

## **RICERCHE EFFETTUATE**

### **SANITA' ANIMALE**

Albieri A, Puggioli A, De\_Filippo° F, Calzolari° M, Bellini R

#### **Quali-quantitative analysis of mosquito species in the plain area of Emilia-Romagna region (Italy) from 2010 to 2016**

VIIIth Conference European Mosquito Control Association (EMCA) : Mosquito control in a changing environment : 12-16 March 2017, Montenegro : abstract book / [s.l. : s.n., 2017]. - p 95 [Nr. Estr. 7630]

European Mosquito Control Association (EMCA) Conference (8th : Montenegro : 12-16 March 2017)

Mosquito surveillance is essential for the early detection of invasive species with public health importance and for the prevention and control of pathogen's transmission like West Nile virus (WNV). Information on distribution, abundance and population dynamic of the mosquito fauna in the Emilia-Romagna region plain area (around 12,000 km<sup>2</sup>) have been collected in the frame of the Regional Vector-borne diseases surveillance program, from 2010 to 2016. The data presented document the collections of adult mosquitoes by the standardized use of 88-110 CO<sub>2</sub> traps and gravid traps activated every 2 weeks from the end of May to the beginning of October in the plain area of the 9 provinces of the region. During the period 2010-2016, more than 2,400,000 adult mosquitoes belonging to 22 species were identified. The commonest and most abundant species were as follows, in decreasing order: *Culex pipiens*, *Aedes caspius*, *Aedes vexans* and *Aedes albopictus*. The most widespread species was *Cx. pipiens*, with highest densities in agricultural and pen-urban habitats, followed by *Ae. albopictus*, most abundant in urban and pen-urban habitat. The quali-quantitative analysis of data shows that composition of the mosquito populations varied between geographical locations, seasonal period and surveillance years.

Alborali° GL, Ruggeri° J, Pesciaroli M, Martinelli° N, Chirullo B, Ammendola S, Battistoni A, Ossiprandi MC, Corradi A, Pasquali P

#### **Prime-boost vaccination with attenuated *Salmonella Typhimurium* znuABC and inactivated *Salmonella Choleraesuis* is protective against *Salmonella Choleraesuis* challenge infection in piglets**

BMC Vet Res. - Vol. 13 ( 2017). - no 284 (9 p). - 25 bib ref ( ultimo accesso 02/11/2017 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5594465/> ) [Nr. Estr. 7681]

Background *Salmonella enterica* serovar *Choleraesuis* (*S. Choleraesuis*) infection causes a systemic disease in pigs. Vaccination could represent a solution to reduce prevalence in farms. In this study, we aimed to assess the efficacy of an attenuated strain of *Salmonella enterica* serovar *Typhimurium* (*S. Typhimurium* znuABC) against *S. Choleraesuis* infection. The vaccination protocol combined priming with attenuated *S. Typhimurium* znuABC vaccine and boost with an inactivated *S. Choleraesuis* vaccine and we compared the protection conferred to that induced by an inactivated *S. Choleraesuis* vaccine. Methods The first group of piglets was orally vaccinated with *S. Typhimurium* znuABC and boosted with inactivated *S. Choleraesuis*, the second one was intramuscularly vaccinated with *S. Choleraesuis* inactivated vaccine and the third group of piglets was unvaccinated. All groups of animals were challenged with a virulent *S. Choleraesuis* strain at day 35 post vaccination. Results The results showed that the vaccination protocol, priming with *S. Typhimurium* znuABC and boosted with inactivated *S. Choleraesuis*, applied to group A was able to limit weight loss, fever and organs colonization, arising from infection with virulent *S. Choleraesuis*, more effectively, than the prime-boost vaccination with homologous *S. Choleraesuis* inactivated vaccine (group B). Conclusion In conclusion, these research findings extend the validity of attenuated *S. Typhimurium* znuABC strain as a useful mucosal vaccine against *S. Typhimurium* and *S. Choleraesuis* pig infection. The development of combined vaccination protocols can have a diffuse administration in field conditions because animals are generally infected with different

concomitant serovars.

Amadori° M

### **The innate immune response to non-infectious stressors : human and animal models**

Networking : tool for an excellent research: a public veterinary health without borders to face new emergencies : atti 4° Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria : Roma 6 Aprile 2017 / edited by Marina Bagni, Antonio Petri, Antonio Lavazza. - [Teramo] : Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, 2017. - (Veterinaria Italiana. Collana di Monografie ; Anno 53, Monografia ; 26) p 28-30. - 1 bib ref [Nr. Estr. 7606]

Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria (4. : Roma : 6 Aprile 2017)

The achievement of high production levels leads to a greater difficulty of farm animals to adapt to the environment. This translates into occurrence of multifactorial diseases and use of drugs. A new conceptual framework must underlie the disease control strategies in this area. This implies the recognition of the innate immune system as a foundation of environmental adaptation after exposure to both infectious and non-infectious stressors. In addition to microbial stressors, the non-microbial ones are recognized because of common signalling pathways based on Damage-Associated Molecular Patterns (DAMPs) and de nova expression of diverse stress proteins on the cell surface. Therefore, sensing, signalling and effector mechanisms of the innate immune system are remarkably similar for both infectious and non-infectious stimuli, albeit differently modulated. Disease may be the final outcome in humans and animals in established models of innate immune response to non-infectious, physical, chemical, metabolic and psychotic stressors. The timely investigation of these coping responses by means of proper clinical immunology and chemistry parameters can be the basis of successful predictive and prognostic approaches. The outlined conceptual framework can be the foundation of a successful "One Health" approach and conducive to fruitful links between pre-clinical and clinical research centres.

Amadori° M, Filipe J, Riva F, Vitali A, Ruggeri° J, Lacetera N

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

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 42. - 1 bib ref [Nr. Estr. 7609]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

Mitogens are diverse compounds of plant and microbial origin, widely employed to test immunocompetence. In healthy, non-immunocompromised hosts, they induce DNA synthesis and division of large leucocyte populations, which can be reasonably associated with the capacity to mount adaptive immune responses. The blastogenic response of bovine peripheral blood mononuclear cells (PBMC) to lipopolysaccharides (LPS) has been investigated for a long time in our laboratories. In particular, a possible correlation between blastogenic response to LPS and disease resistance of periparturient dairy cows had been observed in previous studies (Catalani et al., 2013): low responder cows presented a much higher frequency of disease cases after calving, compared with high responder animals. Owing to the above, different aspects of the blastogenic response to LPS were investigated on PBMC of healthy, dry (2) or lactating (26) Friesian cows, and the extent of the response was evaluated in a 72-hour BrDU incorporation assay, as previously described (Catalani et al., 2013). Unstimulated and BrDU-treated cells were used as negative control. Stimulation with LPS induced little if any increase of cell counts over 72 hours despite

consistent, low to moderate BrDU incorporation in all the PBMC samples under study. Poor replication of LPS-stimulated PBMC was confirmed by cell cycle and cell growth flow cytometry analyses. In particular, LPS stimulation gave rise to very low percentages of S phase cells, sometimes lower than in control, unstimulated cells, as opposed to Concanavalin A-stimulated PBMC. Also, LPS-stimulated and BrDU-treated PBMC were submitted to magnetic separation using B and T cell-specific mAb, and Miltenyi anti-mouse IgG MicroBeads. Analysis of BrDU incorporation after stimulation with LPS showed that both B and CD4 T cells are involved in the blastogenic response to LPS, in contrast with current data based on human and murine models. Finally, as opposed to control cells, LPS-stimulated PBMC maintained the expression of IL-1beta and up-regulated both IDO and TD02 genes (kynurenine pathway, endotoxin tolerance). Both control and LPS-stimulated PBMC down-regulated Ig light chain expression. On the whole, our data indicate that differences in the response to LPS could be accounted for by heterogeneity of responding cells (B and T lymphocytes), that could also cooperate with monocytes in induction and regulation of endotoxin tolerance.

Amato B, Capucchio TM, Biasibetti E, Mangano E, Boniotti° MB, Pacciarini° LM, Migliore S, Vitale M, Fiasconaro M, Di\_Marco Lo\_Presti V

**Pathology and genetic findings in a rare case of Mycobacterium caprae infection in a sow**

Vet Microbiol. - Vol. 205 ( 2017). - p 71-74. - 21 bib ref ultimo accesso 06/06/2018

<https://reader.elsevier.com/reader/sd/D75D04AB21AB1CE7E2983C74795EBCF651F19FA568180AE31556F8FF7E57106C84355F2360A040407A69F05B81473234> [Nr. Estr. 7635]

Bovine tuberculosis, a reemerging zoonosis in diverse ecological scenarios, has been reported in the autochthonous Nebrodi black pig breed population used for meat production in Italy. During a routine abattoir inspection in 2013, 24 of 299 carcasses (8%) of Nebrodi black pigs presented tuberculosis-like lesions at pathologic examination. Mycobacterium bovis was isolated from 23 animals and M. caprae from a 3-year-old sow. The sow showed severe diffuse lesions involving the visceral organs, right coxofemoral joint, and mammary glands. Isolation of M. caprae from mammary glands is uncommon, with only one other case involving a sow reported so far; however, Mycobacteria infection of the mammary glands may be transmitted from lactating sows to piglets, contributing to the spread and maintenance of bovine tuberculosis in swine. Genotyping analysis showed M. caprae spoligotype SB0866 and profile 4,1,5,4,4,11,4,2,4,3,8,7 MIRU-VNTR (mycobacterial interspersed repetitive units-variable number of tandem repeats). The worldwide prevalence of this spoligotype is very low. The finding of severe, diffuse tuberculous lesions strongly suggests that Nebrodi black pigs are susceptible for Mycobacterium spp. and that they might act as a distributor for these microorganisms. Since natural ecosystems with multiple contacts among different livestock species and wild animals are very common in Mediterranean regions, current surveillance and eradication plans for bovine tuberculosis will need to be extended to other potential reservoir species in regions where extensive and traditional breeding systems are operated.

Antognetti V, Gomis JR, Puccica S, Formato G, Pietropaoli M, Federico G, Maroni\_Ponti A, Baratto C, Granato A, Mutinelli F, Carra° E, Leto A, Della\_Marta U, Cersini A

**Development of a Real Time PCR method for Aethina tumida DNA detection from beehive debris, brood combs and brood comb swabs**

45th Apimondia International Apicultural Congress : 29 September - 04 October 2017 29 Istanbul, Turkey : abstract book / [s.l. : s.n., 2017]. - p 268 [Nr. Estr. 7751]

"Apimondia" International Apicultural Congress (45th : Istanbul, Turkey : September 29 - October 04, 2017)

Aethina tumida, or Small Hive Beetle (SHB), is an exotic parasite belonging to Coleoptera order,

Nitidulidae family, causative agent of a notifiable parasitic disease in the EU and considered by Regulation (EU) No 206/2010 that governs the import of bees from Third Countries. *A. tumida* has been reported for the first time in southern Italy in September 2014 in the region of Calabria, Reggio Calabria and Vibo Valentia Provinces, and in the region of Sicily, Siracusa Province. Following its detection a nationwide surveillance program has been established based on clinical inspection of beehives. In order to facilitate the surveillance activities, a Real Time PCR method for *A. tumida* DNA detection from different beehive matrices has been developed. For this purpose 1 g of hive debris, 70 g of brood combs and 200 µl of comb swab diluted in PBS respectively, were used to develop a DNA extraction protocol. After specific preparation of each matrix, DNA extraction was performed using commercial kits: kit NucleoSpin® Tissue Macherey-Nagel for hive debris and brood combs, and QlampDNA® Blood Mini kit Qiagen for comb swabs. The extracted DNA was used as template for a specific Real Time PCR for a 109-bp fragment of the cytochrome oxidase 1 gene (*cox1*) of SHB. The developed protocols have been tested by different laboratories on matrix samples circulated by Istituto Zooprofilattico Sperimentale Lazio e Toscana. All the laboratories identified the presence/absence of SHB DNA in the artificially contaminated/not contaminated matrix samples. The limit of detection (LOD) of the molecular protocols has been determined: 10 target molecules/g for hive debris (approx. 0.5 larva/g) 20 target molecules/70 g of brood comb (approx. 0.5 larva/70 g of brood comb) 5 target molecules/0.2 mL of swab (approx. 0.33 larva/0.2 mL of comb swab) The presence of potential inhibitors in the investigated matrices did not seem affecting DNA amplification. In conclusion the application of these protocols for SHB DNA detection in different beehive matrices seems applicable and reliable to support SHB surveillance programs.

