RICERCHE EFFETTUATE

ARGOMENTI VARI

Andreani G, Carpenè E, Canavacciuolo° A, Ferlizza° E, Menotta° S, Fedrizzi° G, Isani G

Environmental exposure to lead in urban bats from Rome : a case of intoxication?

2018 Scientific Meeting Italian Association for the study of Trace Elements in living organisms - AISETOV "The role of trace elements in health: from healthy environments to healthy living organisms" : Ozzano Emilia, Bologna, October 12, 2018 : abstract book / [s.l. : AISETOV], 2018. - p 31 (Poster P-07) [Nr. Estr. 7987]

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

Introduction: Potential toxic effects of inorganic contaminants on wild bat populations are scarcely reported in literature, however for synanthropic species, as Tadarida teniotis living in an urban contest, a particular attention should be paid to the risk of exposure to toxic metals as Pb resulting from human activities. The aim of this work was to determine the concentrations of ten toxic trace elements in tissues of lactant T. teniotis from a nursery colony located in an urban area characterized by high anthropogenic impact. Material and Methods: Bone, liver, kidney were collected from 30 specimens of T. teniotis belonging to a colony located in the "Quartiere Africano" of Rome, living in a cavity between two buildings. Ten toxic trace metals (Al, As, Ba, Cd, Hg, Pb, Sb, Sr, Th, T1) were analysed by ICP-MS after wet digestion. Metal concentrations are reported as p.g/g wet weight (w.w.). Results and Discussion: Metals were present at detectable concentrations in analysed tissues with the exception of Cd and Th. Concentrations of most of the analysed metals were significantly higher in the bone ($p<0.01$), whereas Hg, Sb and Tl were higher in the liver ($p<0.01$). Hg and As concentrations were slightly above the LOQ in most tissues, while Pb levels were present in concentrations as high as 168±53 tig/g w.w. in bone, 66±27 [tg/g w.w. in liver and 5.7±2.9 tig/g w.w. in kidney and were associated with bone lesions found at necroscopy. Toxicological threshold values for Pb in tissues of bats have not been established yet, however the concentrations found in this research are higher than those reported in bats by other authors and two orders of magnitude higher than toxic threshold levels reported for waterfowl. Lead concentrations determined in the analysed specimens could be suggestive of an intoxication status.

Andreotti A, Fabbri I, Menotta° S, Borghesi F

Lead gunshot ingestion by a Peregrine Falcon

Ardeola. - Vol. 65 no 1 (2018). - p 53-58. - 16 bib ref [Nr. Estr. (ultimo accesso 26/02/2019) https://doi.org/10.13157/arla.65.1.2018.sc1 7516]

Lead ammunition represents a source of poisoning for raptors eating game. Although the Peregrine Falcon Falco peregrinus commonly preys on gamebirds, only a few cases of lead poisoning have been recorded, probably owing to the lack of specific investigations. We document an adult female found dead with many lead shot in the digestive tract, mixed with the remains of a feral Pigeon Columba livia domestica and a European Starling Sturnus vulgaris. Concentrations in heart blood clot, liver, kidney and bone suggest that lead poisoning was not the ultimate cause of death. However, lead levels in the blood clot suggest that a small amount of lead may have been absorbed from the shot. The two prey species involved cannot be hunted in Italy but they are intensively shot all year round to prevent damage to crops..

Asfor A, Howe N, Grazioli° S, Brocchi° E, Tuthill T

A simple universal test to quantitate 146S antigen during production of FMD vaccines

OS18 "Global vaccine security" : EuFMD Open session : October 2018, Puglia, Italy : online version, book of abstracts / [s.l. : s.n., 2018]. - Day 3. - p 26 [Nr. Estr. 8016]

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

Introduction Conventional foot-and-mouth disease (FMD) vaccines are produced by the chemical inactivation of virus preparations grown in cell culture. The efficacy of inactivated vaccines is dependent on the presence of intact virus particles, distinguished by their sedimentation of 1465 in sucrose gradient centrifugation. Such intact 1465 antigen is unstable and can dissociate into capsid subunits (pentamers, 12S) during preparation or storage, resulting in a marked reduction in immunogenicity. Yield and stability of 146S antigen is therefore a crucial parameter for vaccine development and must be optimised for each new vaccine strain. The 'gold standard' in vitro test for assessing 146S particles involves analysis of particle sedimentation in sucrose density gradients. This is a laborious and low throughput method. Immunological reagents that specifically recognise 146S antigen have previously been reported but such reagents are specific for a single serotype of FM DV. Here, we describe the characterization of a 146S-spe¬cific monoclonal antibody (5B6) that recognizes all FMDV serotypes and its use in a simple, universal test to quantitate 146S antigen. Materials and Methods The reactivity of monoclonal antibody 5B6 was characterised using ELISA, confocal microscopy and western blot. The 146S test was developed as a sandwich ELISA using recombinant bovine integrin to capture FMDV particles and 5B6 as a 146S specific detector. Preparations of FMDV antigens of various serotypes were used to evaluate the test, including parallel testing of samples before and after heating to induce complete dissociation of antigen. Results The monoclonal antibody reacted with virus of all serotypes. The sandwich ELISA has specificity for 146S of all serotypes tested. Discussion In summary, we have developed a pan serotypic detection system specific for 1465 particles. This has potential to be further validated as a high throughput test for quantitation of FMDV 1465 particles during vaccine manufacture and quality control.

Asfor A, Howe N, Grazioli° S, Wilsden G, Ludi A, Brocchi° E, King D, Parida S, Tuthill T

A novel VP2 peptide ELISA for universal detection of antibodies for FMD sero-surveillance

OS18 "Global vaccine security" : EuFMD Open session : October 2018, Puglia, Italy : online version, book of abstracts / [s.l. : s.n., 2018]. - Day 3. - p 58 [Nr. Estr. 8013]

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

Introduction FMD diagnostics include the use of serological tests to detect FMDV specific antibodies. Con¬ventional serology tests are reliable and rapid but do not detect antibodies against all virus serotypes. The aim of this study was to assess the potential of conserved sequences at the N-terminus of capsid protein VP2 as universal epitopes for the detection of FMDV specific antibodies against multiple FMDV serotypes. Materials and Methods An ELISA was developed using synthetic peptides corresponding to the N-terminus of VP2 as the capture antigen. The ELISA was evaluated using experimental and reference antisera (n=170) from the world reference laboratory for FMDV (WRLFMD, The Pirbright Institute). Results The peptide ELISA based on the highly conserved VP2 peptide detected antibodies to all se¬ven serotypes of FMDV in sera from immunized and convalescent animals. The peptide-ELISA provides sensitive and specific detection of antibodies to all FMDV viruses used in this study. Discussion In summary, this study highlighted the potential of synthetic peptide as a capture antigen in rapid detection of antibodies to all serotypes of FMDV in animal sera. The test is robust, simple and cost effective and may be beneficial for endemic areas as well as for FMDV free countries which do not vaccinate to maintain status of free from FMD. ..

Badi S, Cremonesi P, Abbassi MS, Ibrahim C, Snoussi M, Bignoli G, Luini° M,

Castiglioni B, Hassen A

Antibiotic resistance phenotypes and virulence-associated genes in Escherichia coli isolated from animals and animal food products in Tunisia

FEMS Microbiol Letters. - Vol. 365 no 10 (2018). - 7 p. - 52 bib ref [Nr. Estr. (ultimo accesso 26/02/2019) https://doi.org/10.1093/femsle/fny088 7922]

Livestock and food products of animal origin constitute important reservoirs of intestinal and extraintestinal pathogenic Escherichia coli including antibiotic-resistant E. coli isolates. To assess potential risks to public health related to E. coli strains of animal origin in Tunisia, 65 E. coli isolates recovered from healthy animals and food products of animal origin were studied. Antimicrobial susceptibility was determined according to CLSI guidelines and genes encoding antibiotic resistance as well as virulence factors were investigated by PCR. High rates of antibiotic resistance were observed to kanamycin (78.4%), gentamicin (75.3%) and streptomycin (75.3%, encoded by strA-strB (7 isolates)), amoxicillin (64.6%), amoxicillin/clavulanic acid (60%), tetracycline (44.6%; tetA (8 isolates) and tetB (7 isolates)), nalidixic acid (27.6%, qnrS (3 isolates), qnrB (2 isolates) and qnrA (one isolate)) and sulfonamides (36.9%; sul1 (1 isolate), sul2 (4 isolates), and sul3 (1 isolate)). Virulotypes classified some isolates as STEC (3%), MNEC (1.5%) and atypical EPEC (1.5%). This study demonstrated high rates of antimicrobial resistance and the presence of some pathogenic pathovars from animal origins that are a cause of concern for public health.

Bevacqua D, Quilot_Turion B, Bolzoni° L

A model for temporal dynamics of brown rot spreading in fruit orchards

Phytopathology. - Vol. 108 no 5 (2018). - p 595-601. - 42 bib ref [Nr. Estr. (ultimo accesso 28/02/2019) https://doi.org/10.1094/PHYTO-07-17-0250-R 8137]

Brown rot, caused by Monilinia spp., is a major disease of stone fruit and, in favorable environmental conditions and in the absence of fungicide treatments, it causes important economic losses. In the present work, we propose a modification of classical susceptible, exposed, infectious and removed compartmental models to grasp the peculiarities of the progression of brown rot epidemics in stone fruit orchards in the last stage of the fruit growth (i.e., from the end of the pit hardening to harvest time). Namely, we took into account (i) the lifespan of airborne spores; (ii) the dependence of the latent period on the cuticle crack surface area, which itself varies in time with fruit growth; (iii) the impossibility of recovery in infectious fruit; and (iv) the abrupt interruption of disease development by the elimination of the host fruit at harvest time. We parametrized the model by using field data from a peach Prunus persica orchard infected by Monilinia laxa and M. fructicola in Avignon (southern France). The basic reproduction number indicates that the environmental conditions met in the field were extremely favorable to disease development and the model closely fitted the temporal evolution of the fruit abundance in the different epidemiological compartments. The model permits us to highlight crucial mechanisms undergoing brown rot build up and to evaluate the consequences of different agricultural practices on the quantity and quality of the yield. We found that winter sanitation practices (which decrease the initial infection incidence) and the control of the fruit load (which affects the host fruit density and the single fruit growth trajectory) can be effective in controlling brown rot in conjunction with or in place of fungicide treatments.

