 bib ref [Nr. Estr. 6064]

In order to evaluate the effect of long transport on weaned piglets transported under warm weather conditions, 144 piglets, previously submitted to a backtest during nursing, were monitored during 4 journeys, each lasting 14 h, carried out from May to September 2009. Into the truck, piglets were allocated in 8 pens on the basis of backtest classification identified as High Resisting (HR), Low resisting (LR), Mixed (M) and Mixed at Loading (MAL). During transport, truck air temperature, skin temperatures and postural and behavioural occurrences were recorded. Prior to and after transportation, blood samples and body weight were also recorded. Piglets lost 5% of their body weight. Environmental conditions affected slightly the behaviour of piglets which were more active during the first 4 h of transport. The behaviour of the piglets was significantly influenced by the type of pen since some differences in biting and exploratory behaviours were found in M pens. Conversely, no differences were found between HR and LR pens. Significant variations with respect to the baseline levels were found only for glucose which decreased and for urea which increased after journey as a result of the prolonged fasting. In general, the results suggest that long-lasting journeys did not have consistent effects on physiological and behavioural parameters of early-weaned piglets while grouping and mixing procedures may affect how they cope with transport.

Martelli P, Saleri R, Cavalli V, De\_Angelis E, Ferrari L, Benetti M, Ferrarini G, Meriardi°G, Borghetti P

**Systemic and local immune response in pigs intradermally and intramuscularly injected with inactivated *Mycoplasma hyopneumoniae* vaccines**

Vet Microbiol. - Vol. 168 ( 2014). - p 357-364. - 24 bib ref [Nr. Estr. 5666]

The systemic and respiratory local immune response induced by the intradermal administration of a commercial inactivated *Mycoplasma hyopneumoniae* whole-cell vaccine (Porcilis® MHYO ID ONCE – MSD AH) in comparison with two commercial vaccines administered via the intramuscular route and a negative control (adjuvant only) was investigated. Forty conventional *M. hyopneumoniae*-free pigs were randomly assigned to four groups (ten animals each): Group A = intradermal

administration of the test vaccine by using the needle-less IDAL® vaccinator at a dose of 0.2 ml; Group B = intramuscular administration of a commercially available vaccine (vaccine B); Group C = intramuscular administration of the adjuvant only (2 ml of X-solve adjuvant); Group D = intramuscular administration of a commercially available vaccine (vaccine D). Pigs were vaccinated at 28 days of age. Blood and bronchoalveolar lavage (BAL) fluid samples were collected at vaccination (blood only), 4 and 8 weeks post-vaccination. Serum and BAL fluid were tested for the presence of antibodies by ELISA test. Peripheral blood mononuclear cells (PBMC) were isolated to quantify the number of IFN- $\gamma$  secreting cells by ELISpot. Moreover, cytokine gene expression from the BAL fluid was performed. Total antibodies against *M. hyopneumoniae* and specific IgG were detected in serum of intradermally and intramuscularly (vaccine B only) vaccinated pigs at 4 and 8 weeks post-vaccination. *M. hyopneumoniae* specific IgA were detected in BAL fluid from vaccinated animals (Groups A and B) but not from controls and animals vaccinated with the bacterin D ( $p < 0.05$ ). Significantly higher gene expression of IL-10 was observed in the BAL fluid at week 8 post-vaccination in the intradermally vaccinated pigs ( $p < 0.05$ ). The results support that the intradermal administration of an adjuvanted bacterin induces both systemic and mucosal immune responses. Moreover, the intramuscularly administered commercial vaccines each had a different ability to stimulate the immune response both systemically and locally.

Massi°P, Fiorentini°L, Barbieri°I, Casadio°M, Tosi°G

#### **Identificazione mediante sequenziamento genomico dei ceppi di virus della malattia di Gumboro (IBDV) isolati nel pollo da carne in Italia e in paesi esteri negli anni 2012, 2013 e 2014**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 155-167. - 15 bib ref [Nr. Estr. 5970]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*Viral population dynamics of very virulent infectious bursal disease virus (vvIBDV) and four non-very virulent IBDV isolated in Italy since 2012 have been analysed and compared to IBDV reference strains. The strains were collected by the intensive farm of five Regions (North, Center and South of Italy). The viral population structure of vvIBDV strains showed a strong relationship between geography and phylogeny, with one main group of vvIBDV observed. The low variability and the geographical pattern observed indicate that the virus evolves slowly, occupying the same geographical niche for a long time as for example in Romagna Region. Probably it is dependent to the biological features of the virus: being able to remain viable for long periods of time due to a strong environmental resistance, and as an immunosuppressive agent, capable to elude temporarily the immune system of the host. About the four non-vvIBDV in commercial broiler, tree of origin in Romagna, is needed, for the future, to evaluate their circulation, pathogenicity and the protection offered against them by commercial IBDV vaccines. In the second part of this study 52 IBDV strains were genetically characterized and compared with reference IBDV strains. Results of the genetic characteristics revealed that the majority of virus (82%) were related to vvIBDV strains. Diagnostic assays that can reliably identify vvIBDV and non-vvIBDV strains are needed for surveillance programs designed to monitor the spread of these viruses.*

Massi°P, Fiorentini°L, Barbieri°I, Giovannetti L, Casadio°M, Tosi°G

#### **Monitoraggio della colonizzazione e persistenza del vaccino vivo attenuato per *Mycoplasma synoviae* in riproduttori pesanti**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 168-171. - 4 bib ref [Nr. Estr. 5971]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*Mycoplasma synoviae is an important causative agent of avian mycoplasmosis. In the present study*

*vlhA gene sequence analysis was applied and verified for typing the Mycoplasma synoviae live vaccine MS-H strain and field isolates from farm breeder chickens. This tool was applied for 42 field strains of M. synoviae isolated from breeder chickens. This analysis revealed that all the strains presented 100% similarity with M. synoviae vaccine strain Mycoplasma synoviae –H. Nobody M. synoviae field strain was identified. In the same time nobody Mycoplasma s. was identified in the second pullets period of life.*

Massi°P, Fiorentini°L, Longoni C, Russo E, Casadi o°M, Tosi°G

### **Riproduzione sperimentale dell'infezione da Salmonella enteritidis in due gruppi di galline ovaiole di 45 e 65 settimane di vita sottoposte a due differenti programmi vaccinali**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 172-176. - 5 bib ref [Nr. Estr. 5972]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*The aim of this study was to examine the duration of immunity of different vaccination schemes using a S. enteritidis live vaccine and a S. enteritidis inactivated vaccine in one group and a S. enteritidis live vaccine and a S. enteritidis-S. typhimurium inactivated vaccine in a second group of layers. Two groups of Lohman Brown chickens were used. Group one (A) was vaccinated two times orally with S. enteritidis live vaccine and once i.m. with S. enteritidis-S. typhimurium inactivated vaccine with an half dose at 14 weeks. Group two (B) was vaccinated two times orally with S. enteritidis live vaccine at 3 and 30 days and once i.m. with S. enteritidis inactivated vaccine at 14 weeks. Ten layers randomly selected from each of the groups were transferred in laboratory and challenged with a S. enteritidis field strain in 45 and 65 weeks of age. In one month we had got an estimation of the impact of challenge on faecal shedding and organ and egg contamination for Salmonella enteritidis.*

Mazzone P, Corneli S, Di\_Paolo A, Maresca C, Biagetti M, Sebastiani C, Ciullo M, Pezzotti G, Ricchi°M, Savi°R, Arrigoni° N

### **Evaluation of Mycobacterium avium subsp. paratuberculosis (MAP) survival in two biogas plants in Italy**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 217 (Poster P-07.7) [Nr. Estr. 5775]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

New legislation on renewable energies allows the use of animal manure in biogas plants. The final product of anaerobic digestion process (AD), namely digestate (DG), could be used as crop fertilizer. Although positively contributing to energy and environmental problems, this use raises health-related concerns regarding bacteria potentially resistant to AD treatment. In particular, MAP, the causative agents of Paratuberculosis (PTB), is a very resistant agent, widespread in cattle manure of affected herds. We here report results relative to the survival of MAP in two different Italian biogas plants, both operating in mesophilic conditions (38-40°C). The first plant was characterized by a double-stage Digester, supplied with manure from 29 bovine herds, 7 of which (24.1%) resulted infected by serological or culture tests. Samples were collected at Pre-tank (PT), Primary Digester (D1) and Secondary Digester (D2). Out of 230 samples submitted to culture, 27 were positive: 12/18 (55.5%) in PT, 14/70 (20.0%) in D1, 1/70 in D2 (1.4%), 0/36 in solid and 0/36 in liquid DG. MAP load showed a significant decrease from PT to D1 and D2; moreover it has never been detected in final DG. This might be due both to AD inactivation and to dilution effect. The second plant was characterized by a single-stage Digester, supplied with manure from a single herd, affected by PTB (apparent prevalence: serology 14.3%; culture 11.6%). Out of 112 samples analyzed, 65 were positive to culture: 36/48 (75.0%) in PT, 26/48 (54.2%) in Digester, 1/13 in solid DG and 2/3 in liquid DG. The higher survival rate of viable MAP reported in this second study might suggest that this single-stage system is more permissive than the double-stage plant. We conclude that the use of DG as a fertilizer can increase the risk of MAP spreading on agricultural soil. To reduce this risk, we suggest, especially for single-stage digesters, the use of manure from PTB negative herds.

Mazzone P, Corneli S, Vitale N, Di\_Paolo A, Biagetti M, Maresca C, Ricchi°M, Mangili P, Papa P, Pezzotti G, Raeber AJ, Arrigoni° N, Cagiola M

**Use of new Johnin in gamma-IFN test to detect Mycobacterium avium subsp. paratuberculosis (MAP) infected cattle : preliminary data**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 127 (Oral O-04.3) [Nr. Estr. 5767]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

Traditional diagnosis of Paratuberculosis (PTB) is based on serology and fecal culture. Gamma Interferon ( $\gamma$ -IFN) and Skin Test (ST), already used for ante-mortem diagnosis of bovine Tuberculosis (bTB) could be also useful for PTB diagnosis.  $\gamma$ -IFN test detects cytokine production of T lymphocytes after stimulation with purified protein derivatives (PPDs), from *M. bovis* (PPDB) and from *M. avium* (PPDA). In order to achieve an early detection of subjects exposed to MAP infection, 3 new PPDs extracted from culture of MAP (PPDJ a, b, c) were produced, assessed in the  $\gamma$ -IFN test, compared to classic PPDs. In the ongoing study, 68 cattle from bTB officially free herds and with previous PTB clinical cases were tested by IDVet ELISA, PCR and culture. PPDJs were used in  $\gamma$ -IFN test (BOVIGAM ELISA) at dilutions 1:5 and 1:10. Samples have been considered positive when Optical Density (OD) value was, at least, twice OD of Nihil. Relative specificity (Sp) of PPDB and PPDJ in bTB diagnosis was calculated using ST as Gold Standard. Relative sensitivity (Se) of PPDJ in PTB diagnosis was calculated using, as GS, the positivity in at least one of classical tests and PCR. Out of 55 animals PTB positive, 46 reacted to both PPDA and PPDJ, 4 only to PPDJ. For bTB diagnosis, the comparative use of PPDA and PPDJ against PPDB in 68 bTB negative cows yielded a 100% Sp. For PTB diagnosis, 1:5 dilution of PPDJ a, b and c showed respectively 83.6%, 76.4% and 74.5% Se. Relatively low Se in PTB diagnosis is influenced by tests used as GS, which are able to detect only advanced stages of disease. In fact 4 out of 9 subjects, previously classified as PTB negative, but positive to PPDJ, became positive 8 months later to serological and culture tests. Our preliminary results highlight the ability of our  $\gamma$ -IFN to avoid false positivity for bTP. For PTB diagnosis we plan to include more subjects with a suitable follow up.

Mazzone P, Gavaudan S, Duranti A, Perugini G, Corneli S, Di\_Paolo A, Cagiola M, Biagetti M, Ciullo M, Boniotti°MB, Pacciarini°M

**Molecular characterisation of Mycobacterium bovis isolated in wild boars and cattle from an Italian pasture between 2002 and 2013**

VI International *M. bovis* conference : Cardiff, Wales, Great Britain, 16th - 19th June 2014 / [s.l. : s.n., 2014]. - p 129 [Nr. Estr. 5899]

International *M. bovis* conference (6th : Cardiff, Wales : 16th - 19th June 2014)

Introduction Bovine tuberculosis (bTB) in wild animals is a serious problem in many parts of the world. Between 2002 and 2013, *Mycobacterium bovis* was isolated from wild boars and from cattle that shared the same pasture in Marche region, central Italy. Two different cattle herds were involved in bTB outbreaks: the first occurred in 2005 and the second in 2013, respectively. With the aim to investigate the role played by wild boars in this area, since 2005 we have started a surveillance activity that led us finding infected wild boars in the environment studied. Materials and methods In the last ten years, samples with suspected lesions from hunted wild boars were collected at slaughterhouse. Lymph nodes, mainly from head and thorax, were processed for histological and culture examination. Cattle positive to skin test or at post mortem inspection were submitted to culture isolation for diagnostic confirmation of bTB. All isolates were typed by spoligotyping and analysis of 12 variable number tandem repeat (VNTR) loci (ETR A-E, 2163a, 2163b, 4052, 1895, 3155, 3232 and 2996) at National Reference Centre for Bovine Tuberculosis.

Results Overall, 29 *M.bovis* were isolated, 16 from wild boars and 13 from cattle belonging to bTB outbreaks. All isolates were characterised by Spoligotype SB0120 and VNTR type 3.3.5.3.3.10.4.4.4.3.6.5. Discussion and conclusion. The finding of a single cluster in the same area between cattle and wild boars confirmed the existence of wildlife-livestock interaction and its potential role in the bTB epidemiology.

Mazzoni C, Scollo A, Tonon F, Gherpell M, Bonilauri°P, De\_Rensis F

**Effetti dell'impiego del seme morto sulla fertilità e sulla prolificità della scrofetta** = Effect of the use of dead semen, on fertility and prolificacy in gilts

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 233-240. - 16 bib ref [Nr. Estr. 5672]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

Lo scopo del presente lavoro, è stato quello di confrontare gli effetti dell'impiego del seme morto sulla fertilità dell'inseminazione al successivo estro ed il suo effetto sul numero dei suinetti nati totali e mummificati, in scrofette adeguatamente condizionate alla gabbia gestazione. Nello studio sono state incluse 525 scrofette divise nei seguenti gruppi: Gruppo I (n=423): scrofette inseminate al momento dell'estro e messe subito nella gabbia gestazione. Gruppo II (n=56): scrofette messe in gabbia gestazione al rilevamento dell'estro, non inseminate subito, ma inseminate con seme vivo al ciclo estrale successivo. Gruppo III (n=74): scrofette messe in gabbia gestazione al rilevamento dell'estro, inseminate con seme morto al primo estro, ed inseminate con seme vivo all'estro del ciclo successivo. Il test di Cuzick per la ricerca del trend fra gruppi ordinati, indica un significativo trend positivo ( $p < 0.05$ ) nel numero dei suinetti nati totali maggiore di 13 passando dal gruppo I al gruppo III. Questa differenza, singolarmente confrontata, fra i gruppi II e rispettivamente I e III, non risulta significativa. Il numero dei suinetti nati totali è stato più alto per le scrofette trattate con il seme morto (gruppo III) di quanto non sia stato per il gruppo I. La differenza fra i gruppi II e rispettivamente I e III, non risulta significativa. Per la fertilità (numero di scrofe che hanno partorito rispetto a quelle fecondate), non si sono riscontrate differenze fra i tre gruppi. I risultati di questo studio, suggeriscono che l'inseminazione con seme morto all'estro precedente a quello servito con il seme vivo, ha un effetto positivo sul successivo numero di nati totali. Questo fatto può essere attribuito anche alla riduzione dei mummificati nel gruppo III rispetto al gruppo I.

*The present study has been conducted to compare the effects of intrauterine infusion of dead boar semen on the reproductive performance at succeeding estrus and its effect of the number of piglets born and born mummify in properly gestation crate adapted gilts. In this study 525 gilts have been utilized and the animals have been divided in three groups: Group I (n=399): Inseminated at first heat detection and immediately located into the gestation crate (Control). Group II (n=53) Immediately located into gestation crate at first heat detection and not yet inseminated but served with alive semen at second heat during the succeeding estrous cycle. Group III: (n=73) immediately located into gestation crate at first heat detection and inseminated with dead semen and served with alive semen at the succeeding estrous cycle (Dead Semen treatment). The Cuzick test for a trend across ordered groups indicate a significant positive trend ( $p < 0.05$ ) in the number of total borne piglets greater 13 between for Groups. While, the difference, between Groups II with Group I and III respectively, individually compared, were not significant. The number of total piglets born was higher in the gilts treated with dead semen (Group III) compared gilts of Group I (Control). There were not differences between Group II and Group I and III Fertility (the number of gilts inseminated that farrowed) was not different across groups. The data of this study suggest that the treatment with dead semen at the estrus cycle before insemination has a positive effect on the following number of piglets born. This is in part due a decrease in the number of mummify piglets in Group III compared Group I.*

Mazzoni C, Tonon F, Borri E, Raffi V, Scollo A, Donna R, Bonilauri°P, Gherpelli M, De\_Rensis F

**Distribution of regular and irregular inter-oestrus interval in sows during different months of**

## the year in Northern Italy

Large Anim Rev. - Vol. 20 no 6 ( 2014). - p 255-257. - 16 bib ref [Nr. Estr. 5996]

The objective of this study was to determine the incidence of regular or irregular inter-oestrus interval in sows during different months of the year in Northern Italy. In this study, 5,103 sows that returned to oestrus were inseminated and assigned to different groups based on the length of their inter-oestrus interval:

- Regular inter-oestrus interval type 1 (Rint-1): sows with inter-oestrus interval of 18-23 days
  - Regular inter-oestrus interval type 2 (Rint-2): sows with inter-oestrus interval of 36-48 days
  - Irregular inter-oestrus interval (IRint group): inter-oestrus interval of 24-35.
- Based on meteorological data of the area in which the farms are located, animals were considered to be under heat stress during the high temperature months of June to August while animals were not considered to be under heat stress during September to May. The proportion of sows with Rint-1 was greater ( $P<0.05$ ) during the high temperature months compared than during the rest of the year. There was no difference between months for the proportion of sows with Rint-2. The proportion of sows with IRint was greater ( $P<0.05$ ) during the months of September and October than during the other months of the year. Results from this study indicate that, in Northern Italy, during the high temperature months of June-August, there was an increase in the proportion of sows with regular inter-oestrus interval, suggesting a failure of conception or an increase of early embryos losses, while there was an increase in the proportion of sows with irregular inter-oestrus interval during September-October, suggesting later embryo losses.

McDonald J, Leo°S, Paternoster°G, Tamba°M, Bonte mpi°G, Arrigoni°N

### **iRAMP : an Italian risk assessment and management planning tool and just-in-time learning within one practical application**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 97 (Oral O-03.3) [Nr. Estr. 5770]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

In 2012 Italy decided to institute a control program for paratuberculosis. As with any control program, education for veterinarians and producers is key to the program's success, so the Italian program created online mod-ules for veterinarians who in turn educate producers. Eleven years before, the US made a similar decision to develop a control program for paratuberculosis. With primary funding from the US Department of Agriculture, through JDIP (John's Disease Integrated Program) and the Wisconsin Department of Trade and Consumer Protection, an alternative to time and place-bound training was created with a national online John's Disease Veterinary Certificate Program as well as virtual farm visits and modules for producers. The latest addition was JD-RAP (jdrap.org), a simulation for producers that embeds education within a risk assessment process where users enter their own data resulting in customized results and specific recommendations. Creating quality education can be an expensive endeavor. Through an Italian-US collaboration we created new tools and modules for Italian veterinarians and producers in a cost-effective way by reusing and repurposing parts of existing, proven US modules. Funded by a US Fulbright award and the Italian Ministry of Health (Re-search Project 2012/001, "Creating an informative integrated system for Paratuberculosis"), the main focus of the project is an electronic version for tablets, both iPad and Android, of a risk assessment for veterinarians to use on the farm and facilitate record maintenance. We mined and revamped elements from JD-RAP and other US modules and embedded mini-lectures, explanations, and examples, accessed through help buttons at strategic points, for just-in-time learning and review of key concepts. We are also creating Italian versions of JD-RAP and other select modules, editing for Italy-specific rules, regulations, and management practices, to add to the educational arsenal for the diagnosis, control, and management of paratuberculosis in Italy. The resulting Italian online education modules will be unveiled at the 12th ICP.

Mohamad KJ, Kaltenboeck B, Rahman KS, Magnino°S, S achse K, Rodolakis A

**Host adaptation of *Chlamydia pecorum* towards low virulence evident in co-evolution of the *ompA*, *incA*, and ORF663 Loci**

PLoS One. - Vol. 9 no 8 ( 2014). - p e103615 (16 p). - 43 bib ref (ultimo accesso 02/09/2014  
<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0103615>) [Nr. Estr. 5794]

*Chlamydia (C.) pecorum*, an obligate intracellular bacterium, may cause severe diseases in ruminants, swine and koalas, although asymptomatic infections are the norm. Recently, we identified genetic polymorphisms in the *ompA*, *incA* and ORF663 genes that potentially differentiate between high-virulence *C. pecorum* isolates from diseased animals and low-virulence isolates from asymptomatic animals. Here, we expand these findings by including additional ruminant, swine, and koala strains. Coding tandem repeats (CTRs) at the *incA* locus encoded a variable number of repeats of APA or AGA amino acid motifs. Addition of any non-APA/AGA repeat motif, such as APEVPA, APAVPA, APE, or APAPE, associated with low virulence ( $P < 10^{-4}$ ), as did a high number of amino acids in all *incA* CTRs ( $P = 0.0028$ ). In ORF663, high numbers of 15-mer CTRs correlated with low virulence ( $P = 0.0001$ ). Correction for *ompA* phylogram position in ORF663 and *incA* abolished the correlation between genetic changes and virulence, demonstrating co-evolution of *ompA*, *incA*, and ORF663 towards low virulence. Pairwise divergence of *ompA*, *incA*, and ORF663 among isolates from healthy animals was significantly higher than among strains isolated from diseased animals ( $P = 10^{-5}$ ), confirming the longer evolutionary path traversed by low-virulence strains. All three markers combined identified 43 unique strains and 4 pairs of identical strains among all 57 isolates tested, demonstrating the suitability of these markers for epidemiological investigations.

Monini M, Zaccaria G, Ianiro G, Lavazza°A, Vaccari G, Ruggeri FM

**Full-length genomic analysis of porcine rotavirus strains isolated from pigs with diarrhea in Northern Italy**

Infect Genet Evol. - Vol. 25 ( 2014). - p 4-13. - 41 ref bib [Nr. Estr. 5684]

Group A rotaviruses (RVA) cause acute dehydrating diarrhea in young of man and many animal species, including pigs. Swine RVA has an important economic impact on the farming industry, and pigs represent a potential reservoir for zoonotic transmission of RVA to humans. To investigate the genetic diversity of porcine RVA strains in Italy and identify their possible zoonotic characteristics, 25 RVA-positive feces were collected from diarrheic pigs in Northern Italy, in 2009–2010; all viral strains were characterized by G and P genotyping RT–PCR. Three samples were selected for full genome sequencing. Sequencing of the NSP3 genes of all samples was also performed. Rotavirus diagnosis was carried out by ELISA and electron microscopy. RT–PCR and Sanger sequencing were performed in a one-tube format, using primer sets specific for each of the 11 genome segments. Analysis of the G (VP7) and P (VP4) genotypes showed that all strains identified were typical porcine RVAs (G4, G5, G9; P[6], P[13], P[23]). Full-length genome sequencing was performed on selected G9 isolates. Most segments belonged to the genotype constellation 1 (Wa-like), which is shared by most human RVA strains, but gene types such as I5 (VP6) and A8 (NSP1), which are typical of porcine and rare among human RVAs, were also detected. We identified RVA strains showing the T7 genotype, an NSP3 gene type that was previously reported in unusual strains of possible porcine or bovine origin from children with diarrhea. Recent reports suggested that G9 RVA may have been introduced from swine to human populations involving gene reassortment events. The observation that some of the RVA genotypes from swine in Italy were similar to viruses characterized in children underlines the importance of animal RVA surveillance, to clarify and monitor the role of animals as genetic reservoirs of emerging RVA strains pathogenic for humans.