Armas F, Camperio C, Coltella L, Selvaggini S, Boniotti° MB, Pacciarini° ML, Di\_Marco Lo\_Presti V, Marianelli C

**Comparison of semi-automated commercial rep-PCR fingerprinting, spoligotyping, 12-locus MIRU-VNTR typing and single nucleotide polymorphism analysis of the *embB* gene as molecular typing tools for *Mycobacterium bovis***

J Med Microbiol. - Vol. 66 no 8 ( 2017). - p 1151-1157. - 36 bib ref [Nr. Estr. 7693]

**Purpose.** Highly discriminatory genotyping strategies are essential in molecular epidemiological studies of tuberculosis. In this study we evaluated, for the first time, the efficacy of the repetitive sequence-based PCR (rep-PCR) DiversiLab *Mycobacterium* typing kit over spoligotyping, 12-locus mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing and *embB* single nucleotide polymorphism (SNP) analysis for *Mycobacterium bovis* typing. **Methodology.** A total of 49 *M. bovis* animal isolates were used. DNA was extracted and genomic DNA was amplified using the DiversiLab *Mycobacterium* typing kit. The amplified fragments were separated and detected using a microfluidics chip with Agilent 2100. The resulting rep-PCR-based DNA fingerprints were uploaded to and analysed using web-based DiversiLab software through Pearson's correlation coefficient. **Results.** Rep-PCR DiversiLab grouped *M. bovis* isolates into ten different clusters. Most isolates sharing identical spoligotype, MIRU-VNTR profile or *embB* gene polymorphism were grouped into different rep-PCR clusters. Rep-PCR DiversiLab displayed greater discriminatory power than spoligotyping and *embB* SNP analysis but a lower resolution power than the 12-locus MIRU-VNTR analysis. MIRU-VNTR confirmed that it is superior to the other PCR-based methods tested here. **Conclusion.** In combination with spoligotyping and 12-locus MIRU-VNTR analysis, rep-PCR improved the discriminatory power for *M. bovis* typing.

Arrigoni° N

**Strumenti diagnostici e linee guida per l'uso responsabile dell'antibiotico nelle patologie mammarie**

Atti Soc Ital Buiatria. - Vol. 49 ( 2017). - p 34-36 [Nr. Estr. 7757]

Congresso Nazionale della Società Italiana di Buiatria (49. : Parma : 23-24 Novembre 2017)

Bedendo G, Panzarin V, Fortin A, Zamperin G, Salogni° C, Pascoli F, Toffan A

### **Caratterizzazione molecolare del Rhabdovirus del pesce gatto in Italia**

Atti del XXIII Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) : Lecce 5-6 Ottobre 2017 / [s.l. : s.n., 2017]. - p 18 (O2) . - 3 bib ref [Nr. Estr. 7671]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (23. : Lecce : 5-6 Ottobre 2017)

A seguito dell'identificazione del rhabdovirus del pesce gatto (Ictalurid rhabdovirus - IcRV) isolato durante un grave episodio di mortalità di *Ameiurus melas* verificatosi nel 2016, si è proceduto alla sua caratterizzazione molecolare, unitamente ad altri 14 virus „isolati nella medesima specie nel corso di episodi di mortalità simili avvenuti dal 1993 ad oggi. L'isolato 16ITT-V/75 precedentemente descritto da Bedendo et al. (2016), è stato sottoposto a Whole Genome Sequencing (WGS) utilizzando la tecnologia Illumina® MiSeq. La sequenza del genoma completo è stata ottenuta mediante de novo assembly ed annotata utilizzando come riferimento la sequenza completa del rhabdovirus della tinca (TenchRV), il genoma più simile a 16ITT-V/75 presente in GenBank. La sequenza consenso generata ha una lunghezza di 11.086 bp ed è costituita da cinque open reading frames (ORF) con segnale di start 3'-UUGUC-5' e segnale di stop/polyA 3'-AUAC(U)7-5', e intervallate da brevi sequenze intergeniche. Le OREs identificate codificano per le classiche proteine dei rhabdovirus nell'ordine: 3'- nucleoproteina (N), fosfoproteina (P), proteina di matrice (M), glicoproteina (G) e RNA polimerasi (L) — 5'.

Al fine di classificare i virus isolati da pesce gatto all'interno della famiglia Rhabdoviridae, per ciascun isolato è stata ottenuta la sequenza parziale del gene N (670 bp) (Talbi et al., 2011) e la sequenza completa del gene G (1630 bp). Le sequenze nucleotidiche ed aminoacidiche dedotte sono state sottoposte ad analisi filogenetica mediante metodo maximum likelihood (ML). I risultati ottenuti indicano che i IcRV appartengono al genere *Spri.vivirus* e presentano la più alta similarità nucleotidica (gene N: 97%; gene G: 98%) con TenchRV. Le sequenze nucleotidiche degli isolati italiani presentano tra loro una scarsa variabilità genetica (100-99.5% di identità per il gene N e 100-98.6% di identità per il gene G). Le sequenze aminoacidiche dedotte della glicoproteina confermano una percentuale di identità elevata tra i ceppi analizzati (100-99%). È interessante notare che uno degli isolati di pesce gatto identificato all'esame virologico come rhabdovirus, risultava negativo al test 1:T4-CR per l'amplificazione del gene G degli IcRV descritte nel presente lavoro. Questo ceppo è stato poi caratterizzato sulla base della sequenza parziale del gene N come SVCV. Il pesce gatto è infatti suscettibile anche ad infezione da parte di SVCV (OIE 2017). Risulta pertanto estremamente importante la corretta caratterizzazione genetica dei rhabdovirus che infettano il pesce gatto poiché la sola identificazione su base virologica può essere difficile. La produzione di pesce gatto in Italia è crollata drasticamente negli ultimi anni passando da 600 t a meno di 200 t (FEAP 2016) a causa delle perdite economiche inflitte a questo settore produttivo dalle virosi. Implementare i metodi diagnostici e le conoscenze relative alla patogenesi di queste malattie può portare sicuramente giovamento a questo delicato settore.

Bertelloni F, Tosi° G, Massi° P, Fiorentini° L, Parigi° M, Cerri D, Ebani VV

### **Some pathogenic characters of paratyphoid Salmonella enterica strains isolated from poultry**

Asian Pac J Trop Med. - Vol. 10 no 12 ( 2017). - p 1161-1166. - 31 bib ref [Nr. Estr. 7749]

Objective: To investigate some pathogenic characters of *Salmonella enterica* strains isolated from poultry. Methods: Twenty-three genetically distinct *Salmonella enterica* strains, of different serovars and pulsotype, were examined for virulence traits. Resistance to gastric acid environment was estimated by measuring the percentage of survived bacterial cells after exposure for 2 h to a synthetic gastric juice. Strains were analyzed with PCR for the presence of the following virulence genes: *mgtC* and *rhuM* located on SPI-3, *sopB* and *pipB* located on SPI-5, *Salmonella* virulence plasmid (*spvR*)(*spvR*), *spvB* and *spvC* located on *Salmonella* plasmid virulence and *sodCI*, *sopE*, and *gipA* located on prophage. Finally, resistance to 21 antibiotics was tested with Kirby-Bauer method. Results: A percentage of 82.60% of strains were resistant to gastric environment after

induction and 60.87% of the strains exhibited constitutive resistance too. Nineteen different virulence profiles were detected. The phage related genes *sodCI* and *sopE* and the plasmid mediated operon *spvR*, *spvB* and *spvC* (*spvRBC*) were detected in 82.60%, 47.82% and 52.17% of strains, respectively. Typhimurium and Enteritidis strains showed the highest number of virulence genes. Twenty-one different antibiotic resistance profiles were obtained and two isolates (Typhimurium and Enteritidis) resulted sensible to all the tested molecules. The ampicillin, streptomycin, sulfonamide and tetracycline resistance profile was detected in seven isolates (30.43%). Conclusion: Our results show that paratyphoid Salmonella strains with several characters of pathogenicity, that may be cause of severe pathology in animals and humans, are circulating among poultry.

Bertocchi° L

#### **La valutazione del benessere animale nel vitellone da carne**

19. Congresso Internazionale SIVAR : 10-12 Maggio 2017, Cremona / [Cremona : s.n., 2017]. - p 8 [Nr. Estr. 7721]

Congresso Internazionale SIVAR (19. : Cremona : 10-12 Maggio 2017)

Bianchi° A, Giacomelli° S, Archetti° I, Rota\_Nodari° S

#### **Habitat influence on haematological parameters of red deer (*Cervus elaphus*)**

11th International Conference on Behaviour, Physiology and Genetics of Wildlife : October 4th-7th, 2017, Berlin, Germany / edited by Pruegel J, Lenz S. - [s.l. : s.n., 2017]. - p 30 [Nr. Estr. 7762]

International Conference on Behaviour, Physiology and Genetics of Wildlife (11th : Berlin, Germany : October 4th-7th, 2017)

The relation between haematological parameters and habitat type was investigated in an alpine population of red deer. The study was carried out on 202 red deer culled for a population management control plan in the period from 31 October 2014 to 28 January 2015 in a selected area (about 25.100 ha) of the Stelvio National Park. The area was divided into ten smaller zones with different environmental characteristics, classified through geolocalisation, Corine Land Cover 2006 (CLC). Demographic and habitat use data was gathered by census and GPS collar data recorded by the park institution. Haematological parameters (HP) (total protein, urea, creatinine, albumin, globulin, A/G ratio, haptoglobin, haemolytic complement, lysozyme, cortisol and protein electrophoresis) were tested on 61 suitable samples. According to culling records and census data five higher density zones (over 13 deer/100 ha estimated) were identified, representing approximately one third of the study area. These zones were located at the centre of the study area, characterized by a greater presence and continuity of CLC category "permanent pastures" and were massively grazed by domestic livestock. Female deer culled in these zones were older than in the others (Mann-Whitney,  $p = 0.005$ ) and their prevalence of pregnancies (presence of corpus luteum) were significantly lower ( $\chi^2$ ,  $p = 0.05$ ). This last evidence could validate the hypothesis of higher density. Moreover, their home ranges, recorded by the park staff showed also a behaviour mostly non-migratory. The comparison of the HP between the animals in the high density area and the others suggested different body conditions and immunological status. In particular, adults (> 1 year old) living in higher density zones had higher total protein (ANOVA,  $p = 0.003$ ), gamma globulin (ANOVA,  $p < 0.001$ ), urea (ANOVA,  $p = 0.003$ ) and a lower A/G ratio (ANOVA,  $p = 0.002$ ). No significative differences were found on young subjects (< 1 year old). These results demonstrate the influence of good pasture on HP. In particular protein and urea concentration were closely related to pasture's quality as previously described. The simultaneous increment of gamma globulin could be interpreted as a major allocation of energy in immunocompetence (antibodies) or as an effect of the higher density. More probably this could be a result of the homeostatic trade-off between higher trophic availability and higher inter-specific competition, represented also by higher pathogen exchanges. Our results could be helpful to develop an integrated approach to population management and epidemiological surveillance.

