

## **RICERCHE EFFETTUATE SANITA' ANIMALE**

Amadori°M, Zanotti°C

### **Immunoprophylaxis in intensive farming systems : the way forward**

Vet Immunol Immunopathol. - Vol. 181 ( 2016). - p 2-9. - 65 bib ref [Nr. Estr. 7383]

High levels of production in intensive farming systems are associated with increased replacement rates as a result of multifactorial diseases. The so-called “production diseases” may include low-grade infection reducing profitability without increased morbidity. Such infections are sustained by low pathogenic viral and bacterial agents which give rise to full-blown disease in association with poor environmental conditions. In these farms, the results of vaccination may be disappointing. Therefore, fundamental issues should be dealt with toward successful immunoprophylaxis. High lean meat and milk production are associated with chronic inflammation and activation of the innate immune system vis-à-vis cellular stress. This may negatively affect adaptive immune responses. A negative modulation of the host microbiome by farm management practices and drug treatments is a further risk factor. The immune response to stressed cells questions the usual correlates of protection investigated after vaccination. In particular, there is evidence that specific and non-specific immune responses may overlap in vitro as a result of a high level of innate immune responses to Damage-Associated Molecular Patterns (DAMPs) and stress antigens. A vigorous adaptive immune response to microbial agents may be sometimes counterproductive, as suggested in porcine reproductive and respiratory syndrome virus (PRRSV) infection. Alternative outcomes should be sometimes pursued: a better homeostatic control of the inflammatory response, effective and self-limiting innate immune responses, and even tolerance induction. On the whole, successful immunoprophylaxis in intensive farming systems demands co-ordinated and multi-disciplinary efforts in terms of animal breeding, farm management and hygiene, correct choice and harmonization of the prophylactic tools (vaccines, immunomodulators, pre- and probiotics). Finally, there is evidence that disease-predicting parameters of the innate immune response may greatly ease the identification of herds and animals at risk, and contribute to reduced antibiotic usage on farm.

Amato B, Mignacca SA, Pacciarini°ML, Vitale M, Antoci S, Cucinotta S, Puleio R, Biasibetti E, Fiasconaro M, Capucchio MT , Di\_Marco Lo\_Presti V

### **An outbreak of bovine tuberculosis in a fallow deer herd (Dama dama) in Sicily**

Res Vet Sci. - Vol. 106 ( 2016). - p 116-120. - 40 bib ref [Nr. Estr. 7263]

Wild ruminants have an important role in the epidemiology of bovine tuberculosis (bTB). This study describes an outbreak of bovine tuberculosis occurring in a fallow deer herd in Sicily. In 2012 a Sicilian herd of 47 animals was referred for cachexia. Pathological examination of 2 dead animals revealed disseminated granulomas predominantly involving the skin and subcutaneous tissues. Tissue samples were submitted for histological analysis, bacteriological culture, and biomolecular assay. PCR analysis identified Mycobacterium strains. Genotyping by spoligotyping and MIRU-VNTR profiles identified Mycobacterium bovis spoligotype SB0120 in both animals. In 2014, bTB skin testing of 28 fallow deer from the same group was positive in 4 and inconclusive in another 4. All 8 positive/inconclusive reactors were euthanized. Disseminated granulomatous lesions were noted in 6 of these animals, 3 of which (2 positive and 1 negative to skin tests) also presented cutaneous lesions. M. bovis spoligotype SB0120 was identified from all animals in which tuberculous-like lesions were observed, including 2 negative reactors. Many of the animals involved in this outbreak presented diffuse skin lesions, a potential route of transmission of M. bovis infection. Given the epidemiological role wildlife play in the maintenance of bTB infection and its potential risk for humans, a comprehensive monitoring plan for this zoonosis in wildlife species in Sicily is needed.

Amato L, Pacciarini<sup>o</sup>M, Schiavon E, Zanoni B, Boniotti<sup>o</sup>MB, Ferronato A, Montagna A, Costa S, Bricchese S, Bonfanti L

**Identification of *Mycobacterium caprae* in a dairy farm in north-eastern Italy**

Int J Infect Dis. - Vol. 53 Suppl ( 2016). - p 33 [Nr. Estr. 7684]

International Meeting on Emerging Diseases and Surveillance (IMED) (6th : Vienna, Austria : November 3 to 7, 2016)

**Purpose:** Enhanced cross-sectorial collaboration and sharing of surveillance information between the animal and the public health sectors are key to improve the management of zoonotic threats. However, there is little evidence on the costs and benefits of One Health (OH) surveillance for zoonoses. An integrated and multi-disciplinary West Nile virus (WNV) surveillance system (SS) has been implemented in Emilia-Romagna since 2009. The SS includes surveillance activities in the public health and in the animal health sectors. From 2013, surveillance information generated in the two sectors is shared, guiding targeted public health interventions to mitigate the risk of WNV transmission via blood transfusion. The objective of this work was to estimate the cross-sectorial costs and benefits associated with the OH approach to surveillance information of this SS. **Methods & Materials:** We applied a conceptual framework to identify the cross-sectorial links between WNV surveillance and triggered interventions, and the associated costs and benefits. Cost items included costs of human, animal, and entomological surveillance, linking of information, and triggered interventions. Benefits were quantified as the averted costs of potential human cases of West Nile neuroinvasive disease associated to infected blood transfusions. Evaluation of costs and benefits of surveillance designs was conducted considering two scenarios: OH and a uni-sectorial approach that does not integrate animal health information. **Results:** The OH scenario was estimated to represent a reduction of 184'619 EUR in the overall costs of surveillance in the 2009-2015 period. The main cost components were blood donation screening activities in both the OH and uni-sectorial scenario. The OH approach allowed savings of 1.24 million EUR in blood donations screening activities. These savings compensated the cost of animal health surveillance and linking of information. Benefits of the OH approach due to avoided short term cost-of-illness and avoided compensation for transfusion-transmitted infections were estimated to be 3.0 million EUR. **Conclusion:** Overall, the OH approach to WNV surveillance in Emilia-Romagna region is estimated to be economically beneficial. These results can further contribute to bring evidence on the economic aspects of OH surveillance for zoonoses and contribute for the prioritization of resource allocated to zoonoses mitigation.

Angelucci<sup>o</sup>A, Fusi<sup>o</sup>F, Lorenzi<sup>o</sup>V, Zanardi<sup>o</sup>G, Bertocchi<sup>o</sup>L

**An investigation on the efficacy of a vaccine against *Staphylococcus aureus* under field conditions in Northern Italy**

Proceedings of the 29th World Buiatrics Congress 2016 : Theme "Cattle Health - Tomorrow's Thinking Today" : Dublin, Ireland 2016 : oral communication and poster abstracts / [s.l. : s.n., 2016]. - p 689-690 [Nr. Estr. 7341]

World Buiatrics Congress (WBC) (29th : Dublin, Ireland : 2016)

**Objectives:** The aim of this study was to evaluate the efficacy of a commercial vaccine (Startvac<sup>®</sup>, Hipra Spain) aimed at reducing intramammary infections (IMI) by *Staphylococcus aureus* and coagulase-negative staphylococci under field conditions. **Materials and Methods:** This study was carried out in a farm in Northern Italy, which was selected basing on the size of the herd (450 lactating cows). Cows were randomly allocated in two groups. The first group received the vaccine following the label regime, and the second group was left unvaccinated to act as controls. The two groups were housed in the same pen. Four aspects of vaccine effectiveness were considered: the first includes the impact of vaccination on the rate of new infections, second it concerns the impact of vaccination on the reduction of shedding of *S. aureus* from an infected animal, third it addresses the impact of vaccination on the rate of cure of the infection; and the finally, the fourth aspect concerns

the reduction of clinical symptoms related to infection due to vaccine effectiveness. Vaccination of animals was made according to the registration protocol of the vaccine for a total of three doses, with the first dose at 45 days before the estimated date of calving, the second administration at 10 days before the date of calving, and the third dose at 52 days post-calving. Results: Results refers to about half of the trial duration (18 months). The prevalence of *S. aureus* in the studied herds was about 3% at single quarter level for vaccinated cows, while the prevalence of coagulase-negative staphylococci amounted to approximately 6%. The incidence of new staphylococcal IMI was lower in the vaccinated group than in the control group. Results showed a moderate difference in *S. aureus* prevalence in milk samples examined between vaccinated and control animals (3% in vaccinated group; 4% in control group). However, the average duration of infection by *S. aureus* was significantly shorter in vaccinated animals compared to control animals. We detected also a lower number of cases of clinical or subclinical mastitis in the vaccinated group than in the control group. Conclusions: The results indicate that the vaccine can be an effective tool to control staphylococcal mastitis, by reducing the incidence and duration of the infection. As reported in other studies, mastitis vaccination can play an important role in the mastitis control programs (Schukken et al, 2014; Bradley et al, 2015).

Arrigoni°N, Garbarino°C, Boldini°M, Ruocco L, Gemma\_Brenzoni L, Gradassi°M, Leo°S, Paternoster°G, Tamba°M

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

Proceedings of the 5th ParaTB Forum : Nantes, France, 19 June 2016 / [s.l. : s.n., 2016]. - p 11-16. - 9 bib ref [Nr. Estr. 7500]

ParaTB Forum (5th : Nantes, France : 19 June 2016)

Asfor A, Grazioli°S, Brocchi°E, King DP, Parida S , Tuthill TJ

**Detection of antibodies against conserved capsid epitopes provides a universal serology assay for diagnosis of FMDV**

Europic2016 : Les\_Diablerets, Switzerland, September 4-8, 2016 / [s.l. : s.n, 2016]. - 7509]

Europic2016 : Les\_Diablerets, Switzerland : September 4-8, 2016)

Foot-and-mouth disease virus (FMDV) causes one of the most economically important livestock diseases worldwide. FMDV exists as 7 serotypes and evolves rapidly so that circulating viruses display high levels of antigenic variation. Diagnosis of FMD can use various approaches including serological tests to detect FMDV specific antibodies. Conventional serology based diagnostics are reliable and rapid but require tests specific to each virus serotype. A number of monoclonal antibodies (mAbs) have previously been reported with cross-reactivity against multiple FMDV serotypes. Some of these mAbs have been mapped to the highly conserved N terminus of FMDV capsid protein VP2, suggesting that such conserved sequences might be useful diagnostic reagents. The aim of this study was to assess the potential of conserved N-terminal sequences of capsid proteins VP2 and VP4 as universal epitopes for the detection of FMDV specific antibodies against multiple FMDV serotypes. Synthetic peptides of various lengths were used to represent the conserved target epitopes at the N terminus of VP2 (VP2N) and N terminus of VP4 (VP4N). A panel of mAbs with existing evidence for cross-serotype activity and sera from cattle infected with each of the 7 serotypes of FMDV were tested for reactivity against the peptides by indirect peptide ELISA. Three mAbs showed strong reactivity to VP2N peptides, including to the shortest peptide tested (the first N-terminal 15 amino acids) suggesting this contained the epitope for these antibodies. Cattle sera against all 7 serotypes of FMDV reacted strongly with VP2N peptides and also with VP4N peptides demonstrating the peptides are indeed able to function as universal detection reagents for FMDV specific antibodies. This study demonstrates that conserved peptide epitopes in the FMDV capsid can be used as serotype-independent antigens for FMD serology. This may have utility in the development of new universal and rapid laboratory or field-based diagnostic tests.

Bacci C, Vismarra A, Passeri B, Sciarrone F, Mangia C, Genchi°M, Fabbi°M, Vicari°N, Bruini I, Brindani F, Kramer L

**Detection of Toxoplasma gondii and Sarcocystis tenella in indigenous Cornigliese sheep in Italy using serological and molecular methods**

Small Rumin Res. - Vol. 135 ( 2016). - p 13-16. - 18 bib ref [Nr. Estr. 7239]

The aim of the present study was to determine seroprevalence for Toxoplasma gondii by meat juice ELISA and evaluate the presence of T. gondii and Sarcocystis spp. within host tissues by histology, PCR and in vitro isolation, in the indigenous Cornigliese sheep breed in northern Italy. Seventeen out of 24 (70.8%) sheep were positive for T. gondii by meat juice ELISA. Twenty sheep (83.3%) were positive by PCR for T. gondii, while 24/24 sheep (100%) were positive by PCR for Sarcocystis spp. Tissues cysts compatible with Sarcocystis spp. were visible in all animals on histology. PCR confirmed the presence of T. gondii after three weeks of in vitro culture on Vero cells in only one sample. Genotyping of T. gondii by RLFP with 5 markers showed a predominance of genotypes II/III. Sequence analysis of Sarcocystis spp. showed only the presence of Sarcocystis tenella. T. gondii and S. tenella are present in a high percentage of Cornigliese sheep in northern Italy. Future studies should concentrate upon the reproductive and economic effects of these parasitic infections, in light of the necessary conservation of this local, indigenous sheep breed.

Baldo°V, D'Incau°M, Salogni°C, Giovannini°S, Rossi L, Acquarone F, Boniotti°MB, Pasquali P, Alborali°GL

**Caratterizzazione biomolecolare, tipizzazione sierologica e resistenza agli antimicrobici in ceppi di Escherichia coli produttori di Shiga tossina (STEC) isolati da suini**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 125-131. - 21 bib ref [Nr. Estr. 7231]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

L'obiettivo del lavoro è di valutare i profili di antibiotico-resistenza, le caratteristiche biomolecolari e sierologiche dei ceppi di Escherichia coli produttori di Shiga tossina (STEC) isolati da suini in accrescimento con diarrea e sintomatologia riconducibile a malattia degli edemi. Sono stati isolati 50 ceppi di E. coli risultati essere produttori di Shiga tossina STX2e provenienti da suini in svezzamento (72%) ed in magronaggio (26%). Le lesioni anatomo-patologiche riscontrate erano caratterizzate da edema (32%), enterite catarrale-emorragica (26%) ed enterite catarrale (24%) e linfoadenite (6%). Il 92% dei ceppi di STEC hanno presentato il fattore fimbriale F18 e il 34% appartenevano al sierogruppo O139. Una elevata percentuale di ceppi è risultata essere resistente a Tetraciclina (92%), Amoxicillina (92%), Neomicina (90%), Doxicilina (90%), Sulfamidico/Trimethoprim (86%), Cefaloridina (82%), Sulfadiazina (82%). Il 74% dei ceppi STEC si sono dimostrati resistenti contemporaneamente ad un numero superiore a 9 antimicrobici e il 64% appartenenti alle classi di multiresistenza comprese tra 10 e 14.

*The aim of this study is to determine the antimicrobial susceptibility profiles of Shiga-toxin producing Escherichia coli (STEC) isolated in growing pigs clinically affected by edema disease and diarrhea. 50 STEC strains have been isolated in weaning (72%) and growing-finishing pigs (26%) with anatomo-pathological lesions related to edema (32%), catarrhal-hemorrhagic enteritis (26%), catarrhal enteritis (24%) and lymphadenitis (6%). The STEC often have the F18 fimbrial factor (92%) and sometimes belong to serogroup O139 (34%). A high percentage of strains was resistant to Tetracycline (92%), Amoxicillin (92%), Neomycin (90%), Doxycilina (90%), Sulfonamide / Trimethoprim (86%), Cephaloridine (82%), Sulfadiazine (82%). 74% of STEC were simultaneously resistant to antimicrobials number greater than 9 and 64% is included in multidrug resistance patterns between 10 and 14.*

Bano L, Tosi°G

**La caratterizzazione batterica nello studio di alcune malattie emergenti e riemergenti del pollame**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) 2016 : LV Convegno nazionale, Tavola rotonda : Padova, 5-6 maggio 2016 - Parma, 23 settembre 2016 / [s.l. : s.n., 2016]. - p 107-109 [Nr. Estr. 7476]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (55. : Padova : 5-6 maggio 2016)

Barberio A, Flaminio B, De\_Vlieghe S, Supré K, Kromker V, Garbarino°C, Arrigoni°N, Zanardi°G, Bertocchi°L, Gobbo F, Catania S, Moroni P

**Short communication : In vitro antimicrobial susceptibility of Mycoplasma bovis isolates identified in milk from dairy cattle in Belgium, Germany, and Italy**

J Dairy Sci. - Vol. 99 ( 2016). - p 6578-6584. - 22 bib ref [Nr. Estr. 7264]

The objective of this study was to assess the in vitro antimicrobial susceptibility of 73 isolates of *Mycoplasma bovis* isolated from milk of dairy cattle herds of Belgium, Germany, and Italy. Minimal inhibitory concentration (MIC) values were determined by the microbroth dilution method for the following antimicrobials: erythromycin, spiramycin, tilmicosin, tylosin, lincomycin, enrofloxacin, doxycycline, oxytetracycline, florfenicol, and tiamulin. Macrolides, florfenicol, oxytetracycline, and enrofloxacin, were chosen because they represent antimicrobials families commonly used in several countries for treatment of *M. bovis*, and their MIC values in cattle population are reported in several studies, allowing a comparison with previous data. Doxycycline and tiamulin were selected to assess the susceptibility of *M. bovis* to new antimicrobials, because they are not registered in the European Union for the treatment of dairy cattle. Among the agents of the different antimicrobial classes, the macrolides showed the highest concentration to inhibit 90% of isolates (MIC<sub>90</sub>), all above the highest concentration tested: >8 µg/mL for erythromycin, >16 µg/mL for spiramycin, and >32 µg/mL for tilmicosin and tylosin. Also the MIC<sub>90</sub> of lincomycin was above the highest concentration tested (>32 µg/mL), but the distribution of the MIC values was almost perfectly bimodal: 41 isolates had a MIC =0.5 µg/mL and 30 isolates >32 µg/mL. Oxytetracycline had a 2-fold higher concentration to inhibit 50% of isolates (2 vs. 0.5 µg/mL) and 1-fold higher MIC<sub>90</sub> (4 vs. 2 µg/mL) than doxycycline. Enrofloxacin and florfenicol had both a MIC<sub>90</sub> of 2 µg/mL, whereas tiamulin had a MIC<sub>90</sub> of 0.5 µg/mL. Significant differences on the MIC values were found among the 3 countries for several antimicrobials: compared with Germany, Belgium and Italy showed significantly higher MIC for lincomycin, spiramycin, and tylosin, and lower for oxytetracycline and florfenicol. The Belgian isolates showed the lowest MIC for enrofloxacin compared with Germany and Italy. The MIC results obtained in our study suggest the presence of a high level of resistance of *M. bovis* isolates originating from milk to macrolides in all countries involved in this study. On the contrary, a low level of resistance was found against the antimicrobials that are not used in cattle, such as tiamulin and doxycycline, highlighting a possible link between antimicrobial treatments and development of resistance in the studied *M. bovis* population.

Barbieri°I, Faccini°S, Boniotti°MB, Alborali°GL , Rosignoli°C, Franzini°G, Nigrelli°A

**Circolazione di PCV2 in allevamenti suini del Nord Italia : verso un nuovo shift genetico?**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 157-164. - 41 bib ref [Nr. Estr. 7228]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Circovirus suino tipo 2 (PCV2) a un importante agente patogeno responsabile di diverse sindromi nei suini collettivamente denominate malattie da PCV (PCVD). Sulla base della sequenza di ORF2, i

ceppi di PCV2 sono attualmente classificati in quattro genotipi: PCV2a, PCV2b, PCV2c e PCV2d. Quest'ultimo è considerato "emergente", essendo sempre più frequentemente isolato a livello mondiale, facendo ipotizzare un imminente shift genetico tra PCV2b e PCV2d. Lo studio riporta i risultati di una attività di monitoraggio di due anni sui ceppi di PCV2 circolanti in allevamenti suini prevalentemente del nord d'Italia. Sono stati effettuati 79 sequenziamenti di ORF2 di ceppi da casi sospetti di PCVD da 58 allevamenti. PCV2b è risultato il genotipo prevalente, circolando nel 63.8% degli allevamenti, seguito dal genotipo PCV2d (29.3%) e PCV2a (6.8%). Un importante incremento del PCV2d si è riscontrato nel corso del 2015. Nel 2014 su 30 allevamenti di provenienza dei ceppi sequenziati, nell'86.7% era presente PCV2b e in uno solo si è riscontrato PCV2d (3.33%). Nel 2015 invece, dei 28 allevamenti campionati il 37,9% aveva PCV2b e il 55,2% PCV2d. I dati rafforzano l'ipotesi di PCV2d come genotipo emergente e di uno shift genetico imminente o addirittura in corso tra PCV2b e PCV2d. In questo contesto sostenere i dati diagnostici con l'analisi di sequenza è estremamente importante.

*Porcine Circovirus Type 2 (PCV2) is an important pathogen related to several disease syndromes in pigs collectively named PCV diseases (PCVD). Based on ORF2 sequences, PCV2 strains are currently classified into four genotypes: PCV2a, PCV2b, PCV2c and PCV2d. The latter is considered an emergent genotype as it has been increasingly isolated worldwide. In this work we report a two-year monitoring activity on PCV2 in swine farms located in the North of Italy. Seventy-nine sequences have been obtained from cases of suspect PCVD. PCV2b is the most widespread genotype: it has been detected in 63.8% of farms, followed by PCV2d (29.3%) and PCV2a (6.8%). A considerable increase in PCV2d frequency was registered during 2015. Indeed in 2014 PCV2b was present in 86.7% of 30 herds and PCV2d circulated in only one (3.33%). On the contrary, in 2015 in 37,9% of the herds were infected by PCV2b, and 55,2% by PCV2d. Data strengthen the hypothesis of PCV2d as an emergent genotype and of an imminent or even ongoing genetic shift between PCV2b and PCV2d. In this context sustaining diagnostic data with sequence analysis is extremely important.*

Bassi E, Ferloni M, Bianchi A, Cannavacciuolo A, Fedrizzi G, Facchetti R

#### **Saturnism in avian scavengers in relation to hunting modalities : the tip of the iceberg**

Atti del III Congresso nazionale fauna problematica : 24-26 novembre 2016 Cesena (FC) : riassunti delle comunicazioni orali e dei poster / [s.l. : s.n., 2016]. - p 18-19 [Nr. Estr. 7442]

Congresso nazionale fauna problematica (3. : Cesena (FC) : 24-26 novembre 2016)

Wildlife may be exposed to lead contamination as a consequence of hunting activities. Waterfowls and grouses may directly assume lead from the ground, while in raptors lead intoxication may be due to the ingestion of preys with elevated lead concentrations in the tissues. Viscera of shot ungulates are quite often directly contaminated with lead due to bullet fragmentation. The practice of leaving on the ground the ungulate viscera after the shot is still frequent, representing a threat for scavenger species and golden eagles and, moreover, hunting season partially overlaps with the most limiting season for birds, in terms of food availability, for raptors. The extraordinary conservation value of the bearded vulture (*Gypaetus barbatus*) and golden eagle (*Aquila chrysaetos*) population living in Lombardy, and Trentino Alto Adige led the Stelvio National Park, and Sondrio Province to implement a study for monitoring the risk of lead intoxication for large raptors tied to the practice of ungulate viscera deposition. The viscera of 153 ungulates shot in the Sondrio Province (Italian Alps) during hunting season 2009-2010 have been collected and examined to detect and quantify lead presence. Ancillary data as type of ammunition, condition and the outcome of the shot have been collected. Lead fragments in the samples have been investigated through CAT (computed axial tomography) and digital radiography and subsequently manually collected. Overall, in 62.1% of samples lead fragments have been detected. On a sample of 147 viscera of roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), chamois (*Rupicapra rupicapra*), wild boar (*Sus scrofa*) and mouflon (*Ovis orientalis musimon*) higher frequencies have been recorded in roe deer (77.7%), chamois (69.6%) while lower in red deer (50%). These outcomes confirm the high risk of lead intoxication for large raptors in areas where ungulates are commonly hunted that might even amplify in the proximity of the protected areas where ungulates and birds of prey are distributed with higher density.

Bassi° S, Carpana° E

**The examination of bees, honey and hive debris as a tool to foresee the onset of American foulbrood**

The 7th European Conference of Apidology (EurBee) : Cluij - Napoca (Romania) 7-9 September 2016 / [s.l. : s.n., 2016]. - p 252 [Nr. Estr. 7457]

European Conference of Apidology (EurBee) (7th : Cluij - Napoca (Romania) : 7-9 September 2016)

Bees, honey and hive debris were collected in winter from 125 hives belonging to 10 apiaries and examined with culture method for the detection of Paenibacillus larvae spores. In the following spring the colonies were inspected for American foulbrood symptoms from the beginning of March to the end of May at 3 weeks intervals. The aims were to compare the efficiency of bees, honey and debris in detecting the infected colonies and evaluate the relationship between the wintry contamination by Paenibacillus larvae in these three materials and the development of the American foulbrood in the same colonies in the spring. The examination of the bees has identified a greater number of infected colonies (117/125) compared to the examination of honey (87/125) and debris (62/125). Regarding the relationship between presence of Paenibacillus larvae and onset of American foulbrood 16 out of 62 (25.8%) colonies in which the debris were positive for Paenibacillus larvae showed disease-symptoms in spring. The disease occurred in 13 out of 70 (18.6%) and 16 out of 117 (13.7%) colonies in which honey and bees were positive for Paenibacillus larvae respectively. None of the colonies with bees and debris negative for Paenibacillus larvae developed the disease while three colonies where the honey was negative have fallen ill in the spring. The onset of the disease in spring is related to the level of wintry contamination by Paenibacillus larvae: for all materials the higher the number of spores detected the greater the probability that the colony becomes ill.

Bassi° S, Galletti° G

**Detection of Paenibacillus larvae from beehive debris : a culture method based on watery extraction of spores**

The 7th European Conference of Apidology (EurBee) : Cluij - Napoca (Romania) 7-9 September 2016 / [s.l. : s.n., 2016]. - p 253 [Nr. Estr. 7460]

European Conference of Apidology (EurBee) (7th : Cluij - Napoca (Romania) : 7-9 September 2016)

The detection of Paenibacillus larvae spores in debris collected on the hive bottom is a non-invasive tool for the identification of infected colonies. We developed a quick and simple culture method for the detection of Paenibacillus larvae in beehive debris based on the extraction of the spores in water (Water method - WM). Briefly, 1 g of debris was placed in a 15 ml test tube with a sealing cap containing 9 ml of sterile distilled water. The suspension was shaken for 30 seconds and then heated in a water bath at 85-90 °C for 15 min. After the heat treatment, the suspension was poured into a Stomacher bag with a lateral filter and the filtered liquid was transferred with a disposable pipette in another test tube. The sample was plated onto 5 plates (100 pi/plate) of MYPGP agar supplemented with nalidixic acid. The plates were incubated at 37 °C in an atmosphere with 10% CO<sub>2</sub> and examined after 8 days. We examined 50 samples of beehive debris with this method and compared the results with the same samples using the Tween Method (TM), which currently is the most effective culture method for the detection of Paenibacillus larvae in beehive debris. WM has an excellent agreement with TM. The use of WM compared to TM presents practical advantages: it is less expensive, it is easier to perform and the sample preparation time is shorter (approximately 25 - 30 min with WM vs. approximately 4 - 5 hrs with TM).

Bassi°S, Loglio°G

**Comparison between the examination of powdered sugar and adult bees for the detection of honeybee colonies infected by Paenibacillus larvae**

The 7th European Conference of Apidology (EurBee) : Cluij - Napoca (Romania) 7-9 September 2016 / [s.l. : s.n., 2016]. - p 254 [Nr. Estr. 7459]

European Conference of Apidology (EurBee) (7th : Cluij - Napoca (Romania) : 7-9 September 2016)

The detection of Paenibacillus larvae infected colonies is imperative for an effective prevention and control of American foulbrood. How already reported in a previous communication the bacteriological examination of powdered sugar can be a useful tool for the identification of these colonies. From 101 beehives, belonging to apiaries with different situations regarding the presence of American foulbrood, we collected samples of both powdered sugar and adult bees. The powdered sugar was dusted over the frames and collected on a sheet of paper placed on the tray in the hive bottom. The bees were collected in the brood nest. Both materials were examined with bacteriological method for the detection and enumeration of Paenibacillus larvae spores. The results were expressed as CFU/g (powdered sugar) or CFU/bee (bees). Concordant results between powdered sugar and bees analysis was observed in a total of 82/101 (81.2%) beehives: in 51 colonies both materials were positive and in 31 both were negative. Discordance was observed in 19/101 beehives (18.8%): in 17 colonies the powdered sugar was positive and the bees were negative, whereas in two colonies the bees were positive and the sugar negative. In the colonies (n. 51) where bees and powdered sugar were both positive, the number of spores was higher in the powdered sugar compared to bees in 44 colonies, conversely in seven colonies bees showed higher spores number. These preliminary results show that powdered sugar could be more effective than adult bees in detecting colonies with Paenibacillus larvae infection.

Baumann J, Kouassi NM, Foni°E, Klenk HD, Matrosovi ch M

**H1N1 swine influenza viruses differ from avian precursors by a higher pH optimum of membrane fusion**

J Virol. - Vol. 90 no 3 ( 2016). - p 1569-1577. - 75 bib ref [Nr. Estr. 7220]

The H1N1 Eurasian avian-like swine (EAsw) influenza viruses originated from an avian H1N1 virus. To characterize potential changes in the membrane fusion activity of the hemagglutinin (HA) during avian-to-swine adaptation of the virus, we studied EAsw viruses isolated in the first years of their circulation in pigs and closely related contemporary H1N1 viruses of wild aquatic birds. Compared to the avian viruses, the swine viruses were less sensitive to neutralization by lysosomotropic agent NH<sub>4</sub>Cl in MDCK cells, had a higher pH optimum of hemolytic activity, and were less stable at acidic pH. Eight amino acid substitutions in the HA were found to separate the EAsw viruses from their putative avian precursor; four substitutions—T492S, N722D, R752K, and S1132F—were located in the structural regions of the HA2 subunit known to play a role in acid-induced conformational transition of the HA. We also studied low-pH-induced syncytium formation by cell-expressed HA proteins and found that the HAs of the 1918, 1957, 1968, and 2009 pandemic viruses required a lower pH for fusion induction than did the HA of a representative EAsw virus. Our data show that transmission of an avian H1N1 virus to pigs was accompanied by changes in conformational stability and fusion promotion activity of the HA. We conclude that distinctive host-determined fusion characteristics of the HA may represent a barrier for avian-to-swine and swine-to-human transmission of influenza viruses.

Belardo V, Audisio P, Pietropaoli M, Bassi°S, Arte se F, Formato G

### **Considerazioni pratiche sulle fonti di alimentazione di *Aethina tumida***

Apitalia. - Vol. no 7-8 ( 2016). - p 28-32. - 26 bib ref [Nr. Estr. 7461]

*Aethina tumida* (*A. tumida*) o piccolo coleottero dell'alveare (Small Hive Beetle - SHB) un parassita delle api, appartenente alla famiglia dei Nitidulidi, originario dell'Africa tropicale e subtropicale Rundle 1940; Schmolke 1974), rinvenuto e parzialmente acclimatato per la prima volta in Europa nel 2014 in Italia (Calabria, Piana di Gioia Tauro) (Palmeri et al. 2014; Mutinelli 2015). I maggiori danni apportati alle api da questo parassita sono provocati dalle sue forme larvali che scavano gallerie nei favi nutrendosi e contaminando tutto ciò che incontrano fino a determinare, nei casi più gravi, la fermentazione del miele e la distruzione del nido (Lundie 1940). Il presente articolo vuole approfondire quanto ad oggi è noto in merito alla alimentazione di *A. tumida*.

Bellini R, Bonilauri°P, Puggioli A, Lelli°D, Gaibani P, Landini MP, Carrieri M, Michaelakis A, Papachristos D, Giatropoulos A, Badieritakis E, Maccagnani B, Calzolari°M, Dottori°M

### **Chikungunya and Dengue risk assessment in Greece**

Vector Biol J. - Vol. 1 no 2 ( 2016). - (6 p). - 28 bib ref (ultimo accesso 11/07/2016  
doi:10.4172/vbj.1000108 ) [Nr. Estr. 7293]

To assess the mutated CHIKV E1-A226V and DENV II infection and dissemination rates of an *Ae. albopictus* population established in Athens (Greece) (2) To assess the risk of outbreaks in four Greek localities based on *Ae. albopictus* population density whose estimate was based on the number of eggs laid in ovitraps. Methods: Under laboratory conditions females were offered blood meal infected with the CHIKV titer of 1X10<sup>6</sup> TCID 50/mL and DENV II titer of 1.76 X10<sup>6</sup> TCID 50/mL; at day 11 after oral infection, females were sacrificed, legs were removed and processed for PCR analysis to assess the presence of viral replicates. In order to evaluate the risk of outbreak of CHIKV and DENV II, a pilot monitoring program was started in three Greek localities and in Chania (Crete), to estimate the adult female population density on the base of the number of eggs in the ovitraps. Results: We proved the vector competence of the Greek *Ae. albopictus* strain for E1-A226V mutated CHIKV and DENV II. Combining the data on the vector competence with those on the female population density, based on the egg density data, the estimated risk of outbreak was relatively low but not negligible. Conclusion: As the vector competence estimated under laboratory conditions was obtained by offering females moderately low initial virus titers, it can be expected a higher vector competence in the field. This consideration, together with a possible increase of the mosquito population due to the global warming effects, make the quantitative ovitrap-based monitoring a necessary and useful tool to estimate the risk of outbreaks.

Bellini°S

### **Compartmentalisation : general principles and potential applications in African swine fever control**

African swine fever : recent research advances and strategies to combat the disease in Europe : COST Action CA15116 : Understanding and combating African Swine Fever in Europe (ASF-STOP) : 6-8 December 2016, Pulawy, Poland : conference proceedings / [s.l. : s.n., 2016]. - p 17 [Nr. Estr. 7454]

COST Action CA15116 : Understanding and combating African Swine Fever in Europe (ASF-STOP) : Pulawy, Poland : 6-8 December 2016)

The OIE Terrestrial Animal Health Code (Terrestrial Code) sets out the standards for the improvement of terrestrial animal health and welfare and veterinary public health worldwide, and for safe international trade in terrestrial animals and their products. The health measures in the

Terrestrial Code should be used by the Veterinary Authorities for early detection, reporting and control of agents pathogenic to terrestrial animals and for humans, and to prevent their transfer via international trade in terrestrial animals and their products, while avoiding unjustified sanitary barriers to trade. - Zoning and compartmentalisation are procedures implemented by a Member Country for defining subpopulations of distinct health status within its territory for the purpose of disease control and/or international trade. While zoning applies to an animal subpopulation defined primarily on a geographical basis, compartmentalisation applies to an animal subpopulation defined primarily by management and husbandry practices related to biosecurity. In practice, spatial considerations and good management including biosecurity plans play important roles in the application of both strategies. Compartmentalisation is not a new concept for Veterinary Services; in fact, it has been applied for a long time in many disease control programs that are based on the concept of disease free herds/flocks. The Terrestrial Code chapter on OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization provides a process for trading partners to follow in achieving recognition of the health status of such subpopulations. These procedures are best implemented by trading partners through establishing parameters and gaining agreement on the necessary measures prior to disease outbreaks, rather than during outbreaks. Zoning and compartmentalisation are not applicable to all diseases and in all situations but separate requirements are developed for each disease for which the application of zoning or compartmentalisation is considered appropriate. Indeed, the effective implementation of compartmentalisation depends, amongst others, on the epidemiology of the disease, country factors, environmental factors, the biosecurity measures, which may be applicable, the health status of animals in adjacent areas, surveillance and the relationship between the public and private sectors. The proposed revised Terrestrial Code chapter on African swine fever (ASF) gives detailed technical recommendations for a country, zone or compartment to be considered as free from ASF, and addresses the disease in domestic and captive wild pigs, wild and feral pigs and African wild suid species.

Bellini°S, Cerioli°MP, Giacomini°E

### **Implementation of control and biosecurity measures to avoid African Swine Fever spread in pig premises**

African swine fever : recent research advances and strategies to combat the disease in Europe : COST Action CA15116 : Understanding and combating African Swine Fever in Europe (ASF-STOP) : 6-8 December 2016, Pulawy, Poland : conference proceedings / [s.l. : s.n., 2016]. - p 83 [Nr. Estr. 7452]

COST Action CA15116 : Understanding and combating African Swine Fever in Europe (ASF-STOP) : Pulawy, Poland : 6-8 December 2016)

African swine fever (ASF) is one of the most serious diseases of pigs that can severely affect and disrupt regional and international trade in animals and animal products with a serious socio-economic impact on pig farming system. ASFV of genotype II, was introduced in Georgia in 2007 and since then ASF is still reported in the Russian Federation where, in certain areas, the disease became endemic representing a constant threat for the neighbouring countries. In 2013, from the Russian Federation, the virus reached Belarus and Ukraine and later, in 2014, it spread to Lithuania, Poland, Latvia and Estonia affecting mainly wild boar and backyard's pigs. Based on the characteristics of the virus and on the epidemiological findings, the introduction into the Baltic countries and into Poland was most probably from Belarus. The ASFV strain that is currently circulating in the Eastern European countries and Baltic States is a highly virulent and highly lethal strain, which has 100% sequence homology with the ASFV identified in Belarus in June 2013. The current ASF situation in the Eastern part of Europe represents a serious threat to the EU livestock sector, particularly if the infection pressure remain high at the Eastern border of the EU. No vaccine or drugs are available to prevent ASF infection. Therefore, it is extremely important to prevent the introduction of the disease in free areas and to reduce, as much as possible, the persistence of the virus in the infected ones. Prevention and early detection play a key role in the control strategy for ASF and enhancing early detection would also improve the efficacy of the disease control measures. The basic elements of biosecurity derive from the knowledge of the epidemiology of the disease, the

duration of pathogen excretion in infected animals, the main routes of excretion, survival of the pathogen in the environment and its routes of infection. Some basic principles of biosecurity apply to all farming systems and all diseases. However, in order to better address preventive and control measures, the main practical biosecurity measures need to be tailored to the targeted disease and to the farming systems in which they are to be implemented. Worth to mention that backyards with poor biosecurity in place are currently playing an important role in the maintenance and spread of ASF in the eastern European Countries. Indeed, in this sector of the pig production system feeding pigs with kitchen waste is common practice and the main biosecurity measures are not easy to implement, due to the minimal investment in infrastructure typical for this type of pig production system. However, there is a set of basic preventive measures applicable also in backyard holdings and, if they are properly implemented, they are effective in minimizing the risk of ASFV spread. The final responsibility of controlling ASF belongs to the veterinary authority. However, in risk areas, pig producers have to understand the risk posed by the presence of the disease and they have to adopt all the necessary precautionary measures to protect their own herds. To achieve this, veterinary services shall provide basic information to pig holders through appropriate communication campaigns and by promoting the adoption of preventive measures.

Bellini°S, Rutili D, Guberti V

**Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems**

Acta Vet Scand. - Vol. 58 ( 2016). - no 82 (10 p). - 53 bib ref [Nr. Estr. 7437]

African swine fever (ASF) is one of the most severe diseases of pigs; it has a drastic impact on the pig industry, causing serious socio-economic consequences to pig farmers and pork producers. In Europe, there are currently two main clusters of infection; one in Sardinia caused by strains of African swine fever virus (ASFV) belonging to genotype I and another in Eastern Europe caused by strains of ASFV belonging to genotype II. The latter is inducing an acute form of ASF and it represents a serious threat to the pig sector. ASF is a disease for which there is no effective vaccine; therefore, prevention has a pivotal role in the control strategy of the disease. This review describes the main preventive measures to adopt to mitigate the risk of ASF spread in pig farming systems.

Ben\_Said M, Abbassi MS, Bianchini°V, Sghaier S, Cr emonesi P, Romanò°A, Gualdi V, Hassen A, Luini°MV

**Genetic characterization and antimicrobial resistance of Staphylococcus aureus isolated from bovine milk in Tunisia**

Lett Appl Microbiol. - Vol. 63 ( 2016). - p 473-481. - 48 bib ref [Nr. Estr. 7451]

Staphylococcus aureus is a major agent of bovine mastitis in dairy herds, causing economic losses in dairy industry worldwide. In addition, milk and milk-products contaminated by Staph. aureus can cause harmful human diseases. The aim of this study was to characterize Staph. aureus strains isolated from dairy farms in Tunisia. Bulk tank milk (n = 32) and individual cow milk (n = 130) samples were collected during the period of 2013–2014. Forty-three Staph. aureus isolates were recovered and typed by spa typing, 16S-23S rRNA intergenic spacer (RS-PCR) and multiplex PCRs for 22 virulence genes. Antimicrobial resistance was also investigated with a disc diffusion test. A selected subsample of 22 strains was additionally genotyped by multilocus sequence typing. Seventeen spa types were recovered, and t2421 (n = 10), t521 (n = 6) and t2112 (n = 5) were the most common. Fourteen different RS-PCR genotypes grouped into 11 clusters were detected in our study, with predominance of the RVI genotype (n = 24). Eight sequence types were identified and Clonal Complex 97, corresponding to RS-PCR cluster R, was the most common (n = 10), followed by CC1 (n = 4), CC15 (n = 3) and other four accounting for one or two strains. Different combinations of virulence genes were reported, and enterotoxin genes were present in few strains

(seh, n = 4; sea, n = 2; sea and seh, n = 2; sec and sel, n = 2). The majority of strains were resistant only to penicillin; only one strain was found to be multiresistant and no methicillin-resistant *Staph. aureus* was demonstrated. Our study reported the isolation of CC97 from bovine milk in Tunisia for the first time and confirmed the relevance of this lineage in intramammary infection in cows.

Bertasio<sup>°</sup>C, Giacomini<sup>°</sup>E, Lazzaro<sup>°</sup>M, Perulli<sup>°</sup>S, Lavazza<sup>°</sup>A, Lelli<sup>°</sup>D, Alborali<sup>°</sup>GL, Boniotti<sup>°</sup>MB

**Fecal shedding and antibody response in four PEDV infected swine breeding farms**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - p 514 [Nr. Estr. 7299]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Bertasio<sup>°</sup>C, Giacomini<sup>°</sup>E, Lazzaro<sup>°</sup>M, Perulli<sup>°</sup>S, Papetti<sup>°</sup>A, Lavazza<sup>°</sup>A, Lelli<sup>°</sup>D, Alborali<sup>°</sup>G, Boniotti<sup>°</sup>MB

**Porcine epidemic diarrhea virus shedding and antibody response in swine farms : a longitudinal study**

Front Microbiol. - Vol. 7 ( 2016). - Article 2009 (9 p). - 31 bib ref [Nr. Estr. 7456]

The porcine epidemic diarrhea virus (PEDV) causes an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea, and a high mortality rate in seronegative neonatal piglets. In the last few years, PED had a large economic impact on the swine industries in Asia and the US, and in 2014, the PEDV also re-emerged in Europe. Two main PEDV variants circulate worldwide but only the S INDEL variant, considered a mild strain, is spreading in Europe. To gain insights into the pathogenicity of this variant, its viral load and temporal shedding pattern were evaluated in piglets from infected farms. Quantitative real-time PCR (qPCR) targeting the spike gene, was validated according to the minimum information for quantitative real-time PCR experiments guidelines. The qPCR was applied to longitudinal studies conducted in four swine farms naturally infected with the PEDV S INDEL variant. Clinical data, fecal swabs, and blood samples were collected from 103 piglets at 15–30-day intervals for 2–5 months. On all four farms, diarrhea was observed in sows during gestation and in farrowing units, and the mortality rates of piglets were 18, 25, 30, and 35%. Different clinical pictures (0-50% of diarrhea positivity), viral titer levels (mean 5.3-7.2 log<sub>10</sub> genome copies/mL), and antibody conditions (30-80% of positivity) were registered among sows on the four farms. The percentage of qPCR positive piglets varied greatly from the beginning (63–100%) to the end (0%) of the infection course. Clinical signs were present in 96% of the qPCR positive animals. Viral loads ranged from 8.5 log<sub>10</sub> to 4 log<sub>10</sub> genome copies/mL in suckling pigs at 3–6 days of age and were not statistically different among farms, despite the different patterns observed in sows. After 2–3 weeks, only a few piglets still showed detectable viral levels and clinical signs, and they developed antibody responses. Moreover, co-infections with other pathogens and biosecurity procedures limiting the circulation of the virus could have influenced the severity of PED infection. QPCR and clinical data were useful in understanding the dynamics of PEDV infections and, therefore, in implementing appropriate control measures.

Bertasio<sup>°</sup>C, Giacomini<sup>°</sup>E, Lazzaro<sup>°</sup>M, Perulli<sup>°</sup>S, Papetti<sup>°</sup>A, Lavazza<sup>°</sup>A, Lelli<sup>°</sup>D, Alborali<sup>°</sup>G, Boniotti<sup>°</sup>MB

**Diarrhea epidemica del suino : studio longitudinale in quattro allevamenti del Nord Italia**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : libro degli atti / [s.l. : s.n., 2016]. - p 55 (Poster 18) [Nr. Estr. 7398]

Introduzione. La diarrea epidemica del suino (PED) è una malattia virale causata da un coronavirus appartenente al genere Alphacoronavirus. Nel suino, la malattia si manifesta con diarrea, vomito, disidratazione e alta mortalità nei suinetti lattanti. Negli ultimi anni, la PED ha avuto un notevole impatto economico in Asia e negli USA e, a partire dal 2014, è ricomparsa anche in Europa. In questo lavoro viene descritto uno studio longitudinale condotto su quattro aziende suinicole del nord Italia, colpite da PED nel 2015. Materiali e Metodi. In ogni azienda sono state selezionate 8-10 scrofe e 19-30 suini neonati per la raccolta dei dati clinici, prelievi di sangue, feci e tamponi rettali. Le scrofe sono state campionate solo al primo prelievo; i suinetti a 3-6 giorni di età e ad intervalli di 15-30 giorni, nel corso di 2-4 mesi (prelievo 1-6: P1-6). I sieri sono stati analizzati con cELISA per valutare la presenza di anticorpi, mentre le feci e tamponi rettali sono stati sottoposti a qPCR Real-Time per la quantificazione del titolo virale. Risultati. Il 96% degli animali positivi alla PCR hanno evidenziato segni clinici. Le scrofe hanno mostrato titoli virali molto eterogenei con valori massimi di 6.4, 8.3, 7.6 e 6.5 logio copie/mL nell'azienda 1, 2, 3 e 4 rispettivamente. Nei suinetti, la presenza del virus in P1 tramite PCR è stata del 63-100%, in P2 è scesa al 10-22% e in P3 solo pochi animali hanno mostrato quantità di virus rilevabili. In P1 i titoli virali hanno mostrato picchi fino a 8.5 logio copie/mL, con medie sovrapponibili tra i quattro allevamenti. Il 54% delle scrofe ha rivelato positività attestata sierologica, mentre in P1 la percentuale di suinetti con anticorpi anti-PEDV è stata del 12%. Solo il 3% dei suinetti positivi al test cELISA mostrano assenza di segni clinici evidenti. La percentuale di animali con anticorpi rilevabili raggiunge il picco massimo entro le tre settimane di età, per poi rimanere stabile fino alla fine dello studio. Conclusioni. La valutazione dei sintomi clinici e l'analisi dei titoli virali e anticorpali degli animali hanno permesso di comprendere la dinamica dell'infezione di PEDV in azienda. Essi possono essere considerati validi indicatori per definire lo stadio della malattia all'interno di un allevamento oltre che rappresentare degli utili strumenti per comprendere la trasmissione e la circolazione del virus tra i vari settori, informazione fondamentale per l'adozione di misure di controllo e di contenimento.

Bertasio<sup>o</sup>C, Papetti<sup>o</sup>A, D'Incau<sup>o</sup>M, Tagliabue<sup>o</sup>S, Boniotti<sup>o</sup>MB

#### **Leptospirosi nella popolazione suina del Nord Italia : sieroprevalenza e caratterizzazione molecolare**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Società Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 327-328. - 5 bib ref [Nr. Estr. 7377]

Congresso Nazionale Società Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*Swine are one of the most significant source of Leptospira infections for man. The aim of this study was to investigate the leptospira infections in the pig population and to understand the genetic characteristic of serogroup Pomona circulating in Northern Italy during 2002-2015. The prevalent serovars detected by microscopic agglutination test were Bratislava and Pomona. All the isolated strains (125) were identified as serogroup Pomona. Multi Locus Sequence Typing showed that 40/42 Pomona isolates had the ST 140 profile and two isolates had 99,9% of nucleotide identity with serovar Mozdock (ST 117). By Multi Locus Variable Number Tandem Repeat Analysis, 38/40 Pomona isolates showed the same profile as the Italian reference strain and 2/40 showed a new MLVA profile. These results are very important to detect new variants of Leptospira in the pig population, in order to monitor their circulation and the possible transmission to other species.*

Bertocchi<sup>o</sup>L

#### **Attenti all'asciutta : quarta puntata: Dal rischio di infezione alla mastite clinica : piani preventivi**

Allev Magazine. - Vol. no 4 ( 2016). - p 2-3 [Nr. Estr. 7467]

Bertocchi°L

**Attenti all'asciutta : terza puntata: Così cambia il rischio di infezione mammaria durante l'asciutta**

Allev Magazine. - Vol. no 3 ( 2016). - p 2-3 [Nr. Estr. 7466]

Bertocchi°L

**Attenti all'asciutta : seconda puntata: Attenzione al benessere animale anche nella messa in asciutta**

Allev Magazine. - Vol. no 1 ( 2016). - p 2-3 [Nr. Estr. 7465]

Bertocchi°L

**Attenti all'asciutta : prima puntata: Asciutta un periodo delicato per la lattifera**

Allev Magazine. - Vol. no 1 ( 2016). - p 48-49 [Nr. Estr. 7464]

Bertocchi°L

**Attenti all'asciutta : quinta puntata: La giusta alimentazione per una corretta messa in asciutta**

Allev Magazine. - Vol. no 5 ( 2016). - p 2-3 [Nr. Estr. 7472]

Biagetti M, Mazzone P, Ciullo M, Sebastiani C, Pacciarini°ML, Lauterio C, De\_Montis A

**A DNA chip-based method for rapid characterization of Mycobacterium isolates**

Large Anim Rev. - Vol. 22 ( 2016). - p 121-126. - 10 bib ref [Nr. Estr. 7305]

Introduction - One of the major problems for the diagnosis of mycobacteriosis is represented by the slow growth of the causative agents and by biochemical identification tests that often are difficult to interpret and sometimes not decisive. The development of molecular techniques (i.e. PCR) has reduced the time required for the identification of isolates. However, a series of additional analysis such as PCR, PCR RFLP, PFGE, spoligotyping or sequencing are necessary to correctly identify the Mycobacterium species. Aim - The aim of this study was to develop and validate a chip for the rapid identification of Mycobacterium isolates. Materials and methods - A total of 88 mycobacterial isolates from human and animal samples, including 4 ATCC strains and two reference strains, used for the production of bovine and avian tubercolins, were examined by chip-based method. All strains were previously characterized by biochemical and molecular methods. The chip-based method relies on a multiplex PCR followed by the PCR product hybridization on a chip. *M. kansasii*, *M. gordonae*, *M. chelonae*, *M. malmoense*, *M. flavescens*, *M. szulgai*, *M. simiae*, *M. abscessus*, *M. lentiflavum*, *M. porcinum*, *M. nonchromogenicum*, *M. terrae*, *M. termoresistibile*, *M. chi-tae* and *M. gadium*, were amplified and used as negative controls. Results and discussion - The developed device was able to recognize Mycobacterium genus (MYC), Mycobacterium tuberculosis complex (MTBC), Mycobacterium avium complex (MAC), *M. fortuitum* (FOR), *M. marinum* (MAR), *M. smegmatis* (SME), *M. scrofulaceum* (SCR) and *M. xenopi* (XEN). The concordance between the expected and observed results was 100% with regard to identification of Mycobacterium genus, as well as to the identification of Mycobacterium tuberculosis complex. Regarding to Nontuberculosis Mycobacteria the concordance was 96%. All the isolates were correctly recognized by the chip with exception of one strain identified as *M. fortuitum* by 16S rRNA gene sequencing. As expected no signal was obtained analysing the negative controls. Conclusion - The developed device was able to characterize Mycobacterium isolates in a single step. It is easy to use, requires basic knowledge of molecular biology and no special equipment is needed. Moreover, it is rapid, sensitive and specific. The device was able to distinguish the MTBC, MAC and NTM. Within the group of MTBC the assay

was able to differentiate *M. tuberculosis* from *M. bovis* / *M. caprae* / *M. microti* group. Within MAC it was possible to differentiate the *M. avium* sub. *paratuberculosis* / *M. avium* sub. *avium* group from *M. avium* subsp. *intracellulare*. The device was also able to identify some important pathogenic NTM, such as *M. fortuitum*, *M. marinum*, *M. smegmatis*, *M. scrofulaceum* and *M. xenopi*.