Biasiotto G, Zanella I, Predolini F, Archetti° I, Cadei M, Monti E, Luzzani M, Pacchetti B, Mozzoni P, Andreoli R, De_Palma G, Serana F, Smeds A, Di_Lorenzo D

7-Hydroxymatairesinol improves body weight, fat and sugar metabolism in C57BJ/6 mice on a high-fat diet

Br J Nutr. - Vol. 120 no 7 (2018). - p 751-762. - 50 bib ref [Nr. Estr. (ultimo accesso 10/01/2019)

https://doi.org/10.1017/S0007114518001824 8074]

7-Hydroxymatairesinol (7-HMR) is a plant lignan abundant in various concentrations in plant foods. The objective of this study was to test HMRLignan™, a purified form of 7-HMR, and the corresponding Picea abies extract (total extract P. abies; TEP) as dietary supplements on a background of a high-fat diet (HFD)-induced metabolic syndrome in mice and in the 3T3-L1 adipogenesis model. Mice, 3 weeks old, were fed a HFD for 60 d. Subgroups were treated with 3 mg/kg body weight 7-HMR (HMRLignan™) or 10 mg/kg body weight TEP by oral administration. 7-HMR and TEP limited the increase in body weight (-11 and -13 %) and fat mass (-11 and -18 %) in the HFD-fed mice. Epididymal adipocytes were 19 and -12 % smaller and the liver was less steatotic (-62 and -65 %). Serum lipids decreased in TEP-treated mice (-11 % cholesterol, -23 % LDL and -15 % TAG) and sugar metabolism was ameliorated by both lignan preparations, as shown by a more than 70 % decrease in insulin secretion and insulin resistance. The expression of several metabolic genes was modulated by the HFD with an effect that was reversed by lignan. In 3T3-L1 cells, the 7-HMR metabolites enterolactone (ENL) and enterodiol (END) showed a 40 % inhibition of cell differentiation accompanied by the inhibited expression of the adipogenic genes PPAR , C/EBPa and aP2. Furthermore, END and ENL caused a 10 % reduction in TAG uptake in HEPA 1–6 hepatoma cells. In conclusion, 7-HMR and TEP reduce metabolic imbalances typical of the metabolic syndrome and obesity in male mice, whereas their metabolites inhibit adipogenesis and lipid uptake in vitro.

Bilato° D, Molica_Colella° E, Pascucci L, Ferrari° M, Dotti° S

Equine adipose-derived mesenchymal stem cells : preliminary results of micro-vesicles and growth factors secretion

Annual Meeting Gruppo Italiano Staminali Mesenchimali (GISM) : April 12-13, 2018, Assisi (PG) / [s.l. : s.n., 2018]. - p 42 (P-04) [Nr. Estr. 7847]

Annual Meeting Gruppo Italiano Staminali Mesenchimali (GISM) : Assisi (PG) : April 12-13, 2018)

OBJECTIVE: Mesenchymal Stromal Cells (MSCs) have shown therapeutic potential in regenerative medicine. Increasing evidence suggests that their effects depend upon the release of extracellular vesicles (EVs) containing growth factors (GFs) and different species of genetic material. In response to changes in the surrounding environment, MSC adapt EVs content to fine-tune their biological effects. In order to speculate on possible applications in regenerative medicine, the aim of the study was to evaluate the ability of equine adipose-derived Mesenchymal Stromal Cells (e-AdMSC) to modulate the release of EVs and to adjust their GFs cargo in response to normoxia and hypoxia conditions. MATERIALS AND METHODS: AdMSCs were isolated from equine adipose tissue. The cells were cultured at 37°C, 5% CO2 with 20% 02 and 3% 02, respectively, for normoxia and hypoxia conditions. After reaching a confluence of 80%, the supernatants of cells were collected and centrifuged at 2500g for 20 min at 4°C to remove floating cells and debris, and subsequently ultra-centrifuged at 100.000g for 70 min at 4°C. Each pellet obtained was re-suspended in 100 p L of phosphate buffer and analyzed by Transmission Electron Microscopy (TEM) at CUME (University Centre of Electron Microscopy, University of Perugia). At the same time, one aliquot was stored at -80 °C for ELISA tests. Once assessed the presence of EVs through TEM, ELISA assays were performed to quantify the GFs secretion in the aforementioned two conditions. Tests were preliminary performed on samples diluted 1:10 and 1:20. The equine and human kits were applied according to the manufacturer's instructions to detect TGFB I, PDGF-BB, IGF1, IGF2, EGF, MET, VEGF. RESULTS: In both samples, TEM analysis demonstrated the presence of EVs ranging in size from 30 to 1000 nm and displaying a variable electron density. No differences were observed between normoxic and hypoxic samples in terms of number and morphology of EVs. Preliminary results obtained by ELISA tests, demonstrated that MET and IGF I content were higher under hypoxia condition. On the other hand, the content of TGF131 and IGF2 resulted higher under normoxia condition. PDGF-BB and EGF showed equal level in both samples. VEGF content was less than the detection range. CONCLUSION: In the present study we isolated EVs from AdMSCs and measured the amount of specific GFs. Morphological analysis evidenced the presence of a mixture of exosomes and microvesicles as revealed by the wide dimensional range. However, no

differences in EVs ultrastructure were detected. Preliminary results revealed that the stressful condition of hypoxia does not seem to be relevant for the secretion of specific GFs. In particular, according to Madrigal et al. 2014. MET and IGF I demonstrated higher level under hypoxia. Moreover, differences between equine and human kits were revealed for TGFB1. Further studies will be necessary to investigate our hypothesis.

Bolotta A, Visconti P, Fedrizzi° G, Ghezzo A, Marini M, Manunta P, Messaggio E, Posar A, Vignini A, Abruzzo PM

Na+, K+-ATPase activity in children with autism spectrum disorder : searching for the reason(s) of its decrease in blood cells

Autism Res. - Vol. 11 (2018). - p 1388-1403. - 62 bib ref [Nr. Estr. (ultimo accesso 19/02/2019) https://doi.org/10.1002/aur.2002 8138]

Na+, KtATPase (NKA) activity, which establishes the sodium and potassium gradient across the cell membrane and is instrumental in the propagation of the nerve impulses, is altered in a number of neurological and neuropsychiatric disorders, including autism spectrum disorders (ASD). In the present work, we examined a wide range of biochemical and cellular parameters in the attempt to understand the reason(s) for the severe decrease in NKA activity in erythrocytes of ASD children that we reported previously. NKA activity in leukocytes was found to be decreased independently from alteration in plasma membrane fluidity. The different subunits were evaluated for gene expression in leukocytes and for protein expression in erythrocytes: small differences in gene expression between ASD and typically developing children were not apparently paralleled by differences in protein expression. Moreover, no gross difference in erythrocyte plasma membrane oxidative modifications was detectable, although oxidative stress in blood samples from ASD children was con¬firmed by increased expression of NRF2 mRNA. Interestingly, gene expression of some NKA subunits correlated with clinical features. Excess inhibitory metals or ouabain-like activities, which might account for NKA activity decrease, were ruled out. Plasma membrane cholesterol, but not phosphatidylcholine and phosphatidlserine, was slighty decreased in erythrocytes from ASD children. Although no compelling results were obtained, our data suggest that alteration in the erytrocyte lipid moiety or subtle oxidative modifications in NKA structure are likely candidates for the observed decrease in NKA activity. These findings are discussed in the light of the relevance of NKA in ASD.

Brites D, Loiseau C, Menardo F, Borrel S, Boniotti° MB, Warren R, Dippenaar A, Parsons SDC, Beisel C, Behr MA, Fyfe JA, Coscolla M, Gagneux S

A new phylogenetic framework for the animal-adapted Mycobacterium tuberculosis complex

Front Microbiol. - Vol. 9 (2018). - Article no. 2820 (14 p). - 101 bib ref [Nr. Estr. (ultimo accesso 11/01/2019) https://doi.org/10.3389/fmicb.2018.02820 8077]

Tuberculosis (TB) affects humans and other animals and is caused by bacteria from the Mycobacterium tuberculosis complex (MTBC). Previous studies have shown that there are at least nine members of the MTBC infecting animals other than humans; these have also been referred to as ecotypes. However, the ecology and the evolution of these animal-adapted MTBC ecotypes are poorly understood. Here we screened 12,886 publicly available MTBC genomes and newly sequenced 17 animal-adapted MTBC strains, gathering a total of 529 genomes of animal-adapted MTBC strains. Phylogenomic and comparative analyses confirm that the animal-adapted MTBC members are paraphyletic with some members more closely related to the human-adapted Mycobacterium africanum Lineage 6 than to other animal-adapted strains. Furthermore, we identified four main animal-adapted MTBC clades that might correspond to four main host shifts; two of these clades are proposed to reflect independent cattle domestication events. Contrary to what would be expected from an obligate pathogen, MTBC nucleotide diversity was not positively

correlated with host phylogenetic distances, suggesting that host tropism in the animal-adapted MTBC seems to be driven more by contact rates and demographic aspects of the host population rather than host relatedness. By combining phylogenomics with ecological data, we propose an evolutionary scenario in which the ancestor of Lineage 6 and all animal-adapted MTBC ecotypes was a generalist pathogen that subsequently adapted to different host species. This study provides a new phylogenetic framework to better understand the evolution of the different ecotypes of the MTBC and guide future work aimed at elucidating the molecular mechanisms underlying host specificity.