Biondi R, Muthusamy R, Rigamonti S, Vicari° N, Magnino° S, Donati M

**Antimicrobial susceptibility of a Chlamydia avium isolate recovered in Italy from a pigeon**

4th European Meeting on Animal Chlamydiosis (EMAC) : 2017, Zagreb : book of abstract / [s.l. : s.n., 2017]. - 7666]

European Meeting on Animal Chlamydiosis (EMAC) (4. : Zagreb : 2017)

The single genus Chlamydia in the family Chlamydiaceae comprises nine known species and the two recently described C. avium, including strains from pigeons and psittacine birds, and C. gallinacea, comprising strains from poultry (1). Chlamydial avian infections are often asymptomatic, but in some cases they can cause enteric, respiratory and ocular diseases, and low to high mortality. The zoonotic potential and pathogenicity of the two newly recognized species have yet to be systematically investigated. Up to now, few reports suggest a possible role of C. avium in causing respiratory disease in parrots and pigeons. In the present study we investigated the antimicrobial susceptibility of the C. avium isolate PV4360-2/99, isolated by the NRL for Animal Chlamydioses from a pigeon in Italy in 1999, and identified with specific primers and protocols (2). The isolate was grown in LLC-MK2 cells. As the in vitro susceptibility of C. avium to antimicrobials has not been studied yet, we tested the activity of doxycycline, roxithromycin and azithromycin, levofloxacin and moxifloxacin against the C. avium isolate, by using a fluorescein-conjugated monoclonal antibody against Chlamydia genus-specific antigen, to detect an infectivity reduction induced by these drugs (3). In addition, in order to investigate active C. avium infection in pigeons, 10 sera with detectable antibodies to C. avium by MIF were examined by the neutralization test with C. avium EBs. Heat-inactivated pigeon sera, incubated for 30 min at 37°C with C. avium EBs diluted in SPG to contain 2x10<sup>4</sup> inclusion-forming units (WU) mL<sup>-1</sup> and fresh rabbit serum, as a source of complement, were inoculated into LLC-MK2 cells; the cell monolayers were centrifuged, incubated for 48 h and subsequently stained for the detection of serum neutralization activity, represented by a 50% reduction from control IFU in infectivity. Finally, in order to detect the presence of plasmid in our isolate, we tested C. avium-infected cells with a rabbit hyperimmune serum, containing specific antibodies to plasmid-encoded protein pgp3, by indirect immunofluorescence technique. Moxifloxacin was the most active quinolone drug against C. avium, with MIC and MBC values of 0.06 mg/L, in comparison with levofloxacin showing MIC and MBC of 0.5 mg/L. The MIC and MBC of doxycycline were 0.125 and 0.5 mg/L, respectively. Roxitromycin and azithromycin showed MIC and MBC values of 0.5 and 1 mg/L, respectively. A neutralizing activity against C. avium was detected in 6 out of the 10 pigeon sera tested, at dilution ranging from 1:10 to 1:40, in the presence of complement. Interestingly, the PV4360-2/99 isolate showed the presence of plasmid, since C. avium-infected cells strongly reacted with the anti-pgp3 serum.

Bonilauri° P, Defilippo° F, Dottori° M, Luppi° A, Massi° P

**Forensic case : when human medicine, veterinary and entomology work together the truth has more chance**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 304 (Poster 162). - 1 bib ref [Nr. Estr. 7601]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

Bregoli° A, Pezzoni° G, Grazioli° S, Calzolari° M, Foglia° EA, Chiapponi° C, Brocchi° E

**Phylogenetic study and evolution of the Swine Vesicular Disease Virus over 25 years of circulation in Italy**

11th Epizone Annual Meeting "Crossing barriers" : 19-21 September 2017, Paris, France :

programme / [s.l. : s.n., 2017]. - p 144 (Poster P3 11) [Nr. Estr. 7660]

Annual meeting Epizone (11. : Paris, France : 19-21 September 2017)

Swine Vesicular Disease Virus (SVDV) belongs to the genus Enterovirus, family Picomaviridae; its genome consists of a positive strand RNA of about 7400 nt codifying for a polyprotein processed, during viral replication, in structural and non-structural proteins by viral proteases. Although only one serotype has been described, four SVDV distinct antigenic variants have been circulating in Italy from 1966 to 2015, identified by using panels of Monoclonal Antibodies (MAbs). The first variant corresponds to the first virus isolated in 1966 in Italy, the second includes viruses circulated in Europe and Far-East during the '70s, the third includes viruses circulating from 1988 to 1992 only in Italy and the fourth variant persisted from 1992 to 2015. The aim of this work was to study the molecular evolution of SVD virus in Italy, with focus for viruses of the fourth variant, using complete genome sequences of 191 representative strains. Two different amplification techniques were used to prepare the samples to be submitted to the Miseq Illumina Instrument: Sequence-Independent Single-Primer Amplification (SISPA) and the production of two amplicons of 3315nt and 5012nt covering all the viral genome length. For each strain, nearly complete sequences of 7335 nt were obtained. The Bayesian phylogenetic analysis performed on the polyprotein coding region shows a clear separation of the four antigenic variants in four distinct genetic lineages. Within the fourth variant two main sub-lineages can be distinguished, both derived from a unique common ancestor dated back to 1990-91. The first sub-lineage, hereafter named "Italian sub-lineage A", includes viruses evolved exclusively in Italy from 1995 to 2010. After a first period of co-circulation in both North and South Italy, from 2000 this sub-lineage was maintained in Southern Italy causing almost exclusively sub-clinical forms. However, a new incursion in North Italy, affecting mainly the Lombardy region, occurred in 2006-2007 with two distinct epidemic waves. Viruses of the second sub-lineage, named "Italian sub-lineage B", are organized in two distinct sub-groups: one includes viruses circulated from 1992 to 1999 mainly in Southern Italy and closely related to isolates detected in Spain and Portugal in 1993 and 1995 respectively; the second sub-group is composed by viral strains detected in Italy from 2004 and more closely related to those found in Portugal in 2003, suggesting a probable reintroduction in Italy following trades with Iberian Peninsula. SVDV evolution in Italy shows a neutral selection, with a uniform distribution of the synonymous mutations throughout the genome. Differently, non-synonymous mutations occurred with higher frequency in the VP1 capsid protein and in the non-structural proteins 2A and 3A, involved in viral replication. Another event that contributed to SVDV evolution in Italy was the recombination between sub-lineage A and B, which occurred in 2007. A total of 20 recombinant strains have been identified and sequenced, the breakpoint for each of them was calculated by Simplot analysis and resulted within the range of 3761-3821nt position. All the recombinant strains are likely to originate from a unique recombination event, as suggested by phylogenetic analysis performed on the recombined genome portions. In conclusion, the fourth antigenic variant lasted in Italy for over 25 years, unlike the previous variants that were temporarily more limited; co-circulation of different genetic sub-lineages occurred along several years, probably promoted by trade relationships with other countries; this enabled a recombination event in 2007 and, after three years of simultaneous circulation of the parental and recombinant viruses, from 2010 only recombinant viruses have been detected.

Brocchi° E, Grazioli° S, Pezzoni° G

**FMD : technical international network for performing diagnosis and control of the disease in endemic countries**

Networking : tool for an excellent research: a public veterinary health without borders to face new emergencies : atti 4° Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria : Roma 6 Aprile 2017 / edited by Marina Bagni, Antonio Petrini, Antonio Lavazza. - [Teramo] : Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, 2017. - (Veterinaria Italiana. Collana di Monografie ; Anno 53, Monografia ; 26) p 69 [Nr. Estr. 7927]

Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria (4. : Roma : 6 Aprile 2017)

The National Reference Centre for FMD with the Biotechnology Laboratory has acquired wide

expertise in the production and characterization of monoclonal antibodies (mAbs) and their use in the development of diagnostic immunoassays. Through consecutive National Grants and EU-funded Consortia projects, extending over 30 years, more than 500 well-characterized mAbs were produced against the seven serotypes and multiple variants of FMDV, and the corresponding hybridoma are stored in the IZSLER Biobank. MAb-based in-house diagnostic ELISAs were initially developed, followed by a significant improvement derived from their conversion in stabilized, ready-to-use kits. The IZSLER portfolio includes a complete spectrum of kits for FMD viruses and antibodies detection and serotyping. In addition, a collaborative study led to the development of the rapid test for field diagnosis (LFD). All these products are being currently used in many endemic countries in Africa, Asia and Middle East, where they enable confirmation of suspect cases with identification of FMDV serotypes, country serosurvey to define infection prevalence, post-vaccination monitoring. In addition to this established research branch, further potent tools like NGS were implemented for phylogenetic reconstruction of recent outbreaks in North Africa. Future perspectives include continuous improvement of FMD diagnostics and strengthening relationships with endemic regions.

Bruun\_Rasmussen T, Boniotti<sup>o</sup> MB, Grasland B, Frossard JP, Dastjerdi A, Hulst M, Hanke D, Pohlmann A, Blome S, Van\_der\_Poel WHM, Steinbach F, Blanchard Y, Lavazza<sup>o</sup> A, Botner A, Belsham GJ

**Variations in the full-length genome sequences of porcine epidemic diarrhoea virus strain CV777 as determined by NGS**

11th Annual Meeting Epizone "Crossing barriers" : 19-21 September 2017, Paris, France : programme and abstracts / [s.l. : s.n., 2017]. - p 132 (C39) [Nr. Estr. 7656]

Annual meeting Epizone (11th : Paris, France : 19-21 September 2017)

The prototypic porcine epidemic diarrhoea virus (PEDV), strain CV777, was initially characterized in 1978 after the disease was first identified in the UK in 1971. This coronavirus has subsequently been widely distributed among different laboratories and has been passaged both within pigs and in cell culture. To determine the variability between different stocks of the PEDV strain CV777 (and its close relatives), sequencing of the full-length genome (ca. 28kb) has been performed in 6 different European laboratories, using different NGS protocols. Not surprisingly, each of the different full genome sequences were distinct from each other and from the published reference sequence (Accession number AF353511) but they are >99% identical. The unique and shared differences between the individual sequences have been identified. The region of the genome encoding the surface exposed spike (S) protein showed the highest proportion of variability including both point mutations and small deletions. The predicted expression of the ORF3 gene product was more dramatically affected in three different variants of this virus strain through either the loss of the initiation codon or the gain of a premature termination codon. The genome of one virus isolate had a substantially rearranged 5'-terminal sequence. This rearrangement was validated through the analysis of sub-genomic mRNAs from infected cells. This work has implications for understanding the basis of differences in virulence of this porcine coronavirus.

Busi<sup>o</sup> C, Martella V, Papetti<sup>o</sup> A, Sabelli<sup>o</sup> C, Lelli<sup>o</sup> D, Alborali<sup>o</sup> GL, Gibelli<sup>o</sup> L, Gelmetti<sup>o</sup> D, Lavazza<sup>o</sup> A, Cordioli<sup>o</sup> P, Boniotti<sup>o</sup> MB

**Group A rotavirus associated with encephalitis in red fox**

Emerg Infect Dis. - Vol. 23 no 9 ( 2017). - p 1535-1538. - 15 bib ref [Nr. Estr. 7705]

In 2011, a group A rotavirus was isolated from the brain of a fox with encephalitis and neurologic signs, detected by rabies surveillance in Italy. Intracerebral inoculation of fox brain homogenates into mice was fatal. Genome sequencing revealed a heterologous rotavirus of avian origin, which could provide a model for investigating rotavirus neurovirulence.