Biffani S, Del\_Corvo, Capoferri R, Pedretti A, Luini<sup>o</sup> M, Williams JL, Pagnacco G, Minvielle F, Minozzi G

**An alternative experimental case-control design for genetic association studies on bovine mastitis**

Animal. - Vol. 2016). - 6 p. - 38 bib ref (ultimo accesso 31/10/2016  
<http://dx.doi.org/10.1017/S1751731116001750> [Nr. Estr. 7351]

The possibility of using genetic control strategies to increase disease resistance to infectious diseases relies on the identification of markers to include in the breeding plans. Possible incomplete exposure of mastitis-free (control) animals, however, is a major issue to find relevant markers in genetic association studies for infectious diseases. Usually, designs based on elite dairy sires are used in association studies, but an epidemiological case-control strategy, based on cows repeatedly field-tested could be an alternative for disease traits. To test this hypothesis, genetic association results obtained in the present work from a cohort of Italian Holstein cows tested for mastitis over time were compared with those from a previous genome-wide scan on Italian Holstein sires genotyped with 50k single nucleotide polymorphisms for de-regressed estimated breeding values for somatic cell counts (SCCs) on *Bos taurus* autosome (BTA6) and BTA14. A total of 1121 cows were selected for the case-control approach (cases = 550, controls = 571), on a combination of herd level of SCC incidence and of within herd individual level of SCC. The association study was conducted on nine previously identified markers, six on BTA6 and four on BTA14, using the R statistical environment with the 'qtscore' function of the GenABEL package, on high/low adjusted linear score as a binomial trait. The results obtained in the cow cohort selected on epidemiological information were in agreement with those obtained from the previous sire genome-wide association study (GWAS). Six out of the nine markers showed significant association, four on BTA14 (rs109146371, rs109234250, rs109421300, rs109162116) and two on BTA6 (rs110527224 and rs42766480). Most importantly, using mastitis as a case study, the current work further validated the alternative use of historical field disease data in case-control designs for genetic analysis of infectious diseases in livestock.

Bonilauri<sup>o</sup> P, Defilippo<sup>o</sup> F, Calzolari<sup>o</sup> M; Luppi<sup>o</sup> A, Torri<sup>o</sup> D, Marzani<sup>o</sup> K, Dottori<sup>o</sup> M

**Comparison of two different Real Time PCRs for detecting Zika virus and results obtained on *Ae. albopictus* collected during Summer 2015 in Italy**

10th Annual Meeting Epizone "Going viral" : 27-29 September 2016, Madrid, Spain : programme and abstracts / [s.l. : s.n., 2016]. - p 115 (Poster 24) [Nr. Estr. 7326]

Annual meeting Epizone (10th : Madrid, Spain : 27-29 September 2016)

Zika virus (ZIKV) is an emerging mosquito-transmitted virus in the family Flaviviridae and genus Flavivirus. It was initially isolated in 1947 from blood of a febrile sentinel rhesus monkey during a yellow fever study in the Zika forest of Uganda and was subsequently isolated from a pool of *Aedes africanus* mosquitoes collected in 1948 from the same region. In the recent outbreaks that occurred in Latin America, *Aedes aegypti* is believed to be the main vector but other species of *Aedes* mosquitoes such as *Ae. albopictus* (tiger mosquitoes) are suspected to be a less efficient but competent vector of the virus. Different Real time PCR protocols are available in literature. The most used protocols was originally proposed by Lanciotti et al. (Emerging Infectious Diseases, Vol. 14, No. 8, August 2008) applied on human specimens and recently defused thought molecule panel test for Zika virus organize by Centers for Disease Control and Prevention (CDC), Fort Collins,

Colorado, in the aim of arbovirus testing proficiency program for 2016. Another, quite diffused in literature, protocol proposed by Faye et al. 2013 (Virology 2013 Oct 22;10:311. doi: 10.1186/1743-422X-10-311.) was describe to be developed and evaluated with field-caught mosquitoes in Africa. The aims of this work were to compare the efficiency and sensitivity of these 2 Real Time PCR with reference samples and positive RNA kindly provided by CDC (Center for Disease Control and Prevention, Fort Collins, USA) and evaluate their performance on field-caught *Aedes albopictus* mosquitoes in Italy. Briefly, samples were extracted with BioSprint 96 semi-automated workstation with One-For-All kit (Qiagen,Hilden, Germany) and cDNA synthesis was achieved using random hexamer (Roche Diagnostics, Mannheim, DE) and SuperScript® II Reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturers' instructions. Real Time PCRs in comparison were run on the same cDNAs in triplicate and CT results were confronted. To test relative efficiency (%) and sensitivity (LOD) of the 2 PCRs , a reference positive RNA were diluted up to 1:1M and PCR reactions were made in quadruplicate. Finally the two PCRs were applied on filed captured mosquitoes in the 9 Provinces of Emilia Romagna region by modified CDC traps baited by CO2 (CO2 traps). The three positive reference samples were correctly identified by the two protocols. In the efficiency (%) and sensitivity (LOD) evaluations both the methods detect in quadruplicate positive control diluted 1:100'000, both method shown a good linearity ( $R^2=0.989$  and  $R^2=0.991$ ) with efficiency of 103.1% and 118.3% for Lanciotti et al. and Faye et al., respectively. The mean Delta CT between the two methods was 2.2 cycles less for Faye et al. protocol. Between June and October 2015, a total of 3312 *Aedes albopictus* mosquitoes were collected. Mosquitoes were grouped in 189 pools according to date, location and species, with a maximum of 150 individuals per pool. Both the Real Time PCRs in comparison were applied on tiger mosquitoes pools and no positive reaction were detected. In conclusion both the methods tested show good sensitivity and get the 100% satisfactory results in molecular panel test. The method of Faye et al. seem to be slightly more sensitive but differences observed falls in the interval of 3 cycles where PCRs are usually considered equivalent. The research of Zika virus in tiger mosquitoes collected during Summer 2015 confirm that Zika virus didn't circulate in our Region. Nevertheless, the very diffused *Ae. albopictus* is in Emilia Romagna Region where it caused an outbreak of Chikungunya virus (CHIKV) in 2007 was recently demonstrate (Di Luca et al. 2016 Eurosurveillance, Volume 21, Issue 18, 05 May 2016) to be susceptible to ZIKV infection. Our results unlighted the importance of keep monitoring arboviruses transmitted by Italian mosquitoes in entomological arbovirus surveillance plan. Acknowledgements: The authors wish to thank Brandy J. Russell and Colleagues of U.S. Department of Health and Human Services, Center for Disease Control and Prevention (CDC), Fort Collins , USA for providing the material used in this study.

Boniotti<sup>°</sup>MB, Giacomini E, Papetti<sup>°</sup>A, Bertasio<sup>°</sup>C, Cerioli<sup>°</sup>M, Lazzaro<sup>°</sup>M, Faccini<sup>°</sup>S, Bonilauri<sup>°</sup>P, Salogni<sup>°</sup>C, Giovannini<sup>°</sup>S, Lavazza <sup>°</sup>A, Alborali<sup>°</sup>GL

#### **Emergence of porcine epidemic diarrhea virus In Italy**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - p 515 [Nr. Estr. 7300]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Introduction: Porcine epidemic diarrhea (PED) is an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea and high mortality in seronegative neonatal piglets. In the last years, PED has had a big economic impact on swine industry in Asia and United States. In 2014, PEDV has also re-emerged in many countries of Europe after about 20 years without PEDV circulation. In Italy, after the last epidemic wave in 2005-2006, different strains of PEDV has been circulating as sporadic outbreaks. This study reports a new epidemic wave in Italy during 2015 caused by the so-called S-INDEL variant. **CC Materials and Methods:** Feces or intestine samples from 2488 pigs showing enteritis were collected from 463 farms during 2015. Most of the samples came from the North of Italy (i.e. the area with the higher density of pig production) and only few from the rest of the country. Mortality and clinical data were collected in 31 and 59 farms, respectively. After RNA extraction, samples were analyzed by a Real time PCR targeting

PEDV/Transmissible Gastroenteritis Virus (TGEV)/Porcine Deltacoronavirus (PDCoV). S1 gene sequence was also obtained to confirm S-INDEL variant in each positive farm. Results: PEDV was detected by Real-time PCR in 205 farms located mainly in Northern Italy and few in the Centre and South of Italy (9). Neither TGEV nor PDCoV were detected. The peak of outbreaks occurred in February-April and decreased in June-September. From October to December the incidence of outbreaks increased again with a 30% of PEDV-reinfection in previously infected farms. Among the positive farms, one was a nursery, 34 were nursery-finisher, 88 finisher, 12 farrow-to-finish and 67 grower-producer. Mortality was higher in the suckling piglets with a mean of 14%, a maximum of 50% and a minimum of 0%. Clinical symptoms were observed at all ages. S1 gene sequence was obtained from 195 farms. All the outbreaks were caused by strains showing > 98% identity with the S-INDEL variant prototype OH-851. The genetic variability among them ranged from 98.7-100%. The phylogenetic analysis showed different clusters consistent with the hypothesis that different entry events could have occurred in Italy. Conclusion: Since January 2015, the S-INDEL PEDV strain rapidly spread in the high-density pig production area in the North of Italy. Most of the outbreaks were in grower-producer, nursery-finisher and finisher farms. Clinical signs and mortality rates were similar to those described in USA and other European countries in the same period.

Boniotti<sup>o</sup>MB, Papetti<sup>o</sup>A, Lavazza<sup>o</sup>A, Alborali<sup>o</sup>G, Sozzi<sup>o</sup>E, Chiapponi<sup>o</sup>C, Faccini<sup>o</sup>S, Bonilauri<sup>o</sup>P, Cordioli<sup>o</sup>P, Marthaler D

**Porcine epidemic diarrhea virus and discovery of a recombinant swine enteric coronavirus, Italy**

Emerg Infect Dis. - Vol. 22 no 1 ( 2016). - p 83-87. - 15 bib ref [Nr. Estr. 7201]

Porcine epidemic diarrhea virus (PEDV) has been detected sporadically in Italy since the 1990s. We report the phylogenetic relationship of swine enteric coronaviruses collected in Italy during 2007–2014 and identify a drastic shift in PEDV strain variability and a new swine enteric coronavirus generated by recombination of transmissible gastroenteritis virus and PEDV.

Borella L, Moretti VM, Alborali<sup>o</sup>GL, Scali<sup>o</sup>F, Salogni<sup>o</sup>CL

**Indagine sulla patogenicità di ceppi mobili di *Aeromonas* spp. isolati da specie ittiche**

Atti del XXII Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) : San Michele all'Adige (TN), 8-9 settembre 2016 / [s.l. : s.n., 2016]. - p 20 [Nr. Estr. 7344]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (22. : San Michele all'Adige, Trento : 8-9 settembre 2016)

Lo studio della patogenicità di *Aeromonas* spp. è caratterizzato da notevole complessità, sviluppandosi in genere su più livelli e comprendendo saggi biologici in vitro (linee cellulari) ed in vivo (modelli animali), esami molecolari ed analisi delle proprietà fenotipiche degli isolati. Nell'ambito di una tesi di specialità (Allevamento, Igiene, Patologia delle Specie Acquatiche e Controllo dei Prodotti Derivati — UNIMI, Sezione Diagnostica di Brescia - IZSLER) sulla caratterizzazione in vitro della virulenza di questi microrganismi, sono stati esaminati 101 ceppi di *Aeromonas* mobili, provenienti da specie ittiche selvatiche (69), allevate (26) ed ornamentali (6). L'isolamento è avvenuto da pesci setticemici (27) ed apparentemente sani (74). Per ciascuno degli isolati, identificati biochimicamente a livello di complex, sono state analizzate le proprietà citotossiche su tre diverse linee cellulari (EPC, BF2, Vero) ed una serie di caratteristiche colturali (crescita a 37°C, emolisi), biochimiche (test VP, decarbossilazione della lisina, fenomeno del suicidio) ed enzimatiche (lipasi, gelatinasi) indicate in letteratura come potenziali fattori di virulenza di *Aeromonas* spp. In particolare, si è indagato sulla correlazione fra questi fattori, la fenospecie dei ceppi (*A. hydrophila*, *A. sobria*, *A. caviae*), la provenienza (selvatica, allevata) ed il quadro clinico delle specie ittiche d'isolamento. In generale, lo studio ha registrato alte prevalenze (54.5-99.0%) dei potenziali indicatori di patogenicità all'interno del campione testato. La produzione di lipasi (98.0%) e gelatinasi

(99.0%) è stata il fattore più frequentemente riscontrato. Anche le caratteristiche colturali/biochimiche hanno mostrato un'ampia diffusione fra i ceppi esaminati, con prevalenze variabili dal 70.3% (crescita a 37°C) al 94.1% (fenomeno del suicidio: attività non suicida). L'attività citotossica ha rappresentato il fattore di virulenza meno prevalente (54.5%). Dai risultati è emersa una correlazione della capacità di crescita a 37°C, dell'attività emolitica e citotossica con la fenotipia dei ceppi, in particolare con i complessi *A. caviae* (crescita a 37°C) ed *A. hydrophila* (attività emolitica e citotossica). Ad esclusione dell'azione decarbossilasica sulla lisina, prevalente negli isolati selvatici, nessuno dei markers di virulenza è risultato associato all'origine dei ceppi. Solamente la crescita a 37°C è stata correlata con presenza di patologia nei pesci, indicando una scarsa significatività dei fattori fenotipici analizzati nella caratterizzazione in tal senso della patogenicità di *Aeromonas* spp. Dei sistemi cellulari tentati, le cellule Vero si sono dimostrate le più sensibili alle citotossine prodotte da *Aeromonas* spp. Si ritiene comunque che l'importanza di utilizzare le cellule Vero sia legata, oltre ad una migliore visualizzazione dell'effetto citopatico, anche alla possibilità di individuare ceppi in grado di esprimere il loro potenziale patogeno nei mammiferi e nell'uomo, il quale andrebbe però confermato mediante l'impiego dei tradizionali modelli animali. Ulteriori studi si rendono necessari per meglio definire il significato clinico dei fattori di virulenza di *Aeromonas* spp. nei pesci, da eseguirsi su un maggior numero di isolati ed associando l'esame dei caratteri fenotipici dei ceppi alle analisi molecolari e, soprattutto, alle prove biologiche in vivo.

Boss R, Cosandey A, Luini M, Artursson K, Bardiau M, Breitenwieser F, Hehenberger E, Lam Th, Mansfeld M, Michel A, Moesslacher G, Naskova J, Nelson S, Podpecan O, Raemy A, Ryan E, Salat O, Zangerl P, Steiner A, Graber HU

**Bovine Staphylococcus aureus : subtyping, evolution, and zoonotic transfer**

J Dairy Sci. - Vol. 99 no 1 ( 2016). - p 515-528. - 60 bib ref [Nr. Estr. 7168]

*Staphylococcus aureus* is globally one of the most important pathogens causing contagious mastitis in cattle. Previous studies using ribosomal spacer (RS)-PCR, however, demonstrated in Swiss cows that *Staph. aureus* isolated from bovine intramammary infections are genetically heterogeneous, with *Staph. aureus* genotype B (GTB) and GTC being the most prominent genotypes. Furthermore, *Staph. aureus* GTB was found to be contagious, whereas *Staph. aureus* GTC and all the remaining genotypes were involved in individual cow disease. In addition to RS-PCR, other methods for subtyping *Staph. aureus* are known, including spa typing and multilocus sequence typing (MLST). They are based on sequencing the spa and various housekeeping genes, respectively. The aim of the present study was to compare the 3 analytic methods using 456 strains of *Staph. aureus* isolated from milk of bovine intramammary infections and bulk tanks obtained from 12 European countries. Furthermore, the phylogeny of animal *Staph. aureus* was inferred and the zoonotic transfer of *Staph. aureus* between cattle and humans was studied. The analyzed strains could be grouped into 6 genotypic clusters, with CLB, CLC, and CLR being the most prominent ones. Comparing the 3 subtyping methods, RS-PCR showed the highest resolution, followed by spa typing and MLST. We found associations among the methods but in many cases they were unsatisfactory except for CLB and CLC. Cluster CLB was positive for clonal complex (CC)8 in 99% of the cases and typically positive for t2953; it is the cattle-adapted form of CC8. Cluster CLC was always positive for t12645 and typically positive for CC705. For CLR and the remaining subtypes, links among the 3 methods were generally poor. Bovine *Staph. aureus* is highly clonal and a few clones predominate. Animal *Staph. aureus* always evolve from human strains, such that every human strain may be the ancestor of a novel animal-adapted strain. The zoonotic transfer of IMI- and milk-associated strains of *Staph. aureus* between cattle and humans seems to be very limited and different hosts are not considered as a source for mutual, spontaneous infections. Spillover events, however, may happen.

Bregoli A, Foglia E, Pezzoni G, Grazioli S, Calzolari N, Chiapponi C, Brocchi E

**Studio dell'evoluzione del virus della malattia vescicolare del suino in Italia attraverso**

## **l'analisi delle sequenze genomiche complete e del profilo antigenico**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 63 (Poster 26) [Nr. Estr. 7415]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

La Malattia Vescicolare del Suino (MVS) 6 causata da un virus appartenente al genere Enterovirus famiglia Picornaviridae, il genoma 6 costituito da RNA a filamento positivo di circa 7400 nt. Il virus 6 presente con un solo sierotipo e 4 distinte varianti genomico-antigeniche. Il virus 6 stato riscontrato per la prima volta nel 1966 in Italia, poi in vari paesi europei negli anni 70-80; in seguito 6 persistito solo in Italia fino al 1992, quando una nuova variante introdotta dall'Olanda ha dato origine a sporadici focolai in Belgio, Spagna, Portogallo e si 6 insediata in Italia fino al 2015. Un obiettivo di questo lavoro 6 stato lo studio dell'evoluzione del virus MVS circolanti in Italia dal 1992 al 2015 (variante genomico-antigenica piu recente), attraverso l'analisi del genoma completo, ottenuto con piattaforma MiSeq (Illumina), di 184 ceppi rappresentativi dell'arco temporale considerato ed inclusivi di alcuni rappresentanti delle 3 varianti precedenti. Per ogni ceppo sono state ottenute sequenze quasi complete della lunghezza di 7335 nt. L'analisi filogenetica bayesiana della porzione codificante, mostra la separazione dei ceppi italiani dell'ultima variante in due cluster principali, con un probabile progenitore comune risalente al 1990-91. Un primo cluster comprende due sub-lineaggi distinti, di cui uno composto da virus isolati nel periodo piu lontano (1992-98) e l'altro composto da virus isolati dal 2004 in poi, probabilmente derivati da una re-introduzione dal Portogallo (lineaggio "portoghese"). Il secondo cluster comprende ceppi evoluti in Italia dal 1995 al 2007 (lineaggio italiano). A partire dal 2007, sono stati individuati prevalentemente ceppi generati da un evento di ricombinazione tra lineaggio italiano e portoghese. L'evoluzione antigenica di 54 ceppi rappresentativi dell'ultima decade 6 stata analizzata utilizzando un pannello di 25 Anticorpi Monoclonali (AcM) di cui 18 neutralizzanti. L'analisi del profilo antigenico, basata su 5 diversi siti target degli AcM neutralizzanti e confrontata con gli isolati degli anni precedenti gia caratterizzati, rileva una sostanziale uniformita dei ceppi ma con alcuni trend evolutivi, in particolare rispetto al sito Ib (VP1, B-C loop) soggetto a mutazione e retromutazione che modificano nel tempo la reattivit  dei rispettivi AcM target, e rispetto al sito IV (VP1 C-end), che muta perdendo gradualmente la reattivit  dell'AcM target. Sono in corso analisi comparative tra le variazioni antigeniche e genetiche.

Cacciamali°A, Zanotti°C, Bilato°D, Stoppani°E, Lombardo°T, Villa°R

### **Development of diagnostic tools for rodent health status evaluation**

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 2 p [Nr. Estr. 7308]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Despite the continuous development of human and veterinary biotechnology, the use of mice and rats as laboratory animals is still common in several fields of research. Different viruses and other pathogens may infect those animals, affecting experimental conditions and invalidating test results. In this perspective, monitoring mice and rats health conditions is mandatory for research studies and could be also useful to improve animal welfare in experimental facilities, in agreement with 3R policy. The Laboratory of Cell Cultures of IZSLER, as the Italian Reference Centre for alternative methods to animal testing, developed a panel of diagnostic tools, according to FELASA recommendations for the health monitoring of laboratory animals in breeding and experimental units (Rehbinder et al., 1996). ELISA and Real-Time PCR have been set up to identify Pneumonia Virus of Mice, Mouse Parvovirus, Theiler's Murine Encephalomyelitis Virus, Murine Hepatitis Virus, Murine Polyomavirus, Ectromelia virus, Reovirus-3, Murine Adenovirus, Murine Norovirus and Murine Cytomegalovirus. Indirect sandwich ELISA were developed using specific immunoglobulins directed against the Minute Virus of Mice (MVM) and Ectromelia virus presented in a solid phase by hyperimmune rabbit sera, previously produced at IZSLER. Sera and feces were obtained from different strains of mice and rats

collected from several experimental units and evaluated with the above-mentioned techniques. The results showed the massive presence of Parvovirus in apparently pathogen-free murine colonies facilities, according to previous published data. ELISA results were compared to those obtained by commercial kits. These diagnostic tests, based on both serology and molecular biology, proved to be efficacious in identifying virus infection and the correlated immune response. This could be a valid tool in order to prevent introduction and spread of pathogens into experimental facilities. The diagnostic panel described could be available at IZSLER as diagnostic service to check health status of mice and rats colony for commonly infectious agents in public and private facilities. In order to implement this panel, pathogens such as bacteria and parasites will be enclosed as well as other laboratory animals species.

Caloni F, Chiari<sup>o</sup> M, Albonico M, Faggionato<sup>o</sup> E, Luciani<sup>o</sup> M, Piazza<sup>o</sup> P, Biancardi<sup>o</sup> A, Cortinovis C

#### **Animal poisoning by baits in Italy**

Toxicol Lett. - Vol. 258 Suppl (2016). - p S294 [Nr. Estr. 7473]

Congress of the European Societies of Toxicology (EUROTOX) (52nd : Seville, Spain : 04th-07th September 2016)

Although the improper or malicious use of poisoned baits is banned in Italy, incidents of intentional animal poisoning are still widespread representing a serious threat to pets as well as wildlife species. Data from this retrospective study were taken from 44 pesticide-based baits sent for toxicological analysis to the laboratory of the Lombardy and Emilia Romagna Experimental Zootechnic Institute (IZSLER) from January 2010 to December 2014. Analyses were performed, in relation to the toxicant, in GC-MS, LC-MS, TLC or physical chemical (zinc phosphide). In 14% of the analysed baits more than one pesticide was present. The use of a combined toxicant is probably due to its assume higher lethal toxicity. Anticholinesterase insecticides (carbamates and organophosphates) proved to be the most common pesticides detected (36%) followed by organochlorine insecticides (30%), anticoagulant rodenticides (20%) and molluscicides (11%). The non-anticoagulant rodenticides alpha-chloralose (5%), strychnine (5%) and zinc phosphide (2%) were also detected. Among anticholinesterase insecticides, analysis of data identified the organophosphate methamidophos (18%) and the carbamate carbofuran (9%) as the main poisoning agents. Despite the organochlorine insecticide endosulfan has been withdrawn from the market in 2011, the active ingredients of its commercial formulation (α-endosulfan and 13-endosulfan) occurred in 30% of the analysed baits. Anticoagulant rodenticides included brodifacoum (5%), bromadiolone (9%), coumatetralyl (7%), difenacoum (2%) and flocoumafen (2%).

Calzolari<sup>o</sup> M

#### **Mosquito-borne diseases in Europe : an emerging public health threat**

Reports Parasitol. - Vol. 5 (2016). - 12 p. - 81 bib ref [Nr. Estr. 7217]

Mosquito-borne pathogens cause some of the more deadly worldwide diseases, such as malaria and dengue. Tropical countries, characterized by poor socioeconomic conditions, are more exposed to these diseases, but Europe is experiencing an increasing number of human cases of mosquito-borne diseases, both imported and indigenous. Some of these cases are due to recrudescence of pathogens already present in the territory, particularly the West Nile virus. However, other neglected mosquito-borne pathogens remain present in Europe, and could produce human cases sustained by local mosquitoes (such as the Tahyna and Sindbis viruses). Native mosquitoes are still able to transmit pathogens eliminated from Europe and reimported by the sick (such as malaria plasmodia), as well as new imported pathogens. An increasing number of large epidemics involving arboviruses, for which humans could be reservoir hosts (eg, Dengue virus, Chikungunya virus, and Zika virus), seasonally concordant with the activity period of European

vectors, poses an expanding risk for potential introduction of these viruses. More autochthonous cases of exotic diseases were reported in Europe, including dengue and chikungunya, raising the potential for the establishment of those pathogens which can be transmitted vertically in vectors. These episodes were often responsible for the establishment of exotic mosquitoes, such as tiger mosquito, imported into Europe by trade and now present in adequate numbers to transmit these pathogens. This actually occurred for chikungunya in Italy in 2007, with more than 200 cases of this disease. Other mosquitoes, potentially vectors of pathogens, can use the same means of entry into Europe, posing new potential risks for health. Dealing with mosquito-borne pathogens, characterized by a complex cycle, will require the establishment of interdisciplinary measures and an internationally coordinated approach, since these diseases do not recognize borders.

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**Phleboviruses circulating in sand flies in Emilia-Romagna region (Northern Italy) in 2013-2015**

Parasite. - Vol. 23 ( 2016). - p 58-59 [Nr. Estr. 7619]

International Symposium on Phlebotomine Sandflies (ISOPS) (9th : Reims, France : June 28th-July 1st, 2016)

Phlebotomine 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, defining the phleboviruses circulating in a particular area is an important health issue. To monitor the presence of phleboviruses, a surveillance system, based on sampling and testing of sand flies, was activated in the Emilia-Romagna region (Northern Italy). The system detected the co-circulation of three different phlebovirus: the well-known Toscana virus and other two previously unreported phleboviruses, tentatively named Ponticelli virus and Sole virus. Sandflies were sampled in 151 geo-referenced sites by suction traps, baited with carbon dioxide, and activated overnight. Sites were placed mainly in the hilly areas of the Region, which are characterized by ecological conditions particularly favorable to sand-flies. Between 2013 and 2015 a total of 90,506 sandflies were sampled. A subsample of 2,527 specimens were morphologically identified: 2,441 (96.5%) were *Phlebotomus peifiliewi* and 86 (3.5%) were *Ph. perniciosus*. This result is consistent with previous results obtained in Emilia-Romagna, which show the overwhelming presence of the *Ph. peifiliewi* compared to *Ph. perniciosus*. The largest number of sand flies (82,181) was collected in 2013. In this year we also caught the largest number of specimens per trap per night, with more than 10,000 sand flies in two different sites. A total of 87,492 sandflies, sorted in 321 pools, were submitted to a Real Time PCR analysis that targeted the Toscana virus, and 35 of these pools, from 17 sites, tested positive. Moreover 26,853 sandflies (in 110 pools) were tested with a pan-phlebovirus PCR followed by the sequencing of produced amplicons, giving 52 positive pools. The phylogenetic analysis made with the homologous sequences of other phleboviruses available in Genbank, suggest the presence of two previously unreported phleboviruses, highlighted by the presence of two well supported clades in the resultant tree. One of these clades falls with the Salehabad serocomplex, and the respective virus has been tentatively named Ponticelli virus, the other clade falls in the Sand fly fever Naples serocomplex, and the respective virus has been tentatively named Sole virus. Both viruses were detected in all the three years of survey in different locations, Ponticelli virus in 11 sites and Sole virus in 10 sites. Interestingly the sequence ascribed to Sole virus was also detected in sand flies from the neighboring Lombardia Region. The isolation and detection of non-described phlebovirus is consistent with the wide variety of new phleboviruses reported in last ten years, especially in Mediterranean basin. Despite Toscana virus having been described as the major cause of summer meningitis in Italy, France and Spain, this virus remains a neglected pathogen. Moreover the discovering of new phleboviruses, reported in this study, and in the Mediterranean basin, raises the issue of their infectious potential, since several of these viruses are serologically detected in vertebrates and show the ability to grow on Vero cells. The sympatric co-circulation of different phlebovirus reported in this study, indicate a very dynamic and complex situation, which deserves a more detailed investigation to characterize the circulation and the possible pathogenicity to humans and animals of these uncharacterized viruses.

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**Wide recognition of Culex pipiens and lack of detection of Culex torrentium through biomolecular differentiation of mosquitoes in the Emilia-Romagna region, Northern Italy**

Med Vet Entomol. - Vol. 30 ( 2016). - p 435-438. - 20 bib ref [Nr. Estr. 7307]

The *Culex pipiens* complex includes species with reported differences in vector competence for arthropod-borne viruses, many of which are of significant importance to human health such as the West Nile virus and the Sindbis virus. This group of mosquitoes is difficult to distinguish morphologically; particularly as adult females. In Europe, the two species of the complex, *Culex pipiens* Linnaeus 1758 and *Culex torrentium* Martini 1925, are often found sympatrically. With the aim to characterize the presence and spread of both species in the Emilia-Romagna region, Northern Italy, mosquitoes of the complex – collected during the West Nile virus surveillance plans – were tested by multiplex real-time PCR for the detection of the two species *Cx. pipiens* and *Cx. torrentium*. A total of 24 165 mosquitoes, collected between 2012 and 2014 from 105 sites, and sorted in 204 pools, were tested. All tested pools were found to be composed of *Cx. pipiens*, whereas *Cx. torrentium* was not detected. These results indicate a likely absence of *Cx. torrentium* mosquitoes within the surveyed territory, whereas *Cx. pipiens* is widely distributed in the area mentioned. This is in line with previous reports, which describe a pre-alpine distribution of *Cx. torrentium* in Italy.

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**Sympatric circulation of two Usutu virus strains in Northern Italy between 2009 and 2014**

13th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID) : Antwerp, Belgium, 10-13 May 2016 / [s.l. : s.n., 2016]. - 1 p (S5.A.01) [Nr. Estr. 7541]

International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID) (13th : Antwerp, Belgium : 10-13 May 2016)

**Introduction** The Usutu virus is a flavivirus circulating between mosquitoes and wild birds, in which it can cause mass mortality. It has been increasingly reported in Europe, raising concerns for its possible pathogenic potential for humans. This virus has been continuously detected from 2009 to 2014 in Emilia-Romagna and Lombardia regions (Northern Italy) through an active surveillance plan. The whole genomes of USUV isolates, obtained during this plan, are analyzed with the aim to characterize the genetic, geographical and temporal spread of USUV in Northern Italy. **Methods** The whole genome sequences (VGSs) of 15 isolates between 2009 and 2014 have been analysed with other VGSs available on Gene Bank database (n= 106). Moreover two dataset, obtained by sequences of the NS5 and E genes has been separately analysed. Phylogenetic interactions and time of origins of the different clades has been inferred by Bayesian Markov Chain Monte Carlo approach (MrBayes 3.2 and Beast 2). **Results** The largest part of European sequences (97/99) group together in a well supported branch of the obtained tree. Sequences of this branch are divided in four clades: one from Austria and Hungary, one from Germany, one from Emilia-Romagna and one from Lombardia. Analysis on the two gene datasets estimated time of the more recent ancestor of European strains in 1991 (NS5) and 1993 (E). **Discussion** The phylogenetic analysis of whole sequences demonstrate that large part of European strains belong to one clade and strongly suggests an autochthonous evolution of these strains from a common ancestor, for which the time of arrival in Europe can be estimated at the beginning of the 90s. In Italy, the presence of two strains of the virus in surveyed area might be the result of two different routes of introduction one from North and one from East.

Calzolari<sup>°</sup>M, Zé-Zé L, Vázquez A, Seco MPS, Amaro F , Dottori<sup>°</sup>M

### **Insect-specific flaviviruses, a worldwide widespread group of viruses only detected in insects**

Infect Genet Evol. - Vol. 40 ( 2016). - p 381-388. - 68 bib ref [Nr. Estr. 7287]

Several flaviviruses are important pathogens for humans and animals (Dengue viruses, Japanese encephalitis virus, Yellow-fever virus, Tick-borne encephalitis virus, West Nile virus). In recent years, numerous novel and related flaviviruses without known pathogenic capacity have been isolated worldwide in the natural mosquito population. However, phylogenetic studies have shown that genomic sequences of these viruses diverge from other flaviviruses. Moreover, these viruses seem to be exclusive of insects (they do not seem to grow on vertebrate cell lines), and were already defined as mosquito-only flaviviruses or insect-specific flaviviruses. At least eleven of these viruses were isolated worldwide, and sequences ascribable to other eleven putative viruses were detected in several mosquito species. A large part of the cycle of these viruses is not well known, and their persistence in the environment is poorly understood. These viruses are detected in a wide variety of distinct mosquito species and also in sandflies and chironomids worldwide; a single virus, or the genetic material ascribable to a virus, was detected in several mosquito species in different countries, often in different continents. Furthermore, some of these viruses are carried by invasive mosquitoes, and do not seem to have a depressive action on their fitness. The global distribution and the continuous detection of new viruses in this group point out the likely underestimation of their number, and raise interesting issues about their possible interactions with the pathogenic flaviviruses, and their influence on the bionomics of arthropod hosts. Some enigmatic features, as their integration in the mosquito genome, the recognition of their genetic material in DNA forms in field-collected mosquitoes, or the detection of the same virus in both mosquitoes and sandflies, indicate that the cycle of these viruses has unknown characteristics that could be of use to reach a deeper understanding of the cycle of related pathogenic flaviviruses.

Caminiti°A, Pelone F, La\_Torre G, De\_Giusti M, Sau Ile R, Mannocci A, Sala M, Della\_Marta U, Scaramozzino P

### **Control and eradication of tuberculosis in cattle: a systematic review of economic evidence**

Vet Rec. - Vol. 179 ( 2016). - p 70-75. - 39 bib ref [Nr. Estr. 7395]

Bovine tuberculosis (TB) is a disease of zoonotic importance for which control and eradication programmes have been carried out in many countries for decades. While the impact of these programmes on public health is still uncertain, the impact on trade is significant because of movement restrictions for animals, costs of testing and culling. The objective of this systematic review was to provide a contribution to the general debate over costs against benefits for the control and eradication of bovine TB in cattle. The search strategy was performed on four electronic databases following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The selection process, data abstraction and quality appraisal were carried out according to the Cochrane Collaboration guidelines. The search identified 66 articles out of which eight fulfilled the inclusion criteria. The evidence gathered in this review by combining the conclusions of the most methodologically sound articles supports the idea that, when multiple cost and benefit components are taken into account, efforts to control or eradicate bovine TB may be effective in reducing disease prevalence, economically viable and worth doing.

Campana L, Giacomelli°S, Polloni°A, Rota\_Nodari° S, Archetti°L, Bianchi°A, Moscati LP

### **Valori di riferimento biochimico clinici di cinghiali (Sus scrofa) allevati e a vita libera**

XVII Congresso Nazionale SIDI LV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDI LV), 2016]. - p 104-105. - 9 bib ref [Nr. Estr. 7365]

Congresso Nazionale Società Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*About serum biochemistry and haematology of wild boar there are few data. Studying these values in such difficult managing species would allow to improve better ways of breeding and a more mindful approach to the animals. In this study, reference ranges for wild boars are presented; they are established from blood samples on 137 boars slaughtered between September 2005 and March 2006. Animals were in good conditions, aged 1 to 3 years. Some of them were captive bred, some wild caught, both in winter and in spring. These values reveal the metabolic status of the animals, mainly related to the body condition and nutritional status.*

Capucci°L, Cavadini°P, Schiavitto M, Lombardi°GI , Lavazza°A

### **Evoluzione dell'RHDV2 verso l'alta patogenicità : urgenza di un vaccino omologo per la tutela delle produzioni cunicole**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 69 ( Poster 32) [Nr. Estr. 7406]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

Introduzione. La malattia emorragica del coniglio (Rabbit Hemorrhagic Disease — RHD) è un'epatite acuta dell'animale adulto causa di mortalità >80%. L'agente eziologico è un calicivirus (RHDV, genere Lagovirus) identificato per la prima volta in Cina nel 1984, diffuso in breve in tutti i continenti e causa di ingenti danni per la zootecnia. Nel 2010 è stato identificato per la prima volta in Francia un nuovo virus (RHDV2) correlato geneticamente all'RHDV, ma diverso per profilo antigenico e patogenicità (mortalità media del 20%). Qui presentiamo i dati di un'infezione sperimentale con ceppi RHDV2 italiani isolati nel 2014 e 2015. Metodi. L'infezione sperimentale è stata eseguita in area BL3, previa autorizzazione del Ministero della Salute e nel rispetto delle norme nazionali (DM 4/3/2014 n°26) ed Europe (2010/63/EU) sulla sperimentazione animale. Tre gruppi di 5 conigli adulti, sieronegativi per RHDV ed RHDV2, sono stati infettati per via orale con 1 ml di un omogenato di fegato allo 0,5% w/v rispettivamente dei ceppi: 1) RHDVBs89, 2) RHDV2Ta14, 3) RHDV2Ch15. I conigli sono stati tenuti sotto osservazione clinica fino alla comparsa dei sintomi di RHD. Alla necropsia sono seguiti test ELISA ed in PCR sul fegato per il rilevamento dell'RHDV ed RHDV2. Risultati e discussione. I due isolati RHDV2 hanno causato RHD acuta in 4 dei 5 animali infettati, con risultati identici a quello del ceppo di riferimento RHDVBs89. Il tempo medio di mortalità è stato di 70 ore per l'RHDV2Ta14, identico a quello dell'RHDVBs89, e di 85 ore per l'RHDV2Ch15. L'esame necroscopico e gli alti titoli virali rilevati in ELISA hanno confermato l'RHD acuta in tutti gli animali. Un animale per ciascun gruppo è deceduto per una enterite batterica e non per RHD; l'esame anatomo-patologico non ha evidenziato lesioni specifiche di RHDV e i fegati erano positivi solo in PCR. I dati dimostrano che gli isolati più recenti di RHDV2 hanno un grado di patogenicità (~80% di mortalità simile a quello dell'RHDV e di 4 volte superiore ai primi isolati del 2010 e 2011. I dati trovano conferma anche dalle osservazioni di focolai di RHD in campo da RHDV2 con mortalità in aumento negli anni. Poiché sia risultati sperimentali che osservazioni sul campo hanno dimostrato che conigli vaccinati con l'RHDV sono protetti solo in minima parte dall'RHD causata dall'RHDV2, si sottolinea l'urgenza di poter disporre di un vaccino RHDV2 autorizzato per l'uso sul campo. Dal punto di vista scientifico, i dati avvalorano l'ipotesi che RHDV2 non sia una variante dell'RHDV ma piuttosto un virus di nuova emergenza.

Cardeti G, Mariano V, Eleni C, Aloisi M, Grifoni G, Sittinieri S, Dante G, Antognetti V, Foglia°EA, Cersini A, Nardi A

### **Encephalomyocarditis virus infection in *Macaca sylvanus* and *Hystrix cristata* from an Italian rescue centre for wild and exotic animals**

Virology J. - Vol. 13 (2016). - no 193 (6 p). - 15 bib ref [Nr. Estr. 7444]

Background: The Encephalomyocarditis virus (EMCV) is a small, non enveloped, positive sense

single-stranded RNA virus in the genus *Cardiovirus*, family *Picornaviridae*, with two known serotypes. It is spread worldwide and infects a huge range of vertebrate hosts with zoonotic potential for humans. The pig is the mammal most likely to be impacted on with the disease, but EMCV occurrence has also been reported in non-human primates and in a variety of domestic, captive and wild animals. Until now, human cases have been very rare and the risk appears to be almost negligible in spite of human susceptibility to the infection. Case presentation: Between September and November 2012 a fatal Encephalomyocarditis virus outbreak involving four Barbary macaques and 24 crested porcupines occurred at a rescue centre for wild and exotic animals in Central Italy. In this open-field zoo park located near Grosseto, Tuscany about 1000 animals belonging to different species, including various non-human primates were hosted at that time. Sudden deaths were generally observed without any evident symptoms or only with mild nonspecific clinical signs. The major gross change was characterised by grey-white necrotic foci in the myocardium and the same EMCV strain was isolated both in macaques and crested porcupines. Phylogenetic analysis has confirmed that only one EMCV strain is circulating in Italy, capable of infecting different animal species. Conclusions: This report confirms the susceptibility of non-human primates to the EMCV infection and describes the disease in porcupine, a common wild Italian and African species. No human cases were observed, but given the zoonotic potential of EMCV these findings are of importance in the context of animal-human interface.

Caruso C, Vitale N, Prato R, Radaelli MC, Zoppi S, Possidente R, Dondo A, Moreno\_Martin°A, Masoero L

**Fattori di rischio e prevalenza sierologica della malattia di Aujeszky in popolazioni di cinghiali del Nord-Ovest Italia, al fine di favorire una sorveglianza territoriale risk - based**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 172-173. - 5 bib ref [Nr. Estr. 7367]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*PrV circulation in wild boars remains a threat far a re-incursion into the currently "low prevalence" swine population. We reported prevalence data in two north- west Italian wild boars populations under different management regimes (free range vs enclosure) and associated risk factors. 1425 wild boars sera were collected (2012/2015); overall, raw seroprevalence rate was 30.39% (433/1425). In contrast, analysis of the two distinct populations showed a significant difference In prevalence rate: In the free ranging was 9.98% (90/902; CI95%; 8.10-12.12%), that is much lower compared to 65.58% (343/523 CI95%; 61.51-69.65%), recorded In population living In La Mandria. Adults and females were observed to be statistically significant In both populations. For this final stage of Aujeszky's disease eradication in Italy, it's essential to set specific epidemiological scenario in wild boars for guiding risk-based measures.*

Casarin E, Lucchese L, Grazioli°S, Facchin S, Rea I don N, Brocchi°E, Morpurgo M, Nardelli S

**A new ELISA using the ANANAS technology showing high sensitivity to diagnose the bovine rhinotracheitis from individual sera to pooled milk**

PLoS One. - Vol. 11 no 1 ( 2016). - p e0145912 (12 p). - 24 bib ref ( Ultimo accesso : 20/01/2016 <http://www.plosone.org/article/fetchObject.action?uri=info:doi/10.1371/journal.pone.0145912&representation=PDF> ) [Nr. Estr. 7174]

Diagnostic tests for veterinary surveillance programs should be efficient, easy to use and, possibly, economical. In this context, classic Enzyme linked ImmunoSorbent Assay (ELISA) remains the most common analytical platform employed for serological analyses. The analysis of pooled samples instead of individual ones is a common procedure that permits to certify, with one single test, entire herds as "disease-free". However, diagnostic tests for pooled samples need to be particularly

sensitive, especially when the levels of disease markers are low, as in the case of anti-BoHV1 antibodies in milk as markers of Infectious Bovine Rhinotracheitis (IBR) disease. The avidin-nucleic-acid-nanoassembly (ANANAS) is a novel kind of signal amplification platform for immunodiagnosics based on colloidal poly-avidin nanoparticles that, using model analytes, was shown to strongly increase ELISA test performance as compared to monomeric avidin. Here, for the first time, we applied the ANANAS reagent integration in a real diagnostic context. The monoclonal 1G10 anti-bovine IgG1 antibody was biotinylated and integrated with the ANANAS reagents for indirect IBR diagnosis from pooled milk mimicking tank samples from herds with IBR prevalence between 1 to 8%. The sensitivity and specificity of the ANANAS integrated method was compared to that of a classic test based on the same 1G10 antibody directly linked to horseradish peroxidase, and a commercial IDEXX kit recently introduced in the market. ANANAS integration increased by 5-fold the sensitivity of the 1G10 mAb-based conventional ELISA without losing specificity. When compared to the commercial kit, the 1G10-ANANAS integrated method was capable to detect the presence of anti-BHV1 antibodies from bulk milk of gE antibody positive animals with 2-fold higher sensitivity and similar specificity. The results demonstrate the potentials of this new amplification technology, which permits improving current classic ELISA sensitivity limits without the need for new hardware investments.

Cavadini°P, Molinari°S, Chiari°M, Pezzoni°G, Brocchi°E, Stalder G, Posautz A, Capucci°L, Lavazza°A

**Presenza e diffusione in Europa del hare calicivirus (HACV), un lagovirus non patogeno della lepre bruna (*Lepus europaeus*)**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 54 (Poster 17) [Nr. Estr. 7414]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

Introduzione. La Sindrome della lepre bruna europea (EBHS) è un'epatite acuta causata da un calicivirus (EBHSV) appartenente, come l'RHDV del coniglio, al genere Lagovirus. Oltre a virus patogeni, il genere include diversi virus non patogeni, tutti identificati nel coniglio, di cui il Rabbit Calicivirus (RCV) è il prototipo. Poiché dati sierologici indicavano la presenza di lagovirus non patogeni anche nella lepre bruna europea, uno studio recente ci ha permesso di identificare in via preliminare tale virus (Hare Calicivirus — HaCV) nell'intestino di lepri allevate. Scopo di questo lavoro è confermare nel tempo la presenza dell'HaCV nell'allevamento di origine e verificare la sua diffusione nelle lepri selvatiche in alcuni Paesi Europei. Metodi. Dal 2010 al 2015 sono stati campionati 323 intestini da lepri selvatiche provenienti da Emilia Romagna e Lombardia e da lepri di un allevamento in provincia di Brescia, unitamente a 210 intestini di lepri selvatiche cacciate nel 2011 in Austria e Germania. I campioni sono stati analizzati in RT-PCR, utilizzando dei primers universali per lagovirus ed i campioni positivi caratterizzati filogeneticamente per sequenziamento del gene della proteina capsidica. L'analisi sierologica sugli animali presenti in allevamento si è basata su metodiche ELISA in grado di evidenziare sia anticorpi anti-EBHSV specifici sia lagovirus cross-reattivi. Risultati e discussione. Il gene della VP60 dell'HaCV è stato identificato in 71 campioni di diversa origine. L'HaCV mostra un'identità nucleotidica media del 73% e amminoacidica dell'82% con le sequenze di EBHSV e rispettivamente del 68% e 76,3% con le sequenze di RHDV. L'analisi filogenetica ha mostrato come tutte le sequenze identificate si raggruppino in uno specifico ramo dell'albero divergente da quello dell'EBHSV. Per quanto riguarda l'allevamento all'origine dell'isolamento (primo caso positivo retrospettivo del 2012), successivi isolamenti, anche in lepri rilasciate su territorio, e ripetute analisi sierologiche hanno confermato la persistenza del virus a tutto il 2015. Le identificazioni multiple in Italia, paesi Europei confermano la probabile elevata diffusione dell'HaCV nelle lepri selvatiche. L'assenza di malattia con quadri simil-EBHS negli animali presenti in allevamento come pure negli animali selvatici, conferma la non patogenicità di questo nuovo lagovirus.

Cavadini°P, Molinari°S, Pezzoni°G, Chiari°M, Br occhi°E, Lavazza°A, Capucci°L

### **Identification of a new non-pathogenic lagovirus in brown hares (*Lepus europeus*)**

5th World Lagomorph Conference : Turlock, California, USA, July 11-15, 2016 : proceedings / edited by Patrick Kelly... [et al.] organized by California State University Stanislaus, Lagomorph Specialist Group, World Lagomorph Society. - [Turlock : California State University Stanislaus], 2016. - p 81 [Nr. Estr. 7319]

World Lagomorph Conference (5th : Turlock, California, USA : July 11-15, 2016)

European Brown Hare Syndrome (EBHS) is a viral disease mainly observed in European brown hare (EBH) (*Lepus europeus*). Its causative agent (EBHSV) belongs to the Lagovirus genus and is highly related to Rabbit Hemorrhagic Disease Virus (RHDV). A third group of Lagoviruses, named rabbit caliciviruses (RCVs), detected in rabbits in Europe and Australia, cause silent infection of the intestinal tract without clinical signs and relevant lesions. The existence of a non-pathogenic EBHSV-like virus in hares was put forward for explaining some "unexpected" positive serological results obtained in EBH and other *Lepus* species from areas (Australia, South American and Central Africa) where the disease has never been detected. We report the results of investigations performed to seek this putative virus eventually resulted in the first identification of a non-pathogenic Lagovirus in hares. On the basis of the present lagovirus nomenclature, we propose to preliminary name the new virus as "Hare Calicivirus" (HaCV). Faecal and blood samples from 37 healthy hares, 1-2 months of age, born and reared in cages in a breeding hare farm of Brescia province (North Italy), were collected during summer 2014. The serological survey was repeated one year after (2015) testing 18 young hares from 7 separate cages to check the persistence of infection and virus circulation. Total RNA was extracted from feces of hares and analyzed by RT-PCR using the universal primers for lagovirus able to amplify a conserved region of the vp60 gene. Three hares resulted RT-PCR positive for the presence of a lagovirus. The sequence analysis of the entire capsid protein vp60 gene showed an average of nucleotide identity of approximately 73% and 68% compared respectively to the EBHSV and RHDV sequences present in GenBank. To perform the phylogenetic analysis the entire vp60 gene sequences were aligned with those of lagoviruses available in GenBank. Thus, the three isolates were shown to be distinct from all previously described members of the genus Lagovirus and to form a new, separate genetic group in the clade of EBHSV. Serological analysis was performed on blood samples using distinct MAb-based ELISAs: i) a competition ELISA for Ab-EBHSV (EBHS specific); ii) an IgG ELISA able to detect EBHSV cross-reactive antibodies (lagovirus specific); iii) and iv) specific direct ELISAs for IgG and IgM anti-HaCV by using a specific recombinant antigen produced in baculovirus, adsorbed on the solid phase. By using serological ELISAs contrasting results were obtained: in the case of competition ELISA most sera resulted negative with just few positive at very low titers, whereas most sera resulted positive in IgG ELISA with medium-high titers for cross-specific antibodies. Medium-high levels of IgM and/or IgG against the homologous HaCV were found in the sera of most hares tested both on 2014 and 2015, thus suggesting that HaCV was probably present since some time and was still circulating in the farm. This aspect and the lack of any sign of disease among the hares show that the viral infection has likely a subclinical course and thus the virus could be considered non-pathogenic at least since the final demonstration given by experimental infection.

Ceglie L, Guerrini E, Boniotti°MB, Cunico G, Schia von E, Lucchese L, Natale A

### **Pilot survey on brown rats as possible reservoirs of Q fever and leptospirosis in cattle**

Int J Infect Dis. - Vol. 53 Suppl ( 2016). - p 60 [Nr. Estr. 7491]

International Meeting on Emerging Diseases and Surveillance (IMED) (6th : Vienna, Austria : November 3 to 7, 2016)

Purpose: Q fever is a widespread zoonosis caused by *Coxiella burnetii* (*C. burnetii*). Previous studies suggested a possible epidemiological role of synanthropic rodents in the spread and maintenance of the infection among ruminants. On the other hand, genotyping studies are showing species-specific associations of *Coxiella* strains to animal species, in particular to bovine. As no data

are available on direct transmission of *Coxiella* strains from rats to ruminants, the first goal of this study was to investigate the presence of *C. burnetii* in brown rats captured in dairy farms' surroundings. The characterization of rat's strains may confirm or exclude their epidemiological role in cattle infection. Brown rats are also well known reservoirs of Leptospirosis, mainly for the serovar Icterohaemorrhagiae, but their role in bovine Leptospirosis is little investigated. Methods 82  
Materials: Since January 2016, 48 brown rats were conferred to the laboratory for necropsy analysis. Real-time PCR for *Coxiella burnetii* (targeting the ISM 1 insertion) and *Leptospira* spp (targeting the genetic fragment rrs, 16S) were respectively performed on spleens and kidneys taken from 48 brown rats; the sampling is still ongoing. *Coxiella* positive samples were submitted to MLVA and SNP analysis to attempt genotyping of bacterial DNAs and compare them to the strains already present in international available data bases, including the ones circulating in Italian ruminant farms. *Leptospira* positive samples were submitted to MLST and MLVA analysis to identify genospecies and, possibly, their respective serogroups and serovars. Results: So far, diagnostic molecular investigations for *Coxiella burnetii* and *Leptospira* spp. have been respectively performed on spleens and kidneys taken from 48 brown rats. Four out of fifty spleens resulted positive for *Coxiella*, three of them unfortunately with Ct greater than 32. The fourth positive sample had a Ct value of 22 and it has been already partially typed with MLVA method. Ten out of 48 kidneys tested positive to *Leptospira* spp. and MLST and MLVA analyses are currently underway. Conclusion: Brown rats are confirmed to be susceptible to *Coxiella burnetii* and *Leptospira* spp. infection, but the genotyping results will clarify their epidemiological role as reservoirs for cattle population.