Monini M, Zaccaria G, Ianiro G, Lavazza°A, Vaccari G, Ruggeri FM

## **Sequenziamento e analisi filogenetica dell'intero genoma di ceppi di rotavirus suini**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 69 [Nr. Estr. 5744]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

I Rotavirus di gruppo A (RVA) sono causa di gastroenterite acuta negli individui giovani di molte specie animali. L'infezione da rotavirus ha un importante impatto economico sul settore agricolo, i suini inoltre possono rappresentare un potenziale serbatoio per la trasmissione zoonotica di rotavirus all'uomo. Per studiare la diversità genetica di ceppi suini di RVA in Italia e individuarne le possibili caratteristiche zoonotiche, 25 campioni fecali provenienti da suini con diarrea, campionati tra il 2009 e il 2010 in tre province del nord Italia, sono stati caratterizzati mediante genotipizzazione delle proteine capsidiche VP7 e VP4 (genotipo G e P) tramite RT-PCR e sequenziamento. Tre campioni sono stati selezionati per effettuare il sequenziamento dell'intero genoma virale e l'analisi filogenetica sugli 11 segmenti. In seguito all'individuazione di ceppi con genotipo T7 del gene NSP3 (identificato per la prima volta in Italia), è stato effettuato anche il sequenziamento dei geni NSP3 di tutti i campioni. La diagnosi di RVA è stata effettuata mediante ELISA e microscopia elettronica. I ceppi analizzati mostravano genotipi G e P tipici di RVA suini (G4, G5, G9; P[6], P[13], P[23]). Il sequenziamento full-length degli 11 segmenti del genoma è stato effettuata su tre isolati G9 (genotipo emergente nell'uomo che in molti paesi risulta essere il genotipo principalmente individuato). La maggior parte dei segmenti genici appartenevano alla costellazione di genotipo 1 (Wa-like), cui appartengono principalmente i ceppi di RVA isolati nell'uomo, ma sono stati individuati anche genotipi come I5 (VP6) e A8 (NSP1), tipici dei RVA suini e identificati raramente tra i RVA umani. Di particolare interesse è stata l'individuazione di ceppi di RVA con genotipo T7 del gene NSP3. Questo genotipo è stato precedentemente individuato in ceppi di rotavirus insoliti, di possibile origine suina o bovina, isolati da bambini con diarrea. Recenti studi suggeriscono che il genotipo G9 potrebbe essere stato introdotto dal suino nella popolazione umana mediante eventi di riassortimento genico. L'osservazione che alcuni genotipi di RVA isolati nel suino risultano simili ai virus individuati nei bambini, sottolinea l'importanza della sorveglianza dei RVA negli animali al fine di poter chiarire e controllare un possibile loro ruolo di serbatoio genico di ceppi di RVA emergenti patogeni per l'uomo.

Monne I, Fusaro A, Nelson MI, Bonfanti L, Mulatti P, Hughes J, Murcia PR, Schivo A, Valastro V, Moreno<sup>o</sup> A, Holmes EC, Cattoli G

### **Emergence of a highly pathogenic avian influenza virus from a low-pathogenic progenitor**

J Virol. - Vol. 88 no 8 ( 2014). - p 4375-4388. - 81 bib ref [Nr. Estr. 5680]

Avian influenza (AI) viruses of the H7 subtype have the potential to evolve into highly pathogenic (HP) viruses that represent a major economic problem for the poultry industry and a threat to global health. However, the emergence of HPAI viruses from low-pathogenic (LPAI) progenitor viruses currently is poorly understood. To investigate the origin and evolution of one of the most important avian influenza epidemics described in Europe, we investigated the evolutionary and spatial dynamics of the entire genome of 109 H7N1 (46 LPAI and 63 HPAI) viruses collected during Italian H7N1 outbreaks between March 1999 and February 2001. Phylogenetic analysis revealed that the LPAI and HPAI epidemics shared a single ancestor, that the HPAI strains evolved from the LPAI viruses in the absence of reassortment, and that there was a parallel emergence of mutations among HPAI and later LPAI lineages. Notably, an ultradeep-sequencing analysis demonstrated that some of the amino acid changes characterizing the HPAI virus cluster were already present with low frequency within several individual viral populations from the beginning of the LPAI H7N1 epidemic. A Bayesian phylogeographic analysis revealed stronger spatial structure during the LPAI outbreak, reflecting the more rapid spread of the virus following the emergence of HPAI. The data generated in this study provide the most complete evolutionary and phylogeographic analysis of epidemiologically intertwined high- and low-pathogenicity viruses undertaken to date and highlight the importance of implementing prompt eradication measures against LPAI to prevent the appearance of viruses with fitness advantages and unpredictable pathogenic properties.

Moreno<sup>o</sup> A, Ceruti R, Boniotti<sup>o</sup> B, Gavazzi L, Fasoli P, Cordioli<sup>o</sup> P

### **Circolazione di un nuovo genotipo di virus della bronchite infettiva aviare in Italia**

Atti della Societa' Italiana di Patologia Aviare (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 177-181. - 6 bib ref [Nr. Estr. 5978]

Convegno annuale Societa' Italiana Patologia Aviare (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*This study reported for the first time the circulation in Italy since 2012 of IBV strains closely related to IBV/Guandong/Xindadi/0903 isolated in China. The description of clinical forms, virus isolation, sequencing of S1 gene and phylogenetic and molecular analysis of four cases detected in the period 2012 - 2014 were here described. The phylogenetic analysis showed that the Italian and Chinese strains formed a clearly distinguishable group within the A2 genotype, which included the QX strain widely isolated in Europe and China. The major part of strains belonging to this genotype were nephropathogenic, however the group here described was associated with respiratory forms without renal involvement. The full-length S1 gene of two Italian strains was sequenced and along with other full-length IBV genomes available from GenBank, IBV/ Guandong/Xindadi/0903 included, was analyzed for recombination. Evidence of recombination was found in the Italian and Chinese strains in the S1 protein being identified the QX and 4/91 sequences as major and minor parents. These results demonstrated the circulation of a new IBV variant closely related to IBV strains isolated in China probably originated as result of a recombination event between QX and 4/91 strains. Permanent monitoring of circulating strains would be advisable in order to rationally modify vaccination strategies to make them appropriate to the field situation.*

Moreno<sup>o</sup> A, Sozzi<sup>o</sup> E, Lelli<sup>o</sup> D, Chiari<sup>o</sup> M, Prati<sup>o</sup> P, Alborali<sup>o</sup> L, Boniotti<sup>o</sup> B, Cordioli<sup>o</sup> P, Lavazza<sup>o</sup> A

### **Genomic characterization of Pseudorabies virus strains isolated from wild boar and dogs in Italy**

8th Annual Epizone Meeting "Primed for tomorrow" : 23 - 25 September 2014 Copenhagen, Denmark : Posters "Domestic-wildlife animal interactions" / [s.l. : s.n., 2014]. - 1 p (Poster WILD01). - 2 bib ref [Nr. Estr. 5951]

Annual meeting Epizone (8th : Copenhagen, Denmark : 23 - 25 September 2014)

Pseudorabies virus (PRV) is the agent of Aujeszky's disease (AD), one of the most economically important diseases of pigs for which suids are the natural hosts. Wild boars can act as reservoirs for viral agents and may represent a potential threat for domestic animals. To deeper investigate the epidemiology of PRV in Italy, we performed the genomic characterization of one PRV strain isolated from wild boar in 2011 and 17 Italian strains originated from dogs isolated in the period 1993-2012. Out of these, ten were hunting dogs, five were working dogs living in or close to pig farms, and for the other two no data was available but they were unable to hunt. Viruses were isolated from tonsils of the wild boar and brains of dogs showing neurological symptoms by inoculation onto PK15 cell lines. Phylogenetic and molecular analysis was performed by partial sequencing of gC gene including reference and field wild boar and pig strains representatives of all the previously described clades (1, 2). Phylogenetic analysis showed that the Italian strains were divided in three clades: 1- it included three strains from working dogs closely related to pig strains isolated in Europe and America in the last 20 years, which belonged to clade B; 2 – it included ten strains from hunting dogs and the wild boar strain closely related to another wild boar Italian strain (ITA561) isolated in 1993. 3- it was formed by two working dogs and two dogs that were unable to hunt closely related to the reference strain S66 and one Brazilian strain, which were previously not included elsewhere; The clade 2 formed a separate Italian group clearly distinguishable from the clades A and B previously described (2) that included the PRV European feral strains; Clade 2 was indeed characterized by a different amino-acid deletion pattern in the gC protein (2 deletions at positions 25 and 185). These results show a clear distinction between viral strains belonging to the first clade isolated from farm

dogs and those belonging to the second clade isolated from dogs used for hunting and then traced back to the wild boar. These strains of clade 2 showed high homology to PRV strains circulating in the 70's and 80's, which today have almost disappeared in the swine population. However, the Italian group formed a clade clearly differentiate from other European wild boar strains. The third clade included four strains isolated from not hunting dogs and one isolated from an Italian pig that was not related neither to feral pig nor to recently pig strains. This study, conducted mainly on hunting dog isolates and then related to wild boars, may deepen our understanding on the epidemiology of AD in wildlife populations.

Moreno<sup>o</sup> A, Vaccari G, Boni A, Lelli<sup>o</sup> D, Lavazza<sup>o</sup> A, Zaccaria G, Di Trani L, Alborali<sup>o</sup> GL, Cordioli<sup>o</sup> P

**Evolutionary and genomic analyses of the pandemic H1N1 viruses in Italian pig farms**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 257 - 3 bib ref [Nr. Estr. 5968]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

Mughini-Gras L, Bonfanti L, Mulatti P, Monne I, Guberti V, Cordioli<sup>o</sup> P, Marangon S

**Environmental correlates of H5N2 low pathogenicity avian influenza outbreak heterogeneity in domestic poultry in Italy**

PLoS One. - Vol. 9 no 1 ( 2014). - p e86788 (13 p). - 34 bib ref [Nr. Estr. 5633]

Italy has experienced recurrent incursions of H5N2 avian influenza (AI) viruses in different geographical areas and varying sectors of the domestic poultry industry. Considering outbreak heterogeneity rather than treating all outbreaks of low pathogenicity AI (LPAI) viruses equally is important given their interactions with the environment and potential to spread, evolve and increase pathogenicity. This study aims at identifying potential environmental drivers of H5N2 LPAI outbreak occurrence in time, space and poultry populations. Thirty-four environmental variables were tested for association with the characteristics of 27 H5N2 LPAI outbreaks (i.e. time, place, flock type, number and species of birds affected) occurred among domestic poultry flocks in Italy in 2010–2012. This was done by applying a recently proposed analytical approach based on a combined non-metric multidimensional scaling, clustering and regression analysis. Results indicated that the pattern of (dis)similarities among the outbreaks entailed an underlying structure that may be the outcome of large-scale, environmental interactions in ecological dimension. Increased densities of poultry breeders, and increased land coverage by industrial, commercial and transport units were associated with increased heterogeneity in outbreak characteristics. In areas with high breeder densities and with many infrastructures, outbreaks affected mainly industrial turkey/layer flocks. Outbreaks affecting ornamental, commercial and rural multi-species flocks occurred mainly in lowly infrastructured areas of northern Italy. Outbreaks affecting rural layer flocks occurred mainly in areas with low breeder densities in south-central Italy. In savannah-like environments, outbreaks affected mainly commercial flocks of galliformes. Suggestive evidence that ecological ordination makes sense genetically was also provided, as virus strains showing high genetic similarity clustered into ecologically similar outbreaks. Findings were informed by hypotheses about how ecological interactions among poultry populations, viruses and their environments can be related to the observed patterns of H5N2 LPAI occurrence. This may prove useful in enhancing future interventions by developing site-specific, ecologically-grounded strategies.

Mutze GJ, Sinclair RG, Peacock DE, Capucci<sup>o</sup> L, Kova liski J

**Is increased juvenile infection the key to recovery of wild rabbit populations from the impact**

### **of rabbit haemorrhagic disease?**

Eur J Wild Res. - Vol. 60 ( 2014). - p 489-499. - 51 bib ref [Nr. Estr. 5788]

The frequency and timing of rabbit haemorrhagic disease (RHD) epizootics and their impact on different age groups of rabbits were studied for 15 years in a recovering rabbit population in South Australia. We recorded the number and body size of rabbits dying during RHD epizootics, collected tissue for genetic analysis of rabbit haemorrhagic disease virus variants and compared the number of carcasses found to the number of susceptible rabbits present at the beginning of each epizootic. All RHD epizootics occurred between late winter and spring, but, progressively, epizootics started earlier and became more frequent and prolonged, fewer susceptible adult rabbits were present during epizootics, and the age of rabbits dying of RHD declined. Increased infection and virus shedding in juvenile rabbits offers the most plausible explanation for those epidemiological changes; the disease is now increasingly transmitted through populations of kittens, starting before young-of-the-year reach adult size and persisting late in the breeding season, so that most rabbits are challenged in their year of birth. These changes have increased juvenile mortality due to RHD but reduced total mortality across all age groups, because age-specific mortality rates are lower in young rabbits than in older rabbits. We hypothesise that this may be the proximate cause of recovery in rabbit populations across Australia and possibly elsewhere.

Nielsen BH, Angelucci A, Scalvenzi A, Forkman B, Fusi F, Tuytens F, Houe H, Blokhuis H, Sorensen JT, Rothmann J, Matthews L, Mounier L, Bertocchi L, Richard MM, Donati M, Nielsen PP, Salini R, De\_Graaf S, Hild S, Messori S, Nielsen SS, Lorenzi V, Boivin X, Thomsen PT

### **Use of animal based measures for the assessment of dairy cow welfare ANIBAM : external scientific report**

Use of animal based measures for the assessment of dairy cow welfare ANIBAM : external scientific report / Bodil Hojlund Nielsen ... [et al.]. - [Parma : EFSA, 2014]. - p 1-340. Disponibile on line <http://www.efsa.europa.eu/it/supporting/doc/659e.pdf> [Nr. Estr. 5810]

The overall aim of the project was to evaluate the use of routinely collected animal based measures (ABMs) for an evaluation of the overall animal welfare in dairy cow herds. ABMs being able to detect worst adverse effects in relation to animal welfare were identified based on the existing literature and expert opinion. The validity and robustness of these ABMs were evaluated and cow mortality, somatic cell count and lameness were selected for further study. A number of factors of variation were selected using expert opinion and used in a model to collate routinely collected data from Italy, Belgium and Denmark on selected ABMs. The routinely collected data was uploaded to the Data Collection Framework platform at EFSA and the data management in this process was evaluated. Five research datasets from Italy, Belgium, Denmark and France including information on ABMs as well as a measure of 'overall animal welfare' at herd level were analysed to evaluate the association between the ABMs (individually or in combination) and overall welfare. The measure of 'overall animal welfare' were not the same for all datasets. Except from the Italian data, the association between the ABMs and the different overall welfare measures were generally weak. Likewise, combining more than one ABM only improved the prediction of the overall welfare in the Italian dataset. Analyses of the other datasets could not confirm this finding. Finally, suggestions for future recordings of ABMs not routinely collected at the moment were given with a special focus on lameness. In conclusion, the relationship between selected ABMs and overall welfare at the herd level is complex and still not sufficiently studied. Therefore, a system using routinely collected ABMs to predict the overall welfare at herd level in dairy herds does not seem realistic based on the results from the present study.

Nigrelli AD, Rosignoli C, Faccini S, Gamba F, Franzini G, Sarli G

**Focolai di infezione da virus dell'encefalomiocardite (EMCV) in tre allevamenti da riproduzione appartenenti alla stessa azienda di produzione suinicola** = Outbreaks of encephalomyocarditis virus (EMCV) infection in three breeding herds of the same pig production farm

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 269-278. - 27 bib ref [Nr. Estr. 5670]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

Nel presente lavoro vengono riportati i dati relativi a focolai di mortalità improvvisa in suinetti in lattazione e aumento di nati-morti e feti mummificati al parto, comparsi contemporaneamente in tre allevamenti da riproduzione (A, B e C), appartenenti allo stessa azienda suinicola. Le tre unità produttive, due nella provincia di Mantova e una nella provincia di Brescia, erano dislocate a più di 20 Km di distanza l'una dall'altra. In un allevamento (A) la mortalità ha coinvolto anche il reparto delle scrofette da rimonta, nella fase di peso vivo compresa tra i 40 e i 70 Kg. Le indagini di laboratorio effettuate su suini e feti colpiti dalla patologia hanno evidenziato la presenza di una infezione da virus dell'encefalomiocardite (EMCV). EMCV è stato rilevato mediante RT-PCR in campioni di tessuto cardiaco delle diverse categorie di animali. La diagnosi è stata confermata dalla presenza di caratteristiche lesioni istopatologiche miocardiche. La contemporaneità dell'insorgenza dei focolai di infezione nei 3 allevamenti rende plausibile l'ipotesi di una trasmissione dell'EMCV dal sito produttivo A agli altri due (B e C) mediante il trasferimento delle scrofette da rimonta. La stima delle perdite globali causate dalle infezioni da EMCV, calcolata sullo scostamento rispetto agli indici aziendali di performance riferiti ai 4 mesi che hanno preceduto i focolai, si è attestata su 1.295 feti, 569 suinetti lattanti e 42 scrofette da rimonta. Il danno economico stimato complessivo ha superato i 76.000 euro.

*The present work shows data related to outbreaks, characterized by sudden death in lactating piglets and increased stillbirth-rate and mummified fetuses, appeared simultaneously in three breeding herds (A, B and C) of the same production farm, placed at more than 20 Km of distance. In one herd (A), mortality involved also the replacement gilts, at 40 -70 kg of live weight. Laboratory investigations, carried out on pigs and fetuses affected by the disease, showed the presence of encephalomyocarditis virus (EMCV) infection. The virus has been detected by RT-PCR on samples of heart tissue of different animal categories (fetuses, piglets, gilts). The diagnosis was confirmed by the presence of characteristic myocardial histopathological lesions. The simultaneous onset of the outbreaks of infection in all the production units makes plausible the hypothesis of a EMCV transmission from the herd A to the others (B and C) through the transfer of replacement gilts. The estimated losses due these EMCV infections outbreaks, calculated on the deviation of the performance data from those of the previous four months, amounted to 1.295 fetuses, 569 piglets and 42 replacement gilts, for an economic damage exceeding 76.000 euro.*

Nogaro C, Bertolotti L, De\_Carlo E, Masoero L, Caruso C, Profiti M, Martucciello A, Galiero G, Cordioli P, Lelli D, Nardelli S, Ingra valle F, Rosati S

**Expression and antigenic characterization of bubaline herpesvirus 1 (BuHV1) glycoprotein E and its potential application in the epidemiology and control of alphaherpesvirus infections in Mediterranean water buffalo**

J Virol Methods. - Vol. 207 ( 2014). - p 16-21. - 19 bib ref [Nr. Estr. 5790]

Bubaline herpesvirus 1 (BuHV1) is a member of ruminant alphaherpesviruses antigenically related to bovine herpesvirus 1 (BoHV1). The impact of BuHV1 infection in infectious bovine rhinotracheitis control program is difficult to establish, due to the lack of specific diagnostic test. The ectodomain of glycoprotein E of BuHV1 was expressed as recombinant secreted protein and used in indirect ELISA as well as in a discriminatory test using the BoHV1 counterpart. A panel of monoclonal antibodies was produced against BuHV1; 6 out of 7 anti-gE monoclonal antibodies specifically recognized the BuHV1 gE. Results indicated BuHV1 gE as a sensitive marker of infection compared to seroneutralization (SN) test or blocking ELISA. When BoHV1 and BuHV1 gEs were immobilized in different wells of the same ELISA microplate, bovine and water buffalo sera were more reactive against the respective infecting virus. About one third of seropositive buffaloes with no history of contact with cattle and having higher SN titres, reacted in BoHV1 gE blocking ELISA, possibly

because of steric hindrance. Since in two occasions BuHV1 was also isolated from water buffalo scoring gB+/gE+ BoHV1 blocking ELISA, we conclude that the combination of the two blocking ELISAs is not suitable to differentiate between BoHV1 and BuHV1.

Pacciarini<sup>o</sup>ML, Loda<sup>o</sup>D, Zanoni<sup>o</sup>M, D'Incau<sup>o</sup>M, Tagliabue<sup>o</sup>S, Boniotti<sup>o</sup>MB

### **Clustering and geographical distribution of Mycobacterium caprae genotypes isolated from Bovine tuberculosis outbreaks in Italy**

VI International M. bovis conference : Cardiff, Wales, Great Britain, 16th - 19th June 2014 / [s.l. : s.n., 2014]. - p 111 [Nr. Estr. 5898]

International M. bovis conference (6th : Cardiff, Wales : 16th - 19th June 2014)

**Introduction** M. caprae is a neo-species of Mycobacterium tuberculosis complex that can cause bovine tuberculosis (bTB). In Europe M. caprae infections are present in central continental countries with different distribution in cattle, goats and wildlife. In Italy the presence of M. caprae has been found in livestock since 2000. In this study we describe the genetic characterisation of M. caprae strains isolated in Italy and the geographical distribution of the main significant genotypes. **Materials and methods** The study included 388 M. caprae strains isolated from 228 bTB outbreaks in cattle and buffalo from 2000 to 2013. Isolates were characterised by spoligotyping and Variable Number Tandem Repeat (VNTR) analysis including 5 ETR loci (A-E) and 6 additional markers: VNTR2163a, VNTR2163b, VNTR2996, VNTR3155, VNTR4052, VNTR1895. **Results** In Italy M. caprae is present in 12 spoligotypes. The most common are SB0415, SB0416 and SB0866 localised respectively in Sardinia, Po Valley, South Italy, and SB0418 isolated either in North or South Italy which represents the 81% of M. caprae infected herds. Additional typing with VNTR markers differentiates >SB0418 into 50 genotypes. The 5 most representatives have a specific geographical localisation. **Discussion and conclusion** M. caprae has been isolated in more than 10% of bTB positive herds in Italy. The most frequent genotypes show an evident geographical clustering. Epidemiological investigation and genotyping of two clusters widespread in North Italy supports the hypothesis that a portion of M. caprae population originates from recent introduction of infected animals from central Europe.