Cabassi CS, Sala A, Santospirito D, Alborali° GL, Carretto E, Ghibauda G, Taddei S

**Activity of AMP2041 against human and animal multidrug resistant *Pseudomonas aeruginosa* clinical isolates**

Ann Clin Microbiol Antimicrob. - Vol. 16 ( 2017). - no 17 (9 p). - 31 bib ref (ultimo accesso 23/10/2018 <https://ann-clinmicrob.biomedcentral.com/articles/10.1186/s12941-017-0193-1> ) [Nr. Estr. 7545]

Background: Antimicrobial resistance is a growing threat to public health. *Pseudomonas aeruginosa* is a relevant pathogen causing human and animal infections, frequently displaying high levels of resistance to commonly used antimicrobials. The increasing difficulty to develop new effective antibiotics have discouraged investment in this area and only a few new antibiotics are currently under development. An approach to overcome antibiotic resistance could be based on antimicrobial peptides since they offer advantages over currently used microbicides. Methods: The antimicrobial activity of the synthetic peptide AMP2041 was evaluated against 49 *P. aeruginosa* clinical strains with high levels of antimicrobial resistance, isolated from humans (n = 19) and animals (n = 30). In vitro activity was evaluated by a microdilution assay for lethal dose 90% (LD90), while the activity over time was performed by time-kill assay with 12.5 µg/ml of AMP2041. Evidences for a direct membrane damage were investigated on *P. aeruginosa* ATCC 27853 reference strain, on animal isolate PA-VET 38 and on human isolate PA-H 24 by propidium iodide and on *P. aeruginosa* ATCC 27853 by scanning electron microscopy. Results: AMP2041 showed a dose-dependent activity, with a mean (SEM) LD90 of 1.69 and 3.3 µg/ml for animal and human strains, respectively. AMP2041 showed microbicidal activity on *P. aeruginosa* isolates from a patient with cystic fibrosis (CF) and resistance increased from first infection isolate (LD90 = 0.3 µg/ml) to the mucoid phenotype (LD90 = 10.4 µg/ml). The time-kill assay showed a time-dependent bactericidal effect of AMP2041 and LD90 was reached within 20 min for all the strains. The stain-dead assay showed an increasing of membrane permeabilization and SEM analysis revealed holes, dents and bursts throughout bacterial cell wall after 30 min of incubation with AMP2041. Conclusions: The obtained results assessed for the first time the good antimicrobial activity of AMP2041 on *P. aeruginosa* strains of human origin, including those deriving from a CF patient. We confirmed the excellent antimicrobial activity of AMP2041 on *P. aeruginosa* strains derived from dog otitis. We also assessed that AMP2041 antimicrobial activity is linked to changes of the *P. aeruginosa* cell wall morphology and to the increasing of membrane permeability.

Calzolari° M, Calvi G, Bonilauri° P, Tamba° M, Albieri A, Angelini P, Dottori° M

**Effetto diluizione della comunita ornitica sulla circolazione del virus West Nile in Emilia-Romagna**

Tichodroma. - Vol. no 6 ( 2017). - p 112-113. - 1 bib ref [Nr. Estr. 7687]

Convegno italiano di ornitologia (19. : Torino : 27 settembre - 1 ottobre 2017)

Il virus West Nile (WNV) è un Flavivirus che circola fra le zanzare (vettori) e gli uccelli (serbatoi). Questo virus può infettare come ospiti a fondo cieco cavalli e persone, arrivando a causare sindromi neuro-invasive gravi. La circolazione del virus compromette la sicurezza di trasfusioni e donazioni d'organo in una data area, per questo oggetto di una sorveglianza sanitaria integrata, volta alla precoce individuazione della sua circolazione in Emilia-Romagna (Calzolari et al. 2015). Una parte importante di questa sorveglianza è quella entomologica che prevede la ricerca del virus nelle zanzare catturate in stazioni fisse (una per quadrante in una griglia di 1 cm<sup>2</sup>). Allo scopo di valutare eventuali correlazioni fra comunita ornitica e circolazione del virus i dati del progetto MIT02000 relativi agli anni 2013-15 sono stati estratti in un buffer di 3 km attorno ai siti della sorveglianza (per un totale di 267 stazioni di campionamento, 10.736 individui di 91 specie). Questi dati sono stati incrociati con i dati della sorveglianza relativi a 53 siti attivi dal 2013-16 (507.264 zanzare testate,

208 pool WNV-positivi). La circolazione del WNV è stata valutata come persistenza del virus (anni con almeno un pool di zanzare positive nel periodo) ed in termini di intensità di circolazione del virus (somma dei tassi di infezione ottenuti per i diversi campionamenti del periodo). La comunità ornitica è stata caratterizzata calcolando il numero medio di individui per ogni specie registrata per osservazione intorno ai diversi siti. Il numero di specie attorno ai siti è risultato inversamente correlato alla persistenza del virus ( $p < 0,01$ ;  $R^2 = 0,94$ ), suggerendo che la circolazione del WNV è più intensa in presenza di comunità ornitiche semplificate (effetto diluizione). Inoltre la presenza di alcune specie di uccelli, legate in particolare agli habitat acquatici ed ai seminativi, è risultata significativamente associata alla circolazione del virus mentre alcune tra le specie maggiormente sinantropiche o legate ad habitat eterogenei sono negativamente correlate alla sua circolazione. Questo lavoro indica come la condivisione di dati ottenuti in progetti diversi (uno di sorveglianza sanitaria ed uno di monitoraggio delle specie di uccelli nidificanti comuni) possano produrre evidenze utili a chiarire l'ecologia di un importante virus di interesse sanitario.

Calzolari ° M, Chiapponi° C, Bonilauri° P, Lelli° D, Baioni° L, Barbieri° I, Lavazza° A, Pongolini° S, Dottori° M, Moreno° A

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

Infect Genet Evol. - Vol. 51 ( 2017). - p 255-262. - 49 bib ref [Nr. Estr. 7540]

Usutu virus is an arbovirus closely related to West Nile virus (genus Flavivirus), which circulates between mosquitoes and wild birds. This virus has been increasingly reported in Europe, raising concerns for its possible pathogenic potential for wild birds and humans. This study reports the whole genome sequences of 15 strains of USUV, isolated between 2010 and 2014 from mosquitoes and wild birds in the course of West Nile virus surveillance in the Emilia-Romagna and Lombardy regions of Northern Italy. Both whole and 656 partial genome sequences, obtained from isolated viruses and field samples (mosquitoes and wild birds), were analyzed to describe the temporal and geographical spread of USUV in the surveyed area. The detected sequences belonged to two groups, with one circulating primarily in the northwestern part of the area and the other in the southeastern part. This pattern is likely the result of different routes of introduction from the North (over the Alps) and from the East, respectively. The phylogenetic analysis of obtained sequences and other European sequences demonstrated that the majority of European strains belonged to one main clade, while less common strains, mainly from Western Europe, fell in other two clades. This analysis strongly suggested an autochthonous evolution process of strains of the main clade from a common ancestor with an estimated time of arrival in Europe at the beginning of the 1990s. In addition to causing mass mortality in wild birds, Usutu virus can infect humans and can sporadically cause disease. These factors and the endemization of the Usutu virus in a large area of Europe, sustained by the obtained data, strongly support the need to adequately survey Usutu virus in areas in which its circulation is detected.

Cambuli C, Puglisi R, Fusi° F, Bertocchi° L, Galli A, Bongioni G, Montedoro M

**Identification of differentially expressed microRNAs in the peripartum of dairy cows : potential indicators of welfare and metabolic efficiency**

Ital J Anim Sci. - Vol. 16 Suppl 1 ( 2017). - p 193-194 [Nr. Estr. 7714]

Animal Science and Production Association ( ASPA ) Congress (22nd : Perugia : June 13-16, 2017)

Genetic selection pressure on dairy cows has increased the incidence of metabolic disorders with important consequences on welfare and longevity. MicroRNAs (miRNA), a family of small non-coding RNAs with a key role in the regulation of gene expression, are interesting for characterizing the molecular basis of immune imbalances. The aim of this work was to investigate differences in miRNAs expression in dairy cattle during the highly susceptible to stress period of the

peripartum. Forty metabolic and immunological parameters, classically used as indicators of increased risk of peripartum-related diseases, were recorded in six Holstein Friesian cows during 4 time-points of the peripartum (-21 ± 2; -3 ± 1; +3 ± 1; +21 ± 2, days). Animals were housed in a highly producing farm monitored according to the model of the National Reference Centre for Animal Welfare. Based on the simultaneous variation found in the levels of non-esterified fatty acids, albumin, cholesterol and haptoglobin, cows were separated into a control group (values within the reference range) and a group with altered metabolic values. The miRNAs were extracted from the buffy coat of 24 blood samples relatively to the 4 time-points and sequenced by Illumina HiSeq 2000. Standard count based bioinformatic pipelines (EdgeR) were applied to reveal expression changes in miRNAs between the two groups. Results identified 30 differentially expressed miRNAs in the group with altered metabolic values compared to control with False Discovery Rate (FDR) at  $p < .05$ . Among them, the bta-mir-2415, bta-mir-126, bta-mir-6522, bta-mir-423, and bta-mir-10b are of particular interest as potential early indicators of a risk of imbalance because they resulted to be differentially expressed 3 weeks before the birth. The corresponding Fold Change (FC) were: logFC = -2.98 (FDR = 0.0039); logFC = 3.93 (FDR = 0.0040); logFC = 2.14 (FDR = 0.003); logFC = -1.32 (FDR = 0.016); logFC = 3.25 (FDR = 0.021). Preliminary bioinformatic analysis indicated putative target genes involved in pathways of cytokines, protein kinase C, membrane transport, and mitochondrial processing (total score >250, total energy < -10). The miRNAs identified in the present work are candidates as potential biomarkers associated with critical moments of the production cycle and can enhance the understanding on the ability to respond more efficiently to critical phases.

Cambuli C, Puglisi R, Fusi<sup>o</sup> F, Bertocchi<sup>o</sup> L, Galli A, Bongioni G, Montedoro M

#### **Differential expression of microRNA in the peripartum of Holstein Friesian cattle**

36th International Society for Animal Genetics Conference ISAG 2017 : 16th-21st July 2017, Dublin, Ireland "Genomes to phenomes" : abstract book / [s.l. : s.n., 2017]. - p 161 (Abstract MT352) [Nr. Estr. 7720]

International Society for Animal Genetics Conference (ISAG) (36th : Dublin, Ireland : 16th-21st July 2017)

Intensive genetic selection in dairy cows has affected the onset of a variety of metabolic disorders. Moreover, it is known that the peripartum period is of particular relevance with respect to metabolic and oxidative stress and general increased susceptibility to disorders. MicroRNAs (miRNA), small non-coding RNAs with a role in regulating gene expression, are of interest in understanding the different capacity of response of the immune system. The aim of this study was to investigate miRNAs differential expression in order to characterise the molecular basis of immune imbalances in dairy cattle during the peripartum. Forty metabolic and immunological parameters classically used as indicators of increased risk of peripartum-related diseases were recorded in 100 Holstein Friesian cows during 4 time-points of the peripartum (-21 ± 2; -3 ± 1; +3 ± 1; +21 ± 2, days). Animals were housed in a farm monitored according to the model of the National Reference Centre for Animal Welfare. Based on the simultaneous variation found in the levels of non-esterified fatty acids, albumin, cholesterol and haptoglobin, six cows were separated into a control group (values included in the corresponding reference range) and a group with altered values. The miRNAs were extracted from buff' coat of 24 blood samples relatively to the 4 time-points and sequenced by Illumina HiSeq 2000. Statistical analysis, performed with EdgeR, identified 30 differentially expressed miRNAs in the group with altered values respect to control group (False Discovery Rate, FDR. at  $P < 0.05$ ). Among them, the bta-mir-2415 (logFC = -2.98, FDR = 0.004), bta-mir-126 (logFC = 3.93, FDR = 0.004), bta-mir-6522 (logFC = 2.14, FDR = 0.003) and bta-mir-10b (logFC = 3.25, FDR = 0.02) were differentially expressed 3 weeks before the birth. The bta-mir-6522 (logFC = 3.032, FDR = 0.01) and bta-mir-6531 (logFC = -3.535, FDR = 0.03) were differentially expressed 3 days after birth. The bta-mir-10b (log FC = -2.744, FDR = 0.007) and bta-mir-143 (logFC = -1.687, FDR = 0.06) were differentially expressed 3 weeks after the birth. The miRNAs identified in the present work are potential candidates to improve the understanding on the ability to respond more efficiently to critical phases.