Cerutti F, Luzzago C, Lauzi S, Ebranati E, Caruso C, Masoero L, Moreno<sup>o</sup>A, Acutis PL, Zehender G, Peletto S

#### **Phylogeography, phylodynamics and transmission chains of bovine viral diarrhoea virus subtype 1f in Northern Italy**

Infect Genet Evol. - Vol. 45 ( 2016). - p 262-267. - 38 bib ref [Nr. Estr. 7353]

Bovine viral diarrhoea virus (BVDV) type 1 in Italy is characterized by high genetic diversity, with at least 20 subtypes. Subtype 1f is endemic in a restricted geographic area, meaning that it has local distribution. We investigated the population dynamics of BVDV-1f in Northern Italy and characterized the transmission chains of a subset of samples from Piedmont and Aosta Valley regions. A total of 51 samples from 1966 to 2013 were considered and 5' UTR sequences were used for phylogeography. A subset of 12 samples was selected for Npro gene sequencing and further characterization of the transmission chains using both molecular and epidemiological data. Phylogeography estimated the root of BVDV-1f tree in Veneto in 1965. Four significant subclades included sequences clustering by region: Lombardy (n = 3), Lombardy and Emilia-Romagna (n = 7), Piedmont (n = 17), Piedmont and Aosta Valley (n = 21). The Piedmont-only subclade has a ladder-like branching structure, while the Piedmont and Aosta Valley subclade has a nearly complete binary structure. In the subset, the outbreak reconstruction identified one sample from Piedmont as the most probable source of infection for the Aosta Valley cases. An ad hoc questionnaire submitted to public veterinarians revealed connections between sampled and non-sampled farms by means of trades, exhibitions and markets. According to the phylogeography, BVDV-1f moved westward, entering from Veneto, and spreading to Lombardy and Emilia-Romagna in the early 1990s, and finally to Piedmont and Aosta Valley in the first decade of 2000s. Both phylogeographic analyses on the whole dataset and on the selection of Npro dataset pointed out that subtype 1f entered Aosta Valley from Piedmont. The integration of molecular and epidemiological data revealed connections between farms, and such approach should be considered in any control plan. In Aosta Valley, the study showed that BVDV1f can be controlled only monitoring the introduction of cattle from Piedmont region.

Cerutti F, Luzzago C, Lauzi S, Ebranati E, Caruso C, Masoero L, Moreno<sup>o</sup>A, Acutis PL, Zehender G, Peletto S

## **Filogeografia, filodinamica e catene di contagio di bovine viral diarrhea virus sottotipo 1-f in Nord Italia**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 18 [Nr. Estr. 7409]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

Il virus della diarrea virale bovina (Bovine viral diarrhea virus, BVDV) tipo 1 in Italia è caratterizzato da alta variabilità genetica, con almeno 20 sottotipi presenti (1a-1t). Il sottotipo 1f è endemico in una ristretta area geografica, ed è quindi caratterizzato da una distribuzione locale. In questo lavoro si sono studiate la filodinamica e la filogeografia di BVDV-1f nel nord Italia e sono state determinate le catene di contagio in un sottogruppo di campioni provenienti dal Piemonte e dalla Valle d'Aosta. Per la filogeografia sono state analizzate le sequenze 5'UTR di 51 campioni distribuiti in un arco temporale compreso tra il 1966 e il 2013. Successivamente è stato selezionato un sottogruppo di 12 campioni per il sequenziamento del gene Npro per caratterizzare le catene di contagio mediante dati molecolari ed epidemiologici. L'analisi filogeografica ha stimato la radice dell'albero nel 1965 in Veneto. Quattro subclade contengono sequenze raggruppate per regione: Lombardia (n=3), Lombardia ed Emilia-Romagna (n=7), Piemonte (n=17), Piemonte e Valle d'Aosta (n=21). Il subclade piemontese ha una struttura "ladder-like", mentre il subclade di Piemonte e Valle d'Aosta ha una struttura quasi completamente binaria. Nel sottogruppo Npro, la ricostruzione dell'outbreak ha identificato in un campione del Piemonte la fonte più probabile di infezione per la Valle d'Aosta. Un apposito questionario inviato ai veterinari competenti ha rivelato connessioni epidemiologiche e flussi commerciali diretti o indiretti (compravendite, fiere, mercati) tra allevamenti campionati e non campionati. La filogeografia ha stimato che BVDV-1f, dopo la sua comparsa in Veneto, si è propagato verso ovest, diffondendosi in Lombardia ed Emilia-Romagna nei primi anni '90, e successivamente in Piemonte e Valle d'Aosta nel primo decennio del 2000. Sia l'analisi filogeografica sull'intero dataset che sul sottogruppo di Npro hanno indicato che BVDV-1f è stato introdotto in Valle d'Aosta a partire dal Piemonte.

Chiapponi°C, Faccini°S, Bolzoni°L, Merenda°M, Bionioni°L, Manfredi°R, Zanni°I, Rosignoli°C, De\_Mattia°A, Nigrelli°A, Foni°E

### **Circulation of influenza D virus in cattle in Italy**

6th European Congress of Virology : Hamburg, Germany, October 19-22, 2016 / [s.l. : s.n., 2016]. - P26-3 [Nr. Estr. 7482]

European Congress of Virology (6th : Hamburg, Germany : October 19-22, 2016)

Background: A new member of the Orthomyxoviridae family, provisionally named Influenza D virus (IDV), was first reported in swine in the USA. This virus has been detected in bovine in USA, China, France and recently in Italy. Since cattle seems to be the reservoir of this new virus, this study aimed to investigate the IDV epidemiology in the Italian bovine population. Methods: In the period August 2015-April 2016 we tested, for IDV presence, 563 samples (nasal swabs and lungs) from 305 farms by PCR and virological methods. The study was conducted in Northern Italy, area with high density of swine and cattle farms. Samples were collected both from farms affected by respiratory disease and from asymptomatic animals. Genetic characterization of 10 IDVs was performed by NGS sequencing. At the same period, we performed a serological study, by Hemagglutination Inhibition test, using the Italian isolate D/swine/Italy/199724/2015. A total of 945 serum samples collected for routinely diagnostic activities from 20 symptomatic and 45 asymptomatic Italian farms were analyzed. Besides, 420 sera samples were randomly selected from 42 farms in Mantua province, where the virus was detected at first. Results: The prevalence of IDV positive swabs among symptomatic and asymptomatic animal was 12.7% and 6.1% respectively and none of the positive lungs came from asymptomatic animals. The positivity for IDV from swabs was significantly higher than from lungs. Ten viruses were isolated and their genetic analysis showed that all clustered with the first detected virus 0/swine/Oklahoma/1334/2011. Serology data showed that all the tested farms from Mantua province were positive to IDV, with an animal prevalence of 92.4%. The statistical analysis performed on serological data from diagnostics showed that the prevalence in samples from

symptomatic farms was significantly higher than in sera from asymptomatic farms, 66.7% vs. 52.4% respectively. Conclusion: IDV circulates actively among Italian bovine population. The upper respiratory tract seems to be the election site for virus detection, but further investigation is needed to monitor its pathogenic role and its epidemiology.

Chiapponi° C, Faccini° S, De\_Mattia° A, Baioni° L, Barbieri° I, Rosignoli° C, Nigrelli° A, Foni° E

**Detection of influenza D virus among swine and cattle, Italy**

Emerg Infect Dis. - Vol. 22 no 2 ( 2016). - p 352-354. - 8 bib ref [Nr. Estr. 7223]

Chiari° M, Calzolari° M, Prosperi° A, Perulli° S, F accin° F, Avisani° D, Cerioli° M, Zanoni° M, Tironi° M, Bertolotti° M; Defilippo° F, Moreno° A, Farioli M, Piatti A, Dottori° M, Lelli° D, Lavazza A

**Surveillance of mosquitoes and selected arthropod-borne viruses in the context of Milan EXPO 2015**

Int J Environ Res Public Health. - Vol. 13 no 7 ( 2016). - no 689 (7 p). - 23 bib ref ( ultimo accesso 06/09/2016 <http://www.mdpi.com/1660-4601/13/7/689> ) [Nr. Estr. 7294]

From 1 May 2015 to 31 October 2015 over 20 million visitors from all over the world visited the Universal Exhibition (EXPO) hosted by Milan (Lombardy region, Italy), raising concerns about the possible introduction of mosquito-borne diseases from endemic countries. The entomological surveillance protocol performed in Lombardy over the last three years was implemented in the EXPO area and in the two major regional airports using both Center for Disease Control CO2 and Biogents Sentinel traps. This surveillance aimed to estimate the presence and densities of putative vectors, and also to support investigations, including the vector species involved and area of diffusion, on the local spread of Chikungunya, Dengue and West Nile viruses (WNV) by competent vectors. From 3544 mosquitoes belonging to five different species, 28 pools of *Culex* spp. and 45 pools of *Aedes* spp. were screened for the presence of WNV, and for both Chikungunya and flaviviruses, respectively. The entomological surveillance highlighted a low density of potential vectors in the surveyed areas and did not reveal the presence of Chikungunya or Dengue viruses in the local competent vectors inside the EXPO area or in the two airports. In addition, the surveillance reported a low density of *Culex* spp. mosquitoes, which all tested negative for WNV.

Chiari° M, Ferrari° N, Giardiello° D, Avisani° D, P acciarini° ML, Alborali° L, Zanoni° M, Boniotti° MB

**Spatiotemporal and ecological patterns of Mycobacterium microti infection in wild boar (Sus scrofa)**

Transbound Emerg Dis. - Vol. 63 no 5 ( 2016). - p e381-e388. - 46 bib ref [Nr. Estr. 7356]

*Mycobacterium microti* has recently been described as the causative agent of tuberculosis-like lesions in wild boar (*Sus scrofa*), a reservoir specie of *Mycobacterium tuberculosis* complex (MTBC) in some European Mediterranean ecosystem. Through a five-year survey on tuberculosis in free-living wild boars, the epidemiological trend of *M. microti* infections and the host and population risk factors linked with its occurrence were described. Retropharyngeal and mandibular lymph nodes of 3041 hunted wild boars from six different districts were macroscopically inspected. The sex and age of each animal were registered, as well as the animal abundance in each district. Lesions compatible with tuberculosis (190) were collected and analysed using a *gyrB* PCR-RFLP assay. *M. microti* was identified directly in 99 tissue samples (Prev = 3.26%; 95% CI: 2.67–3.97%), while neither *Mycobacterium bovis*, nor other members of the MTBC were detected. The probability of

being *M. microti* positive showed spatio-temporal variability, with 26% of increase of risk of being infected for each year. Moreover, a positive effect of wild boar abundance and age on the prevalence was detected. The generalized increase in the European wild boar population, coupled with its sensitivity to *M. microti* infection, poses a future concern for the identification and management of MTBC members in wild boar.

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

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

Atti del III Congresso nazionale fauna problematica : 24-26 novembre 2016 Cesena (FC) : riassunti delle comunicazioni orali e dei poster / [s.l. : s.n., 2016]. - p 69-70 [Nr. Estr. 7440]

Congresso nazionale fauna problematica (3. : Cesena (FC) : 24-26 novembre 2016)

The life cycle of Taeniids represents an example of multi-host system. The complexity of these parasites can therefore cover the epidemiological issues of the interface wild-domestic animals, especially once spatial overlap between wild and domestic definitive and intermediate hosts occurs. In particular, Taeniids are a family of tapeworms and the adult parasite infects the small intestines of the definitive host, characteristically carnivores, while the larval stage (*Cysticercus*) occurs in the musculature, lung, liver, brain, etc. (extraintestinal sites) of herbivores, that are the intermediate hosts. Definitive hosts become infected through a predator-prey relationship while the foraging on pasture contaminated with eggs of this parasite, shed through definitive host faeces, is the primary cause of infection in the intermediate hosts. Two adult female roe deer (*Capreolus capreolus*), hunted in March 2015 and March 2016, respectively, in apparently good body condition showed numerous oval, white cysts of approximately two to four mm in diameter in the muscles of their whole bodies. In July 2015 an adult tapeworm was observed in the faeces of a semi-stray dog (*Canis lupus familiaris*). These samples came from two contiguous areas in Italian Northern Apennines, where roe deer is the most abundant among wild ruminants with a density of 18 - 20 subjects/100 ha in area 1 and 2, respectively; recently, re-established populations of wolves (*Canis lupus*) are present in both these areas. Following the field detection of parasites, adult tapeworm and cysts (from both thigh and back muscles) were collected and stored at 4°C for successive examinations. In laboratory, both adult and larval stages were analysed by visual examination under both optical and dissecting microscope. The DNA of both adult tapeworm and cysts was extracted using a commercial kit and a fragment of 450 bp of the mitochondrial cytochrome c oxidase I (*coxI*) gene was amplified with specific primers JB3 (5'TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4.5 (5'TAAAGAAAGAACATAATGAAAATG-3'). Sequencing was performed in an ABI Prism 3130 genetic analyser. *Cysticerci* and adult tapeworm of *Taenia ovis krabbei* were identified. *T. krabbei* cysticercosis was recorded for the first time in Italy supporting the role of roe deer as intermediate host of this parasite in Italian territory. Although the role of dogs in the parasite's life cycle emerges, the overlap between wild and domestic definitive hosts of the study area raises concerns about the temporal (old undetected or new) introduction and the spread of this parasite by one of the canid species (wolf or dog). Indeed, although wolf has been reported as original definitive host of *T. krabbei*, domestic populations 'at risk' (i.e. hunting dogs, shepherd dogs) can be infected and contribute to spreading the infection. Although *T. krabbei* is not a public health issue, economic concerns for hunters and meat producers related to the cysticerci damage of carcasses emerged. Therefore, the need is to evaluate the spread of *T. krabbei* in the intermediate and definitive host populations and to plan a proper sanitary education for hunters in order to avoid practices that could favour the maintenance of its life cycle.

Chiari°M, Molinari°S, Cavadini°P, Bertasi°B, Capucci°L, Lavazza°A

**Praying hares is the sole method used by carnivores for causing their death?**

5th World Lagomorph Conference : Turlock, California, USA, July 11-15, 2016 : proceedings / edited by Patrick Kelly... [et al.] organized by California State University Stanislaus, Lagomorph Specialist Group, World Lagomorph Society. - [Turlock : California State University Stanislaus], 2016. - p 82 [Nr. Estr. 7320]

World Lagomorph Conference (5th : Turlock, California, USA : July 11-15, 2016)

Carnivores come into contact with many infectious agents due to their position at the top of the food chain. Thus, they could be potential carriers of infectious agents that occur in their prey species, even if they are not susceptible and not infected and do not develop clinical signs. This is the case of lagoviruses, which are specific agents of severe disease in rabbits (Rabbit Hemorrhagic Disease RHD) and hares (European brown hare syndrome-EBHS). EBHS is a highly contagious disease of brown hares (*Lepus europaeus*) and, with lower frequency, of mountain hare (*Lepus timidus*) and Italian hare *Lepus corsicanus*). When introduced into a naive brown hare population, EBHSV achieves almost 100% morbidity. Mortality is about 50% in the adult age class but absent in young individuals <2-3 months of age, which become infected, do not exhibit clinical signs but seroconvert developing a long-lasting immunity. The virus is considered endemic in all European countries, forcing the adoption of surveillance programs for controlling the diffusion of the disease and the dynamics of hare populations. During the annual serological surveillance of hares captured for restocking in a protected open area, managed as 'breeding-for-restocking' ground, one dead hare was found, collected and examined. Few days after, four red foxes were hunted in close proximity of the same area and then delivered to IZSLER for laboratory investigations, according to the Regional program for wildlife monitoring. At necropsy the typical lesions suggestive of EBHS were found in the hare carcass i.e. petechial lung haemorrhages, friable, fatty and discolored liver, splenomegaly. The diagnosis of EBHSV was then confirmed in the liver and spleen homogenates by sandwich ELISA and RT-PCR. All the hare sera sampled during the capture operation tested positive in serological c-ELISA with medium-high titers, thus indicating an active circulation of EBHSV in that area. Thereafter, in order to elucidate the epidemiological role of predators and to confirm previous experimental data indicating the possibility that carnivores, after having predated diseased or dead lagomorphs, can excrete infectious lagoviruses with feces, we tested by RT-PCR for the presence of EBHSV the liver, spleen, mesenteric lymph nodes and contents of different part of the gut (duodenum, cecum and rectum) of the four red foxes. The intestinal content of one fox resulted virologically positive, whereas the other organs and all samples of the remaining three foxes resulted negative. Among the food debris present in the gastrointestinal contents of the positive fox we found some materials that were genetically identified as of hare origin. The 4 foxes were also serologically tested for EBHSV antibodies by c-ELISA with negative results. The identified EBHSV strains from hare and red fox were amplified, sequenced and compared. Partial sequence of VP60 gene amplified from the positive fox, showed a nucleotide identity of 96% compared to the Italian reference strain EBHSV\_BS89 (X98002). These results proved, in natural condition, the possible epidemiological role of carnivores as passive vectors of EBHSV. In particular, red fox feeding on infected hares might contribute to spread infectious viral particles, thus promoting the persistence and occurrence of EBHS cases.

Chiari°M, Molinari°S, Cavadini°P, Bertasi°B, Lavazza°A, Capucci°L

### **Preying hares is the sole method used by carnivores for causing their death?**

Hystrix Ital J Mamm. - Vol. 27 Suppl ( 2016). - p 87 [Nr. Estr. 7258]

Congresso Italiano di Teriologia (10. : Acquapendente (VT) : 20-23 Aprile 2016)

Carnivores come into contact with many infectious agents due to their position at the top of the food chain. Thus, they could be potential carriers of some infectious agents that occur in their prey species even if they are not susceptible, and are not infected nor develop clinical signs. This is the case of lagoviruses, which are specific agents of severe disease in rabbit and hares respectively Rabbit Hemorrhagic Disease (RHD) and European brown hare syndrome (EBHS). EBHS is a highly contagious disease of brown hares (*Lepus europaeus*) and, with lower frequency, of other hare species (*Lepus timidus* and *Lepus corsicanus*). When introduced into a naive brown hare population, EBHSV infection achieves almost 100% morbidity. Mortality is about 50% in the adult age class but

absent in young individuals less than about 2– 3 months of age. These young individuals when come in contact with the virus, become infected, do not exhibit any clinical signs but seroconvert developing a long-lasting immunity with low/medium antibody titers. The virus is considered endemic in all European countries including Italy, forcing the adoption of surveillance programs in order to control the diffusion of the disease and the dynamics of hare populations. During the annual serological surveillance of hares captured for restocking in a protected but open (i.e. not fenced) area, managed as “breeding for restocking” ground, one dead hare was found, collected and conferred to IZSLER for laboratory examination. Few days after, four red foxes were hunted in close proximity of the same area and then delivered to IZSLER for laboratory investigations, according to the Regional program for wildlife monitoring. At necropsy the typical lesions suggestive of EBHS were found in the hare carcass i.e. petechial lung haemorrhages, friable, fatty and discolored liver with an accentuated lobular pattern. The diagnosis was then confirmed by detecting positive results for EBHSV in the liver and spleen homogenates by both sandwich ELISA and RT-PCR. All the hare sera sampled during the capture operation tested positive in serological c-ELISA with high titers. This result is indicative of an active circulation of EBHSV in that area. Thereafter, in order to elucidate the epidemiological role of predators and to confirm previous experimental data indicating the possibility that carnivores, after having predated diseased or dead lagomorphs, can excrete infectious lagoviruses with feces, we examined by RT-PCR for the presence of EBHSV the liver, spleen, mesenteric lymph nodes and intestinal contents (collected from the duodenum, cecum and rectum tract) of the four red foxes. The intestinal content of one fox resulted virologically positive, whereas the other organs and all the viscera of the remaining three foxes resulted negative. Among the food debris present in the gastrointestinal contents of the positive fox we found the presence of materials genetically identified as of hare origin. The 4 foxes were also serologically tested for EBHSV antibodies by c-ELISA with negative results. The identified EBHSV strains from both species were amplified, sequenced and compared. Partial sequence of VP60 gene amplified from the positive fox, showed a nucleotide identity of 96% compared to the Italian reference strain EBHSV\_BS89 (X98002). These results proved, for the first time in natural condition, the possible epidemiological role of carnivores as passive vectors of EBHSV. In particular, red fox feeding on infected hares might contribute to spread infectious viral particles, thus promoting the persistence and occurrence of EBHS cases. However, further studies are needed to verify the impact of this specific epidemiological role of the red fox in the complex epidemiology of EBHSV.

Chiari°M, Molinari°S, Cavadini°P, Bertasi°B, Zanoni°M, Capucci°L, Lavazza°A

**Red foxes (*Vulpes vulpes*) feeding brown hares (*Lepus europaeus*) infected by European brown hare syndrome virus (EBHSV) might be involved in the spread of the virus**

Eur J Wildl Res. - Vol. 62 ( 2016). - p 761-765. - 23 bib ref ( ultimo accesso 03/01/2017  
<http://rd.springer.com/article/10.1007%2Fs10344-016-1055-4> ) [Nr. Estr. 7397]

Carnivores are potential carriers of agents that infect their prey species, even though they themselves are not susceptible, such as the lagovirus that causes European brown hare syndrome (EBHS), a severe disease of brown hares endemic in Europe. During our wildlife surveillance in Lombardy, we identified an EBHS outbreak in a protected area by both virological analyses (sandwich ELISA and RT-PCR) of the target organs from one dead hare and serological examinations (competitive ELISA) of captured animals. Since four red foxes were contemporarily hunted in the same area, we examined their organs by RT-PCR for the EBHS agent (EBHSV). The intestinal content of one fox tested positive, while the fox' other organs (liver, spleen, and mesenteric lymph nodes), and all of the samples from the remaining three foxes, tested negative. Moreover, in the gastrointestinal content of the positive fox, we found food debris that was genetically identified as being of hare origin. The competitive ELISA test for EBHSV antibodies gave negative results in all of the fox sera. Genetic analyses of the EBHSV amplicons obtained by RT-PCR in the hare and the fox indicated a full homology (99.9 % nucleotide and 100 % amino acid identity). These results support the fact that red fox, as other predators, feeding on EBHSV infected hares may have genetic prints of the virus in their gut contents. Even if we did not prove that lagovirus particles remained infective in the excreted feces and, thus, contaminated the ground in the outbreak area, these eventualities cannot be excluded, and we could at least conclude that red foxes might assume a potential role in the indirect transmission of lagovirus, as EBHSV.

Chiari°M, Molinari°S, Cavadini°P, Bertasi°B, Zanoni°M, Capucci°L, Lavazza°A

**Red foxes (*Vulpes vulpes*) feeding brown hares (*Lepus europaeus*) infected by European brown hare syndrome virus (EBHSV) might be involved in the spread of the virus?**

Atti del III Congresso nazionale fauna problematica : 24-26 novembre 2016 Cesena (FC) : riassunti delle comunicazioni orali e dei poster / [s.l. : s.n., 2016]. - p 25-26 [Nr. Estr. 7441]

Congresso nazionale fauna problematica (3. : Cesena (FC) : 24-26 novembre 2016)

Generalist predators are at the top of the food chain and they can come in contact with many micro and macro parasites that occur in their prey species. Thus, they could be considered both potential carriers and sentinel of these infectious agents, even if they are not susceptible species, without developing both infection and clinical signs. This is the case of European brown hare syndrome (EBHS), a lagovirus endemic in Europe that causes severe disease in brown hares. During the annual wildlife surveillance in force in Lombardy, we identified an outbreak of EBHS in a protect area by both serological examination (c-ELISA) on captured animals and virological analyses (sandwich ELISA and RT-PCR) on target organs of one dead hare. In addition, we examined by RT-PCR for EBHSV the liver, spleen, mesenteric lymph nodes and three intestinal tracks of four red foxes hunted in the same area. The intestinal content of one fox resulted positive, whereas its other organs and all the viscera of the remaining three foxes resulted negative. Moreover, among the food debris, present in the gastrointestinal contents of the positive fox, we found materials genetically identified as of hare origin. Then, we serologically tested the foxes for EBHSV antibodies by c-ELISA with negative results. The nucleotide identity of the EBHSV strains isolated in the hare and the fox was 99.9%, and the amino acid identity was 100%, indicating the presence of the same viral strain in the two species. Our results proved, for the first time in natural condition, the possible epidemiological role of carnivores as passive vectors of EBHSV. It should be underlined that the course of EBHS is often acute and death suddenly occurs within few days, without clear clinical symptoms or only behavioural changes that make the hares more vulnerable. Therefore, during one outbreak of EBHS, foxes, as generalist predators, may consume carcasses of death hares due to this disease, as well as infected animals that became easy prey. This situation is certainly realistic in the North Italy area since the density population of foxes is known to be high and lagomorphs are considered an important food resource for them, even if consumption is largely determined by their local abundance and availability. As a consequence, it is theoretically possible that foxes that have consumed EBHS-infected hares may carry the virus spreading it through their droppings. Nevertheless, the capability of transmission of the disease could be finally demonstrated only by experimental infection of negative hares using such faecal material as inoculum. If the elimination of infectious EBHSV virions by red foxes in field condition will be shown, similarly to what was experimentally demonstrated in dogs for RHDv, red foxes could be definitively enrolled as contributors to the maintenance of EBHSV in the environment without the intervention of the defined reservoir species, thereby supporting its endemicity and geographical spread among different hare populations. In conclusion, since predatory activities and EBHS are considered two main causes of the hare population's decline in Europe, the present data could contribute to improve the understanding of EBHS epidemiology and particularly its mechanisms of diffusion by supporting the hypothesis that red fox preying and feeding with EBHS-positive hares can passively carry and eliminate EBHSV virions with their feces, and as consequence they can induce the death of hares not only preying them.

Chiari°M, Molinari°S, Cavadini°P, Bertasi°B, Zanoni°MG, Capucci°L, Lavazza°A

**Preying brown hares (*Lepus europaeus*) is the sole method used by red fox (*Vulpes vulpes*) for causing their death?**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) :

August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 110 (Poster no 22) [Nr. Estr. 7333]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

Generalist predators are at the top of the food chain and they can come in contact with many micro and macro parasites that occur in their prey species. Thus, they could be considered both potential carriers and sentinel of these infectious agents, even if they are not susceptible species, without developing both infection and clinical signs. This is the case of European brown hare syndrome (EBHS), a lagovirus endemic in Europe that causes severe disease in brown hares. During the annual wildlife surveillance in force in Lombardy, we identified an outbreak of EBHS in a protect area by both serological examination (c-ELISA) on captured animals and virological analyses (sandwich ELISA and RT-PCR) on target organs of one dead hare. In addition, we examined by RT-PCR for EBHSV the liver, spleen, mesenteric lymph nodes and three intestinal tracks of four red foxes hunted in the same area. The intestinal content of one fox resulted positive, whereas its other organs and all the viscera of the remaining three foxes resulted negative. Moreover, among the food debris, present in the gastrointestinal contents of the positive fox, we found materials genetically identified as of hare origin. Then, we serologically tested the foxes for EBHSV antibodies by c-ELISA with negative results. The nucleotide identity of the EBHSV strains isolated in the hare and the fox was 99.9 %, and the amino acid identity was 100 %, indicating the presence of the same viral strain in the two species. These results proved, for the first time in natural condition, the possible epidemiological role of carnivores as passive vectors of EBHSV. In particular, asymptomatic foxes that have consumed EBHS-infected hares can carry the virus and spread it with their droppings, maintaining active the viral circulation without the contribution of the defined reservoir species. Predatory activity and EBHS are considered two of the main causes of the hare population decline in Europe. The evidence of the concrete possibility that red fox can be a "mechanical" carrier of EBHSV contributes to improve the comprehension of the epidemiology of EBHS and to specify that foxes can induce the death of hares not only preying them.

Chiari°M, Prosperi°A, Avisani°D, Zanoni°M, Lelli°D, Perulli°S, Moreno°A, Dottori°M, Lavazza°A

#### **The integrate West Nile Disease surveillance in Lombardy region, Italy**

Atti 8° Congresso nazionale societa italiana di medicina tropicale e salute globale (SIMET) : 21 Ottobre 2016 Brescia / [s.l. : Societa Italiana di Medicina Tropicale e Salute Globale (SIMET), 2016]. - (Quaderni della societa italiana di medicina tropicale e salute globale ; 2) p 3 [Nr. Estr. 7428]

Congresso nazionale societa italiana di medicina tropicale e salute globale (SIMET) (8. : Brescia : 21 Ottobre 2016)

Introduction In 2013, the circulation of West Nile virus (WNV) was detected in Lombardy and in the following years (2014- 2015) a more targeted surveillance program was activated with the aim to early detect WNV circulation in mosquitoes and wild birds before the occurrence of disease in humans. In addition, a specific survey in the context of EXPO 2015 was carried out. Materials and Methods The Lombardy plan territory was split into square areas of 20 Km<sup>2</sup> and one CO<sub>2</sub>-CDC trap was placed in each square in order to performed night trap sessions every 15 days from May to September. In addition, monitoring of hunted wild birds was spatially and temporally distributed, according to the dimension of each province and monthly captures from April to October were carried out. After identification, Culex spp. mosquitoes were divided in 628 pools of maximum of 100 specimens each. Organs (brain, spleen, heart, kidneys) from each bird were taken at necropsy and pooled. The samples were subjected to two real-time PCRs for the detection of WNV and Usutu virus and a screening PCR for the presence of flaviviruses, which targeted 250 nucleotides of the NS5 gene conserved region. The obtained amplicons were sequenced for virus identification. Results More than 100,000 Culex spp., divided in 628 pools, and 2800 wild birds were tested. The WNV was detected in 30 pools of Culex spp. and in 23 birds. This surveillance was able to detect the viral circulation at a minimum of two weeks before the occurrence of onset of human clinical cases. As recommended by the Ministry of Health, human blood transfusion tests for WND

(NAT-PCR) started in each province after the notification of WNV circulation detected by the integrated surveillance system. Conclusions The results demonstrated the endemic presence of WNV in Lombardy and confirmed the entomological and wild birds surveillance as an effective measure for the early identification of WNV circulation in infected areas, thus providing a useful and cost-effective tool for the public health authorities starting the human blood transfusion tests after the notification of WNV circulation and in the application of measures to prevent human infection and the blood.

Chiari<sup>o</sup>M, Prosperi<sup>o</sup>A, Faccin<sup>o</sup>F, Zanoni<sup>o</sup>M, Moren<sup>o</sup> A, Farioli M, Lelli<sup>o</sup>D, Sozzi<sup>o</sup>E, Lavazza<sup>o</sup>A

#### **The integrate West Nile Disease surveillance in Lombardy region, Italy**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 25 [Nr. Estr. 7330]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

In 2013, the circulation of West Nile virus (WNV) was detected in Lombardy and between 2014 and 2015 a surveillance programme was activated with the aim to detect WNV circulation in mosquitoes and wild birds as early as possible and before the occurrence of human cases. We present the results of the integrate WND surveillance adopted in Lombardy, based on both entomological and wild birds screening. The plan territory of Lombardy was split into square areas of 20 km<sup>2</sup> and one CO<sub>2</sub>-CDC trap was placed in each square for performing night trap sessions every 15 days from May to September. In addition, the monitoring of hunted wild birds (*Pica pica*, *Corvus corone cornix*, *Garrulus glandarius*) was performed and distributed either spatially, according to the size of each province, and temporally from April to October. After identification, *Culex* spp. mosquitoes were divided in pools, each of 100 specimens maximum. Organs (brain, spleen, heart, kidneys) from each bird were taken at necropsy and pooled. The samples were subjected to two distinct real-time PCR protocols for the detection of WNV and USUV respectively and to a screening PCR for the presence of flaviviruses, which targeted 250 nucleotides of the conserved region of the NS5 gene. The obtained amplicons were sequenced for virus identification. More than 100.000 *Culex* spp., divided in 628 pools, and 2,800 wild birds were tested in 2014 and 2015. The virus were detected in 30 pools of *Culex* spp. and in 23 wild birds. This WNV surveillance system precisely identified the areas and provinces affected by the virus and detected the viral circulation at least two weeks before the occurrence of onset of human cases. For this reason, as recommended by the Ministry of Health, human blood transfusion tests for WND (NAT-PCR) started immediately after the notification of WNV circulation detected by the integrated surveillance system. Note that the average time taken from sampling to getting the final results was less than five days. The capability of detection of WNV through mosquitoes and birds surveillance programmes is coherent with the One health approach and gives benefits in terms of cost-effectiveness and public health safety. In particular, it enables a more targeted blood unit testing strategy and reduces the probability of virus transmission via blood and organ donation systems, totally in line with a One Health methodology. Considering the endemic presence of WNV in the Lombardy, the entomological and wild birds surveillance represents an effective support for the early identification of WNV circulation, thus providing a useful tool for the public health authorities in the application of measures to prevent human infection.

Consoli<sup>o</sup>M, Faccin<sup>o</sup>F, Sozzi<sup>o</sup>E, Lelli<sup>o</sup>D, Lavazza<sup>o</sup>A, Piccirillo A, Moreno<sup>o</sup>A

#### **Tipizzazione del virus della laringotracheite infettiva aviare tramite protocolli di End-point PCR**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 180-181. - 4

bib ref [Nr. Estr. 7497]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

Infectious laryngotracheitis (ILT) is an acute highly contagious respiratory disease of chickens caused by Gallid Herpesvirus 1. The disease is controlled by vaccination with 2 types of live attenuated vaccines, which are produced by sequential passages in cell cultures (tissue culture origin, TCO) or embryonated eggs (chicken embryo origin, CEO). Strong evidence indicate that ILTV epizootics may originate from CEO-derived strains, which can regain virulence and persist in the field. We report the development of 3 new PCR methods, able to differentiate vaccine and CEO related field strains, targeting ORFE, ORFD and UL36 genes. Two profiles were identified: one typical of CEO vaccines (no insertion G R K) and the other related to field strains (insertion A H R). We tested 89 ILTV PCR positive samples collected from broilers, capons, layers and broiler breeders during the period 2004-2016, and identified two distinct profiles (CEO vaccine and field profiles) without observe mixed forms.

Cortimiglia<sup>°</sup>C, Luini<sup>°</sup>M, Bianchini<sup>°</sup>V, Marzagalli<sup>°</sup> L, Vezzoli<sup>°</sup>F, Avisani<sup>°</sup>D, Bertoletti<sup>°</sup>M, Ianzano A, Franco A, Battisti A

**Prevalence of Staphylococcus aureus and of methicillin-resistant S. aureus clonal complexes in bulk tank milk from dairy cattle herds in Lombardy Region (Northern Italy)**

Epidemiol Infect. - Vol. 144 no 14 ( 2016). - p 3046-3051. - 12 bib ref [Nr. Estr. 7321]

Staphylococcus aureus is the most important causative agent of subclinical mastitis in cattle resulting in reduced milk production and quality. Methicillin-resistant S. aureus (MRSA) strains has a clear zoonotic relevance, especially in the case of occupational exposure. The aim of the study was to evaluate the prevalence of S. aureus and MRSA in bulk tank milk (BTM) from dairy cattle herds in the Lombardy Region (Northern Italy) and to identify the main MRSA circulating genotypes. MRSA strains were characterized by susceptibility testing, multi-locus sequence typing (MLST), spa typing and SCCmec typing. A total 844 BTM samples were analysed and S. aureus and MRSA were detected in 47.2% and 3.8% of dairy herds, respectively. MLST showed that the majority (28/32) of isolates belonged to the typical livestock-associated lineages: ST398, ST97 and ST1. Interestingly, in this study we report for the first time the new ST3211, a single locus variant of ST(CC)22, with the newly described 462 aroE allele. Our study indicates high diffusion of S. aureus mastitis and low, but not negligible, prevalence of MRSA in the considered area, suggesting the need for planning specific control programmes for bovine mastitis caused by S. aureus, especially when MRSA is implicated.

Cosandey A, Boss R, Luini<sup>°</sup>M, Artursson K, Bardiau M, Breitenwieser F, Hehenberger E, Lam Th, Mansfeld M, Michel A, Moesslacher G, Naskova J, Nelson S, Podpecan O, Raemy A, Ryan E, Salat O, Zangerl P, Steiner A, Graber HU

**Staphylococcus aureus genotype B and other genotypes isolated from cow milk in European countries**

J Dairy Sci. - Vol. 99 no 1 ( 2016). - p 529-540. - 26 bib ref [Nr. Estr. 7170]

Staphylococcus aureus is globally one of the most important pathogens causing contagious mastitis in cattle. Previous studies, however, have demonstrated in Swiss cows that Staph. aureus isolated from bovine intramammary infection is genetically heterogeneous, with Staph. aureus genotype B (GTB) and GTC being the most prominent genotypes. In addition, Staph. aureus GTB was found to be contagious, whereas Staph. aureus GTC and all the remaining genotypes were involved in individual cow disease. The aim of this study was to subtype strains of Staph. aureus isolated from bovine mastitic milk and bulk tank milk to obtain a unified view of the presence of bovine

staphylococcal subtypes in 12 European countries. A total of 456 strains of Staph. aureus were subjected to different typing methods: ribosomal spacer PCR, detection of enterotoxin genes, and detection of gene polymorphisms (lukE, coa). Major genotypes with their variants were combined into genotypic clusters (CL). This study revealed 5 major CL representing 76% of all strains and comprised CLB, CLC, CLF, CLI, and CLR. The clusters were characterized by the same genetic properties as the Swiss isolates, demonstrating high clonality of bovine Staph. aureus. Interestingly, CLB was situated in central Europe whereas the other CL were widely disseminated. The remaining 24% of the strains comprised 41 genotypes and variants, some of which (GTAM, GTBG) were restricted to certain countries; many others, however, were observed only once.

D'incau°M, Grassi°A, Giovannini°S, Salogni°C, Zanoni°M, Ruggeri°J, Pasquali P, Alborali°GL

### **Resistenza agli antimicrobici di ceppi di Salmonella typhimurium e della sua variante monofasica isolati da suini in accrescimento**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 115-119. - 13 bib ref [Nr. Estr. 7233]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Da indagini batteriologiche, eseguite nel periodo 2012 — 2014, su campioni di feci, visceri, linfonodi e carcasse di suino sono stati isolati 245 ceppi di Salmonella typhimurium di cui 202 appartenenti alla sua variante monofasica. Per tutti i ceppi 6 stata valutata la sensibilita in vitro agli antimicrobici con il metodo Kirby — Bauer. I risultati ottenuti mettono in evidenza il tipo fondamentale di multiresistenza gia riscontrato in precedenti lavori (amoxicillina, tetraciclina, streptomycina e sulfonamidi). Inoltre, il 24,49% degli isolati 6 risultato resistente a 10 o pia molecole contemporaneamente.

*245 strains of Salmonella typhimurium (202 belonging to its monophasic variant) were isolated in the period 2012 - 2014, from fecal samples, viscera, lymph nodes and pig carcasses. All strains were tested in vitro for antimicrobial susceptibility by Kirby — Bauer method. The results highlight the fundamental type of multidrug resistance showed in previous works (amoxicillin, tetracycline, streptomycin, sulfonamides). Furthermore, 24,49% of the isolates were resistant to 10 or more antibiotics.*

De\_Prado A, Appiani A, Velthuis A, Bertocchi°L, Be cvar O, Davidek J, Bay D, Le\_Page P, Holstege M, Veenkamp A, Van\_Werven T, Dalez B, Paduch JH, Kromker K, Piepers S, Schukken Y, Jimenez L

### **An investigation of the incidence of milk leakage after dry-off in commercial dairy herds around Europe**

Proceedings of the 29th World Buiatrics Congress 2016 : Theme "Cattle Health - Tomorrow's Thinking Today" : Dublin, Ireland 2016 : oral communication and poster abstracts / [s.l. : s.n., 2016]. - p 667-668 [Nr. Estr. 7481]

World Buiatrics Congress (WBC) (29th : Dublin, Ireland : 2016)

Objectives: The objective of the study was to investigate the incidence of cows leaking milk during the first two days post dry-off on commercial dairy farms around Europe and to investigate the relationship between milk leakage and milk production. Materials and Methods: This study was carried out in commercial dairy farms in France, Germany, Italy, The Netherlands, Belgium, Spain, Czech Republic and Denmark. A total of 1,142 cows from 41 farms were included in the study. All cows had a satisfactory general health based on a physical examination including clinical mastitis, four functional quarters and were not treated with internal or external teat seal. Milk leakage was observed at three visits (V): between 20 to 24 hours (V1), 30 to 34 hours (V2) and 48 to 52 hours (V3) after the dry-off. Cows had stand in headlocks and were observed twice per visit for 30

seconds each. Milk leakage was considered to be present when a stream of milk was coming from a teat, when a milk drop on the teat was present or when milk was present on the ground under the udder. Milk production data during the last 24 hours before dry-off was obtained from the records on the milking system. The individual cow was the experimental unit, whereas the quarter was the data collection unit. Generalized linear models with random effects for farm were used to test the relation between milk leakage and production. Results: Milk leakage within two days after dry-off was observed in 279 out of the 1142 cows, resulting in a mean milk leakage incidence per farm of 24.4% (95% CI: 21.9% - 26.9%). Cows with observed milk leakage, had on average 1.6 leaking teats (95% CI: 1.56 - 1.66). In 55.3% of the cows leaking milk one quarter was leaking, in 32.3% two quarters, in 8.3% three quarters and in 4.1% four quarters. Rear quarters turned out to have 4.3 times as many odds on milk leakage than front quarters (95% CI: 3.3 - 5.7). Most milk leakage (on average 30,1% of the cows per farm) was observed between 30 and 34 hours after dry-off compared to on average 14,7% of the cows per farm between 20 to 24 hours and 17,2% between 48 to 52 hours, respectively. The last recorded yield 24h before dry-off was significantly associated with milk leaking. Cows with a higher last recorded yield had 2.6 (95% CI: 1.61 - 4.27) higher odds on milk leakage than cows with a lower last recorded yield, even though the association was not completely linear. Conclusions: Results show that the incidence of milk leakage in these European dairy farms is 24.4% average. Due to the link between the cow milk production the last day before dry-off and the incidence of milk leakage after dry-off, the data indicate that higher milk production at dry-off is a predictor of milk leaking after dry off.

De\_Prado AI, Velthuis A, Bertocchi<sup>o</sup>L, Appiani A, B ecvar O, Davidek J, Bay D, Jimenez L, Kromker K, Paduch JH, Piepers S, Van\_Werven T, Veenkamp A, Dalez B, Le\_Page P, Holstege M, Schukken Y

**Relationship between milk leakage the first two days after dry-off and new intramammary infection around the dry period and at calving in commercial dairy herds around Europe**

IDF FIL Mastitis Conference : 7-9 September 2016 Nantes, France / [s.l. : s.n., 2016]. - 1 p [Nr. Estr. 7468]

IDF FIL Mastitis Conference : Nantes, France : 7-9 September 2016)

Delvecchio A, Tosi<sup>o</sup>G, Gandon N, Gauthier S, Prandi ni F, Herrmann A, Lemiere S

**Evaluation of in vivo replication of a Newcastle disease live attenuated vaccine strain in commercial broiler chicks : a comparison of drinking-water, spray and eye drop vaccination methods**

American Association Of Avian Pathologists (AAAP) Symposium : "Emerging and reemerging zoonotic disease" : August 6-9, 2016 San Antonio, TX / [s.l. : s.n., 2016]. - 2 p [Nr. Estr. 7463]

American Association of Avian Pathologists (AAAP) Symposium : San Antonio, TX : August 6-9, 2016)

Newcastle disease virus (NDV) is one of the most important infectious agents in the poultry industry causing huge economic losses worldwide. Vaccination in endemic countries is widely used in order to keep NDV controlled. Several live attenuated vaccines are available on the market and the efficacy of these vaccines has to be tested in laboratory conditions in order to validate their use in the field. In the present studies a commercial live attenuated vaccine against ND (VG/VA AVINEW) was administered in commercial broiler chicks by spray, drinking water and eye-drop under controlled conditions. Target organs for vaccine virus replication were collected at different days post vaccination (DPV); the vaccine virus was recovered by RT-PCR. The detection of the NDV vaccine was compared between the different routes of administration at different ages. The NDV vaccine replication patterns were correlated with validation of vaccine virus tropism, respiratory and digestive, whatever the route of administration. As already described in the literature for IB and aMPV live attenuated vaccines, replication patterns of eye drop or drinking water routes were slightly different from the spray.

Dezfuli BS, Zeppellini L, Rubini°S, Manera M, Castaldelli G, Giari L

**Intestinal histopathology due to an acanthocephalan in two corvids species from North Italy**

XXIX Congresso Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College "Parasites, Poverty and Social commitment" : Bari, June 21-24, 2016 / [s.l. : s.n., 2016]. - p 219. - 5 bib ref [Nr. Estr. 7303]

Congresso Nazionale Societa' Italiana di Parassitologia European Veterinary Parasitology College (29. : Bari : June 21-24, 2016)

Di\_Bartolo I, De\_Sabato L, Marata A, Martinelli°N, Magistrali CF, Monini M, Ponterio E, Ostanello F, Ruggeri FM

**Serological survey of hepatitis E virus infection in farmed and pet rabbits in Italy**

Arch Virol. - Vol. 161 ( 2016). - p 1343-1346. - 24 bib ref [Nr. Estr. 7216]

The recent identification in rabbits of hepatitis E viruses (HEV) related to viruses infecting humans raises the question of the role of this species as possible HEV reservoir. A serological survey on rabbit HEV infection was conducted in Italy during 2013-2014, including both farmed and pet rabbits. We found an anti-HEV antibody seroprevalence of 3.40 % in 206 farmed rabbits (collected on 7 farms) and 6.56 % in 122 pets. RNA was extracted from IgG-positive sera and analyzed by HEV-specific realtime RT-PCR. None of the samples were positive, confirming that no viremia was present in the presence of IgG. Only one serum sample from a farmed rabbit was positive for IgM, but no HEV RNA was detected in it. Pet rabbit feces were also tested for HEV RNA, with negative results. This finding suggests that HEV is circulating in rabbits in Italy.

Dore S, Liciardi M, Amatiste S, Bergagna S, Bolzoni°G, Caligiuri V, Cerrone A, Farina G, Montagna CO, Saletti MA, Scatassa ML, Sotgiu G, Cannas EA

**Survey on small ruminant bacterial mastitis in Italy, 2013–2014**

Small Rumin Res. - Vol. 141 ( 2016). - p 91-93. - 11 bib ref [Nr. Estr. 7486]

Mastitis is the most important disease of dairy small ruminants affecting animal welfare, agricultural economy, and food safety. Only a few investigations on the bacterial epidemiology of udder infections have been performed. Aim of the study was to describe the Italian epidemiology of bacterial mastitis in small ruminant dairy herds. An ad hoc electronic data collection module was created by the National Reference Center for Sheep and Goat Mastitis (C.Re.N.M.O.C). Public health veterinary laboratories of the Experimental Zooprophyllactic Institutes (EE.ZZ.II) (n = 10) were selected. Nine (90.0%) EE.ZZ.II. participated to the survey and 8 (87.5%) provided a full report. Bacteriological culture results from 30,232 sheep and goat milk samples collected in 1,795 herds between 2013 and 2014 were analyzed. Coagulase-negative staphylococci (CNS) were the most frequently isolated bacteria in dairy sheep and goats, followed by Staphylococcus aureus; other bacterial species were Pseudomonas spp., Streptococcus uberis, Enterobacteriaceae, Enterococcus spp., Streptococcus spp. and Coryneiforms. Italian results confirm previous findings described in other countries; CNS are the most prevalent bacteria, probably due to subclinical symptoms, whereas Staphylococcus aureus is the most prevalent clinical mastitis etiological agent. The present survey, based on the first, Italian standardized data electronic collection focused on small ruminant mastitis, may represent the backbone for future control and preventive strategies nationwide.

Dotti°S, Bilato°D, Visone R, Zanotti°C, Villa°R , Ferrari°M

**Metodi alternativi alla sperimentazione animale : organ on chip e bioreattore**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 31-32. - 3 bib ref [Nr. Estr. 7357]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*A strategy of 3Rs (ie. reduction, refinement and replacement) Is being applied for laboratory use of animals. Different alternative methods ore applied to implement this strategy. In this terms, recently, 3D cell culture models have gained attention because of their great potential level of application not possible with conventional 2D or 3D static culture systems. New different approaches, such as microfluidic cell culture device (organ-on-a-chip) and bioreactor, create cell cultures that allow to study physiology in an organ specific context and enable development of novel in vitro disease mode) and replacement of animals used in different toxin-drugs testing and diagnostic.*

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

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

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 1 p [Nr. Estr. 7314]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Porcine Reproductive and Respiratory Syndrome (PRRS) is an elusive model of host/virus relationship in which disease is determined by virus pathogenicity, pig breed susceptibility and phenotype, microbial infectious pressure and environmental conditions. Successful disease control corresponds to "stability", i.e. a condition with no clinical signs of PRRS in the breeding-herd population and no viremia in weaning-age pigs. The aim of this work was to compare the profile and time-course of humoral and cell-mediated immunity of replacement gilts in one stable and one unstable herd, respectively. In particular, we investigated PRRS virus (PRRSV) in serum and group oral fluid samples by Real-time RT-PCR, PRRSV-specific IgA and IgG in oral fluids, serum IgG antibody and the cell-mediated response (PRRSV-specific release of interferon-gamma) in whole blood samples. These parameters were measured in order to identify possible discrepancies in the development and kinetics of the immune response against PRRSV. Gilts got regularly infected around 7-9 weeks after entering the stable farm, and at the very beginning in the unstable one. Four main results must be highlighted: A) the precocity of the Ab response in group oral fluids was similar to that seen in sera; B) circulation of PRRSV was consistently detected in the unstable herd, as opposed to the stable one; C) a balanced IgA and IgG response in oral fluids was only observed in the stable herd; D) an IFN-gamma response was regularly observed in the stable herd, whereas gilts of the unstable one were partly positive at arrival day, only (transfer of maternal immunity). The above findings indicate that a peculiar profile of immune response to PRRSV underlies herd stability. Therefore, the outlined immune parameters can represent a useful readout system to evaluate successful adaptation to PRRSV based on acclimatization of breeding animals and management of pig flow. In this respect, failure of disease control measures could be traced back to farm management and/or peculiar virus "immunotypes", affecting the immune response of PRRSV-infected animals.