Pampiglione G, Scaravelli D, Fiorentini<sup>o</sup>L, Tosi<sup>o</sup>G, Massi<sup>o</sup>P

### **Valutazione dell'efficacia in laboratorio di prodotti a base di bicarbonato di sodio e silice per la lotta al Dermanyssus gallinae**

Atti della Società Italiana di Patologia Aviare (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 187-188. - 3 bib ref [Nr. Estr. 5973]

Convegno annuale Società Italiana Patologia Aviare (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*A laboratory experiment was provided to evaluate efficacy of a commercial formulate of a product based on silica and sodium bicarbonate against Dermanyssus gallinae. The solution at 30% was sprayed in plastic capsules where between 30 and 40 mites were transferred, when product dried, in 3 replicas and in 3 replicas without any treatment. Despite the considerable variability in responses at the day 4 all the treated mites died. The formulation promises good reliability and wide interest as it can be used also in organic farming.*

Papa P, Arrigoni<sup>o</sup>N, Caporali A, Mangili P, Maresca C, Scoccia E, Paniccà M, Corneli S, Di\_Paolo A, D'Avino N, Mazzone P

### **Paratuberculosis seroprevalence in dairy cattle in two regions of Central Italy : Umbria and Marche**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 192 (Poster P-06.16) [Nr. Estr. 5760]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiologic agent of Paratuberculosis (PTB). In Europe the prevalence of infected herds varies from 7% to 55% and in northern Italian regions published data report herd-level apparent prevalence (h-AP) ranging between 48% and 65%. Dairy caule herds from two bordering central Italian regions (Umbria and Marche) were the target populations of investigation; they were evaluated together, being similar in consistence and breeding type. All sera of cattle older than 24 months were collected and analyzed as part of monitoring programmes in 2009-2011. Moreover, the farmers were requested to fill a questionnaire, aimed at evaluate risk factors for MAP spreading in herds. MAP specific antibodies in individual serum samples were tested by IDVet ELISA kit. In total, we tested 10524 subjects, coming from 178 herds out of 395 dairy farms present in the two regions (45%); 94 herds were positive with 52.8% h-AP (LC 95% 47.6%-58%). Positive animals were 492, with 4.7% individual-level AP (LC 95% 4.3%-5.1%). We found significant correlation between housing type and presence of infection in herds: in particular free-stall housing is more closely related to infection than tie-stall housing. Other significative risk factors are related to water supply (water trough vs bowl), management (replacement 20%) and previous PTB cases in last 5 years. The heterogeneity of the various studies (analytical technique, age, productive attitude of tested subjects) makes difficult to compare results obtained in whole Italy. However, Umbria and Marche PTB h-AP can be considered similar to that calculated in other Italian regions. The most significant associations between risk factors and the presence of infection in the herd are the type of housing and watering. The high level of h-AP suggests in the future the application of guidelines for PTB control and certification, recently approved at a National level.

Papetti°A, Loda°D, Pacciarini°ML, Boniotti°MB

**Molecular identification of species within the *Mycobacterium tuberculosis* complex by high resolution melting analysis**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 99 - 4 bib ref [Nr. Estr. 5887]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

Pautasso A, Giorda F, Iulini B, Píntore MD, Petrella A, Denurra D, Toffan A, Sholl F, Terracciano G, Latini M, Ferri N, Caracappa S, Rubini°S, Mazzariol S, Di\_Guardo G, Ballardini M, Mignone W, Casalone C

**Italian diagnostic network on cetaceans strandings : a national monitoring program to investigate the causes of death**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 54 - 5 bib ref [Nr. Estr. 5862]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

Pesciaroli M, Alvarez J, Boniotti°MB, Cagiola M, Di\_Marco V, Marianelli C, Pacciarini°M, Pasquali P

**Tuberculosis in domestic animal species**

Res Vet Sci. - Vol. 97 Suppl ( 2014). - p s78-s85. - 126 bib ref

([http://ac.els-cdn.com/S0034528814001623/1-s2.0-S0034528814001623-main.pdf?\\_tid=03747f92-4d2f-11e4-bc67-00000aacb35f&acdnt=1412582684\\_2f7bfc8048f8b89446ed0d8fa2cf654f](http://ac.els-cdn.com/S0034528814001623/1-s2.0-S0034528814001623-main.pdf?_tid=03747f92-4d2f-11e4-bc67-00000aacb35f&acdnt=1412582684_2f7bfc8048f8b89446ed0d8fa2cf654f)) [Nr. Estr. 5871]

*M. bovis* and *M. caprae*, members of the *Mycobacterium tuberculosis* complex (MTC), are the major causative agents of tuberculosis in domestic animals. Notably, *M. bovis* exhibits a wide host range; the infection has been reported in many domesticated animals and free or captive wildlife. Despite most of them acting as spill-over hosts in particular epidemiological scenarios, some domesticated species as pigs, camelids and goats may display high rates of infection and possibly play a role in the inter-species transmission of the disease. The aim of this review is to make an updated overview of the susceptibility and the role in the transmission of the disease of the most common domesticated animals species such as small ruminants, pigs, horses, camelids, dogs and cats. An overview of the diagnostic approaches to detect the infection in each of the species included in the review is also presented.

Pezzoni°G, Caminiti°A, Stercoli°L, Galletti°G, Brocchi°E

### **Comparazione di tre saggi ELISA home-made per la rilevazione di anticorpi anti-HEV nei suini in condizioni di campo**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 76 [Nr. Estr. 5746]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

Il virus dell'Epatite E (HEV) è responsabile di epatiti acute nell'uomo. Il genoma è costituito da un filamento di RNA positivo ed è organizzato in tre Open Reading Frame (ORF). La ORF2, che codifica per la proteina capsidica, è considerata la maggior componente immunogena del virus. Sono noti quattro genotipi, di cui il 3 ed il 4 sono stati isolati dall'uomo, dal suino e da altre specie animali. L'infezione da HEV è una zoonosi, e vi sono numerose evidenze di trasmissione attraverso l'assunzione di carni crude contaminate. Lo scopo del nostro studio è stato lo sviluppo ed il confronto delle performance di tre ELISA home-made per la rilevazione di anticorpi anti-HEV nei suini. I tre saggi fanno uso di Anticorpi Monoclonali (AcM) ed antigeni ricombinanti. L'ELISA 1 e 2 sono di tipo indiretto, ed entrambe utilizzano l'AcM anti-IgG suine 4B6, marcato con perossidasi, per il rilevamento degli anticorpi suini. L'ELISA 3 è di tipo competitivo ed utilizza l'AcM anti-HEV 4E12 come competitore dei sieri in esame per il legame all'antigene: per questa caratteristica l'ELISA 3 rileva anticorpi anti-HEV indipendentemente dalla specie. Rispetto agli antigeni, nell'ELISA 1 è utilizzato l'antigene ricombinante corrispondente alla porzione C-terminale 394aa-660aa della ORF2 prodotto in *E.coli*, direttamente adsorbito, mentre nell'ELISA 2 e 3 la porzione della ORF2 112aa-608aa espressa in baculovirus è catturata dall'AcM anti-HEV 4E12. Sono stati esaminati un totale di 779 sieri di suini allevati in 27 aziende. Data l'indisponibilità di un gold standard, l'accuratezza dei saggi è stata valutata mediante un approccio Bayesiano a classi latenti ed una regressione logistica ad effetti misti (per considerare l'effetto dovuto al raggruppamento dei suini negli allevamenti). I risultati hanno mostrato che i tre test hanno un valore diagnostico simile in termini di sensibilità (mediana della distribuzione a posteriori dall'89% al 94%, ed intervalli di credibilità al 95% (IC 95%) tutti sovrapposti). In termini di specificità, l'ELISA 1 è risultata di poco superiore all'ELISA 3 (mediana ELISA 1: 99%, IC 95%: 98-100%; ELISA 3: 90%, 95%, IC 95%: 86-94%). Tuttavia, questa differenza potrebbe essere dovuta al più ampio spettro di anticorpi (IgG ed IgM) che l'ELISA 3 può rilevare. I nostri risultati suggeriscono che i tre saggi sono strumenti utili per la rilevazione e la gestione dell'infezione da HEV in campo. Tutti i test ELISA sviluppati beneficiano dell'utilizzo di reagenti riproducibili (MAbs e antigeni ricombinanti) i quali rendono le analisi altamente standardizzabili.

Pezzoni°G, Caminiti°A, Stercoli°L, Grazioli°S, Galletti°G, Santi°A, Tamba°M,

Brocchi<sup>°</sup>E

**Comparison of three in-house ELISAs for the detection of hepatitis E virus infection in pigs under field conditions**

J Virol Methods. - Vol. 207 ( 2014). - p 95-103. - 58 bib ref [Nr. Estr. 5785]

Hepatitis E virus (HEV) is a RNA non-enveloped virus that comprises four genotypes. The genome of HEV is organized into three Open Reading Frames (ORFs), and the ORF2 is responsible for encoding capsid proteins. HEV can infect a wide range of hosts, and pigs are considered the main reservoir. HEV infection is considered a zoonosis and it is responsible for acute hepatitis in humans, especially in developing countries. The development of a blocking ELISA would be of high value for screening purpose, because there is no need of species specific reagents. The present study was conducted to assess three in-house ELISAs for the detection of HEV infection in 779 sera collected from breeding and fattening farms under field conditions. Two assays were indirect ELISAs, while the third was a blocking ELISA. Two different recombinant antigens were generated from specific sequences of the HEV-ORF2, and a Latent Class approach in a Bayesian framework was used to evaluate the diagnostic accuracy of each ELISA. Because the three ELISAs cannot be thought of as independent, all possible dependence structures were modelled starting from the general case of conditional independence to the most complex situation of three mutually dependent assays. Results showed that none of the three ELISAs was significantly superior to the others in terms of sensitivity (posterior median value ranging from 89% to 94%, all 95% posterior credible intervals (95%PCI) overlapped). In terms of specificity, one of the indirect ELISAs was superior to blocking ELISA (posterior median indirect ELISA: 99%, 95%PCI: 98–100%; blocking ELISA: 90%; 95%PCI: 86–94%). However, this difference could be due to the potential wider spectrum of antibodies that blocking ELISA can actually detect.

Piralla A, Moreno<sup>°</sup>A, Orlandi ME, Percivalle E, Chi apponi<sup>°</sup>C, Foni<sup>°</sup>E, Luini<sup>°</sup>M, Baldanti F

**Swine influenza H3N2 virus infection in an immunocompromised adult patient, Italy 2014**

17th Annual Meeting of European Society for Clinical Virology : Prague 28/9-1/10 2014 : proceedings / [s.l. : s.n., 2014]. - 1 p (ID 162). - bib ref [Nr. Estr. 5960]

Annual Meeting of European Society for Clinical Virology (17th : Prague : 28/9-1/10 - 2014)

Three principal different subtypes of Influenza A virus are circulating in the Italian pig population: H1N1, H3N2, H1N2. H1N1pdm virus and various reassortant strains have also been detected. A current insight into the epidemiology of swine influenza virus (SIV) in Italian pig farms was considered appropriate because of the antigenic and genetic variability of SIV. In 2011, 2012 and 2013 year a passive virological surveillance in the Northern Italy area pig population was conducted and, respectively 1039, 1186 and 1001 lung samples and nasal swabs from respiratory disease outbreaks in pig farms were collected. We tested clinical samples for influenza A virus by a real-time RT-PCR test targeting the M gene and, for virus subtyping, by Multiplex RT-PCR test. In order to perform SIV isolation, PCR positive samples were inoculated onto various substrates. Antigenic characterization was performed by HI test using optimized protocols and reagents. A total of 3206 clinical samples were tested using real-time RT-PCR. We obtained 146 (14%), 133 (11,4%) and 136 (13,5%) SIV positive cases, from samples collected in 2011, 2012, 2013 respectively. Virological examination performed on the PCR positive samples led to the isolation respectively of 83, 66, and 70 SIVs. Subtype H1N1 was isolated at stable rate during the considered period: 46,9%, 51,5% and 47,1%. H1N1pdm SIV rate raised in 2013 (10%) compared to results obtained in 2011 (4,8%) and 2012 (3%). In 2013 it was recorded a lower circulation rate of H1N2 subtype (7,14%) compared to 2011 and 2012 (26,5% and 28,7% while, in the same year, H3N2 subtype was isolated with higher rate (28,5%) compared to 2011 (18%) and to 2012 (13,6%). In 6 clinical samples (2,6%) it was possible to reveal, by Multiplex RT-PCR test, that two different SIV subtypes were present contemporary in the same sample. Moreover the Multiplex RT-PCR detected also the circulation of 1% of H1N1 reassortant strains (human-like HA), 3,1% of H1N2 reassortant strains (avian-like HA). Only a H1N1pdm reassortant strain was identified in the period. The study highlights that H3N2 SIV

subType seems to circulate more frequently in Italian pig farms recently and that 10% reassortment cases occurred among the SIV lineages. Moreover 2,6% rate of SIV mixed infection was recorded. These data are indicative to persist in SIV surveillance and even to master the studies in genetic characterization of SIVs to trace the genetic changes of SIVs in a so evolving epidemiological picture.

Polloni<sup>o</sup>A, Rota\_Nodari<sup>o</sup>S, Giacomelli<sup>o</sup>S, Archetti<sup>o</sup>L

#### **Lactate dehydrogenase isoenzymes in cows with downer syndrome**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 273 - 3 bib ref [Nr. Estr. 6020]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15, 2014)

Pregel P, Scaglione FE, Ruggeri<sup>o</sup>J, Pesciaroli M, Gaetarelli<sup>o</sup>B, Ammendola S, Battistoni A, Bollo E, Alborali<sup>o</sup>GL, Pasquali P

#### **Parenteral and oral administration of an attenuated Salmonella typhimurium vaccine in piglets : histopathological findings**

LXVIII Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : Convegno SICV : XI Convegno AIPVet : XII Convegno SIRA16-18 Giugno 2014 Pisa / [s.l. : s.n., 2014]. - p 42. - 2 bib ref [Nr. Estr. 5990]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 68 Convegno SICV Convegno AIPVet : 11 Convegno SIRA : 12 : Pisa : 16-18 Giugno 2014)

Salmonellosis is the first cause of gastroenteric diseases in humans, mostly due to contamination of pork products (Boyen et al., 2008). The use of vaccines in pigs could represent a valid method to minimize the spread of Salmonella spp. in the environment (Rostagno, 2011). Aim of this work was to compare the effects on the intestinal mucosa of *S. Typhimurium* znuABC when administered orally or parenterally in piglets. Histopathological characterization was then performed, in order to evaluate the impact of vaccination and infection on intestinal tracts. Twenty-five Salmonella-free weaned piglets were divided into 4 groups: Group A (5 piglets) was intramuscularly vaccinated with  $10^4$  CFU of *S. Typhimurium* strain znuABC (an isogenic mutant strain); groups B (5 piglets) and C (4 piglets) were intragastrically vaccinated with  $5 \times 10^7$  and  $5 \times 10^5$  CFU of *S. Typhimurium* strain znuABC, respectively; group D (11 piglets) was used as control. Six weeks after vaccination, five naïve piglets of group D were challenged with  $4 \times 10^8$  CFU of wild type *S. Typhimurium* ATCC 14028, and all the groups were allocated in the same barn for two weeks to allow the contact of vaccinated and naïve animals with the shedder ones. Piglets were euthanized four weeks. Samples of ileocecal lymph nodes, ileum, caecum and colon were collected and submitted to histological analyses. A numerical value based on the degree of lesions was assigned to each examined intestinal section, taking into account epithelium, submucosa and Peyer's patch conditions, congestion and lesion patterns. Histologically, naïve piglets showed the most severe lesions in all the intestinal tracts. Epithelial conglutination and necrosis were associated to vascular congestion and lymph nodes depletion. Group C, being the most affected of the vaccinated groups, showed microscopical lesions similar to those observed in naïve piglets. Conglutination was present in 94.4% of examined tracts in group D and in 50.0% in group C, whereas only 26.7% and 20.0% of intestinal tracts of group A and B were involved ( $p < 0.0001$ ). Group A and B showed a milder degree of lymphocytic and eosinophilic inflammation. The infection, acquired by direct contact with piglets shedding wild type *S. Typhimurium*, showed marked differences with the pattern observed in animals infected by oral challenge, revealing a clear involvement of the ileum, with evident signs of reactivity. When wild type *S. Typhimurium* is intragastrically administered, a high number of salmonellae reaches the gut and invades the mucosa of the colon and the cecum in a very limited period of time. At the opposite, when piglets are infected by a continuous contact with *S. Typhimurium* released by shedding piglets, they are exposed to a reduced number of salmonellae,

but for a protracted period of time. In such condition, the ileum represents a preferential route of Salmonella entry.

Prosperi°A, Gallina L, Casà G, Savini F, Chiari°M , Alborali°GL, Scagliarini A, Lavazza°A

#### **Caratterizzazione di Fringilla coelebs papillomavirus identificato in Italia**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 78 [Nr. Estr. 5747]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

Il Fringilla coelebs Papillomavirus (FcPV) appartiene alla famiglia delle Papillomaviridae, che comprende diverse specie in grado di infettare sia l'uomo che gli animali, dando luogo a lesioni epiteliali proliferative, solitamente autolimitanti e che spesso esitano in regressione spontanea. Ad oggi sono state isolate solo quattro specie di papillomavirus aviari: Fringilla coelebs papillomavirus (FcPV), Psittacus erithacus papillomavirus (PePV), Francolinus leucoscepus papillomavirus (FIPV) e il non ancora classificato Pygoscelis adeliae papillomavirus 1 (PaCV1). I papillomavirus hanno virioni sferici, con diametro tra 55 e 60 nm, privi di envelope e presentano morfologia costante indipendentemente dal sito e dal tipo delle lesioni. Sono caratterizzati da un genoma circolare dsDNA di circa 8.000 bp, che nei papillomavirus aviari codifica per sei proteine precoci (E1, E2, E4, E6, E7, E9) e due tardive (L1, L2). L'incidenza di questa malattia è solitamente abbastanza ridotta (1,3% nella popolazione), anche se, quando entra in un gruppo, tende ad infettare la maggior parte dei soggetti. Dal punto di vista clinico le lesioni cutanee indotte da FcPV si presentano come papillomi squamosi, prevalentemente a carico del piede e del tarso-metatarso. Le prime segnalazioni di lesioni riferibili a papillomavirus in fringuelli e peppole risalgono al 1969, ma ad oggi sono disponibili un numero limitato di dati relativi al genoma di questi virus ed in particolare non vi sono segnalazioni in bibliografia riguardo ceppi responsabili di infezioni in Italia. Il nostro lavoro, svolto partendo da campioni di fringuelli e peppole pervenuti al servizio di diagnostica dell'IZSLER, è stato incentrato sulla ricerca di particelle virali riferibili a papillomavirus mediante microscopia elettronica e sulla successiva caratterizzazione del genoma virale. Il DNA genomico circolare di FcPV è stato amplificato mediante RCA (Rolling Circle Amplification) ed in seguito analizzato mediante taglio enzimatico. I frammenti ottenuti sono stati in seguito clonati e quindi sequenziati. La regione che comprende i geni E1, E2 ed E7, del FcPV isolato in Italia, ha mostrato un'elevata percentuale d'identità rispetto all'unica sequenza disponibile in banca dati e riferita ad un ceppo virale isolato in USA.

Razzuoli E, Villa°R, Ferrari A, Amadori°M

#### **A pig tonsil cell culture model for evaluating oral, low-dose IFN-alfa treatments**

Vet Immunol Immunopathol. - Vol. 160 ( 2014). - p 244-254. - 44 bib ref [Nr. Estr. 5784]

Oral, low-dose IFN- $\alpha$  treatments proved effective in several models of viral infections and immunopathological conditions. Also, they do not give rise to the serious side effects observed after parenteral inoculation of high doses (105 U/kg b.w. and higher). There is convincing evidence that such treatments work through an early, effective interaction with oral lymphoid tissues before the IFN- $\alpha$  molecules are rapidly destroyed by gut enzymes. Yet, the paucity of detailed information about these crucial interactions and the lack of recognized in vitro models hamper the development of proper administration protocols. On the basis of a previous study, we developed an in vitro model of interaction between different types of human and porcine IFNs- $\alpha$  at low/moderate concentrations and pig tonsil cells. The IFNs- $\alpha$  under study showed different properties with respect to three fundamental control actions: (1) IgA release in culture, (2) release of natural antimicrobial compounds, and (3) homeostatic regulation of the inflammatory response. This was checked in pig

intestinal epithelial cells (IPEC-J2 cell line) treated with supernatants of control and IFN  $\alpha$ -treated tonsil cell cultures, respectively, in terms of inflammatory cytokine and chemokine responses. Some IFNs- $\alpha$  caused a significant inhibition of IL-8 (protein release and gene expression) and beta-defensin 1 (gene expression) probably through second messengers released by IFN  $\alpha$ -treated tonsil cells. Interestingly, a human lymphoblastoid IFN- $\alpha$  under study caused the decrease of polyclonal IgA release by pig tonsil cells and significantly stimulated the in vitro recall antibody response of swine PBMC to Foot-and-Mouth Disease virus. The modulation of IgA and antibacterial compounds was accompanied by an anti-inflammatory control action at the same, low to moderate IFN- $\alpha$  concentrations (1–100 U/ml). This highlights the very foundation of the homeostatic control actions performed by Type I IFNs: to promote an effective host response to infectious and non-infectious stressors and to turn off noxious inflammatory responses associated with tissue damage and waste of metabolic energy. The described tonsil cell model in vitro can be conducive to a further development of oral cytokine treatments in humans and animals in the “one health” conceptual framework.

Rizzo F, Mignone W, Niosi G, Orusa R, Vicari<sup>o</sup>N, Pe letto S, Cerutti F, Monaco F, Savini G, Mandola ML

#### **Agenti patogeni emergenti in volatili selvatici : riscontri nel territorio ligure**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 83 [Nr. Estr. 5776]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

La Regione Liguria, affacciata sul Mar Tirreno, è una zona a rischio elevato per l'introduzione, da parte di volatili migratori, di agenti zoonotici emergenti, sia di natura virale che batterica. Nel corso della migrazione autunnale, uccelli acquatici migratori a lungo raggio, a partire dalla Finlandia e lungo le direttrici di volo del Paleartico occidentale, attraversano Paesi Bassi, nord ovest Italia e Sardegna, fino a raggiungere le aree di svernamento del Nord Africa; nel periodo primaverile si assiste, invece, ad una inversione del flusso migratorio a partire dalle aree africane. In entrambi i casi, le coste liguri rappresentano, per molti di essi, aree di rifugio e sosta temporanea. Il presente lavoro espone i risultati della sorveglianza passiva realizzata in Liguria, con la collaborazione dei Servizi Veterinari della Provincia di Imperia, su volatili selvatici trovati malati, feriti o morti dal 2011 a oggi. Nel corso dello studio 188 esemplari, appartenenti a quindici Ordini aviari, sono stati raccolti e sottoposti a visita autoptica e analisi biomolecolari. Gli acidi nucleici estratti dai tessuti, quali Sistema Nervoso Centrale (SNC), cuore, fegato, reni, polmone e intestino, sono stati amplificati con metodiche real-time PCR per la ricerca di Influenza tipo A, Newcastle virus e Chlamydiaceae. Al fine di rilevare eventuali infezioni da flavivirus nei volatili, è stata utilizzata una PCR classica specifica per una porzione della regione NS5 di Flavivirus. Sono state riscontrate positività per C. psittaci in una tortora e per clamidia atipica in gabbiani reali e fenicottero; un altro gabbiano reale è risultato infetto dal sottotipo influenzale aviario H13N2. I virus influenzali a bassa patogenicità sono comuni in molte specie di Caradriformi, mentre, per quanto ne sappiamo, questo è il primo riscontro di clamidie atipiche in gabbiano reale e fenicottero. Nel mese di maggio 2013 un lodolaio (Falco subbuteo), specie migratrice a lungo raggio, viene trovato morente in territorio collinare in Provincia di Imperia; l'animale muore poco dopo presso il Centro di Recupero di Sanremo. L'autopsia evidenzia cachessia e edemi discrasici. Le analisi di sequenza del frammento NS5 di Flavivirus, ottenuto in PCR, rilevano la presenza di materiale genetico riconducibile a West Nile virus (WNV), Lineage 1, nel rene e nel SNC. La successiva analisi filogeografica, realizzata su un frammento di 400 bp del polyprotein gene di WNV, ha collocato il ceppo nel Kenyan/Western Mediterranean clade, comprendente la maggior parte delle sequenze dell'Europa occidentale e Nord Africana. Il rilevamento di WNV nei tessuti del lodolaio conferma l'importante ruolo giocato dagli uccelli migratori nella diffusione di WNV e, più in generale, dei flavivirus in Europa e nel bacino del mediterraneo. L'Italia, rifugio e sosta temporanea di molte specie di uccelli migratori, ne risulta esposta in maniera continuativa.