Caminiti<sup>o</sup> A, Pelone F, Battisti S, Gamberale F, Colafrancesco R, Sala M, La\_Torre G, Della\_Marta U, Scaramozzino P

**Tuberculosis, brucellosis and leucosis in cattle: a cost description of eradication programmes in the Region of Lazio, Italy**

Transbound Emerg Dis. - Vol. 64 ( 2017). - p 1493-1504 - 48 bib ref [Nr. Estr. 7521]

The eradication of tuberculosis, brucellosis and leucosis in cattle has not yet been achieved in the entire Italian territory. The region of Lazio, Central Italy, represents an interesting case study to evaluate the evolution of costs for these eradication programmes, as in some provinces the eradication has been officially achieved, in some others the prevalence has been close to zero for years, and in still others disease outbreaks have been continuously reported. The objectives of this study were i) to describe the costs for the eradication programmes for tuberculosis, brucellosis and leucosis in cattle carried out in Lazio between 2007 and 2011, ii) to calculate the ratio between the financial contribution of the European Union (EU) for the eradication programmes and the estimated total costs and iii) to estimate the potential savings that can be made when a province gains the certification of freedom from disease. For the i) and ii) objectives, data were collected from official sources and a costing procedure was applied from the perspective of the Regional Health Service. For the iii) objective, a Bayesian AR(1) regression was used to evaluate the average percentage reduction in costs for a province that gained the certification. The total cost for the eradication programmes adjusted for inflation to 1 January 2016 was estimated at 18 919 797 euro (5th and 95th percentiles of the distribution: 18 325 050–19 552 080 euro). When a province gained the certification of freedom from disease, costs decreased on average by (median of the posterior distribution) 47.5%, 54.5% and 54.9% for the eradication programmes of tuberculosis, brucellosis and leucosis, respectively. Information on possible savings from the reduction of control costs can help policy makers operating under budget constraints to justify the use of additional resources for the final phase of eradication.

Canelli E, Catella A, Borghetti P, Ferrari L, Ogno G, De\_Angelis E, Corradi A, Passeri B, Bertani V, Sandri G, Bonilauri<sup>o</sup> P, Leung FG, Guazzetti S, Martelli P

**Phenotypic characterization of a highly pathogenic Italian porcine reproductive and respiratory syndrome virus (PRRSV) type 1 subtype 1 isolate in experimentally infected pigs**

Vet Microbiol. - Vol. 210 ( 2017). - p 124-133. - 58 bib ref [Nr. Estr. 7692]

Highly pathogenic (HP) isolates of the PRRS virus started emerging in North America and Asia in the late 1990s. More recently, they have emerged in Europe. These isolates are characterized by high viral loads, severe general clinical signs and high mortality, in sows, weaners and growers. Their genome shows a discontinuous aminoacids deletion in the non-structural protein 2 (NSP2). The present study was aimed at characterizing the clinical, pathological and immunological features of a highly pathogenetic, Italian PRRSV-1 subtype 1 isolate (PRRSV1\_PR40/ 2014), following experimental infection in conventional 4-weeks-old pigs. The PRRSV1\_PR40/2014 infected group showed severe clinical signs (high fever and dispnoea). Pathological lesions, including severe lymphocytopenia in bronchial lymph-nodes and thymus were also recorded. Higher serum PRRSV genome copies and lower virus neutralizing antibody titer were observed in the PR40 group, when compared to the group infected with a conventional PRRSV strain. The genetic analysis of the strain, and the phenotypic features observed in the field and reproduced in the experimental study, confirmed the high pathogenicity of the Italian PRRSV-1 subtype 1 PR40 isolate.

Cannella V, Purpari G, Mira F, Barreca S, Valenza A, Gucciardi F, Macaluso G, Antoci F, Farina G, Cusumano F, Lavazza<sup>o</sup> A, Guercio A

### **Bovine coronavirus (BCOV) infection in bovines farmed in Sicily**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 148 (Poster 015). - 4 bib ref [Nr. Estr. 7589]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

Cannistra° M, Capelli° G, Gandolfi P, Cammi M, Idropici E, Garbarino° C, Arrigioni° N

### **La diagnosi rapida di mastite bovina in allevamento: uno strumento per ridurre i trattamenti antibiotici**

XLIX Congresso della Societa Italiana di Buiatria (SIB) : 23-24 Novembre 2017 Parma : volume degli abstract / 7756]

Congresso della Societa Italiana di Buiatria (SIB) (49. : Parma : 23-24 Novembre 2017)

Nella comunità scientifica e nell'opinione pubblica è forte la preoccupazione per lo sviluppo di antimicrobico-resistenze, responsabili di infezioni talvolta incurabili nell'uomo. In ambito veterinario, allo scopo di contrastare l'insorgenza del fenomeno, sono state pubblicate le Linee guida Europee sull'uso prudente degli antimicrobici (2). Le Linee guida raccomandano l'utilizzo della terapia antibiotica rispettando le indicazioni d'uso, solo a scopo terapeutico ed a seguito di specifica diagnosi; le stesse Linee guida raccomandano l'utilizzo di sistemi diagnostici rapidi, da utilizzare direttamente in allevamento. Vengono illustrati i risultati di un protocollo on farm culture (OFC), per la diagnosi rapida di mastite clinica, oggi causa del maggior consumo di antibiotici nell'allevamento bovino da latte. Il protocollo, che ha previsto l'utilizzo di tre terreni selettivi (rispettivamente per Streptococchi, Stafilococchi e Coliformi) in piastre a tre settori prodotte da IZSLER, è stato applicato in 5 allevamenti per un periodo di sei mesi. Il dato più rilevante è che, sulla base del risultato della OFC, è stata evitata la terapia antibiotica nel 53% dei casi di mastite clinica riscontrati (dal 44% al 71% nei vari allevamenti), con un importante vantaggio economico per gli allevatori, legato a minor scarto di latte e minor acquisto di farmaci.

*In the scientific community and in public opinion, there is a strong concern for the development of antimicrobial resistance, responsible for human infections, sometimes incurable. In the veterinary field, in order to counteract the onset of this phenomenon, the "European guidelines for the prudent use of antimicrobials" (2) were published. These Guidelines recommend the use of antibiotic therapy in accordance with the indications of use, only for therapeutic purposes and following specific diagnosis; moreover the same Guidelines recommend the use of rapid diagnostic tests to be used directly on the farm. Here we report the results of an on-farm culture (OFC) protocol, for rapid diagnosis of clinical mastitis which is the main cause of antibiotic use in dairy cattle breeding. The protocol, based on the use of Tr-Plates with selective media (three sectors for Streptococci, Staphylococci and Coliforms, respectively) produced by IZSLER, was applied to 5 herds for a period of six months. Based on the result of OFC, antibiotic therapy was avoided in 53% of cases of clinical mastitis (from 44% to 71% in the 5 herds), with a significant economic advantage for breeders, linked to lower milk loss and lower purchase of antimicrobials.*

Capra E, Cremonesi P, Pietrelli A, Puccio S, Luini° M, Stella A, Castiglioni B

### **Genomic and transcriptomic comparison between Staphylococcus aureus strains associated with high and low within herd prevalence of intra-mammary infection**

BMC Microbiology. - Vol. 17 ( 2017). - no 21 (16 p). - 66 bib ref ( ultimo accesso 27/02/2017 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5247818/> ) [Nr. Estr. 7519]

Background: Staphylococcus aureus (Staph. aureus) is one of the major pathogens causing mastitis in dairy ruminants worldwide. The chronic nature of Staph. aureus infection enhances the contagiousness risk and diffusion in herds. In order to identify the factors involved in intra-mammary infection (IMI) and diffusion in dairy cows, we investigated the molecular characteristics of two

groups of *Staph. aureus* strains belonging to ST8 and ST398, differing in clinical properties, through comparison of whole genome and whole transcriptome sequencing. Results: The two groups of strains, one originated from high IMI prevalence herds and the other from low IMI prevalence herds, present a peculiar set of genes and polymorphisms related to phenotypic features, such as bacterial invasion of mammary epithelial cells and host adaptation. Transcriptomic analysis supports the high propensity of ST8 strain to chronicity of infection and to a higher potential cytotoxicity. Conclusions: Our data are consistent with the invasiveness and host adaptation feature for the strains GTB/ST8 associated to high within-herd prevalence of mastitis. Variation in genes coding for surface exposed proteins and those associated to virulence and defence could constitute good targets for further research.

Capucci° L, Cavadini° P, Brocchi° E, Pezzoni° G, Lavazza° A

**From "X disease" to RHDV2 : over 25 years of research on lagoviruses**

Networking : tool for an excellent research: a public veterinary health without borders to face new emergencies : atti 4° Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria : Roma 6 Aprile 2017 / edited by Marina Bagni, Antonio Petrini, Antonio Lavazza. - [Teramo] : Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, 2017. - (Veterinaria Italiana. Collana di Monografie ; Anno 53, Monografia ; 26) p 71 [Nr. Estr. 7926]

Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria (4° : Roma : 6 Aprile 2017)

When we started to work on "X disease" of rabbits on 1986, its aetiology of was not yet defined. We contributed to firstly describe the viral aetiology and to classify such virus. Thereafter, in the new genus Lagovirus of the family Caliciviridae, with RHDV as prototype, some other agents were included as results of our studies i.e. the European Brown Hare Syndrome Virus (EBHSV) on 1988, the first consistent antigenic variant RHDVa on 1996 and the first non-pathogenic lagovirus (Rabbit calicivirus - RCV) on 1997. These important achievements were made possible by the set up and extensive use in field surveys of innovative serological and virological tests, mainly based on specific reagents, including MAbs, produced and characterized in IZSLER. Thanks to these consolidated expertise and the recognition as OIE Reference Lab, multiple collaboration were established worldwide. When the new serotype RHDV2 was firstly reported in France on 2010, the enforced epidemiological surveillance on domestic and wild lagomorphs and the availability of diagnostic methods permitted to quickly detect the same virus in Italy on 2011, and then to better define its antigenic, genomic and pathological characteristics, including the capacity to infect other lagomorph species of the *Lepus* genus. In addition, on 2014 we found another non-pathogenic lagovirus in hares named Hare Calicivirus (HaCV).