Drumo R, Pesciaroli M, Ruggeri J, Tarantino M, Chirullo B, Pistoia C, Petrucci P, Martinelli°N, Moscati L, Manuali E, Pavone S, Pic ciolini M, Ammendola S, Gabai

G, Battistoni A, Pezzotti G, Alborali°GL, Napolioni V, Pasquali P, Magistrali CF  
**Salmonella enterica Serovar typhimurium exploits inflammation to modify Swine Intestinal Microbiota**

Front Cell Infect Microbiol. - Vol. 5 ( 2016). - Article no. 106 (13 p). - 35 bib ref [Nr. Estr. 7214]

Salmonella enterica serovar Typhimurium is an important zoonotic gastrointestinal pathogen responsible for foodborne disease worldwide. It is a successful enteric pathogen because it has developed virulence strategies allowing it to survive in a highly inflamed intestinal environment exploiting inflammation to overcome colonization resistance provided by intestinal microbiota. In this study, we used piglets featuring an intact microbiota, which naturally develop gastroenteritis, as model for salmonellosis. We compared the effects on the intestinal microbiota induced by a wild type and an attenuated S. Typhimurium in order to evaluate whether the modifications are correlated with the virulence of the strain. This study showed that Salmonella alters microbiota in a virulence-dependent manner. We found that the wild type S. Typhimurium induced inflammation and a reduction of specific protecting microbiota species (SCFA-producing bacteria) normally involved in providing a barrier against pathogens. Both these effects could contribute to impair colonization resistance, increasing the host susceptibility to wild type S. Typhimurium colonization. In contrast, the attenuated S. Typhimurium, which is characterized by a reduced ability to colonize the intestine, and by a very mild inflammatory response, was unable to successfully sustain competition with the microbiota.

Fabbi°M, Vismarra A, Faccini°S, Kramer L, Vicari° N, Prati°P, Mangia C, Marino AM, Rosignoli°C, Rigamonti°S, Zannella°G, Genchi M

**Valutazione del rischio di trasmissione di Toxoplasma gondii attraverso il consumo del "prosciutto di Parma" DOP**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 63-64. - 5 bib ref [Nr. Estr. 7361]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*Toxoplasma gondii is considered one of the most common parasitic infections in the world and its cysts represent an important source of Infection for human being. "Prosciutto di Parma DOP" in a typical Italian park product well known all over the world. Little Information is available concerning the effect of curing and survival of T. gondii cysts in ham. The aim of this study was to evaluate the survival and viability of T. gondii cysts in cured hams processed according to the procedural of the "Prosciutto di Parma" Consortium. Sixteen pigs were infected with sporulated T. gondii oocysts and slaughtered 4 months later. Twelve thighs were cured and 12 were immediately analyzed. After 12 months of seasoning, hams were tested by cell culture and bioassay to evaluate the vitality of the parasite. Data obtained from the cured hams indicated no evidence of viable parasites. This is the first study applied to evaluate the influence of processing of cured ham on the viability of T. gondii.*

Faccini°S, Barbieri°I, Rosignoli°C, Franzini°G, Boniotti°MB, Alborali°GL, Nigrelli°AD

**Genetic characterization of Porcine Circovirus 2 field isolates from Italian farms**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - p 484 [Nr. Estr. 7421]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Introduction: Porcine Circovirus Type 2 (PCV2) is an important pathogen related to several disease syndromes in pigs, collectively named PCVD (PCV disease). PCV2 strains are currently classified into four genotypes: PCV2a, PCV2b, PCV2c and PCV2d. The latter is considered an emergent genotype. It has been, indeed, increasingly isolated worldwide, mainly in cases of suspected vaccine failure, rising concerns about vaccine protection and possible ongoing genetic shift. Materials and Methods: In order to study the circulation of PCV2 strains related to PCVD outbreaks in Italian farms, 46 samples with high viral loads, were completely sequenced. Samples had been conferred, between January 2014 and October 2015, to IZSLER diagnostic laboratories from 35 different herds. All the farms except 2 declared to apply a vaccination program against PCV2, and were recording an increase in clinical cases compatible with PCVD. PCV2 detection and quantification were performed by Real-Time PCR. PCV2 full-length genome sequence was achieved by Sanger method from tissue samples with more than 10 exp 8 genome copies/fl or sera and oral fluids with PCV2 loads higher than 10 exp 6 genome copies/mL. Phylogenetic analysis was accomplished using the distance-based Neighbor-Joining method. Results: PCV2a was found in 4 samples, from 2(5,7%) vaccinated herds. PCV2b was the prevalent genotype and was identified in 24 samples from 21 farms (60%); only one was not vaccinated. The emergent genotype PCV2d, was detected in 15 samples from 10 herds (28,6%); only 1 had suspended the vaccination program. Finally, 3 samples from 2 vaccinated farms (5,7%) had mixed sequences of genotypes PCV2b and PCV2d. Considering the distribution of genotypes over the time, a substantial increase of PCV2d circulation in Italy during 2015 can be observed. Indeed, in 2014, among 21 investigated herds, 2 were infected by PCV2a, 21(85,7%) by PCV2b and only 1 by PCV2d. On the contrary, in 2015 none of the investigated herds had PCV2a, PCV2b was recovered in 3 (21.43%), 2 had a concomitant circulation of PCV2d and PCV2b, while 10 (64.2%) were infected by PCV2d. Conclusion: Data strengthen the hypothesis of PCV2d as an emergent genotype. Besides, the considerable increase in proportion of PCV2d infected herds recorded in this study in 2015, suggests the existence of an ongoing genetic shift between PCV2b and PCV2d. The role of vaccination pressure and natural selection is not clear yet and should be further investigated. Supporting diagnostic data with sequence analysis is extremely important in this context.

Favole A, Mazza M, Vallino Costassa E, D'Angelo A, Martinelli°N, Avanzato T, Grifoni S, Orrù C, Hughson A, Acutis PL, Zanusso G, Caughey B, Casalone C, Corona C

#### **I meccanismi di amiloidogenesi come nuovo approccio al test diagnostici nelle malattie da prione della specie caprina**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 200-201. - 3 bib ref [Nr. Estr. 7369]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*The spread of BSE agent to small ruminants is a major Issue in the surveillance of TSEs since BSE passage Into a new host may change strain properties and make it difficult to recognize the original strain. Two natural BSE cases have been reported in goats. On this basis, the development of a new approach for ante mortem diagnosis of small ruminant TSE strains may be a feasible target that would help to reduce the risk of epidemic spread of goat TSEs. In this study was assessed the use of the RT-QuIC assay for the diagnosis of TSEs in goats. The sensitivity and specificity of this method were evaluated for the detection of prion seeding activity In brain and cerebrospinal fluid from goats infected with natural Scrapie or experimental BSE. RT-QuIC revealed a high sensitivity and specificity to detect prion seeding activity In cerebrospinal fluid from symptomatic goats. These data reveal a great diagnostic potential of this method for the ante mortem diagnosis in small ruminants.*

Fernandez\_Pinero J, Pezzoni°G, Cano\_Comez C, Fernandez-Pacheco P, Brocchi° E, Jimenez\_Clavero MA

## **Swine vesicular disease virus**

Molecular detection of animal viral pathogens / edited by Dongyou Liu. - Boca Raton, FL : CRC Press, 2016. - p 89-100. - 92 bib ref [Nr. Estr. 7653]

Filipe J, Trevisi E, Massara M, Minuti A, Bani P, Amadori<sup>o</sup>M, Riva F

### **Rumen fluid, a new diagnostic matrix in dairy cattle farms?**

Int J Health Anim Sci Food Safety. - Vol. 3 no 1s ( 2016). - 1 p. - 3 bib ref [Nr. Estr. 7274]

Veterinary and Animal Science Days : Milan, Italy : 2016, 8th-10th June)

Production diseases of dairy cows are considered man-made problems caused by the inability of cows to achieve a sufficient feed energy intake (Mulligan, 2008). A correct management of production diseases demands early diagnostic and prognostic parameters, in order to improve the management system and reduce the prevalence of clinical cases (Ingvarsen, 2003). A previous study of our group indicated that forestomachs walls express immune receptors and cytokines, and the rumen liquor contains leukocytes able to produce IFN- $\gamma$  (Trevisi, 2014). Our working hypothesis implied that ruminant fluids could be a source of diagnostic information for the identification of herds at risk for production diseases. We first demonstrated that the diet can influence the immune response in forestomachs. Diverse leukocyte populations at low concentrations and IFN- $\gamma$  were revealed in some samples of rumen fluids, with a clear inhibition of the response observed in the animals fed the maize-supplemented diet, compared to a normal and a soy-supplemented diet. We better characterized the leukocytes subpopulations in the rumen liquor, isolating B cells, monocytes and  $\gamma\delta$ T cells. Finally we performed a field survey in order to find correlation among the immune profile of the rumen liquor. Clinically healthy animals showed a farm specific immunologic pattern of the rumen liquor: low CD45 mRNA expression, low IFN- $\gamma$ , few/absent B-cells. We can conclude that the epithelial cells of ruminant forestomachs can react to different stresses (metabolic, infectious, inflammatory) and the inflammatory response can be sustained by infiltrating leukocytes. Our data points into the idea that dairy farms could be ranked according to a risk score using the inflammatory markers in rumen fluids, in addition to the traditional analysis.

Filipe JFS, Trevisi E, Massara M, Minuti A, Bani P, Amadori<sup>o</sup>M, Riva F

### **Rumen fluid, a new diagnostic matrix in dairy cattle farms?**

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 1 p. - 3 bib ref [Nr. Estr. 7313]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Production diseases of dairy cows include several pathologies and are considered manmade problems caused by the inability of cows to achieve a feed energy intake matching their high production requirements (1). A correct management of production diseases demands early diagnostic and prognostic parameters, in order to implement the necessary adjustments in the management system and reduce the prevalence of clinical cases (2). A previous study of our group showed that forestomachs walls express immune receptors and cytokines, and the rumen liquor contains leukocytes able to produce IFN- $\gamma$  (3), suggesting an integrated system including receptors, signaling molecules, cytokines and infiltrating leukocytes. Forestomach immune response could react to "dangers" arising within the forestomach environment, but also act as reporter system of disease conditions arising elsewhere in the body. Our working hypothesis implied that ruminal fluids could be an important source of diagnostic information for the identification of herds at risk for production diseases, in addition to the traditional blood and faecal analysis. We first demonstrated that the diet can influence the immune response in forestomachs. Diverse leukocyte populations at

very low concentrations and IFN- $\gamma$  were revealed in some samples of rumen fluids, with a clear inhibition of the response observed in all the animals fed the maize-supplemented diet, compare to a normal and a soy-supplemented diet. We better characterized the leukocytes subpopulations in the rumen liquor, isolating B cells, monocytes, and  $\alpha$ T cells. We also compared the leukocyte composition in ruminocentesis versus nasal probe sampling, and some differences seem to occur, probably due the fact the samples come from different areas of the rumen, however no significant statistical difference between samples collection techniques was found. Finally we performed a field survey (146 cows from 13 farms) in order to find correlation among the immune profile of the rumen liquor (FACS and molecular analysis), blood, and faecal parameters. Clinically healthy animals showed a farm specific immunologic pattern of the rumen liquor: low CD45 mRNA expression, low or absent IFN- $\gamma$ , few or absent B-cells. Whereas farms at risk for general wellness presented high levels of CD45 and IFN- $\gamma$ , increased numbers of B-cells and other leukocyte populations, such as myeloid cells. This immunological pattern of the rumen liquor seems to be associated to inflammatory markers of acute phase response in blood. We can conclude that the epithelial cells of ruminant forestomachs can react to disturbances of the fermentation processes due to improper diets, and the inflammatory response can be sustained by infiltrating leukocytes, able to release cytokines in the rumen liquor. Our data points into the idea that dairy farms could be ranked according to a risk score using the inflammatory markers in rumen fluids (leukocyte populations, CD45 expression). These markers could integrate the usual, consolidated information (e.g. rumen pH and VFA, milk cell counts, blood/faecal analysis).

Finazzi<sup>o</sup>G, Losio<sup>o</sup>MN, Varisco<sup>o</sup>G

#### **Optimization of microbiological recovery from surfaces for environmental monitoring**

European Symposium on Food Safety : 11-13 May 2016 Athens, Greece / [s.l. : s.n., 2016]. - 7247]

European Symposium on Food Safety : Athens, Greece : 11-13 May 2016)

Introduction: The FLOQSwab is a specimen collection device recognized worldwide for its superior performance in the clinical diagnostics. The aim of this work was to evaluate the FLOQSwab for the recovery of microbiological samples from surfaces compared to the traditional swab (rayon tipped swab) as per ISO 18593:2004 standard. Purpose: The FLOQSwab, thanks to the innovative manufacturing technology, allows improvements to the efficiency of recovery and release of analyte. The study has been divided into 2 experiments. Methods: In the first experiment the two swabs were evaluated for their capacity to recover and release the analyte (three different bacterial loads of *E. coli*). In the second experiment, the two swabs were evaluated for their capacity to recover three different bacterial loads of *E. coli*/from two different surface materials (stainless steel and polypropylene). Results: In all experiments the flocked swab demonstrated a higher recovery rate compared to the traditional rayon tipped swab. Significance: The data obtained from this preliminary study demonstrated that the FLOQSwab can be a good food surfaces collection device that improves the recovery of the analyte and thus produce accurate results. Based on the outcomes of the study, a larger field study is in progress using the FLOQSwab for sample collection to improve both environmental monitoring and the efficacy of the hygiene controls for food safety.

Fiocchi A, Gustinelli A, Gelmini<sup>o</sup>L, Rugna<sup>o</sup>G, Renz i<sup>o</sup>M, Fontana<sup>o</sup>MC, Poglayen G

#### **Helminth parasites of the red fox *Vulpes vulpes* (L.,1758) and the wolf *Canis lupus italicus* Altobello,1921 in Emilia-Romagna, Italy**

Ital J Zool. - Vol. 83 no 4 ( 2016). - p 503-513. - 80 bib ref [Nr. Estr. 7492]

In the period 2013–2014 a survey was carried out on the helminthic fauna of 60 wild canids, 57 red foxes (*Vulpes vulpes*) and three wolves (*Canis lupus italicus*), collected in the Emilia-Romagna region, Italy. The study focused mainly on the gastrointestinal and hepatic helminths. Parasites were recovered in 91.2% of the red foxes and in all the wolves examined. Multiple infections were found in

the majority of the animals (71.9% of the foxes and 100% of the wolves). In total, 14 intestinal helminth species were identified, two trematodes (*Alaria alata*, *Brachylaima* spp.), seven cestodes (*Mesocestoides* spp., *Taenia crassiceps*, *Taenia pisiformis*, *Taenia polyacantha*, *Dipylidium caninum*, *Taenia ovis*, *Taenia hydatigena*) and five nematodes (*Uncinaria stenocephala*, *Toxocara canis*, *Trichuris vulpis*, *Pterigodermatites affinis*, *Ancylostoma caninum*). The heartworm *Dirofilaria immitis* was also recovered in two foxes. No *Echinococcus* spp. were found. Our study shows that foxes are reservoir hosts of zoonotic parasites, including *A. alata*, a rare digenean trematode in the Italian peninsula. Results are compared with those of other surveys on helminths of wild canids carried out in Italy and other European countries.

Foglia°EA, Bregoli°A, Pezzoni°G, Grazioli°S, Brocchi°E

### **Caratterizzazione e diffusione del virus dell'encefalomiocardite virale (EMCV) in Italia in anni recenti**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 42 (Poster 05) [Nr. Estr. 7412]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

L'encefalomiocardite (EMC) è una virosi di varie specie animali, provocata da un virus appartenente alla famiglia Picornaviridae, che si può manifestare con encefaliti, miocarditi o aborti. I suini sono la specie da allevamento più sensibile. La presenza del virus negli allevamenti suinicoli in Italia è stata dimostrata a partire dal 1986, ed in seguito è stata confermata in varie aziende del nord-est. L'EMC ha andamento stagionale ed i roditori sembrano avere un ruolo importante come vettore biologico e nel mantenimento del virus. Il presente lavoro ha fornito aggiornamenti sia sull'evoluzione epidemiologica/molecolare che sulla diffusione del virus. Il profilo antigenico di 50 ceppi isolati tra il 2013 e il 2015 è stato valutato con un test ELISA, utilizzando un pannello di 40 anticorpi monoclonali (AcM) che identificano al meno 7 diverse aree antigeniche. Nonostante una sostanziale stabilità antigenica degli isolati, variazioni sporadiche sono state osservate in alcuni epitopi di due maggiori siti antigenici coinvolti nella neutralizzazione, mentre appare stabilizzata (44/50 ceppi) la mutazione che annulla la reattività di un AcM neutralizzante, già osservata in ceppi isolati prima del 2000. Nuove mutazioni sono state occasionalmente rilevate anche in siti non coinvolti nella neutralizzazione. È invece confermata la stabilità dell'unico sito lineare target di un AcM neutralizzante. L'analisi filogenetica basata sul gene della VP1 mostra identità superiore al 89.7% tra 47 ceppi italiani analizzati; 3 isolati suini di origine spagnola formano un cluster separato ma con elevata vicinanza filogenetica con i virus circolanti in Italia (identità superiore al 87%); 4 ceppi isolati da primati in un parco naturalistico italiano si organizzano tra quelli di origine suina, formando un sottogruppo specifico.

Per l'indagine sulla diffusione del virus sono stati esaminati circa 13.000 sieri prelevati nel 2016 in oltre 500 aziende suine della Lombardia e dell'Emilia Romagna, utilizzando un test ELISA in-house per la ricerca di anticorpi specifici. Rispetto ai dati raccolti nel 2010, è aumentata dal 54% al 71% la proporzione di aziende sieropositive con un trend in aumento della sieroprevalenza intra-allevamento, che è risultata elevata (>50%) nel 9% delle aziende, media (50%-20%) nel 22%, bassa (<20%) nel rimanente 40%. La presenza di livelli di sieropositività simili in aziende focolaio e aziende senza manifestazioni cliniche conferma la circolazione subclinica del virus.

Foni°E, Chiapponi°C, Faccini°S, Baioni°L, Barberi°I, Rosignoli°C, Merenda°M, Zanni°I, Manfredi°R, Sandri G, Nigrelli°AD

### **Sulla circolazione di virus "influenza D" in suini di allevamenti del Nord Italia**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 151-155. - 16 bib ref [Nr. Estr. 7229]

Meeting Annuale della Società Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Tra i virus appartenenti alla famiglia Orthomyxoviridae è stato recentemente individuato un nuovo tipo di virus influenza che è stato proposto di identificare come "influenza D". In questo studio è stata condotta una indagine virologica, nei confronti di virus "influenza D", su campioni diagnostici (n.530) prelevati in suini di allevamenti (n.273) dell'area padana, in corso di forme morbose febbrili-respiratorie, ma anche nell'ambito di piani aziendali di monitoraggio sanitario. Tramite metodiche di tipo biomolecolare è stato possibile accertare la presenza di virus "influenza D" nel 3,2% dei campioni provenienti da 4 allevamenti (1,4%). È stato ottenuto isolamento virale da tre tamponi nasali tramite inoculazione di substrati cellulari (HRT-18 e ST). L'analisi genetica condotta su una selezione dei genomi ottenuti ha permesso di rilevare che i virus suini influenzali D italiani sono correlati fra loro e appartengono al cluster riferibile a D/swine/Oklahoma/1334/2011. Indagini epidemiologiche, virologiche e filogenetiche più accurate ed estese si rendono necessarie per delineare meglio il ruolo patogenetico di questo agente nel suino. Un approfondimento delle conoscenze è imperativo per ottenere elementi di valutazione sull'eventuale ruolo di serbatoio di questa specie nella epidemiologia dell'infezione e soprattutto per capire meglio la genesi e la storia evolutiva del virus "influenza D".

*Among the viruses belonging to the family Orthomyxoviridae a new type of influenza virus was recently identified. It has been proposed to be named "influenza D". To evaluate circulation of "influenza D virus" in Italian pig farms a virological investigation was conducted on diagnostic samples (n.530) collected from pig farms (n.273) in the Po Valley, during febrile-respiratory symptoms, but also on samples collected for health monitoring. By biomolecular tests (RT-PCR) it was possible to ascertain the presence of "influenza D" virus in 3.2% of samples from four monitored farms (1.4%). Virus isolation was obtained from three nasal swabs on cell lines (HRT-18 and ST). Genetic analysis conducted on a selection of available genomes revealed that the Italian swine influenza D viruses are closely related and cluster with D/swine/ Oklahoma/1334/2011. Epidemiological investigations, virological and phylogenetic studies are required to better outline the pathogenic role of this virus in swine and to obtain elements of assessment on the possible role of swine as a reservoir of this virus and above all to better understand the genesis and the evolutionary history of this novel virus.*

Fontanesi L, Di\_Palma F, Flicek P, Smith AT, Thulin CG, Alves PC, Lagomorph Genomics Consortium<sup>o</sup>, [for Lagomorph Genomics Consortium, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna Lavazza Antonio]

**LaGomiCs — Lagomorph Genomics Consortium : an international collaborative effort for sequencing the genomes of an entire mammalian order**

J Hered. - Vol. 107 no 4 ( 2016). - p 295-308. - 78 bib ref (doi:10.1093/jhered/esw010) [Nr. Estr. 7498]

The order Lagomorpha comprises about 90 living species, divided in 2 families: the pikas (Family Ochotonidae), and the rabbits, hares, and jackrabbits (Family Leporidae). Lagomorphs are important economically and scientifically as major human food resources, valued game species, pests of agricultural significance, model laboratory animals, and key elements in food webs. A quarter of the lagomorph species are listed as threatened. They are native to all continents except Antarctica, and occur up to 5000 m above sea level, from the equator to the Arctic, spanning a wide range of environmental conditions. The order has notable taxonomic problems presenting significant difficulties for defining a species due to broad phenotypic variation, overlap of morphological characteristics, and relatively recent speciation events. At present, only the genomes of 2 species, the European rabbit (*Oryctolagus cuniculus*) and American pika (*Ochotona princeps*) have been sequenced and assembled. Starting from a paucity of genome information, the main scientific aim of the Lagomorph Genomics Consortium (LaGomiCs), born from a cooperative initiative of the European COST Action "A Collaborative European Network on Rabbit Genome Biology—RGB-Net" and the World Lagomorph Society (WLS), is to provide an international framework for the sequencing of the genome of all extant and selected extinct lagomorphs. Sequencing the genomes of an entire order will provide a large amount of information to address biological problems not only related to lagomorphs but also to all mammals. We present current and planned sequencing programs and outline the final objective of LaGomiCs possible through broad international collaboration.

Formenti<sup>o</sup>N, Ferrari N, Trogu T, Pedrotti L, Gaffur i<sup>o</sup>A, Lanfranchi P

**Toxoplasma gondii in red deer (*Cervus elaphus*) : epidemiological investigation and alternative sera sampling method**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 24 [Nr. Estr. 7329]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

Red deer (*Cervus elaphus*) can be intermediate host of *Toxoplasma gondii*, representing a potential public health concern. Indeed, its intense culling and the increasing consumption of raw or undercooked meat, may pose risks for humans. The need is therefore to investigate *T. gondii* in this host species. Moreover, the definition of alternative sampling methods for sera may help to solve diagnostic concerns related to logistical field activities and availability/suitability of sera. A sero-epidemiological survey was performed in red deer from Italian Central Alps to investigate (i) the spread and the dynamics of *T. gondii*, (ii) the reliability of sera obtained from blood through intracavernous venipuncture (IV) as an alternative to sera from blood of major vessels (MV) in ELISA test. Overall 242 sera were collected during two culling management plans (2014 and 2015). In 2015, 75 sera were obtained by both IV and MV techniques. Samples were tested by a commercial ELISA kit (IDVET, Montpellier, France) and results were analysed through Generalized Linear Models. An overall prevalence (p) of 21.5 % emerged. Calves (p = 5 %) were less infected than 1-year-old (p = 23.5 %) and > 2-year-old (p = 31.5 %) deer. Subjects of low anthropised area (p = 8.6 %) were less infected than those of the high (p = 33.7 %) and moderate (p = 15.7 %) anthropised ones. Subjects of 2014 (p = 16 %) were less infected than those of 2015 (p = 29 %) and seropositive deer of 2015 showed serological titres higher than those of 2014. Prevalence of 30.7 % and 26.7 % from MV and IV, respectively, showed an "Excellent agreement" (K value = 0.9024) between the two techniques. The effect of age class and anthropisation on infection supports horizontal transmission as the main route. The difference in prevalence and serological titres between study years leads to recent infections of deer of 2015 and thus to a new introduction or reintroduction of *T. gondii* in the study area. This finding should be considered even in relation to the potential zoonotic risk. The concordance between results supports the use of IV as a useful alternative for sera field sampling.

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

**Spread and genotype of *Toxoplasma gondii* in naturally infected alpine chamois (*Rupicapra r. rupicapra*)**

Parasitol Res. - Vol. 115 no 5 ( 2016). - p 2115-2120. - 67 bib ref [Nr. Estr. 7240]

The complex life cycle of *Toxoplasma gondii* involves many animal species, raising zoonotic, economic, and conservation issues. This complexity is reflected in the molecular structure of *T. gondii*, whose different genotypes differ in pathogenicity. Among the intermediate hosts of *T. gondii*, wild ungulates may be a source of human infection. Despite intense hunting activity and the consumption of raw or undercooked meat, little information is available on the spread of *T. gondii* and the distribution of its genotypes in these species, including the alpine chamois (*Rupicapra r. rupicapra*). Ninety-three sera and 50 brain tissues from chamois were sampled (1) to investigate the spread of *T. gondii* with serological and molecular analyses, and (2) to genotype the strains with a restriction fragment length polymorphism analysis of the SAG2 locus. The prevalence of *T. gondii* was low on both serological (3.2 %) and molecular (2 %) analyses, and infections were concentrated in individuals >1 year old. These findings demonstrate the sporadic presence of the protozoan in this species on consistent diagnostic tests. Horizontal transmission seems to be the main route of infection, and cats are the only definitive host in the study area. This prevalence suggests that the

environment of the chamois is less contaminated with oocysts than environments close to human settlements. The SAG2 type II genotype was detected in this species for the first time. Although this genotype is predominant in human toxoplasmosis, these results suggest that the chamois is a minor source of human infection.

Fusaro A, Tassoni L, Milani A, Hughes J, Salviato A, Murcia PR, Massi°P, Zamperin G, Bonfanti L, Marangon S, Cattoli G, Monne I

**Unexpected inter-farm transmission dynamics during a highly pathogenic avian influenza epidemic**

J Virol. - Vol. 90 no 14 ( 2016). - p 6401-6411. - 27 bib ref [Nr. Estr. 7296]

Next-generation sequencing technology is now being increasingly applied to study the within- and between-host population dynamics of viruses. However, information on avian influenza virus evolution and transmission during a naturally occurring epidemic is still limited. Here, we use deep-sequencing data obtained from clinical samples collected from five industrial holdings and a backyard farm infected during the 2013 highly pathogenic avian influenza (HPAI) H7N7 epidemic in Italy to unravel (i) the epidemic virus population diversity, (ii) the evolution of virus pathogenicity, and (iii) the pathways of viral transmission between different holdings and sheds. We show a high level of genetic diversity of the HPAI H7N7 viruses within a single farm as a consequence of separate bottlenecks and founder effects. In particular, we identified the cocirculation in the index case of two viral strains showing a different insertion at the hemagglutinin cleavage site, as well as nine nucleotide differences at the consensus level and 92 minority variants. To assess interfarm transmission, we combined epidemiological and genetic data and identified the index case as the major source of the virus, suggesting the spread of different viral haplotypes from the index farm to the other industrial holdings, probably at different time points. Our results revealed interfarm transmission dynamics that the epidemiological data alone could not unravel and demonstrated that delay in the disease detection and stamping out was the major cause of the emergence and the spread of the HPAI strain.

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

**Italian on-farm welfare assessment protocol for dairy cows : results from the last 4-years activities**

Proceedings of the 29th World Buiatrics Congress 2016 : Theme "Cattle Health - Tomorrow's Thinking Today" : Dublin, Ireland 2016 : oral communication and poster abstracts / [s.l. : s.n., 2016]. - p 265 [Nr. Estr. 7343]

World Buiatrics Congress (WBC) (29th : Dublin, Ireland : 2016)

Objectives: Over the last 10 years farm animal welfare has been of increasing interest for public opinion; consumers are very sensitive to how farm animals are reared and they are becoming more and more distrustful about farmers' practices. Challenge for scientific community and public institutions is to provide an objective tool for identifying if animal welfare is guaranteed or not. The Italian National Animal Welfare Reference Centre (CRenBA) based in the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) in Northern Italy is trying to meet this challenge applying an on-farm welfare assessment protocol for dairy cows. Materials and Methods: The CRenBA dairy cow welfare assessment protocol is based on scientific findings from Welfare Quality Project®, European Food Safety Authority (EFSA) publications on risk assessment related to dairy cow welfare and draft regulation concerning cattle (Strasbourg revised version No 8, 09/2009). The method takes into account also some minimum legal requirements (Council Directives 98/58/EC and 2008/119/EC). It consists of both management- and resource-based measures (Area A-Management and personnel, 23 indicators; Area B-Structures and equipments, 29 indicators) and animal-based measures (Area C, 18 indicators) such as: avoidance distance, body condition score,

cleanliness of animals, lameness, integument alterations, udder health, mortality rate (cows and calves), mutilations. Each assessment parameter has three choice options: unacceptable (hazard), acceptable and excellent (benefit). Parameters have different weighted scores given by an expert opinion consultation, based on their importance in decreasing or improving animal welfare. The overall welfare score results from adding partial scores of each Area and is expressed on a scale 0-100%. The protocol has been checked for inter-operators agreement. Application of this protocol by a skilled operator takes about 2 hours in a 100-lactating-cows-herd. From 2012 to 11/2015, 1047 Italian dairy herds in loose housing system have been assessed for animal welfare by 104 veterinarians that attended a specific training course at CReNBA. Farms were selected all around Italy for research activities and private consultation purposes. Results: Dairy herds were characterized by an average number of 252.7 animals (min 7-max 2736), 116.3 lactating cows (min 4-max 1135) and 27.9 kg/per cow as daily milk production (min 10-max 41). The recorded overall welfare scores (WS) ranged from 36.18% to 95.90%, and most of them were very high (first quartile: 63.84%; median: 70.42%; third quartile: 77.31%). Herds with overall WS under the median value (70.42%) were found to be smaller and less efficient in milk production compared to herds that gained a WS >70.42%: statistically significant differences were found in average herd size (234 vs 271 animals, respectively; P-value = 0.0049), average number of lactating cows (108 vs 124.5 cows, respectively; P-value = 0.0053) and average daily milk production (26.8 vs 29.02 kg milk per cow/day, respectively; P-value <0.001). There were positive Pearson correlations between partial scores given to Area C (animal-based measures) and Area A (indicators about managerial factors;  $r = 0.446$ ) and Area B (indicators about structural factors;  $r = 0.382$ ). Despite very good results on animal welfare assessment, most of the farms (52.05%) had at least one non-compliance with minimum requirements provided by the law in force, mainly for calves under 6 months of age: the most frequent non-compliances were absence of contact between calves in single cages (349 farms) and disbudding performed after 21 days of age (226 farms). Conclusions: The CReNBA dairy cow welfare assessment protocol is a reliable, timesaving, repeatable and scientifically-based tool to evaluate animal welfare level on-farm. Data collected in 1047 dairy herds showed positive information about dairy cow welfare in Italy. Herds with higher welfare levels were found to have more animals and higher production, as dairy cows in good welfare conditions usually have better performances. A stronger correlation was found between managerial factors (Area A) and welfare consequences on cows (Area C) meaning that skilful stockmanship can have larger effects in promoting animal welfare than housing structures (Area B).

Gaffuri°A, Formenti°N, Chiapponi°C, D'incäu°M, Manenti°S, Paterlini°F

**Mycoplasma infections in ibex (*Capra ibex*) and chamois (*Rupicapra r. rupicapra*) : not only *Mycoplasma conjunctivae*!**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 121 (Poster n. 45) [Nr. Estr. 7334]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

Despite the wide knowledge on *Mycoplasma conjunctivae* in wild ungulates, less information are available on other *Mycoplasma* infections in free-ranging ruminants although they may affect these wild species. *Mycoplasma*, acting alone or in association with other infecting agents, can indeed cause severe pneumonia for which particularly free-ranging ibex (*Capra ibex*) and chamois (*Rupicapra r. rupicapra*) may be very susceptible till having effects on populations' dynamics. Here, we report *Mycoplasma* infections recorded in free-ranging ibex and chamois from Italian Central Alps describing gross pathology lesions and the diagnostic trial we used to successfully detect *Mycoplasma* species. Five found-dead ungulates (three ibexes and two chamois) and lungs of an hunted chamois were analysed. Animals were necropsied and a standardised diagnostic trial was performed. For *Mycoplasma* analyses, lung samples were inoculated into both pleuropneumonia-like organism (PPLo) agar and broth. Positive samples were confirmed by PCR and identified by partial sequencing of the 16S rRNA gene. A septicaemia of *M. mycoides* subsp. *capri* with generalised lymph-nodes hyper-plasia, bronchopneumonia and spleen hyperplasia was recorded in a young ibex.

The same Mycoplasma was detected in the lung of a hunted young chamois with pleuritis and bronchopneumonia localised in apical lobes of both lungs. In both the infections, Mycoplasma acted in association with a virus, parapoxvirus in ibex and gamma-herpesvirus in chamois. *M. agalactiae* and *M. ovipneumoniae* were detected in two adults, an ibex and a chamois, respectively. In both cases Mycoplasma were in association with *Pasteurella* sp. and, only in the ibex, also with herpesvirus (BHV4). In other two cases, an ibex and a chamois, Mycoplasma were detected by PCR but the identification was ambiguous. Mycoplasma recorded in ibexes and chamois are among the most spread and pathogenic for small ruminants. As during summer pastures these wild species can have a spatial overlap with flocks, sheep and goats may have a role in the spread of these infections crossing the interface wild-domestic animals.

Galletti°G, Paternoster°G, Santi°A, Tamba°M, Licata E, Guberti V

**A risk index to evaluate avian influenza viruses introduction by wild birds**

Annual Meeting of the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) : 15-18 March 2016 Elsinore, Denmark / [s.l. : s.n., 2016]. - 1 p [Nr. Estr. 7682]

Annual Meeting of the Society for Veterinary Epidemiology and Preventive Medicine (SVEPM) : Elsinore, Denmark : 15-18 March 2016)

Gaspari V, Ortalli M, Moriconi M, Foschini MP, Baldovini C, Lanzoni A, Cagarelli R, Gaibani P, Rossini G, Vocale C, Patrizi A, Rugna°G, Carra E, Landini MP, Varani S

**An emerging cluster of cutaneous leishmaniasis in North-Eastern Italy : is a novel strain circulating in this area?**

44. Congresso Nazionale Società Italiana Microbiologia (SIM 2016) : Pisa, 25-28 September 2016 : abstract book / [s.l. : s.n., 2016]. - p 42-43 (Oral communication C 016) [Nr. Estr. 7622]

Congresso Nazionale Società Italiana Microbiologia (SIM 2016) (44. : Pisa : 25-28 September 2016)

Introduction: Leishmaniasis is a phlebotomine-transmitted infection caused by protozoan belonging to the genus *Leishmania*. A broad spectrum of clinical manifestation, ranging from tegumentary to visceral Leishmaniasis is recognized, the latter being fatal if untreated. Human Leishmaniasis may vary from asymptomatic to clinically evident disease, which can remain localized to the skin (cutaneous Leishmaniasis, CL) or extend to the respiratory mucous membranes or throughout the reticulo-endothelial system (visceral Leishmaniasis). Human Leishmaniasis is on increase in the Mediterranean Europe. Nevertheless, the exact prevalence of cutaneous CL cases is largely unknown as under-diagnosis and underreporting are common. In this study, we evaluated epidemiological, clinicopathological and microbiological aspects of CL cases occurring in the Bologna province, north-eastern Italy. Materials and Methods: We performed a retrospective study on CL cases diagnosed in the Bologna province between January 2013 and December 2015. Results: During 2013-2015, 30 cases of CL were identified in the Bologna province with an average incidence of 1.00/100,000, with a 4- up to 12-fold increase as compared to previous years. Sixteen out of 30 (53%) CL cases presented as single, typical lesions. CL diagnosis was carried out by histological and molecular techniques, nevertheless in 6 out of 29 (21%) PCR-positive cases amastigotes were not visible on histology. Molecular identification of *Leishmania* species by hsp70-based PCR showed signatures of both *L. infantum* and *L. donovani*. Further molecular evaluation of strain characteristics is ongoing. Conclusions: We report an increased number of cases of CL in a focal area of north-eastern Italy in 2013-2015. Our study highlights the importance of CL surveillance in the Mediterranean basin and emphasize the need of molecular laboratory surveillance for CL in endemic areas.

Gasparri°S, Giacomini°E, Pitozzi°A, Lazzaro°M, Guarneri°F, Alborali°GL,

Boniotti<sup>o</sup> MB

**Molecular characterization of *Mycoplasma hyopneumoniae* in a multi-site endemic farm by MLVA**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - p 242 [Nr. Estr. 7423]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Introduction: Multiple Locus Variable Tandem Repeat Analysis (MLVA) is a useful method to characterize bacterial strains and understand the transmission chains and sources of infection in order to implement more effective control measures. The present study is aimed to use MLVA technique to characterize *M.hyo* strains in pigs from wean to finish in the same herd. Materials and Methods: The study was carried out in a three-site herd in the North of Italy with endemic *M. hyo* infection on both vaccinated and unvaccinated animals. Tracheobronchial swabs (TBS) were taken from each pig at the first week of life (T1) and once a month until 9 months old (T2-T10). During the slaughtering, lungs were inspected and the presence and severity of lesions were recorded. TBS and lung samples were analyzed by qPCR directed against the p102 gene of *M. hyo*. Based on qPCR results, 113 positive samples (74 TBS and 39 lungs) belonging to 18 different animals were characterized by MLVA. The method is based on the detection of the number of tandem repeats at four multiple variable number tandem repeat (VNTR) loci within the genome (Locus 1, Locus 2, p 97-1, p 97-2). The monitoring of *M.hyo* population in this specific herd was further deepened analyzing other 8 samples (7 nasal swabs and two lungs), collected at 1 and 2 years after the study. Results: Both in vaccinated and unvaccinated animals, *M.hyo* infection was detected by qPCR from T5 but a general decrease was observed at T10. Furthermore, most of the animals showed fluctuating level of *M.hyo* and only few animals were positive throughout the study until the 10th month. Out of 113 specimens 97 samples were completely characterized by MLVA. Two distinct strains (MLVAtype-1 and MLVAtype-2) were identified: MLVAtype-1 was detected in all the analyzed animals from T5 until the last time point and in the lungs; MLVAtype-2 was detected in five pigs. The second genotype was identified especially in the lungs (10) and in TBS collected from two animals at T9 or T10. Furthermore in one animal a coinfection of two different strains in the same lung samples was observed. The 8 samples collected at 1 and 2 years after the study were characterized by MLVAtype-2. Conclusion: At the beginning of the study a single *M.hyo* genotype was prevalent in the herd infecting all the examined animals. A second genotype, detected at the last time points and in the lungs, could indicate a second wave of infection. This second strain seems to persist in the herd as observed during the following analysis. Moreover, the present study evidenced the presence of two different strains of *M.hyo* in the same herd and even in the same animal.

Gastaldelli M, Stefani E, Fontana<sup>o</sup> S, Boniotti<sup>o</sup> B, Koets A, Koehler H, Bakker D, Vicenzoni G, Pacciarini<sup>o</sup> ML, Pozzato N

**Jd-luminex : ricerca di anticorpi contro antigeni multipli di *Mycobacterium avium* ssp. paratuberculosis mediante array in fase liquida**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 55. - 2 bib ref [Nr. Estr. 7360]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

Serology is an important tool for Johne's disease (JD) control. The luminex suspension array allows the simultaneous detection of antibodies against multiple antigen targets. In this study we optimized the test with 3 recombinant proteins (MAP0210c, MAP2942, MAP2609), coupling each antigen with a bead set and modifying the standard protocol. We analysed 737 sera (510 negative and 227 positive) and defined Cut-off values applying ROC analysis fixing the specificity >99%. We

calculated test performance considering at least one positive antigen result (Se=23.8% and Sp=99.4%). The comparison with a commercial ELISA showed good agreement with comparable Se (23.8 vs 27.3%). We then tested 189 sera from different Tuberculosis (TB) and JD herd status that showed no interference with TE infection or ID vaccination. To resume, we have standardized a 1D-Luminex serological assay for cattle, with comparable performance to current ELISAs that can be easily implemented with additional antigens.

Gastaldelli M, Stefani E, Fontana°S, Boniotti°B, Koets Ad, Koehler K, Pacciarini°L, Pozzato N

#### **Multiplex detection of bovine anti-MAP antibodies by luminex suspension array**

13th International colloquium on paratuberculosis : Nantes, France, 20-24 June 2016 : program & abstracts / [s.l. : s.n, 2016]. - p 92 [Nr. Estr. 7685]

International colloquium on paratuberculosis (13th : Nantes, France : 20-24 June 2016)

ELISA serological tests represent an important tool in Johne's disease (JD) control programs. The Luminex suspension array (Luminex) is a technology platform that allows the simultaneous detection of antibodies against multiple antigen targets. In this study we tested several antigens (3 purified protein derivatives Johnei (PPD-J) preparations, 4 Escherichia coli recombinant Mycobacterium avium ssp. paratuberculosis (MAP) proteins and one MAP peptide) to be applied in this platform. We coupled each antigen with a bead set via a carbodiimide reaction and applied the protocol provided by Luminex with the following modifications and settings. Serum samples were pre-absorbed with Mycobacterium phlei to remove non-specific reactions, plates were read by "high" acquisition mode using the Bioplex™200 (Bio-Rad) and results were expressed as net median fluorescence intensity of 2 replicates. Due to technical issues with some antigens (high background or ineffectiveness) we eventually optimized the 1D-Luminex test with 3 recombinant proteins: Ag1 (MAPo2ioc), Ag2 (MAP2942), Ag7 (MAP2609), all produced at CVI. For test evaluation we analysed 737 sera (510 1D negative from uninfected herds and 227 MAP culture positive of which 165 sera tested positive in ELISA). Cut-off values and Se of each antigen were defined applying ROC analysis and fixing the Sp of each antigen >99%. The performance of the 1D-Luminex test was calculated with the cumulative result of Ag1, Ag2 and Ag7, considering at least one positive antigen (Se=23.8% and Sp=99.4%). The parallel application of a commercial ELISA (IDEXX) demonstrated a good agreement with the 1D-Luminex test with a comparable Se (23.8 vs 27.3%). We further examined 189 sera from different Tuberculosis (TB) herd status: ELISA positive/ MAP culture negative from TB free/JD infected. ID vaccinated (Silirum CZV) from TB free/JD infected. TB culture positive cattle from TB infected/JD unknown. Other cattle from TB infected/JD unknown. These results demonstrated no interference in sera from TB infected and JD vaccinated cattle. To resume, we have standardized a 1D-Luminex serological assay for cattle. The performance of the test is comparable to commercial ELISAs and can be easily implemented with additional antigens, when available, to improve the performance of the test.

Gazzonis AL, Garcia GA, Zanzani SA, Ortega\_Mora LM, Invernizzi°A, Manfredi MT

#### **Neospora caninum infection in sheep and goats from north-eastern Italy and associated risk factors**

Small Rumin Res. - Vol. 140 ( 2016). - p 7-12. - 37 bib ref [Nr. Estr. 7273]

Neospora caninum is worldwide recognized as one of the major abortive pathogens in cattle. Although cases of abortion are also registered in sheep and goats, information and epidemiological data on neosporosis in small ruminants are usually scant. In Italy, but few exceptions, data are limited to sparse reports of abortions. This survey was aimed to i) update information on N. caninum infection in sheep and goats from north-eastern Italy; ii) analyze associated individual and flock risk factors and iii) compare N. caninum positivity with previously obtained data on Toxoplasma gondii.

Four hundred and fourteen goats and 428 sheep from 39 flocks in Lombardy region were enrolled. Blood samples were collected and analyzed by an in-house ELISA followed by a confirmatory Western Blot. The test resulted positive for 5.7% of goats and 19.3% of sheep. As to farms, 32.1% and 89.4% of caprine and ovine flocks showed positive, respectively. Managerial variables, including rearing system and farm size, affected *N. caninum* infection. Small, family-run farms were at higher risk rather than intensive caprine farms or transhumant sheep flocks. Outcomes from the survey mainly indicate that these farms should implement their care about proper sanitary measures and monitor the spread of the infection among small ruminants.

Genchi M, Vismarra A, Mangia C, Kramer L, Vicari°N , Faccini S; Rigamonti°S, Fabbi°M

**Risk assessment of *Toxoplasma gondii* infection through the consumption of "Prosciutto di parma" DOP : preliminary data**

XXIX Congress Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College "Parasites, Poverty and Social commitment" : Bari, June 21-24, 2016 / [s.l. : s.n., 2016]. - p 68 [Nr. Estr. 7554]

Congress Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College (29. : Bari : June 21-24, 2016)

*Toxoplasma gondii* is considered one of the most common parasitic infections in the world due to its impressive range of hosts, widespread environmental contamination and the diverse means by which animals can be infected (EFSA, 2007). "Prosciutto di Parma" is a typical and popular Italian pork product known all over the world, highly valued for its flavor. *T. gondii* cysts in pork are persistent and they represent an important source of infection for human. However, little information is available concerning the effect of curing and salting on *T. gondii* cysts in ham. Furthermore, the scientific community and in particular the public opinion have diverse views on the possibility of transmission that could be observed through the consumption of cured ham. The aim of this study was to evaluate the survival and viability of tissue cysts of *T. gondii* in cured hams according to the procedural guideline of the "Prosciutto di Parma" consortium. Twelve pigs were infected per os with 1000 sporulated *T. gondii* oocysts and slaughtered 4 months later. Twelve thighs were cured and 12 were immediately digested according to Dubey (Vet Parasitol. 15;74:75-7, 1998). To verify the infection, serology, meat juice serology, PCR from muscle tissue, cell cultures, and bioassay in mice were carried out. After 12 months of ageing, hams were digested and processed by cell culture, RT-PCR and bioassay in mice to evaluate the vitality and the presence of parasite DNA. All pigs became infected. Preliminary data from the hams indicated the presence of *T. gondii* DNA, while the cell-cultures were negative. The bioassay in mice is still in progress. This study is the first in which the influence of processing of cured ham on the viability of *T. gondii* has been evaluated.

Gerber PF, Lelli°D, Zhang J, Strandbygaard B, More no°A, Lavazza°A, Perulli°S, Botner A, Comtet L, Roche M, Pourquier P, Wang C, Opriessnig T

**Diagnostic evaluation of assays for detection of antibodies against porcine epidemic diarrhea virus (PEDV) in pigs exposed to different PEDV strains**

Prev Vet Med. - Vol. 135 ( 2016). - p 87-94. - 33 bib ref [Nr. Estr. 7445]

Porcine epidemic diarrhea virus (PEDV) has caused economic losses in the Americas, Asia and Europe in recent years. Reliable serological assays are essential for epidemiological studies and vaccine evaluation. The objective of this study was to compare the ability of five enzyme-linked immunosorbent assays (ELISAs) to detect antibodies against different PEDV strains in pig serum. A total of 732 serum samples from North American or European pigs were tested. Samples included experimental samples from pigs infected with classical (G1a PEDV) or variant genogroup 1 PEDV (G1b PEDV), pandemic genogroup 2 PEDV (G2b PEDV) or non-infected controls. Field samples

from herds with confirmed or unknown PEDV exposure were also used. Three indirect ELISAs based on G2b antigens (ELISAs 1, 2 and 3), a competitive ELISA based on the G2b antigen (ELISA 4) and a competitive ELISA based on the G1a antigen (ELISA 5) were compared. Overall, the tests had a moderate agreement (= 0.61). G1a PEDV infected pigs were earliest detected by ELISA 3, G1b PEDV infected pigs were earliest detected by ELISAs 4 and 5 and the performance of all tests was similar for the G2b PEDV group. ELISA 1 showed the overall lowest detection on experimentally and field derived samples. Diagnostic sensitivity and specificity with a 95% probability interval were estimated to be 68.2% (62.1–74.4%) and 97.5% (95.2–99.0%) for ELISA 1, 73.7% (71.5–79.6%) and 98.4% (96.6–99.5%) for ELISA 2, 86.2% (81.1–90.6%) and 91.6% (87.7–94.8%) for ELISA 3, 78.3% (72.8–83.5%) and 99.7% (98.2–100%) for ELISA 4, and 93.5% (90.3–96.0%) and 91.2% (83.8–97.9%) for ELISA 5. Differences in detection among assays seem to be more related to intrinsic factors of an assay than to the PEDV antigen used.

Giacomini° E

#### **Aggiornamenti sulla situazione della PED in Italia**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 29-37. - 7 bib ref [Nr. Estr. 7227]

Atti della giornata di studio "Evoluzione e tendenze dei mercati internazionali e impatto delle malattie: il caso PED" : Parma : 9 Ottobre 2015)

Giacomini° E, Ferrari° N, Pitozzi° A, Remistani° M, Giardiello° D, Maes D, Alborali° GL

#### **Dynamics of Mycoplasma hyopneumoniae seroconversion and infection in pigs in the three main production systems**

Vet Res Commun. - Vol. 40 ( 2016). - p 81-88. - 29 bib ref [Nr. Estr. 7262]

In this study, we investigated the dynamics of Mycoplasma hyopneumoniae infections in 66 pig farms, with different production systems (one-, two-, and three-site systems), and considered different risk factors. Serological assay was used to detect serum antibodies against M. hyopneumoniae and real time polymerase chain reaction (RT-PCR) was performed to detect M. hyopneumoniae DNA in tracheobronchial swabs. Results demonstrated that M. hyopneumoniae infection status was predominantly influenced by the age of the animals and the type of production system. Infection rates were higher in older animals and the prevalence was higher in the one- and two-site systems than in the three-site systems. Dynamics of infection by RT-PCR showed that earlier M. hyopneumoniae infection on one-site farms occurs earlier, while on two- and three-site farms occurs later but spreads faster, suggesting that contact between animals of different age favors the transmission.

Giacomini° E, Lazzaro° M, Boniotti° MB, Scali° F, Pasquali P, Amadori° M, Ruggeri° J, Bardini R, Gamba F, Leotti° G, Alborali° GL

#### **Oral Fluid sampling as a tool for PRRSV surveillance in gilts**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - p 586 [Nr. Estr. 7419]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Introduction: Gilts are a potential source of porcine reproductive and respiratory syndrome virus (PRRSV) and their introduction into breeding herds is a pivotal step of disease control. Data on the

onset of PRRSV infection in replacement gilts during the acclimatization period would be useful for PRRS control strategy. This study was aimed to compare PRRSV detection and PRRSV-specific antibody responses in Oral Fluid (OF) versus serum samples in order to determine whether pen-based OF sampling could be used as a tool for PRRSV surveillance in gilts in breeding herds in Italy. Materials and Methods: The study was carried out in 11 breeding farms endemically infected with PRRSV in a high pig density area in Italy. Sows had been vaccinated with PRRSV modified-live virus and inactivated vaccines in 4 and 2 farms, respectively. No vaccines were used in gilts in the other 5 farms. OF was sampled in 3 pens with 5 animals each. Those gilts were also bled individually to obtain serum samples. Samplings were performed at the beginning of the acclimatization period (TO), at 4 (T1) and 8 weeks (T2) later. Sera and OF were used in a PRRSV real-time quantitative reverse transcription polymerase chain reaction (qPCR) and a PRRSV-specific serum antibody ELISA. PRRSV IgA and IgG Ab assays were used only in OF. A total of 495 sera and 99 OF samples were analyzed. Results: In one out of 11 herds, all the gilts were completely PRRSV-negative by qPCR, and Ab-negative in the assays on serum and OF samples at the three time points. In the other 10 farms, animals showed PRRSV infection. At 12, serum and OF samples of the 10 farms were Ab-positive, only 6 of them being also qPCR-positive. IgA and IgG Ab assays on OF were negative at TO and positive at 12 in 7 and 3 farms, respectively. The concordance of qPCR results on OF vs sera was 96.9%, 93.9% and 69.7% at TO, T1 and T2, respectively. At T2, in 7 samples qPCR was positive in OF and negative in sera. The concordance of OF vs serum Ab assays was 87.9% at TO, 84.8% at T1 and 93.9% at T2. A comparison of antibody responses in qPCR-positive vs. negative oral fluid samples showed higher S/P ratios in qPCR-positive oral fluid samples (mean S/P 4.18 vs. 3.67). Conclusion: The concordance of qPCR and antibody ELISAs in gilts was high at all the time points (TO, T1 and T2). The prevalence of PRRSV qPCR positive samples in OF was greater when compared with serum at T2. Although the approach should be validated in further field trials, the results of this study showed that gilt oral fluid samples could provide an efficient and sensitive approach to PRRSV surveillance in infected or presumed-negative pig breeding herds.