Rizzo F, Mignone W, Niosi G, Orusa R, Vicari°N, Pe letto S, Cerutti F, Savini G, Mandola ML

### **Surveillance of wild birds infections in Liguria, Italy**

8th Annual Epizone Meeting "Primed for tomorrow" : 23 - 25 September 2014 Copenhagen, Denmark : poster "Emerging diseases" / [s.l. : s.n., 2014]. - 1 p ( EMER08 ) [Nr. Estr. 5896]  
Annual meeting Epizone (8th : Copenhagen, Denmark : 23 - 25 September 2014)

The Liguria region in Northwestern Italy, facing the Ligurian sea, represents a risk area for the introduction of zoonotic infections such as Avian Influenza, Newcastle disease (ND), West Nile disease and avian chlamydiosis, being located along flyways used by migrating waterfowl to cross the Western Palearctic. During spring and autumn migration, birds stop to feed and rest by Italian coasts, before undertaking the flight to Northern Europe or Africa according the flyways. Herein, we present the results of the passive surveillance on wild birds found dead, ill or injured in Liguria region, in collaboration with the Veterinary authority of the Imperia province, from January 2011 onwards. During the study a total of 188 wild birds, belonging to fifteen avian Orders, were collected and underwent autopsy examination. Tissue samples (CNS, lung, kidney, heart, liver, spleen and intestine) were tested for Influenza type A and Newcastle disease virus by real-time RT-PCR assays targeting the matrix genes and for avian chlamydiosis by real-time PCR a 16S rRNA real-time PCR assay. In order to monitor arboviral infections eventually harboured by wild birds, all specimens were checked also with an end point PCR protocol specific for a tract of the NS5 gene of the Flavivirus Genus. Positivities for a not yet classified species of Chlamydiaceae have been found in two yellow-legged gulls and one flamingo, for *C. psittaci* in a turtle dove and another yellow-legged gull was found infected with H13N2 strain of Influenza A virus. Low pathogenic avian Influenza viruses are seasonally common in many Charadriiformes species, while, to our knowledge, these are the first findings of the new chlamydial agent in yellow-legged gull. In May 2013, an Eurasian hobby, dead at the rehabilitation centre of Sanremo and showing cachexy and discrasic edemas at the autopsy examination, resulted infected at the kidney and CNS level with a Lineage 1 strain of the West Nile virus. By the phylogeographic analyses, this strain clustered in the Kenyan/western mediterranean clade, which comprises the majority of western European sequences. In particular the sequence is located in the subclade A', together with other West Nile sequences from Italy and Morocco. This evidence may suggest a re-introduction of the virus in the Italian territory from Northern Africa, considering the migratory habits of the Eurasian hobby, which is used to stop along the Italian west coasts for feeding, during its northward migration from Africa in spring.

Rizzoli A, Bolzoni°L, Cagnacci F, Hauffe HC, Neteler M, Tagliapietra V, Rosà R

### **Global changes and wildlife zoonotic disease emergence : the case of tick-borne encephalitis**

Hystrix Ital J Mammalogy. - Vol. 25 (supplement) ( 2014). - p 59 [Nr. Estr. 5921]

Congresso Italiano di Teriologia (9. : Civitella Alfedena (AQ) : 7-10 Maggio 2014)

Of all the known zoonotic tick borne diseases, tick borne encephalitis caused by TBE virus (TBEV) is the most common tick borne disease transmitted to humans in Europe and eastern and central Asia. It is now endemic in 27 European countries, and has been declared an international public health problem. Since the virus is also transmissible through raw milk and dairy products of infected goats, sheep or cattle TBEV has the potential to make a significant impact on food security and regional economy, especially in areas using traditional methods of milk collection and processing and the use of un-pasteurised milk for the production of typical local dairy products. We analysed pattern of TBE emergence in northern Italy combining eco-epidemiological long term and extensive surveys. Major drivers of disease emergence were identified in changes in forest management and the rise of ungulate population. Spatial and temporal variation in infection risk is driven by the interaction of several factors, including local variation of tick host abundance. Although significant progress have been made in our understanding of TBEV ecology, several other factors need a better understanding to improve our ability to predict how the risk of TBE infection would change in

the near future under a global change scenario.

Rodríguez-Campos S, Smith NH, Boniotti<sup>o</sup>MB, Aranaz A

**Overview and phylogeny of Mycobacterium tuberculosis complex organisms : implications for diagnostics and legislation of bovine tuberculosis**

Res Vet Sci. - Vol. 97 Suppl ( 2014). - p s5-s19. - 278 bib ref [Nr. Estr. 5870]

Members of the Mycobacterium tuberculosis complex (MTBC) cause a serious disease with similar pathology, tuberculosis; in this review, bovine tuberculosis will be considered as disease caused by any member of the MTBC in bovids. Bovine tuberculosis is responsible for significant economic loss due to costly eradication programs and trade limitations and poses a threat to both endangered and protected species as well as to public health. We here give an overview on all members of the MTBC, focusing on their isolation from different animal hosts. We also review the recent advances made in elucidating the evolutionary and phylogenetic relationships of members of the MTBC. Because the nomenclature of the MTBC is controversial, its members have been considered species, subspecies or ecotypes, this review discusses the possible implications for diagnostics and the legal consequences of naming of new species.

Rodríguez-Campos S, Smith NH, Boniotti<sup>o</sup>MB, Aranaz A

**Overview and phylogeny of Mycobacterium tuberculosis complex organisms : implications for diagnostics and legislation of bovine tuberculosis**

World Congress on controversies, debates and consensus in veterinary medicine : October 23-25, 2014, Prague, Czech Republic / [s.l. : s.n., 2014]. - p S29 [Nr. Estr. 5916]

World Congress on controversies, debates and consensus in veterinary medicine : Prague, Czech Republic : October 23-25, 2014)

Romano A, Vitale N, Chiavacci L, Rossi F, Zoppi S, Bergagna S, Gennero MS, Varello K, Richelmi G, Acutis PL, Boin C, Arrigoni<sup>o</sup> N, Gorla M

**Assessing the prevalence of paratuberculosis: a cohort study in two friesian farms in North-Western Italy**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 189 (Poster P-06.10) [Nr. Estr. 5563]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

A prospective longitudinal study in 2 Friesian dairy herds in North-western Italy was carried out in order to improve knowledge about incidence of seroconversion and fecal shedding, complemented with study on genetic resistance and susceptibility to Paratuberculosis (PTB). Three hundred cows, previously positive at PTB serological test, were selected and split into 3 different age categories (from 1.5 to 4.5 years). Each animal was tested every 4 months for antibodies in serum (ELISA test), for MAP isolation and detection respectively by culture and PCR on stool; PCR and culture were as well applied to monitor environmental contamination. Slaughtered cattle were also submitted to post mortem tests, collecting stool and target organs (lymph nodes, rectum, cecum, small intestine and ileo-cecal valve) for culture, PCR and histo pathological examinations. A total of 1789 feces (80 of which from environmental samples), 2345 sera and 249 organ samples were tested during our study. In vita tests have been used to classify animals as "PTB positive" or "PTB negative", using a Bayesian algorithm that assesses sensitivity and specificity of combined tests serially to estimate the prevalence. This classification leads to build the base for genetic study on PTB resistance. The overall prevalence in Farm 1 ranged from 55% (CI95%: 33-78%) by PCR to 21% (CI95%: 14-30%) by culture, while in Farm 2 it ranged from 34% (CI95%: 24-44%) by PCR to 17% (CI95%: 10-25%)

by culture. The estimated prevalence calculated on the basis of post mortem test on slaughtered cattle (n=49) was 57.3% (CI95%: 51-64%). Different infecting pressures were observed in the 2 herds and the highest prevalence occurred with the presence of high-shedders. These data highlight how in both farms, even with different management and exposure conditions, environmental contamination is maintained despite progressive removal of PTB positive animals.

Rosà R, Marini G, Bolzoni<sup>o</sup>L, Neteler M, Metz M, De lucchi L, Chadwick EA, Balbo L, Mosca A, Giacobini M, Bertolotti L, Rizzoli A

**Early warning of West Nile virus mosquito vector : climate and land use models successfully explain phenology and abundance of Culex pipiens mosquitoes in north-western Italy**

Parasites & Vectors. - Vol. 7 no 269 ( 2014). - 12 p. - 58 bib ref [Nr. Estr. 5919]

Background West Nile Virus (WNV) is an emerging global health threat. Transmission risk is strongly related to the abundance of mosquito vectors, typically Culex pipiens in Europe. Early-warning predictors of mosquito population dynamics would therefore help guide entomological surveillance and thereby facilitate early warnings of transmission risk. Methods We analysed an 11-year time series (2001 to 2011) of Cx. pipiens mosquito captures from the Piedmont region of north-western Italy to determine the principal drivers of mosquito population dynamics. Linear mixed models were implemented to examine the relationship between Cx. pipiens population dynamics and environmental predictors including temperature, precipitation, Normalized Difference Water Index (NDWI) and the proximity of mosquito traps to urban areas and rice fields. Results Warm temperatures early in the year were associated with an earlier start to the mosquito season and increased season length, and later in the year, with decreased abundance. Early precipitation delayed the start and shortened the length of the mosquito season, but increased total abundance. Conversely, precipitation later in the year was associated with a longer season. Finally, higher NDWI early in the year was associated with an earlier start to the season and increased season length, but was not associated with abundance. Proximity to rice fields predicted higher total abundance when included in some models, but was not a significant predictor of phenology. Proximity to urban areas was not a significant predictor in any of our models. Predicted variations in start of the season and season length ranged from one to three weeks, across the measured range of variables. Predicted mosquito abundance was highly variable, with numbers in excess of 1000 per trap per year when late season temperatures were low (average 21°C) to only 150 when late season temperatures were high (average 30°C). Conclusions Climate data collected early in the year, in conjunction with local land use, can be used to provide early warning of both the timing and magnitude of mosquito outbreaks. This potentially allows targeted mosquito control measures to be implemented, with implications for prevention and control of West Nile Virus and other mosquito borne diseases.

Rosamilia<sup>o</sup>A, Bonilauri<sup>o</sup>P, GherPELLI<sup>o</sup>Y, Rosignoli <sup>o</sup>C, D'Incau<sup>o</sup>M, Nigrelli<sup>o</sup>AD, Rugna<sup>o</sup>G, Maioli<sup>o</sup>G, Biasi<sup>o</sup>G, Dottori<sup>o</sup>M, Carra<sup>o</sup>E , MeriALDI<sup>o</sup>G, Luppi<sup>o</sup>A

**Antimicrobial resistance of Salmonella enterica serovar Typhimurium var. monophasic 4,[5],12:i:- isolated from pigs**

ESPHM 2014 : proceedings 6th European Symposium on Porcine Health Management : 7th-9th May, 2014, Sorrento, Italy / [s.l. : s.n., 2014]. - p 143 (Poster P26) [Nr. Estr. 6070]

European Symposium on Porcine Health Management (ESPHM) (6th : Sorrento, Italy : 7th-9th May, 2014)

Aim of the present study was to provide preliminary data on the antimicrobial resistance of Salmonella enterica serovar Typhimurium var. monophasic (S.4,[5],12:i:-), isolated from pigs. Eighty-six S.4,[5],12:i:- strains belonging to 2 groups were used in the present study. Forty-five strains (group I) were isolated from ileocecal lymph nodes sampled at slaughterhouse while 41 strains (group II) were obtained from samples collected from clinical pigs sent to the laboratory for

diagnostic purpose. The susceptibility of S.4,[5],12:i:- strains to a panel of antimicrobials was tested using a broth microdilution technique (Sensitre TREK). Fourteen antimicrobials agents were tested: ampicillin (A), chloramphenicol (C), streptomycin (S), sulfamethoxazole (Su), tetracycline (T), gentamicin (GEN), florfenicol (FFN), kanamycin (KAN), trimethoprim (TMP), nalidixic acid (NAL), colistin (COL), cefotaxime (FOT), ceftazidime (TAZ), ciprofloxacin (CIP). Isolates were classified as resistant, susceptible or intermediate to antimicrobials in accordance with the break points proposed by the Clinical and Laboratory Standards Institute (2008). Intermediate isolates were grouped with susceptible isolates. The S.4,[5],12:i:- isolates showed resistance to Su (100%), S (100%), A (91.9%), T (89.5%), GEN (53.5%), KAN (43%), NAL (38.4%), C (36%), TMP (33.7%), FFN (33.7%), COL (20.9%), TAZ (13.9%), FOT (6.9%), CIP (5.8%). Multiple resistance was almost entirely due to the circulation of two phenotypes: the tetra-resistant profile ASSuT (54.6%, n=47) and the penta-resistant profile ACSSuT (34.9%, n=30). Additional resistances to GEN (36.2%) and FFN (83.3%) were observed in ASSuT and ACSSuT strains respectively. The resistance to the antimicrobials did not differ significantly between the two groups of S.4,[5],12:i:- isolated (group I and II) except for CIP (0% group I and 12% group II; p<0.01) and NAL (22% group I and 56% group II; p<0.01). The results of this study showed that S.4,[5],12:i:- strains isolated in Italy from pigs were frequently multi-resistant showing mainly an ASSuT profile while a lower percentage of strains had the ACSSuT profile. Noteworthy, the prevalence of S.4,[5],12:i:- with the ASSuT profile significantly increased in the last years among human isolates in Italy, suggesting a possible relationship between swine and human salmonellosis throughout the food chain, which is of interest for epidemiological, animal health and public health purposes.

Rosamilia<sup>°</sup>A, Carra<sup>°</sup>E, Bonilauri<sup>°</sup>P, GherPELLI<sup>°</sup>Y, Corpus<sup>°</sup>F, Morganti<sup>°</sup>M, Rugna<sup>°</sup>G, Rosignoli<sup>°</sup>C, Nigrelli<sup>°</sup>A, D'Incau<sup>°</sup>M, Biasi<sup>°</sup>G, Dottori<sup>°</sup>M, MeriALDI<sup>°</sup>G, Luppi<sup>°</sup>A

**Prevalenza, antibiotico-resistenza e caratterizzazione molecolare di ceppi di Salmonella enterica serovar 4,[5], 12:i:- isolata da suini al macello e da campioni patologici** = Prevalence, antimicrobial resistance and molecular characterization of Salmonella enterica subsp. enterica serovar 4,[5], 12:i:- isolated from slaughtered pigs and pathological samples

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 171-188. - 30 bib ref [Nr. Estr. 5675]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

Da Febbraio a Novembre 2013, 41 partite di suini regolarmente macellati, provenienti da altrettanti allevamenti del Nord Italia sono state campionate attraverso il prelievo di linfonodi ileocecali (30 suini per partita), per un totale di 1230 suini campionati. Salmonella spp. è stata isolata nel 18,78% dei suini macellati e 21 sierotipi sono stati identificati. S. 4,[5],12:i:- è risultato il sierotipo maggiormente isolato (4,47%), seguito dalla S. Rissen (3,25%) e S. Derby (2,68%) mentre S. Typhimurium ha presentato una prevalenza al macello soltanto dello 0,74%. Novantaquattro ceppi di S. 4,[5],12:i:- (55 isolati dal precedente campionamento al macello e 39 isolati da campioni patologici provenienti da 30 allevamenti) sono stati testati per valutare la loro sensibilità nei confronti di 14 antibiotici. Il 90,42% degli isolati è risultato essere multiresistente evidenziando la circolazione di due fenotipi prevalenti: ASSuT (48,9% dei ceppi) ed ACSSuT (41,5% dei ceppi). Due metodiche di genotipizzazione batterica sono state applicate per valutare la diversità genetica dei ceppi di S. 4,[5],12:i:- appartenenti ai due gruppi (macello e allevamento): il metodo Pulsed Field Gel Electrophoresis (PFGE) e il metodo Multi-Locus Variable-Number Tandem-Repeat Analysis (MLVA). La PFGE ha individuato 40 differenti genotipi, mentre la metodica MLVA ne ha individuati 28. Combinando insieme i diversi profili ottenuti dalle due metodiche il potere discriminante aumenta in modo significativo (Indice di Diversità di Simpson 0,97 p<0.001). I risultati del nostro studio confermano l'alta prevalenza di Salmonella spp. nei suini macellati e sottolineano l'elevata incidenza della S. 4,[5],12:i:- che si conferma come sierotipo preponderante. I dati di antibiotico-resistenza ottenuti dai ceppi di S. 4,[5],12:i:- evidenziano la circolazione di due principali profili fenotipici di multi resistenza (ASSuT ed ACSSuT).

*From February to November 2013, 1230 ileocolic lymph nodes were randomly collected from heavy pigs (9-10 months old, 160 kg BW) belonging to 41 batches coming from as many herds. Salmonella*

*spp. was isolated from 231 out of 1230 lymph nodes (18,78% CI95% 16,60%- 20,96%) and 38 batches out of 41 were positive. A total of 21 different serovars of Salmonella were identified. S. 4,[5],12:i:- was the most prevalent serovar (4,47%) followed by S. Rissen (3,25%) and S. Derby (2,68%) while S. Typhimurium showed a prevalence of 0,74%. Fifty-five strains of S. 4,[5],12:i:- isolated during the survey at the slaughterhouse and 39 strains of S. 4,[5],12:i:- isolated from clinical pigs were tested for their susceptibility to 14 antimicrobials. All strains of S. 4,[5],12:i:- showed resistance to at least one antimicrobial and 90,42% of them showed multiresistance (resistance to 4 or more antibiotics). The most common pattern of resistance profile was the ASSuT profile (48,9% of strains) followed by the ACSSuT profile (41,5% of strains). Strains were characterized by Pulsed Field Gel Electrophoresis (PFGE) and Multi-Locus Variable-Number Tandem-Repeat Analysis (MLVA). PFGE analysis generated 40 different profiles while MLVA showed 28 different profiles. Combining the two methods the discriminatory power increased (Simpson's index of diversity 0,97  $p < 0,001$ ). The combination of different molecular methods can be valuable to characterize Salmonella strains and to investigate their epidemiological relationship. The prevalence of Salmonella serovars in this study showed an overall decline of serovar Typhimurium. To some extent this reduction has been counteracted by an increase in prevalence of the emerging serovar 4,[5],12 $\pm$ -, characterized by isolates with multidrug resistance (ASSuT and ACSSuT profiles) as described in several European countries.*

Rosignoli°C, Faccini°S, Marazzotta°E, Franzini° G, Nigrelli°AD

#### **Prevalence of botulinum neurotoxin-producing Clostridia in intestinal samples from slaughtered dairy and beef cattle in Italy**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 110 - 5 bib ref [Nr. Estr. 6060]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15,2014)

Rosignoli°C, Franzini°G, Sandonà°I, Faccini°S, Nigrelli°AD

#### **Resistenza agli antimicrobici in ceppi di Staphylococcus aureus isolati da mastiti bovine cliniche e subcliniche dal 2004 al 2013**

Buiatria. - Vol. 2014). - 5 p. - 6 bib ref [Nr. Estr. 6062]

Lo scopo del presente studio retrospettivo è stato quello di rilevare il tasso di resistenza agli antimicrobici di ceppi di S. aureus isolati da latte di bovine con mastiti cliniche o subcliniche e di valutarne il trend in un periodo di 10 anni, dal 2004 al 2013. Dall'analisi complessiva dei dati la proporzione dei ceppi non sensibili è risultata pari al 47,8% per penicillina G, 45,2% per tetraciclina, 45,2% per amoxicillina, 19,8% per enrofloxacin, 19,4% per danofloxacin, 12,3% per marbofloxacin, 7,7% per amoxicillina/acido clavulanico, 7,6% per tilosina, 5,8% per cloxacillina, 4,4% per cefapirina, 3,5% per cefquinome e 3,1% per rifaximina. Il trend del tasso di resistenza di S. aureus nei 10 anni presi in esame si è dimostrato in crescita per 10 antimicrobici su 12 esaminati. Solo nei confronti di cefapirina e rifaximina la proporzione di ceppi non sensibili è rimasta statisticamente stabile. I risultati del presente studio sottolineano l'importanza di un continuo monitoraggio della sensibilità agli antimicrobici dei ceppi di S. aureus isolati da casi di mastite nella bovina da latte. Le informazioni che ne derivano sono infatti necessarie per una scelta ragionata del farmaco volta ad una maggior efficacia del trattamento sull'animale e ad una riduzione del rischio di sviluppo di resistenze agli antimicrobici.

Rosignoli°C, Nigrelli°A

#### **Indagini diagnostiche sulla mortalità in stalla di bovine adulte da latte**

Mantovalleva & produce magazine. - Vol. 10 no 3 ( 2014). - p 32-37 [Nr. Estr. 6059]

Rosignoli°C, Sandonà°I, Franzini°G, Faccini°S, Nigrelli°AD

**Indagine sulle cause di morte nelle bovine adulte di allevamenti da latte**

Buiatria. - Vol. 2014). - 7 p. - 5 bib ref [Nr. Estr. 6061]

In questo lavoro vengono riportati i risultati di indagini necroscopiche effettuate per identificare le cause di morte in bovine adulte di allevamenti da latte. Negli anni 2012 e 2013 sono state esaminate in totale 74 bovine appartenenti a 42 allevamenti distribuiti nel territorio della provincia di Mantova. Le categorie utilizzate per classificare le cause di morte e le relative frequenze riscontrate sono state le seguenti: gastro-intestinali (48,6%), metaboliche (17,6%), circolatorie (9,5%), respiratorie (6,8%), genito-urinarie (6,8%), mammarie (6,8%), muscolo-scheletriche (1,4%). Solo in due casi (2,7%) non è stato possibile pervenire alla definizione della patologia responsabile del decesso. Dal nostro studio è emerso che circa la metà delle cause di morte sono attribuibili a eventi patologici controllabili mediante l'applicazione di adeguate misure gestionali. La morte degli animali adulti negli allevamenti da latte può talvolta diventare un rilevante problema economico. In questi casi l'esame necroscopico è in grado di fornire le informazioni necessarie per valutare in modo accurato il problema e impostare un efficace piano di prevenzione.

Rossi G, De\_Leo GA, Pongolini°S, Natalini S, Vince nzi S, Bolzoni°L

**Potential routes of disease transmission : analysis of direct and indirect contact patterns in dairy farm networks**

XXIV Congresso della Società Italiana di Ecologia "L'ecologia oggi : responsabilità e governance" : 15-17 settembre 2014 Ferrara : abstract / [s.l. : s.n., 2014]. - p 70 [Nr. Estr. 5925]

Congresso Società Italiana di Ecologia (S.It.E) (24. : Ferrara : 15-17 settembre 2014)

Infectious diseases in domestic animals can be transmitted between farms through different route that depend on the ecology of host and pathogen and on farming practices in act. Potentially infectious contacts between farms can be divided into direct, as the movement of animals between farms, and indirect, through personnel or fomites. For a better understanding of diseases transmission, many national health agencies collected data on animai exchanges between farms. On the other hand, indirect contacts have been usually overlooked and their estimates rely on limited data from farmer surveys or on functions of distance (transmission kernel). In our study, we investigated the role of both direct and indirect contacts in shaping diseases transmission network in the dairy cattle farm system in Emilia-Romagna (Italy). In particular, our analysis of network features relies on extensive datasets on cattle exchanges and on frequent farm visitors as veterinarians and dairy trucks. We first used different topology metrics, such as in- and out-infection chains for farms and the giant strong and weak components among others, to compare networks formed by different combinations of direct and indirect contacts. Then, we used model selection techniques to compare a null model, in which potential infectious indirect contacts probability rely only on geographical distance, to other models in which this probability depends also on other covariates (farm density, farm size). Our preliminary results suggest that the inclusion of indirect contacts can substantially change the topology of the farm contact network.