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

**Increased pathogenicity in rabbit haemorrhagic disease virus type 2 (RHDV2)**

Vet Rec. - Vol. 180 no 17 ( 2017). - no 426 (2 p). - 14 bib ref (ultimo accesso 04/07/2017 <http://veterinaryrecord.bmj.com/content/180/17/426.2>) [Nr. Estr. 7576]

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

**Novel plasmid-mediated colistin resistance mcr-4 gene in Salmonella and Escherichia coli, Italy 2013, Spain and Belgium, 2015 to 2016**

EuroSurveillance. - Vol. 22 no 31 ( 2017). - p 30589 (5 p). - 10 bib ref ( ultimo accesso 05/12/2017 <http://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2017.22.31.30589>) [Nr. Estr. 7700]

Carra° E,, Bergamini° F, Cirasella° L, Antognetti V, Rivera\_Gomis J, Milito M,

Belardo V, Bassi° S, Formato G, Cersini A

**Development of three Real-Time PCR assays for the differentiation of *Aethina tumida* from other Nitidulidae (Coleoptera) species as alternative to morphological examination**

45th Apimondia International Apicultural Congress : 29 September - 04 October 2017 29 Istanbul, Turkey : abstract book / [s.l. : s.n., 2017]. - p 269 [Nr. Estr. 7750]

"Apimondia" International Apicultural Congress (45th : Istanbul, Turkey : September 29 - October 04, 2017)

Introduction. The small hive beetle (SHB) *Aethina tumida* (Coleoptera: Nitidulidae) is a parasite of the honeybee colonies, detected in two regions of Southern Italy (Calabria and Sicilia) since the second half of 2014. Thanks to the intensive surveillance plans carried out in 2014-2017, the presence of the parasite is so far confined only in Calabria region. Some Nitidulidae species are associated with the honeybees, among these *Cychramus luteus*, whose presence has been reported in Europe, could be confused with SHB. In general, other Nitidulidae species, like *Meligethes aeneus* and *Brachypeplus glaber*, could be present in the territory under SHB surveillance, and it should not be ruled out their interference with the analysis of identification of *Aethina tumida*. To assist the surveillance and control activities of SHB, we developed three distinct Real-Time PCR assays to ease the differentiation of SHB from these mentioned Nitidulidae species, over all when parts of insects are found in hive debris. Materials and methods. For each Nitidulidae species, Cytochrome oxidase subunit 1 gene (C01) sequences, available from Gen Bank, were aligned using the Clustal W within BioEdit (Hall 1999). Three new sets of specific primers and MGB probes, were designed using Primer Express Software (Applied Biosystems, UK). Results. Distinct TaqMan™ Real-Time PCR assays were developed for detecting the presence of DNA of *Cychramus luteus*, *Meligethes aeneus* and *Brachypeplus glaber*. Synthetic DNA taken from reference samples of these species were positive, confirming the sensitivity of these methods; similarly, total DNA extracted from SHB larvae or adults were negative when tested with our Real-Time PCR assays, confirming their specificity. Discussion. The TaqMan™ Real-Time PCR assays we developed, could represent a reliable support to get a quick differential identification of SHB from *Cychramus luteus*, *Meligethes aeneus* and *Brachypeplus glaber*. Above all, this identification is useful, in the presence of parts of insect. More analyses on DNA extracts, taken from beehives of Calabria region containing parts of Nitidulidae that resulted negative to *A. tumida* PCR Real-Time (Ward, 2007), are still ongoing.

Caruso C, Peletto S, Rosamilia A, Modesto P, Chiavacci L, Sona B, Balsamelli F, Ghisetti V, Acutis PL, Pezzoni° G, Brocchi° E, Vitale N, Masoero L

**Hepatitis E virus : a cross-sectional serological and virological study in pigs and humans at zoonotic risk within a high-density pig farming area**

Transbound Emerg Dis. - Vol. 64 ( 2017). - p 1443-1453. - 56 bib ref [Nr. Estr. 7676]

An increase in autochthonous hepatitis E virus (HEV) infections has been recorded in Italy suspected to be zoonotically transmitted from pigs; this study was carried out to determinate the seroprevalence and risk factors associated with hepatitis HEV exposition, both in swine and humans working in pig farms, located within a high-density pig farming area in Piedmont region, north-western Italy. The presence of viral RNA in human and swine samples was also evaluated, and phylogenetic analysis was performed on HEV-positive samples. Forty-two swine farms were sampled; 142 workers were enrolled in the study and classified into two groups: (i) 69 workers with occupational contact with swine (including veterinarians and farmers) recruited in the 42 sampled farms; (ii) 73 without occupational contact with swine. Forty-one of 42 (97%) swine farms resulted positive to enzyme-linked immunosorbent assay test for HEV antibodies (Abs). Overall seroprevalence in swine was 50% (441/879), with seropositivity rate higher in sows (333/469, 71%). HEV RNA in stool samples was detected in animals from 13 of 42 tested farms (31%), and a higher positivity resulted in weaners (40/246, 16.3%). Phylogenetic analysis classified all HEV isolates within genotype 3 (subtypes 3f, 3e, 3c). All humans were negative for HEV viral genome in blood. Five of 142 sera were positive for IgG anti-HEV with an overall prevalence of 3.52% with no

statistically significant differences in prevalence rates between workers at zoonotic risk and the control group (5.7% versus 1.3%). In contrast, a significant difference (OR 10.1) was observed within the subgroup including subjects exposed for short periods (veterinarians) compared with those who worked for long periods (farmers) suggesting a correlation between the time of exposure and the likelihood of HEV infection. Reporting HEV infection is not mandatory in Italy, but a constant epidemiological surveillance should be ensured to clarify the epidemiology of this disease.

Chiari° M, Cortinovis C, Vitale° N, Zanoni° M, Faggionato° E, Biancardi° A, Caloni F  
**Pesticide incidence in poisoned baits : a 10-year report**

Sci Total Environ. - Vol. 601-602 ( 2017). - p 285-292. - 51 bib ref [Nr. Estr. 7569]

The incidence of pesticides in poisoned baits recovered from 2005 to 2014 in Italian northern regions of Emilia Romagna and Lombardy was analysed. A total of 956 baits tested positive for pesticides during the study period. In 9.3% (n = 89) of the baits analysed more than one toxic compound was present. Insecticides (53.2%) proved to be the pesticides most commonly involved followed by rodenticides (34.3%), molluscicides (12.3%) and herbicides (0.2%). Among insecticides, the anticholinesterases (organophosphates and carbamates) were the most frequently detected (59.1%, n = 412) followed by organochlorines (39.8%; n = 277). Anticoagulants proved to be the rodenticides most commonly involved (78.5%; n = 353) with many baits containing brodifacoum or bromadiolone, while metaldehyde was the most used molluscicide (88.2%; n = 142). A high number of baits contained banned pesticides such as endosulfan, methamidophos, carbofuran and strychnine indicating that the restrictions and the bans on the use of toxic and highly toxic pesticides are not accompanied by a lower use in poisoned baits. The high incidence of banned pesticides detected in the present survey is extremely worrying and poses the question of where and how these compounds were obtained. Efforts have to be made to reduce this illegal practice and its environmental impact. Moreover, education and awareness campaigns should be instituted.

Cleton NB, Van\_Maanen K, Bergervoet SA, Bon N, Beck C, Godeke GJ,  
Lecollinet S, Bowen R, Lelli° D, Nowotny N, Koopmans MPG, Reusken CBEM

**A serological protein microarray for detection of multiple cross-reactive flavivirus infections in horses for veterinary and public health surveillance**

Transbound Emerg Dis. - Vol. 64 ( 2017). - p 1801-1812. - 45 bib ref [Nr. Estr. 7763]

The genus Flavivirus in the family Flaviviridae includes some of the most important examples of emerging zoonotic arboviruses that are rapidly spreading across the globe. Japanese encephalitis virus (JEV), West Nile virus (WNV), St. Louis encephalitis virus (SLEV) and Usutu virus (USUV) are mosquito-borne members of the JEV serological group. Although most infections in humans are asymptomatic or present with mild flu-like symptoms, clinical manifestations of JEV, WNV, SLEV, USUV and tick-borne encephalitis virus (TBEV) can include severe neurological disease and death. In horses, infection with WNV and JEV can lead to severe neurological disease and death, while USUV, SLEV and TBEV infections are mainly asymptomatic, however, and induce antibody responses. Horses often serve as sentinels to monitor active virus circulation in serological surveillance programmes specifically for WNV, USUV and JEV. Here, we developed and validated a NS1-antigen protein microarray for the serological differential diagnosis of flavivirus infections in horses using sera of experimentally and naturally infected symptomatic as well as asymptomatic horses. Using samples from experimentally infected horses, an IgG and IgM specificity of 100% and a sensitivity of 95% for WNV and 100% for JEV was achieved with a cut-off titre of 1 : 20 based on ROC calculation. In field settings, the microarray identified 93–100% of IgG-positive horses with recent WNV infections and 87% of TBEV IgG-positive horses. WNV IgM sensitivity was 80%. Differentiation between closely related flaviviruses by the NS1-antigen protein microarray is possible, even though we identified some instances of cross-reactivity among antibodies. However,

the assay is not able to differentiate between naturally infected horses and animals vaccinated with an inactivated WNV whole-virus vaccine. We showed that the NS1-microarray can potentially be used for diagnosing and distinguishing flavivirus infections in horses and for public health purposes within a surveillance setting. This allows for fast, cheap, syndrome-based laboratory testing for multiple viruses simultaneously for veterinary and public health purposes.

Cook B, Springer K, Capucci° L, Mutze G

**Rabbit haemorrhagic disease : Macquarie Island rabbit eradication adds to knowledge on both pest control and epidemiology**

Wildl Res. - Vol. 44 no 2 ( 2017). - p 93-96. - 36 bib ref [Nr. Estr. 7634]

Rabbit haemorrhagic disease virus (RHDV), introduced into in Australia and New Zealand as a biological-control agent for wild rabbits, is least efficacious in cool humid areas where a non-pathogenic calicivirus (RCV-A1) also circulates. Heavy rabbit mortality following release of RHDV on cold sub-Antarctic Macquarie Island, where RCV-A1 was apparently absent, not only complemented the planned rabbit eradication operations, especially by reducing secondary poisoning of sea-birds from aerial baiting, but also ruled out cool or humid climate as a major limiting factor of disease spread. In turn, this has advanced the idea that RCV-A1 antibodies inhibit RHDV spread as well as reducing disease severity and mortality.

Corbella M, Mariani B, Ferrari C, Comandatore F, Scaltriti° E, Marone P, Cambieri P

**Three cases of mcr-1-positive colistin-resistant Escherichia coli bloodstream infections in Italy, August 2016 to January 2017**

EuroSurveillance. - Vol. 22 no 16 ( 2017). - no 30517 (4 p). - 18 bib ref (ultimo accesso 07/06/2017 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5404483/>) [Nr. Estr. 7546]

Curcio L, Luppi° A, Bonilauri° P, Gherpelli° Y, Pezzotti G, Pesciaroli M, Magistrali CF

**Detection of the colistin resistance gene mcr-1 in pathogenic Escherichia coli from pigs affected by post-weaning diarrhoea in Italy**

J Glob Antimicrob Resist. - Vol. 10 ( 2017). - p 80-83. - 17 bib ref [Nr. Estr. 7694]

Objectives: The aim of this study was to investigate the presence of plasmid-mediated colistin resistance genes in Escherichia coli from pigs affected by post-weaning diarrhoea (PWD). Methods: DNA samples collected from 51 E. coli isolates from Italian pigs affected by PWD in 2015– 2016 were studied. Isolates were classified as presumptively resistant to colistin by routine susceptibility testing and were investigated for the presence of the mcr-1 gene of plasmid origin by PCR. E. coli isolates testing negative for mcr-1 were analysed for the presence of a novel plasmid-mediated gene, mcr-2. Isolates were characterised for fimbrial [F4 (k88), F5 (k99), F6 (987P), F18 and F41] and toxin (LT, STa, STb and Stx2e) determinants by PCR as well as for the occurrence of haemolysis by phenotypic observation. Susceptibility to apramycin, cefquinome, enrofloxacin, florfenicol, gentamicin, tetracycline and trimethoprim/sulfamethoxazole (SXT) was also determined by disk diffusion. Results: Most of the isolates showed the presence of at least one virulence factor, confirming their pathogenic potential. The presence of mcr-1 was shown in 37 (72.5%) of the 51 isolates. All of the mcr-1- negative isolates tested negative for the mcr-2 gene. Moreover, 80.4% of the isolates were resistant to apramycin, 9.8% to cefquinome, 54.9% to enrofloxacin, 52.9% to florfenicol, 76.5% to gentamicin, 96.1% to tetracycline and 78.4% to SXT. Conclusions: This is the first report documenting the presence of the mcr-1 gene in pathogenic E. coli isolated from pigs

affected by PWD in Italy.