Giacomini<sup>o</sup> E, Lazzaro<sup>o</sup> M, Boniotti<sup>o</sup> MB, Scali<sup>o</sup> F, Pasquali P, Amadori<sup>o</sup> M, Ruggeri<sup>o</sup> J, Bardini R, Gamba F, Leotti G, Ana<sup>o</sup> M [i.e. Moreno A], Alborali<sup>o</sup> GL

#### **Monitoraggio dei PRRSV e SIV in suini svezzati mediante l'uso di fluidi orali**

Atti Convegno SIPAS. - Vol. 42 (2016). - p 165-168. - 11 bib ref [Nr. Estr. 7236]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Le patologie respiratorie nell'allevamento suino sono la principale causa di perdite economiche. Di conseguenza, negli anni si sono sviluppate pratiche di management e di profilassi per il loro controllo. Questo studio è stato svolto in 8 allevamenti, facendo dei prelievi in suini allo svezzamento di età compresa fra 30-90 giorni. È stato prelevato il sangue venoso da ogni soggetto, assieme a tamponi nasali (TN), poi analizzati in pool da 5 animali l'uno, e la saliva (OF). Le matrici sono state analizzate per la ricerca di virus Porcine Reproductive Respiratory Syndrome (PRRSV) e Virus Influenzale Suino (SIV) tramite real-time RT-PCR. I risultati per PRRSV sul siero di sangue mostrano una maggiore prevalenza di campioni positivi con l'avanzare dell'età, con valori più che raddoppiati da 30 a 90 giorni di vita sia per PRRSV che per SIV. La percentuale di concordanza in RT-PCR fra siero e OF è stata pari al 83% mentre quella fra TN e OF era del 92%.

*Respiratory diseases represent causes of major economic losses in pig production. Therefore, management approaches and prophylaxis plans have been developed for their control and treatments. This study was conducted in 8 farms by sampling 1-3 months old pigs. Blood samples and nasal swabs (NS) were taken from a total of 45 animals and examined as pool of 5 animals. In addition, 3 oral fluids (OF) at each time point were taken. The three types of sample were examined for Porcine Reproductive Respiratory Syndrome Virus (PRRSV) and Swine Influenza Virus (SIV) by real-time RT-PCR. The results of PRRSV test on blood serum indicated an increasing of prevalence related to age, being the values for both PRRSV and SIV more than doubled between 30 and 90 days of age. The agreement with RT-PCR for PRRSV between serum and OF was 83% and that between*

*TN and OF was 92%.*

Guberti V, Masiulis M, Bellini<sup>o</sup>S

**Preventive measures to minimize the risk of African swine fever spread during wild boar hunting**

COST Action CA15116 : Understanding and combating African Swine Fever in Europe (ASF-STOP) : African swine fever : recent research advances and strategies to combat the disease in Europe : 6-8 December 2016 Pulawy, Poland : conference proceedings / [s.l. : s.n., 2016]. - p 69 [Nr. Estr. 7453]

COST Action CA15116 : Understanding and combating African Swine Fever in Europe (ASF-STOP) : Pulawy, Poland : 6-8 December 2016)

African swine fever (ASF) is a severe viral disease of domestic and wild pigs. In Europe, there are currently two main clusters of infection, one in Sardinia caused by strains of ASFV belonging to genotype I, the second in the East part of Europe caused by strains of ASFV belonging to genotype II. It appears that in Sardinia wild boar have a limited role in the spread of ASF whilst in the Baltic countries and Poland wild boar are involved in the spread of the disease, especially at the interface with the backyard's sector. Hunting is practiced in the majority of the forested areas of North East Europe and wild boar are one of the more intensively hunted ungulate species. Wild boar are susceptible to ASFV and they show similar clinical signs and mortality to domestic pigs. The dynamic of the infection in the wild boar shows the same pattern observed in domestic pigs. When wild boar die, infected carcasses, if not promptly removed, remain in the environment and they can, directly or indirectly, infect other susceptible pigs, continuing the epidemiological cycle of the disease. Wild boar can also contribute to spread the virus during the infectious period of the disease, since they eliminate the virus into the environment throughout their excretions and secretions. Considering that a large amount of ASFV is shed during the infectious period of the disease and that the virus is rather resistant into the environment, especially if protected by organic material, it can be expected that in the affected forests, the viral contamination of the environment to be rather high. Indeed, hunting wild boar implies blood contamination of the soil, transportation of dead animals to the dressing facility (when dressing is not performed directly on the field), dressing animals, offal discharge, meat dissection and its conservation. A high viral contamination of the environment increases the likelihood of virus transmission to domestic pigs. In the framework of a control strategy for ASF, hunting is the sole practical mean to collect samples from wild boar. However, wild boar hunting can be a dangerous practice, if appropriate preventive measures are not adopted to minimize the risk of further spread of the disease. Due to the limited density dependent spread of ASFV, hunting should primarily be seen as a tool to decrease the environmental load of the virus rather than at mechanistically decrease wild boar population size.

Henritzi D, Zhao N, Starick E, Simon G, Krog JS, Larsen LE, Reid SM, Brown JH, Chiapponi<sup>o</sup>C, Foni<sup>o</sup>E, Wacheck S, Schmid P, Bee r, Hoffmann B, Harder TC

**Rapid detection and subtyping of European swine influenza viruses in porcine clinical samples by haemagglutinin- and neuraminidase-specific tetra- and triplex real-time RT-PCRs**

Influenza Other Respir Viruses. - Vol. 10 ( 2016). - p 504-517. - 41 bib ref [Nr. Estr. 7396]

Background: A diversifying pool of mammalian-adapted influenza A viruses (IAV) with largely unknown zoonotic potential is maintained in domestic swine populations worldwide. The most recent human influenza pandemic in 2009 was caused by a virus with genes originating from IAV isolated from swine. Swine influenza viruses (SIV) are widespread in European domestic pig populations and evolve dynamically. Knowledge regarding occurrence, spread and evolution of potentially zoonotic SIV in Europe is poorly understood. Objectives: Efficient SIV surveillance programmes depend on sensitive and specific diagnostic methods which allow for cost-effective large-scale analysis. Methods: New SIV haemagglutinin (HA) and neuraminidase (NA) subtype- and lineagespecific multiplex real-time RT-PCRs (RT-qPCR) have been developed and validated with reference virus

isolates and clinical samples. Results: A diagnostic algorithm is proposed for the combined detection in clinical samples and subtyping of SIV strains currently circulating in Europe that is based on a generic, M-gene-specific influenza A virus RT-qPCR. In a second step, positive samples are examined by tetraplex HA- and triplex NA-specific RT-qPCRs to differentiate the porcine subtypes H1, H3, N1 and N2. Within the HA subtype H1, lineages "av" (European avian-derived), "hu" (European human-derived) and "pdm" (human pandemic A/H1N1, 2009) are distinguished by RT-qPCRs, and within the NA subtype N1, lineage "pdm" is differentiated. An RT-PCR amplicon Sanger sequencing method of small fragments of the HA and NA genes is also proposed to safeguard against failure of multiplex RT-qPCR subtyping. Conclusions: These new multiplex RT-qPCR assays provide adequate tools for sustained SIV monitoring programmes in Europe.

Knowles NJ, Bachanek-Bankowska K, Wadsworth J, Mioulet V, Valdazo-Gonzalez B, Eldaghayes IM, Dayhum AS, Kammon AM, Sharif MA, Waight S, Shamia AM, Tenzin S, Wernery U, Grazioli S, Brocchi E, Subra maniam S, Pattnaik B, King DP  
**Outbreaks of foot-and-mouth disease virus in Libya and Saudi Arabia during 2013 due to an exotic O/ME-SA/Ind-2001 lineage virus**

Transbound Emerg Dis. - Vol. 63 ( 2016). - p e431-e435. - 13 bib ref [Nr. Estr. 5912]

Foot-and-mouth disease viruses are often restricted to specific geographical regions and spread to new areas may lead to significant epidemics. Phylogenetic analysis of sequences of the VP1 genome region of recent outbreak viruses from Libya and Saudi Arabia has revealed a lineage, O-Ind-2001, normally found in the Indian subcontinent. This paper describes the characterization of field viruses collected from these cases and provides information about a new real-time RT-PCR assay that can be used to detect viruses from this lineage and discriminate them from other endemic FMD viruses that are co-circulating in North Africa and western Eurasia.

Lavazza A, Cavadini P, Chiari M, Capucci L

### **Lagovirus e lagomorfi : un rapporto in continua evoluzione**

Atti del III Congresso nazionale fauna problematica : 24-26 novembre 2016 Cesena (FC) : riassunti delle comunicazioni orali e dei poster / [s.l. : s.n., 2016]. - p 142-143 [Nr. Estr. 7439]

Congresso nazionale fauna problematica (3. : Cesena (FC) : 24-26 novembre 2016)

La Malattia Emorragica del coniglio (Rabbit Haemorrhagic Disease = RI-ID) e la Sindrome della lepre bruna europea (European Brown Hare Disease = EBHS) sono due malattie clinicamente e patologicamente simili, causate da agenti virali a RNA a singola catena appartenenti al genere Lagovirus della famiglia Caliciviridae tra loro correlati ma filogeneticamente distinti. RHDV, segnalato per la prima volta in Cina nel 1984, si è rapidamente diffuso in tutto il mondo dagli anni '80 in conigli domestici e selvatici della specie *Oryctolagus cuniculus*. Evoluzione spaziale e temporale di RHDV ha generato sei distinti genotipi, di cui il 6 corrisponde ad un sottotipo antigenico (RHDVa) prevalente in alcuni Paesi tra cui l'Italia. In sostanza RHDV sembra in grado di co-evolvere molto rapidamente per "superare" l'aumento di resistenza alla malattia, dovuto all'immunità di popolazione ma forse anche ad una resistenza genetica. Infatti, nel 2010 è stato identificato in Francia un nuovo lagovirus con un'identità genetica della proteina capsidica di "solo" 80%, ma in grado di indurre una malattia del tutto sovrapponibile a RHD, anche in conigli già vaccinati e di età inferiore a 40gg, che viceversa pur infettandosi, non venivano a morte se infettati da RHD "classica". Questo nuovo virus, chiamato RHDV2, si è rapidamente diffuso in conigli selvatici e domestici in tutta Europa e in Australia, oltre ad un caso in Canada, in apparenza "rimpiazzando" i ceppi precedenti (RHDV/RHDVa). Ha un profilo antigenico molto distinto, ed è in rapida evoluzione, potendo causare nella specie ospite d'elezione mortalità variabili dal 5 al 70% in funzione dei ceppi virali, tra cui quelli isolati più recentemente (2014-15) sono i più virulenti, anche in condizioni sperimentali. Anche l'epidemiologia del virus si è modificata: RHDV2 è in grado di causare malattia con

caratteristiche sovrapponibili all'EBHS in alcune specie di lepri: ripetuti casi sono stati segnalati nella lepre sarda (*Lepus capensis mediterraneus*) nello stesso momento in cui RHDV2 è comparso in conigli selvatici, mentre episodi sporadici sono stati osservati nella lepre appenninica (*Lepus corsicanus*) in Sicilia e più recentemente nella lepre europea (*Lepus europaeus*) in Italia, Spagna, Francia e Australia. Queste caratteristiche specifiche suggeriscono che RHDV2 potrebbe rappresentare una nuova emergenza virale da una sorgente sconosciuta, e tra le ipotesi vi è anche quella di un possibile salto di specie di lagovirus tra specie diverse di lagomorfi. Infatti, oltre ai virus patogeni RHDV nel genere *Lagovirus* sono ricompresi anche una serie di agenti virali non patogeni RHDV-like (RCVs) identificati nel coniglio domestico e selvatico sia in Europa sia in Australia. La diversità genetica all'interno del gruppo degli RCVs varia da non protettivi a parzialmente o totalmente protettivi verso RHDV. Sin dalla prima descrizione in Svezia (-1980), la distribuzione dell'EBHS è stata limitata all'Europa, riconoscendo come ospite primario la lepre europea e più raramente la lepre variabile (*Lepus timidus*) e la lepre appenninica (*Lepus corsicanus*), ma non altre specie di lepre come la lepre iberica (*Lepus granatensis*), la lepre cantabrica (*Lepus castroviejoi*) e la lepre sarda (*Lepus capensis mediterraneus*). Inoltre abbiamo verificato in condizioni naturali e sperimentali la suscettibilità a EBHSV, con occasionali quadri di malattia EBHS-like, della minilepre (*Sylvilagus floridanus*), specie invasiva ben rappresentata nel nord-centro Italia, nella quale non sono fino ad oggi stati identificati altri lagovirus. A oggi è conosciuto un solo sierotipo di EBHSV ma sono stati identificati un certo numero di genogruppi differenti. La distribuzione di EBHSVs suggerisce che questo virus è evoluto lentamente nella area di origine (EBHS non è stata mai segnalata in Australia e Sud-America) senza alcuna cambio sostanziale nella struttura e patogenicità. Come nel caso di RHDV, abbiamo proposto e di recente dimostrato l'esistenza di un lagovirus non patogeno EBHSV-like sia in lepri europee selvatiche che allevate dal nostro Paese ma anche in Germania e Austria. Il significato epidemiologico di questo virus che abbiamo chiamato "Hare Calicivirus" (HaCV) deve tuttavia essere ancora chiarito e meglio definito.

Lavazza°A, Cavadini°P, Chiari°M, Molinari°S, Capucci°L

#### **New insights of Lagovirus infection in wild rabbits and hares**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 11 [Nr. Estr. 7328]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

Rabbit Haemorrhagic Disease (RHD) and European Brown Hare Disease (EBHS) are similar diseases caused by highly related but phylogenetically distinct Lagoviruses, family Caliciviridae. RHDV emerged in mid '80 and during its worldwide spread evolved in six genotypes of which genotype 6 is an antigenic subtype (RHDVa) prevalent in some countries, including Italy. RHDV seems to rapidly co-evolving to match increased disease resistance in rabbits. In fact, in 2010, a new lagovirus having a genetic capsid protein identity with RHDV of "only" 80 %, and able to cause RHD also in vaccinated and new-born rabbits was identified in France. This virus spread rapidly within Europe (but it was also reported in Australia), apparently replacing former strains (RHDV/RHDVa). It has a very distinct antigenic profile and variable mortality rates (5 - 70 %) depending on the viral strains, being the recent ones we found the more virulent, also in experimental condition. Additionally, we showed that RHDV2 causes an EBHSV-like disease in Cape hares in Sardinia and a single case in Italian hares in Sicily. More recently we identified some sporadic cases of RHDV2 in Brown hares both in Spain and Italy. These specific features suggest that RHDV2 could represent a new viral emergence from an unknown source, including a possible species jump of lagoviruses between species of lagomorph genus. In addition to virulent RHDVs, non-pathogenic rabbit RHDV-like viruses (RCVs) have been identified in rabbits in Europe and Australia. The genetic diversity among RCVs varies from non-protective, partially or fully protective strains so that there is a gradient of cross-protection in rabbit populations following their circulation. Since first description in Sweden (-1980), EBHS distribution has been restricted to Europe, affecting primarily brown hares and, less frequently, Mountain hare and Italian hare, but not other European species such as Iberian hare, Broom Hare and Cape hare. Moreover we proved the natural and

experimental susceptibility of Eastern cottontail to EBHSV, occasionally resulting in EBHS-like disease. A single viral serotype of EBHSV is known, but a number of different genogroups were identified. The distribution of EBHSVs suggests that they slowly evolved in their area of origin without any dramatic changes in structure and pathogenicity. As in the case of RHDV, the existence of a non-pathogenic EBHSV-like virus was put forward and we recently succeeded in the identification of such non-pathogenic Lagovirus in captive and wild hares, named "Hare Calicivirus" (HaCV). However, the epidemiological meaning of this "new" lagovirus is still to be clarified.

Lavazza<sup>°</sup>A, Chiari<sup>°</sup>M, Nassuato C, Giardiello D, Tittarelli C, Grilli G

**Serological investigation on Encephalitozoon cuniculi in pet rabbits in North-Central Italy**

J Exot Pet Med. - Vol. 25 no 1 ( 2016). - p 52-59. - 34 bib ref [Nr. Estr. 7206]

This study determined the seroprevalence of Encephalitozoon cuniculi in pet rabbits from north-central Italy and correlated it with data (age and clinical signs) obtained from private veterinary practitioners. During the period 2007 to 2010, 826 pet rabbit sera were collected and tested using carbon immunoassay. In total, 310 out of 826 rabbits (37.53%, 95% CI: 34.23 to 40.94) displayed clinical signs consistent with infection, whereas the remaining 516 animals presented for wellness examination or to be vaccinated. The results were analyzed according to age, serological status, and the presence or absence of clinical signs. Seropositive results were observed in 59.56% (95% CI: 56.12 to 62.92) of the examined animals. Seroprevalence in the clinically ill rabbits was 70.65% (95% CI: 65.18 to 75.59), much higher than in healthy animals, which showed a prevalence of 52.91% (95% CI: 48.5 to 57.27). Clinical signs were classified into 4 groups: nonvestibular neurologic signs, vestibular signs (including head tilt), renal failure, and ocular lesions. Odds of animals presenting with clinical disease signs were higher in seropositive rabbits (odds ratio = 2.14, 95% CI: 1.59 to 2.9), and this association was particularly true for vestibular signs (odds ratio = 4.1, 95% CI: 2.48 to 7.13). The prevalence values were different with regard to age, with a peak in 3- to 4-year-old animals. Average titers in both healthy and clinically ill rabbits at various ages were different; rabbits with overt disease signs had higher titers when very young (<3 months) and from 1 to 4 years of age. In conclusion, this study provides more precise indications of the prevalence of E. cuniculi in pet rabbits in Italy, underlying growing interest and concern among veterinarians and pet owners regarding this organism.

Lecchi C, Marques AT, Redegalli M, Meani<sup>°</sup>S, Vinco<sup>°</sup> LJ, Bronzo V, Cecilian F

**Short communication : Circulating extracellular miR-22, miR-155, and miR-365 as candidate biomarkers to assess transport-related stress in turkeys**

Animal. - Vol. 10 no 7 ( 2016). - p 1213-1217. - 10 bib ref [Nr. Estr. 7355]

MicroRNA (miRNA) have been identified in circulating blood and might have the potential to be used as biomarkers for several pathophysiological conditions. To identify miRNA that are altered following stress events, turkeys (*Meleagris gallopavo*) were subjected to 2 h of road transportation. The expression levels of five circulating miRNA, namely miR-22, miR-155-5p, miR-181a-3p, miR-204 and miR-365-3p, were detected and assessed by quantitative polymerase chain reaction using TaqMan<sup>®</sup> probes, as potential biomarkers of stress. The areas under the receiver operating characteristic curves were then used to evaluate the diagnostic performance of miRNA. A panel of three stress-responsive miRNA, miR-22, miR-155 and miR-365 were identified; their expression levels were significantly higher after road transportation and the area under the curve (AUC) were 0.763, 0.71 and 0.704, respectively. Combining the three miRNA a specificity similar to the one found for the three miRNA separately was found. The AUC of the weighted average of the three miRNA was 0.763. This preliminary study suggests that the expression levels of circulating miR-22, miR-155 and miR-365 are increased during transport-related stress and that they may have diagnostic value to discriminate between stressed- and unstressed animals.

Lelli°D, Beato MS, Cavicchio L, Lavazza°A, Chiapp oni°C, Leopardi S, Baioni L, De\_Benedictis P, Moreno°A

**First identification of mammalian orthoreovirus type 3 in diarrheic pigs in Europe**

Virology J. - Vol. 13 ( 2016). - no 139 (5 p). - 15 bib ref ( ultimo accesso 30/09/2016  
<http://virologyj.biomedcentral.com/articles/10.1186/s12985-016-0593-4> ) [Nr. Estr. 7322]

Mammalian Orthoreoviruses 3 (MRV3) have been described in diarrheic pigs from USA and Asia. We firstly detected MRV3 in Europe (Italy) in piglets showing severe diarrhea associated with Porcine Epidemic Diarrhea. The virus was phylogenetically related to European reoviruses of human and bat origin and to US and Chinese pig MRV3.

Lelli°D, De\_Benedictis P, Decaro N, Prosperi°A, L eopardi S, Priori P, Boniotti°MB, Papetti°A, Scaravelli D, Rosti E, Chiapponi °C, S ozzi°E, Bonilauri°P, Perulli°S, Moreno°A, Lavazza°A

**I chiropteri come reservoir di virus zoonotici emergenti in Italia: implicazioni per la salute pubblica e la conservazione biologica**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 26 [Nr. Estr. 7408]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

I pipistrelli sono universalmente conosciuti come reservoir di virus emergenti a rischio zoonosico. Considerando la carenza di dati eco-epidemiologici sulla circolazione di virus nelle popolazioni di pipistrelli in Italia, nel 2014 il Ministero della Salute ha finanziato nell'ambito del Bando Ricerca finalizzata/giovani ricercatori un progetto dal titolo "An epizootiological survey of bats as reservoirs of emerging zoonotic viruses in Italy: implications for public health and biological conservation" finalizzato ad approfondire sia gli aspetti di sanità pubblica che di conservazione biologica attraverso lo studio delle principali infezioni virali in pipistrelli autoctoni in Italia. L'indagine è principalmente orientata all'identificazione di coronavirus (CoVs), lyssavirus (LYSVs) e orthoreovirus (MRVs), ma, attraverso un protocollo diagnostico ad ampio spettro, mira anche ad investigare la presenza di altri virus potenzialmente zoonosici o patogeni per gli stessi pipistrelli. Nel corso della sorveglianza passiva attuata nei primi 18 mesi di progetto (2014-2016) sono stati raccolti 247 campioni da 7 differenti specie, suddivisi in 142 carcasse e 105 campioni fecali. L'attività di sorveglianza attiva è stata eseguita attraverso il campionamento di 5 colonie miste di *M. myotis* e *M. blythii* localizzate nel territorio altoatesino e prevedeva la raccolta di campioni di sangue, tamponi orali e feci da un numero statisticamente significativo di soggetti. I risultati ottenuti dimostrano che l'infezione da CoVs è largamente diffusa tra le popolazioni di pipistrelli in Italia con dati di prevalenza globali del 5-7% in 5 diverse specie. Le sequenze del genoma virale ottenute appartengono al genere Alpha-Coy e Beta-Coy clade 2c (MERS-like Coy) e 2b (SARS-like CoV). Positività sierologiche per LYSVs sono state rilevate in due colonie miste di *M. Myotis* e *M. blythii* mentre non si è ottenuta alcuna positività virale. Lo studio conferma inoltre il ruolo dei pipistrelli come carrier di una grande varietà di MRVs, permettendo l'isolamento di ceppi riassortanti dei sierotipi 1 e 2 e di ceppi MRV3 correlati a virus responsabili di forme enteriche nell'uomo e suino. Positività per astrovirus e adenovirus sono state inoltre rilevate rispettivamente in 2 campioni fecali (*Pip. kuhlii*) e in un campione d'organo (*H. savii*). Lo studio fornisce un quadro significativo ancorché preliminare sulla distribuzione delle principali infezioni virali nei pipistrelli in Italia.

Lelli°D, De\_Benedictis P, Decaro N, Prosperi°A, L eopardi S, Scaravelli D, Rosti E.

Moreno<sup>o</sup> A, Lavazza<sup>o</sup> A

**An epizootiological survey of bats as reservoirs of emerging zoonotic viruses in Italy : implications for public health and biological conservation**

Hystrix Ital J Mamm. - Vol. 27 Suppl ( 2016). - p 25 [Nr. Estr. 7259]

Congresso Italiano di Teriologia (10. : Acquapendente (VT) : 20-23 Aprile 2016)

Bats are being increasingly recognized as the reservoir of highly pathogenic and zoonotic emerging viruses in tropical regions, however, little is known on the human-pathogenic viruses, which may be present in bats in European countries. Considering the poor eco-epidemiological data on the circulation of viruses in bats in Italy, in 2014 the Italian Ministry of Health funded a project entitled "An epizootiological survey of bats as reservoirs of emerging zoonotic viruses in Italy: implications for public health and biological conservation" with the leadership of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna and involving both the University of Bari and the Reference Centre for Rabies and for Infectious Diseases at the Animal-Human Interface based at Istituto Zooprofilattico Sperimentale delle Venezie, Italy. This project aims to cover both human health and biological conservation issues by promoting and implementing a passive and active surveillance system for viral infections in Italian bat populations. The survey is mainly targeted on the detection of coronaviruses, lyssaviruses and orthoreoviruses; of notice, diagnostic protocols broadly targeting viral pathogens will also be implemented, to investigate other newly-emerging viruses with a potential zoonotic importance or proving dangerous for bats. We are herewith describing the activities performed so far within the framework of the first year of the project and presenting the overall data on the virological load in Italian bat populations obtained from our previous investigations in bats, which have been crucial to provide valid background results for a credible project proposal in the fields of viral zoonoses, veterinary virology and chiroptera conservation. Passive surveillance involved the analyses of dead animals collected from bat rehabilitation centers or from known roost sites. Fresh carcasses were fully necropsied, and tissue specimens from different organs were analyzed. Fecal samples collected from injured and hospitalized individuals were also sampled and tested. Organs and fecal samples were examined through a broadly reactive PCR-based protocol for the presence of viral agents with zoonotic potential. Next generation sequencing (NGS), virus isolation techniques and electron microscopy were also used. Active surveillance involved live sampling of freeranging animals from selected colonies. Bats were captured with hand nets and samples were collected under physical restraint, including blood, salivary swabs, feces/anal swabs, and wing biopsies. Serum samples were analyzed for the presence of antibodies against European bat lyssavirus 1 (EBLV1), while molecular biology was applied to saliva and fecal material to investigate the presence of lyssaviruses, coronaviruses and orthoreoviruses. A diagnostic protocol for investigating the major viral infections of bats was developed and applied on field samples originating from different parts of Italy. A total of 580 fecal and tissue samples belonging to 12 different bat species (*Pipistrellus kuhlii*, *Hypsugo savi*, *Pipistrellus pipistrellus*, *Nyctalus noctula*, *Rhinolophus hipposideros*, *Tadarida teniotis*, *Vespertilio murinus*, *Plecotus auritus*, *Miniopterus schreibersii*, *Myotis blythii*, *Myotis myotis*, *Eptesicus serotinus*) were analysed between 2009–2015. We demonstrated that coronaviruses, both AlphaCoVs and Beta-CoVs, are widespread among Italian bat populations. The whole-genome sequence of two strains belonging to the clade 2c betacoronavirus related to MERSC-CoV was obtained and characterized. Bat lyssavirus infection has been detected in *Myotis myotis* and *Myotis blythii* during the active surveillance in two mixed colonies, the results will be presented by Leopardi et al., in the course of this same congress. The survey also provides evidence that insectivorous bats carry a wide variety of Mammalian orthoreoviruses (MRVs) with members of the type 3 mostly represented. Three representative MRVs belonging to serotype 1, 2 and 3 were selected and fully-sequenced through NGS. In particular, BatMRV3- 5515/2/IT2012 showed the highest similarity (99%) with a virus (SL-MRV01) recently detected from a child with acute gastroenteritis in Slovenia and its zoonotic potential deserves further investigation. Positivity for astroviruses and adenoviruses were also detected in fecal and organ samples, respectively. Results offer a preliminary dataset on the distribution of major viral infections in bats in Italy, an achievement so far never obtained, which improves our understanding on their spread and evolution. The project also proves to have enhanced the methods for detecting the viruses that may emerge from bats and has strengthened the cooperation between human and veterinary virologists and bat specialists.

Leo°S, Calamari L, Arrigoni°N, Garbarino°CA, Ric chi°M, Amadori°M

**Effect of paratuberculosis infection on welfare parameters of dairy cows : preliminary results**

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 2 p. (Poster 29P) [Nr. Estr. 7312]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

The aim of this study was to evaluate the immunological and metabolic conditions of cows infected by map, compared with healthy cows reared in the same herd, to verify the role of map infection as predisposing factor for other infectious and metabolic diseases occurring in the peripartum. for this purpose, we adopted a case-control study in which cases were defined as animals testing positive in serological elisa and/or fecal culture for map. the controls were selected among healthy animals of the same herd (testnegative and asymptomatic). blood samples of 13 positive and 13 negative cows were collected 5 times, at days -30, +3, +10, +30, +100 with respect to calving. a metabolic profile was measured in each sample, including positive and negative indicators of the acute phase response (app, i.e. haptoglobin, bilirubin, ceruloplasmin, albumin, cholesterol, paraoxonase). the app values were used to calculate the liver functionality index (LFI), which defines the inflammatory response during the first month of lactation. the cell-mediated immune response to map was investigated by an ifn-gamma release assay on whole blood samples. flow cytometry immunophenotyping was performed on isolated peripheral blood mononuclear cells (pbmc) using a panel of monoclonal antibodies to bovine monocytes and sub-populations of lymphocytes. we could not calculate the lfi of 4 cases out of 13, because they were culled before 30 days post partum. Reasons of early culling were left-displaced abomasum, milk fever and mastitis. The mean LFI was -3.15 and -2.84 for cases and controls, respectively, with no significant differences between the two groups under study; interestingly, lower values of ca, albumin and creatinine were measured in case animals. the most common abnormalities in the prevalence and fluorescence intensity of surface marker expression were a decreased prevalence of cd8+ t cells and mhci+ pbmc. some pbmc samples also showed an abnormal concentration of nkp46+ (nk) cells. no significant differences were observed between map-positive and control cattle. however, by including in the control group only the 5 LFI gamma-negative cows, increased prevalence of b cells and a reduced prevalence of cd45-positive pbmc were only observed in some map-positive animals. the negative LFI values highlight an altered metabolic profile in both groups. this was worse in the map-infected group, which also produced less milk. however, drug usage was greater in the control group, because map-negative cows were treated for metritis, mastitis and lameness, whereas the positive ones were culled as soon as they showed symptoms of illness or decreased milk production. a greater number of observations are badly needed though to corroborate the initial hypothesis and draw an inference as to a peculiar influence of map infection on welfare parameters and homeostasis of bovine pbmc.

Leo°S, Zago C, Garbarino C, Calza L, Cammi M, Pera zzi M, Idropici E, Arrigoni°N

**Applicazione delle linee guida per la paratubercolosi in un caseificio sociale produttore di Grana Padano**

Buiatria. - Vol. 2016). - 6 p. - 12 bib ref [Nr. Estr. 7432]

Congresso della Societa Italiana di Buiatria (SIB) : Montichiari (BS) : 8-9 Settembre 2016)

Following the approval of the "National guidelines for the control of bovine paratuberculosis and for assigning the health ranking of the herds" in 2013, the National Reference Centre for Paratuberculosis (PTNRC) promoted their adoption in the dairy cattle farms of Piacenza's Province, whose main production is the Grana Padano cheese. This activity, coordinated by PTNRC, actively involved dairy farmers, veterinary surgeons, Veterinary Health Service and Farmers' Association (ARAER). We described here the experience of paratuberculosis control and certification, carried out

in the period 2014-2016 in a cheese factory, involving 22 dairy herds, with an average of 176 adult cows per farm (from 20 to 380 cows over 24 months of age). During the first meeting, farmers were involved and motivated by explaining the expected advantages by joining the plan (lowering the losses due to the disease, qualification of herd productions such as milk, cheese and replacements). The key points of the intervention were: serological tests on all the cows over 24 months of age (paid by the dairy industry), risk assessment for introduction and transmission of paratuberculosis in each herd, coordinated by PTNRC in collaboration with veterinary practitioners and farmers' association (ARAER), periodic meetings involving all the stakeholders, to maintain the focus on the disease control plan. The adopted measures determined a dropping in sero-prevalence from 4,8% (187 positive animals out of 3890) to 3,2% (129 positives out of 3887). In 2015, after the updating of the health management plans, the first official health rankings were obtained: nowadays three farms are negative (PT2), 14 farms are classified as low risk (sero-prevalence < 5%, corresponding to PT1), and 5 farms result without clinical cases (PT0). The pivotal point for the success of the plan is the synergic cooperation of the stakeholders (practitioners, Public Health Services, farmers, dairy industry) through the creation of a work group, able to support the participation of the farmers in the program.

Locatelli C, Cremonesi P, Bertocchi L, Zanoni MG, Barberio A, Drigo I, Varisco G, Castiglioni B, Bronzo V, Moroni P

#### **Methicillin-resistant *Staphylococcus aureus* in bulk tank milk of dairy cows and effect of swine population density**

J Dairy Sci. - Vol. 99 (2016). - p 1-6. - 35 bib ref [Nr. Estr. 7193]

The methicillin-resistant *Staphylococcus aureus* (MRSA) has recently frequently been reported in dairy cattle, usually with low prevalence. The livestock-associated MRSA (LA-MRSA) ST398 is especially involved in cases of subclinical and clinical mastitis. Swine carry LA-MRSA without clinical symptoms and are considered its reservoir and shedder. People exposed to swine are particularly at risk of LA-MRSA colonization. Environments with relevant livestock density are a demonstrated risk factor for humans to be carriers of a LA-MRSA. This work investigated dairy farms located in an area with a high livestock density, mainly represented by swine. Bulk tank milk samples from 224 dairy farms were collected, and their status was defined as MRSA-positive or MRSA-negative based on culture on chromogenic medium. The number of fattening swine and of fattening swine herds was calculated in an area of 3 km around each dairy farm through georeferencing. The probability of a *Staphylococcus aureus*-positive dairy farm to be MRSA positive based on the extent of potential infective pressure due to swine density was calculated. Both the number of swine herds and the number of swine were associated with the MRSA status of dairy herds. The 9 MRSA isolated were typed by multi-locus sequence typing and spa-typing, and characterized for their virulence factors and antimicrobial resistance profiles. The ST and spa-types detected are consistent with those present in the Italian swine population. Virulence and resistance profiles are mostly consistent with the types detected. This work provides the first evidence of the epidemiological challenge exerted by the density of the swine population on MRSA in dairy cows.

Loglio G, Bassi S

#### **The examination of powdered sugar to detect *Paenibacillus* larvae infections in honeybee colonies**

The 7th European Conference of Apidology (EurBee) : Cluj - Napoca (Romania) 7-9 September 2016 / [s.l. : s.n., 2016]. - p 246 [Nr. Estr. 7458]

European Conference of Apidology (EurBee) (7th : Cluj - Napoca (Romania) : 7-9 September 2016)

The common strategy to control the American foulbrood is based on the application of correct

preventive measures and early clinical diagnosis. Nevertheless, some honeybee colonies can be infected by *Paenibacillus* larvae spores, even in very large number, without showing clinical symptoms of American foulbrood. The detection of such colonies is crucial for the application of effective preventive measures. In this work was evaluated the use of the powdered sugar to identify colonies with symptomatic or asymptomatic infections by *Paenibacillus* larvae. Twenty-eight honeybee colonies were included in the trial: seven colonies with American foulbrood symptoms (Group A), seven colonies without symptoms of disease but belonging to apiaries in which were present diseased colonies (Group B) and 14 colonies coming from an apiary where no cases of American foulbrood were reported in the last years -(Group C). The sugar was dusted on the top bars of brood combs, brushed between the frames and collected on a sheet of paper placed in the hive bottom. The sugar samples were examined with culture method. All the sugar samples from Group A were positive with a very high load of *Paenibacillus* larvae spores, comprised between 40,400 and 2,000,000 UFC/g. In Group B, *Paenibacillus* larvae spores were found in six colonies out of seven with values between 140 and 8,100 UFC/g. All the samples taken from Group C were negative. Based on the results of this preliminary trial the examination of the powdered sugar seems to be a good indicator of *Paenibacillus* larvae infection.

Loli\_Piccolominil L, Rangoni R, Santi°A, Bonfanti L, Marangon S, Finarelli AC, Natalini S

**Descrizione di un'epidemia di influenza aviare ad alta patogenicità H7N7 in Emilia- Romagna**  
= Description of an highly pathogenic H7N7 avian influenza epidemic in Emilia-Romagna Region  
Not Ist Superiore Sanita'. - Vol. 29 no 1 ( 2016). - p iii-iv. 6 bib ref [Nr. Estr. 7219]

During the 2013 summer an epidemic of highly pathogenic avian influenza (HPAI) H7N7 occurred in Emilia-Romagna region. This paper describes the epidemic, the results of surveillance on farms and in humans, the measures taken. The origin of epidemic was attributed to infection by wild ducks. Six outbreaks were identified and overall were culled about 1,5 million birds with total direct damage amounting to almost 15 million euro. Three human cases were detected.

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

#### **The importance of dry cow welfare for a healthier dairy herd**

Proceedings of the fourth DairyCare Conference 2016 "Lifelong sensing of health and welfare and big data and the internet of things" : Lisbon, October 13th and 14th 2016 / editor, C.H. Knight. - [s.l. : DairyCare COST Action FA1308, 2016]. - p 35 (P.13) [Nr. Estr. 7469]

DairyCare Conference (4th : Lisbon : October 13th and 14th 2016)

Management of the dry period has a key role in the protection of cow health and welfare. In fact, the dry period is a very important stage for the mammary gland health, for the success of the following lactation and for the prevention of post-partum diseases. In particular, the dry period can play an important role for the control of mastitis, that is still the most common and costly disease and the main cause of antibiotic consumption in the dairy industry. The aim of the present study was to assess the level of welfare of dry cows in Italy and to investigate its relationship with on-farm mortality of adult cows, bulk somatic cell count and number of antibiotic treatments for mastitis. The survey was conducted in 1432 Italian dairy farms, with an average size of 246 cows (range 7- 2736 cows) and a mean milk production of 27.8 kg/day per cow (range 10-41 kg/day per cow). The welfare of dry cows was assessed using management, resource and animal base measures listed in Table 1. In the investigated farms, the main welfare hazards linked to dry cows management were found to be: non-use of calving pen or use of a calving pen with poor hygienic conditions (26.96%), presence of dirty floors (17.88%) and poor bedding hygienic condition (11.38%). 20.95% of the investigated farms had an insufficient number of water points in the dry cow pen (less than 1 water bowl for 10 cows or less than 6 cm/cow of trough) and in 14.18% of the farms dry cows were

overstocked. Concerning animal based measures, 38.76% of the studied herds showed more than 20% of dirty animal, instead in 11.10% of the dairy farms more than 30% of the animals displayed integument alterations. Overall, 177 (12.36%) dairy farms were found with a scarce level of dry cow welfare and these herds show higher rates of mortality, BSCC and antibiotic treatments than the other studied herds (Figure 1). The obtained results underlie the importance of a good dry cow management and welfare for the safeguarding of dairy cows health.

Lucchese L, Boniotti<sup>°</sup>B, D'Incau<sup>°</sup>M, Furlanello T, Mazzotta E, Ceglie L, Marchione S, Bertasio<sup>°</sup>C, Natale A

**Isolation and typing of *Leptospira* strains : a three-year passive surveillance on suspected clinical cases in dogs**

27th ECVIM-CA, European College of Veterinary Internal Medicine Congress 2016, 8th-10th Settembre 2017 [i.e. 2016], Goteborg, Sweden / [s.l. : s.n., 2016]. - 7489]

European College of Veterinary Internal Medicine Congress (ECVIM-CA) (27th : Goteborg, Sweden : 8th-10th Settembre 2016)

Leptospirosis is a widespread zoonotic disease which diagnosis is traditionally based on serological tests. The more recent development of PCR techniques allow the direct detection of Leptospiral DNA on clinical samples, but not the targeting of serovar/genospecies. The molecular genotyping (MLST, VNTR) can characterize the strains, but in most cases these techniques are effective only after strain isolation. Isolation is therefore still of primary importance, despite it is difficult, time-consuming and requiring specialised laboratories. An increasing interest on Leptospirosis in recent years led to the commercialization in Europe of vaccines including serovars Australis and Grippotyphosa as antigens in addition to Icterohaemorrhagiae and Canicola. Studies report the reemergence of the infection, but results usually lack the support of the strains isolation. From 2013 to 2016, the collaboration between the IZSVE and Italian veterinary practitioners allowed the collection of samples from 329 suspected cases of Leptospirosis in dog. The inoculums of urine in culture medium were performed in sterile conditions by the clinicians and sent to the laboratory. A real-time PCR targeting a portion of the pathogenic leptospire gene rrs (16S) was performed on refrigerated urine or whole blood as well as the microscopic agglutination test (MAT) on serum for the antibodies detection when available. Among the 329 cases, 42 were confirmed by PCR and 78 by serology (titre >1:400); in 8 cases the isolation of *Leptospira* strains from urine had been possible. The cultured strains were identified by means of serotyping and genotyping at the IZSLER. The genotyping based on MLST and VNTR profiles identified 6 of the strains as *L. interrogans* serogroup Icterohaemorrhagiae, 1 as *L. kirschneri* serogroup Pomona serovar Mozdock, 1 as *L. interrogans* serogroup Australis. The serotyping identified the same serogroups, with a complete concordance. Serology, when present (5 out of 8), revealed a positivity with the highest titre against the identified etiological serogroup except in one case. The 6 dogs positive for Icterohaemorrhagiae were not or irregularly vaccinated. The two dogs non-Icterohaemorrhagiae resulted regularly vaccinated with the bivalent vaccine for *Leptospira*. Icterohaemorrhagiae and Australis confirmed their role in the clinical leptospirosis in dog and the importance of a correct vaccination to prevent the symptoms, while Pomona appeared as a risk for dogs and the absence of an effective vaccine protection suggests that leptospirosis should be taken into account in the differential diagnosis of any inflammatory illness, even when dogs are regularly vaccinated.

Luppi<sup>°</sup>A, Bonilauri<sup>°</sup>P, Dottori<sup>°</sup>M, Rosina S, Casap<sup>°</sup>pa P, Rugna<sup>°</sup>G, Krejci R, Mazerolles P, Maioli<sup>°</sup>G, Catelli E, Meriardi<sup>°</sup>G, Martelli P

**Survey of pulmonary lesions and pleuritis in slaughter-aged pigs in Italy**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - 1 p (poster PO-PW1-021) [Nr. Estr. 7618]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Luppi° A, Bonilauri° P, Maioli° G, Gherpelli° Y, Do ttori° M

### **Resistenza agli antibiotici in ceppi di Streptococcus suis isolati nel suino nel periodo 2004-2014**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 133-137. - 7 bib ref [Nr. Estr. 7230]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Trecento-ventiquattro ceppi di Streptococcus suis isolati da casi di streptococcosi nel suino in Italia, nel periodo 2004-2014, sono stati sottoposti alla valutazione della sensibilita a diversi antibiotici impiegando il metodo della disco diffusione (Kirby-Bauer). I ceppi sono stati ana-lizzati per la loro suscettibilita a 8 antimicrobici: lincomicin (2 jig), tilosina (30 jig), ampicillina (10 jig), penicillina (6 jig), tetraciclina (30 jig), trimetoprim-sulfametossazolo (1,25/23,75 jig), cefalexina (30 1.1g) e ceftiofur (30 jig). La maggior parte degli isolati erano sensibili alla ampicillina, penicillina, amoxicillina + acido clavulanico, cefalexina e ceftiofur, mentre bas-si livelli di sensibilita sono stati registrati per lincomicina, tilosina e tetraciclina. Il tasso di suscettibilita dei ceppi S.suis al trimetoprim-sulfametossazolo ha mostrato un certo grado di variabilita a seconda dell'anno di isolamento. Le tendenze relative alla suscettibilita dei ceppi di S.suis isolati nel periodo 2004-2014 ai singoli antimicrobici, non hanno mostrato alcuna variazione statisticamente significativa nel periodo in esame.

*The antimicrobial susceptibility of 324 Streptococcus suis strains isolated from diseased pigs in Italy in the period 2004-2014 was determined by disc diffusion method (Kirby-Bauer). The strains were analysed for their susceptibility to 8 antimicrobials: lincomycin (2 jig), tylosin (30 jig), ampicillin (10 jig), penicillin (6 jig), tetracycline (30 jig), trimethoprim-sulfamethoxazole (1,25/23,75 jig), cephalexin (30 jig) and ceftiofur (30 jig). Most of the S.suis isolates were susceptible to ampicillin, penicillin, amoxicillin+clavulanic acid, cephalexin and ceftiofur, while very low levels of sensitivity were recorded for lincomycin, tylosin and tetracycline. The rate of susceptibility of S.suis strains to trimethoprim-sulphamethoxazole showed a certain degree of variability according to the year of isolation. The trends in susceptibility of S.suis strains isolated from 2004 to 2014 to individual antimicrobials did not show any statistically significant variation over the period considered.*

Luppi° M, Gibellini MV, Gin T, Vangroenweghe F, Van denbroucke V, Bauerfeind R, Bonilauri° P, Labarque G, Hidalgo A

### **Prevalence of virulence factors in enterotoxigenic Escherichia coli isolated from pigs with post-weaning diarrhoea in Europe**

Porcine Health Manag. - Vol. 2 ( 2016). - no 20 (6 p). - 22 bib ref [Nr. Estr. 7352]

Post-weaning diarrhoea (PWD), due to Escherichia coli, is an important cause of economic losses to the pig industry primarily as a result of mortality and worsened productive performance. In spite of its relevance, recent data about the prevalence of virulence genes and pathotypes among E. coli isolates recovered from cases of PWD in Europe are scarce. Results This study investigates the prevalence of fimbrial and toxin genes of E. coli by PCR among 280 farms with PWD across Europe. A total of 873 samples collected within the first 48 h after the onset of PWD (occurring 7–21 days post weaning) were submitted to the laboratory for diagnostic purposes. Isolation and identification of E. coli were performed following standard bacteriological methods and PCR assays for the detection of genes encoding for fimbriae (F4, F5, F6, F18 and F41) and toxins (LT, STa, STb and Stx2e). The prevalence of fimbriae and toxins among E. coli isolates from cases of PWD was: F4 (45.1 %), F18 (33.9 %), F5 (0.6 %), F6 (0.6 %), F41 (0.3 %), STb (59.1 %), STa (38.1 %), LT (31.9 %) and Stx2e (9.7 %). E. coli isolates carrying both fimbrial and toxin genes were detected in 52.5 % of the cases (178 out of 339 isolates), with 94.9 % of them being classified as enterotoxigenic E. coli (ETEC). The most common virotype detected was F4, STb, LT. Conclusions This study confirms that ETEC is frequently isolated in pig farms with PWD across Europe, with F4-

and F18-EPEC variants involved in 36.1 % and 18.2 % of the outbreaks, respectively.

Maisano°AM, Lorenzi°V, Angelucci°A, Fusi°F, Rom anò°A, Spelta°C, Vezzoli°F, Bertocchi°L, Luini°M

#### **Diagnosi di mastite da Staphylococcus aureus : metodi di campionamento a confronto**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 218-219. - 3 bib ref [Nr. Estr. 7370]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*The present study aimed at evaluating the efficacy of individual composite milk samples analysis compared to quarter milk samples analysis to find a cheaper and time-saving method for the detection of Staphylococcus aureus intra-mammary infections (IMI). 2644 quarter milk samples (aseptically collected) and 661 composite milk samples (not aseptically collected) were obtained from 661 cows in 5 herds. quarter milk samples analysis, considered as the gold standard, was able to detect 236 cows with IMI, while the analysis of composite milk samples identified 229 infected cows. Methods concordance was 95% with an excellent Cohen's  $\kappa$  (0.89). Sensitivity (Se) and specificity (Sp) of the composite milk samples were  $91.5 \pm 2.1$  and  $96.9 \pm 1.3$  (IC 95%), respectively. In addition, the Se of the composite samples grew as the number of infected quarter for cow increased. Composite milk sampling was found to be a reliable and feasible screening tool for the identification of S. aureus infected cows.*

Marques AT, Lecchi C, Grilli G, Giudice C, Nodari° SR, Vinco°LJ, Cecilianì F

#### **The effect of transport stress on turkey (Meleagris gallopavo) liver acute phase proteins gene expression**

Res Vet Sci. - Vol. 104 ( 2016). - p 92-95. - 25 bib ref [Nr. Estr. 7222]

The aim of this study was to investigate the effects of transport-related stress on the liver gene expression of four acute phase proteins (APP), namely  $\alpha$ 1-acid glycoprotein (AGP), C-Reactive Protein (CRP), Serum Amyloid A (SAA) and PIT54, in turkeys (Meleagris gallopavo). A group of seven BUT BIG 6 commercial hens was subjected to a two-hour long road transportation and the quantitative gene expression of APP in the liver was compared to that of a non transported control group. The expression of AGP and CRP mRNA was found to be increased in animals slaughtered after road transport. The presence of AGP protein was also confirmed by immunohistochemistry and Western blotting. The results of this study showed that road-transport may induce the mRNA expression of immune related proteins. The finding that AGP and CRP can be upregulated during transport could suggest their use as for the assessment of turkey welfare during transport.

Martelli P, Saleri R, Ferrarini G, De\_Angelis E, Cavalli V, Benetti M, Ferrari L, Canelli E, Bonilauri°P, Arioli E, Caleffi A, Nathu es H, Borghetti P

#### **Impact of maternally derived immunity on piglets' immune response and protection against porcine circovirus type 2 (PCV2) after vaccination against PCV2 at different age**

BMC Vet Res. - Vol. 12 ( 2016). - no 77 (12 p). - 32 bib ref ( ultimo accesso 28/06/2016 <https://bmcvetres.biomedcentral.com/articles?query=saleri&volume=12&searchType=&tab=keyword> ) [Nr. Estr. 7286]

Background This study was aimed at evaluating the clinical protection, the level of Porcine circovirus

type 2 (PCV2) viremia and the immune response (antibodies and IFN- $\gamma$  secreting cells (SC)) in piglets derived from PCV2 vaccinated sows and themselves vaccinated against PCV2 at different age, namely at 4, 6 and 8 weeks. The cohort study has been carried out over three subsequent production cycles (replicates). At the start/enrolment, 46 gilts were considered at first mating, bled and vaccinated. At the first, second and third farrowing, dams were bled and re-vaccinated at the subsequent mating after weaning piglets. Overall 400 piglets at each farrowing (first, second and third) were randomly allocated in three different groups (100 piglets/group) based on the timing of vaccination (4, 6 or 8 weeks of age). A fourth group was kept non-vaccinated (controls). Piglets were vaccinated intramuscularly with one dose (2 mL) of a commercial PCV2a-based subunit vaccine (Porcilis® PCV). Twenty animals per group were bled at weaning and from vaccination to slaughter every 4 weeks for the detection of PCV2 viremia, humoral and cell-mediated immune responses. Clinical signs and individual treatments (morbidity), mortality, and body weight of all piglets were recorded. Results All vaccination schemes (4, 6 and 8 weeks of age) were able to induce an antibody response and IFN- $\gamma$  SC. The highest clinical and virological protection sustained by immune reactivity was observed in pigs vaccinated at 6 weeks of age. Overall, repeated PCV2 vaccination in sows at mating and the subsequent higher levels of maternally derived antibodies did not significantly interfere with the induction of both humoral and cell-mediated immunity in their piglets after vaccination. Conclusions The combination of vaccination in sows at mating and in piglets at 6 weeks of age was more effective for controlling PCV2 natural infection, than other vaccination schemas, thus sustaining that some interference of MDA with the induction of an efficient immune response could be considered. In conclusion, optimal vaccination strategy needs to balance the levels of passive immunity, the management practices and timing of infection..