Brookes SM, Everett HE, Van_Diemen PM, Byrne AMP, Ramsay A, Watson S, Nunez A, Moreno° A, Chiapponi° C, Foni° E, Brown IH

Assessment of zoonotic transmission of swine influenza A viruses to naive or vaccinated ferrets

4th International Symposium on Neglected Influenza Viruses : Brighton, UK,18-20 April 2018 / organized by the International Society for Influenza and other respiratory virus diseases (ISIRV). - [s.l. : s.n., 2018]. - p 13. - 1 bib ref (Oral O1) [Nr. Estr. 8110]

International Symposium on Neglected Influenza Viruses (4th : Brighton, UK : 18-20 April 2018)

An in vivo study was conducted to assess the infection dynamics of two swine-origin H1N1 influenza A viruses, specifically, a swine influenza A pandemic 2009 (pdnn09) virus strain and human influenza A virus.isolate (A/Pavia/65/16)1 that is phylogenetically indistinguishable from avian-like Eurasian swine influenza A lineage viruses currently circulating amongst pigs in Italy. As swine influenza A viruses exhibit greater genetic diversity than influenza A viruses circulating in the human population, we also assessed whether the human 2016-17 seasonal influenza vaccine could afford protection against the two swine influenza A virus strains, using ferrets as a human model. This vaccine incorporates one H1N1 antigen, A/California/07/09 that is representative of human pdm09 viruses. The study design used two groups of five pigs, with each group housed in separate rooms and infected with 2.4x107TCID50 of one strain. The infected pigs were co-housed with a group of five naive ferrets and a group of five vaccinated ferrets held in separate cages. Both virus strains readily infected pigs and produced mild, pathogenesis profiles. Analysis of pig nasal swabs showed that both virus strains reached peak shedding levels at 2-4dpi and shedding ceased by 7dpi. Daily ferret nasal wash samples were analysed to assess potential zoonotic transmission of virus. All ferret groups, except the group that had been vaccinated and exposed to the swine-origin pdm09 virus, had a viral shedding profile in nasal wash samples characteristic of infection. Seroconversion produced antibodies that detected the pdm09 and avian-like Eurasian swine influenza A virus lineages. In contrast, the ferret group that had been vaccinated and exposed to the swine-origin pdm09 virus showed a significant reduction in viral shedding in nasal secretions. An increased influenza-specific antibody response was not detected following infection, perhaps indicating a lack of productive infection because of immunity afforded by the vaccine. All infected ferrets exhibited mild clinical signs and controlled the infection. This study confirms that vaccine and challenge strains must be highly matched in order to afford protection and also indicates that pre-existing immunity to pdm09 strains may not provide protective immunity to all currently circulating swine influenza A virus H1N1 strains. The strain that had been associated with human clinical disease was found in this study to produce mild clinical signs in pigs, the natural host and in ferrets, representing a human model. There was no evidence of increased risk in comparison to the swine-origin pdm09 strain assessed in parallel.

Byrne AMP, Snowden J, Coward V, Chiapponi° C, Foni° E, Everett HE, Brookes SM, Brown IH

Optimisation of the culture and detection methods for influenza D viruses

4th International Symposium on Neglected Influenza Viruses : Brighton, UK,18-20 April 2018 /

organized by the International Society for Influenza and other respiratory virus diseases (ISIRV). - [s.l. : s.n., 2018]. - p 35 (Poster P4) [Nr. Estr. 8112]

International Symposium on Neglected Influenza Viruses (4th : Brighton, UK : 18-20 April 2018)

Introduction: Since the identification and isolation of influenza D from swine in the United States in 2011, this novel virus has been detected in swine, cattle, horses, ruminants and humans in Europe, Africa, North America and Asia and has been associated with respiratory disease in some cases. However, only a limited survey has been conducted to assess the circulation of influenza D in livestock within the United Kingdom. Methods: Using a representative strain of influenza D from Europe, D/swine/Italy/199724-3/2015, three different cell lines were assessed for their ability to support the replication of these strains. The cell lines used were a human colorectal adenocarcinonna (Caco2) cell line, the Madin-Darby canine kidney (MDCK) cell line and the new-born pig tracheal (NPTr) cell line. The growth of these virus strains in the different cell lines was monitored using a haemagglutination assay. Serum raised against influenza D was also used to detect the virus using a haemagglutination inhibition (HI) assay. A RRT-PCR method to detect both D/swine/Italy/199724-3/2015 and D/swine/Oklahoma/1334/2011 was also developed based on previous work and the ability of several other influenza diagnostic RRT-PCR methods to detect influenza D was also assessed. Results: It was found that the Caco2 cell line was superior to both MDCK and NPTr cell lines for the amplification of D/swine/Italy/199724-3/2015 virus, which was actively detected by both haemagglutination and HI assays. It was also found that the Nagy (Nagy et al. 2010), Spackman (Spackman et al. 2002) and Spackman "Perfect Match" (Slomka et al. 2010) M-gene RRT-PCR methodologies currently used for influenza A screening in livestock were not able to detect either influenza D strains. Based on this we adapted a previously published RRT-PCR method (Hause etal. 2011), which was able to detect both D/swine/Italy/199724-3/2015 and D/swine/Oklahonna/1334/2011 with comparable sensitivity. Conclusions: By optimising the viral culture and detection methods of influenza D viruses, we are now in a position to perform a large-scale survey of swine and cattle in the United Kingdom to assess the prevalence of this novel pathogen in livestock.

Caloni F, Sambuy Y, Lombardi° G, Dotti° S, De_Angelis I

3Replacement Winter School "Out of the barriers : in vitro models in toxicology"

Altex Alternative Anim Exp. - Vol. 35 no 4 (2018). - p 520-521 [Nr. Estr. (ultimo accesso 11/03/2019) https://doi.org/10.14573/altex.1807231 8035]

Calzolari° M, Ferrarini° P, Bonilauri° P, Lelli° D, Chiapponi° C, Bellini R, Dottori° M

Co-circulation of eight different phleboviruses in sand flies collected in the Northern Apennine Mountains (Italy)

Infect Genet Evol. - Vol. 64 (2018). - p 131-134. - 15 bib ref [Nr. Estr. (ultimo accesso 11/03/2019) https://doi.org/10.1016/j.meegid.2018.06.014 7878]

The sand flies are the biological vectors of a variety of viruses belonging to the Phlebovirus genus. As several of these viruses, like Toscana virus, are important agents of diseases in humans, the definition the phleboviruses circulating in a particular area is an important health issue. This work presents results obtained between 2013 and 2016, by testing 25,853 field-collected sand flies, sampled at 50 sites in the Northern edge of the Apennine Mountains, in Emilia-Romagna and Lombardy regions (Italy). Isolation of the three reassortant Ponticelli I, II, and III viruses was successful from five pools of sand flies. These results, and phylogenetic analysis made by obtained sequences, suggest the presence of eight different viruses: Toscana virus, Fermo-like virus, Corfou virus, Ponticelli viruses, and two unknown putative viruses. The co-circulation of different phlebovirus reported in this study, indicate a very dynamic and complex situation, which deserves a more detailed investigation to characterize their possible pathogenicity to humans and animals.

Carretto E, Brovarone F, Nardini P, Russello G, Barbarini D, Pongolini° S, Gagliotti C, Carattoli A, Sarti M

Detection of mcr-4 positive Salmonella enterica serovar Typhimurium in clinical isolates of human origin, Italy, October to November 2016

EuroSurveillance. - Vol. 23 no 2 (2018). - no. 17-00821 (3 p). - 15 bib ref [Nr. Estr. (Ultimo accesso 11/01/2019) https://doi.org/10.2807/1560-7917.ES.2018.23.2.17-00821 8075]

In the context of an analysis into the epidemiology of mobile colistin determinants, we investigated a collection of 106 human clinical isolates of Enterobacteriaceae. These isolates were obtained between January 2016 and October 2017 in two main hospitals in Emilia Romagna, Italy, namely IRCCS Arcispedale Santa Maria Nuova, Reggio Emilia and Sant'Agostino-Estense Hospital of Baggiovara. These isolates had originally been selected on the basis of their reduced susceptibility to colistin (MIC = 2 mg/L). Among the 67 Escherichia coli, 27 Klebsiella pneumoniae, six Salmonella species and six other Enterobacteriaceae collected, we detected the mcr-4 gene in two human isolates of S. enterica subsp. enterica monophasic variant of serovar Typhimurium. The mcr-4 gene was not detected in any other isolate of the above collection; further analyses for other mcr genes are ongoing in these and additional isolates.

Chiapponi° C, Foni° E

Full-genome sequencing dei virus respiratori : i virus influenzali come modello : workflow e studi molecolari

XLVII Congresso Nazionale AMCLI : Rimini, 10-13 Novembre 2018 / [s.l. : s.n., 2018]. - 1 p. (ID: 17604) [Nr. Estr. 8121]