Curcio L, Luppi° A, Orsini S, Cucco L, Bonilauri° P, Pesciaroli M, Massacci FR, Pezzotti G, Magistrali CF

**Prevalence of plasmid families in pathogenic colistin resistant Escherichia coli isolated from pigs in Spain, Italy and Belgium**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 164 (Poster 031). - 5 bib ref [Nr. Estr. 7592]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

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

**Physiological characterization of dry and post-partum period in Holstein and Rendena breeds**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 36. - 1 bib ref [Nr. Estr. 7607]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

In the last 60 years genetic selection in Holstein cattle has been mainly focused on the milk yield criterion, and this mono-aptitude selective criterion has led to their high propensity to develop critical physiological conditions in the pregnancy to lactation transition period with repercussions on reproductive efficiency, longevity in farm and resistance to stress and diseases [1][2]. A different scenario appears to happen with autochthonous Italian dairy breeds, which are typically characterized by much better fertility and higher resistance and resilience against disease [3]. Unfortunately, less is known about the biological mechanisms behind this rusticity. In this study, a multidisciplinary approach was applied to compare different physiological parameters related with the metabolism, milk protein profiles and the milk microbiota in Holstein Friesian (HF) and Rendena (REN) breed, reared in the same farm and under the same management conditions. The cows (6 HF and 4 REN) were all among 2 and 4 lactations. The average milk yield was significantly higher in HF compared to REN (HF=5,366 kg vs REN=3,769;  $p=0.0147$ ). The percentage of milk fat (HF=3.52% vs REN=3.37%) and protein (HF=3.02% vs REN=3.08%) content was comparable in the two breeds. Quarter milk samples and venous blood were collected from each animal at the following time points: dry-off (Ti), 1 day after calving (T2), 7-10 days after calving (T3) and 30 days after calving (T4). Blood samples were used for the analysis of plasma metabolites such as: glucose, total cholesterol, urea, inorganic phosphorus, total protein, albumin, total bilirubin, aspartate aminotransferase (GOT),  $\gamma$ -glutamyltransferase (GGT), creatinine, NEFA, 0-0H-butyric acid (BHBA), thiol groups (SHp) and ferric reducing antioxidant power (FRAP). Bacteriological analyses, somatic cell counting, protein profiles and characterization of the milk microbiota were performed on milk samples. Plasma metabolic parameters were analyzed using a pair wise comparison (least significant difference test). A spatial power covariance structure was used. HF cows showed a more severe fat mobilization (NEFA and BHBA  $p<0,05$ ) and systemic inflammatory response at T2 and T3 in comparison with REN cows. We also observed a lower risk of oxidative stress (FRAP  $p<0.05$ ) and an increased amino acid mobilization (creatinine  $p<0.05$ ) in Rendena cows immediately after calving. Upon bacteriological analysis, contagious bacteria such as Staph. aureus and S. agalactiae were not found, but significant differences were assessed in the general composition of the milk microbiota of the two breeds. Concerning the milk protein abundance profile, several bands associated to immunoglobulin components were present in consistently higher amounts in REN colostrum when compared to HF cows. In addition, the relative abundance of caseins between the

two breeds did also vary at all time points, prompting further investigations about its implications on cheesemaking properties. In conclusion, several differences were observed in the two breeds, in spite of the same farming conditions. The physiological observations reported in this work present numerous hints on the factors that may provide autochthonous, more rustic breeds with a better ability to face the critical post-partum period.

Daminelli° P, Cosciani-Cunico° E, Dalzini° E, Monastero° P, Finazzi° G, Losio° MN  
**Fate of indigenous verocytotoxin-producing Escherichia coli in uncooked, raw milk cheeses**

European Symposium on Food Safety : 29-31 May 2017 Brussels, Belgium / [s.l. : s.n., 2017]. - p 98-99 (poster P1-40) [Nr. Estr. 7573]

European Symposium on Food Safety : Brussels, Belgium : 29-31 May 2017)

Introduction: Verocytotoxin-producing Escherichia coli (VTEC) is a foodborne pathogen that can cause severe illness and death. Cattle is a natural reservoir of VTEC; consequently, they may be isolated from raw cow milk and non-pasteurized dairy products, like Uncooked Raw Milk Cheeses (URMC), typically produced in many Italian regions. Purpose: The aim of this study was to assess a survey of VTEC in URMC, produced in the Northern Italy, and to study the fate of indigenous VTEC during seasoning. Methods: From 2013 to 2016, a total of 1,029 curd samples of URMC were analyzed to check for the presence of VTEC using the standard method, ISO/TS 13136:2012. Cheeses produced with positive curds were collected and the fate of the pathogen was studied. Results: VTEC were detected in 28 out of 1,029 curd samples (2.72%, with 1.82% – 3.91% Confidence Interval at 95% significance level). The proportion of positive samples decreased during cheese ripening. After 8 months at 14°C, the water activity (aw) decreased to between 0.977 and 0.919 and no VTEC was detected in the samples. Significance: These data confirm that the cheese ecosystem, especially aw, affects VTEC survival. This study can help safety authorities improve the risk analysis of URMC.

De\_Lorenzi° G, Gherpelli° Y, Bonilauri° P, Marzani° K, Dottori° M, Torri° D, Pangallo° G, Luppi° A

**Escherichia coli enterotossigeni isolati in focolai di PWD in Italia, Belgio-Olanda e Spagna : prevalenza dei fattori di virulenza** = Enterotoxigenic Escherichia coli isolated during PWD outbreaks in Italy, Belgium-the Netherlands and Spain: prevalence of virulence factors

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 125-131. - 17 bib ref [Nr. Estr. 7557]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo 2017)

Nel presente studio sono stati inclusi 543 ceppi di Escherichia coli enterotossici (ETEC) isolati nel periodo compreso tra gennaio 2015 e dicembre 2016, da 495 focolai di diarrea post svezzamento (PWD) occorsi in aziende suinicole in Italia, Belgio-Olanda e Spagna. I ceppi sono stati genotipizzati impiegando una metodica multiplex PCR per i geni codificanti per le fimbrie F4, F5, F6, F18, F41 e per le tossine (tossine termostabili, STa e STb, tossina termolabile, LT e tossina Shiga-like, Stx2e). La prevalenza delle principali fimbrie e tossine tra i ceppi inclusi nello studio risultata essere: F4 (45,4%), F18 (44,8%), STa (37,9%), STb (35,4%), LT (25,4%), Stx2e (1,3%) in Italia; F4 (52,3%), F18 (40,3%), STa (37,7%), STb (38,9%), LT (22,2%), Stx2e (1,2%) in Belgio-Olanda; F4 (45,8%), F18 (44,2%), STa (34,1%), STb (34,5%), LT (31,1%), Stx2e (0,3%) in Spagna. Nella maggior parte dei casi è stato isolato un solo vrotipo per ogni focolaio, tuttavia nel 2,4%, nel 18,5% e nel 3,5% dei casi, rispettivamente in Italia, Belgio-Olanda e Spagna, più di un vrotipo era coinvolto nello stesso focolaio. Per gli allevamenti in cui è stato possibile seguire focolai ricorrenti di PWD da ETEC nel periodo considerato si rileva che in circa la metà di questi, sia in Italia, sia in Belgio sono stati isolati nella stessa azienda diversi vrotipi di ETEC, in tutti i casi con un'alternanza di ceppi presentanti fimbrie F4 e F18.

*This study investigated the prevalence of fimbrial (F4, F5, F6, F18 and F41) and toxin (LT, STa, STb and Stx2e) genes of 543 enterotoxigenic Escherichia coli (ETEC) by PCR among 495 outbreaks of enteric colibacillosis occurred in the post weaning period in Italy, Belgium-The Netherlands and Spain in a period of two years (from January 2015 to December 2016). The prevalence of the main fimbriae among ETEC isolated from the outbreaks occurred in the different countries included in the study were: F4 (45,4%), F18 (44,8%) in Italy; F4 (52,3%), F18 (40,3%) in Belgium-The Netherlands; F4 (45,8%), F18 (44,2%) in Spain. The prevalence of genes encoding for the toxins was: STa (37,9%), STb (35,4%), LT (25,4%), Stx2e (1,3%) in Italy; STa (37,7%), STb (38,9%), LT (22,2%), Stx2e (1,2%) in Belgium-The Netherlands; STa (34,1%), STb (34,5%), LT (31,1%), Stx2e (0,3%) in Spain. In most of the cases one virotype have been isolated in each outbreak, but more than one virotype has been involved during the same outbreak in 2.4%, 18.5% and 3.5% of them in Italy, Belgium-The Netherlands and Spain respectively. In about half of the farms, where recurrent outbreaks of ETEC PWD occurred, both in Italy (6/13) and in Belgium-The Netherlands (10/17), different virotypes of ETEC in subsequent outbreaks have been isolated (in all cases with an alternation of strains presenting fimbriae F4 and F18).*

De\_Lorenzi° G, Raffi V, Gibelli° L, Dottori° M, Bonilauri° P, Luppi° A

#### **Infezione congenita da Pox virus in due suinetti**

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 175-178. - 5 bib ref [Nr. Estr. 7563]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo 2017)

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

#### **Identification and full genome characterization of new unclassified alfa-cov viruses from bats in Italy**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 253 (Poster 114). - 4 bib ref [Nr. Estr. 7596]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

Defilippo° F, Grisendi° A, Meriardi° G, Bertasi° B, Dottori° M, Bonilauri° P

#### **Metodo macroscopico per la rilevazione e il recupero di artropodi vivi infestanti gli alimenti : il selettore di Berlese**

Le sinergie tra grande distribuzione organizzata, industria, piccole produzioni locale e controllo ufficiale : tutela del consumatore, difficoltà e prospettive : XXVII Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) : Perugia, 13-14-15 Settembre 2017 / [s.l. : s.n., 2017]. - p 37 (P029-93) [Nr. Estr. 7860]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (27. : Perugia : 13-14-15 Settembre 2017)

La possibilità di individuare un eventuale infestazione da artropodi nei prodotti alimentari e nei loro locali di stoccaggio è essenziale per far sì che si possa dimostrare che l'alimento offerto sia sano e adatto al consumo. Numerosi sono i metodi utilizzati per rilevare artropodi interi o loro frammenti (es: il livello dell'acido urico nelle materie prime, filth-test, ecc.), la scelta dipende dalla sensibilità richiesta, le risorse disponibili e il fattore tempo. L'obiettivo di questo studio è quello di verificare l'efficacia dell'estrazione di artropodi vivi da matrici alimentari quali cereali e loro derivati e semi oleosi attraverso un selettore di Berlese. Nella sua versione più semplice il selettore di Berlese consiste in un imbuto in cui viene deposto un setaccio (8mesh) con all'interno una garza (diametro 16cm) sulla quale viene posto il campione del peso di 50g. Al di sotto dell'imbuto viene posta un recipiente di raccolta. Una fonte di calore (nel nostro caso una lampada 250W) appena sopra il campione provoca lo spostamento progressivo della parte attiva della fauna infestante verso il

basso, che sfugge all'essiccamento, fino a cadere nel recipiente di raccolta. In questo lavoro, una colonia stabile di *Oryzaephilus surinamensis* e acari appartenenti al genere *Tyroglyphus*, tipici infestanti di prodotti cerealicoli e semi oleosi, è stata mantenuta in laboratorio a temperatura, umidità e fotoperiodo costante. Per validare il metodo sono stati preparati artificialmente dei campioni contaminati con 3 e 5 larve di *O. surinamensis* (peso di una singola larva 0,0015 g) e 5 acari di farina tipo 00, farina di soia, farina di mais, farina integrale, crusca, semi di lino e semi di sesamo. Per ogni prova è stata valutata la capacità di recupero dell'infestante e le tempistiche per il completamento della prova. I dati ottenuti dimostrano che per gli acari otteniamo il recupero completo entro 60' dall'inizio della prova. Per quanto riguarda le larve e gli adulti di *O. surinamensis* il recupero è vincolato alla granulometria della matrice alimentare usata, infatti è più lento per matrici quali farina (tempi di recupero tra 120-180') e più rapido per matrici quali semi, crusca e farina di mais. Lo studio conferma l'efficacia del metodo anche se necessita di ulteriori studi di applicabilità in caso di infestazione di altri gruppi di insetto quali i lepidotteri.