Martinelli° C, Giovannini° S, Salogni° C, D'Incau° M, Bonardi S, Brindani F, Birbes° L, Terrini° A, Acquarone F, Guadagnini M, Pasquali P, Alborali° GL

#### **Caratterizzazione genomica e profili di antibiotico resistenza di E.coli isolati da bovini con forme enteriche**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 133-134. - 5 bib ref [Nr. Estr. 7366]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*In this study we investigate distribution of virulence genes and antibiotic resistance profile of E.coli strains isolated from calves during 2006-2015. The virulence gene cadine F5 was found in 57 isolates (42,86%). All 133 E. coli strains were MDR (Multidrug Resistant). E.coli FS positive were more resistant to flumequine, danofloxacin, enrofloxacin and amoxlcillin than FS-negative strains. FS-positive strains isolated from carcasses were more amoxicillin+clavulanic acid resistant than F5-positive strains from faeces; FS-negative strains from fecal samples, on the contrary, were more cefaloridine e apramicin resistant than FS-negative strains from carcasses.*

Mescolini G, Lupini C, Bellinati C, Felice V, Listorti V, Massi° P, Tosi° G, Rossi G, Presente P, Cecchinato M, Catelli E

#### **Epidemiologia molecolare del virus della malattia di Marek in Italia nel 2014-2016**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 47 (Poster 10) [Nr. Estr. 7413]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

La Malattia di Marek 6 una patologia del polio a carattere linfoproliferativo, diffusa a livello mondiale e causata dal Gal/id alphaherpesvirus 2 (denominato anche MDV) in cui si riconoscono diversi patotipi (mild, virulent, very virulent e very virulent plus). Essa si manifesta con diverse forme cliniche, fra cui le pit) rilevanti sono la "classica" e l'"acuta". A tutt'oggi dati relativi alla

caratterizzazione dei ceppi MDV circolanti in Italia sono carenti. 18 ceppi MDV, evidenziati nel periodo 2014 -2016 in allevamenti intensivi (n.8 — in corso forma "acuta") e rurali (n.8 — in corso di forma "classica" e n.2 - in corso di forma "acuta"), sono stati caratterizzati dal punto di vista molecolare tramite sequenziamento e analisi del gene meq. Tale gene 6 il principale oncogene virale ed alle sue caratteristiche molecolari, in particolare al numero di ripetizioni di 4 proline (PPPP) nel dominio di transattivazione della proteina, sembra essere correlata la virulenza. Il gene meq 6 è stato amplificato mediante PCR e sequenziato dopo estrazione del DNA virale da penne o organi di animali colpiti. Le sequenze nucleotidiche e amminoacidiche sono state allineate e confrontate, mediante il software Clustal W, con sequenze omologhe di ceppi MDV a diversa patogenicità, presenti in GenBank. L'analisi filogenetica è stata condotta utilizzando l'algoritmo Neighbor-Joining. I ceppi evidenziati in corso di forma "classica" formavano un unico cluster e presentavano percentuali di identità nucleotidica alte con patogeni mild o attenuati. I ceppi evidenziati in corso di forma "acuta" invece si raggruppavano in un altro cluster, con alcuni isolati polacchi, e presentavano elevata identità nucleotidica con ceppi virulenti, very virulent e very virulent plus. In conclusione ceppi MDV ad elevata virulenza, già segnalati in Europa, circolano anche nel nostro paese soprattutto nel settore avicolo industriale, nonostante la vaccinazione sia eseguita di routine in tutte le categorie produttive. Sporadicamente questi virus sono stati evidenziati, per superamento delle barriere di biosicurezza, anche nel settore rurale dove sembrano invece prevalenti ceppi a bassa virulenza associati a forme "classiche".

Miarelli M, Drumo R, Signorelli F, Marchitelli C, Pavone S, Pesciaroli M, Ruggieri° J, Chirullo B, Ammendola S, Battistoni A, Alborali°GL , Manuali E, Pasquali P

#### **Salmonella Typhimurium infection primes a nutritive mechanism in piglets**

Vet Microbiol. - Vol. 186 ( 2016). - p 117-125. - 36 bib ref [Nr. Estr. 7225]

*Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) is an important cause of acute food-borne zoonoses worldwide, typically carried by pigs. It is well known that *Salmonella* has evolved a wide array of strategies enabling it to invade the host, but little information is available on the specific host responses to *Salmonella* infections. In the present study, we used an in vivo approach (involving piglets infected with a virulent or an attenuated *S. Typhimurium* strain) coupled to histological and proteomic analysis of the cecum mucosa, to highlight the host pathways activated during *S. Typhimurium* infection. We confirm the complex host-pathogen interaction. Our data showed that the metabolic and the cytoskeleton organization functions were the most significantly altered. In particular, the modifications of energy metabolic pathway could suggest a "nutritive" mechanism, in which the host reduces its metabolic and energetic status to limit *Salmonella* infection. This study could represent a preliminary approach, providing information useful to better understand the host-*Salmonella* interaction.

Mignone G, Lazzara F, Vencia W, Vito G, Ferrari A, Amadori°M, Razzuoli E  
**Gut innate immune response to cadmium exposure**

Atti Convegno Nazionale della Società Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 2 p. - 4 bib ref [Nr. Estr. 7310]

Convegno Nazionale della Società Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Cadmium (Cd) is a toxic and carcinogenic heavy metal widely distributed in the environment. The ingestion of contaminated food and drinking water is the major source of exposure to Cd for humans and animals and the gastrointestinal tract is the first target of interaction. The toxicity of Cd is related

to its ability to modulate the activity of cellular enzymes, to initiate oxidative stress, to suppress mitochondrial functions and disrupt calcium homeostasis. However, little is known about Cd interaction with the intestinal tract (1). Owing to the above, the aim of our study was to investigate the effects of Cd on innate immunity using swine jejunal IPEC-J2 cells (2). Cells were seeded into 12-well tissue culture plates (2 mL per well,  $2 \times 10^5$  cells/mL) and incubated at 37°C in 5% CO<sub>2</sub> until confluence. Cells were then treated for 3 hours with 1  $\mu$ M and 10  $\mu$ M Cd solutions at 37°C in 5% CO<sub>2</sub>. We tested five wells for each concentration and untreated wells were used as negative control. Total RNA was extracted and following the reverse transcription step (2) the change in mRNA expression profiles of porcine cytokines IL-1 $\beta$ , IL-6, IL-8, Nf- $\kappa$ B1, Nf- $\kappa$ B-p65, MYD88, IL-18, IFN- $\beta$ , P38,  $\beta$ 2-M, TLR4, TLR5, MD2, CD14, TNF- $\alpha$ , bD1, bD2, bD3, bD4, JNK, STAT3 and SOCS1 was investigated using primer sets described in previous studies (2). HPRT1 and GAPDH were used as housekeeping control genes (3). In each sample of IPEC-J2 cells, the relative expression of the selected genes was calculated using the formula  $Ct = Ct(\text{target gene}) - Ct(\text{housekeeping})$ . The average intensity of expression (ct sample - Ct negative control) of the genes under study was compared among treatment groups by one-way analysis of variance (ANOVA). The threshold for significance was set at  $P < 0.05$ . In cells treated with 1  $\mu$ M Cd we showed a significant increase ( $P < 0.05$ ) of IL-6, IL-8, Nf- $\kappa$ B1 and Nf- $\kappa$ B-p65 gene expression and down-regulation of TNF- $\alpha$  expression ( $P = 0.002$ ). These data are in agreement with previous studies (1) and highlight a pro-inflammatory effect of low concentrations of Cd. Concerning the treatment with 10  $\mu$ M Cd we observed up-regulation ( $P < 0.05$ ) of bD1, bD2, bD3, IFN- $\beta$ , IL-18, TNF- $\alpha$  and  $\beta$ 2-M and down-regulation of IL-8, Nf- $\kappa$ B1 and STAT3 gene expression. These results suggest activation of the Type I IFNs system; in particular we observed an IFN- $\beta$  response after treatment with 10  $\mu$ M of Cd. Moreover, we also observed up-regulation of  $\beta$ 2-M, indicated as an in vivo marker of Cd exposure in previous studies (4). In conclusion, our results support the hypothesis that Cd exposure may modify the basal level of cytokine expression; specifically, different concentrations of this heavy metal seem to influence different compartments of the innate immune response. These data confirm the ability of non-infectious stressors to modulate innate immunity; hence, they might cause an alteration of gut interaction with bacteria.

Mignone G, Lazzara F, Vencia W, Vito G, Ferrari A, Bozzetta E, Amadori M, Razzuoli E

#### **Esposizione al cadmio e modulazione dell'immunità intestinale : studi in vitro**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 81-82. - 4 bib ref [Nr. Estr. 7363]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*Foodborne is one of the main source of cadmium (Cd) exposure in animal and humans; the gastrointestinal tract (GIT) is the first target, but few data on Cd/enterocytes interaction, are available to date. Here, the aim was to investigate the Cd effects on innate immunity using, as experimental model, swine jejunal IPEC-12 cell. Cells were treated with Cd solutions (111M and 10 $\mu$ M for 3 hours). Changes in mRNA expression profiles of important porcine cytokines were studied by RT-Real Time PCR. ELISA assay was used to evaluate the IL-8-release. Cell Immunomodulation in a model of Salmonella Typhimurium infection was also verified. Our results confirm the important role of Cd, acting as non-infectious stressor, in the modulation of the innate-immunity. Furthermore, the obtained data show a decrease, induced by Cd, of the 5. Typhimurium celi invasion potential. In conclusion, it's possible to suggest that this heavy metal is able to alter the interaction GIT/normal microbial population.*

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

#### **The fourth state of matter and its biocidal effect : plasma vs coccidia**

XXIX Congresso Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary

Parasitology College "Parasites, Poverty and Social commitment" : Bari, June 21-24, 2016 / [s.l. : s.n., 2016]. - p 133 [Nr. Estr. 7480]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College (29. : Bari : June 21-24, 2016)

*Eimeria* spp. are obligate intracellular parasites, the oocysts are. their environmental resistance forms. The oocysts wall is extremely robust, it is resistant to mechanical and chemical damage. In this regard *Eimeria* spp. can serve as the prototype of pathogen elements with a very high resistance similar, in this aspect, to *Toxoplasma gondii* and *Cryptosporidium parvum* important zoonotic agents\_ Therefore, the occasion to test a non-chemical agent appear to be an interesting interdisciplinary opportunity. Dielectric Barrier Discharge (DBD) is a non-thermal plasma, that could be also used for sanitization purpose. The synergy of energetic electrons and ions, reactive species (such as atomic oxygen, ozone and nitric oxides and hydrogen peroxide), UV photons and intense electric fields is a key point in non-thermal plasma sanitization treatment. The efficacy of other kinds of plasma technology has already been demonstrated against bacteria, spores, virus, yeasts/moulds and parasites. Petri dishes filled with 2.0 ml of water containing *Eimeria* spp. oocysts have been subjected to different DBD direct treatments and exposure times. After the treatment, the efficacy has been evaluated by the sporulation capacity of coccidia compared with untreated specimen. The number of sporulated and damaged oocysts has been counted considering a representative population of 100 oocysts both in control and in treated samples. Preliminary results have shown a statistically significant decrease of sporulated oocysts with long exposure times and high discharge voltages. Absence of possible resistance phenomena and pollution linked to the perspective of a good devitalization level is an incentive to go on in this way.

Moreno<sup>o</sup> A, Lelli<sup>o</sup> D, Beato MS, Chiapponi<sup>o</sup> C, Cavicc hio L, Leopardi S, De\_Benedictis P, Lavazza<sup>o</sup> A

#### **First identification of Mammalian orthoreovirus type 3 in diarrheic pigs in Europe**

10th Annual Meeting Epizone "Going viral" : 27-29 September 2016, Madrid, Spain : programme and abstracts / [s.l. : s.n., 2016]. - p 40-41 (Oral 10) [Nr. Estr. 7323]

Annual meeting Epizone (10th : Madrid, Spain : 27-29 September 2016)

Mammalian orthoreoviruses (MRVs) have long be considered non-pathogenic, although mild respiratory and enteric diseases have occasionally been reported in young animals and children. Several evidences have recently shown that MRVs can cause severe diseases. Cases of neonatal diarrhea and neurological signs in children were associated with both MRV2 and MRV3 in Europe and North America. MRV3 has been recently isolated from piglets with severe diarrhea and respiratory signs in China, Korea and the US, also in association with Porcine Epidemic Diarrhea (PEDV), Transmissible Gastroenteritis (TGEV) and Porcine A-C rotaviruses. In particular, MRV3 was proven to be pathogenic to pigs. In 2015, an important epidemic of PED occurred in Italy caused by S-INDEL strains very closely related to each other and to the US Ohio851 strain. A first attempt to isolate PEDV using VERO cells was conducted collecting 11 swine fecal samples at the beginning of the epidemic, between February and March 2015. Cytopathic effect (CPE) was detected after the first cell passage in one sample. Cell culture supernatant was examined through Electron Microscopy (EM) and RNA was extracted from cell culture and fecal sample and analyzed for MRV using RT-PCRs targeting the L1 and S1 genes. The EM examination of CPE positive cell culture and molecular analyses confirmed the presence of MRV. Full genome sequencing of the isolated virus (MRV3/Sw/It/224660-4/2015) was conducted using an Illumina MiSeq platform. Based on S1 phylogeny, the novel swine MRV strain belonged to the lineage III of the MRV3 and was closely related to human and bat strains and two US porcine MRV3s recently described as associated to PED outbreaks (Figure 1). It share the highest nucleotide identity with MRV3 SI-MRV01 (98,4%) detected from a child with acute gastroenteritis in Slovenia and with a bat isolate T3/Pipistrellus Kuhlii/It/5515-2/2012 (98,2%). The S2 and S3 phylogeny indicated monophyletic groups with US and Chinese pig MRV3 strains and human T1L whereas the S4 revealed a separated group formed by Italian, US and Chinese pig MRV3 strains. The other segments L1, L3, M1, M3, S2, S3 and S4 of the Italian strain were related to US porcine MRV3. Interestingly, the last segments L2 and M2 were

closely related to MRVs of bat origin, MRV3 and MRV1 respectively. We here describe the finding of a MRV3 associated with a PED outbreak in Italy. A similar association was reported in the US during the 2013-2015 PED epidemic, with mortality up to 100% in affected farms. Porcine MRV3s, placed in lineage IV and frequently associated with other enteric viruses, were also described in pigs suffering diarrhea in South Korea. To highlight that Italian and US porcine MRV3 associated to PED outbreaks were characterized by a S1 gene highly related to European bat strains and both fall into lineage III, differently from the Asian MRV3 porcine isolates. The study of potential synergic effects between PEDV and MRV3 is crucial, considering the PED impact on the swine industry. Based on the L2 and S1 genetic distances, it appears that the swine and bat Italian MRV3 are highly correlated. Such evidence arises questions on the epidemiological link between pigs and Kuhl's pipistrelle common in urban environments. The absence of data on the MRVs distribution and genetic characteristics in Europe prevents any hypothesis on the most likely epidemiological links between bats, pigs and humans. The distribution of MRV3 among pigs and bats could probably be widespread in Europe, although it still needs to be further investigated.

Moreno<sup>o</sup> A, Lelli<sup>o</sup> D, Cela E, De\_Sabato L, Lavazza<sup>o</sup> A, Ciccozzi M, Sozzi<sup>o</sup> E, Vaccari G

### **Detection and full genome sequencing of Beta-CoV viruses related to Middle East Respiratory Syndrome from two bats in Italy**

10th Annual Meeting Epizone "Going viral" : 27-29 September 2016, Madrid, Spain : programme and abstracts / [s.l. : s.n., 2016]. - p 98 (Poster 10) [Nr. Estr. 7325]

Annual meeting Epizone (10th : Madrid, Spain : 27-29 September 2016)

Since the early 70's a variety of pathological conditions in domestic and wild animals has been attributed to Coronaviruses (CoVs) infections. Currently six different CoV strains are known to infect humans. Two of these belonged to Beta-CoV genus, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), and caused severe respiratory diseases with case fatality rates of 9% and 36% respectively. The evolutionary origin of SARS-CoV, which was firstly detected in 2002, involved bat hosts, possibly with civets as intermediate host and the source of human infection. The origin of the MERS-CoV is currently not well known although more recent studies point for camels as possible reservoirs or intermediate hosts. Bats have also been suspected as the source of MERS-CoV because of genetic likenesses between Beta-CoV found in bats and the MERS virus in humans. Recently, a CoV originated from an South African bat (SA bat) was identified as highly related to MERS-CoV, and a fragment of a CoV showing 100% of identity to MERS-CoV was found in a fecal sample from an Egyptian tomb bat (*Taphozous perforatus*). In this study, we described the detection and full genome sequencing of CoVs from two Italian bats (IT bats) of different species, *Pipistrellus kuhlii* and *Hypsugo savii*. Bat carcasses were obtained from the Modena Wildlife Recovery Center (North Italy). During necropsy, no macroscopic lesions suggesting infectious diseases but traumatic injuries were observed. Pools of viscera (lung, heart, spleen and liver) and intestine were tested separately using a pan-coronavirus one-step RT-PCR, which used neoCoV specific primers that amplified 180bp of the RdRp gene. For phylogenetic analysis, full genome sequencing was conducted directly on pan-coronavirus positive intestines using the Ion Torrent NGS technology. Complete genome sequences of MERS-CoV and Alfa- and Beta-CoVs from bats, human and camels (n. 131) were downloaded from ViPR system and a multiple sequence alignment was calculated using the MUSCLE algorithm. Maximum likelihood phylogenetic tree was performed using the ViPR implementation of the RAxML algorithm with the GTR model. Genetic relationships between Italian and SA bats and MERS-CoV were confirmed by comparison of the sequence distances of MERS-CoV and bat-BCoV 2c (SA bat and IT bats) using SSE v1.2. IT bats BCoVs showed 80% overall nucleotide identity across the whole genome to MERS-CoV. The nucleotide distances respect to MERS-CoV were higher in the genomic region encoding the S protein for all bat sequences including SA bat and in the ORF3 and ORF5 coding regions for the Italian sequences (fig.1A). Phylogenetic tree of the complete genome showed a monophyletic group formed by MERS sequences originated from humans and camels that are related to bat sequences. The most related sequences are those originated from SA, Italy and China (fig. 1B). Bats, with extensive geographical

distribution and capability of flight have been documented as natural hosts of large number of diverse viruses such as lyssaviruses, paramyxoviruses and filoviruses. The genetic diversity of CoVs in bats exceeds that known for other hosts, which is compatible with bats being the major reservoir of mammalian CoVs. The emergence of MERS-CoV probably involved genetic exchanges between different viral ancestors that may have occurred either in bat ancestors or in camels acting as mixing vessels for viruses from different hosts.

Moreno LZ, Silva GFR, Gomes VTM, Matajira CEC, Silva APS, Mesquita RE, Lotto NP, Ferreira TSP, Christ APG, Sato MIZ, Gherpelli<sup>o</sup> Y, Dottori<sup>o</sup> M, Bonilauri<sup>o</sup> P, Luppi<sup>o</sup> A, Moreno AM

**Application of protein profiling of virulent *Haemophilus parasuis* by MALDI-TOF mass spectrometry**

J Infect Dev Countr. - Vol. 10 no 6 ( 2016). - p 678-681. - 15 bib ref [Nr. Estr. 7315]

Mulatti P, Monne I, Vieira JT, Dorotea T, Terregino C, Lorenzetto M, Loli\_Piccolomini L, Santi<sup>o</sup> A, Massi<sup>o</sup> P, Bonfanti L, Marangon S

**H7N7 highly pathogenic avian influenza in poultry farms in Italy in 2016**

10th Annual Meeting Epizone "Going viral" : 27-29 September 2016, Madrid, Spain : programme and abstracts / [s.l. : s.n., 2016]. - p 132 (Poster 40) [Nr. Estr. 7327]

Annual meeting Epizone (10th : Madrid, Spain : 27-29 September 2016)

Following the 1999-2000 H7N1 Highly Pathogenic (HP) Avian Influenza (AI) epidemic, involving over 16 million birds, no HP viruses were detected in domestic poultry in Italy until 2013. In August 2013 a H7N7 HPAI virus was identified in a free-range layer farm belonging to a large production company with numerous farms in several Italian regions. The affected farm was located in close proximity to important resting sites for migratory wild birds. The prompt adoption of strict control measures limited the spread of the disease, preventing the virus from spreading to neighbouring Densely Populated Poultry Areas (DPPAs). Only 6 farms were infected and the epidemic was contained in less than a month. After an interval of over a year, a H5N8 HPAI virus was isolated in a single fattening turkey farm in December 2014, in proximity to the same wetland areas as the 2013 outbreaks. The virus was closely related to the H5N8 strain that circulated in wild birds in Europe in 2014-2015, with repeated incursions into domestic poultry. On 30 April 2016, a H7N7 HPAI virus was confirmed in an organic free-range layer farm located in the same area as the 2013 outbreak. Control measures provided in Directive 94/2005/CE were promptly applied, and enhanced surveillance activities were implemented in the DPPAs. Epidemiological data and phylogenetic analyses indicated that the virus likely originated from wild birds as a Low Pathogenicity (LP) AI strain, introduced into the farm through direct contacts. Phylogenetic analyses indicated the H7N7 HPAI virus regrouped with viruses belonging to different subtypes identified in wild and domestic birds in Europe, Africa and Asia between 2009 and 2015. The haemagglutinin (HA) gene had low genetic similarity with other HPAI viruses previously identified in Italy and Europe, and the H7N7 LP AI virus detected on 15 April 2016 in an ornamental birds farm located in the same province as the outbreak (sequence similarity < 93%). Further analyses of HA sequences showed the co-circulation of at least two distinct highly pathogenic viruses with a different insertion at the HA cleavage site. Moreover, the HA cleavage site motif typical of an LP AI virus was identified in swabs collected from live birds. A HPAI H7N7 virus was also identified on 16 May 2016 in a fattening turkey farm located within the Protection Zone of the previous outbreak. The epidemiological investigation did not allow identification of the possible source of the virus, and contact tracing led to the preventive culling of a fattening turkey farm belonging to the same production company and located within the Protection Zone. Control measures included enhanced surveillance in turkey farms belonging to the same company. In the phylogenies of the eight gene segments, the virus resulted closely related to the H7N7 HPAI virus identified during the previous outbreak. The limited number of HPAI outbreaks recorded in Italy after the large epidemic of 1999-2000 points to an improved coordination of efforts to contain the spread of AI in the poultry sector, possibly in addition to a shift in the ecology and dynamics of the disease.

However, the occurrence of repeated introductions of both HPAI and LPAI strains from wild birds, along with the potential for successive mutation within domestic poultry, highlights the need of a targeted improvement of biosecurity, and the importance of developing a surveillance framework to enable prompt detection of asymptomatic AI infections in wild birds before spillover can threaten domestic farms.

Natale A, Lucchese L, Boniotti<sup>°</sup>MB, D'Incau<sup>°</sup>M, Fur lanello T, Ceglie L, Guerrini E, Marchione S, Bertasio<sup>°</sup>C, Gagliazzo L, Toson M, Adler B

**Trying to better understand the epidemiology of leptospirosis in dogs : strain genotyping**

Vetpath 2016 : Prato, Italy, 11-14 October 2016 / [s.l. : s.n., 2016]. - p 4 [Nr. Estr. 7490]

Vetpath : Prato, Italy : 11-14 October 2016)

Leptospirosis is a widespread zoonosis. Different *Leptospira* serovars are prevalent in many countries and are correlated to disease in dogs, but it is difficult to evaluate their pathogenicity and to describe clinical signs related to specific serovars, due to the multiple cross-reactions seen with the MAT serological screening and to the inability of current molecular diagnostic techniques to identify the *Leptospira* serovar. The development of molecular genotyping (MLST, VNTR) allows characterization of strains, but in most cases, these techniques are effective only if performed on isolated strains; isolation is therefore still of primary importance. From January 2013 to June 2016, a total of 876 samples were collected from 352 suspected cases of leptospirosis in dogs : 270 urine samples were analysed by culture and by real time PCR targeting the 16S rrs gene; 214 whole blood samples and 47 organ samples were analysed by PCR; 345 serum samples were tested by MAT. Sixty-six samples were confirmed by PCR and 78 by serology (titre >400); in 8 cases the isolation of *Leptospira* strains from urine was achieved. The cultured strains were identified by serotyping and genotyping. The genotyping based on MLST and VNTR profiles identified 6 of the strains as *L. interrogans* serogroup Icterohaemorrhagiae, 1 as *L. kirschneri* serogroup Pomona serovar Mozdok, 1 as *L. interrogans* serogroup Australis. The serotyping identified the same serogroups, with complete concordance. Serology, when available (5 out of 8 times), revealed a positivity with the highest titre against the identified etiological serogroup except in one case. Identification of *Icterohaemorrhagiae* and *Australis* confirmed their role in the clinical leptospirosis in dogs; *Pomona* appeared as a risk for dogs and the absence of a registered *Pomona* vaccine suggests that leptospirosis should be taken into account in the differential diagnosis of any inflammatory illness, even when dogs are regularly vaccinated.

Papetti<sup>°</sup>A, Giacomini<sup>°</sup>E, Bertasio<sup>°</sup>C, Cerioli<sup>°</sup>M, Lazzaro<sup>°</sup>M, Faccini<sup>°</sup>S, Bonilauri<sup>°</sup>P, Vezzoli<sup>°</sup>F, Tironi<sup>°</sup>M, Salogni<sup>°</sup>C, Giovannini<sup>°</sup>S, Lavazza<sup>°</sup>A, Alborali<sup>°</sup>GL, Boniotti<sup>°</sup>MB

**Nuova ondata epidemica di diarrea epidemica suina in Italia**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 39-40. - 9 bib ref [Nr. Estr. 7358]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*Porcine Epidemic Diarrhea Virus (PEDV) causes watery diarrhea, dehydration and a high mortality among suckling pigs. In the last years, PED had a large economic impact on swine industry in Asia and United States of America (USA). In Europe, the last reported outbreak occurred in 2005-2006 in Italy. In 2014, PEDV has also re-emerged in many European countries. This study reports a new epidemic wave in Italy during 2015-2016. PEDV was detected by Real-time PCR in 335 farms located mainly in the North of Italy and few in the Centre and South of Italy. Most of the outbreaks*

*were In grower-producer, nursery-finisher and finisher farms. Clinical signs and mortality rates were similar to those described in USA and other European countries in the same period. 51 gene sequence was obtained from 285 samples. These strains have a high nucleotide identity to the PEDVS-INDEL strains from United States. Based on S1 gene sequencing more than one virus entry could have occurred In Italy.*

Parigi°M, Fregnani°G, Caminiti°A, Fiorentini°L, Massi°P, Tosi°G

**Isolamento di Avian nephritis virus (ANV) su uova embrionate di pollo SPF : risultati preliminari**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) 2016 : LV Convegno nazionale, Tavola rotonda : Padova, 5-6 maggio 2016 - Parma, 23 settembre 2016 / [s.l. : s.n., 2016]. - p 222-226. - 7 bib ref [Nr. Estr. 7477]

Tavola rotonda Societa' Italiana Patologia Aviaria (SIPA) : Parma : 23 Settembre 2016)

*Avian Nephritis Virus (ANY) is an astrovirus considered cause of diarrhea, interstitial nephritis, uricosis and dead in different avian species. In this study we reported the preliminary results of isolation, propagation and pathological characteristics of ANV in specific pathogen free (SPF) chicken embryonated eggs (CEE) from chickens and guinea-fowls with enteric and renal lesions. The CEE were inoculated via the yolk sack route at 14 days of incubation and the viral isolation was confirmed using PCR techniques. The embryos were small and presented several abnormalities in the internal organs, as livers enlarged and yellow, enlarged intestines filled with water and prominent ureter and urate deposition in the kidney. The viral replication was confirmed by RT-PCR in all the second-passage inocula.*

Parigi°M, Massi°P, Tosi°G, Fiorentini°L, Kovacs A, Vandi L

**How to counteract an Escherichia coli challenge in turkeys through an antibiotic alternative strategy**

Proceedings of the 10th Turkey Science and Production Conference : Chester, UK, March 10th - 11th, 2016 / [s.l. : s.n., 2016]. - p 10-12. - 6 bib ref [Nr. Estr. 7261]

Turkey Science and Production Conference (10th : Chester, UK : March 10th - 11th, 2016)

Paternolli S, Lavazza°A, Dellamaria D, Cavadini°P, Bano L, Natale A, Obber F, Tezzele R, Luchesa L, Capucci°L

**Three year follow-up of an outbreak of rabbit haemorrhagic disease due to RHDV2 in wild rabbits (*Oryctolagus cuniculus*) in North-Eastern Italy**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 159 (Poster n. 23) [Nr. Estr. 7335]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

Rabbit haemorrhagic disease is a fatal disease of European rabbits (*Oryctolagus cuniculus*) caused by a lagovirus (RHDV). Since 2010, RHDV2, a new strain with a specific antigenic profile, is spreading worldwide in domestic and wild rabbits. We describe the first outbreak of RHDV2 in Italy in wild rabbits living in a natural preserved area close to Trento (North-Eastern Italy), and the results of a virological and serological follow-up (2012 - 2014). In January 2012, due to unusual mortality among wild rabbits, we planned passive and active surveillance. During the three-year period, 296 carcasses (19 found dead and 277 hunted) were submitted to the lab. At necropsy, organ samples were collected from 292 individuals, including when possible, sera from cardiac clots. Specific sandwich MAbs ELISAs and RT-PCRs for the different RHDV strains were performed on liver/spleen samples. Out of 292, 28 samples (9.6 %) were RHDV positive: 26 for RHDV2 and 2 for RHDV1. All positives were detected on early 2012 but in November 2015 two more rabbits found dead in the

same area resulted positive for RHDV2. 224 sera, 121 in 2012 (P1), 41 in 2013 (P2) and 62 in 2014 (P3), were tested by specific cELISAs and isotype ELISAs for RHDV and RHDV2 (P3 only) antibodies. The seroprevalences differed along the time, being: 1) quite low in P1 (18.2 % positives and 27.3 % not conclusive (N.C), close to the threshold value < 1/10) in sera collected contemporarily to the outbreak; 2) sharply increasing in P2 (51.2 % positives and 19.5 % N.C) with higher titres (1:160-1:320), showing an active spread of the infection among the population; 3) decreasing in P3 (38.7 % positive and 21 % N.C), indicating a reduction of viral dissemination. The titres found in P3 were 4-8 times higher for RHDV2 than for RHDV. The detection of specific IgM in P 1-P2 and IgA in P2-P3 confirmed the active circulation of RHDV2 in the area. We could conclude that: (a) the epidemic event caused low mortality rates and ended within 2/3 months (P1); (b) the infection quickly became endemic during P2-P3 with high prevalence and without evident mortality; (c) different RHDV strains were circulating at the same time; (d) the re-occurrence of the disease after 3 - 4 years is likely due to the rabbit population's cyclic renewal and to the RHDV easy transmission.

Paternoster<sup>o</sup>G, Babo\_Martins S, Mattivi A, Cagarell i R, Angelini P, Bellini R, Tamba<sup>o</sup>M, Santi<sup>o</sup>A, Rushton J, Stark K

### **Economics of one health : evidence of substantial benefits of integrated West Nile virus surveillance**

Int J Infect Dis. - Vol. 53 Suppl ( 2016). - p 33 [Nr. Estr. 7493]

International Meeting on Emerging Diseases and Surveillance (IMED) (6th : Vienna, Austria : November 3 to 7, 2016)

Purpose: Enhanced cross-sectorial collaboration and sharing of surveillance information between the animal and the public health sectors are key to improve the management of zoonotic threats. However, there is little evidence on the costs and benefits of One Health (OH) surveillance for zoonoses. An integrated and multi-disciplinary West Nile virus (WNV) surveillance system (SS) has been implemented in Emilia-Romagna since 2009. The SS includes surveillance activities in the public health and in the animal health sectors. From 2013, surveillance information generated in the two sectors is shared, guiding targeted public health interventions to mitigate the risk of WNV transmission via blood transfusion. The objective of this work was to estimate the cross-sectorial costs and benefits associated with the OH approach to surveillance information of this SS. Methods & Materials: We applied a conceptual framework to identify the cross-sectorial links between WNV surveillance and triggered interventions, and the associated costs and benefits. Cost items included costs of human, animal, and entomological surveillance, linking of information, and triggered interventions. Benefits were quantified as the averted costs of potential human cases of West Nile neuroinvasive disease associated to infected blood transfusions. Evaluation of costs and benefits of surveillance designs was conducted considering two scenarios: OH and a uni-sectorial approach that does not integrate animal health information. Results: The OH scenario was estimated to represent a reduction of 184'619 EUR in the overall costs of surveillance in the 2009-2015 period. The main cost components were blood donation screening activities in both the OH and uni-sectorial scenario. The OH approach allowed savings of 1.24 million EUR in blood donations screening activities. These savings compensated the cost of animal health surveillance and linking of information. Benefits of the OH approach due to avoided short term cost-of-illness and avoided compensation for transfusion-transmitted infections were estimated to be 3.0 million EUR. Conclusion: Overall, the OH approach to WNV surveillance in Emilia-Romagna region is estimated to be economically beneficial. These results can further contribute to bring evidence on the economic aspects of OH surveillance for zoonoses and contribute for the prioritization of resource allocated to zoonoses mitigation.

Piccirillo A, Lavezzo E, Niero G, Moreno<sup>o</sup>A, Massi<sup>o</sup> P, Franchin E, Toppo S, Salata C, Palù G

## **Full genome sequence-based comparative study of wild-type and vaccine strains of infectious Laryngotracheitis virus from Italy**

PLoS One. - Vol. 11 no 2 ( 2016). - p e0149529 (19 p). - 48 bib ref ( ultimo accesso 03/11/2016 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0149529> ) [Nr. Estr. 7215]

Infectious laryngotracheitis (ILT) is an acute and highly contagious respiratory disease of chickens caused by an alphaherpesvirus, infectious laryngotracheitis virus (ILTV). Recently, full genome sequences of wild-type and vaccine strains have been determined worldwide, but none was from Europe. The aim of this study was to determine and analyse the complete genome sequences of five ILTV strains. Sequences were also compared to reveal the similarity of strains across time and to discriminate between wild-type and vaccine strains. Genomes of three ILTV field isolates from outbreaks occurred in Italy in 1980, 2007 and 2011, and two commercial chicken embryo origin (CEO) vaccines were sequenced using the 454 Life Sciences technology. The comparison with the Serva genome showed that 35 open reading frames (ORFs) differed across the five genomes. Overall, 54 single nucleotide polymorphisms (SNPs) and 27 amino acid differences in 19 ORFs and two insertions in the UL52 and ORFC genes were identified. Similarity among the field strains and between the field and the vaccine strains ranged from 99.96% to 99.99%. Phylogenetic analysis revealed a close relationship among them, as well. This study generated data on genomic variation among Italian ILTV strains revealing that, even though the genetic variability of the genome is well conserved across time and between wild-type and vaccine strains, some mutations may help in differentiating among them and may be involved in ILTV virulence/attenuation. The results of this study can contribute to the understanding of the molecular bases of ILTV pathogenicity and provide genetic markers to differentiate between wild-type and vaccine strains.

Polloni°A, Archetti°I, Avanzini C, Vinco°LJ, Gia comelli°S, Lombardi°G, Cecilian F, Lecchi C, Rota\_Nodari°S

## **Benessere dei suinetti alla castrazione: risultati preliminari dell'espressione di alcuni mirna e cortisolo nella saliva**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 61-68. - 28 bib ref [Nr. Estr. 7235]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Per questo studio sono stati selezionati un totale di 35 suinetti sottoscrofa di 3-4 giorni di vita, suddivisi in 3 gruppi. Il gruppo A ha ricevuto un anestetico locale e un antidolorifico prima della castrazione, il gruppo B è stato castrato senza utilizzo di farmaci e il gruppo C stato esclusivamente manipolato. Per valutare il dolore/stress indotto dalla castrazione e/o dalla manipolazione nei diversi gruppi di trattamento, da ogni soggetto è stato prelevato un campione di saliva prima dell'intervento di castrazione o di manipolazione (F1) e dopo 30-45 minuti (F3) al fine di dosare il cortisolo e alcuni miRNA (miR-19b, miR-27b e miR-365). Durante la castrazione sono stati rilevati dati di tipo comportamentale mediante una scheda validata. Il delta di cortisolo e la rilevazione comportamentale hanno evidenziato un punteggio di dolore inferiore nel gruppo trattato e un minor incremento di cortisolo anche se statisticamente le differenze tra gruppi sono apparse non significative. Per quanto riguarda i miRNA, l'analisi statistica ha evidenziato un incremento significativo di miR-19b nel gruppo B da F1 a F3, e di miR-365 nel gruppo Ada F1 a F3 mentre la differenza non è risultata statisticamente significativa tra prima e dopo il trattamento per il gruppo B e C. Osservando i valori di AUC delle curve ROC miR-19b si è dimostrato essere il più efficace a discriminare tra gruppo di animali castrati senza utilizzo di farmaci e con utilizzo di anestetico locale e antinfiammatorio.

*A total of 35 piglets 3-4 days old, divided into 3 groups were selected for this study. Group A received a local anesthetic and a nonsteroidal anti-inflammatory drug (NSAID) before castration, group B was castrated without use of drugs and the group C was only manipulated (control group). All subjects were sampled by salivary swabs placed in the mouth prior to surgery castration (F1) and 30-45 minutes after surgery (F3) or after been only manipulated to quantify cortisol and some miRNAs*

(miR-19b, miR-27b and miR-365). During castration behavioral data were recorded on a validated form. Delta cortisol (cortisol in F1-F3) and behavioral observations showed a minor pain score and raise in cortisol in the treated group compared to the others, even if differences between groups were not statistically significant. Regarding miRNA, the statistical analysis showed significant increase of miR-19b in group B from F1 to F3, and of miR-365 in group A from F1 to F3 while the difference before and after treatment between group B and C was not statistically significant. The values of AUC (Area Under the Curve) of ROC curve (Receiver Operating Characteristic), showed that miR-19b is the most useful miRNA to discriminate between the groups of castrated animals with or without the use of drugs.

Pozzi PS, Alborali°GL

**Animal welfare regulations for swine keeping in Israel : a comparison with the EU directive 120 of 2008 “Laying down minimum standards for the protection of pigs”**

Isr J Vet Med. - Vol. 71 no 1 ( 2016). - p 10-14. - 19 bib ref [Nr. Estr. 7251]

In February 2015, Israel approved the new Animal Welfare Law – Animal Protection – “Regulations for Swine Keeping for Agricultural Purposes”, which was implemented since May 2015. In comparison with European Union (EU) Legislation on swine protection (Council Directive 2008/120/EC of 18 December 2008), Israeli Regulations are ameliorative in terms of reduction of days in insemination stalls for gilts and sows; reduction of days in restraint during lactation; available floor area to each animal; pain management and relief in the course of castration, tail docking and corner-teeth clipping.

Procopio°A, Caminiti°A, Prosperi°A, Perulli°S, Lelli°D, Galletti°G, Moreno°A, Paternoster°A, Santi°A, Licata E, Gelmini°C, Rugna°G, Lavazza°A, Tamba°M

**Stima dell'accuratezza di un test ELISA indiretto per la diagnosi di West Nile nei corvidi. Un modello a classi latenti**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 59 (Poster 22) [Nr. Estr. 7404]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

Il virus West Nile (WNV) è uno dei più diffusi arbovirus e negli ultimi anni è diventato endemico in alcune regioni italiane. L'avifauna selvatica migratoria e stanziale è implicata nella diffusione e nel mantenimento del WNV in un territorio. Lo studio si propone di stimare la Sensibilità (Se) e la Specificità (Sp) diagnostica di un test ELISA indiretto per il rilevamento di anticorpi nei confronti del WNV nei corvidi. Il test è stato messo a punto presso il Laboratorio di Virologia dell'IZSLER, per disporre di uno strumento di screening rapido ed economico. Tra il 2013 e il 2014 sono stati prelevati 137 campioni di siero (67 nel 2013 e 70 nel 2014) da gazze (*Pica pica*), di età inferiore a un anno, abbattute nelle province di Ferrara e Modena. I campioni sono stati testati in parallelo con il test ELISA e con il test di sieroneutralizzazione (SN), secondo la metodica OIE (cut-off 1:10). Il test ELISA impiega un antigene ricombinante corrispondente al dominio III della proteina E dell'envelope di WNV (Istituto Pasteur), adsorbito su piastra, posto a reazione con sieri in esame alla diluizione 1:500. Per il rilevamento delle immunoglobuline aviari si è utilizzato un anticorpo monoclonale "pan-avian" coniugato con perossidasi. I risultati dei due test sono stati analizzati con modelli bayesiani a classi latenti, per stimarne Se e Sp diagnostica a diversi cut-off del test ELISA. I modelli sono stati implementati con e senza termini di dipendenza condizionale tra ELISA e SN. Il Bayesian P-value è stato utilizzato per valutare la bontà di adattamento del modello, il DIC (Deviance Information Criterion) per la scelta del modello. Le elaborazioni sono state svolte con WinBUGS. Per tutti i cut-off è stato selezionato il modello che non contiene i termini di dipendenza condizionale. Risultati: con il cut-off 10% OD il test ELISA ha Se 93% (=mediana; 95%PCI: 83-100%) e Sp 79% (95%PCI: 55- 99%), il test SN ha Se 86% (95%PCI: 74-99%) e Sp 84% (95%PCI: 65-99%). Con il cut-off 30% OD, la Se del test ELISA è 78% (95% PCI: 52-99%) e la Sp 94% (95% PCI: 83-100%), il test SN ha Se 95% (95%PCI: 84-100%) e Sp 63% (95%PCI: 48-94%). Come atteso, all'aumentare

del cut-off diminuisce la Se ed aumenta la Sp del test ELISA, che sembra avere delle buone performance in termini di accuratezza a tutti i cut-off considerati. I sieri sono stati raccolti in un'area in cui circola anche il virus Usutu, stesso sierogruppo del WNV, e questo può avere influito sui valori di specificità stimati.

Procopio<sup>o</sup>A, Galletti<sup>o</sup>G, Santi<sup>o</sup>A, Paternoster<sup>o</sup>G, Licata E, Guberti V, Tamba<sup>o</sup>M  
**Valutazione del rischio di introduzione dell'influenza aviaria in Emilia-Romagna tramite contatto con avifauna selvatica**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 58 (Poster 21) [Nr. Estr. 7403]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

Il piano nazionale di sorveglianza dell'influenza aviaria (PNIA) individua le province da controllare sulla base della densità di allevamenti e sulla presenza di zone umide. Le regioni possono individuare altre aree a rischio di introduzione del virus per contatto con l'avifauna selvatica, serbatoio del virus. A tale scopo in Emilia-Romagna è stato sviluppato un indice di rischio di introduzione per contatto con avifauna selvatica (IRIS), che viene determinato dalla vicinanza ad aree umide, dall'abbondanza di anatidi, dall'estensione dei loro movimenti e dalla prevalenza di Influenza Aviaria (IA). Il territorio regionale è stato suddiviso in celle da 1 Km<sup>2</sup>, utilizzando la griglia di riferimento europea fornita dalla European Environment Agency, e per ciascuna cella è stato calcolato il relativo IRIS, che rappresenta la densità per Km<sup>2</sup> di anatidi infetti. I dati utilizzati di prevalenza (5%), censimento e distanze di volo sono stati raccolti nel periodo invernale. Per individuare valori soglia attraverso i quali stabilire delle classi di rischio è stata analizzata la distribuzione di IRIS nelle celle attorno a 33 focolai primari di IA (6 HPAI, 27 LPAI), rilevati in Emilia-Romagna dal 2000 al 2015. Sono stati individuati tre livelli di rischio (basso, medio, alto) utilizzando il 33esimo percentile (0.25) e la mediana (0.40) di questa distribuzione; in tal modo è stato possibile definire le aree a rischio nelle quali applicare eventuali misure preventive. Poiché i parametri utilizzati nel calcolo di IRIS sono riferiti al periodo di svernamento dell'avifauna selvatica (nov-feb), abbiamo valutato separatamente la distribuzione di IRIS attorno ai focolai invernali e non invernali. Le distribuzioni non appaiono differenti, suggerendo che, anche durante il periodo in cui avvengono le migrazioni e cambiano le dinamiche della prevalenza di IA nell'avifauna selvatica, IRIS permette comunque l'individuazione delle aree dove è più probabile il contatto tra anatidi infetti e pollame. Dalla classificazione ottenuta risultano ad alto rischio introduzione ampie porzioni delle province di Forlì-Cesena, Ravenna, Bologna e Ferrara. Quest'ultima è stata aggiunta all'elenco delle province a rischio del PNIA sulla base del presente studio ed è stata sede nella primavera 2016 di un focolaio HPAI H7N7 in un allevamento biologico di ovaiole. L'indagine epidemiologica ha evidenziato che il virus IA molto probabilmente è entrato in allevamento attraverso contatti con la fauna selvatica.

Prosperi<sup>o</sup>A, Chiari<sup>o</sup>M, Zanoni<sup>o</sup>M, Gallina L, Casà G, Scagliarini A, Lavazza<sup>o</sup>A  
**Identification and characterization of Fringilla coelebs papillomavirus 1 (FcPV1) in free-living and captive birds in Italy**

J Wild Dis. - Vol. 52 no 3 ( 2016). - p 756-758. - 16 bib ref [Nr. Estr. 7382]

A papillomavirus (PV) was identified by negative-staining electron microscopy in skin lesions of two bird species (Fringillidae) in Italy. Genetic analyses revealed an FcPV1 with a low genetic variability in the E6, E7, E1, E2, and L1 genes and the long control region when compared to the FcPV1 reference strain.

Prosperi°A, Lelli°D, Moreno°A, Sozzi°E, Licata E, Chiari°M, Gelmini°L, Tamba°M, Lavazza°A

**West Nile Virus circulation in Emilia-Romagna region, Italy : serological survey in Eurasian magpies (2013 - 2014)**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 165 (Poster n. 9) [Nr. Estr. 7336]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

The West Nile virus (WNV) is nowadays the most widespread arbovirus in the world, being present in all continents exception made for the Antarctica. Migratory and resident wild birds are highly implicated in spreading and maintaining WNV in a territory. Eurasian magpie (*Pica pica*), well known reservoir for WNV, is strongly preferred by *Culex pipiens* and may play a crucial role in WNV seasonal spill-over events to humans and horses in most European countries. The objective of this study was the titration of "WNV neutralising antibodies in fledgling magpies (less than one year old), using serum samples collected in Emilia-Romagna region (Italy), during two consecutive vectorial seasons (2013 and 2014). Samples were collected from 135 animals (46 in 2013 and 89 in 2014) and then analysed using a serum neutralisation (SN) test. The SN assay was set up according to the OIE recommended protocol, using a WNV field strain isolated during the 2014 season. The 46 samples from 2013 were collected between the 5th of June and the 20th of September. Out of them 16 (34.8 %) were negative whereas 30 (65.2 %) presented WNV-neutralising antibodies with a neutralisation titre ranging from 1:10 and 1:320. Such a result was interesting taking into account that the surveillance programme in this area did not evidence any WNV circulation during the 2012. The 89 samples from 2014 were collected from the 20th of May to the 18th of June. Forty-three (48.3 %) of the 89 sera analysed were negative and 46 (51.7 %) were positive with a neutralisation titre between 1:10 and 1:640. Since 2014 WNV has become clearly endemic in some Italian areas (as Emilia-Romagna, Lombardy, Friuli-Venezia-Giulia, Veneto, Sicily and Sardinia regions), but there are no data available about the WNV seroprevalence in the avian reservoirs, although the viral circulation was well demonstrated. This study reports the detection of WNV neutralising antibodies in Eurasian magpie, adding data about the seroprevalence of this virus in one of its wild birds reservoirs, giving also an idea of the degree of WNV endemisation in Italy.

Prosperi°A, Lelli°D, Moreno°A, Sozzi°E, Pezzotti R, Figuerola J, Soriguer R, Capucci°L, Brocchi°E, Lavazza°A

**Production and characterisation of pan-avian monoclonal antibodies and their application in serological monitoring of wild birds**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 50-51 [Nr. Estr. 7332]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

The role of the wild fauna as a reservoir for several infectious agents, including viruses, bacteria and macroparasites, may have a great impact on human and animal health. Wild birds may take part in the maintenance and dissemination of several pathogens, among which West Nile virus (WNV), Usutu virus (USUV), Avian Influenza virus (AIV), Newcastle Disease virus (NDV), *Salmonella* spp. and *Campylobacter* spp. might be some examples. Furthermore migratory birds could introduce emerging or neglected disease moving through different and distant areas. Serological surveys may be important in the identification of a pathogen circulation and in the knowledge of its epidemiology, although the lack of immunoreagents availability for the wildlife species often affects the feasibility of this type of investigations. The aim of our work was the production and the characterisation of

pan-avian monoclonal antibodies (mAbs), reactive against various avian species, in order to develop indirect ELISA tests, which may be useful in wildlife surveillance programmes. In the present study chicken's purified immunoglobulins and a pool of purified avian immunoglobulins belonging to four species (*Columba livia*, *Pica pica*, *Gallus gallus* and *Coturnix coturnix*) were used to immunise Balb/c mice, in order to produce hybridomas following a standardised protocol. These hybridomas were firstly screened via an indirect ELISA test, using the homologous antigen. Then selected ones were characterised with an ELISA test, using immunoplates coated with avian immunoglobulins belonging to 39 serum samples of 14 avian orders (Anseriformes, Galliformes, Pelecaniformes, Ciconiiformes, Phoenicopteriformes, Struthioniformes, Falconiformes, Gruiformes, Charadriiformes, Columbiiformes, Piciformes, Accipitriformes, Podicipediformes and Passeriformes) and also with mammalian immunoglobulins (using cattle, horse, sheep, goat, dog, cat, swine and human sera). This procedure allowed the characterisation of 105 hybridomas producing reactive mAbs against avian immunoglobulins, 31 of these were made with the avian immunoglobulins' pool antigen whereas the others 74 mAbs with the chicken's one. Two out of these cross-species reactive mAbs, which showed the higher reactivity (in OD value) with the avian sera and no or low reactivity with the mammalian ones, were selected, cloned, purified and then horseradish peroxidase (HRP) conjugated. In conclusion, the main result of our study was the production of pan-avian HRP conjugated mAbs, which were used to set up indirect ELISA tests for the identification of specific antibodies against WNV, USUV and AIV in serum samples, which may be useful introduced into wildlife surveillance programmes.

Prosperi°A, Lelli°D, Moreno°A, Sozzi°E, Pezzotti R, Figuerola J, Soriguer R, Capucci°L, Brocchi°E, Lavazza°A

#### **Sorveglianza sierologica nell'avifauna selvatica: impiego di anticorpi monoclonali pan-avian**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 34 [Nr. Estr. 7411]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

L'avifauna selvatica svolge un ruolo fondamentale come reservoir di diversi agenti virali, tra cui West Nile virus (WNV) ed Usutu virus (USUV). Gli uccelli migratori rappresentano inoltre una via d'introduzione e disseminazione dei virus nell'arco delle loro rotte migratorie. Pertanto le indagini sierologiche nell'avifauna selvatica sono uno strumento cruciale per acquisire informazioni epidemiologiche, ma la loro applicabilità spesso limitata dalla carenza di saggi sierologici indiretti, funzionali al ridotto volume del campione reperibile in queste specie. Gli obiettivi di questo lavoro sono stati la produzione e la caratterizzazione di mAb pan-avian, cross-reattivi nei confronti delle immunoglobuline (Ig) di diverse specie aviari, e la valutazione della loro applicabilità in test ELISA indiretti. Topi Balb/c sono stati quindi immunizzati utilizzando due diversi antigeni: IgG di polio purificate ed IgG purificate provenienti da 4 specie aviari (*Columba livia*, *Pica pica*, *Gallus gal/us*, *Coturnix coturnix*) appartenenti a 4 differenti ordini. Gli ibridomi, prodotti con un protocollo standardizzato, sono stati prima sottoposti a screening, usando l'antigene o mologo, e quindi caratterizzati usando piastre ELISA rivestite sia con le Ig di 39 specie aviari appartenenti a 14 ordini diversi, sia con Ig di mammiferi per valutarne l'eventuale reattività aspecifica. Ciò ha permesso l'identificazione e la caratterizzazione di 105 ibridomi con un differente pattern di reattività nei confronti delle Ig aviari. Il mAb con il più ampio spettro di cross-reattività verso le specie aviari e nessuna cross-reattività per i mammiferi, sono stati clonati, purificati e coniugati HRP. Il coniugato col miglior quadro di reattività è stato impiegato per lo sviluppo di test ELISA indiretti, per l'identificazione di anticorpi aviari multispecie nei confronti di WNV ed USUV. Sono state sensibilizzate piastre ELISA con un antigene ricombinante corrispondente al DIII della proteina E di WNV od USUV (Institut Pasteur), posto quindi a contatto con sieri aviari noti. L'avvenuta reazione antigene-anticorpo è stata rilevata impiegando il mAb-HRP pan-avian selezionato. I risultati ottenuti sono stati quindi confrontati con quelli ricavati dal test sierologico di riferimento (sieroneutralizzazione), per una preliminare validazione. In conclusione, i mAb pan-avian sono reagenti potenzialmente impiegabili in reazioni ELISA per lo studio delle infezioni virali degli uccelli domestici e selvatici.