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

Le tecniche di sequenziamento di nuova generazione (NGS) hanno rivoluzionato negli ultimi anni le conoscenze, to studio e la diagnostica dei patogeni virali. Inoltre queste metodologie, adeguatamente sviluppate si stanno rivelando preziose quando usate in un approccio metagenomico che si discosta dal metodo "a target" caratteristico delta diagnostica tradizionale tramite PCR. Nell'ambito dei virus respiratori, ampio utilizzo di queste tecniche e stato fatto con i virus influenzali umani ed animali. L'esempio del virus influenzale pu6 essere considerato emblematico net rappresentare approcci metodologici e vantaggi applicativi dell'NGS. It virus influenzale di tipo A (IAV), in particolare, manifesta caratteristica di elevata variabilita genetica, legata alla peculiare frammentarieta del suo genoma ma anche a fattori esterni, quale la pressione immunitaria di popolazione e alcuni aspetti ecologici, ambientali e sociali. In tale scenario, IAV e riconosciuto una delle principali minacce per i sistemi sanitari globali sia in ambito umano che in ambito veterinario. E' importante tenere ben presente la minaccia dell' insorgenza di eventi pandemici e del loro impatto sanitario ma anche economico sulla attuale struttura sociale globalizzata. La caratterizzazione genetica tradizionale di IAV passa attraverso molteplici reazioni di RT-PCR specifiche per i diversi segmenti genomici con successiva analisi multipla di sequenziamento Sanger. Grazie allo sviluppo delle tecniche NGS è ora possibile invece preparare librerie di sequenziamento a partire da RNA virale con analisi simultanea di tutti i geni presenti nello stesso campione. L'analisi bioinformatica dei dati di sequenziamento svolge un ruolo cruciale nel processo, sono infatti disponibili vane pipeline, commerciali o gratuite, per l'assemblaggio delle sequenze. Le informazioni the si ottengono analizzando i genomi completi permettono di conoscere i cambiamenti genetici di ogni segmento genico per i ceppi virali protagonisti delle diverse stagioni influenzali. Questi dati inoltre si possono rendere disponibili su un elevato numero di osservazioni, in modo tale che i dati possono avere anche un inquadramento statisticamente significativo. Va inoltre osservato come l'NGS sia in grado di fomire in tempi ridotti una quantita di informazioni notevolmente superiore a quanto possibile in precedenza. In questo contesto la tempestiva identificazione di mutazioni associate ad una maggiore severita clinica o ad una diffusione pandemica è un aspetto cruciale delta epidemiologia di questa infezione. Poter considerare

puntualmente le caratteristiche genetiche di virus influenzali circolanti permette di inquadrare le rispettive caratteristiche antigeniche e di conseguenza anche quelle di resistenza ai farmaci. Infine, l'NGS rende pii rapido to studio della corrispondenza antigenica tra i ceppi inclusi net vaccino stagionale e quelli circolanti.

Dotti° S, Villa° R, Ferrari° M, Lombardi° G

MSCS in practice : preparation, distribution, quality control and regulation

Annual Meeting Gruppo Italiano Staminali Mesenchimali (GISM) : April 12-13, 2018, Assisi (PG) / [s.l. : s.n., 2018]. - p 30 (Abstract S-21) [Nr. Estr. 7848]

Annual Meeting Gruppo Italiano Staminali Mesenchimali (GISM) : Assisi (PG) : April 12-13, 2018)

In recent years, cell therapy has evolved quickly gaining great interest in both human and veterinary field. In particular, it has evoked considerable excitement in the animal-owning public because of the promise that stem cell technology could deliver tissue regeneration for injuries, for which natural repair mechanisms do not allow functional recovery and for which current therapeutic strategies have minimal effectiveness. Before tackling the therapeutic approach, and due to the fact that mesenchymal stem cells (MSCs) are identified as a pharmaceutical drug, it is necessary to consider the laboratory process that allows to obtain a suitable product to the medical employment. The clinical application of cell therapy involves different critical aspects that must be considered during laboratory process. The first step to consider is represented by the manipulation of the sample during in vitro amplification; this aspect is particularly thorny and demanding, because the sample is submitted to different procedures that can result in contamination or cells damages. After the isolation, all manipulation must be performed in certified laboratories and with standardized procedures approved by the quality management system. Furthermore, quality controls represent a crucial point in order to guarantee a safe and functional final product. Quality testing should be performed in each batch in order to ensure the purity of the final product resulting from the production process. The quality controls, are made in accordance with European Pharmacopiea and EMA regulations and consist in the evaluation of sterility against bacteria and mycoplasma, the potential viral contaminations by either inoculation of the sample in cell cultures or by molecular biology assays and the maintenance of the amplified cells of the differentiation ability towards the osteogenic, adipogenic and chondrogenic cell line. Furthermore, the evaluation of the absence of tumorigenicity carried out either by in vitro or in vivo tests plays a critical role. In veterinary field, only autologous MSCs are currently approved although the results of experimental clinical trials already performed and transmitted to Ministry of Health have demonstrated the safety of allogeneic MSCs and no apparent differences in their injury repair capacity. The use of allogeneic MSCs offers the possibility to store quality tested batches of cells available at each time. Only a strong cooperation among the laboratory staff, the regulatory agencies and, mainly, the veterinarians in the field, will allow to improve the application of the innovative regenerative medicine based on MSCs in veterinary practice.

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

Relationship between some animal-based measures collected at herd-level and the exposure of dairy cows to poor management and housing conditions

Proceedings of the fifth DairyCare Conference 2018 : Thessaloniki, March 19th and 20th 2018 / editor, C.H. Knight. - [s.l.] : DairyCare COST Action FA1308, 2018. - p 47 (Poster 10) [Nr. Estr. 7818]

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

Nowadays, public awareness of farm animal welfare (AW) issues is increasing and several countries have implemented different onfarm welfare assessment systems to better tackle them. To foster continuous monitoring, the use of some routinely collected animal-based-measures (ABMs) as

“iceberg indicators” of overall AW could represent a good tool to early identify at-risk herds and focus further controls on them. For investigating this hypothesis, the results from 1516 dairy cow welfare assessments (years 2014-2017) were selected from the Italian Animal Welfare Reference Centre (CReNBA) database. It includes the outputs from the application of the CReNBA protocol1 used at national level for on-farm controls. The frequencies of four herd-level ABMs, categorized in three levels (see Table1 for details), were compared with the scores gained by the farms in terms of management and housing conditions. Herds with poorer scenarios (scores =60.00%) showed more often, in a statistically significant way, worse levels of ABMs (Table1), suggesting that cows found it hard to adapt to such conditions. Then, the number of herds with the worst category of each ABM was tested, using 2x2 contingency tables, against their exposure to each managerial and housing hazard (n=52) measured in the protocol. Some of the strongest associations were seen for i) lameness >8% and poor feeding (OR=2.42; 95% CI=1.40-4.18; P=0.0015), lack of foot trimmings (OR=1.74; 95% CI=1.18-2.56; P=0.0051), slippery floors for lactating-cows (OR=2.47; 95% CI=1.43-4.27; P=0.0012), long waiting-time in the holding area (OR=1.76; 95% CI=1.12-2.76; P=0.014); ii) BTSCC >400,000 cells/ml and poor hygienic conditions in lactating-cow lying area (OR=3.22; 95% CI=1.10-9.47; P=0.033), poor calving-pen management (OR=2.91; 95% CI=1.44-5.87; P=0.0029), poor milking hygiene (OR=4.01; 95% CI=1.36-11.87; P=0.012), overstocking in lactating-cows (OR=2.70; 95% CI=1.26-5.78; P=0.011), overstocking in calving pen (OR=2.45; 95% CI=1.04-5.77; P=0.040); iii) No of mastitis antibiotic treatments >80% and overstocking in lactating-cows (OR=1.84; 95% CI=1.08-3.14; P=0.026), overstocking in drycows (OR=1.98; 95% CI=1.16-3.39; P=0.012), long waiting-time in the holding area (OR=2.09; 95% CI=1.12-3.90; P=0.020). These preliminary results suggested that some ABMs routinely monitored could be indicators of herds at risk of poor welfare; however further analyses should be performed.

Gaffuri° A, Angelucci° A, Lorenzi° V, Fusi° F, Bertocchi° L

Benessere e biosicurezza nelle capre da latte : il metodo di valutazione del Centro di Referenza Nazionale per il Benessere Animale = Welfare and biosecurity in dairy goats : the assessment protocol developed by the Italian National Reference Centre for Animal Welfare

XXIII Congresso Nazionale Societa' Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) : Napoli 12-14 settembre 2018 / edited by Antonio Bosco ... [et al.]. - [Naples : University of Naples Federico II, 2018]. - (Mappe parassitologiche ; 24) p 55-56. - 5 bib ref [Nr. Estr. 7947]

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

L’allevamento della capra da latte sta assumendo sempre più importanza nella zootecnia italiana e rappresenta un settore in espansione. Negli ultimi anni il sistema di allevamento ha subito delle profonde e rapide modificazioni, soprattutto per le razze a spiccata attitudine lattifera, affiancando ai sistemi estensivi, quelli intensivi e semi-intensivi. Cie ha comportato una maggiore attenzione verso le problematiche connesse al benessere e, in particolare, allo studio dei fattori ambientali e tecnico-gestionali che possono rappresentare un pericolo per il benessere degli animali. Dato questo nuovo scenario, il Centro di Referenza Nazionale per il Benessere Animale (CReNBA), nell'ambito del progetto di ricerca ministeriale RuminantWelfare, ha sviluppato un protocollo (check-list) per la valutazione del benessere nell'allevamento della capra da latte. Ii sistema, gia validato per la valutazione del benessere della bovina da latte (Bertocchi etal., 2018), si caratterizza per l'efficace rilievo delle carenze dell'allevamento e permette di individuare lo stretto collegamento fra condizioni di allevamento ed effetti sugli animali, attraverso le cosi dette "animal based measures" (ABMs). 11 metodo inoltre utilizza parametri oggettivi e misurabili e i tempi di lavoro (visita aziendale, compilazione della check-list, invio della stessa al CReNBA ed ottenimento del risultato) sono contenuti. La valutazione e eseguita da veterinari che hanno frequentato un corso di formazione organizzato dal CreNBA.