Rota\_Nodari°S, Polloni°A, Giacomelli°S, Vezzoli° F, Galletti°G

**Assessing pig welfare at stunning in Northern Italy commercial abattoirs using electrical method**

Large Anim Rev. - Vol. 20 no 2 ( 2014). - p 87-91. - 16 bib ref [Nr. Estr. 5699]

According to Council Regulation 1099/2009 regular checks at stunning are compulsory to ensure

that the animals do not pre-sent any signs of consciousness or sensibility in the period between the end of the stunning process and death. So far there are no data available on the prevalence of correctly electrically stunned pigs in Italy and on the signs more effective to use in practice to evaluate poorly stunned animals or return of consciousness. The aim of this study was to evaluate the prevalence of correctly stunned pigs by electrical stun in Italian commercial abattoirs through the clinical analysis of signs of consciousness. Four commercial abattoirs using three different electric stunning systems were studied to assess the efficiency of stunning. The abattoirs A and C used head-only manual stunning with an intensity 1.3A; the abattoir D used head-only manual stunning with an intensity 0.4 - 2A and the abattoir B used head-heart automatic stunning with an intensity 2.5A and 1A for the head and the chest respectively. A total of 1620 heavy pigs were evaluated from stunning to complete bleeding to evaluate the presence of signs of consciousness (animal alert, presence of rhythmic breathing, head uplift, pain reaction) and other signs (vocalizations, tongue movements, etc.). At the end of the observation the evaluator expressed a judgment concerning the state of consciousness for each pig conscious: presence of rhythmic breathing and/or pain reaction and/or righting reflex and/or the animal appeared alert; unconscious: presence of loss of posture, tonic phase, and clonic phase (not for head-heart automatic stunning); absence of rhythmic breathing, pain reaction, righting reflex; doubtful: not clear presence of rhythmic breathing or pain reaction or righting reflex. A statistically significant difference was observed in the distribution of the state of consciousness between the different abattoirs ( $p=0.01$ ) and between the three different stunning systems ( $p=0.04$ ). Abattoir B resulted to be more efficient (0% conscious; 0.25% doubtful) in stunning, compared to the others: abattoir A (0.5% conscious; 1.5% doubtful); C (2% conscious; 0.5% doubtful); D (1.7% conscious; 0.7% doubtful). Pain reaction, rhythmic respiration and head uplift could be used as practical key parameters for a regular evaluation of stunning, however future investigations are needed to better clarify their effectiveness taking into consideration the site of application of tongs and the current applied to each animal. The importance of tongue movements in apparently unconscious animals should be also investigated with more attention. No correlation was found between the state of consciousness and any of the anamnestic variables considered (signs of recent fighting; weight higher or lower than the range; signs of stress).

Rubini°S, Fico R, Rasera\_Berna°G, Govoni G, Tassi nari M, Lorenzini R

**Predator attack on flamingos (*Phoenicopterus roseus*) kept in captivity solved by the analysis of salivary DNA**

3rd European Association of Veterinary Laboratory Diagnosticians (EAVLD) Congress : Pisa (Italy), 12-15 October, 2014 : abstract book and final programme / [s.l. : s.n., 2014]. - p 276 - 3 bib ref [Nr. Estr. 5861]

European Association of Veterinary Laboratory Diagnosticians Congress (EAVLD) (3rd : Pisa (Italy) : October 12-15,2014)

**INTRODUCTION:** The present case study concerns four flamingos that, unable to fly, were kept in a conservation oasis in Ferrara, Emilia Romagna Region. All birds have found dead, some of them headless. Although a fox (*Vulpes vulpes*) was initially considered responsible of the killing, the birds have been taken to the laboratory for further investigations. The present study aims at identifying the predator through analysis of its behaviour, the pathology investigation and the DNA analysis.

**MATERIALS AND METHODS:** All four flamingos found dead on the morning of 14th January 2013 likely have been killed by a predator during the night before and therefore the bodies were still well preserved. The inspection of the area could not reveal some breakages in the fence, reinforcing the hypothesis that a predator had to climb the fence to enter the centre and reach the flamingos. After killing, only few parts of the birds have been eaten and two heads have been removed and not found. All these elements have been considered expression of behaviour of a fox. In the laboratory the carcasses of the birds have been classified and a pathology examination took place. Intercanine distance were measured. Swabs from the predator's saliva have been collected from the edge of the lesions referable to bites. Six swabs have been collected for identification of fox (*Vulpes vulpes*) DNA. The analysis have been carried out at the National Reference Centre for Forensic Veterinary.

**RESULTS:** The predator's DNA has been successfully extracted from two out of the six salivary samples. Amplification and following sequencing of a portion of ND1 gene showed, through

comparison with control sequences and international databases (GenBank), that the predator belonged to species *Canis lupus* instead of species *Vulpes vulpes* as it was initially thought based on first findings. The exam of mitochondrial DNA does not allow distinction between wolf and dog (both belonging to *C. lupus* species) and therefore an analysis of nuclear DNA through amplification of 20 STR loci has been required. The individual genotype of the predator has thus been obtained. The statistical analysis showed then that the salivary DNA belonged to a dog, excluding further the possibility that a wolf was the predator. (Lorenzini et al., 2014). **DISCUSSION AND CONCLUSIONS:** A literature research concerning the interdental distance of the lesions as described in the pathology, has been carried out. The intercanine distance is a species-specific element and therefore it can be used for identification of the predator (Ratz et al., 1999). With special reference to adult fox, the average intercanine distance is 24-27 mm (Hart et al., 1982), greater than those recorded through pathology. The lacci prese, claimed that a fox was the predator involved, but an accurate investigation brought full light on what had happened. It is worthwhile to highlight that genetic investigation, pathology and field observations have been effective in clarifying the whole episode.

Ruggeri° J, Martinelli° N, Pesciaroli M, Scaglione FE, Pregel P, Ammendola S, Battistoni A, Bollo E, Pasquali P, Alborali° L

**Efficacy of attenuated *Salmonella typhimurium* delta $\nu$ abc vaccine against *Salmonella choleraesuis* infection in piglets : a comparison with *Salmonella choleraesuis* killed vaccine**

LXVIII Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : Convegno SICV : XI Convegno AIPVet : XII Convegno SIRA16-18 Giugno 2014 Pisa / [s.l. : s.n., 2014]. - p 204. - 2 bib ref [Nr. Estr. 5989]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 68 Convegno SICV Convegno AIPVet : 11 Convegno SIRA : 12 : Pisa : 16-18 Giugno 2014)

Recently, we produced a mutant strain of *Salmonella Typhimurium*, deleted of *ZnuABC* genes (*S. Typhimurium*  $\nu$ ABC) and we proved its safety and efficacy either in mouse or pig model infection with virulent *S. Typhimurium*. In this study we assess the efficacy of attenuated *S. Typhimurium*  $\nu$ ABC vaccine and killed *S. choleraesuis* vaccine during *S. choleraesuis* infection. Clinical symptoms of *S. choleraesuis* infection in pigs are septicemia, pneumonia, enterocolitis, hepatitis, meningo-encephalitis and abortion. Animals are routinely treated with antibiotics to prevent infection. This practice can lead to an increase of multi-drug-resistant strains. Bio-safety programs and good management practices, are applied for infection control. However, vaccination could represent a complementary solution to reduce prevalence. 18 weaned piglets, born in *Salmonella*-SPF farm were divided in 3 groups. 6 piglets were vaccinated by a gavage with  $5 \times 10^7$  CFU of *S. Typhimurium*  $\nu$ ABC (group A), 6 piglets were intramuscularly vaccinated by killed *S. choleraesuis* vaccine (group B) and 6 piglets were naïve (group C). Groups were challenged with  $5 \times 10^8$  CFU of virulent *S. choleraesuis* day 25 post vaccination. Animals were weighted during vaccination and killing. After challenge, temperature was recorded day 3, 4, 5, 7, 11 and fecal samples were collected day 3, 7, 11. Tonsils, lymph nodes, intestinal content of caecum and colon were collected for microbiological analysis during necropsy. The results reported show that group A presents a temperature 1°C lower than groups B and C until 7 days after infection. Afterwards mean temperature of each group is range among 39.5°C and 40 °C. Any difference in weight gain is recorded among vaccinated and naïve groups. However, microbiological results are significant. *S. choleraesuis* is not detectable in faeces of piglets vaccinated with attenuated strain of *S. Typhimurium* (group A) day 11 post infection. Furthermore, *S. choleraesuis* colonization is limited in ileocaecal lymph nodes, caecum and colon of group A piglets. In conclusion, these findings extend the validity of attenuated *S. Typhimurium*  $\nu$ ABC strain as a useful mucosal vaccine either in *S. Typhimurium* or *S. choleraesuis* pigs infection. Furthermore, these data corroborate the greater competency of attenuated vaccine than killed one to reduce intestinal colonization. Schwarz P1, Kich JD, Kolb J, Cardoso M. Use of an avirulent live *Salmonella Choleraesuis* vaccine to reduce the prevalence of *Salmonella* carrier pigs at slaughter. *Vet Rec.* 2011 Nov 19;169(21):553. Selke M, Meens J, Springer S, Frank R, Gerlach GF. Immunization of pigs to prevent disease in humans: construction and protective efficacy of a *Salmonella enterica* serovar *Typhimurium* live negative-marker vaccine.

Ruggeri° J, Martinelli° N, Pitozzi° A, Pasquali P, Alborali° G

**Development of *Brachyspira hyodysenteriae* infections model in pigs**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 236 (Poster P202) [Nr. Estr. 5725]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

*Brachyspira hyodysenteriae* is a gram negative bacterium, agent of Swine Dysentery. The disease is characterized by mucohaemorrhagic colitis and rapid loss of weight. Surveillance programs are envisaged for identification of infected herds, hence controlling the spreading of infection. Different experimental infection models have been already studied to understand *B. hyodysenteriae* infection and pathogenesis. Unfortunately, many studies failed. Stress facilitates experimentally infection and, recently, interest of many researchers was focused on feed. The effects of different microbial fermentations induced by various substrates, such as rice and soy, are evaluated in their capability to facilitate *B. hyodysenteriae* colonization. Aim of this study was to investigate the pathogenicity of *B. hyodysenteriae* Spanish strain in experimentally infected pigs fed with soy for 7 days before infection. Four groups of weaned piglets were enrolled in this study. Group A and D were inoculated by gavage with 50 ml of *B. hyodysenteriae* broth, contained 10<sup>8</sup> CFU/ml, for three consecutive days. Group B and D were fed with soy and group C was the control group. Animals were monitored every day, analyzing clinical signs and fecal consistency and were weighted at autopsy. Feces were plated onto selective *Brachyspira* agar plates (Reparto produzione Terreni, Izsler-Brescia) and incubated for 5 days at 37°C in anaerobic conditions (GENbag anaer, Biomérieux). Hemolytic zones associated with *Brachyspira* growth were analyzed by end-point multiplex PCR. Animals were euthanized at three different time-points to understand the disease progression. Differences of growth among groups were observed at 19 and 29 days post inoculation. Animals, inoculated with *B. hyodysenteriae*, grew less than untreated groups. At the same time points, differences of fecal consistency were recorded, all animals of group A and D had diarrhea and *B. hyodysenteriae* was isolated in this samples. During necropsy, no lesions were observed in piglets of group B and C. Piglets of group D, fed with soy, manifested serious lesions at colon and caecum starting from two weeks after infection, while piglets of group A, infected but not fed with soy, at three weeks after infection. In conclusion, these data show that soy diet favored the progression of the infection and worsened gut lesions.

Ruggeri° J, Pesciaroli M, Gaetarelli° B, Scaglione FE, Pregel P, Ammendola S, Battistoni A, Bollo E, Alborali° GL, Pasquali P

**Parenteral administration of attenuated *Salmonella Typhimurium* znuABC is protective against salmonellosis in piglets**

Vaccine. - Vol. 32 ( 2014). - p 4032-4038. - 27 bib ref [Nr. Estr. 5718]

A major cause of salmonellosis in humans is the contamination of pork products. Infection in pigs can be controlled using bio-security programs, but they are not sufficient in countries where a high level of infection is recorded. In this context, the use of vaccines can represent a valid supplementary method of control. Recently, we have demonstrated that an attenuated strain of *Salmonella enterica* serovar *Typhimurium* (*Salmonella Typhimurium* znuABC) is protective against systemic and enteric salmonellosis in mouse and pig infection models, candidating this strain as an oral attenuated vaccine. In this study, we compared the efficacy of this attenuated *Salmonella Typhimurium* strain when administered orally or parenterally. Furthermore, in order to reproduce a pseudo-natural infection model, vaccinated pigs were allocated in the same pen with animals shedding virulent *Salmonella Typhimurium*. Animals were monitored weekly after vaccination and contact with infected piglets. Diarrhea and ataxia were recorded and *Salmonella* shedding was

tested individually through bacterial culture. After four weeks of cohousing, piglets were euthanized, after which lymph nodes reactivity and gross lesions of the gut sections were scored at necropsy. Organs were submitted to microbiological and histological analyses. The data reported herein show that parenterally vaccinated animals do not shed the attenuated strain, and at the same time the absence of symptoms and decrease in virulent strain shedding in feces from day 6 after challenge demonstrated protection against infection induced by virulent Salmonella Typhimurium. In conclusion, our findings suggest that this is an alternative route of Salmonella Typhimurium znuABC administration, without ignoring the advantages associated with oral vaccination.

Ruggeri° J, Pesciaroli M, Gaetarelli° B, Scaglione FE, Pregel P, Ammendola S, Battistoni A, Bollo E, Alborali° GL, Pasquali P

**Attenuated S. typhimurium ZnuABC is protective against salmonellosis in piglets**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014 Mexico / [s.l. : s.n., 2014]. - v. 2. - p 260. - 9 bib ref [Nr. Estr. 5729]

International Pig Veterinary Society Congress (IPVS) (23rd : Mexico : June 8 - 11, 2014)

Ruggeri° J, Pesciaroli M, Martinelli° N, Gradassi° M, Scaglione FE, Ammendola S, Battistoni A, Magistrali CF, Pasquali P, Alborali° GL

**Efficacia di un vaccino attenuato e di uno inattivato per la profilassi di Salmonella typhimurium nei suini in accrescimento** = Efficacy of an attenuated and killed (inactivated) Salmonella typhimurium strains against salmonellosis in growing pigs

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 189-196. - 10 bib ref [Nr. Estr. 5674]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

La contaminazione della carne di suino da Salmonella spp. è una delle cause d'infezioni enteriche dell'uomo e la vaccinazione dei suini può ridurre la contaminazione lungo la catena produttiva. Recentemente abbiamo dimostrato che S.Typhimurium deleva del trasportatore ZnuABC (S.Typhimurium AznuABC) è un vaccino attenuato promettente in alcuni modelli murini d'infezione da S.Typhimurium (Ammendola, 2007; Pasquali, 2008; Pesciaroli, 2011). In questo studio, abbiamo confermato la sicurezza e l'efficacia di S.Typhimurium AznuABC somministrato per via orale nei suini. La sicurezza di S.Typhimurium AznuABC è stata testata monitorando le condizioni cliniche degli animali ed eseguendo analisi microbiologiche e sierologiche della risposta immunitaria umorale e cellulare nei campioni di feci e di sangue. Abbiamo testato l'efficacia di S.Typhimurium AznuABC in 2 gruppi di suini vaccinati con 2 diversi dosaggi del ceppo, paragonando i risultati con quelli ottenuti da un gruppo di suini vaccinati con un ceppo inattivato e da un gruppo non vaccinato. Dopo l'infezione con il ceppo omologo virulento, i suini vaccinati con S.Typhimurium AznuABC non presentavano segni clinici e la colonizzazione del tratto intestinale e l'eliminazione fecale del ceppo virulento di S. Typhimurium era ridotta rispetto ai controlli. Questi risultati suggeriscono che S.Typhimurium AznuABC è attenuato e immunogeno nei suini e potrebbe essere un promettente vaccino mucosale attenuato.

*Pork meat contamination by Salmonella spp. is a major cause of human enteric infections in industrialized countries and vaccination of pigs may represent an effective instrument in reducing Salmonella burden through the food chain. We have previously demonstrated that S Typhimurium lacking the ZnuABC transporter (S. Typhimurium AznuABC) is a promising candidate live vaccine in different mouse models of S. Typhimurium infection (Ammendola, 2007; Pasquali, 2008; Pesciaroli, 2011). In this study, we confirmed in pigs the safety and immunogenicity of S.Typhimurium AznuABC orally administered. We have tested the safety of S.Typhimurium AznuABC monitoring clinical conditions of animals and we conducted a microbiological culture and a quantification of the humoral and cellular immune response, respectively, on fecal and blood samples of pigs. We have tested the protective effects of S.Typhimurium AznuABC in four groups of pigs: animals vaccinated with S. Typhimurium AznuABC (two dosages tested), controls vaccinated with a formalin-inactivated*

*virulent strain and unvaccinated controls. After the challenge, pigs vaccinated with the attenuated S. Typhimurium AznuABC strain did not display clinical signs of salmonellosis. The vaccine reduced intestinal tract colonization and fecal shedding of the fully virulent Salmonella strain. These results suggest that S. Typhimurium AznuABC is attenuated and immunogenic in pigs and it could be a promising attenuated live mucosal vaccine.*

Rugna°G, Bonilauri°P, Garbarino°C, Licata E, Tam ba°M, Merialdi°G

**Monitoring zoonotic diseases in the wild boar (*Sus scrofa*) population of the Emilia-Romagna Region (Northern Italy)**

Trends in game meat hygiene : from forest to fork / edited by P. Paulsen, A. Bauer and F.J.M. Smulders. - Wageningen, The Netherlands : Wageningen Academic Publishers, 2014. - p 145-149. - 21 bib ref [Nr. Estr. 6045]

Knowledge of the diseases circulating in wildlife populations is significant not only for conservation and livestock production but also to ensure public health. Here we report the results of a seven year monitoring programme of wildlife diseases carried out in the Emilia-Romagna region, Italy, between 2006 and 2012. Samples of muscular tissue were examined for the presence of *Trichinella* spp. larvae and *Toxoplasma* spp. and viscera were analysed for *Mycobacterium* spp. and *Brucella* spp. Seroprevalence of *Toxoplasma gondii* was 16.7% and *Trichinella pseudospiralis* larvae were detected in 1/70,948 wild boar. The survey showed the circulation of *Brucella suis* biovar 2 in the regional wild boar population and the absence of *Mycobacterium bovis*. The monitoring programme confirms the very low circulation of *Trichinella* spp. in the regional wildlife population. Further studies are necessary to evaluate the role of wild boar in the epidemiology of toxoplasmosis and brucellosis.

Rugna°G, Forlenza J, Bonilauri°P, Carra°E, Luppi °A, Martelli P, Merialdi°G

**Use of molecular typing (MLST) and serology to study the epidemiology of *Brachyspira hyodysenteriae* infection in a large scale herd**

ESPHM 2014 : proceedings 6th European Symposium on Porcine Health Management : 7th-9th May, 2014, Sorrento, Italy / [s.l. : s.n., 2014]. - p 181 (Poster P95) [Nr. Estr. 6068]

European Symposium on Porcine Health Management (ESPHM) (6th : Sorrento, Italy : 7th-9th May, 2014)

Introduction. A successful control program for Swine dysentery (SD), especially in large-scale herds, relies on the accurate understanding of the intra-farm epidemiology. The aim of the study was to attempt the identification of the infection's sources and the transmission patterns of *B. hyodysenteriae* in an Italian pig farm affected by SD by using a molecular typing method (MLST) and serology. Materials and methods. The study was conducted in a herd based on 5 units. Grand-parent sows are reared in unit A from which gilts are moved to farrow-to-finish units (B and C). A portion of growers are moved from units B and C to be fattened in units D and E. Occasionally growers are purchased from an outer farm, declared free of SD, and fattened in unit E. Recurrent episodes of SD are observed in the farm, but units A and D have no history of SD in the last 2 years. Fecal samples were collected from pigs suffering from clinical signs of SD in a period of 18 months. Blood samples from at least 40 pigs were collected from each unit. Fecal samples were cultivated for *B. hyodysenteriae* and the isolates were submitted to MLST. Two hundred blood samples were tested with the PrioCHECK® *Brachyspira* Ab porcine ELISA (Prionics AG, Switzerland) for the detection of *B. hyodysenteriae* antibodies based on a novel recombinant antigen. Results. A total of 18 faecal samples from different 3 units were examined and 13 *B. hyodysenteriae* strains were isolated. The MLST results showed the circulation of isolates belonging to the same sequence type (ST 77). ELISA serology showed positivity in all the units, A and D included. A group of pigs purchased from the outer farm was tested twice and remained seronegative. Discussion and conclusion. The MLST showed that the source of infection was unique and probably endogenous. The ELISA test showed that *B. hyodysenteriae* antibodies were present in all groups including those with no recent history of clinical SD. Based on the case history and the serologic results it is possible

to hypothesize that grandparent sows (unit A), even though not affected by clinical SD, are the probable source of *B. hyodysenteriae* spreading. MLST can be of great usefulness in studying the epidemiology of *B. hyodysenteriae* infection in large scale multisite herds and serology can allow to check the SD status of pigs purchased from a supplier farm detecting subclinically infected animals. The combined use of both techniques could be of great help for the correct implementation of control strategies.

Sachse K, Laroucau K, Riege K, Wehner S, Dilcher M, Creasy HH, Weidmann M, Myers G, Vorimore F, Vicari°N, Magnino°S, Liebler \_Tenorio E, Ruettger A, Bavoil PM, Hufert FT, Rosselló\_Móra R, Marz M

**Evidence for the existence of two new members of the family Chlamydiaceae and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov.**

Syst Appl Microbiol. - Vol. 37 ( 2014). - p 79-88. - 42 bib ref [Nr. Estr. 5683]

The family Chlamydiaceae with the recombined single genus *Chlamydia* currently comprises nine species, all of which are obligate intracellular organisms distinguished by a unique biphasic developmental cycle. Anecdotal evidence from epidemiological surveys in flocks of poultry, pigeons and psittacine birds have indicated the presence of non-classified chlamydial strains, some of which may act as pathogens. In the present study, phylogenetic analysis of ribosomal RNA and *ompA* genes, as well as multi-locus sequence analysis of 11 field isolates were conducted. All independent analyses assigned the strains into two different clades of monophyletic origin corresponding to pigeon and psittacine strains or poultry isolates, respectively. Comparative genome analysis involving the type strains of currently accepted Chlamydiaceae species and the designated type strains representing the two new clades confirmed that the latter could be classified into two different species as their average nucleotide identity (ANI) values were always below 94%, both with the closest relative species and between themselves. In view of the evidence obtained from the analyses, we propose the addition of two new species to the current classification: *Chlamydia avium* sp. nov. comprising strains from pigeons and psittacine birds (type strain 10DC88T; DSMZ: DSM27005T, CSUR: P3508T) and *Chlamydia gallinacea* sp. nov. comprising strains from poultry (type strain 08-1274/3T; DSMZ: DSM27451T, CSUR: P3509T).