Puzelli S, Rizzo C, Fabiani C, Facchini M, Gaibani C, Landini MP, Gagliotti C, Moro ML, Rangoni R, Piccolomini LL, Finarelli AC, Tamba° M, Rezza G, Declich S, Donatelli I, Castrucci MR

**Influenza A(H7N7) virus among poultry workers, Italy, 2013**

Emerg Infect Dis. - Vol. 22 no 8 ( 2016). - p 1512-1513. - 5 bib ref [Nr. Estr. 7391]

Radaelli E, Castiglioni V, Recordati C, Gobbi A, Capillo M, Invernizzi° A, Scanziani E, Marchesi F

**The pathology of aging 129S6/SvEvTac mice**

Vet Pathol. - Vol. 53 no 2 ( 2016). - p 477-492. - 75 bib ref [Nr. Estr. 7242]

The 129 mouse strain is commonly used for the generation of genetically engineered mice. Genetic drift or accidental contamination during outcrossing has resulted in several 129 substrains. Comprehensive data on spontaneous age-related pathology exist for the 129S4/SvJae substrain, whereas only limited information is available for other 129 substrains. This longitudinal aging study describes the life span and spontaneous lesions of 44 male and 18 female mice of the 129S6/SvEvTac substrain. Median survival time was 778 and 770 days for males and females, respectively. Tumors of lung and Harderian gland were the most common neoplasms in both sexes. Hepatocellular tumors occurred mainly in males. Hematopoietic tumors were observed at low frequency. Suppurative and ulcerative blepharoconjunctivitis was the most common nonneoplastic condition in both sexes. Corynebacteria (primarily *Corynebacterium urealyticum* and *C. pseudodiphtheriticum*) were isolated from animals with blepharoconjunctivitis and in some cases from unaffected mice, although a clear causal association between corynebacterial infections and blepharoconjunctivitis could not be inferred. Polyarteritis occurred only in males and was identified as the most common nonneoplastic contributory cause of death. Eosinophilic crystalline pneumonia occurred in both sexes and was a relevant cause of death or comorbidity. Epithelial hyalinosis at extrapulmonary sites was noted at higher frequency in females. This study contributes important data on the spontaneous age-related pathology of the 129S6/SvEvTac mouse substrain and is a valuable reference for evaluation of the phenotype in genetically engineered mice obtained with this 129 substrain.

Razzuoli E, Lazzara F, Bilato° D, Ferraris M, Venci a W, Vito G, Amadori° M, Ferrari A

**Salmonella serovar interaction with jejunal epithelial cells**

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 2 p. - 4 bib ref [Nr. Estr. 7311]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13 Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Salmonella spp infections are an important source of foodborne illnesses and therefore a major public health concern (1). Important studies highlighted the molecular basis of pathogenesis of *S. Typhimurium* infection, while scanty data are available about other environmental serotypes, often isolated in cases of foodborne disease but not included in pathogenicity studies. Owing to the above, the aim of our work was to verify invasiveness in the IPEC-J2 swine jejunal cell line and the modulation of intestinal innate immunity by six different environmental Salmonella strains. In our

study, overnight cultures of 7 different *Salmonella enterica* strains: *S. Coeln*, *S. Ablogame*, *S. enterica* sub-specie *diarizonae* (Strain 1), *S. Veneziana*, *S. enterica* sub-specie *diarizonae* (strain 2), *S. Typhimurium* and *S. Thompson* isolated from wild boar livers were sub-cultured for 2 h at 37 °C in BHI medium. Each bacterial strain was re-suspended at  $1 \times 10^8$  CFU/ml in DMEM/F12 medium (2) and used to infect IPEC-J2 cells; untreated cells were employed as negative control. Bacterial penetration and innate immune responses were evaluated as previously described (2, 3). Differences between data sets were checked for significant differences by Kruskal-Wallis test, followed by a Dunn's post-test. The significance threshold was set at  $P < 0.05$ . All the strains were able to penetrate inside IPEC-J2 cells. In particular, our results demonstrated greater penetration of *S. Coeln* ( $P < 0.0001$ ) and *S. Thompson* ( $P = 0.0059$ ) compared with *S. Typhimurium* (control strain). *S. Diarizonae* 1 ( $P = 0.0408$ ) showed lesser penetration with respect to the control strain. Concerning innate immunity, our results showed different abilities to modulate gene expression by the strains under study. In particular, in accordance with another study (2), *S. Typhimurium* infection determined a pro-inflammatory effect characterized by upregulation of IL-8 ( $P = 0.022$ ), TNF- $\alpha$  ( $P = 0.0003$ ), IL-1b ( $P < 0.0001$ ), p38 MAPK ( $P = 0.0027$ ) and IL-18 ( $P = 0.041$ ) and an increase of antimicrobial peptide gene expression: bD1 ( $P = 0.001$ ), bD2 ( $P = 0.002$ ), bD4 ( $P = 0.0006$ ). At the same time we observed down regulation of IL-4 ( $P = 0.03$ ) and MD2 ( $P = 0.0018$ ). On the contrary, *S. Coeln* caused a significant decrease of p38 MAPK and CD14 ( $P = 0.0157$ ,  $P = 0.0431$ ) gene expression and no modulation of antimicrobial peptides. *S. Thompson* caused a significant increase of JNK1 ( $P = 0.0196$ ), NFK- $\kappa$ p65 ( $P = 0.0046$ ) gene expression. *S. Ablogame* down-regulated p38 MAPK ( $P = 0.03$ ), TLR4 ( $P < 0.05$ ) and TLR5 ( $P < 0.05$ ). Treatment with *S. Diarizonae* strain 1 caused a significant decrease of p38 MAPK ( $P = 0.0412$ ), MD2 ( $P = 0.0044$ ) and bD4 ( $P = 0.0344$ ) gene expression. The adopted cell line had been shown to give valuable information about pathogenicity of *Salmonella* spp. (4). Our data suggest a potential pathogenic role of all the strains under study and different interactions with the host. In particular, our findings about *S. Coeln* and *S. Thompson* are in agreement with an EFSA report.

Razzuoli<sup>°</sup>E, Olzi E, Calà P, Cafazzo S, Magnani D, Vitali A, Lacetera N, Archetti<sup>°</sup>L, Lazzara L, Ferrari A, Costa L, Amadori<sup>°</sup>M

#### **Innate immune responses of young bulls to a novel environment**

Vet Immunol Immunopathol. - Vol. 172 ( 2016). - p 9-13. - 22 bib ref [Nr. Estr. 7237]

Animal welfare during transportation has been investigated in several studies, as opposed to post-transportation phases. In this study, we evaluated the effect of a novel environment after transportation on 26 Friesian bulls,  $242 \pm 42$  day-old, from ten different dairy farms. Animals were shipped to a breed-ing center in different seasons, and selected parameters of innate immunity (serum bactericidal activity, hemolytic complement, serum albumin (Alfa), (Beta), and (Ypsilon)-globulins, interleukin-6, TNF- $\alpha$ ) were monitored before and after the arrival at days—4/0/4/15/30. Our results showed significant differences of IL-6 and TNF-(alfa) \_ protein levels at destination in December ( $94 \pm 1.3$  pg/ml) and June ( $+788$  pg/ml), respectively. Moreover, the serum levels of these cytokines increased between days 0 and 15 after the arrival, the modulation of IL-6 being in agreement with established models of physical and/or psychological stress. Concerning the modulation of albumin, alpha and beta-globulins, the highest levels were detected in April, whereas a significant decrease was observed between day 15 and 30 after arrival; on the contrary, (ypsilon)-globulin levels significantly increased after day 15. The results of this study highlight the occurrence of innate immune responses of young bulls to the combined effects of climate (season) and novel farming conditions.

Rizzo C, Napoli C, Venturi G, Pupella S, Lombardini L, Calistri P, Monaco F, Cagarelli R, Angelini P, Bellini R, Tamba<sup>°</sup>M, Piatt i A, Russo F, Palù G, Chiari<sup>°</sup>M, Lavazza<sup>°</sup>A, Bella A, the Italian WNV surveillance working group [for Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna Lelli D,

Prosperi A, Faccin Francesca]

**West Nile virus transmission : results from the integrated surveillance system in Italy, 2008 to 2015**

EuroSurveillance. - Vol. 21 no 37 ( 2016). - p 10-17. - 31 bib ref ( ultimo accesso 28/02/2017 <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=22580> ) [Nr. Estr. 7354]

In Italy a national Plan for the surveillance of imported and autochthonous human vector-borne diseases (chikungunya, dengue, Zika virus disease and West Nile virus (WNV) disease) that integrates human and veterinary (animals and vectors) surveillance, is issued and revised annually according with the observed epidemiological changes. Here we describe results of the WNV integrated veterinary and human surveillance systems in Italy from 2008 to 2015. A real time data exchange protocol is in place between the surveillance systems to rapidly identify occurrence of human and animal cases and to define and update the map of affected areas i.e. provinces during the vector activity period from June to October. WNV continues to cause severe illnesses in Italy during every transmission season, albeit cases are sporadic and the epidemiology varies by virus lineage and geographic area. The integration of surveillance activities and a multidisciplinary approach made it possible and have been fundamental in supporting implementation of and/or strengthening preventive measures aimed at reducing the risk of transmission of WNV through blood, tissues and organ donation and to implementing further measures for vector control.

Rizzo F, Bertolotti L, Robetto S, Lelli°D, Rosati S, Culasso P, Calvini M, Toffoli R, Orusa R, Mandola ML

**Circolazione di coronavirus in quattro specie di chiroteri del piemonte**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 60 (Poster 23) [Nr. Estr. 7405]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

In seguito alla comparsa di zoonosi altamente virulente, è stato dimostrato il ruolo dei pipistrelli nel loro mantenimento e la capacità di questi mammiferi di ospitare virus geneticamente anche molto diversi. Il presente studio si propone di indagare la circolazione di virus nelle popolazioni di chiroteri del Piemonte e Liguria. Qui riportiamo i risultati ad oggi raggiunti nell'identificazione di alpha coronavirus (aCoVs) in tre specie di chiroteri in Piemonte. Tamponi orali, urina, feci e carcasse in buono stato di conservazione sono stati raccolti da chiroterologi autorizzati nel periodo di attività dei chiroteri dal 2013 al 2015. Lo screening molecolare per la famiglia delle Coronavirinae è stato realizzato con una PCR end point specifica per il gene per la RNA polimerasi virale (RdRp). I campioni positivi sono stati caratterizzati filogeneticamente e messi in coltura sulle linee continue Vero, Mark 145 e TB1Lu. Ad oggi 184 chiroteri appartenenti a 18 specie diverse sono stati analizzati. La positività per Coronavirus è risultata in 11 esemplari delle specie *Myotis nattereri*, *Myotis daubentonii*, *Pipistrellus kuhlii* e *Pipistrellus pipistrellus*. La caratterizzazione filogenetica è stata possibile per nove sequenze del frammento RdRp (382 bp) di Coronavirus ottenute da campioni di feci e urina appartenenti alle specie *M. nattereri* (n=3), *P. kuhlii* (n=2) e *P. pipistrellus* (n=4). Le tre sequenze di aCoV riscontrate in *M.nattereri* formano un unico clade divergente per il 15% da sequenze di aCoV in *M.nattereri* identificate in Inghilterra. La determinazione di specie sulla base della sequenza del Citocrom B ha rivelato che i tre esemplari appartenevano alla specie *M.nattereri* SpA, una nuova specie in via di definizione all'interno del complesso di specie criptiche *M. nattereri*. Tre sequenze di aCoV ritrovate in *P. pipistrellus* formano un unico clade, mentre la quarta risulta divergente per il 28% dalle altre. Inaspettatamente le due sequenze di aCoV in *P.kuhlii* risultano al 25% divergenti tra di loro. Sulla base della filogenesi del frammento RdRp risulta che tutte le sequenze identificate in questo studio appartengono al genere alpha coronavirus. Inoltre, un lineage divergente di aCoV è stato caratterizzato in *M.nattereri* SpA. In conclusione, i nostri risultati confermano l'associazione specie specifica tra coronavirus e specie ospite, ma resta da chiarire la notevole divergenza riscontrata in aCoV di chiroteri della stessa specie.

Romagnoli N, Zaghini A, Fedrizzi<sup>o</sup>G, Sala A, Babbin i S, Barbarossa A

**Disposition of Stanozolol in plasma after intra-articular administration in the horse**

J Equine Vet Sci. - Vol. 47 ( 2016). - p 16-19. - 8 bib ref [Nr. Estr. 7348]

The purpose of this study was to provide data on the disposition of stanozolol after bilateral intra-articular injection in the tarsal joints, to discover the length of time for which the drug can be detected in plasma after administration. Fourteen horses were included in the study. After aseptically preparing the injection site, 1 mL of an aqueous suspension containing 5 mg of stanozolol was injected into both the right and left tarsal joints of 12 horses; the two remaining animals were not treated and were used as a control group. Five milliliters of blood was collected immediately before stanozolol administration (t0) and at 1, 2, 4, 6, 12, 18, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, and 168 hours after injection. The plasma concentration was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) after solid phase extraction. The maximum plasma concentration was 1.7 ng/mL (range, 0.5–3.0 ng/mL), measured at 6 hours (range, 4–12 hours). The plasma elimination half life varied between 4 and 12 hours, whereas the plasma clearance per fraction of dose absorbed was in the 257.85–820.88 L/h range. The results of the present study make a preliminary contribution toward understanding the elimination profile of intra-articularly administered stanozolol in the horse. The drug passes rapidly into the systemic circulation, is eliminated rapidly, and is detected in plasma for no more than 36 hours after local administration.

Roman\_Sosa G, Brocchi<sup>o</sup>E, Schirrmeyer H, Wernike K, Schelp C, Beer M

**Analysis of the humoral immune response against the envelope glycoprotein (Gc) of Schmallenberg virus reveals a domain located at the amino terminus targeted by monoclonal antibodies with neutralizing activity**

J Gen Virol. - Vol. 97 ( 2016). - p 571-580. - 29 bib ref [Nr. Estr. 7248]

Orthobunyaviruses are enveloped viruses that are arthropod-transmitted and cause disease in humans and livestock. Viral attachment and entry are mediated by the envelope glycoproteins Gn and Gc, and the major glycoprotein, Gc, of certain orthobunyaviruses is targeted by neutralizing antibodies. The domains in which the epitopes of such antibodies are located on the glycoproteins of the animal orthobunyavirus Schmallenberg virus (SBV) have not been identified. Here, we analysed the reactivity of a set of mAbs and antisera against recombinant SBV glycoproteins. The M-segment-encoded proteins Gn and Gc of SBV were expressed as full-length proteins, and Gc was also produced as two truncated forms, which consisted of its amino-terminal third and carboxyl-terminal two-thirds. The sera from convalescent animals reacted only against the full-length Gc and its subdomains and not against the SBV glycoprotein Gn. Interestingly, the amino-terminal domain of SBV-Gc was targeted not only by polyclonal sera but also by the majority of murine mAbs with a neutralizing activity. Furthermore, the newly defined amino-terminal domain of about 230 aa of the SBV Gc protein could be affinity-purified and further characterized. This major neutralizing domain might be relevant for the development of prophylactic, diagnostic and therapeutic approaches for SBV and other orthobunyaviruses.

Romanò<sup>o</sup>A, Maisano<sup>o</sup>AM, Spelta<sup>o</sup>C, Cremonesi P, Gar barino<sup>o</sup>C, Arrigoni<sup>o</sup>N, Vezzoli<sup>o</sup>N, Luini<sup>o</sup>M

**Indagini ambientali in allevamenti bovini con infezioni intramammarie da MRSA**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 343-344. - 4

bib ref [Nr. Estr. 7378]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. :  
Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*The present study was conducted In dairy herds were MRSA presence was previously demonstrated with the aim to (i) evaluate the prevalence of MRSA intra-mammary infections (IMI) and (ii) Identify the specific environmental contamination. MRSA IMI's were detected at low prevalence (less than 5%), except for one herd where it reached the 24,8%. The circulating MRSA genotypes were different for each herd and In two herds two different genotypes were found with one of them predominant. Four and 11 out of 18 cows, among those with MRSA IMI, were positive for nasal and udder skin swabs, respectively. The environmental analyses showed that milking parlor was systematically contaminated in different sites as well as the young calves mangers and buckets, whereas the heifers pens and mangers were not. Thus, in MRSA positive herds, especially milkers and personnel to young calves should be considered at risk of occupational infection.*

Rosignoli°C, Barisani C, Ubertini S, Grazioli°S, Franzini°G, Nigrelli°AD

**Stomatite ulcerativa in vitelle alimentate con fieno contenente pabbio (*Setaria spp*)**

Buiatria. - Vol. 2016). - 3 p. - 2 bib ref [Nr. Estr. 7380]

Viene riportato un episodio di stomatite ulcerativa che ha coinvolto 10 vitelle di circa 3 mesi di età appartenenti ad una stalla di bovine da latte della provincia di Mantova. La forma clinica si è manifestata con moderato peggioramento della normale condizione corporea e lieve perdita di saliva dalle commessure labiali. L'ispezione della cavità orale permetteva di rilevare la presenza di estese lesioni ulcerose sulla lingua, sul palato e sulla mucosa delle guance. I soggetti colpiti non erano depressi e mostravano una temperatura corporea nella norma. L'esame microscopico a basso ingrandimento di campioni di tessuto prelevati dalle lesioni evidenziava la presenza, al loro interno, di numerosi filamenti vegetali dotati di uncini particolarmente vulneranti e in grado di ancorare saldamente il corpo estraneo al tessuto mucosale. Tali filamenti vegetali sono stati identificati come setole di infiorescenze di "Pabbio" (*Setaria spp*), presente in quantità elevata nel fieno somministrato agli animali. Dopo la rimozione dalla dieta del fieno contaminato la guarigione delle lesioni è avvenuta in circa 3 settimane.

Rosignoli°C, Merenda°M., Faccini°S, Chiapponi°C , De\_Mattia°A, Bufalo G,  
Garbarino°C, Baioni°L, Nigrelli°AD, Foni°E

**Virus influenza D e malattia respiratoria del bovino : indagine in allevamenti italiani**

Buiatria. - Vol. 2016). - 6 p. - 16 bib ref [Nr. Estr. 7379]

Un nuovo genere di virus influenzale, provvisoriamente definito Influenzavirus D (IDV), è stato recentemente rilevato prima nel suino e successivamente nel bovino, negli USA, Cina, Francia, Italia, Giappone e Messico. Attualmente si ritiene che proprio il bovino possa fungere da serbatoio naturale di IDV. Evidenze sperimentali depongono, inoltre, a favore di un ruolo primario di questo virus nell'eziologia del complesso della malattia respiratoria del bovino. L'obiettivo di questo studio è stato quello di indagare sull'epidemiologia di questa nuova infezione negli allevamenti bovini italiani. A tale scopo tra Aprile 2014 e Luglio 2016, sono stati sottoposti ad un test RT PCR, specifico per IDV, 771 campioni, principalmente tamponi nasali e tessuti polmonari, prelevati da bovini con o senza patologia respiratoria provenienti da 459 diversi allevamenti. Sono risultati positivi a IDV 56 campioni (7,3%). Tra questi, 46 erano stati prelevati da soggetti con patologia respiratoria e 10 da bovini con altre patologie. Sono stati individuati 30 focolai di malattia respiratoria con presenza di positività per IDV, 4 in allevamenti per la produzione di carne e 26 in allevamenti da latte. Le rispettive aziende zootecniche erano distribuite in 12 differenti province del nord Italia. In 19 focolai (63,3%), IDV si dimostrava l'unico virus respiratorio rilevato con i test di laboratorio, mentre in 11 focolai (36,7%) IDV si presentava in associazione con altri importanti patogeni virali respiratori (BVDV, BRSV, BHV-1, PI3V e BCV). Questo studio non solo fornisce l'evidenza della diffusa

circolazione di IDV nei bovini in Italia, ma raccoglie elementi di supporto alla dimostrazione del coinvolgimento di questo virus nell'eziologia delle sindromi respiratorie infettive di questa specie animale. Ulteriori indagini nel campo dell'epidemiologia, virologia e patobiologia sono necessari, nel prossimo futuro, per chiarire i numerosi aspetti ancora sconosciuti di questa nuova infezione respiratoria del bovino.

Rossi L, Soares\_Filipe JF, Lombardi A, Gottardo D, Demartini E, Alborali°GL, Reggi S, Crotti A, Baldi A

#### **Piglets fed seed-based oral vaccine against verocytotoxic Escherichia coli – in vivo study**

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - (2p) p. - 2 bib ref [Nr. Estr. 7579]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Verocytotoxic Escherichia coli (VTEC) is responsible of severe enterotoxaemia in swine. VTEC pathogenicity is strictly related to VT2e toxin and F18 adhesive fimbriae. Nowadays no vaccines are available and an outbreak of the disease requires antibiotic medication, therefore novel strategies, alternative to antibiotics, are required. Plantbased oral vaccines offer an innovative approach to vaccination with the main advantages of avoidance of injections and ability to induce specific antibodies in the mucosa, where the major pathogens gain access to the body. The aim of this study was to evaluate the efficacy of tobacco seeds-based oral vaccines directed against VTEC infection (ethical authorization: 102/2015-PR). A competitive indirect ELISA was developed to measure respectively the F18 adhesive fimbriae and the B subunit of verocytotoxin, antigens expressed in Nicotiana tabacum seeds previously produced (Rossi et al. 2013). 36 weaned piglets were divided randomly into 4 experimental groups (CC, challenged control; CT, challenged treated; UC, unchallenged control; UT, unchallenged treated). Treated piglets were fed five times, on day 0, 1, 2, 7 and 14, with 20 g of engineered milled tobacco seeds (expressing 6.6-7.4 µg of F18 plus 34-37 µg of VT2eB) mixed with 20 g of milk powder. Controls received 20 g of wild type of tobacco seeds. The animals were challenged at day 20 with 10E10 CFU of O138 VTEC E. coli. During the entire experimental period body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion (FC) were registered individually. From day 20 to 30, animals were evaluated for the general health status and scored daily for specific clinical signs (respiratory, palpebral oedema, epiphora, vitality, faecal consistency, and rectal temperature) with a point-score scale described by Rossi et al. (2014). The oral delivery strategy guaranteed the total consumption of the treated feed. The uninfected piglets did not show any VTEC-related clinical sign. In the first postchallenge period (days 21-25) CT showed a reduction of ADG and FI lower than CC. CT showed an average total score (from day 1 to day 9 post-challenge) significantly lower than CC for oedema, epiphora, vitality and depression. Death, respiratory and neurologic signs were not observed. These results show that piglets fed tobacco seeds expressing VTEC antigens have overall a better clinical status. This oral delivery strategy appeared effective in reducing the development of clinical signs after challenge with O138 VTEC E. coli strain.

Rota\_Nodari°S, Gaffuri°A, Cova MP, Bencetti F, Ar chetti°I, Polloni°A, Santi°A, Galletti°G

#### **Reference intervals for serum haptoglobin, cortisol and lysozyme in immediate post-partum and lactating dairy goats**

Large Anim Rev. - Vol. 22 no 6 ( 2016). - p 267-270. - 21 bib ref [Nr. Estr. 7505]

Introduction - In Northern Italy the most common breeds reared for milk production are Saanen and Chamois Colored. Delivery and peak of lactation are two critical points during the life of a dairy goat and serum lysozyme activity, haptoglobin and cortisol are indicators of non specific immunity and stress that could be easily applicable in field evaluations. Aim - The objective of this study was to calculate Reference Intervals (RIs) in according to the International Federation of Clinical Chemistry (IFCC) and Clinical and Laboratory Standard Institute (CLSI). Materials and methods - 132 animals were sampled immediately postpartum and at peak of lactation. Sera obtained in laboratory were stored in aliquots at -80°C until a nalysis. Serum lysozyme was assessed by the lyso-plate assay, serum haptoglobin was measured by a colorimetric kit and serum cortisol was measured by a solid-phase, competitive chemiluminescent enzyme immunoassay. Results and discussion - RIs for lysozyme were 0.7-6.4 vg/mL for Chamois Colored in the immediate postpartum and 0.6-4.7 pg/mL for Saanen. RIs for haptoglobin were 0-0.2 mg/mL and 0-0.8 for Chamois Colored and Saanen respectively in the immediately postpartum while increased slightly in Chamois Colored and decreased for Saanen at the peak of lactation. Cortisol serum level were 0.1-3.4 pg/dL for Chamois Colored and 0.1-3.5 for Saanen in the immediately postpartum and they were 0.1- 2.9 and 0-3.3 at the peak of lactation respectively. Conclusions - The results provide a well-established, statistically defined RIs for haptoglobin, lysozyme and cortisol for the two of the most typical dairy breeds goats raised in Northern Italy in two critical stages of goat breeding and could be indicators of animals health and welfare.

Rubini°S, Faggionato°E, Berardelli C, Faggioli P, Fico R, Meriardi°G, Fontana° MC, Barbieri S, Talarico A, Frisoni P, Gaudio RM

#### **Avvelenamento di animali domestici e selvatici in provincia di Ferrara dal 2008 al 2015 : approccio multidisciplinare**

XI Convegno Nazionale del Gruppo Italiano di Patologia Forense (GIPF) : Ferrara 20-22 Ottobre 2016 / [s.l. : s.n., 2016]. - p 85 [Nr. Estr. 7401]

Convegno Nazionale del Gruppo Italiano di Patologia Forense (GIPF) (9. : Ferrara : 20-22 Ottobre 2016)

Background Il 8.12.2008 il Ministero della Salute ha emanato la prima di una serie di Ordinanze relative a "Norme sul divieto di utilizzo e di detenzione di esche o di bocconi avvelenati". Materiali e metodi Dal 2008 al 2015 la sezione di Ferrara dell'IZSLER ha ricevuto al fine di confermare la presenza di sostanze tossiche: 204 campioni di esche/bocconi sospetti, 123 tra carcasse e contenuti gastrici di animali domestici, 45 carcasse di animali selvatici appartenenti a 19 specie diverse, per un totale di 372 campioni. Le esche sono state sottoposte prima ad un esame ispettivo e poi, se necessario, indirizzate al laboratorio chimico per ricerca di sostanze tossiche. Sulle carcasse si è proceduto con l'esame necroscopico e, sulla base dei rilievi, sono stati prelevati materiali da sottoporre ad analisi chimiche. Risultati Il 37,6% delle esche (140 campioni), è risultato positivo per presenza di sostanze tossico/nocive. Le sostanze tossiche più spesso rilevate sono stati i ratticidi anticoagulanti (39,7%), seguiti da associazione carbammati/pesticidi fosforati (15,7%), carbammati (12,8%) e pesticidi clorurati (9,3%). Nel 5% delle esche erano presenti corpi estranei (chiodi o aghi). Il 3,6% dei campioni positivi conteneva stricnina. Le carcasse o i contenuti gastrici di animali domestici conferiti appartenevano soprattutto a cani (52,8%) e gatti (45,5%). I cani risultati avvelenati sono stati 28 (43,1%), i gatti 25 (44,6%). I tossici responsabili di questi avvelenamenti sono stati, nel cane, i ratticidi anticoagulanti (39,3%) e nel gatto i ratticidi anticoagulanti (24%) e i carbammati (24%). Il 66,7% del selvatici è risultato avvelenato; tra le diverse specie si evidenzia che 4 rapaci su 4 sono risultati positivi per pesticidi mentre negli animali sinantropi i tossici ritrovati sono stati prevalentemente ratticidi anticoagulanti. Conclusioni Molte sostanze tossiche sono di facile reperimento sul mercato ma in cinque casi, inviati al laboratorio nel 2008, 2009 e 2012, è stata rilevata anche stricnina, prodotto bandito dal commercio da anni. L'Ordinanza impone il coinvolgimento del Servizio Veterinario dell'AUSL e del IZSLER ma, questa norma rimane spesso disattesa, perciò il numero di casi segnalati all'Autorità Giudiziaria è certamente inferiore a quello reale. Sebbene si sia raggiunto un buon livello diagnostico da parte dei laboratori chimici degli IZZSS, i margini di miglioramento restano ampi e risulta quindi importantissima la collaborazione tra le diverse competenze, come quella in atto con il Laboratorio di Tossicologia Forense dell'Università

di Ferrara, al fine dell'ampliamento del numero e della categoria di sostanze tossiche individuabili.

Rubini°S, Ravaioli C, Previato S, D'Incau°M, Tass inari M, Guidi E, Lupi S, Meriardi°G, Bergamini M

**Prevalence of Salmonella strains in wild animals from a highly populated area of North-Eastern Italy**

Ann Ist Super Sanita'. - Vol. 52 no 2 ( 2016). - p 277-280. - 17 bib ref [Nr. Estr. 7302]

Introduction. Salmonella is a ubiquitous pathogen that can infect host species, like wild birds, rodents, and/or arthropods, which may transmit infection to domestic animals and human population. Aim. In order to assess the related risk, a cross-sectional study was performed on 1114 carcasses of wild animals from a north-eastern area of the Emilia-Romagna Region, Italy. Materials and methods. During post mortem examination, intestine samples were cul-tured. A statistical analysis demonstrated that there is no correlation between the presence of sub-clinically infected animals and greater human population density. In contrast, a significant correlation between the number of carcasses positive for Salmonella spp. and greater spatial density of pig, poultry. and cattle farms was observed ( $p < 0.01$ ). Results. The results of the present study show that wild animals with omnivorous feeding habits are particularly exposed to Salmonella colonization and, consequently, to spreading the organism. Regarding drug resistance, this study confirms the resistance to anti microbials is increasing in commensal and environmental isolates.

Ruggeri°J, Chirullo B, Martinelli°N, Drumo R, Sca glione EF, Prege P, Ammendola S, Battistoni A, Corradi A, Ossiprandi MC, Bollo E, Pasquali P, Alborali°GL

**Attenuated vaccine of Salmonella typhimurium monophasic variant in piglets : evaluation of efficacy in homologous and heterologous challenge infection**

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 2 p. - 3 bib ref [Nr. Estr. 7580]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Salmonella Typhimurium Monophasic variant was identified during the second part of nineties and its incidence is increased during the last two decades. As S. Typhimurium, is widely spread worldwide and clinically indistinguishable (Echeita et al., 1999). Pigs could be chronically infected and introduce bacteria into slaughterhouse contaminating the food chain process with a high risk for human health (Hauser et al., 2010; EFSA, 2015). The aim of this study was to analyze efficacy of two attenuated vaccines, S. Typhimurium znuABC and S. Typhimurium Monophasic variant znuABC in homologous and heterologous challenge infections in piglets, because, these two strains are the most common isolated in pig farms and they could be simultaneously present in piggeries. Twenty-eight animals were divided in 6 groups, two unvaccinated (5 in group E and 5 in group F), two vaccinated with attenuated Salmonella Typhimurium znuABC (3 in group A and 5 in group B) and two vaccinated with attenuated Salmonella Typhimurium znuABC Monophasic variant (5 in group C and 5 in group D). Three groups were infected with virulent S. Typhimurium (A, D, E) and three with S. Typhimurium Monophasic variant (B, C, F) at day 36 after vaccination. Clinical investigations were weight and temperature measurement. Fecal samples were collected at day 1, 4, 9, 15, 23, 29 and 35 after vaccination and at day 2, 7, 10, 14 and 20 after infection for microbiological analysis. At day 20 after infection, piglets were euthanized and samples of tonsils, ileocecal lymph nodes, spleen, ileum, caecum and colon were collected for microbiological and histological investigations. Results have underlined that weight was not affected by vaccination and

infection. The trend of vaccines shedding was similar among groups and particularly, a sharp decline was present in both groups during the first week after vaccination. Vaccination with both attenuated strains was more effective in reducing shedding of mST than shedding of *S. Typhimurium*. Tonsils were the most colonised organs and mST znuABC was more effective in reducing heterologous colonization. In conclusion, vaccination with mST znuABC seems to be the preferable choice reducing faecal shedding of homologous and heterologous virulent strains significantly. Furthermore, it determines a reduction in organs of immune system, as tonsils and lymph nodes, during heterologous infection with virulent *S. Typhimurium*.

Ruggeri°J, Martinelli°N, Chirullo B, Drumo R, Oss iprandi M, Corradi A, Alborali°GL, Pasquali P

**S. Typhimurium and S. Typhimurium Monophasic variant attenuated vaccines. A comparison of efficacy in homologous and heterologous infection in piglets**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - p 417 [Nr. Estr. 7422]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Salogni°C, Lazzaro°M, Giacomini°E, Giovannini°S , Zanoni°M, Giuliani°M, Ruggeri°J, Pozzi P, Pasquali P, Boniotti°MB, Albo rali°GL

**Infectious agents identified in aborted swine fetuses in a high density breeding area: a tree year of study**

J Vet Diagn Invest. - Vol. 28 no 5 ( 2016). - p 550-554. - 32 bib ref [Nr. Estr. 7316]

Reproductive failure in sows is one of the most important factors affecting pig breeding. Many reproductive disorders are linked to both environmental factors and infectious agents. The goal of our study was to determine the presence of pathogens that are known to cause abortion, considering a set of conditioning factors, such as seasonality and pregnancy period. A large number of aborted fetuses (1,625 fetuses from 140 farms) from a high-density breeding area in northern Italy was analyzed for a period of 3 years. The pigs were diagnosed based on direct (culture, PCR) or indirect (enzyme-linked immunosorbent assay) evidence. An infectious etiologic agent was found in 323 of 549 cases of abortion (58.8%). These included viral agents (Porcine circovirus-2, 138/323; Porcine reproductive and respiratory syndrome virus, 108/323; porcine parvovirus, 20/323; pseudorabies virus, 6/323; and Encephalomyocarditis virus, 3/323) and bacteria (*Escherichia coli*, 64/323; *Streptococcus* sp., 63/323; *Staphylococcus* sp., 5/323; *Pasteurella* sp., 3/323; *Shigella* sp., 1/323; and *Yersinia* sp., 1/323). This study describes the prevalence of infectious agents involved in reproductive failure in a high-density swine population. The data can be useful to swine breeders, practitioners, and medical specialists in monitoring animal health and in supervising the breeding process.

Salogni°C, Lazzaro°M, Giovannini°S, Giuliani°M, Giacomini°E, Pasquali P, Alborali°GL

**Mycoplasma hyorinis, Haemophilus parasuis e co-infezioni batteriche nelle polisierositi del suino**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 121-124. - 7 bib ref [Nr. Estr. 7232]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Scopo del presente lavoro è quello di valutare le associazioni tra infezioni con *Haemophilus parasuis* and *Mycoplasma hyorhinis* ed altri patogeni batterici in suini che all'esame anatomico patologico presentavano polisierosite. Sono stati individuati 74 soggetti appartenenti a tre diverse fasi di allevamento così suddivise: 49 svezzamento (76%), 17 magronaggio (16%) e 8 sottoscrofa (8%). Da ogni soggetto sono state effettuate indagini batteriologiche e PCR. È stato riscontrato *H. parasuis* in 37 casi (58%), *M. hyorhinis* in 31 (48%), *Streptococcus* sp. in 20 (31%), *Pasteurella multocida* in 12 (19%), *Escherichia coli* in 9 (14%), *Actinobacillus pleuropneumoniae* in 8 (13%) e *Actinomyces pyogenes* in 2 (3%). È stato inoltre riscontrato un elevato numero di coinfezioni. Questo è stato particolarmente evidente per *M. hyorhinis* (87%) ed *H. parasuis* (57%). Non è stata invece osservata nessuna relazione tra microrganismo evidenziato, categoria di animali colpiti e sierosa colpita.

*Polyserositis is identified as an inflammation fibrinous or fibrinous-purulent frequently but not exclusively related to infection by Haemophilus parasuis and Mycoplasma hyorhinis. The most affected animals, in more virulent and acute form, are typically pigs in the weaning phase and to a lesser extent the growing-finishing. The objective of this study is to determine the associations among H. parasuis, M. hyorhinis and bacterial pathogens in 74 pigs with polyserositis. 49 post-weaning pigs (76%), 17 growing-finishing (16%) and 8 piglets (8%) were included. From each one we sampled swabs and tissues for further bacteriological and PCR investigations. It has been observed H. parasuis in 37 cases (58%), M. hyorhinis in 31 (48%), Streptococcus sp. in 20 (31%), Pasteurella multocida in 12 (19%), Escherichia coli in 9 (14%), Actinobacillus pleuropneumoniae in 8 (13%) and Actinomyces pyogenes in 2 (3%). It has been observed a high number of co-infections. This was particularly evident for M. hyorhinis (87%) and H. parasuis (57%). It was however observed no relationship between pathogen, category of affected animals or affected serosa.*

Salvatore D, Di\_Francesco A, Poglayen G, Rugna°G, Santi°A, Morandi B, Baldelli R

#### **Molecular characterization of *Leishmania infantum* strains by kinetoplast DNA RFLP-PCR**

Vet Ital. - Vol. 52 no 1 (2016). - p 71-75. - 25 bib ref [Nr. Estr. 7254]

Il metodo più frequentemente utilizzato per la classificazione di *Leishmania* spp. è ancora rappresentato dalla caratterizzazione isoenzimatica sebbene sia un metodo laborioso e in alcuni casi non sufficientemente discriminante. Nel presente studio abbiamo utilizzato una reazione di amplificazione del DNA kinetoplastico seguita da digestione enzimatica, al fine di caratterizzare 15 ceppi di *Leishmania infantum* MON-1, isolati da altrettanti cani ospitati in canili pubblici di 7 differenti aree geografiche del nord Italia. Il ceppo di riferimento MHOM/ TN/1980/IPT1 di *L. infantum* è stato incluso nello studio. A conferma del potere discriminante di questa metodica, sono stati osservati 6 differenti profili enzimatici, anche all'interno di uno stesso zimodema. A nostro avviso, kDNA RFLP-PCR può pertanto trovare un'applicazione utile per discriminare i casi di reinfezioni da quelli dovuti a ricadute, piuttosto che in studi epidemiologici su larga scala.

*Multilocus enzyme electrophoresis is the tool most frequently used to classify Leishmania spp., although it is time consuming and, sometimes, a not enough discriminative method. In the present study a kinetoplast DNA polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to characterize 16 zymodeme MON-1 Leishmania infantum strains: 15 were from dogs housed in public kennels of 7 geographical areas in the Emilia-Romagna region, Northern Italy, 1 was the L. infantum reference strain MHOM/TN/1980/IPT1. Six enzymatic patterns were observed. Kinetoplast DNA RFLP-PCR confirmed to have a good discriminatory power within the same zymodeme and proved to be useful for comparing few strains or discriminating between relapse and reinfection in the same host. We therefore recommend its use for discriminating between relapse and reinfection in the same host rather than supporting large-scale epidemiological studies.*

Salvioli°M, Chiari°M, Lavazza°A, Pasquali S, Zani°M, Capucci°L, Gilioli G  
**An epidemiological model for the European brown hare syndrome (EBHS)**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 47 [Nr. Estr. 7331]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

In the last decades a progressive decline in the hare populations has occurred in Italy and other European countries. Among many other causes, this decline has been associated with the occurrence of the EBHS, a highly contagious disease of brown hare, emerged in the '80s and currently considered endemic in Europe. The EBHS infection can achieve almost 100 % morbidity, when it is introduced into a naive brown hare population, and the mortality can be about 60 % in infected adult. The disease is not observed in new-born hares that are infected without exhibiting any clinical sign and develops a long-lasting immunity. In order to gain new insights on the EBHS epidemiology, we developed a mathematical model considering both the hare biology and the EBHS infection dynamics in an age-structured population. The model aimed at investigating the influence of the hare population density on the EBHS epidemiology. A set of eight ordinary differential equations was used to describe a compartmental system, where individuals were classified into different age categories (new born = less than 3 months, young 3 - 6 months, and adult = older than 6 months) and status with respect to the infection (susceptible/seronegative, infected and recovered/seropositive). The fluxes between different compartments were described by transfer functions representing development, fecundity, infection and recovery rates. The functions' parameters, including natural and EBHS-related mortality, were estimated from literature and from available observations. The model showed that EBHS transmission had a complex dynamic, strongly affected by hare density. In particular, at high-density value the virus was endemically maintained in the population, with a low mortality rate since most new born or survivals had acquired the infection becoming seropositive. On the contrary, for low-density values, no constant transmission of EBHS between susceptible individuals was observed in the simulation and the virus was pushed to extinction within a short time. Therefore, low density hare populations did not support EBHS endemic persistence and were exposed to recurrent EBHS outbreaks due to a low or absent immunity. An intermediate epidemiological pattern was observed in the transition from low to high population density. These simulation results were consistent with the serological data collected in different protected but open areas in the province of Brescia (Northern Italy) over the last years. Model results suggested that the strategy of promoting the endemic circulation of the virus through density control mechanisms seems to be the best way to reduce the EBHS impact.

Salvioli°M, Lavazza°A, Zanoni°M, Chiari°M, Gili oli G

#### **An epidemiological model for the European brown hare syndrome (EBHS)**

Hystrix Ital J Mamm. - Vol. 27 Suppl ( 2016). - p 23 [Nr. Estr. 7257]

Congresso Italiano di Teriologia (10. : Acquapendente (VT) : 20-23 Aprile 2016)

In the last decades a progressive decline in the hare populations has occurred in Italy and Europe generally. It has been associated, among the other hypothetical causes, also with the occurrence of European brown hare syndrome (EBHS), a highly contagious disease, emerged in the '80s and nowadays considered endemic in all European countries. EBHSV infection can achieve almost 100% morbidity, when it is introduced into a naive brown hare population and the mortality can be about 50– 70% in the adult age class. The disease is not observed in young hares (less than about 2-3 months) that are infected without exhibiting any clinical sign and develop a long-lasting immunity usually characterized by low-medium antibody titers when compared to high titers shown by convalescent hares which survived to the disease. In order to gain new insights on the disease epidemiology in brown hare population, we developed a mathematical model trying to describe the EBHS infection dynamics in an agestructured population. The model aims in particular at understanding the influence of hare population density in the EBHS epidemiology. In a previous described EBHS model density values greater than 15 hares/km<sup>2</sup> have been considered necessary to promote an endemic situation. Our epidemiological model takes into consideration both hare biology and population structure, as well as the EBHS infection dynamics. The system is described

by a compartmental model, where individuals are classified into different age categories (newborn= $<3$  months, young= $3-6$  months and adult= $\geq 6$  months) and stages of infection (susceptible/seronegative, infected and recovered/seropositive). The fluxes between different compartments are described by rate functions representing the development, the infection and the recovery. Natural mortality and well as mortality due to EBHS are also considered. The whole system is described by a set of eight ordinary differential equations. The parameters in the demographic rate functions have been estimated from data reported in the literature and from own observations, the same is for the recovery rate. Numerical tools allow us to investigate prevalence of overt disease and its impact (mortality and morbidity) during an EBHS outbreak. The model has been used to investigate possible disease epidemic scenarios. In detail, if the virus has an epidemic behavior into the hare population, the infection dynamic could be characterized by possible recurrent outbreaks with high mortalities. These outbreaks begin when the population immunity is low (high number of susceptible/seronegative individuals) and susceptible/seronegative young and adult hares are exposed to EBHS virus. As a consequence of EBHS outbreaks, surviving young and adult hares will develop protective immunity against the virus (i.e. seropositive). In addition we consider the case in which there is a constant recruitment of a sufficient number of new receptive/seronegative individuals (represented by newborn hares): here the virus is maintained endemically with low mortality rate due to EBHS and most adult individuals become recovered/seropositive. Future studies are needed to compare the obtained data with field ones, this modelling approach providing a new strategy for evaluation of the influence of population density and landscape characteristics on EBHS seroprevalence.

Savini F, Gallina L, Di\_Marco P, Vicari D, Puleio R, Lavazza<sup>o</sup>A, Purpari G, Guercio A, Scagliarini A

#### **Co-infezioni da Papillomavirus e parapoxvirus in lesioni cutanee di bovini in Sicilia**

VI Workshop Nazionale di Virologia Veterinaria : Torino 13-14 Ottobre 2016 : Libro degli atti / [s.l. : s.n., 2016]. - p 25 [Nr. Estr. 7410]

Workshop Nazionale di Virologia Veterinaria (6. : Torino : 13-14 Ottobre 2016)

La papillomatosi del bovino è una malattia infettiva contagiosa virale che causa neoformazioni cutanee diffuse fino ad assumere quadri clinici di estrema gravità causando ricadute economiche rilevanti soprattutto quando le lesioni sono localizzate a livello mammario e degli organi genitali. Nelle lesioni sono fino ad oggi stati identificati e completamente sequenziati 15 tipi di Papillomavirus bovini (BPVs), in grado di colpire epidermide e derma. La patologia è distribuita a livello mondiale e la diagnosi è perlopiù effettuata clinicamente o mediante osservazione delle particelle virali al microscopio elettronico. La profilassi negli allevamenti bovini può essere eseguita con vaccini stabulogeni. La diagnosi molecolare consente di identificare tipi e varianti virali con differente potere patogeno e di caratterizzarne il genoma. Inoltre, è stato già dimostrato che le lesioni proliferative possono albergare co-infezioni con virus potenzialmente zoonotici appartenenti alla famiglia Poxviridae. La nostra indagine si è posta l'obiettivo di valutare la presenza e distribuzione di virus epiteliotropi in lesioni papillomatose di bovini da latte e da carne di differenti province della Sicilia. Campioni patologici sono stati analizzati tramite ME e le lesioni classificate istologicamente. In seguito sono state eseguite PCR con primer BPVs, orthopoxvirus e parapoxvirus specifici e Rolling Circle Amplification (RCA). In tutti i campioni è stato identificato almeno uno dei quattro generi di BPVs, tra cui i Delta tipo 1 e 2 sono risultati i più rappresentati. Le co-infezioni sono risultate più frequenti rispetto alle infezioni singole inoltre, nel 50% delle lesioni positive alla ME per il solo papillomavirus, è stato rilevato anche DNA di virus appartenenti al genere parapoxvirus. La possibilità di instaurare infezioni subcliniche nel bovino da parte di agenti zoonotici appartenenti alla famiglia Poxviridae è stata riportata anche da altri autori e suggerisce l'importanza della diagnosi molecolare accanto a quella clinica per evitare la trasmissione dell'infezione all'uomo e ad altri animali.

Scagliarini A, Casà G, Trentin B, Gallina L, Savini F, Morent M, Lavazza°A, Puleio R, Buttaci C, Cannella V, Purpari G, Di\_Marco P, Piquemal D, Guercio A

**Evidence of zoonotic Poxviridae coinfections in clinically diagnosed papillomas using a newly developed mini-array test**

J Vet Diagn Invest. - Vol. 28 no 1 ( 2016). - p 59-64. - 26 bib ref [Nr. Estr. 7192]

Our study describes a newly developed mini-array test for the rapid detection of poxviruses in animals and humans. The method is based on detection that combines target nucleic acid amplification by polymerase chain reaction and specific hybridization, using enzyme-linked antibodies, allowing identification of zoonotic orthopoxviruses and parapoxviruses in animal and human biological samples. With 100% specificity, the test rules out the possibility of cross-reactions with viral agents causing look-alike diseases. The assay was employed in the field to investigate the causes of several outbreaks of a malignant proliferative skin disease that affected domestic ruminants in Sicily during 2011–2014. Due to specific aspects of the lesions, the animals were clinically diagnosed with papillomatosis. The mini-array test allowed the identification of coinfections caused by more than 1 viral species belonging to the Parapoxvirus and Orthopoxvirus genera, either in goats or in cattle. Our study suggests that the so-called “papillomatosis” can be the result of multiple infections with epitheliotropic viruses, including zoonotic poxviruses that cannot be properly identified with classical diagnostic techniques.

Scali°F, Alborali°GL, Giacomini°E, Lazzaro°M, C andela L, Nigrelli°A, Vezzoli°F, Rosignoli°C, Paterlini°F, Boldini°M, Prati°P, P asquali P, Vitali A, Borrello S

**Monitoring antimicrobials consumptions in pig farms with different approaches**

Atti Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), joint meeting REEV-Med, XVI Convegno S.I.C.V, XIV Convegno S.I.R.A, XIII Convegno AIPVet, XIII Giornata Studio So.Fi.Vet, III Convegno RNIV : 13-16 Giugno 2016, Palermo / [s.l. : s.n., 2016]. - 2 p. - 3 bib ref [Nr. Estr. 7581]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 70 REEV-Med  
Convegno SICV : 16 Convegno SIRA : 14 Convegno AIPVet : 13 Convegno So.Fi.Vet : 13  
Convegno RNIV : 3 : Palermo : 13-16 Giugno 2016)

Veterinary medicinal products (VMPs) are widely used in pigs including antimicrobials critically important for human medicine (CIA) [1]. Bacteria may develop antimicrobial resistance (AMR) that affect both animal and human health [2]. Decreasing antimicrobials consumption is pivotal to slow down AMR spread and proper standards to monitor VMPs usages are essential. However, a unique standard for veterinary medicine is still under discussion. Prioritisation of antimicrobial classes for human medicine is also debated and different classifications were proposed [1, 3]. The aim of this study was to test different approaches for monitoring antimicrobials usage in pig farms. 42 fattening farms, with at least 2000 pig produced per year, were selected from herds included in a project implemented by Ministry of Health in collaboration with IZSLER, Lombardy Region and Official Veterinary Services. Data for 2014 were collected retrospectively. Consumptions were calculated using a Defined Daily Dose Animal standard (DDDAit) and a mass-based standard: milligrams of active ingredient (AI) consumed per kilogram of live weight produced (mg/kg meat). DDDAit were used to calculate, for each farm, mean days of therapy per pig per year (average weight at treatment 100 kg) with two different approaches. One considered each used AI independently; the other considered VMPs with a combination of AIs as a single antimicrobial. CIAs were identified according to WHO ranking [1] and further stratified with EMA classification [3]. The 42 farms produced 220,569 pigs (mean 5368, range 2056-15798) and 37,797 tonnes of live weight (mean 920, range 354-2686). Mean consumptions, at farm level, were 196 mg/kg meat (range 20-530), 27.69 days/pig/year for single AIs (range 3.41- 81.52) and 22.71 days/pig/year for combinations (range 3.41-81.52). Days/pig/year differed significantly (p-value<0.0001) between the two standards, mean difference was 18.36% (range 0.00%-48.95%). 24.69% of consumed DDDAit were CIAs. According to EMA classification, 38.90% of these CIAs were category 1 antimicrobials and 61.10% category 2. Although practical to calculate, mass-based standards do not consider AIs power and should be

discontinued. DDD-based approaches can be more accurate but collecting data without an electronic prescription system is time consuming. Widely accepted standards are strongly needed for both consumptions calculation and AIs prioritisation. Particular attention should be posed when monitoring usages of combinations VMPs. Considering AIs separately increases combinations VMPs impact on overall consumptions, and so, it may discourage abuses. Nevertheless, these VMPs can reduce risks of AMR via their AIs synergic effects against some bacteria.