Gaibani P, Ambretti S, Scaltriti° E, Cordovana M, Berlingeri A, Pongolini° S, Landini

MP, Re MC

A novel IncA plasmid carrying blaVIM-1 in a Kluyvera cryocrescens strain

J Antimicrob Chemother. - Vol. 73 no 11 (2018). - p 3206-3208. - 11 ref bib [Nr. Estr. (ultimo accesso 11/01/2019) https://doi.org/10.1093/jac/dky304 7917]

Gladue DP, Largo E, De_La_Arada I, Aguilella VM, Alcaraz A, Arrondo JLR, Holinka LG, Brocchi° E, Ramirez-Medina E, Vuono EA, Berggren KA, Carrillo C, Nieva JL, Borca MV

Molecular characterization of the viroporin function of foot-and-mouth disease virus nonstructural protein 2B

J Virol. - Vol. 92 no 23 (2018). - p e01360-18 (19 p). - 40 bib ref [Nr. Estr. (ultimo accesso 29/01/2019) https://doi.org/10.1128/JVI.01360-18 8104]

Nonstructural protein 2B of foot-and-mouth disease (FMD) virus (FMDV) is comprised of a small, hydrophobic, 154-amino-acid protein. Structurefunction analyses demonstrated that FMDV 2B is an ion channel-forming protein. Infrared spectroscopy measurements using partially overlapping peptides that spanned regions between amino acids 28 and 147 demonstrated the adoption of helical conformations in two putative transmembrane regions between residues 60 and 78 and between residues 119 and 147 and a third transmembrane region between residues 79 and 106, adopting a mainly extended structure. Using synthetic peptides, ion channel activity measurements in planar lipid bilayers and imaging of single giant unilamellar vesicles (GUVs) revealed the existence of two sequences endowed with membrane-porating activity: one spanning FMDV 2B residues 55 to 82 and the other spanning the C-terminal region of 2B from residues 99 to 147. Mapping the latter sequence identified residues 119 to 147 as being responsible for the activity. Experiments to assess the degree of insertion of the synthetic peptides in bilayers and the inclination angle adopted by each peptide regarding the membrane plane normal confirm that residues 55 to 82 and 119 to 147 of 2B actively insert as transmembrane helices. Using reverse genetics, a panel of 13 FMD recombinant mutant viruses was designed, which harbored nonconservative as well as alanine substitutions in critical amino acid residues in the area between amino acid residues 28 and 147. Alterations to any of these structures interfered with pore channel activity and the capacity of the protein to permeabilize the endoplasmic reticulum (ER) to calcium and were lethal for virus replication. Thus, FMDV 2B emerges as the first member of the viroporin family containing two distinct pore domains.

Gomez_Morales MA, Della_Casa G, Licata E, Merialdi° G, Amati A, Rugna° G, Cherchi S, Tonanzi D, Ramini° M, Marucci G, Interisano M, Ludovisi A, Faeti V, Pozio E

Long term study on Trichinella muscle larvae and circulating IgG in pigs

XXX Congresso Nazionale Societa' Italiana di Parassitologia (SOIPA) "Mutamenti ambientali e parassiti" : 26-29 Giugno 2018, Milano / [s.l. : s.n., 2018]. - p 165 [Nr. Estr. 8117]

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

INTRODUCTION. In the European Union, trichinellosis in humans is strongly reduced to less than 150 infections in 2016 (EFSA, 2017, EFSA J, 15:5077). However, pork from domestic pigs and wild boar still represents the main source of human infections worldwide (Murrell and Pozio, 2011, Em Inf Dis, 17:2194-2202). Even if parasites of the genus Trichinella are disappeared from most of pig farms of EU, these pathogens are still circulating among backyard and free-ranging pigs of five EU countries including Italy. To monitor Trichinella infections in domestic and wild swine, serology has been proposed as a cheap and fast method. However, our knowledge on circulating IgG kinetic in relation to the larval burden in muscles and age of the infection, is limited. The aim of the present

study was to monitor the relationship between the IgG kinetic in serum samples and the larval burden in muscles of domestic pigs infected by Trichinella spiralis, T. britovi and T. pseudospiralis for a two-year-period. MATERIALS AND METHODS. Sixty pigs of about 40 kg were infected per os with 10,000 larvae/animal of T. spiralis (20 animals), T. britovi (20 animals) and T. pseudospiralis (20 animals). Blood was collected one day before infection and one per month up to slaughtering. Four animals of each of the three groups were slaughtered at 2, 6 and 12 months post infection. At slaughtering, 100 g were collected from eight muscles (diaphragm pillars, tongue, masseter, intercostal, loin, shoulder, anterior and posterior leg muscles). Serum samples were tested by ELISA and Western blot for confirmation. Muscle samples were digested and larvae per gram counted. RESULTS AND CONCLUSIONS. Infecting T. spiralis larvae were still present in all the eight pig muscles one year p.i. A small amount (0.2 larvae/g in the diaphragm) of T. britovi larvae was detected up to 6 months p.i., but no larvae were detected 12 months p.i. T. pseudospiralis larvae were detected in the muscles 2 months p.i., but no larvae were detected 6 months p.i. All animals seroconverted between 35 and 40 days p.i., and the highest IgG level was detected 2 months p.i. irrespective of the Trichinella species. One year p.i., circulating IgG were no more detectable in sera of T. pseudospiralis infected animals. The level of detected circulating IgG was still high (>50% ELISA index) in T. britovi and T. spiralis infected pigs, even if no larvae were detected in T. britovi infected pigs. The experimental study is still in progress.

Gori M, Ebranati E, Scaltriti° E, Huedo P, Ciceri G, Tanzi E, Pontello M, Zehender G, Pongolini° S, Bolzoni° L

High-resolution diffusion pattern of human infections by Salmonella enterica serovar Napoli in Northern Italy explained through phylogeography

PLoS One. - Vol. 13 no 8 (2018). - p e0202573 (16 p). - 49 bib ref [Nr. Estr. (ultimo accesso 11/03/2019) https://doi.org/10.1371/journal.pone.0202573 7944]

Salmonella enterica serovar Napoli (serovar Napoli) is an emerging cause of human salmonellosis in Northern Italy. No specific reservoirs of serovar Napoli have been identified in Italy, so far. However, the environment, especially surface waters, has been hypothesized as an important source of infection based on the observation that genotypically different clusters of serovar Napoli are detected in different geographical macro-areas. To further support the hypothesis of a spatially-restricted pattern of serovar Napoli diffusion, a spatial segregation of serovar Napoli lineages should be observed also at smaller geographical scale. However, classical genotyping techniques used for Salmonella, such as pulsed-field gel electrophoresis (PFGE), did not possess enough discriminatory power to highlight spatial clustering of serovar Napoli within the macro-areas. To this purpose, we performed phylogeographical analyses based on genome-wide single nucleotide polymorphisms to test whether spatio-temporal evolution patterns of serovar Napoli in Northern Italy could be recognized with high geographical resolution, i.e. at local level. Specifically, we analyzed the local spread of the main PFGE clonal group, responsible for more than 60% of human infections in the study area, that did not show any geographical differentiation by PFGE within Northern Italy, i.e. the macro-area considered in the study. Both discrete and continuous phylogeography highlighted the existence of two main geographically-restricted clades: a Southern clade corresponding to the Po Valley and a Northern clade corresponding to the Pre-Alps area. Furthermore, the phylogeographical analyses suggested that the most probable site of origin of the clone was in an area of the Po Valley at the confluence of the Po and Ticino rivers, one of the most important Italian wetlands. These findings provide further support to the hypothesis that environmental transmission may play an important role in the ecology of serovar Napoli.

Henry L, Morris A, Mioulet V, Wood BA, Gray A, King DP, Grazioli° S, Pezzoni° G, Brocchi° E

Comparative performance of monoclonal and polyclonal-based antigen ELISAs for FMDV detection

OS18 "Global vaccine security" : EuFMD Open session : October 2018, Puglia, Italy : online version, book of abstracts / [s.l. : s.n., 2018]. - Day 3. - p 34 [Nr. Estr. 8015]

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

Introduction Serotyping assays are integral for the detection and characterisation of foot-and-mouth disease virus (FMDV). The WRLFMD currently uses a rabbit/guinea-pig polyclonal antibody-based indirect sandwich ELISA (PAb ELISA) to serotype diagnostic submissions. In recent years, the number of samples that could not be typed with this assay has increased. Thus, the IZSLER/Pirbright kit based on monoclonal antibodies (MAb ELISA) was validated with the view to include in the WRLFMD portfolio of ISO/IEC 17025 tests. Methods The serotypes common between both assays and therefore selected for testing were 0, A, C, Asia1, SAT1 and SAT2. The MAb ELISA sensitivity was evaluated with FMDV isolates that represent serotypes, topotypes and lineages circulating between 1964 and 2018, representing all seven serotypes 0 (n=99), A (n=52), C (n=5), Asia1 (n=22), SAT1 (n=23) and SAT2 (n=52). The MAb ELISA limit of detection was evaluated using isolates and original epithelium suspension. The results obtained were compared with those reported by the WRLFMD using the PAb ELISA. The MAb ELISA was also evaluated with isolates that gave borderline or negative results on the PAb ELISA 0 (n=60), A (n=18), Asia1 (n=1) and SAT2 (n=1). Lastly, the MAb ELISA specificity was assessed with viruses that cause similar clinical signs to FMD. Results Overall, there was good concordance between the assays; however, the MAb ELISA demonstrated an improved sensitivity with both isolates and original suspension. The MAb assay detected all isolates missed by the PAb ELISA, apart from two recent 0/CATHAY samples. The pan-FMD test included in the MAb ELISA detected all type 0, A, C and Asia1 isolates, but demonstrated reduced sensitivity for the SATs. No cross-reactivity was observed with other vesicular disease viruses. Discussion The MAb ELISA is a simple and robust kit for serotyping with an overall sensitivity of 90% compared to 83% for the PAb ELISA.