Sait M, Livingstone M, Clark EM, Wheelhouse N, Spalding L, Markey B, Magnino° S, Lainson FA, Myers GSA, Longbottom D

**Genome sequencing and comparative analysis of three *Chlamydia pecorum* strains associated with different pathogenic outcomes**

BMC Genomics. - Vol. 15 no 23 ( 2014). - 15 p. - 74 bib ref [Nr. Estr. 5923]

Background *Chlamydia pecorum* is the causative agent of a number of acute diseases, but most often causes persistent, subclinical infection in ruminants, swine and birds. In this study, the genome sequences of three *C. pecorum* strains isolated from the faeces of a sheep with inapparent enteric infection (strain W73), from the synovial fluid of a sheep with polyarthritis (strain P787) and from a cervical swab taken from a cow with metritis (strain PV3056/3) were determined using Illumina/Solexa and Roche 454 genome sequencing. Results Gene order and synteny was almost identical between *C. pecorum* strains and *C. psittaci*. Differences between *C. pecorum* and other chlamydiae occurred at a number of loci, including the plasticity zone, which contained a MAC/perforin domain protein, two copies of a >3400 amino acid putative cytotoxin gene and four (PV3056/3) or five (P787 and W73) genes encoding phospholipase D. *Chlamydia pecorum* contains an almost intact tryptophan biosynthesis operon encoding *trpABCDFR* and has the ability to sequester kynurenine from its host, however it lacks the genes *folA*, *folKP* and *folB* required for folate metabolism found in other chlamydiae. A total of 15 polymorphic membrane proteins were identified, belonging to six *pmp* families. Strains possess an intact type III secretion system

composed of 18 structural genes and accessory proteins, however a number of putative inc effector proteins widely distributed in chlamydiae are absent from *C. pecorum*. Two genes encoding the hypothetical protein ORF663 and IncA contain variable numbers of repeat sequences that could be associated with persistence of infection. Conclusions Genome sequencing of three *C. pecorum* strains, originating from animals with different disease manifestations, has identified differences in ORF663 and pseudogene content between strains and has identified genes and metabolic traits that may influence intracellular survival, pathogenicity and evasion of the host immune system.

Salogni°C, Gibelli°L, Gelmetti°D, Nieddu G, Alborali°GL

**Descrizione di un caso di micobatteriosi in ciclidi ornamentali del lago Malawi**

XX Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) : Certosa di Calci (PI), 18-19 settembre 2014 / [s.l. : s.n., 2014]. - p 30 [Nr. Estr. 5827]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (20. : Certosa di Calci (PI) : 18-19 settembre 2014)

Nell'estate del 2013 un allevatore di pesci ornamentali segnalava che da più di un anno in alcuni gruppi di ciclidi del lago Malawi erano osservabili lesioni cutanee che ne pregiudicavano la commercializzazione. Le lesioni erano presenti esclusivamente in pesci riproduttori di età superiore ai 1-2 anni, si manifestavano come alterazioni della livrea (comparsa di aree cutanee di color brunastro) e/o come lesioni proliferative/ulcerate localizzate per la maggior parte a livello della cute, delle pinne o del cavo orale. Sintomi di malessere generale, quali lieve dimagrimento ed esoftalmo erano altresì osservabili. L'incidenza della patologia era bassa (10% circa dei soggetti) così come la mortalità (1-2% circa). I pesci più gravemente colpiti venivano poi spostati in una vasca comune ed esclusi dalla vendita. Venti pesci con lesioni cutanee ed i fanghi del filtro biologico della vasca di isolamento sono stati sottoposti ad esame necroscopico, istologico, batteriologico routinario e per ricerca micobatteri, virologico, micologico e parassitologico. L'esame anatomopatologico ha evidenziato lesioni cutanee in tutti i soggetti e lesioni nodulari di piccole dimensioni (diametro massimo di circa 1 mm) , più raramente nel fegato (4 soggetti), nella milza e nel rene (2 soggetti). L'esame istologico ha evidenziato la presenza di granulomi multipli in cute, milza, fegato, stomaco e peritoneo. I granulomi erano circondati da una spessa capsula connettivale e contenevano aggregati di bacilli acido-resistenti compatibile con una infezione da micobatteri. Gli esami batteriologico, virologico, micologico e parassitologico hanno dato esito negativo. L'esame batteriologico per micobatteri è risultato negativo da pool di organi e positivo dai fanghi del filtro biologico, con l'isolamento in purezza *Mycobacterium fortuitum*. Il mancato isolamento di *M fortuitum* nelle lesioni viscerali può essere imputato alla riduzione di vitalità dei micobatteri a seguito della reazione immunitaria dell'ospite, oppure all'inefficace processazione del campione. La porta d'ingresso dell'infezione può essere imputabile alle peculiari caratteristiche di aggressività dei ciclidi che sono causa di ferite cutanee facilmente infettabili da microrganismi patogeni presenti nell'ambiente, come nel caso specifico è avvenuto per *M fortuitum*. Questa condizione ha permesso il mantenimento nel tempo dell'infezione.

Salogni°C, Grassi°A, Cervellione F, Alborali°GL, Gibelli°LR, Gelmetti°D

**Sindrome gastroenterica della trota iridea (RTGE) : descrizione di un focolaio atipico di malattia** = Rainbow Trout Gastro Enteric Syndrome (RTGE) : description of an atypical outbreak of disease

Ittiopatologia. - Vol. 11 ( 2014). - p 43-50. - 12 bib ref [Nr. Estr. 5910]

La sindrome gastroenterica della trota iridea (RTGE) è una sindrome che interessa la trota iridea (*Oncorhynchus mykiss*) il cui agente causale è il batterio *Candidatus arthromitus*. Nel mese di gennaio del 2012 in una trota coltura della pianura padana alimentata con acqua di risorgiva a 13°C si è verificato un episodio di gastroenterite che ha colpito esemplari di trota iridea di 50-60 grammi.

Sono stati campionati alcuni pesci che mostravano un'evidente sintomatologia e quindi sottoposti ad esame anatomopatologico e ad esami di laboratorio di tipo parassitologico, colturale, virologico e istologico. Un quadro autoptico caratterizzato da distensione addominale, congestione ed edema delle pareti intestinali, presenza di feci mucose biancastre, unitamente all'evidenza in sede istologica di un'enterite desquamativa con batteri filamentosi segmentati (SFB) Gram positivi, ha permesso di fare diagnosi di gastroenterite della trota iridea (RTGE). Il caso descritto si discosta dal quadro classico di RTGE degli allevamenti italiani per la categoria di animali colpiti e le condizioni termiche riscontrate. Tale atipicità è da riferire a fattori predisponenti che hanno alterato l'equilibrio tra ospite e patogeno.

*Rainbow Trout Gastro Enteric Syndrome (RTGE) is a pathology that affected farmed rainbow trout (Oncorhynchus mykiss) due to the infectious bacteria Candidatus arthromitus. In January 2012, a gastroenteritis case occurred in a trout farming in Pianura Padana (Northern Italy) powered by spring water at 13°C. The weigh of reared trouts was about 50-60 g. On symptomatic fish necropsy examination and other laboratory tests as parasitological, bacteriology, virological and histological investigation have been performed. The autopsy showed swollen abdomen, congestion and edema of intestinal wall. Histological examination showed the presence of whitish mucous stools and evidence of desquamative enteritis with presence of Gram positive segmented filamentous bacteria (SFB). There fire, based on the results obtained, it was possible to make a diagnosis of RTGE. This case is different from classic RTGE usually detected in Italian farms due to the smaller size of fish affected and the lower water temperature. This anomaly is probably due to predisposing factors that have altered the balance between post and pathogen.*

Santi°A, Renzi°M, Baldelli R, Calzolari°M, Camin iti°A, Dell'Anna°S, Galletti°G, Lombardini A, Paternoster°G, Tamba°M

#### **A surveillance program on canine Leishmaniasis in the public kennels of Emilia-Romagna Region, Northern Italy**

Vector Borne Zoonotic Dis. - Vol. 14 no 3 ( 2014). - p 206-211. - 27 bib ref [Nr. Estr. 5789]

Since 2007, a canine leishmaniasis (CanL) surveillance program has been carried out in public kennels of the Emilia-Romagna region with the aim of providing health guarantees for dog adoptions. According to this program, monitoring activities were performed to verify the presence of sandflies and infected dogs, and a specific CanL risk class was assigned to each kennel, resulting in different control approaches (entomological and/or serological monitoring, clinical surveillance, therapeutic treatment of infected dogs, protections against vector bites). From 2007 to 2012, 20,931 dogs, 89.8% of which were identified by microchip and housed in 73 kennels, were examined using an indirect fluorescent antibody test. In all, 528 (2.8%) dogs tested positive, and 43.0% of these were asymptomatic. The authors used monitoring results, in particular serological tests performed on dogs at admittance to the kennel and annual controls of sentinel dogs, to estimate CanL risk in the whole region and to evaluate the efficacy of the preventive measures adopted. CanL seroprevalence in dogs tested at the admittance in kennels increased significantly from 2010 (1.0%; 29/2858) to 2012 (2.4%; 69/2841). In contrast, the number of seroconversions in sentinel dogs was stable in 2010 (1.2%; 11/896) and 2011 (1.6%; 13/825) and decreased in 2012 (0.9%; 8/850), suggesting the efficacy of the preventive measures applied.

Scaravelli D, Massi°P, Tosi°G, Fiorentini°L

#### **Casi insospettati di patologie scheletriche: displasia del femore in Threskiornis aethiopicus e di frattura del femore in Ara macao**

Atti della Societa' Italiana di Patologia Aviare (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 196-197. - 7 bib ref [Nr. Estr. 5974]

Convegno annuale Societa' Italiana Patologia Aviare (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*In an adult male of Threskiornis aethiopicus was found a displasia of the femur with diffuse periartritis areas and new-formations and in adult female Ara macao the fracture of the femur. Despite the gravity of the fracture both the two case go under silence in the two collections of derivation of the specimens. A strong attention and training have to be paid to the welfare of animals.*

Sebastianelli M, Ciuti F, Manfredini°A, Vicari°N, Gobbi M, D'Avino N, Fabbi°M, Filippini G

**Aborto enzootico : indagine molecolare in aborti ovini e caprini di 13 regioni italiane** =  
Enzootic abortion of ewes : molecular survey in sheep and goats of 13 italian region

Large Anim Rev. - Vol. 20 no 4 (Suppl 1) ( 2014). - p 159. - 8 bib ref [Nr. Estr. 5902]

Congresso Nazionale Societa Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) (21. : Foggia : 9-12 Settembre 2014)

Serraino A, Arrigoni°N, Ostanello F, Ricchi°M, Marchetti G, Bonilauri°P, Bonfante E, Giacometti F

**A screening sampling plan to detect Mycobacterium avium subspecies paratuberculosis-positive dairy herds**

J Dairy Sci. - Vol. 97 ( 2014). - p 3344-3351. - 47 bib ref [Nr. Estr. 5787]

Mycobacterium avium ssp. paratuberculosis (MAP) is the etiological agent of paratuberculosis, a chronic contagious bacterial disease primarily affecting dairy cattle. Paratuberculosis represents a dual problem for the milk production chain: in addition to economic losses to affected herds, MAP may have zoonotic potential. Infected herds must be identified in order to implement programs designed to reduce the incidence of disease within and between herds and to prevent MAP from entering the food chain. The objective of this study was to evaluate the sensitivity and specificity of a screening sampling plan (SSP) to detect MAP-positive dairy herds by repetitive analysis of bulk tank milk (BTM) samples by ELISA and in-line milk filter (ILMF) samples by PCR. Samples from BTM and ILMF were collected twice from 569 dairy herds in southern Italy. Additionally, 12,016 individual milk samples were collected: 9,509 from 102 SSP-positive herds (SSP MAP-positive) and 2,507 from 21 randomly selected SSP-negative herds (SSP MAP-negative). There was a total of 126 SSP MAP-positive herds (i.e., 21.3% SSP MAP-positive herds; 95% confidence interval = 18.0–24.9); the within-herd apparent prevalence (AP) ranged between 0.00 and 22.73% (mean 6.07%). A significant difference in within-herd AP was shown between SSP MAP-positive herds and SSP MAP-negative herds. A highly significant association was shown between the median AP herd status (>5%) and positivity to at least one ILMF or BTM sample. The SSP detected a minimum of 56.25% of low AP herds (AP =2.0%) up to a maximum of 100% of herds with a within-herd AP =8.0%. Overall, the SSP detected 85.57% of herds in which at least one individual milk sample was positive by ELISA. The proposed SSP was an inexpensive and useful tool to detect MAP-positive herds with a higher risk of infection diffusion and milk contamination. Although the SSP cannot be used for MAP-free certification of herds, it could be useful to prioritize appropriate control measures aimed at reducing the prevalence of infection in dairy herds and milk contamination.

Simon G, Larsen LE, Duerrwald R, Foni°E, Harder T, Van\_Reeth K, Markowska-Daniel I, Reid SM, Dan A, Maldonado J, Huovilainen A, Billinis C, Davidson I, Aguero M, Vila T, Herve S, Breum SO, Chiapponi°C, Urbaniak K, Kyriakis CS, ESNIP3 Consortium, Brown IH, Loeffen W

**European surveillance network for influenza in pigs : surveillance programs, diagnostic tools and swine influenza virus subtypes identified in 14 European countries from 2010 to 2013**

PLoS One. - Vol. 9 no 12 ( 2014). - p e115815 (21 p.). - 75 bib ref - ultimo accesso 08/01/2015

Swine influenza causes concern for global veterinary and public health officials. In continuing two previous networks that initiated the surveillance of swine influenza viruses (SIVs) circulating in European pigs between 2001 and 2008, a third European Surveillance Network for Influenza in Pigs (ESNIP3, 2010–2013) aimed to expand widely the knowledge of the epidemiology of European SIVs. ESNIP3 stimulated programs of harmonized SIV surveillance in European countries and supported the coordination of appropriate diagnostic tools and subtyping methods. Thus, an extensive virological monitoring, mainly conducted through passive surveillance programs, resulted in the examination of more than 9 000 herds in 17 countries. Influenza A viruses were detected in 31% of herds examined from which 1887 viruses were preliminary characterized. The dominating subtypes were the three European enzootic SIVs: avian-like swine H1N1 (53.6%), human-like reassortant swine H1N2 (13%) and human-like reassortant swine H3N2 (9.1%), as well as pandemic A/H1N1 2009 (H1N1pdm) virus (10.3%). Viruses from these four lineages co-circulated in several countries but with very different relative levels of incidence. For instance, the H3N2 subtype was not detected at all in some geographic areas whereas it was still prevalent in other parts of Europe. Interestingly, H3N2-free areas were those that exhibited highest frequencies of circulating H1N2 viruses. H1N1pdm viruses were isolated at an increasing incidence in some countries from 2010 to 2013, indicating that this subtype has become established in the European pig population. Finally, 13.9% of the viruses represented reassortants between these four lineages, especially between previous enzootic SIVs and H1N1pdm. These novel viruses were detected at the same time in several countries, with increasing prevalence. Some of them might become established in pig herds, causing implications for zoonotic infections.

Sozzi°E, Moreno°A, Lelli°D, Cinotti°S, Alborali °GL, Nigrelli°A, Luppi°A, Bresaola°M, Catella°A, Cordioli°P

#### **Genomic characterization of Pseudorabies virus strains isolated in Italy**

Transbound Emerg Dis. - Vol. 61 no 4 ( 2014). - p 334-340. - 17 bib ref [Nr. Estr. 5368]

In this study, we undertook the genomic characterization of 54 pseudorabies virus (PRV) strains isolated in Italy during 1984–2010. The characterization was based on partial sequencing of the UL44 (gC) and US8 (gE) genes; 44 strains (38 for gene gE and 36 for gC) were isolated on pig farms; 9 originated from dogs and 1 from cattle. These porcine PRV strains, which were closely related to those isolated in Europe and America in the last 20 years, and the bovine strain bovine/It/2441/1992 belong to cluster B in both phylogenetic trees. Six porcine strains that do not belong to cluster B are related in both gE and gC phylogenetic trees to the ‘old’ porcine PRV strains isolated in the 1970s and 1980s. In the last two decades, the presence of these strains in domestic pig populations has been reduced drastically, whereas they are prevalent in wild boar. The two remaining strains have an interesting genomic profile, characterized by the gC gene being closely related to the old porcine PRV strains, and the gE gene being similar to that of recently isolated strains. Three strains originating from working dogs on pig farms are located in cluster B in both phylogenetic trees. Five strains isolated from hunting dogs have a high degree of correlation with PRV strains circulating in wild boar. The last isolate has a gC gene similar to that in the two porcine strains mentioned previously, and the gE gene is correlated with the strains isolated from hunting dogs. These results provide interesting insight into the genomic characterization of PRV strains and reveal a clear differentiation between the strains isolated from hunting dogs that are related to the wild boar strains and those originating from domestic pigs.

Sozzi°E, Papetti°A, Lelli°D, Boniotti°B, Moreno °A, Brocchi°E, Alborali°L, Lavazza°A, Cordioli°P

#### **Diagnosis and investigations on PED in Northern Italy**

8th Annual Epizone Meeting "Primed for tomorrow" : 23 - 25 September 2014 Copenhagen, Denmark : Posters "Intervention strategies and legislation" / [s.l. : s.n., 2014]. - 1 p. [Nr. Estr. 5888]  
Annual meeting Epizone (8th : Copenhagen, Denmark : 23 - 25 September 2014)

In Italy and likely in Europe, the last epidemic of Porcine Epidemic Diarrhoea (PED) affecting pigs of all ages was described on 2005-2006, when PED coronavirus was identified in 63 herds by electron microscopy, PCR and serology (1). Watery diarrhoea without mucus and blood was associated with a reduction of feed consumption. In farrowing-to-weaning herds, diarrhoea affected the sows and suckling piglets, and the mortality in piglets was up to 34%. In growers and fatteners the morbidity ranged from 20 to 80%, but there was either no mortality or it was very low. Thereafter a systematic diagnostic approach was implemented to screening the presence of PEDV in Northern Italy. Two immunoassays were developed, based on Monoclonal antibodies (MAbs) produced against the European CV777 reference strain. An ELISA MAbs based "antigen-capture" to detect anti-PEDV antibodies, was compared with the immune-peroxidase monolayer assay test (IPMA) by testing 296 samples from 11 commercial swine farms. IPMA test and ELISA demonstrated a very good agreement, suggesting the use of ELISA as useful test for serosurveys (2). A double antibody sandwich enzyme-linked immune-sorbent assay (DAS-ELISA) was developed and firstly compared with RT-PCR in the examination of 506 specimens (faecal samples and intestinal contents) collected during 2006-2007 from pigs originating from different farms located in the Po valley (3,4). The correlation between the two methods was higher when testing faecal samples ( $K = 0.97$ , 95% CI: 0.94-1.00) than testing intestinal samples ( $K = 0.62$ , 95% CI: 0.35-0.89). Such methods were then employed as screening tools in field surveys to evaluate the presence and circulation of PEDV in pig farms in the North Italy. During the period 2008-2014 a total of 1563 samples from clinical cases of pig enteritis were investigated and PEDV was diagnosed in 61 outbreaks. A total of 21 positive ELISA cases and 5 positive samples previously detected in 2007 were then confirmed by RT-PCR, using PEDV specific primers (4). The amplified products were sequenced to establish genetic relationship of the partial S1 gene of field strains, and to perform phylogenetic analysis. The nucleotide and amino acid sequences were aligned and compared to selected PEDV sequences available from the GenBank database. Phylogenetic trees (generated by the neighbor-joining method by MEGA 5) showed that PEDV strains were divided into two groups: the first comprised the PEDV isolates from 2006 to the beginning of 2009, while the second contained the isolates from mid-2009 to 2012. Finally, to determine the actual sero-prevalence, over 500 pig sera from 15 farms were checked for the presence of antibodies. On the whole these results indicate the endemic presence of "PEDV European-like strains" in Italy since its last important epidemic occurrence around ten years ago.

Stacchiotti A, Rovetta F, Ferroni M, Corsetti G, Lavazza<sup>o</sup> A, Sberveglieri G, Aleo MF

#### **Taurine rescues cisplatin-induced muscle atrophy in vitro : a morphological study**

Oxidative Med Cell Longev. - Vol. Article ID 840951 ( 2014). - p. (11) - 54 bib ref ( ultimo controllo 15/05/2014 : <http://dx.doi.org/10.1155/2014/840951> ) [Nr. Estr. 5687]

Cisplatin (CisPt) is a widely used chemotherapeutic drug whose side effects include muscle weakness and cachexia. Here we analysed CisPt-induced atrophy in C2C12 myotubes by a multidisciplinary morphological approach, focusing on the onset and progression of autophagy, a protective cellular process that, when excessively activated, may trigger protein hypercatabolism and atrophy in skeletal muscle. To visualize autophagy we used confocal and transmission electron microscopy at different times of treatment and doses of CisPt. Moreover we evaluated the effects of taurine, a cytoprotective beta-amino acid able to counteract oxidative stress, apoptosis, and endoplasmic reticulum stress in different tissues and organs. Our microscopic results indicate that autophagy occurs very early in 50  $\mu$ M CisPt challenged myotubes (4 h–8 h) before overt atrophy but it persists even at 24 h, when several autophagic vesicles, damaged mitochondria, and sarcoplasmic blebblings engulf the sarcoplasm. Differently, 25 mM taurine pretreatment rescues the majority of myotubes size upon 50  $\mu$ M CisPt at 24 h. Taurine appears to counteract atrophy by restoring regular microtubular apparatus and mitochondria and reducing the overload and the localization of

autophagolysosomes. Such a promising taurine action in preventing atrophy needs further molecular and biochemical studies to best define its impact on muscle homeostasis and the maintenance of an adequate skeletal mass in vivo.

Sternberg\_Lewerin S, Good M, Saéz\_Llorente J, Zanardi°G, Evans L, Preto I, Bezos\_Garrido J, Pacciarini°M, Piazza V

### **Bovine tuberculosis eradication programmes in the EU : experiences from balancing science and policy**

VI International M. bovis conference : Cardiff, Wales, Great Britain, 16th - 19th June 2014 / [s.l. : s.n., 2014]. - p 34-35 [Nr. Estr. 5897]

International M. bovis conference (6th : Cardiff, Wales : 16th - 19th June 2014)

Introduction The EU Commission's Task Force for monitoring animal disease eradication was set up in 2000, the subgroup for bovine tuberculosis was among the first created. The subgroup revised the Working Document on the eradication of bovine tuberculosis in the EU in 2012 (published 2013).  
Materials and methods Experiences, as documented in the reports from visits to member states and in the Working document were compiled by the subgroup. Results and discussion Some general issues that are important to all eradication programmes include disease definition, programme organisation, training and education, quality control, data analyses, enforcement and stakeholder involvement. Specific issues that must be adapted to the regional situation include definition and application of the epidemiological unit, use and interpretation of different diagnostic tests, management of infected herds and surveillance. The subgroup suggests that bovine tuberculosis be defined as "infection in all Bos species by any disease-causing species in the M. tuberculosis-complex". Education, training and quality assurance is important on all levels of the control programme but the specific needs vary depending on the structure of the organisation in different member states. We have seen a need for many local and regional science informed adaptations of control and eradication tools such as the use of different test combinations, testing frequency, test interpretation, targeted surveillance and eradication strategy in individual herds. A key to success is the access to, and continuous epidemiological analyses of, relevant data to monitor, assess and strengthen the programmes.

Tamba°M, Natalini S, Paternoster°G, Santi °A, Galletti°G, Caminiti°A, Licata E, Garbarino°C, Arrigoni°N

### **Bulk-tank milk ELISA for estimating the prevalence of paratuberculosis in dairy herds of a Northern Italian Region**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 200 (Poster P-06.26) [Nr. Estr. 5759]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

The aim of this study was to estimate the herd prevalence of paratuberculosis in dairy herds of the Emilia-Romagna Region, Northern Italy. Between 2011 and 2012, a monitoring plan was carried out in 2859 dairy herds, 80% of all regional dairy herds. We tested bulk tank milk every four months, for a total of three samplings per herd, using a commercial ELISA kit (ID VET, France). On the whole, bulk milk tested positive at least once in 440 herds (15.4%). Adjusting for test sensitivity (Se=0.35) and specificity (Sp=0.995), the estimated real herd prevalence was 43.2% (95%CI: 39.4%-47.2%). Apparent herd prevalence increased according to herd size, reaching 50% among herds larger than 500 cattle. Conversely, we found no association between sampling season (four-month periods) and probability to have a positive result. An additional sampling was performed in 175 herds, randomly selected from those always negative to ELISA bulk milk test: in each herd we collected 30 serum samples from cows older than 36 months for ELISA, and six environmental faecal samples for PCR

and culture. Out of these 175 herds, 79 (45.1%, 95%CI: 37.6%-53.0%) were MAP infected (at least one positive serum and/or environmental sample). Five herds tested positive for environmental samples only. In 60 out of these 79 positive herds within-herd seroprevalence was lower than 5%. Also for this additional sampling, as for ELISA bulk milk results, herd-level prevalence increased according to herd size; in fact 8 out of the 10 investigated herds over 500 cattle were infected. In the Emilia-Romagna region, the estimated MAP herd prevalence was about 45%, but it is likely to be higher among large-size herds. As found in other researches, repeated bulk milk ELISA testing seems a useful tool to screen herds with high prevalence.