Scali°F, Giacomini°E, Lazzaro°M, Nigrelli°A, Bontempi°G, Pasquali P, Borrello S, Bonati S, Perella A, Candela L, Vitali A, Alborali° GL

**Monitoring antimicrobial consumptions in fattening pigs in Italy: preliminary findings towards an integrated approach**

24th International Pig Veterinary Society (IPVS) Congress, 8th European Symposium of Porcine Health Management : 7th-10th June, 2016 Dublin, Ireland : abstracts book / [s.l. : s.n., 2016]. - p 185 [Nr. Estr. 7420]

International Pig Veterinary Society Congress (IPVS) : 24th European Symposium of Porcine Health Management : 8th : Dublin, Ireland : 7th-10th June, 2016)

Introduction: Italy is a large pig producer and a system to monitor active ingredients (AIs) consumptions of veterinary medicinal products (VMPs) at farm level is needed. The aims of this study were to develop a tool to record these consumptions and to compare Ala usages with production losses, biosecurity levels and health statuses. Materials and Methods: A data collection software, an XML database and an interactive dashboard were developed to store data and perform calculations. The system was tested with a convenience sample of 20 fattening farms (mean pig slaughtered per year 4780). Data were collected retrospectively for 2014 or 2013. Ala consumptions were calculated yearly as milligrams of AI used per kilogram of meat produced (mg / kg meat). In addition, defined daily and course dose animal for Italy (DDDAit and DCDAit), based on national prescriptions, were established. Mean days and courses of therapy per pig were also calculated using 100 kg as average weight at treatment. Biosecurity levels were evaluated with a survey and losses as sum of modality and cull. Correlations between AIs usages and losses or biosecurity were investigated. To further assess differences in Ala consumptions, farms were grouped according to clinical reports, presence or absence of *Brachyspira hyodysenteriae* and *Actinobacillus pleuropneumoniae* (APP). Results: Average usages were 114 mg/kg meat (range; 20-222), 17.7 days (range; 4.1-37.9) and 3.3 cycles (range; 0.6-6.9) per pig. Administration routes were 4.1% injectable (of total DDDAit), 22.6% oral powder, 10.9% oral solution and 62.4% premix. The top five used AIs were lincomycin (20.4% of consumed DDDAit), doxycycline (16.5%), tiamulin (15.6%), amoxicillin (12.9%) and colistin (10.1%). Mean biosecurity score was 63.0% (range; 48.9%- 73.9%). Mean losses were 5.2% (range; 2.0%-10.0%). AIs consumption and biosecurity or losses were not significantly correlated. 35% of the farm reported respiratory signs, 20% enteric and 45% both. 30% were positive to *B. hyodysenteriae*, 25% to APP, 10% to both and 35% were negative. AIs consumptions did not significantly differ between groups. Conclusion: An XML database allows changing bases of calculation, when new standards are established, without affecting stored data. Interactive dashboards offer an intuitive depiction of Ala consumptions, via charts, with different levels of aggregation. Evaluations on national consumptions and comparisons between AIs usages, losses, biosecurity and health status require further studies with a larger sample size. Data on animal welfare, slaughterhouse and other pathogens should be included to improve the health status assessment and the integrated approach.

Scali°F, Giacomini°E, Lazzaro°M, Vezzoli°F, Rosignoli°C, Paterlini°F, Nigrelli°A, Boldini°M, Prati°P, Paolo P, Vitali A, Alborali°G

**Indagine su biosicurezza e consumo di antimicrobici in 25 allevamenti suini da ingrasso: confronto tra diversi standard di misurazione**

Atti Convegno SIPAS. - Vol. 42 ( 2016). - p 97-102. - 12 bib ref [Nr. Estr. 7234]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (42. : Montichiari (BS) : 10-11 Marzo 2016)

Gli antimicrobici trovano largo impiego nell'allevamento suinicolo ed i fenomeni di antibiotico resistenza destano sempre più preoccupazione. La riduzione dell'uso di antimicrobici è possibile soltanto attraverso un efficace sistema di monitoraggio, basato su standard affidabili, che consideri anche di altri parametri che potrebbero influenzare i consumi. Lo scopo di questo studio è identificare potenziali standard e valutare il rapporto tra consumi, dimensioni aziendali e biosicurezza. I consumi di 25 allevamenti da ingrasso sono stati analizzati utilizzando tre unità di misura: milligrammi di principio attivo consumati per produrre un chilogrammo di carne (mg PA / kg carne), Defined Daily Doses Animal for Italy (DDDAit) e Defined Course Doses Animal for Italy (DCDAit). Quattro indicatori derivati da DDDAit/DCDAit sono stati identificati: giorni/anno, cicli/ anno, DDDAit e DCDAit consumate per suino prodotto. La biosicurezza è stata analizzata con un questionario. I consumi medi sono risultati: 20,19 giorni/anno, 3,73 cicli/anno, 134 mg Pa / kg carne, 2.112 DDDAit e 390 DCDAit per suino. Nessuna correlazione è stata individuata tra biosicurezza, consumi e dimensioni aziendali. Gli indicatori basati su DDDAit/DCDAit forniscono utili informazioni sul consumo di antibiotici, tuttavia, sono necessarie ulteriori indagini con un campione più ampio ed un maggior approccio integrato che consideri nuovi parametri da analizzare ed ampliare i dati raccolti su quelli già inclusi.

*Antimicrobials (Ams) are widely used in pig farms and antimicrobial resistance in an increasingly serious menace. A monitoring system is pivotal to reduce Ams consumption. An efficient system must be based on reliable standards and it should consider other parameters that can increase Ams usages. The aim of this study was to identify potential standards of measurement. Correlations between Ams consumption, biosecurity and farm size were also evaluated. Three units of measurement were used to assess Ams consumption in 25 fattening pig farms: milligrams of active ingredient consumed to produce a kilogram of meat (mg AI / kg meat), Defined Daily Doses Animal for Italy (DDDAit) e Defined Course Doses Animal for Italy (DCDAit). Four indicators, based on DDDAit/ DCDAit, were also identified: days/year, cycles/year, DDDAit and DCDAit used per pig produced. Biosecurity levels were evaluated via questionnaire. Mean Ams consumptions were: 20.19 days/year, 3.73 cycles/year, 134 mg AI / kg meat, 2,112 DDDAit and 390 DCDAit per pig. No correlations were founded between Ams consumption, biosecurity and farm size. Indicators based on DDDAit/DCDAit can provide useful data on Ams usages. Further studies with a larger sample size are required to assess correlations between Ams usages and other parameters. In addition, an integrated approach should be used, with the inclusion of new parameters analyzed and more data collected on the ones already considered in this study.*

Scaravelli°D, Tosi°G, Fiorentini°L, Parigi°M, Caminiti°A, Massi°P

### **I corvidi quali specie sentinella per le "avian emerging diseases" in Romagna**

Atti della Societa' Italiana di Patologia Aviare (SIPA) 2016 : LV Convegno nazionale, Tavola rotonda : Padova, 5-6 maggio 2016 - Parma, 23 settembre 2016 / [s.l. : s.n., 2016]. - p 233-236. - 13 bib ref [Nr. Estr. 7474]

Tavola rotonda Societa' Italiana Patologia Aviare (SIPA) : Parma : 23 Settembre 2016)

*Among wild birds three species of Corvids, Magpie Pica pica, Hooded crow Corvus corone cornix and Jay Garrulus glandarius were used as sentinel species for the presences of Flavivirus, West Nile, Usutu and Newcastle viruses in the territories of Ravenna, Rimini and Forli Provinces, the Romagna area, during year 2013- 2015 as part of the comprehensive monitoring effort going on in Lombardia and Emilia Romagna regions by Istituto Zooprofilattico Sperimentale sections. The three corvids confirm they role as potential host for the different virus. Hooded crown have 1,2% positive sample in 2014 for Flavivirus, any positive for New-castle, 1,2% positive for Usutu in 2014 as well as 1,2% positive in 2014 for West Nile virus. Magpie was positive all the years for Flavivirus reaching the 23% of positive in 2015, any positive for Newcastle, till 7% of positive for Usutu and 3,7 positive for West Nile virus. Jay reach 10% of positive for Flavivirus as well for Usutu, but had no positive for Newcastle and West Nile virus. The species differ for susceptibility and dissemination among years and can represent a better model if the collection and transfer procedures will be improved and more*

*precisely configured with the territory.*

Scaravelli°D, Tosi°G, Parigi°M, Fiorentini°L, M. Massi°P

**Studies on carnivores conferred to Istituto Zooprofilattico of Forli in 2013-2015**

Hystrix Ital J Mamm. - Vol. 27 Suppl ( 2016). - p 143 [Nr. Estr. 7462]

Congresso Italiano di Teriologia (10. : Acquapendente (VT) : 20-23 Aprile 2016)

Carnivore represent the typical umbrella species in terms of conservation and for the human dimension in conservation biology. This role can be also considered for the environmental health as they tend to accumulate, in some respects, the parasitic diversity, contaminants and also to be very sensitive to human related pressures. To evaluate this role and as a part of the project here we report on the last three years of investigations carried out on specimens of wild carnivores received by the Institute in Forli. In this first report we do not consider foxes as their status and management cases, related to control plans and the regional wildlife health control plan need a different approach. From 2011 to 2015 15 wolves *Canis lupus* were brought to the laboratory for analysis, the 19% of the whole number for the same years in both regions. They were 1 in 2011, 2 in 2012 and 2013, 4 in 2014 and 6 in 2015. The increase in number is related both to the wide spreading of the species to lower parts of the Apennine's valleys, in more close contact with human activities, and also due to the increase of attention to evaluate the animals found dead in the territory. Like for others wildlife also wolves have a lot of vehicle casualties but the poisoning is still the main cause of death, with different active ingredients, especially organochlorine pesticide. 8 cases resulted positive for *Escherichia coli* in intestine, as well as in 15 intestinal helminths were recognized. Also sarcoptic mange was found on carcasses coming from the border with the Marche region, where is also widespread on *Vulpes vulpes*. Among ectoparasites were found *Ixodes ricinus* and *Rhipicephalus turanicus*. Among Mustelidae, the *Meles meles* numbers are between 3 and 1 in 2011 and 2012 respectively to 22 in 2015, summing 40 specimens, 13% of the total of the 2 regions. All specimens arrived from road casualties. In 9 cases etiological agents were identified: *Citrobacter freundii* (2), *Edwardsiella tarda*, *Enterococcus faecalis*, *Enterococcus faecium/durans*, *Escherichia coli* (6), *Genus Shigella*, *Serratia liquefaciens* and also 1 case with *Salmonella typhimurium* (no evidence of symptoms) and one of *Yersinia pseudotuberculosis*. Just one *Martes foina* arrived in 2015, an adult male crashed on road, around 1% of the whole number checked in the two regions in 5 years. In the specimen *Citrobacter koseri* was isolated. In these years in all the sections 98 specimens were checked, the majority in Sondrio and Binago, as ever mostly from car traumas. Other 16 different etiological agents were also identified in the other cases, as well as sarcoptic mange in Alps. Unexpected also a *M. putorius* carcass came in late 2015 near Ravenna from the road. The species was considered in rarefaction and this new case bring back the attention to the actual status. No *Mustela vison* were checked despite the species still has a stable population on the Ronco river and for which it is not in place unfortunately any control plan. Only one *Felis silvestris* was checked, anyway an important witness of the local increasing population, also found dead on the road, where one of the *E. coli* strain was found. The attempt to collect new information about the potential role of wildlife in the increasing "new" diseases as well as the diffusion of zoonosis and relationship along with the livestock sector and the presence in urban centers are discussed in the light of the "one health" concept. Thanking all the crew helping in analysis and data management, this work is part of the project "Inurbazione della fauna selvatica, sinantropi e possibile rischio zoonotico" currently in progress.

Shimmon G, Wood BA, Morris A, Mioulet V, Grazioli° S, Brocchi°E, Berryman S, Tuthill T, King DP, Burman A, Jackson T

**Truncated bovine integrin Alpha-v/Beta-6 as a universal capture ligand for FMD diagnosis**

PLoS One. - Vol. 11 no 8 ( 2016). - p e0160696 (132 p). - 28 bib ref ( ultimo accesso 03/11/2016 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0160696> ) [Nr. Estr. 7381]

Foot-and-mouth disease (FMD) is endemic in many regions of the world and is one of the most

prevalent epizootic animal diseases. FMD affects livestock, such as cattle, sheep, goats and pigs, and causes enormous economic losses due to reduced productivity and trade restrictions. Preparedness and early diagnosis are essential for effective control of FMD. Many diagnostic assays are dependent on raising high-affinity, anti-FMD virus (FMDV) serotype-specific antibodies in small animals (rabbits and guinea pigs) that give broad virus coverage. Here we show that soluble, truncated forms of bovine  $\alpha\beta 6$  bind FMDV in an authentic RGD and divalent cation dependent interaction and can be used as the trapping reagent in a FMDV sandwich ELISA. In addition, inclusion of FLAG or His tags facilitates simple purification without the loss of virus binding. We also provide evidence that when combined with a guinea pig polyclonal serum, or serotype-specific monoclonal antibodies, the integrin can be used to detect viruses representative of all FMDV serotypes. We also show that recombinant FMDV empty capsids, with stabilising disulphide bonds, can serve as an antigen in the ELISA and can therefore replace inactivated virus antigen as a positive control for the assay. Our results demonstrate the potential use of bovine  $\alpha\beta 6$  and FMDV empty capsids in FMD diagnostic assays.

Sozzi° E, Moreno° A, Lelli° D, Prosperi° A, Perulli ° S, Giacomini° E, Alborali° GL, Brocchi° E, Lavazza° A

**Sviluppo e validazione di una metodica ELISA competitiva per la ricerca di anticorpi nei confronti del virus della diarrea epidemica suina (PEDV)**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 243-244. - 6 bib ref [Nr. Estr. 7372]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*A monoclonal antibody (MAb)-based competitive ELISA for detecting antibodies against PED virus was developed and validated. The diagnostic performance of the test was evaluated by three steps: 1 comparison with the immunoperoxidase monolayer assay test (IPMA) by testing 296 field samples; 2 ROC analysis using a panel of 762 known sera; 3 collection of sera from experimental infections. The competitive ELISA had excellent diagnostic performance and discriminatory power with high Se and Sp values (Se=96.5%, 95%IC 94.1-98,1; Sp=98.2%, 95%IC 96.3-99.3). In addition PEDV ELISA was able to detect positive experimental sera starting from 5-7 days post-infection.*

Steinrigl A, Cay B, Nauwynck H, Desmarests L, Christiaens I, Theuns YV, Van\_De\_Stede Y, Botner A, Strandbygaard B, Brnic D, Nurmoja I, Tedersoo T, Laine T, London L, Grasland B, Rose N, Evain L, Marce C, Biome S, Schwarz BA, Kritas S, Fortomaris P, Balint A, Dan A, Moriarty J, Ryan E, Lavazza° A, Alborali° G, Boniotti° B, Cerioli° M, Masiulis M, Vilhena\_Clemen te PI, Vaz Y, Correia MJ, Van\_Der\_Poel W, Van\_Der\_Wolf P, Grontvedt CA, Tafjord\_Heier B, Ondrejкова A, Mojzis M, Polak D, Gonzalo\_Martinez D, Huken C, Steinbach F, Roberts H, Williamson S, Franssen P, Garrido GC

**Collection and review of updated scientific epidemiological data on porcine epidemic diarrhoea**

European Food Safety Authority (EFSA) / [s.l. : s.n, 2016]. - EFSA J. - Vol. 14 no 2 ( 2016). - p 1-52. - 53 bib ref [Nr. Estr. 7238]

Porcine epidemic diarrhoea (PED) is a non-zoonotic viral disease of pigs caused by a coronavirus and characterised by watery diarrhoea and weight loss. PED is not notifiable to the EU or World Organisation for Animal Health listed but it is notifiable at the national level in Finland, France, Ireland and Sweden. PED case reports from seven countries and PED surveillance and monitoring activities in thirteen countries were reported. This information was combined with an extensive literature review to provide an update on global PED occurrence, circulating strains and impact in

2014-2015. PED confirmed cases have been reported in North America, South America, Asia and Europe. PED virus (PEDV) sequences originating from EU pig herds indicate that the strains currently in circulation share nearly 100% sequence identity and have greater than 99% sequence identity with the reference INDEL (insertion/deletion) strain USA/0H851/2014. In 2014-2015, greater genetic variability has been reported in strains circulating in Asia compared with EU Member States and a non-INDEL strain has been detected in the Ukraine in 2014. Data on impact confirms that mortality is higher in suckling piglets and diarrhoea is observed in all age groups. The reported impact is in agreement with that reported in EFSA AHAW Panel (2014) indicating that the impact of recently reported PED outbreaks in Asia and the USA seems to be more severe than that described in EU countries, although the impact of different PEDV strains is difficult to compare between one country and another, as impact is dependent not only on pathogenicity but also on factors such as biosecurity, herd size, farm management, sanitary status or herd immune status.

Strandbygaard B, Lavazza° A, Lelli° D, Blanchard Y, Grasland B, Le\_Poder S, Rose N, Steinbachh F, Van\_der\_Poel WHM, Widén F, Belsham GJ, Botner A

**Inter-laboratory study to characterize the detection of serum antibodies against porcine epidemic diarrhoea virus**

Vet Microbiol. - Vol. 197 ( 2016). - p 151-160. - 29 bib ref [Nr. Estr. 7494]

Porcine epidemic diarrhea virus (PEDV) has caused extensive economic losses to pig producers in many countries. It was recently introduced, for the first time, into North America and outbreaks have occurred again in multiple countries within Europe as well. To assess the properties of various diagnostic assays for the detection of PEDV infection, multiple panels of porcine sera have been shared and tested for the presence of antibodies against PEDV in an inter-laboratory ring trial. Different laboratories have used a variety of "in house" ELISAs and also one commercial assay. The sensitivity and specificity of each assay has been estimated using a Bayesian analysis applied to the ring trial results obtained with the different assays in the absence of a gold standard. Although different characteristics were found, it can be concluded that each of the assays used can detect infection of pigs at a herd level by either the early European strains of PEDV or the recently circulating strains (INDEL and non-INDEL). However, not all the assays seem suitable for demonstrating freedom from disease in a country. The results from individual animals, especially when the infection has occurred within an experimental situation, show more variation.

Taddei° R, Renzi° M, Procopio° A, Canelli E, Gallet ti° G, Gentile A, Merialdi° G, Tamba° M, Schares G

**Diagnosi sierologica di besnoitiosi bovina : tre test di conferma a confronto**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 245-246. - 9 bib ref [Nr. Estr. 7373]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*Bovine besnoitiosis is a chronic disease caused by protozoal Besnoitia besnotti, recognized t in 2010 by EFSA as an emerging disease in Europe. In order to increase the significance of serological surveys, the use of ELISA kits as screening test with subsequent confirmation analysis is recommended. The objective of this work is to compare the performance of three confirmatory test (two Western Blotting protocols and one IFAT test) on a panel of 38 serum samples reactive to the commercial ELISA PrioCHECK Besnoitia Ab2.0 kit (Prionics). Our study corroborates that ELISA screening results needs to be further confirmed. The confirmation of reactive results could be limited to the analysis of positive samples not including the inconclusive results. IFAT test showed higher performance. Taking into account the lower laboriousness, the greater ease of access to less*

*specialized laboratories and the lower cost, the IFAT would be the preferable test among the three evaluated in this work.*

Tagliabue°S, Figaroli°BM, D'Incau°M, Foschi G, Gennero MS, Giordani R, Natale A, Papa P, Ponti N, Scaltrito D, Spadari L, Vesco G, Ruocco L

### **Serological surveillance of Leptospirosis in Italy : two-year national data (2010-2011)**

Vet Ital. - Vol. 52 no 2 ( 2016). - p 129-138. - 41 bib ref [Nr. Estr. 7290]

La leptospirosi è una malattia infettiva riemergente diffusa in tutto il mondo la cui prevalenza è spesso sottostimata. Il Centro Nazionale di Referenza per la Leptospirosi (NRCL), presso l'Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia (Italia), con la collaborazione di tutti gli altri Istituti Zooprofilattici Sperimentali (IZZSS), ha valutato la diffusione di tale importante zoonosi in Italia. I dati sierologici, ottenuti tra il 2010 e il 2011, da ciascun Istituto, sono stati raccolti dal NRCL e valutati. I campioni di siero raccolti da 43.935 soggetti di varie specie animali sono stati analizzati con la tecnica di agglutinazione microscopica (MAT), utilizzando un pannello di 8 antigeni di diverso sierogruppo (Australis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Sejroe, Tarassovi). Per identificare le positività sierologiche è stato utilizzato il valore soglia di 1:100. Sono stati rilevati titoli positivi per 6.279 sieri. I campioni da bovini (46,9%), da suini (27,5%), da ovini e caprini (7,4%), da cani (6,9%) e da cinghiali (4,5%) sono stati più numerosi rispetto a quelli da equini e da altre specie. L'analisi dei dati ha mostrato che i sierogruppi più comuni in Italia sono: Australis presente in cani, cinghiali, cavalli, lepri, suini, volpi e roditori; Sejroe rilevato in bovini, ovini, caprini e bufali; Icterohaemorrhagiae presente in cani, caprini e volpi; Pomona rilevato in suini, bovini, e specie selvatiche; Grippotyphosa è stato riportato nelle lepri.

*Nowadays, leptospirosis is a re-emerging widespread infectious disease often underestimated worldwide. The National Reference Centre for Leptospirosis (NRCL), at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia (Italy), with the cooperation of all the other Istituti Zooprofilattici Sperimentali (IZZSS), evaluated the distribution of such important zoonosis in Italy. Serological data obtained between 2010-2011 by each laboratory were collected by the NRCL and discussed. Serum samples collected from 43,935 animal specimens were analysed by the Microscopic Agglutination Test (MAT), using a panel of 8 serogroups as antigens (Australis, Ballum, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Sejroe, Tarassovi). A MAT cut-off of 1:100 was used to identify the serological positivities, 6,279 sera showed positive titers. Bovine (46.9%), swine (27.5%), ovine and goat (7.4%), dog (6.9%), and wild boar (4.5%) samples were delivered to the Laboratories more frequently than equine and other species sera. Data analysis showed that the most common serogroups in Italy are: Australis present in dogs, wild boars, horses, hares, swine, foxes, and rodents; Sejroe detected in cattle, sheep, goats, and buffaloes; Icterohaemorrhagiae present in dogs, goats, and foxes; Pomona detected in swine, cattle, and wild species; Grippotyphosa reported in hares.*

Tosi°G, Caminiti°A, Fiorentini°L, Massi°P

### **Uso di acidi organici a corta catena e riduzione delle lesioni plantari nel broiler : risultati preliminari**

Atti della Società Italiana di Patologia Aviare (SIPA) 2016 : LV Convegno nazionale, Tavola rotonda : Padova, 5-6 maggio 2016 - Parma, 23 settembre 2016 / [s.l. : s.n., 2016]. - p 246-249. - 9 bib ref [Nr. Estr. 7475]

Tavola rotonda Società Italiana Patologia Aviare (SIPA) : Parma : 23 Settembre 2016)

*Short-chain fatty acids (SCFA) have several positive effects on broilers as they improve animal production parameters and show a considerable antimicrobial activity. In this study, the effect of dietary supplementation with SCFA has been evaluated on the growth of intestinal Clostridium perfringens and Escherichia coli populations, and on the severity of lesions observed on the plantar surface of broilers. For the first objective, a negative binomial regression was used to evaluate the differences in the number of C. perfringens and E. coli cell forming units per gram (CFUs/g) in the*

*ileum between broilers of the control group and broilers fed the same diet supplemented with SCFA (treatment group). For the second objective, a multinomial logistic regression was used to evaluate whether the type of diet (basal diet or diet supplemented with the additive) significantly affected the severity of lesions observed on the plantar surface. The statistical analysis was performed in a Bayesian framework. Results showed that the number of C. perfringens CFUs/g significantly decreased by 77.8% (=median of the posterior distribution) in broilers receiving the feed additive, whereas the number of E. coli CFUs/g decreased by 97.3%. Regarding the severity of lesions observed on the plantar surface, controls were 3.55 times as likely to have mild lesions compared with broilers of the treatment group. Dietary supplementation with SCFA significantly reduced specific intestinal microbial populations and decreased the prevalence and severity of lesions on the plantar surface.*

Tosi°G, Fiorentini°L, Parigi°M, Massi°P, Scaravelli°D, Tabanelli E, Howe D, Paz-Silva A

**Preliminary results of exposure to Sarcocystis spp. in horses from Italy using Sarcocystis neurona as antigen**

XXIX Congresso Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College "Parasites, Poverty and Social commitment" : Bari, June 21-24, 2016 / [s.l. : s.n., 2016]. - p 159 [Nr. Estr. 7479]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College (29. : Bari : June 21-24, 2016)

Sarcocystis spp. are coccidian parasites that can infect a wide range of animals and that need two hosts to complete their life cycle. Horses serve as intermediate hosts for several species of Sarcocystis, included S.neurona, that is responsible of the neurologic disease equine protozoal myeloencephalitis (EPM). To date, S.neurona is reported only in the Western Hemisphere, where it is restricted the definitive host, the opossum. In 2015, a horse located in the Forli-Cesena district suddenly showed severe neurological signs, as pelvic limb and neck tremors and stiffness, decline of vigilance and lack of appetite and dead in few days. No presence of heavy metals and pesticides was found either in the beverage water, feed and the gastric content. Few weeks later, four horses from the same stable showed the same acute neurological signs; thus, they were tested for West Nile virus and botulinum toxin, resulting negative. Although none of the horses travelled abroad, but considering the protozoal myeloencephalitis-like symptoms evidenced, we serologically investigated the 4 symptomatic animals plus two asymptomatic ones from the same stable, using a S. neurona-specific rSnSAG2 ELISA. Considering a percent positivity (PP%) of 25%, all sera resulted positive with highest PP values found in symptomatic horses. However, when sera have been further evaluated by an rSnSAG4/3 ELISA and a Western blot analysis that used S.neurona SN3 whole-merozoite as antigen, they tested negative. The serological reactivity evidenced could be related to infection with other apicomplexan parasites, although it seems more likely that tested animals have been infected by other European Sarcocystis species closely related to S.nettrona. Using the same serological methods, in Spain, a large proportion of horses resulted exposed to Sarcocystis spp. Although these serological findings cannot neurological clinical symptoms of the animals, they can be considered the start for further studies Sarcocystis.

Tosi°G, Fiorentini°L, Parigi°M, Scaravelli°D, Leotti G, Ostanello F, Massi°P

**Control of infection by Eimeria Tenella, Eimeria Maxima ed Eimeria Acervulina in broilers with the association sulfadimethoxine and trimethoprim**

XXIX Congresso Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College "Parasites, Poverty and Social commitment" : Bari, June 21-24, 2016 / [s.l. : s.n., 2016]. - p 201 [Nr. Estr. 7478]

Congresso Nazionale Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College (29. : Bari : June 21-24, 2016)

*Eimeria* spp. infections in broiler still cause huge economic losses. An experiment was conducted to evaluate the efficacy of a combination of drugs in the control of multiple infections of these parasitic protozoans in chickens experimentally infected. Five groups of 20 Ross308 broilers at 21st day of life were infected per os with 1 ml of a suspension containing 5000 oocysts of *Eimeria tenella*, 5000 of *E. maxima* and 10.000 of *E. acervulina* per liter. At 35th day, the therapy with the association of 200 mg di Sulfadimethoxine and 40 mg of Trimethoprim per ml was subdivided in 4 groups: "A" control; "B" with 0.5 ml/L in drinking water for 5 days; "C" with 1 ml/L for the first day and 0.5 ml/L for 4 days; "D" with 1 ml/L for 5 days, group "E" 2 ml/L for 1 day and 1 ml/L for 4 days. All dead specimens were necropsied and intestinal lesion score was evaluated using Johnson et al. (1970) method. Every day for each group oocyst number were counted on a pool of feces by McMaster method as well as direct observation and enumeration of oocyst were performed on the subjects who died. Clinical signs attributable to Coccidiosis have appeared at 6th day after inoculation and first mortality was observed after 14 days. In the same day the treatments started and mortality go further for 2 days only in the control group. The lesion scores show how the inoculation causes macroscopic lesions referable to *E. acervulina* and *E. tenella*, but less obvious for *E. maxima*, in the relative portions of the gut. The groups treated with different dosages had a highly significative reduction ( $p < 0.001$ ) of lesions compared to the control group. No significative differences ( $p > 0.05$ ) were found between constant dosage (B and D) or variable (C and E). The treatment has seen a good efficacy in reducing mortality, opening a good perspective for the control of these parasites.

Trogu T, Formenti<sup>o</sup>N, Berrilli F, Marangi M, Gianga spero A, Ferrari N, De\_Liberato C, Viganò R, Lanfranchi P

#### **Detection of *Giardia duodenalis* in free ranging Alpine cervids**

XXIX Congress Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College "Parasites, Poverty and Social commitment" : Bari, June 21-24, 2016 / [s.l. : s.n., 2016]. - p 231 [Nr. Estr. 7556]

Congress Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College (29. : Bari : June 21-24, 2016)

Survival and diffusion strategy of *Giardia duodenalis* consists of its being a generalist pathogen infecting a wide range of animal hosts in different environments, including humans. Recently, this protozoan has been detected in alpine chamois (*Rupicapra rupicapra rupicapra*). In order to provide further knowledge on circulation of this protozoa in alpine environment, we investigate the occurrence and genetic identity of *G. duodenalis* in red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*). The study was carried in the Lepontine Alps in 2013-2014. Faecal samples were collected from soil in the protected area of the Alpe Veglia-Alpe Devero Natural Park and from culled animals in the contiguous VCO2 hunting district. A total of 196 faecal samples were collected from red deer and 119 from roe deer. Faeces were frozen at -20° and a commercial ELISA kit (RIDASCREENOGiardia) was used to detect protozoan copro-antigens. Positive samples were subjected to a nested PCR for molecular characterization. *G. duodenalis* prevalence was 2.5% (5/196) (95%CI=0,3-4,7) in red deer and 8.4% (10/119) (95%CI=3.4-13.4) in roe deer. *G. duodenalis* was molecularly confirmed in 4 red deer and 3 roe deer. Zoonotic assemblage A was identified in red deer, while sequencing of PCR fragments from roe deer samples was not possible due to the poor DNA quality. This study shows that wild alpine cervids harbour *G. duodenalis*, contributing to its spread in the alpine environment. Considering that *G. duodenalis* prevalence detected is not negligible and that zoonotic assemblage A was isolated, the increase of deer populations, outdoor and zootechnical activity suggest a potential zoonotic and economic risk. Moreover, assuming that the highest protozoan emission by deer occurs in the first weeks/months of life (at least for livestock), logistical difficulties due to late-spring field conditions in the protected area and sampling in autumn hunting season mean that real prevalence could actually be underestimated.

Trogu T, Formenti<sup>o</sup>N, De\_Liberato C, Berrilli F, Ma rangi M, Giangaspero A,

Ferrari N, Lanfranchi P

**Comparison of diagnostic tests for Giardia detection in wild ungulates : is Elisa test a good choice?**

XXIX Congress Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College "Parasites, Poverty and Social commitment" : Bari, June 21-24, 2016 / [s.l. : s.n., 2016]. - p 230 [Nr. Estr. 7555]

Congress Societa' Italiana di Parassitologia SOIPA & European Veterinary Parasitology College (29. : Bari : June 21-24, 2016)

Giardiasis is one of the most common parasite intestinal infections in humans, wild and domestic animals worldwide, and therefore constitutes a potential zoonotic and zoo-economic risk. However, little information is currently available about its presence or its effect on wildlife populations. Immunofluorescence (IF) is the most widely used assay for Giardia detection, also in wild bovids. In the present study, IF was used as a comparative test in order to evaluate the performance of immunoenzymatic testing (ELISA) as a diagnostic option. Fecal samples were collected from 166 alpine chamois (*Rupicapra rupicapra rupicapra*), culled during the 2013-2015 hunting season in the Central Italian Alps. Samples were divided into two portions; the first was stored in potassium dichromate (2.5%) and subjected to immunofluorescence analysis (MERIFLUOR® Cryptosporidium/Giardia), while the second was frozen at -20°C and subjected to immunoenzymatic testing. A commercial ELISA kit (RIDASCREEN® Giardia) was used, and the agreement between the analytical approaches was assessed by calculating the Kappa (K) value (EpiTools, Ausvet; CI 95%). A Giardia prevalence of 7.8% (13/166) and 6.6% (11/166) was recorded by IF and ELISA respectively, thus showing a substantial agreement (k-value = 0.73) between the two tests. The ELISA test could therefore represent a good choice and an alternative tool for direct giardiasis diagnosis in wild ungulates because it has several advantages: it is cheaper, can be used to carry out simultaneous screening of numerous samples, and also provides objective spectrophotometer reading and antigen detection. Molecular analyses will make it possible to obtain further data in order to evaluate the actual performances of the ELISA test for Giardia detection, and support its application in a context involving wildlife.

Vaccari G, De\_Sabato L, Cella E, Lavazza°A, Zaccar ia G, Boni A, Lo\_ Presti A, Sozzi°E, Ciccozzi M, Prosperi°A, Lelli°D, Moreno °A

**Full genome characterization of beta-cov viruses related to middle east respiratory syndrome from two bats in Europe with NGS**

XVII Congresso Nazionale SIDiLV : Pacengo di Lazise (VR), 28-30 Settembre 2016 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2016]. - p 87-88. - 5 bib ref [Nr. Estr. 7364]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV) (17. : Pacengo di Lazise (VR) : 28-30 Settembre 2016)

*The emerging MERS-CoV in 2012 causing lethal respiratory infections underlined the zoonotic potential of Betacoronavirus (B-CoVs) already evidenced by the SARS-CoV causing a pandemic in 2002, 2003. The discovery of B-CoVs in bats, with high similarity to MERS and SARS CoV, suggested that common ancestors may have evolved in these species. In this study we developed a method, based on an NGS approach, able to sequence the entire genome of R-CoVs in some bat species in order to study the zoonotic potential of isolates circulating in Italy. The method was able to identify, in two out of three positive samples to a pan-coronavirus RT-PCR, the entire genome of B-CoVs. Genome analysis identified the expected ORFs, the transcription regulatory sequences, the slippery-sequence and cleavage site of the p1ab common in R-CoVs. Phylogenetic analysis revealed that Italian, along with other China and South Africa sequences of bat viruses represent the more closely related isolates to the MERS-CoV.*

Vecchio D, Bertocchi<sup>o</sup>L, De\_Rosa G, Napolitano F, Neglia G, Grassi C, Rossi P, Iemma L, Palladino M, D'Ausilio F, Natale A, De\_Carlo E

**Ruminant welfare : development of a new approach to evaluate Buffalo welfare, work in progress**

The 11th World Buffalo Congress "From the tropics to the world" : Cartagena, Colombia, 23 al 26 de Noviembre 2016 / [s.l. : s.n., 2016]. - 1 p [Nr. Estr. 7470]

World Buffalo Congress (11th : Cartagena, Colombia : 23-26 Noviembre 2016)

Velarde\_Nieto R, Cavadini<sup>o</sup>P, Neimane A, Chiari<sup>o</sup>M, Cabezòn O, Lavin S, Gaffuri<sup>o</sup>A, Grilli G, Gavier\_Widen D, Lavazza<sup>o</sup>A, Capucci<sup>o</sup>L

**Detection of the new emerging rabbit hemorrhagic disease type 2 virus (RHDV2) in European brown hares (*Lepus europaeus*) from Italy and Spain**

5th World Lagomorph Conference : Turlock, California, USA, July 11-15, 2016 : proceedings / edited by Patrick Kelly... [et al.] organized by California State University Stanislaus, Lagomorph Specialist Group, World Lagomorph Society. - [Turlock : California State University Stanislaus], 2016. - p 66 [Nr. Estr. 7318]

World Lagomorph Conference (5th : Turlock, California, USA : July 11-15, 2016)

Rabbit Hemorrhagic Disease virus (RHDV), a member of the genus Lagovirus, causes a fatal hepatitis (rabbit hemorrhagic disease = RHD) with a mortality of 80-95%. Since its first occurrence in Europe on 1986, RHDV has caused several outbreaks in wild and domestic rabbits, but never in European brown hares (*Lepus europaeus*) (EBH) and only once in two Iberian hares (*Lepus granatensis*). In 2010, a new RHDV-related virus, called RHDV2, emerged and rapidly spread in Europe, giving origin to extended epidemics, thanks to its specific antigenic profile that allowed RHDV2 to largely escape the immunity previously generated by RHDV in rabbit populations. During a 5-year spreading in Europe, RHDV2 was identified as cause of disease and mortality also in Cape hare (*Lepus capensis mediterraneus*) and Italian hare (*Lepus corsicanus*). Here we describe two distinct incidents of RHDV2 infection in EBH occurred in Italy and Spain in fall 2012 and spring 2014, respectively. One hare in North-Italy and two hares in Catalonia were found dead and the macro- and microscopic lesions found were highly suggestive of lagovirus infection. After necropsy, laboratory investigations were focused on diagnosis of EBHS. To better define the nature of the infection and confirm the initial diagnosis of lagovirus, two independent ELISAs, both based on specific MAbs produced against RHDV, RHDVa, RHDV2 and EBHSV were used to examine the livers of the three hares: i) the "typing ELISA" is a sandwich ELISA employing a panel of monoclonal antibodies (MAbs) able to identify the lagovirus species present in the samples (RHDV or EBHSV); ii) the "subtyping ELISA" based on a group of RHDV2 specific MAbs to specifically identify RHDV2. In addition the presence of EBHSV and RHDV2 RNAs was tested by RT-PCR using different sets of primers. In all three hares the identified viruses were characterized as RHDV2, genetically related to the other RHDV2 strains identified in Europe in the same areas and periods. EBH is the most common hare species in Europe, often sharing the same living environments with rabbits. As consequence, EBH populations have been subjected to a high challenge by RHDV2 in the last years. The detection of just three hares affected by RHDV2 suggests that EBH is not a specific host of RHDV2 but, presumably it could be a spillover due to the high infection pressure of RHDV2 and the limited barriers existing between lagomorphs species. Thus, EBH may occasionally become infected with RHDV2, and also die showing typical EBHS-like lesions. To confirm that, a serological survey was conducted on hare sera and precisely 149 collected from North-Italy between 2007 and 2010 (pre RHDV2 period), 106 collected in the same period and areas in Spain where the two cases of RHDV2 in hares were identified, 154 and 253 collected from North-Italy respectively during 2012 and 2013. On the whole the seroprevalence for RHDV2 was very low with also generally low titres, thus suggesting that the RHDV2 infection was not widespread among hares. In conclusion, our findings contribute to improve the knowledge about the distribution and epidemiological characteristics of RHDV2.

Velarde R, Lavazza<sup>o</sup>A, Cavadini<sup>o</sup>P, Chiari<sup>o</sup>M, Neim anis A, Cabezón O, Lavin S, Gaffuri<sup>o</sup>A, Grilli G, Gavier\_Widén D, Capucci<sup>o</sup>L

**Detection of the new emerging rabbit hemorrhagic disease type 2 virus (RHDV2) in European brown hares (*Lepus europaeus*) from Spain and Italy**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - 1 p [Nr. Estr. 7338]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : August 27th-31st, 2016)

Rabbit hemorrhagic disease virus (RHDV), a member of the genus Lagovirus, causes rabbit hemorrhagic disease (RHD), a fatal hepatitis of rabbits. In 2010, a new RHDV — related virus, called RHDV2, emerged in Europe. In addition to rabbits, this lagovirus causes disease and mortality in *Lepus capensis* (Cape hare) and *Lepus corsicanus* (Italian hare). However, RHDV2 infection has not been re-reported in European brown hare (*Lepus europaeus*), the most common hare species in Europe. Since arrival of the virus to Italy and Spain (probably in 2011) we have found four cases of mortality in this species caused by RHDV2. These cases had macroscopic and microscopic lesions consistent with RHD or European Brown Hare Syndrome. Macroscopic findings included epistaxis, disseminated visceral haemorrhages, moderate splenomegaly and a discoloured pale liver. Microscopic findings were most severe in the liver in the three hares examined. and consisted in extensive to massive hepatic necrosis with both widespread single-cell coagulative necrosis and multifocal areas of lytic necrosis. Acidophilic bodies also were scattered throughout the sections. There was moderate to marked fatty degeneration of hepatocytes and multinucleate hepatocytes occasionally were seen (8 per 10 high power fields). Also, occasional necrotic hepatocytes containing intracytoplasmic basophilic granules were seen. Two independent ELISA tests, both based on the use of specific MAbs produced against RHDV, RHDVa, RHDV2 and EBHSV were first used in order to narrow down the etiology. Second, the presence of EBHSV and RHDV2 RNA was tested by RT-PCR. We found that different RHDV2 strains caused a RHD-like disease in European brown hares. Our findings expand the known host range of RHDV2 and provide further support for the capability of RHDV2 to infect hosts other than rabbits. They also improve our knowledge about the distribution and epidemiological characteristics of this new lagovirus.

Veldkamp T, Hocking P, Vinco<sup>o</sup>LJ

**Effect of dietary electrolyte balance and crude protein content on foot pad dermatitis in commercial turkeys**

The proceedings of XXV World's Poultry Congress 2016 : September 5-9, 2016, Beijing, China : abstracts / [s.l. : s.n., 2016]. - p 617 (S13-0013) [Nr. Estr. 7448]

World's Poultry Congress (25. : Beijing, China : 5-9 September, 2016)

Factors such as dietary electrolyte balance (EB) and crude protein (CP) content, age, and strain may affect the prevalence of foot pad dermatitis (FPD). The objective of the study was to evaluate the effect of decreasing EB (high EB (HEB) vs. low EB (LEB)) and CP (high CP (HP) vs. low CP (LP)) in two turkey strains on growth performance, litter quality and FPD in a 2x2x2 factorial block design. A total of 1920 male poults were housed in 64 pens (3 m wide x 4 m deep) littered with wood shavings at a stocking rate of 30 poults/pen. Diets were formulated isocaloric for 5 phases (0-28, 28-56, 56-84, 84-112 and 112-134 days of age) and containing per phase 290 vs. 260, 270 vs. 240, 230 vs. 200, 200 vs. 170, 170 vs. 140 g CP/kg, respectively; and EB (240 vs. 130 mEq/kg) in all phases. Free amino acids were supplemented to the diets according to breeder recommendations. LEB diets were formulated by exchange of soya bean meal by maize gluten meal, peas, potato protein, rapeseed meal and sunflower seed meal. Water and feed were provided ad libitum. Body weight, feed intake, litter moisture and FPD scores were recorded at 28, 56, 84, 112 and 134 days of age. Body weight gain was not affected by CP and FCR was significantly higher on LP than on HP diets (2.56 vs. 2.50; P=0.002). FPD score of turkeys fed on LP was lower than on HP until 84 days (P<0.001). LEB resulted in a significantly lower feed intake (420 vs. 435 g/d) and body weight gain

(166 vs. 172 g/d) over the period 28 to 124 days and lower body weight (18588 vs. 19405 g) at 134 days of age compared with HEB whereas FCR was not affected. Litter was significantly dryer on LEB than HEB diets ( $P < 0.001$ ). FPD score on LEB was significantly lower than on HEB diets ( $P < 0.001$ ). FPD was not affected by turkey strain. It is concluded that litter quality can be improved and FPD can be decreased in turkeys fed on diets containing lower CP and EB levels.

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**Effect of dietary protein source and crude protein content on growth performance, litter quality and foot pad dermatitis in two commercial turkey strains**

Proceedings of the 10th Turkey Science and Production Conference : Chester, UK, March 10th - 11th, 2016 / [s.l. : s.n., 2016]. - p 54-57. - 7 bib ref [Nr. Estr. 7260]

Turkey Science and Production Conference (10th : Chester, UK : March 10th - 11th, 2016)

The objective of the study was to evaluate the effect of decreasing dietary electrolyte balance EB (high EB (HEB) vs. low EB (LEB)) and crude protein CP (high CP (HP) and low CP (LP)) in two turkey strains on growth performance, litter quality and FPD. The experimental treatments were evaluated in a 2x2x2 factorial block design. Diets were formulated to be isocaloric for 5 phases (0-28, 28-56, 56-84, 84-112 and 112-134 days of age) and containing per phase 290 vs. 260, 270 vs. 240, 230 vs. 200, 200 vs. 170, 170 vs. 140 g CP/kg, respectively; and EB (240 vs. 130 mEq/kg) in all phases. Free amino acids were supplemented to the diets according to breeder recommendations. LEB diets were formulated by exchange of soya bean meal by maize gluten meal, peas, potato protein, rapeseed meal and sunflower seed meal. Water and feed were provided ad libitum. Body weight, feed intake, and FPD were recorded at 28, 56, 84, 112 and 134 weeks of age. Overall results indicate that daily feed intake in turkeys fed on LP was numerically higher than on HP diets. Body weight gain was not affected by CP and FCR was significantly higher in turkeys fed on LP diets than in turkeys fed on HP diets (2.56 vs. 2.50;  $P = 0.002$ ). FPD in turkeys fed on LP diets was significantly lower than FPD in turkeys fed on HP diets until 84 days ( $P < 0.001$ ). Turkeys fed on LEB diets showed a significantly lower feed intake (420 vs. 435 g/d) and body weight gain (166 vs. 172 g/d) over the period 28 to 134 days of age and a lower body weight (18588 vs. 19405 g) at 134 days of age compared with turkeys fed on HEB diets. Feed conversion ratio was not affected by EB. Litter was significantly dryer in pens with turkeys fed on LEB diets than in pens with turkeys fed on HEB diets ( $P < 0.001$ ). FPD in turkeys fed on LEB diets was significantly lower than in turkeys fed on HEB diets ( $P < 0.001$ ). FPD was not affected by turkey strain. It is concluded that litter quality can be improved and FPD can be decreased in turkeys fed on diets containing lower CP and EB levels.

Velo E, Kadriaj P, Mersini K, Shukullari A, Manxhari B, Simaku A, Hoxha A, Caputo B, Bolzoni<sup>o</sup>L, Rosà R, Bino S, Reiter P, Della\_Torre A

**Enhancement of Aedes albopictus collections by ovitrap and sticky adult trap**

Parasites & Vectors. - Vol. 9 ( 2016). - no 223 (5 p). - 19 bib ref (ultimo accesso 12/05/2016 <http://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-016-1501-x>) [Nr. Estr. 7253]

Background: In the last decades, *Aedes albopictus* has become an increasing public health threat in tropical as well as in more recently invaded temperate areas due to its capacity to transmit several human arboviruses, among which Dengue, Chikungunya and Zika. Enhancing the efficiency of currently used collection approaches, such as ovitraps and sticky traps, is desirable for optimal monitoring of the species abundance, for assessment of the risk of arbovirus transmission and for the optimisation of control activities. Findings: Two sets of 4 x 4 Latin-square experiments were carried out in Tirana (Albania) to test whether modifications in ovitrap shape and size and in oviposition substrate would increase collections of *Ae. albopictus* eggs and whether hay-infusion would increase adult catches by sticky trap. Generalized Linear Mixed Models with negative binomial error distribution were carried out to analyse the data. Cylindrical ovitraps lined with germination

paper yielded significantly higher egg catches than those exploiting either the (commonly used) wooden paddles or floating polystyrene blocks as oviposition substrates. No difference was observed between cylindrical and conical shaped ovitraps. Ovitrap and sticky traps baited with hay infusion yielded significantly higher egg and adult catches than un-baited ones. A significant relationship between ovitrap and sticky trap catches was observed both in the absence and in the presence of attractants, with ovitrap catches increasing more than sticky trap catches at increasing adult female densities. Conclusions: This study provides grounds for optimisation of ovitraps and sticky traps as monitoring tools for *Ae. albopictus* by (i) supporting use of germination paper as most appropriate oviposition substrate; (ii) suggesting the possible use of stackable conical ovitraps for large scale monitoring; (iii) confirming the use of hay-infusion to increase egg catches in ovitraps, and showing that hay-infusion also significantly increases adult catches by sticky traps.

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#### **Influence of crate height on the welfare of broilers during transport**

J Vet Behav. - Vol. 14 ( 2016). - p 28-33. - 20 bib ref [Nr. Estr. 7416]

Poultry transport systems are currently under investigation with a particular focus on design and dimensions of commercially available transport crates. The height of the crates currently used is debated and considered by some parties to be insufficient to fulfill animal welfare needs. The opposing view is that the welfare of birds transported in higher crates is even worse. The European Food Safety Authority highlighted the lack of scientific evidence supporting recommendations on the height of crates used for poultry transportation. To fill this gap and provide scientific knowledge on the effect of crate height on the welfare of poultry during transport, a field trial was carried out on a commercial journey of 2,618 broilers, comparing the welfare of birds transported in commercial crates with that of others transported in crates of doubled height. Animal welfare was evaluated through the use of animal-based parameters, such as behavior, physiologic variables, dead on arrivals, and postmortem lesions observed at slaughter. None of the parameters assessed proved advantages of the higher crates over those currently used. On the other hand, several parameters underlined favorable aspects of current crates toward the modified ones. Although based on the results of 1 single test, it appears from this trial that the suggestion to replace the transport crates commercially in use at present with crates doubled in height is not supported by improvements in animal welfare. On the contrary, this replacement would have a negative effect on the welfare of broilers during transport.

Zanet S, Chiari<sup>o</sup> M, Battisti E, Tizzani P, Triscioglio A, Taricco L, Lavazza<sup>o</sup> A, Ferroglio E

#### **Leishmania infantum in wild lagomorphs : an epidemiological survey in Italy**

Contributions to the 12th Conference of the European Wildlife Disease Association (EWDA) : August, 27th-31st, 2016, Berlin, Germany / edited by Anke Schumann ... [et al.]. - [s.l. : s.n., 2016]. - p 213 (Poster n. 50) [Nr. Estr. 7337]

Conference of the European Wildlife Disease Association (EWDA) (12th : Berlin, Germany : 27th-31st August 2016)

*Leishmania infantum* is the etiological agent of zoonotic visceral leishmaniasis (ZVL). In Northern Italy the epidemiology of ZVL has experienced profound changes in the last decades as far as territorial expansion and involvement of different animal species. We tested by PCR, the spleen of 222 lagomorphs (n = 10 wild rabbits *Oryctolagus cuniculus*, n = 108 hare *Leptis europaeus* and n = 104 Eastern cottontails *Sylvilagus floridanus*). The animals were culled during regular hunting activities between 2008 and 2014 and come from 10 different regions of Italy. A specific PCR protocol was used to amplify a 145bp fragment of the kinetoplastid DNA of *L. infantum*. All positive amplicons were sequenced to confirm species identification. A total of 51 animals tested positive by

PCR with an overall prevalence (P) of infection of 22.97 % (CI 95 % 17.93 - 28.94). The highest prevalence was recorded in rabbits: P = 30 % (CI95 % 10.78 % - 60.32 %) followed by *S. floridanus* with 28 of 104 animals infected and a prevalence of 26.92 % (CI95 % 19.33 % - 36.16 %). The lowest prevalence was recorded in brown hare (P = 18.52 %; CI95 % 12.32 % - 26.88 %) with 20 infected animals out of 108. Despite not being statistically significant, the higher prevalence recorded in rabbits and cottontails can be attributed to the more limited home-range of these two species compared to *L. europaeus* which can favor the spread of infection as it better copes with the behavioural attitude of phlebotomine sandflies vectors.

Zanzani SA, Di\_Cerbo A, Gazzonis AS, Epis S, Invernizzi°A, Tagliabue°S,  
Manfredi MT

**Parasitic and bacterial infections of *Myocastor coypus* in a metropolitan area of Northwestern Italy**

J Wild Dis. - Vol. 52 no 1 ( 2016). - p 126 -130. - 18 bib ref [Nr. Estr. 7221]

Coypus (*Myocastor coypus*) are widespread throughout Europe. In northern Italy, they are abundant in the flatland areas, and their high population densities can cause economic loss and ecosystem damage. We examined 153 coypus for selected parasitic and bacterial infections. We found *Strongyloides myopotami* (63.4% prevalence), *Trichostrongylus duretteae* (28.1%), *Eimeria coypi* (86.3%), and *Eimeria seideli* (6.8%), but did not find *Giardia duodenalis* or *Cryptosporidium* spp. We also isolated *Staphylococcus aureus* (10.1%), *Escherichia coli* (4.5%), and *Streptococcus* spp. (3.4%) from lung samples; no *Salmonella* spp. were isolated from fecal samples. Coypus had antibodies to *Toxoplasma gondii* (28.9%) and to four serovars of *Leptospira interrogans* (44.9%); Australis/Bratislava was the serovar most frequently detected. It is clear that coypu can be infected with pathogens of human and veterinary importance.