Hocking PM, Vinco° LJ, Veldkamp T

Soya bean meal increases litter moisture and foot pad dermatitis in maize and wheat based diets for turkeys but maize and non-soya diets lower body weight

Br Poult Sci. - Vol. 59 no 2 (2018). - p 227-231 - 14 bib ref [Nr. Estr. (ultimo accesso 11/03/2019) https://doi.org/10.1080/00071668.2018.1423675 7807]

1. An experiment was conducted to investigate the effect of crude protein (CP) concentration and dietary electrolyte balance (DEB) on growth performance, processing yields, litter quality and foot pad dermatitis (FPD) in male turkeys from two commercial hybrids. Soya bean meal was replaced by vegetable protein sources selected for lower K concentrations to lower DEB in order to improve litter quality and subsequent quality of foot pads. 2. Effects of CP on litter friability and wetness were not consistent during the production period. FPD in turkeys fed on diets with low CP was significantly lower than FPD in turkeys fed on diets with high CP until 84 d. Growth performance was adversely affected at low CP. Processing yields were not affected by CP. 3. Litter was significantly dryer in pens of turkeys fed on diets with low DEB than in pens of turkeys fed on diets with high DEB. FPD in turkeys fed on diets with low DEB was significantly lower than in turkeys fed on diets with high DEB. Growth performance and processing yields were adversely affected at low DEB. 4. FPD in turkey hybrid A was higher than in turkey hybrid B at 28 d of age. Thereafter, no differences in FPD between turkey hybrids were observed. Growth performance and processing yields were not affected by turkey hybrid. 5. Overall, a significant interaction effect of CP × DEB was observed for FCR: in turkeys fed on the high DEB treatment, FCR of turkeys fed on the high CP diets was lower than FCR of turkeys fed on the low CP (LCP) diets whereas on the low DEB treatment, FCR was not affected by CP treatment. 6. It was concluded that litter quality can be improved and FPD may be decreased in turkeys fed on diets containing lower CP and DEB levels.

Kuch A, Goc A, Belkiewicz K, Filipello° V, Ronkiewicz P, Golebiewska A, Wrobel I,

Kiedrowska M, Wasko I, Hryniewicz W, Lomonaco S,Skoczynska A

Molecular diversity and antimicrobial susceptibility of Listeria monocytogenes isolates from invasive infections in Poland (1997-2013)

Sci Rep. - Vol. 8 (2018). - Article no. 14562 (11 p). - 52 bib ref [Nr. Estr. (ultimo accesso 011/01/2019) https://doi.org/10.1038/s41598-018-32574-0 8078]

The epidemiology of invasive listeriosis in humans appears to be weakly characterized in Poland, the sixth most populous member state of the European Union. We obtained antimicrobial susceptibility data, PCR-serogroups and genotypic profiles for 344 invasive isolates of Listeria monocytogenes, collected between 1997 and 2013 in Poland. All isolates were susceptible to the 10 tested antimicrobials, except one that was resistant to tetracycline and minocycline and harbored the tet(M), tet(A) and tet(C) genes. Overall, no increasing MIC values were observed during the study period. Four PCR-serogroups were observed: IVb (55.8%), IIa (34.3%), IIb (8.1%) and IIc (1.8%). We identified clonal complexes (CCs) and epidemic clones (ECs) previously involved in outbreaks worldwide, with the most prevalent CCs/ECs being: CC6/ECII (32.6%), CC1/ECI (17.2%), CC8/ECV (6.1%) and CC2/ECIV (5.5%). The present study is the first extensive analysis of Polish L. monocytogenes isolates from invasive infections.

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

Are farmers taking care about the future of their dairy herd?

Proceedings of the fifth DairyCare Conference 2018 : Thessaloniki, March 19th and 20th 2018 / editor, C.H. Knight. - [s.l.] : DairyCare COST Action FA1308, 2018. - p 55 (Poster 23) [Nr. Estr. 7820]

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

Heifers represent the future of a dairy cow herd, but farmers' attention is usually focused on the producing animals rather than on the young stock. However, management and housing of heifers could have an important impact on the expected milk production and quality as well as on herd health. The goal of the present study was to verify and analyse the differences between farmers' practices towards lactating cows (LC) and towards heifers (HF). During the two-year period 2016-2017, 854 Italian dairy farms (range: 5–1,300 lactating cows and 1–910 heifers) were visited by trained assessors to collect data about rearing routines of LC and HF. The management-, resource- and animal-based measures listed in Table 1 were recorded in each herd for both LC and HF groups. The obtained data were analysed using odds ratios with 95% confidence intervals. "To be HF" or "not to be HF (i.e. to be LC)" was the exposure variable, while on-farm measure answers were the outcomes. Results are reported in Table 1. Frequencies of poor hygiene and inadequate type of bedding material were found to be higher in HF group than in LC group. Also cleanliness of floor in the walking area and space availability in the lying area were found to be insufficient more frequently in the HF group than in the LC group. Considering the animal-based measures, HF were found to be more difficult to approach and dirtier than LC. On the other hand, LC group suffered for an inadequate number of feeding places and of water points and was found to have integument alterations with higher frequency than HF group. Cleanliness and inadequate lying area were the two main problems of HF group. This lack of attention could cause problems, such as mastitis, in freshly calved heifers with important welfare and economic consequences.

Mazzone P, Corneli S, Di_Paolo A, Maresca C, Felici A, Biagetti M, Ciullo M, Sebastiani C, Pezzotti G, Leo° S, Ricchi° M, Arrigoni° N

Survival of Mycobacterium avium subsp. paratuberculosis in the intermediate and final digestion products of biogas plants

J Appl Microbiol. - Vol. 125 (2018). - p 36-44. - 40 bib ref [Nr. Estr. (ultimo accesso 11/03/2019)

https://doi.org/10.1111/jam.13762 7895]

Aims: To evaluate the survival of Mycobacterium avium subsp. paratuberculosis (MAP) during anaerobic digestion (AD), we studied two different biogas plants loaded with manure and slurry from paratuberculosis-infected dairy herds. Methods and Results: Both plants were operating under mesophilic conditions, the first with a single digester and the second with a double digester. Mycobacterium avium subsp. paratuberculosis detection was performed by sampling each stage of the process, specifically the prefermenter, fermenter, liquid digestate and solid digestate stages, for 11 months. In both plants, MAP was isolated from the prefermenter stage. Only the final products, the solid and liquid digestates, of the one-stage plant showed viable MAP, while no viable MAP was detected in the digestates of the two-stage plant. Conclusions: Mycobacterium avium subsp. paratuberculosis showed a significant decrease during subsequent steps of the AD process, particularly in the two-stage plant. We suggest that the second digester maintained the digestate under anaerobic conditions for a longer period of time, thus reducing MAP survival and MAP load under the culture detection limit. Significance and Impact of the Study: Our data are unable to exclude the presence of MAP in the final products of the biogas plants, particularly those products from the single digester; therefore, the use of digestates as fertilizers is a real concern related to the possible environmental contamination with MAP.

Moreno° A, Lelli° D, Lavazza° A, Sozzi° E, Zanni° I, Chiapponi° C, Brocchi° E, Foni° E

Mab-based competitive ELISA for the detection of antibodies against influenza D virus

4th International Symposium on Neglected Influenza Viruses : Brighton, UK,18-20 April 2018 / organized by the International Society for Influenza and other respiratory virus diseases (ISIRV). - [s.l. : s.n., 2018]. - p 29-30 (Oral O25) [Nr. Estr. 8111]

International Symposium on Neglected Influenza Viruses (4th : Brighton, UK : 18-20 April 2018)

Objectives: Influenza D virus (IDV) was first reported in 2011 in swine in Oklahoma and consequently found in cattle, sheep and goats across North America and Eurasia. Cattle have been proposed as the natural reservoir. In this study, we developed and validated a MAb-based competitive ELISA for the detection of antibodies against IDV virus (IDV¬ELISA). Methods: Hybridomas specific to IDV were generated using Balb/C mice immunized with purified IDV/Swine/Italy/199724-3/2015. The specificity of MAbs was determined by comparing their reactivity with the homologous and other influenza A viruses along with additional bovine and swine viruses (BHV1, PI3, RSV, BVDV, PEDV PRRSV). IDV-ELISA was performed using the partially purified antigen coated to the plate, two serum dilutions (1/10 and 1/20) and addition of peroxidase-conjugated MAb. Results were expressed as percentage of inhibition (PI) with respect to the non-inhibited reaction. To evaluate the diagnostic performances of IDV-ELISA, we used 618 sera (204 HI test negative and 414 HI test positive) from different species: bovine (478), swine (79), wild ruminants (47), pheasant (9) and chickens (5). The agreement between IDV-ELISA and HI test was assessed by Cohen's Kappa value (K). ROC analyses were performed to enable the selection of best cut-off value and estimation of diagnostic specificity and sensitivity. Results: Thirty-one anti-IDV MAbs were characterized using different ELISAs, imnnunofluorescence and HI assays. Out of nine MAbs positive by HI one showing wide intra-type cross-reactivity was selected as competitor MAb in the IDV¬ELISA. K analysis showed an almost perfect agreement (K=0.93; 95%CI -0.899-0.961) between HI test and IDV-ELISA. ROC analysis evidenced that IDV-ELISA was accurate with an Area Under Curve AUC=0.996 (95%CI 0.988 to 0.999) and high sensitivity (Se: 99.75; 95%CI 98.6 - 100.0) and specificity (Sp: 98.52; 95%CI 95.7 - 99.7). The cut-off value representing the optimal balance of Se and Sp at the first dilution 1/10 was 65% percentage of inhibition. The subsequent dilution 1/20 could be used to estimate the antibody level. Conclusions: These results proved excellent diagnostic performances of IDV-ELISA, which compared to HI test presented mayor advantages, such as suitability for automation, low dependence to individual skills, spectrophotonnetric reading and easy interpretation of the results. This assay can potentially be exploited to detect antibodies against IDV in different animal species.