Tamba°M, Paternoster°G, Formigoni A, Garbarino°C , Santi°A, Galletti°G, Fustini M, Caminiti°A, Natalini S, Arrigoni°N

### **Estimation of economic losses associated with Johne's disease in dairy herds of Northern Italy**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 118 (Poster P-03.27) [Nr. Estr. 5768]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

The aim of this study was to estimate the economic losses related to *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection in dairy herds of the Emilia-Romagna Region, Northern Italy. Results of a monitoring plan carried out on bulk milk samples in 2011-2012 were used to recruit case (n=40) and control dairy herds (n=46). Herds were tested using a commercial ELISA every four months, for a total of three tests. Cases were selected among herds positive to at least one bulk milk test. In these herds, serological testing was carried out on heads older than 36 months using the same ELISA to confirm MAP infection and estimate prevalence. In 175 herds where all the bulk milk tests were negative, we serologically tested a sample of 30 cows older than 36 months, and collected six fecal environmental samples for PCR and cultural tests. If all these tests were negative, the herd was considered as control. Each case was matched with at least one control according to the geographic location and herd size. For the selected herds, we collected data on cows older than 24 months reared during the two years preceding the individual serological testing. Data such as ear-tag, sex, breed, type of movement, date of birth, date of death/culling were extracted from the farm register of the Cattle National Database. A total of 19,215 cows, mostly Friesian, were considered. For cases we found a shorter life of culled cows (62.3 vs 64.5 months; p<0.01), a higher culling rate (31.7% vs 27.3%; p=0.02), and a higher mortality rate (3.6% vs 2.6%; p=0.07). We used a spreadsheet to quantify costs due to the observed differences. For case herds we estimated a loss of more than 200€/cow/year: 60% of costs were related to reduced milk production due to the shorter life of cows. This study, the first on economic impact of Paratuberculosis in Italy, suggests that MAP is associated with herd-level economic losses, supporting the implementation of control programs to recover producers profitability.

Tamba°M, Paternoster°G, Formigoni A, Garbarino°C , Santi°A, Galletti°G, Fustini M, Caminiti°A, Natalini S, Arrigoni°N

### **Perdite economiche associate alla presenza di paratubercolosi in allevamento** = Estimation of economic losses associated with Johne's Disease in dairy herds of Northern Italy

Large Anim Rev. - Vol. 20 no 4 ( 2014). - p 147-151. - 14 bib ref [Nr. Estr. 5815]

Allo scopo di valutare le perdite economiche correlate all'infezione da *Mycobacterium avium* subsp. *paratuberculosis* (MAP) ne-gli allevamenti da latte della Regione Emilia-Romagna, i risultati di un piano di monitoraggio effettuato nel 2011-2012 su cam-pioni di latte di massa sono stati usati per reclutare aziende caso (n=40) e controllo (n=46). Gli allevamenti sono stati testati tre volte con cadenza quadrimestrale tramite test ELISA. I casi sono stati selezionati tra gli allevamenti positivi ad almeno una prova sul latte di massa. Negli allevamenti con tutti i test di latte di massa negativi, è

stato effettuato un approfondimento diagnostico costituito da un controllo sierologico di 30 vacche di età superiore a 36 mesi e dal prelievo di sei pool di feci ambientali, sulle quali è stato ricercato MAP mediante coltura e qPCR. La mandria è stata considerata come controllo se tutti questi test erano negativi. A ogni caso è stato abbinato almeno un controllo considerando la posizione geografica e le dimensioni dell'allevamento. Per gli allevamenti arruolati, sono stati estratti dall'Anagrafe Bovina Nazionale i dati (identificativo, sesso, razza, tipo di movimentazione, data di nascita, data di morte/macellazione) delle vacche (età >24 mesi) allevate nei due anni precedenti il controllo sierologico individuale. Complessivamente sono entrate nello studio 19.215 vacche, per lo più di razza Frisona. Negli allevamenti caso è stata rilevata una vita più breve delle vacche riformate (62,3 vs 64,5 mesi,  $p<0,01$ ), un tasso di riforma più elevato (31,7% vs 27,3%,  $p=0,02$ ) e una mortalità più elevata (3,6% vs 2,6%;  $p=0,07$ ) rispetto ai controlli è stato utilizzato un foglio di calcolo per quantificare i costi correlati alle differenze osservate. Negli allevamenti infetti da MAP è stata stimata una perdita di oltre 200 €/vacca/anno: il 60% del danno è legato alla minor durata della vita produttiva delle vacche riformate. Questo studio, il primo sull'impatto economico della paratubercolosi in Italia, mostra che MAP è associato a perdite economiche significative che consigliano l'attuazione di programmi aziendali di controllo finalizzati ad un recupero di redditività delle aziende..

*Introduction - Quantification of the economic losses related to Mycobacterium avium subsp. paratuberculosis (MAP) infection is essential to encourage participation of dairy cattle producers in Paratuberculosis control programs. The aim of this study was to estimate the loss of income related to MAP infection in dairy herds of the Emilia-Romagna Region, Northern Italy. Materials - Results of a monitoring plan carried out on bulk milk samples in 2011-2012 were used to recruit case (n=40) and control dairy herds (n=46). Herds were tested using a commercial ELISA every four months, for a total of three tests. Cases were selected among herds positive to at least one bulk milk test. In these herds, serological testing was carried out on heads older than 36 months using the same ELISA to confirm MAP infection and estimate prevalence. In 175 herds tested a sample of 30 cows older than 36 months, and collected six fecal environmental samples for PCR and cultura) tests. If all these tests were negative, the herd was considered as control. Each case was matched with at least one control according to the geographic location and herd size. For the selected herds, we collected data on cows older than 24 months reared during the two years preceding the individual serological testing. Data such as eartag, sex, breed, type of movement, date of birth, date of death/culling were extracted from the farm register of the Cattle National Database. Results - A total of 19,215 cows, mostly Friesian, were considered. For cases we found a shorter life of culled cows (62.3 vs 64.5 months;  $p<0.01$ ), a higher culling rate (31.7% vs 27.3%;  $p=0.02$ ), and a higher mortality rate (3.6% vs 2.6%;  $p=0.07$ ). We used a spreadsheet to quantify costs due to the observed differences. For case herds we estimated a loss of more than 200 €/cow/year: 60% of costs were related to reduced milk production due to the shorter life of cows. Conclusions - This study, the first on economic impact of Paratuberculosis in Italy, suggests that MAP is associated with herd-level economic losses, supporting the implementation of control programs to recover producers profitability.*

Tosi°G, Fiorentini°L, Ceroni S, Casadio°M, Compagnoni E, Massi°P

#### **Andamento della contaminazione da deossinivalenolo (DON) e da tossina T-2 in mangimi destinati a specie avicole e descrizione di casi clinici correlati nel pollo da carne**

Atti della Società Italiana di Patologia Aviare (SIPA) : Salsomaggiore Terme (PR) 8 - 9 Maggio 2014 : LIII Convegno annuale / [s.l. : s.n., 2014]. - p 198-203. - 7 bib ref [Nr. Estr. 5976]

Convegno annuale Società Italiana Patologia Aviare (SIPA) (53. : Salsomaggiore Terme (PR) : 8 - 9 Maggio 2014)

*A 2-year survey was carried out in order to evaluate the incidence of mycotoxins in poultry feed. Fusarium mycotoxins tested were those known for their impact on feed industry and poultry husbandry, namely deoxynivalenol (DON) and T-2 toxin. A total of 235 feed samples were analyzed. The data obtained showed an increase in the levels of DON and T-2 toxin in 2013 and, in particular, in the summer-autumn period. In the same period some outbreaks linked to a trichotecenes mycotoxicosis were observed in broiler chicken flocks. The toxic effects (clinical signs and lesions) of trichotecenes observed in these cases are described.*

Trevisi E, Amadori°M, Riva F, Bertoni G, Bani P

**Evaluation of innate immune responses in bovine forestomachs**

Res Vet Sci. - Vol. 96 ( 2014). - p 69-78. - 40 bib ref [Nr. Estr. 5669]

Previous studies had indicated an active role of bovine forestomachs in the response to alimentary disorders as well as to inflammatory and infectious processes in both the gastro-intestinal (GI) tract and elsewhere. We investigated the potential of bovine forestomachs to receive, elaborate and produce signals and mediators of the innate immune response. Indeed, we detected the expression of Toll IL-1R8/single Ig IL-1-related receptor (TIR8/SIGIRR) and other receptors and cytokines, such as Toll-like receptor (TLR)4, interleukin (IL)-1 $\beta$ , IL-10 and Caspase-1 in the forestomach walls of healthy cows. Their presence suggests an active role of forestomachs in inflammatory disorders of the GI tract and other body compartments. Moreover, interferon (IFN)- $\gamma$  was revealed in ruminal content. We confirmed and further characterized the presence of leukocytes in the rumen fluids. In particular, T-, B-lymphocytes and myeloid lineage cells were detected in the ruminal content of both rumen-fistulated heifers and diseased cows. An acidogenic diet based on daily supplements of maize was shown to inhibit leukocyte accumulation, as opposed to a control, hay-based diet, with or without a soy flour (protein) supplement. On the whole, results indicate that bovine forestomachs can receive and elaborate signals for the immune cells infiltrating the rumen content or other organs. Forestomachs can thus participate in a cross-talk with the lymphoid tissues in the oral cavity and promote regulatory actions at both regional and systemic levels; these might include the control of dry matter intake as a function of fundamental metabolic requirements of ruminants.

Trevisi E, Zecconi A, Cogrossi S, Razzuoli°E, Gros si P, Amadori°M

**Strategies for reduced antibiotic usage in dairy cattle farms**

Res Vet Sci. - Vol. 96 ( 2014). - p 229-233. - 43 bib ref [Nr. Estr. 5663]

The need for antibiotic treatments in dairy cattle farms can be reduced by a combined intervention scheme based on: (1) timely clinical inspections, (2) the assessment of animal-based welfare parameters, and (3) the use of predictive laboratory tests. These can provide greater insight into environmental adaptation of dairy cows and define animals at risk of contracting disease. In the long-term, an improved disease control justifies the adoption of such a combined strategy. Many antibiotic treatments for chronic disease cases are often not justified with a cost/benefit analysis, because the repeated drug administration does not give rise to the expected outcome in terms of animal health. In particular, compared with untreated cases, antibiotics may not lead to greater cure rates for some forms of mastitis. Lastly, a substantial reduction of antibiotic usage in dairy farms can be achieved through the proper use of immunomodulators, aimed at increasing immunocompetence and disease resistance of cows.

Trevisi P, Colombo M, Priori D, Motta V, La\_Torre R, Archetti°I, GherPELLI°Y,  
D'InCà R, Bosi P

**Effects of live yeast added to the weaning diet on health, immunity and gastrointestinal functionality, of susceptible weaning pigs orally challenged with E. coli F4ac**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - p 283. - 1 bib ref [Nr. Estr. 5755]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Tucci T, Roveri M, Alborali°G, Ferrari°M, Tecli M E, Dereu A, Doncecchi P, Pineiro C, Morales J

**Intensive immunization of gilts improves health status of their progenies**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 208 (Poster 149) [Nr. Estr. 5721]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

The aim of this study was to evaluate the effect of intensive immunization of gilts on productive performance and health status of their progenies. The experiment was conducted in a farrow-to-finish commercial farm in Mantova (Italy). A total of 48 sows (32 gilts and 16 mature sows) from two consecutive batches (24 sows per batch) were used and managed as follows: 16 gilts (GILT) under usual vaccination program (Aujeszky, PRRS, Erysipela, Parvovirus); 16 gilts (H-GILT) were more intensively immunized adding Circovirus and colibacillosis vaccines; and mature sows (SOW), parities 3-6, under usual vaccination program. After weaning (21 days of age), pigs were distributed in 36 pens of 12 pigs each (12 pens per treatment). Pigs were weighed at birth, weaning, end of nursery phase (63 days of age) and every two months until slaughtering at 270 days of age (about 160 kg BW). At slaughtering, lung pneumonia lesions and presence of pleuritis were individually evaluated. Data were analysed by GLM models of SAS. No differences were found between treatments at birth weight (1.5 kg). At weaning and at the end of nursery phase, the SOW group had significantly higher body weight (BW) than the other two groups, while no differences were found between both gilts groups. At the end of the fattening period, no differences were observed in BW between groups, but unexpectedly final BW was numerically higher in GILT (166.4 kg) than in SOW (163.0 kg) and in H-GILT (161.0 kg) groups. Percentage of mortality was higher ( $P<0.05$ ) in the GILT group (39.2%) than in H-GILT (21.8%) and in SOW (18.3%) groups. Mortality was especially higher in the growing-fattening period, due to a respiratory disease outbreak. Higher mortality in GILT group caused higher space allowance at the end of the fattening period in this group, which could be associated with higher final BW. Presence of lung lesions, pneumonia and/or pleuritis, was higher ( $P<0.05$ ) in GILT (83.5% lungs affected) than in H-GILT (65.1) and in SOW (68.2%) groups. We conclude that intensive immunization of gilts decreased mortality and the presence of lung lesions at the slaughtering, although did not affect productive performance. We can conclude that, in a low-health status farm, intensive immunization of gilts improves health status of their progenies.

Vallini C, Annibale O, Menotta°S, Rubini°S, Tarri cone L

**Two more cases of Green Turtles (*Chelonia mydas*) in the Italian Waters of the Northwestern Adriatic Sea and an inorganic contaminant investigation**

Marine Turtle Newsletters. - Vol. 143 ( 2014). - p 7-9. - 14 bib ref [Nr. Estr. 5866]

Veronesi F, Morganti G, Di\_Cesare A, Lepri E, Cassini R, Zanet S, Deni D, Chiari° M, Ferroglio E

***Eucoleus boehmi* infection in red fox (*Vulpes vulpes*) from Italy**

Vet Parasitol. - Vol. 206 ( 2014). - p 232-239. - 47 bib ref [Nr. Estr. 5933]

In the last decade an increase of the number of red foxes in anthropized habitats across European countries, including Italy, has been observed. This poses implications in terms of disease transmission between wildlife and domestic animals; in fact, there are evidences of the role of foxes as reservoirs and amplifiers of a broad spectrum of parasites infecting pets. The present study evaluated the prevalence of *Eucoleus boehmi*, an emerging extra-intestinal nematodes of the Capillariinae subfamily, in red foxes. The nasal passages and sinuses of 179 red foxes culled from several areas of northern and central Italy were inspected and the mucosal surfaces were scrapped and examined for adult nematodes and eggs, microscopically and genetically identified. Overall 55

foxes (30.7%) were found to be infected with *E. boehmi*, i.e. 27 on inspection of the nasal passages and sinuses and 28 on mucosal flush and scraping. The occurrence of *E. boehmi* was significantly ( $p < 0.05$ ) correlated to the sampling location, the age and gender of the animals examined; the higher rates of prevalence were observed in animals culled in Piedmont (43.3%) and in female (60.6%) and adult (38.1%) subjects. A total of 184 adult parasites were recovered, with a mean intensity of infection of 3.34, and a more frequent localization of *E. boehmi* in the nasal passages rather than in the sinuses. A significant ( $p < 0.05$ ) relationship was found between the parasite burden and body condition and age of the animals; the intensity of infection was significantly higher in juveniles (mean: 6.3 specimens) and in animals showing poor fox body condition (mean: 7.8 specimens). These results show that *E. boehmi* is highly prevalent in the red fox populations of certain areas of Italy. Epidemiological implications are discussed, with a special focus on the role that this wild canid may have in the increasing transmission of nasal eucoleosis to domestic dogs.

Veronesi G, Faccini<sup>o</sup>S, Barbieri<sup>o</sup>I, Cominotti F, Nigrelli<sup>o</sup>AD

**Effects of management strategies on abortion episodes and PRRSV circulation in an endemically infected breeding farm**

6th European Symposium on Porcine Health Managements : 7th - 9th May, 2014 Sorrento, Italy : proceedings / [s.l. : s.n., 2014]. - p 181 (Poster P96). - 3 bib ref [Nr. Estr. 5940]

European Symposium on Porcine Health Managements (ESPHM) (6th : Sorrento, Italy : 7th - 9th May, 2014)

This field study describes the dynamics of PRRSV circulation in an endemically infected breeding farm, before, during and after a significant management change, aimed to decrease the abortions in late gestation stages. The farm was a farrow-to-nursery of more than 700 productive sows, located in a high density swine area of northern Italy. The farm (A) was part of a group, of a same owner, constituted by another farrow-to-nursery facility (B) and a quarantine unit, both placed at more than 5 Km. The history of PRRSV presence in the farms was documented by an archive of sequences collected since 2008. The study covers the period from the beginning of 2011 to September 2013. The reorganization, which began at the end of 2011, was completed in the second half of 2012. The purpose of the change was to better separate animals of different age, immune condition, parity and care necessities, a measure known to be useful for improving both production performance and PRRS control. In practice: sows were segregated from gilts (sows in farm A and gilts in B), reducing in the meantime the number of the latter, and weaned pigs were decreased in farm A by transferring them to farm B. Periodic blood samplings were performed (>600 samples) for serological and virological analyses. About 95% of sows, in farm A, were found PRRS seropositive (mean S/P $\pm$ SD 2.2 $\pm$ 0.98), but none were demonstrated viremic by Real-Time PCR. In contrast, 59% of blood samples collected from sows at time of abortion, during outbreaks, were PCR positive. PRRSV isolates were sequenced (orf5 and orf7) in order to acquire epidemiological information. Production and reproductive data, recorded by the farm software, were evaluated together with laboratory results on PRRSV circulation. The proportion of abortions over farrowings, either in farm A or in the whole group, was significantly ( $P < 0.01$ ) reduced after the introduction of the new management system, while the ratio remained almost the same in farm B. No significant change could be reported for other production and reproductive parameters. These results have been achieved despite 2, of the 4 abortion peaks recorded, occurred after the beginning of reorganization. Sequence analysis revealed that all abortion outbreaks were related to the entry of new PRRSV strains, suggesting the presence of breaks in the biosecurity system. The study demonstrates the effectiveness of the new management strategies in reducing abortions, but at the same time highlights, once again, how a strict biosecurity is an unavoidable requirement for PRRSV control.

Veronesi G, Faccini<sup>o</sup>S, Barbieri<sup>o</sup>I, Cominotti F, Rosina S, Beccalossi M, Nigrelli<sup>o</sup>AD

**Effects of management strategies on abortion episodes and PRRSV circulation in an**

### **endemically infected breeding farm**

Proceedings of the 23rd International Pig Veterinary Society (IPVS) Congress : June 8 - 11, 2014, Cancun, Mexico / [s.l. : s.n., 2014]. - p 623. - 4 bib ref [Nr. Estr. 5754]

International Pig Veterinary Society Congress (IPVS) (23rd : Cancun, Mexico : June 8 - 11, 2014)

Vezzoli°F, Benedetti°V, Sinelli M, Luini°M

#### **L'esame ispettivo ante-mortem per la valutazione del benessere delle scrofe in allevamento**

= Ante-mortem inspection for the evaluation of the farm welfare in sows

Atti Convegno SIPAS. - Vol. 40 ( 2014). - p 113-120. - 14 bib ref [Nr. Estr. 5678]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (40. : Montichiari (BS) : 27-28 Marzo 2014)

Il nostro studio nasce all'interno di un Progetto di Ricerca Ministeriale in corso che ha come obiettivo quello di verificare se sia possibile utilizzare al macello le stesse osservazioni animali basate che possono essere rilevate in allevamento utilizzando la metodologia proposta dal Welfare Quality®. In questa fase preliminare è stata presa in considerazione la categoria scrofe. È stata costruita una scheda per la registrazione dei rilievi riferibili al benessere animale, osservabili al macello durante la visita ispettiva ante-mortem. Tali rilievi sono elencati in 4 gruppi riferiti al comportamento (Good housing), all'alimentazione (Good feeding), alla appropriatezza dei ricoveri (Good Housing) e alla salute degli animali (Good health) per presenza di lesioni o di malattie. Sono state effettuate osservazioni su 758 scrofe pervenute al macello direttamente da 3 allevamenti (rispettivamente n. 263, 60 e 90) e da un centro di raccolta (n. 345). Su un totale di 404 osservazioni di "non conformità", oltre il 40% sono riferite a Good health (lesioni), il 36,3% a Good housing, per la presenza di bursiti e ulcere o di imbrattamento fecale soprattutto in un allevamento. Un BCS insufficiente (Good feeding) è stato rilevato in una piccola percentuale delle scrofe, soprattutto in un allevamento. Problemi di Good health (malattia) hanno interessato l'11,2% delle osservazioni. Il nostro studio evidenzia che le osservazioni al macello effettuate in fase ante mortem, soprattutto se completate da rilievi ispettivi post-mortem, possono rappresentare, con alcune limitazioni, un efficace sistema per il monitoraggio del benessere a livello di allevamento.

*Our study is part of a in progress Research Project that aims to verify whether it is possible to use at slaughter the same observations proposed by the Welfare Quality O. In this preliminary phase was taken into account the category sows. A check list for recording the findings related to animal welfare, observed at the slaughterhouse during ante-mortem inspection has been prepared. These findings are listed in 4 principles regarding behavior (Appropriate behavior), feeding (Good feeding), housing (Good Housing) and animal health (Good health) for the presence of injury or diseases. Observations were made on 758 sows received directly to slaughter from 3 herds ( respectively n . 263 , 60 and 90 ) and from a holding pens (n. 345) . Out of a total of 404 observations of " non-compliance" , more than 40% are related to Good health (lesions), 36.3% to Good housing, due to the presence of bursitis and ulcers or fecal soiling especially in one herd. An inadequate BCS (Good feeding ) has been detected in a small percentage of sows, especially in one herd. Good health problems (diseases) ) are involved in the 11,2 % of the observations. Our study shows that the observations made during the ante mortem inspection, especially if supplemented by post-mortem inspection findings, with certain limitations, may be an effective system for monitoring the welfare at farm level.*

Vicari°N, Bianchi°A, Manfredini°A, Giacomelli°S , Genchi°M, Bertoletti°I, Fabbio°M

#### **A survey on Francisella tularensis in Sondrio Province after importation of infected hares**

Proceedings International conference on diseases of zoo and wild animals 2014 : May 28-31, Warsaw, Poland / [s.l. : s.n., 2014]. - p 214 [Nr. Estr. 5779]

International conference on diseases of zoo and wild animals : Warsaw, Poland : May 28-31, 2014)

Tularemia is a rare zoonotic disease caused by *Francisella tularensis*. This bacterium has been recovered from numerous animal species and can be transmitted to humans through different routes of infections. In Europe, only *F. tularensis* subsp. *holarctica* (type B) was reported. In Italy, most human infections are caused by ingestion of contaminated drinking water and more rarely after contacts with infected animals such as hares or arthropod bites. In Italy, infected hares are found in different regions in particular in Northern and Central Italy representing a potential risk for exposed humans such as hunters. In Sondrio Province (Italian Alps), the emergence of tularemia started in 2005 due to imported infected European brown hares (*Lepus europaeus*). Since that year, a survey on susceptible species (lagomorphs, ungulates, foxes and arthropods) was carried out in the area. We report the results of the survey. Over the past decade a total of 803 samples collected from mammals (n = 747) and ticks (n = 56) have been tested (535 by PCR and 273 by serum agglutination). No positive results were found except for two ticks (*Ixodes ricinus*) collected in spring 2013 from the carcass of a dead roe deer. The ticks were found *F. tularensis* (type B) positive by PCR. The genotyping performed by MLVA analyses shows a genotype typical for Eastern Europe. These preliminary results suggest to continue in monitoring the spread of *F. tularensis* in Sondrio Province focusing the investigation on arthropod-vectors rather than on susceptible animals to clarify if this genotype has been settled in the area.

Vicari°N, Manfredini°A, Mandola ML, Rizzo F, Laba Iestra°I, Prati°P, Magnino°S, Fabbi°M

#### **Investigation for new chlamydial species in wild birds**

12th German Chlamydia Workshop : 2-4 Aprile 2014 Berlino / [s.l. : s.n., 2014]. - p 21 [Nr. Estr. 5934]

German Chlamydia Workshop (12th : Berlino : 2-4 Aprile 2014)

The family Chlamydiaceae encompasses nine well-known species in the single genus *Chlamydia*. However, two new species, *C. avium* and *C. gallinaceae* (ACC) have been recently proposed. These new species have been detected in pigeons, chickens and Ratearle birds in Italy, Germany and France. In order to investigate the presence of these novel chlamydiae in wild birds, a retrospective study was performed applying two different specific real-time PCRs, one for *C.91.aM2ta* (ACC) and one for *C. avium* (ACP) on ghiaia t/Aste-positive samples obtained from different species. Samples (viscere, faeces and cjp,acal swabs) had been submitted between 2009 and 2013 to the National Reference Laboratory for Animal Chlamydioses. The DNA extracts were reanalysed with both a real-time PCR targeting the 23S gene and a PCR-RFLP assay targeting the 16S gene for the Chlamydiaceae family. All positive confirmed samples were then analysed with the new specific real-time PCRs mentioned above. Out of 41 samples, eight (seven from pigeons, and one from a magpie) tested positive with the real-time PCR specific for ACP, while no sample tested positive for ACC. These results suggest epidemiological importance of the new chlamydial species.

Viglietti A, Fonti P, De\_Francesco I, Genchi C, Piazza C, Ferrari N, Genchi°M

#### **Study of *Aelurostrongylus abstrusus* in a spontaneous infection in cats**

XXVIII Congresso Nazionale Societa' Italiana di Parassitologia SOIPA : Roma, 24-27 giugno 2014 / [s.l. : s.n., 2014]. - p 98 [Nr. Estr. 5821]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA (28. : Roma : 24-27 giugno 2014)

**INTRODUCTION:** *Aelurostrongylus abstrusus* is a lungworm of the domestic and wild felids distributed worldwide. Prevalence rates of *A. abstrusus* in cats are often variable. Global prevalence rate varies from 50% free roaming cats in Albania (Knaus et al., Wien. Klin. Wochenschr.,

123:31-35) to 1% in a mixed cat population in Spain (Miró et al., 2004, *Vet. Parasitol.*, 126: 249-55). The clinical manifestations are highly variable. Radiographic findings are dependent on the age of animal, on the period of illness and the chronicity of infection. The aim of this study was to define the effects of the number of larvae in faeces (LPG) on respiratory clinical signs and their relation with radiographic findings. **MATERIALS AND METHODS:** 196 cats, referred to the veterinary clinic for routine examinations, vaccinations, or different conditions and or hospitalization were included in the study. All cats come from the island of San Pietro, Sardinia, Italy. The history of each cat and clinical symptoms were recorded. For each cats, fecal samples were collected on 3 consecutive days and examined by Bearmann technique and for quantifying larva (LPG) by FLOTAC. Four age groups and 4 groups of severity of symptoms were formed: 1) 2-6 months; 2) 7-11 months; 3) 1-5 years; 4) > 6 years; 0) asymptomatic; 1) mild symptoms; 2) moderate symptoms; 3) severe symptoms. Thorax radiographs in double orthogonal position were performed and radiographs, were evaluated to giving a score depending on the severity of the lesions. All radiographs were reviewed blinded by 3 coauthors one clinician, one expert radiologist and one veterinary radiology from academy. Positive cats were treated with emodepside 2.1%/praziquantel 8.6% spot-on (Profender®, Bayer). After 15 and 28 days a fecal examination with FLOTAC was performed to assess the treatment efficacy. **RESULTS:** 52 out of the 196 cats (26.5%) were positive for *A. abstrusus* at fecal examination. The prevalence in lifestyle was: indoor 3.3%; outdoor 32.3% and mixed 25.6%. In age groups was: group 1) 28.1%; group 2) 23%; group 3) 24.3%; group 4) 23%. Asymptomatic cats were 27%, 33% presented mild symptoms, 29% moderate symptoms and 11% severe symptoms. The number of L1 ranged from a minimum of 25 LPG to a maximum of 144800 LPG. Increasing numbers of larvae were associate with higher probabilities to develop high symptoms scores while cats age, their Rx patterns and their interactions did not show significant effects. After 15 days and 28 days post-treatment with emodepside 2.1%/praziquantel 8.6% spot-on (Profender®, Bayer), 29.4% and 4.3% cats were still positive, respectively. **CONCLUSIONS:** Our data show that all age groups were infected equally and increasing of numbers of LPG were associated with higher probabilities to develop more severe symptoms. Moreover infestations by *A. abstrusus* are poorly defined and prevalences were high even in asymptomatic cats.

Vitale N, Possidente R, D'Errico V, Dondo A, Bergagna S, Barbero R, Meistro S, Bozzetta E, Peletto S, Cerrutti F, Romano A, Gorla M, Arrigoni N, Chiavacci L

### **Estimating diagnostic accuracy of paratuberculosis (PTB) diagnostic test with latent class models**

12th International Colloquium on Paratuberculosis : Parma, Italy, 22/26 June 2014 : program and abstracts / [s.l. : s.n., 2014]. - p 67 (Poster P-02.10) [Nr. Estr. 5772]

International Colloquium on Paratuberculosis (ICP) (12th : Parma, Italy : 22/26 June 2014)

A prospective longitudinal study was carried out on 2 dairy herds in order to evaluate diagnostic test for PTB. A cohort of 300 dairy cattle (from 1.5 to 4.5 years of age) were selected and tested 7 times from July 2010 to July 2012. At each sampling, every 4 months, serum ELISA, faecal culture (FC) and PCR on individual faeces were performed. Different latent class models were compared by the method of Maximum Likelihood and Bayesian inference. Maximum Likelihood (ML) analysis was performed by IEM (log-linear and event history analysis with missing data using the EM algorithm, J. K. Vermunt), using the Deviance Information Criterion (DIC), starting with the simplest model under conditional independence and gradually increasing the complexity by including conditional covariances between test pairs. Bayesian analysis was performed by WINBUGS, comparing different models: assuming conditional independence, including conditional dependence between pairs of tests (Elisa- FC, Elisa -PCR, FC-PCR). Priors for sensitivity and specificity of tests were based on data reported by literature. For ML analysis, according to the deviance information criterion, the best model was the one allowing a dependency between ELISA and FC. Also for Bayesian analysis, the best model accounted for conditional dependence between ELISA and FC. Results of ML analysis overlapped to Bayesian analysis. Estimated mean within-herd prevalence ranged from 21% to 44%. FC test highlighted the presence of 5 high shedders. ELISA overall sensitivity resulted 82.7% (CI95%:70.5%-91.7%), FC Se: 83.8% (CI95%: 69.7%-95.4%) and PCR Se: 91.8% (CI95%: 81.9%- 97.5%). ELISA overall Specificity resulted 97.7% (CI95%: 96.8%-98.4%); FC Sp: 99.8%

(CI95%: 99.5% - 100%) and PCR Sp: 88.7% (CI95%: 86.9%-90.2%). Results from this study are similar to that reported in literature for high paratuberculosis within-herd prevalence. The dependence found between ELISA and FC was reported in literature to be correlated with the disease stage.

Vitali A, Lana E, Amadori M, Bernabucci U, Nardone A, Lacetera N

**Analysis of factors associated with mortality of heavy slaughter pigs during transport and lairage**

J Anim Sci. - Vol. 92 ( 2014). - p 5134-5141. - 23 bib ref [Nr. Estr. 5865]

The study was based on data collected during 5 yr (2003–2007) and was aimed at assessing the effects of the month, slaughter house of destination (differing for stocking density, openings, brightness, and cooling device types), length of the journey, and temperature–humidity index (THI) on mortality of heavy slaughter pigs (approximately 160 kg live weight) during transport and lairage. Data were obtained from 24,098 journeys and 3,676,153 pigs transported from 1,618 farms to 3 slaughter houses. Individual shipments were the unit of observation. The terms dead on arrival (DOA) and dead in pen (DIP) refer to pigs that died during transport and in lairage at the abattoir before slaughtering, respectively. These 2 variables were assessed as the dependent counts in separate univariate Poisson regressions. The independent variables assessed univariately in each set of regressions were month of shipment, slaughter house of destination, time traveled, and each combination of the month with the time traveled. Two separate piecewise regressions were done. One used DOA counts within THI levels over pigs transported as a dependent ratio and the second used DIP counts within THI levels over pigs from a transport kept in lairage as a dependent ratio. The THI was the sole independent variable in each case. The month with the greatest frequency of deaths was July with a risk ratio of 1.22 (confidence interval: 1.06–1.36;  $P < 0.05$ ) and 1.27 (confidence interval: 1.06–1.51;  $P < 0.05$ ) for DOA and DIP, respectively. The lower mortality risk ratios for DOA and DIP were recorded for January and March ( $P < 0.05$ ). The aggregated data of the summer (June, July, and August) versus non-summer (January, March, September, and November) months showed a greater risk of pigs dying during the hot season when considering both transport and lairage ( $P < 0.05$ ). The mortality risk ratio of DIP was lower at the slaughter house with the lowest stocking density (0.64 m<sup>2</sup>/100 kg live weight), large open windows on the roof and sidewalls, low brightness (40 lx) lights, and high-pressure sprinklers as cooling devices. The mortality risk ratio of DOA increased significantly for journeys longer than 2 h, whereas no relationship was found between length of transport and DIP. The piecewise analysis pointed out that 78.5 and 73.6 THI were the thresholds above which the mortality rate increased significantly for DOA and DIP, respectively. These results may help the pig industry to improve the welfare of heavy slaughter pigs during transport and lairage.

Wallerstroem S, Lagerqvist N, Temperton NJ, Cassmer M, Moreno A, Karlsson M, Leijon M, Lundkvist A, Falk KI

**Detection of antibodies against H5 and H7 strains in birds : evaluation of influenza pseudovirus particle neutralization tests**

Infect Ecology Epidemiol. - Vol. 4 ( 2014). - no 23011 (8 p). - 56 bib ref (ultimo accesso 23/02/2015 <http://dx.doi.org/10.3402/iee.v4.23011> ) [Nr. Estr. 5986]

Introduction: Avian influenza viruses circulate in bird populations, and it is important to maintain and uphold our knowledge of the viral strains that are currently of interest in this context. Here, we describe the use of hemagglutinin-pseudotype retroviruses based on highly pathogenic influenza viruses for the screening of avian sera for influenza A antibodies. Our aim was also to determine whether the pseudovirus neutralization tests that we assessed were sensitive and simple to use compared to the traditional methods, including hemagglutination inhibition assays and

microneutralization tests. Material and methods: H5 and H7 pseudovirus neutralization tests were evaluated by using serum from infected rabbits. Subsequently, the assays were further investigated using a panel of serum samples from avian species. The panel contained samples that were seropositive for five different hemagglutinin subtypes as well as influenza A seronegative samples. Results and discussion: The results suggest that the pseudovirus neutralization test is an alternative to hemagglutination inhibition assays, as we observed comparable titers to those of both standard microneutralizations assays as well as hemagglutinin inhibition assays. When evaluated by a panel of avian sera, the method also showed its capability to recognize antibodies directed toward low-pathogenic H5 and H7. Hence, we conclude that it is possible to use pseudoviruses based on highly pathogenic avian influenza viruses to screen avian sera for antibodies directed against influenza A subtypes H5 and H7.

Zaccaria G, Lelli°D, Boni A, Di\_Trani L, Papetti° A, Boniotti°MB, Cordioli°P, Moreno°A, Vaccari G

**Analisi Full genome mediante Next generation sequencing (su piattaforma ION TORRENT PGM™) di coronavirus isolato da pipistrello in Italia**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 92 [Nr. Estr. 5751]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

I chiroteri rappresentano il serbatoio naturale di numerosi patogeni virali trasmissibili all'uomo. Le analisi filogenetiche di coronavirus isolati da pipistrello hanno dimostrato l'origine zoonotica dell'epidemia di SARS-CoV, virus appartenente al genere  $\beta$ coronavirus ( $\beta$ -CoVs), ed evidenziato nel *Rhinolophus sinicus* l'ospite naturale più probabile. Dal 2012 sono stati segnalati 536 casi umani di infezione da un nuovo  $\beta$ -CoV, Mers-CoV, caratterizzati da elevata mortalità (145 morti confermate fino ad oggi). Tutti i casi riportati, compresi quelli denunciati in Europa, Asia sud-orientale e negli Stati Uniti, sono stati collegati a casi primari di infezione provenienti dal Medio Oriente. Gli studi epidemiologici e virologici hanno evidenziato nel cammello la probabile fonte di infezione primaria per l'uomo; inoltre numerose ricerche ipotizzano che alcune specie di chiroteri possano rappresentare il serbatoio naturale di un progenitore di Mers-CoV. Il nostro studio si propone di definire un protocollo per il sequenziamento del genoma di Coronavirus, utilizzando nuove tecnologie di sequenziamento (NGS). A tale scopo, è stato analizzato un campione di contenuto intestinale prelevato da un pipistrello appartenente alla famiglia dei Vespertilionidi, riscontrato positivo con RT-PCR specifica per CoV. Il genoma virale è stato amplificato utilizzando la tecnica sequence independent single primer amplification (SISPA) e sequenziato con sequenziatore Ion PGM™. L'analisi bioinformatica è stata realizzata con i software Ion Torrent suite e CLCbio utilizzando assemblaggio "de novo" e/o mapping. Le analisi bioinformatiche hanno permesso di definire il genoma completo del nostro isolato (circa 30 kb) e di identificare le principali regioni codificanti relative ai geni di ORF1ab, spike, envelope, membrana e nucleocapside, oltre a 5 regioni omologhe a Orf4a, Orf4b, Orf5 ed Orf8. Mediante l'analisi filogenetica della sequenza della RNA polimerasi RNA dipendente, il nostro isolato è stato classificato come appartenente al gruppo C dei  $\beta$ -CoVs. Il confronto della sequenza completa del campione con quelle disponibili in Genbank mostra un'omologia del 80% con un query-coverage del 80% con i Mers-CoV, mentre un'omologia del 78% con un query-coverage del 60% con il gruppo di Bat-CoV HKU5, isolati in Cina. I dati ottenuti nel presente studio dimostrano come la tecnica di NGS messa a punto e le analisi bioinformatiche adottate siano utilizzabili per ottenere il genoma completo di CoVs direttamente da campioni biologici, anche di isolati non strettamente correlati a quelli conosciuti.

Zanardi°G, Caminiti°A, Delle\_Donne°G, Moroni P, Santi°A, Galletti°G, Tamba°M, Bolzoni°G, Bertocchi°L

### **Comparing real-time PCR and bacteriological cultures for Streptococcus agalactiae and Staphylococcus aureus in bulk-tank milk samples**

J Dairy Sci. - Vol. 97 ( 2014). - p 5592-5598. - 20 bib ref [Nr. Estr. 5817]

For more than 30 yr, a control plan for Streptococcus agalactiae and Staphylococcus aureus has been carried out in more than 1,500 dairy herds of the province of Brescia (northern Italy). From 2010 to 2011, the apparent prevalence of Strep. agalactiae has been relatively stable around 10%, but the apparent prevalence of Staph. aureus has been greater than 40% with an increasing trend. The aim of this paper was to estimate and compare the diagnostic accuracy of 3 assays for the detection of Strep. agalactiae and Staph. aureus in bulk-tank milk samples (BTMS) in field conditions. The assays were a qualitative and a quantitative bacteriological culture (BC) for each pathogen and a homemade multiplex real-time PCR (rt-PCR). Because a gold standard was not available, the sensitivities (Se) and specificities (Sp) were evaluated using a Bayesian latent class approach. In 2012 we collected one BTMS from 165 dairy herds that were found positive for Strep. agalactiae in the previous 2-yr campaigns of eradication plan. In most cases, BTMS collected in these herds were positive for Staph. aureus as well, confirming the wide spread of this pathogen. At the same time we also collected composite milk samples from all the 8,624 lactating cows to evaluate the within-herd prevalence of Strep. agalactiae. Streptococcus agalactiae samples were cultured using a selective medium Tallium Kristalviolette Tossin, whereas for Staph. aureus, we used Baird Parker modified medium with added Rabbit Plasma Fibrinogen ISO-Formulation. In parallel, BTMS were tested using the rt-PCR. Regarding Strep. agalactiae, the posterior median of Se and Sp of the 2 BC was similar [qualitative BC: Se = 98%, posterior credible interval (95%PCI): 94–100%, and Sp = 99%, 95%PCI: 96–100%; quantitative BC: Se = 99%, 95%PCI: 96–100%, and Sp = 99%, 95%PCI: 95–100%] and higher than those of the rt-PCR (at 40 cycle threshold, Se = 92%, 95%PCI: 85–97%; Sp = 94%, 95%PCI: 88–98%). Also in case of Staph. aureus, the posterior medians of BC were generally higher than those of rt-PCR. In fact, although the Se of BC was slightly lower (rt-PCR at 40 cycle threshold, median Se = 99%, 95%PCI: 97–100%, and qualitative BC, median Se = 94%, 95%PCI: 87–99%), the Sp was much higher (rt-PCR at 40 cycle threshold, median Sp = 67%, 95%PCI: 38–97%; qualitative BC, median Sp = 95%; 95%PCI: 76–100%). Our study confirms that BC and rt-PCR are reliable diagnostic tools to detect Strep. agalactiae and Staph. aureus, and rt-PCR results should be confirmed by BC carried out on BTMS and possibly on composite milk samples.

Zanardi°G, Caminiti°A, Delle\_Donne°G, Moroni P, Santi°A, Galletti°G, Tamba°M, Bolzoni°G, Bertocchi°L

### **Comparing Real-Time Polymerase Chain Reaction and bacteriological cultures for Streptococcus agalactiae and Staphylococcus aureus in bulk tank milk samples**

Proceedings of the XXVIII World Buiatrics Congress : Cairns, 2014 : oral communication and poster abstracts / editor, David S. Beggs. - Australia : Australian Cattle Veterinarians, 2014. - p 204-205 [Nr. Estr. 5781]

World Buiatrics Congress (WBC) (28th : Cairns : 2014)

Zanotti°C, Lelli°D, Martinelli°N, Amadori°M

### **Parametri immunologici associati a diverse dosi di vaccini anti-porcine circovirus 2**

V Workshop Nazionale di Virologia Veterinaria : Teramo, 26-27 giugno 2014 : riassunti / a cura di Roberto Delogu ... [et al.]. - Roma : Istituto Superiore di Sanità, 2014. - (ISTISAN congressi ; 14/C3) p 93 [Nr. Estr. 5750]

Workshop Nazionale di Virologia Veterinaria (5. : Teramo : 26-27 giugno 2014)

L'utilizzo estensivo di vaccini contro il PCV2 si è rivelato efficace nel controllo dell'infezione virale e della sindrome multisistemica del deperimento del suino. In questo studio, 20 suini sono stati vaccinati con preparati a contenuto antigenico controllato per identificare parametri di risposta immunitaria umorale e cellulo-mediata predittivi della protezione in vivo nei confronti del virus. Due

gruppi di suini sono stati trattati rispettivamente con il vaccino inattivato Circovac e con solo adiuvante dello stesso vaccino, gli altri 3 con dosi scalari (10X, 5X e 2,5X) di un vaccino inattivato da noi preparato con particelle virali purificate a concentrazione nota. Dopo 4 settimane tutti i suini sono stati infettati per via intranasale con  $2 \times 10^5$  TCID<sub>50</sub> di virus PCV2a. I titoli Ab sono stati valutati mediante test ELISA a competizione e sieroneutralizzazione su cellule PK-15, la viremia tramite real-time PCR. La risposta cellulo-mediata è stata valutata come rilascio Ag-specifico di IFN- $\gamma$  da sangue intero (ELISA), numero di cellule Ag-specifiche secernenti IFN- $\gamma$  (ELISPOT) e proliferazione cellulare (rivelazione dell'antigene Ki-67). Tra il giorno 14 e 21 post-infezione i suini appartenenti al gruppo di controllo hanno sviluppato completa viremia, 3 suini appartenenti rispettivamente ai gruppi 10X, 5X e Circovac hanno mostrato viremia a titolo inferiore mentre i restanti soggetti sono risultati completamente protetti dall'infezione. La vaccinazione ha indotto un iniziale aumento dei titoli Ab ELISA proporzionale alla dose somministrata, un calo in concomitanza dell'infezione e un successivo rialzo dopo 14 giorni. 7 giorni dopo l'infezione si è osservato un aumento significativo di Ab neutralizzanti rispetto ai titoli pre-vaccinazione in tutti i gruppi tranne quello di controllo (aumento al 14° giorno), ma nessuna correlazione dose vaccinale-titoli Ab. I test di rilascio di IFN- $\gamma$  da sangue intero e di ricerca del Ki-67 hanno mostrato una chiara curva dose-risposta: sia nella fase post-vaccinazione che in quella postinfezione i suini trattati con dosaggio vaccinale più basso hanno mostrato una maggiore positività ad entrambi i test, associata ad una più elevata protezione verso la viremia. Il numero di cellule secernenti IFN- $\gamma$  non è risultato in accordo con questi dati. Tutti i dosaggi vaccinali testati erano in grado di azzerare o ridurre sensibilmente la carica virale mediante un meccanismo non correlato al titolo di Ab neutralizzanti. L'effetto protettivo potrebbe essere correlato alla capacità, soprattutto dei vaccini a più basso contenuto antigenico, di polarizzare la risposta immunitaria in senso antivirale, con un ruolo presumibilmente importante di linfociti T CD8 $\beta$ +, citotossici, Classe I-ristretti.

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#### **Modulation of plasma antioxidant activity in weaned piglets by plant polyphenols**

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This study was conducted to evaluate the effect of plant polyphenols (PP) on antioxidant activity in weaned piglets. First, a uniform design, one optimising an experimental technique that can rationally arrange the concentrations of mixture components, was used to obtain the best PP mixture of apple, grape seed, green tea and olive leaf polyphenols based on in vitro antioxidant capacity and inhibitory action on bacterial growth. Second, the optimised PP mixture was tested in vivo with an efficacy trial on piglets. The optimal effects of the mix were observed in vitro when apple, grape seed, green tea, olive leaf polyphenols and a carrier (silicon dioxide) accounted for 16.5, 27.5, 30, 2.5 and 23.5%, respectively, of the mixture. Forty-eight weaned piglets were randomly allocated to two dietary treatments (6 replicates of 4 piglets each per treatment) and fed a control diet (CTR) or CTR supplemented with 0.1% of the optimised PP mixture. Dietary PP did not affect growth performance compared to the CTR group. Plasma total protein, urea nitrogen and lysozyme content were not affected by dietary treatment. No differences of E. coli or Clostridia counts in the faeces and caecum content between the CTR and PP groups were observed. A reduced malondialdehyde concentration in the PP group was observed on day 21 compared to the CTR group (P=0.02). In conclusion, the prepared PP mixture has the potential to improve plasma antioxidant activity