Morris A, Grazioli° S, Pezzoni° G, Wilsden G, Browning C, Gubbins S, Ludi A, King D, Brocchi° E

Investigating cross reactivity of serological enzyme linked immunosorbent assays

OS18 "Global vaccine security" : EuFMD Open session : October 2018, Puglia, Italy : online version, book of abstracts / [s.l. : s.n., 2018]. - Day 3. - p 63 [Nr. Estr. 8012]

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

Introduction Serological assessments are vital in supporting official programmes aimed at monitoring, controlling and assessing the prevalence of foot-and-mouth disease virus (FMDV). The World Organisation for Animal Health (01E) details the virus neutralisation test (VNT) as the 'gold standard' for the detection of antibodies reactive to FMDV structural proteins. However, a number of in-house and commercial serological Enzyme Linked Immunosorbent Assays (ELISAs) are widely employed to indirectly assess the immune status of an animal. The purpose of this study is to determine the extent of cross reactivity that exists for three routinely used serological ELISAs: polyclonal Liquid Phase Blocking ELISA (LPBE), polyclonal Solid Phase Competition ELISA (SPCE) and commercially available kits based on SPCE principle and monoclonal antibodies (IZSLER kits). Method and .Results Three routinely used serological ELISAs for detection of antibodies against five FMDV se-rotypes (0, A, Asia 1, Southern African Territories (SAT) 1 and SAT 2) were employed for comparison: LPBE, SPCE and IZSLER SPCE kits. A selection of 365 monovalent experimental sera, representing all seven serotypes: 0 (n=116), A (n=120), C (n=18), Asia 1 (n=61), SAT 1 (n=14), SAT 2 (n=30) and SAT 3 (n=6) were assayed and analysed according to the validated protocol for each ELISA. Thus far, the presence of cross reactivity is evident in all three ELISAs for the seven serotypes, although higher sensitivity was observed for the sera specific to the serotype of the ELISA. Discussion The presence of cross reactivity using ELISAs prevents serotyping of an individual serum, therefore interpretation should be considered at population level. In the context of a known outbreak scenario the assays are sensitive for that specific serotype.

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

Comparison of welfare assessment results from Welfare Quality® and a national system on Italian dairy farms

Proceedings of the fifth DairyCare Conference 2018 : Thessaloniki, March 19th and 20th 2018 / editor, C.H. Knight. - [s.l.] : DairyCare COST Action FA1308, 2018. - p 58 (Poster 28) [Nr. Estr. 7821]

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

The lack of common legislation for dairy cow welfare in Europe and the growing public concern over the ethics of livestock production have brought several research groups and private industries to develop different on-farm welfare assessment systems. Among these, the Welfare Quality® (WQ) can be considered the gold standard. The Italian Reference Centre for Animal Welfare (CReNBA) has developed a dairy cow welfare assessment protocol based on WQ and EFSA publications to be used routinely at national level for supporting official on-farm controls and for certifying animal-friendly products. This preliminary study tested the compatibility of the CReNBA system with WQ. The welfare of dairy cattle was assessed on 5 loose housed Italian farms (18 – 54 lactating cows) simultaneously by one qualified CReNBA assessor and one trained WQ assessor. The farms' overall welfare score (OWS) and principle scores (PS) obtained from WQ and CReNBA protocols were compared using Pearson's correlation. Only data for lactating and dry cows were considered, except for disbudding practices that concerned calves. In WQ, PS are calculated through measure and criteria scores by applying different weights. CReNBA assessment involves animal-, resource- and management-based measures, that can be related to the WQ principles (Table 1). Each CReNBA measure has a different weight, which was assigned by expert opinion elicitation. The single measure scores are aggregated and normalized between 0-100% to obtain the PS and the OWS. According to both protocols, the OWS was "enhanced" for four farms and "acceptable" for one

farm. However, a different farm was classified “acceptable” according to WQ and CReNBA. The systems agreed well in three of the four PS (Table 1). Differences in the remaining principle might derive from the great importance given to the water provision measures in the WQ system, as underlined in other studies. This preliminary test showed that, on welfare principle level, the CReNBA system can produce results that are compatible with WQ, even though the measures in CReNBA system and WQ are partly different. However, the sample size in this test was very small and thus further experiments are needed to confirm the results.

Peli A, Merialdi° G, Limatola C

Gli Organismi Preposti al Benessere Animate (OPBA) : stato dell'arte, ruoli e responsabilità e risultati della prima indagine conoscitiva in Italia

Prima Convention Nazionale degli Organismi Preposti al Benessere Animale (OPBA) : Roma, 9-10 luglio 2018 / [s.l. : s.n., 2018]. - p 11-16 [Nr. Estr. 8161]

Convention Nazionale degli Organismi Preposti al Benessere Animale (OPBA) (1. : Roma : 9-10 luglio 2018)

Pezzotta R, Scaltriti° E, Vezzoli C, Caruso A, Fiorentini S

Neuroinvasive Streptococcocus group A in paediatric patients : three non-clustered subsequent cases

6th Italian experience in biomedical research : young minds at work : Desenzano del Garda (BS), 12th-13th October, 2018 / [s.l. : s.n., 2018]. - p 39 [Nr. Estr. 8024]

Annual Italian Experience in Biomedical Research : young minds at work (6th : Desenzano del Garda (BS) : 12th-13th October 2018)

Group A streptococcus (GAS) is responsible for a wide spectre of human pathologies. Since 1980s a great increase in the incidence of GAS-related invasive diseases has been observed but GAS neuroinvasion remains a rare event. Here we report a 3-cases serie occurred in Brescia from Febuary to April 2018. METHODS. A total of 4 GAS strains, isolated from nasal swab (SP1), CSFs (SP2 and 5P3) and abscess (SP4), were subjected to Whole Genome Sequencing (WGS) with 250x2bp paired-end runs on a Miseq. Genomes were de-novo as¬sembled and compared to GAS genomes downloaded from international da¬tabases using kSNP3. Phylogenetic distances were inferred by Maximum Likelihood (ML) algorithms using PhyML. Virulence factors were detected by mapping reads against Virulence Factor (VFDB). RESULTS. GAS neuroinvasion occurred in a two-years-old female with menin¬gitis (sample SP1 and SP2), a six-years-old male with meningitis and post-infec¬tion sequelae (sample 5P3) and, a four-years-old female presenting crebellar abscess that leaded to exitus (sample SP4). Among virulence factors (VF), the M protein is crucial and its gene is used as the basis for GAS typing. Strains SP1 and 2 were found to be close related to MGAS 15252 and no exclusive VF were found comparing these isolates with others in this study (SP3 and 4). SP3 resulted to be an M6 type GAS similar to MGAS10394 with an exclusive VF (exoenzyme mf4). 5P4 strain, resulted to be a Manfred° M5 strain, carrying several exclusive VF: exoenzyme sda and exo¬toxins speA e spel. CONCLUSIONS. Genomic investigation elucidated that neuroinvasive GAS iso¬lates belong to different known virulent type (e.g. M5 and M6 type) and highli¬ghted that they possess several significant VFs. According to the detected genes, 5P4, isolated from the patient with the worst outcome, appeared as the most virulent among the analyzed strains.

Piredda I, Palmas B, Noworol M, Tola S, Longheu C, Boniotti° MB, Bertasio° C, Denurra D, Cherchi M, Pintore A, Ponti MN

First isolation of Leptospira SPP from a dolphin (Tursiops truncatus) in the Mediterranean

sea, assessment of halotolerance in seawater

3rd ELS scientific meeting on Leptospirosis and other rodent borne haemorrhagic fevers : 24-26 May 2018, Alghero / [s.l. : s.n., 2018]. - p 70 [Nr. Estr. 7862]

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

The pathogens Leptospira species are very widespread in nature, persisting and multiplying in the kidney tubes of many domestic and wild animals (reservoir). Infected animals shed the pathogen in their urines. Many mammals, including humans, are subject to infection, but information on leptospirosis associated with cetacean species is currently very limited. This paper describes the first isolation of a pathogenic Leptospira interrogans from a dolphin stranded along the coasts of Sardinia, showing that bottlenose dolphin represents a potential source for human leptospirosis in marine environments. The bacteriological survey is conducted sowing 25 mg of renal tissue homogenate in three tube which contained 3 ml of Ellinghausen- McCullough-Johnson-Harris (EMJH) semi-solid medium (Difco) supplemented with 200 pg/mL 5-fluorouracil and incubated at 28-30°C for 60 days and regularly observed under dark-field microscopy every 7 days. Three panels designated as A, B, and C were used in this study to test halotolerance of isolated strain. Real-time PCR targeting highly conserved region of the LipL32 gene was used in the study. Three target genes rrs, secY and rpoB were used for sequencing. For typing, Multilocus Sequence Typing (MLST), Multilocus Variable-Number Tandem- Repeat Analysis (MLVA), and Pulse Field Gel Electrophoresis (PFGE) were performed on dolphin isolate. On June 2016, an adult female bottlenose dolphin (Tursiops truncates species) was found dead and stranded near to the gulf of Orosei Although bad conditions of kidneys were observed, a positive culture of Leptospira spp. was obtained after about three weeks. Real time PCR reported positivity for pathogenic Leptospira. Genetic characterization classified the strain as Leptospira interrogans. Dolphin isolate survived different seawater concentrations (up to 20%). Residual viability thus infectivity after exposure to pure seawater for 3 days was demonstrated by subculturing surviving bacteria in fresh EMJH. Our study provides the first evidence of pathogenic Leptospira classified as belonging to the genus Leptospira interrogans in a dolphin stranded in the Mediterranean Sea. PFGE and MLVA, applied to the dolphin isolate and 2 other wild boar isolates analyzed in parallel, showed the same genetic profile demonstrating that the same strain can be transmitted from terrestrial to marine animals and can contaminate the coastal environment. These findings suggest some questions about the route of infection and the potential role of cetaceans in the epidemiology of leptospirosis. The zoonotic nature of this strain and its diffusion, between both wild and domestic terrestrial animals as well as in marine mammals, does pose a significant risk to human health. These results highlight the need for conducting deeper longitudinal surveys including other animal species living in this area.

Re R, Bertocchi° L, Giovannantonio P, Fusi° F, Lorenzi° V, Angelucci° A, Monteverde VP, Nicolussi P

Protocollo di valutazione del benessere e della biosicurezza nelle pecore da latte = Check list for assessing welfare and biosecurity in dary [i.e. dairy] sheep breeding

XXIII Congresso Nazionale Societa' Italiana di Patologia e di Allevamento degli Ovini e dei Caprini (SIPAOC) : Napoli 12-14 settembre 2018 / edited by Antonio Bosco ... [et al.]. - [Naples : University of Naples Federico II, 2018]. - (Mappe parassitologiche ; 24) p 157-158. - 5 bib ref [Nr. Estr. 7948]

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

L'allevamento semiestensivo degli ovini da latte, più sostenibile rispetto agli allevamenti intensivi, e caratterizzato da un minore impatto sul benessere animale; tuttavia presenta alcune criticita su cui è necessario focalizzare l'attenzione. A livello europeo non esiste una normativa specifica per la salvaguardia del benessere negli allevamenti ovini; la Direttiva 98/58/CE fornisce solo regole generali e non specifiche per questa specie. Ii progetto Welfare Quality ha introdotto una nuova metodica per la valutazione del benessere incentrata sugli ABMs (animal based measures), ma che utilizza anche rilevazioni riguardanti gli N-ABMs (non animal based measures). ABMs e N-ABMs

sono entrambi necessari per ottenere una valutazione completa del benessere in allevamento. EFSA riprende la linea innovativa di Welfare Quality riguardo alle ABMs, ma in più introduce la valutazione dei fattori di rischio che incidono negativamente sull'animale, fornendo un parere scientifico sui maggiori pericoli per il benessere degli ovini produttori di lana, came e latte. Non esiste in Italia un protocollo di valutazione del benessere animale nell'allevamento degli ovini da latte che utilizzi sia gli ABMs che gli N-ABMs unitamente alla valutazione del rischio. L'IZS della Sardegna, su incarico del CRENBA, ha sviluppato un protocollo di valutazione semplice e facilmente utilizzabile, da applicare per i controlli ufficiali in tutto ii territorio nazionale.

Rubini° S, Barbieri S, Gaudio RM, Govoni G, Berna GR, Fico R, Lorenzini R, Fontana° MC, Taddei° R, Tassinari M, Frisoni P, Guidi E, Bergamini M

Veterinary forensic sciences to solve a fatal case of predation on flamingos (Phoenicopterus roseus)

Vet Ital. - Vol. 54 no 2 (2018). - p 175-180. - 25 bib ref [Nr. Estr. (ultimo accesso 11/03/2019) http://www.izs.it/vet_italiana/2018/54_2/175.htm 7887]

The present case study concerns a case of predation of 4 individuals of captive pink flamingo in Emilia Romagna Region, Northeastern Italy. The pink flamingo (Phoenicopterus roseus) is a species included in the Red List of Threatened Species established by the International Union for Conservation of Nature (IUCN) which lists species in danger of extinction. During the Winter of 2013,4 flamingos (2 in the Comacchio area, and 2 from Argenta and Codigoro oases — Ferrara province) were found dead some of them headless, with their bodies severely bitten. At first, a fox (Vulpes vulpes) was suspected to be the predator responsible for the killing and the birds were taken to the laboratory for further investigations. The investigations included: field observations, study of the predator behaviour, necropsy examinations, assessment of the intercanine distance, and genetic analysis on the predator's traces. The intercanine distance indicated that the predator could not have been a fox. The analysis of salivary DNA samples enabled us to establish that the predator was in fact a dog. This case highlights the importance of co-operation among the various branches of forensic sciences and the great usefulness of the roles filled by other veterinary forensic experts involved in solving crime.

Rugna° G, Salvatore D, Carra° E, Bergamini° F, Di_Francesco A, Varani S

Leishmaniasis : a peculiar epidemiological situation in northeastern Italy

2eme Congres de l'Ecole Nationale de Medicine Veterinaire de Sidi Thabet, Tunisie : livres de resumes et programme : Sidi Thabet, 25 et 26 Octobre 2018 / [s.l. : s.n., 2018]. - p 31 [Nr. Estr. 8109]

Congres de l'Ecole Nationale de Medicine Veterinaire de Sidi Thabet, Tunisie (2eme : Sidi Thabet, Tunisie : 25 et 26 Octobre 2018)

Résumé: Visceral leishmaniasis (VL), caused by Leishmonia infantum (L. infantum), is endemic in the Mediterranean basin, where dog is considered the principal domestic reservoir and the infection occurs through the bite of infected female sand fly species of the genus Phlebotomus. In Italy, classical endemic zones for Vt. are the Tyrrhenian littoral, the southern peninsular regions, and the islands. Since the 1990s, the incidence of VL has increased in humans and dogs, both in the traditional endemic areas and in the northern regions previously regarded as non-endemic. From November 2012 to May 2013, a human outbreak of VL occurred in the Emilia-Romagna region (Bologna province), northern Italy (Varani et al., 2013): 14 autochthonous cases of VL were notified. In the same period, an increasing number of VL cases was reported in the neighboring Modena province (Franceschini et al., 2016). Following this increase, surveillance activities have been implemented, involving humans, vectors and dogs (Santi et al., 2014). In this regard, 65 Leishmonia samples (isolates and DNA from biological samples), divided in two subsets, were analyzed (Rugna

at al., 2017, 2018). The first subset, called E¬R, included 55 samples obtained from different provinces of the Emilia-Romagna region during 2013- 2017 (40 samples from dogs, 11 from humans and 4 from sand flies). The second subset, called extra E-R, included 10 isolates obtained from human VL cases occurred in other Italian regions. All the samples were analyzed by molecular methods focused on genetic targets, such as cysteine protease B (cpb) gene, k26-gene and a repetitive nuclear region on chromosome 31. Multilocus microsatellite typing completed the population genetic study. The results suggested a peculiar epidemiological situation in northeastern Italy, with the co-circulation of two distinct populations of L. infantum: one population mainly detected in dogs and the other population detected in humans and in a sand fly. The presence of two sympatric populations could be explained by two overlapping transmission cycles occurring in the Emilia-Romagna region, involving different sand fly vectors and/or reservoirs. A comprehensive surveillance of parasitic strains obtained from vectors, human and non-human hosts, is warranted to confirm the presence of two concurrent epidemiological cycles and to improve control measures for leishmaniasis in this area.

Tambassi° M, Berni° M, Bracchi° C, Morganti° M, Scaltriti° E, Bolzoni° L, Kingsley R, Pongolini° S, Casadei° G

A single SNP in the transcriptional regulator hilD disrupts Salmonella Derby virulence in human cells

International Symposium Salmonella and Salmonellosis : Saint-Malo, France, 24-26 September 2018 / edited by Pierre Colin, Geneviève Clement. - [s.l. : ZOOPOLE Développement - ISPAIA, 2018]. - p 10 [Nr. Estr. 8029]

International Symposium Salmonella and Salmonellosis : Saint-Malo, France : 24-26 September 2018)

In-depth knowledge of the molecular mechanisms that regulate host adaptation and cross-species transmission in Salmonella will improve prevention of pathogens transmission within surveillance systems. We performed an epidemiological and Pulsed-Field Gel Electrophoresis (PFGE) analysis on the —10,000 Salmonella isolates within our Regional Enter-Net Reference Center collection. We found that serovar Derby (115 human and 277 swine isolates) is more prevalent in swine (28.2%) than in human (2.6%), but, considering the two most prevalent PFGE profiles in swine, PFGE_A is significantly less isolated in human than in swine(1 human vs 27 swine isolates) whereas PFGE_B is proportionally isolated in human as well as in swine (15 human vs 26 swine isolates). Invasion and replication assays on 39 isolates from PFGE_A and PFGE_B profiles showed that in human cells the replication efficiency of PFGE_B isolates is up to 4 logs higher than that of PFGE_A isolates while no differences are detected in swine cells. We reasoned that analyzing close PFGE_A and PFGE_B isolates with different host ranges, by whole genome sequencing, could identify the genetic features involved in host adaptation. SNPs-based phylogeny showed that PFGE_A isolates cluster separately from PFGE_B, suggesting different evolutionary paths. We found 18 non-synonymous SNPs differentiating PFGE_A from PFGE_B isolates, including one single SNP in hilD, the main transcriptional regulator of SPI-1 and other virulence genes. In a PFGE_B isolate (ER1175) the virulent-hilD allele was replaced by recombination with the PFGE_A not-virulent-hilD variant (ER1175::nvhi/D): the obtained mutant shows the same low replication efficiency of PFGE_A isolates in human cells, as well as the hi/D-knockout mutant (ER1175Ahi/D). Accordingly, a PFGE_A isolate with virulent-hilD allele shows the same high replication efficiency of PFGE_B isolates. Mutants remained fully pathogenic in swine cells. We extracted bacterial RNA from human cells infected by ER1175, ER1175::nvhi/D and ER1175Ahi/D at 3 different time-points (0, 30 and 60 minutes post-infection) to analyze the expression of hilD and 6 SPI-1 genes over time. We performed qPCR on the other SPI-1 transcriptional activators (hilC, rtsA, hilA and invF) and 2 representative genes of SPI-1 type III secretion system and effector proteins injected into host cells (invA and sipB), respectively. During infection ER1175 increases expression of hilD and SPI-1 genes over time, while almost no expression was recorded for ER1175::nvhi/D and ER1175Ahi/D. Overall this work shows that just one SNP in the transcriptional regulator hilD impairs human cells-specific virulence of Salmonella Derby.

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Identification of a practical and reliable method for the evaluation of litter moisture in turkey production

Br Poult Sci. - Vol. 59 no 1 (2018). - p 7-12. - 9 bib ref [Nr. Estr. (ultimo accesso 11/03/2019) https://doi.org/10.1080/00071668.2017.1381334 7812]

1. An experiment was conducted to compare 5 different methods for the evaluation of litter moisture. 2. For litter collection and assessment, 55 farms were selected, one shed from each farm was inspected and 9 points were identified within each shed. 3. For each device, used for the evaluation of litter moisture, mean and standard deviation of wetness measures per collection point were assessed. 4. The reliability and overall consistency between the 5 instruments used to measure wetness were high (a = 0.72). 5. Measurement of three out of the 9 collection points were sufficient to provide a reliable assessment of litter moisture throughout the shed. 6. Based on the direct correlation between litter moisture and footpad lesions, litter moisture measurement can be used as a resource based on-farm animal welfare indicator. 7. Among the 5 methods analysed, visual scoring is the most simple and practical, and therefore the best candidate to be used on-farm for animal welfare assessment.