Del\_Corvo M, Luini M, Stella A, Pagnacco G, Ajmone\_Marsan P, Williams JL, Minozzi G

#### **Identification of additional loci associated with antibody response to *Mycobacterium avium* ssp. *Paratuberculosis* in cattle by GSEA–SNP analysis**

Mamm Genome. - Vol. 28 ( 2017). - p 520-527. - 64 bib ref ( ultimo accesso 06/06/2018 <https://link.springer.com/article/10.1007%2Fs00335-017-9714-7> ) [Nr. Estr. 7680]

*Mycobacterium avium* subsp. *paratuberculosis*: (MAP) causes a contagious chronic infection results in Johne's disease in a wide range of animal species, including cattle. Several genome-wide association studies (GWAS) have been carried out to identify loci putatively associated with MAP susceptibility by testing each marker separately and identifying SNPs that show a significant association with the phenotype, while SNP with modest effects are usually ignored. The objective of this study was to identify modest-effect genes associated with MAP susceptibility using a pathway-based approach. The Illumina BovineSNP50 BeadChip was used to genotype 966 Holstein cows, 483 positive and 483 negative for antibody response to MAP, data were then analyzed using novel SNP-based Gene Set Enrichment Analysis (GSEA–SNP) and validated with Adaptive Rank Truncated Product methodology. An allele-based test was carried out to estimate the statistical association for each marker with the phenotype, subsequently SNPs were mapped to the closest genes, considering for each gene the single variant with the highest value within a window of 50 kb, then pathway-statistics were tested using the GSEA–SNP method. The GO biological process "embryogenesis and morphogenesis" was most highly associated with antibody response to MAP. Within this pathway, five genes code for proteins which play a role in the immune defense relevant to response to bacterial infection. The immune response genes identified would not have been considered using a standard GWAS, thus demonstrating that the pathway approach can extend the interpretation of genome-wide association analyses and identify additional candidate genes for target traits.

Donati M, Cenacchi G, Biondi R, Papa V, Borel N, Vecchio\_Nepita E, Magnino S, Pasquinelli G, Levi A, Franco OL

#### **Activity of synthetic peptides against *Chlamydia***

Biopolymers. - Vol. 108 ( 2017). - p e23032 (6 p). - 30 bib ref [Nr. Estr. 7765]

The in vitro activity of six synthetic peptides against 36 strains of *Chlamydia* from different origins was investigated. Clavanin MO (CMO) proved to be the most active peptide, reducing the inclusion number of all *Chlamydia* strains from eight different species tested by >50% at 10 µg mL<sup>-1</sup>. Mastoparan L showed an equal activity against *C. trachomatis*, *C. pneumoniae*, *C. suis*, and *C. muridarum*, but did not exert any inhibitory effect against *C. psittaci*, *C. pecorum*, *C. abortus*, and *C.*

avium even at 80 pg mL<sup>-1</sup>. These data suggest that CM0 could be a promising compound in the prevention and treatment of chlamydial infections.

Ebranati E, Lauzi S, Cerutti F, Caruso C, Masoero L, Moreno<sup>o</sup> AM, De\_Mia GM, Peletto S, Zehender G, Luzzago C

#### **Highlighting priority areas for bovine viral diarrhea control in cattle in Italy : a phylogeographic approach**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 198. - 4 bib ref [Nr. Estr. 7614]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

The bovine viral diarrhea virus (BVDV) prevalence and genetic diversity in a geographic area are largely influenced by live animal trade and management practices [1]. Despite control and eradication programs are underway in several European countries, the risk of BVDV spread within and among countries is still present [2]. Two BVDV recognized species have been identified in cattle, BVDV-1 and BVDV-2, and a putative third one, HoBi-like pestivirus, recently associated with sporadic BVDV-like clinical forms [3]. BVDV-1 is the predominant genotype circulating in European cattle population. In this study, a phylogeographic analysis was applied to BVDV-1 most prevalent subtypes in Italy [4] to reconstruct the origin and spatio-temporal distribution and to trace the main viral flows between different locations to highlight priority areas for BVDV control. With this aim a comprehensive dataset of BVDV-1b (n=173) and 1e (n=172) 5'UTR sequences has been analyzed, including both novel and published sequences from Italy and European countries bordering and/or with cattle commercial flows with Italy. A common phylogeographic pattern was observed for BVDV-1b and 1e subtypes: interspersed from multiple areas was widespread until the end of the last century, whereas significant local clusters were observed starting from 2000. These findings support a continuous viral flow among different areas over long time scales with no evidence of significant geographical structure, while a local system of viral evolution is limited to more recent years. Northern Italy has been highlighted as the area of origin of the main clades of both BVDV subtypes at national level, acting both as a crucial area for introduction and a maintenance source for other areas. Other Italian regions mainly of central and southern areas contributed to limited geographical distribution and local BVDV persistence. Interestingly a similar pattern has been recently observed also for BVDV-1f. On the whole, priority control measures should be focused on: i) breaking the gravity-like dynamic of the infection, originating in larger animal populations from Northern regions and diffusing to smaller populations; ii) stopping the dynamic of infections in regions with self-maintenance of BVDV as demonstrated by significant spatial clusters observed in recent years.

Eldaghayes I, Dayhum A, Kammon A, Sharif M, Ferrari G, Bartels C, Sumption K, King DP, Grazioli<sup>o</sup> S, Brocchi<sup>o</sup> E

#### **Exploiting serological data to understand the epidemiology of foot-and-mouth disease virus serotypes circulating in Libya**

Open Vet J. - Vol. 7 no 1 ( 2017). - p 1-11. - 30 bib ref [Nr. Estr. 7526]

Foot-and-mouth disease Sporadic outbreaks of foot-and-mouth disease (FMD) have occurred in Libya for almost fifty years. During the spring of 2013, a countrywide serosurvey was undertaken to assess the level of FMD virus circulation and identify FMD virus serotypes in the country. A total of 4221 sera were collected, comprising samples from large ruminants (LR; n=1428 samples from 357 farms) and small ruminants (SR; n=2793 samples from 141 farms). FMD sero-prevalence of NSP

antibodies determined by ELISA were 19.0% (271/1428) with 95% CI (16.9 – 21.0) and 13.5% (378/2793) with 95% CI (12.3 – 14.8) for LR and SR samples, respectively. The sero-prevalence of NSP antibodies in LR was 12.3% and 19.8% for age group < 1 year and = 1 year, respectively ( $X^2=4.95$ ,  $P=0.026$ ), while in SR was 3.7%, 13.6% and 21.3% for age group < 1 year, 1-2 year and > 2 year, respectively ( $X^2=118.1$ ,  $P=0.000$ ). These observed NSP serologic profiles support the hypothesis of an endemic level of FMD circulation in Libya. All positive sera were tested for SP antibodies for O, A and SAT-2 FMD virus serotypes. Serotype O was the dominant circulating serotype followed by serotype A, while evidence of SAT-2 was not found. These data provide an insight into the wider epidemiology of FMD in Libya, and contribute to field and laboratory investigations that during 2013 serotype O (O/ME-SA/Ind-2001 lineage) was isolated from clinical samples collected from the country.

Faccini° S, Barbieri° I, Gilioli A, Sala° G, Gibelli° LR, Moreno° A, Sacchi° C, Rosignoli° C, Franzini° G, Nigrelli° A

**Detection and genetic characterization of Porcine circovirus type 3 in Italy**

Transbound Emerg Dis. - Vol. 64 ( 2017). - p 1661-1664. - 14 bib ref [Nr. Estr. 7520]

Porcine circovirus type 3 (PCV3) was recently proposed as a new porcine circovirus. It has been described by researchers in the USA and China and associated with porcine dermatitis and nephropathy syndrome, reproductive failure and systemic inflammation disease. The study reports the occurrence of the new virus in Italy. PCV3 was detected in the tissues of foetuses and stillborn piglets coming from two farms located in the Po Valley. The genome sequences of the two Italian strains share 99.7% to 97.8% of nucleotide identity with those available in GenBank. Results strengthen the hypothesis of PCV3 as a new emerging porcine circovirus, widespread all over the world. It follows the urgency of investigating in depth epidemiology and pathogenicity associated with this new virus.

Faccini° S, De\_Mattia A, Chiapponi° C, Barbieri° I, Boniotti° MB, Rosignoli° C, Franzini° G, Moreno° A, Foni° E, Nigrelli° AD

**Development and evaluation of a new Real-Time RT-PCR assay for detection of proposed influenza D virus**

J Virol Methods. - Vol. 243 ( 2017). - p 31-34. - 17 bib ref [Nr. Estr. 7528]

The occurrence of virus belonging to the putative genus Influenzavirus D, has been demonstrated all around the world arousing interest within the scientific community. Most of the published virological surveys are based on the first described Real-Time PCR method, designed on the PB1 gene of the first isolate. The necessity of extending investigation to different animal species and geographic areas, requires a continuous update of molecular tests, considering newly sequenced strains. Moreover, the availability of an alternative assay, is essential either to confirm data, or for ensuring the detection of the widest number of strains. A new Real-Time PCR, specific for influenza D virus (IDV), was developed and evaluated. The target sequences of primers and probe are highly conserved among IDV strains currently known. The specificity of the method was demonstrated in silico by BLAST, and in vitro with a huge panel of common swine and bovine respiratory pathogens. The analytical sensitivity of the Real-Time PCR was estimated through synthetic RNA molecules and the limit of detection was about 20 copies/L. The assay was assessed in field and proved to be a valuable tool for the detection of IDV strains.

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

## **Patterns of detection of main respiratory viruses associated with bovine respiratory disease complex in Italy**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 162 (Poster 029). - 10 bib ref [Nr. Estr. 7591]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

Ferlazzo° G, Meriardi° G, Peli A

### **Abscess disease in small ruminant farms of Emilia-Romagna Region : a preliminary study**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 294. - 5 bib ref [Nr. Estr. 7615]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

Abscesses are widespread in sheep and goat farms, which underlies severe economic losses such as decrease in milk production and weight gain, lameness, drugs and labour for therapies. *Staphylococcus aureus* subsp. *anaerobius* (SAAN) and *Corynebacterium pseudotuberculosis* (CPS), are causative agents of Morel's disease (MD) and Caseous lymphadenitis (CLA), respectively [1], with abscesses occurring in perilymphnodal area (MD) or within lymph nodes (CLA) [2]. Beyond animal suffering and decreased growth and production, it has to be highlighted that both SAAN and CPS are possible zoonotic agents [4][5]. There is confusion in scientific literature as to abscess disease aetiology. Awareness of MD in Italy is limited, and it is likely to be mistaken for the well-known CLA, which shows a very similar clinical picture [3]. The objectives of this study were to evaluate clinical appearance and spread of these diseases in farms of Emilia-Romagna Region and to characterize the aetiology of the clinical cases observed on farm. We visited 8 dairy goat and 2 sheep farms: 14 goats and 2 sheep aging about 7 years with abscess lesions were examined and purulent tissue samples were collected. Each sample was concurrently subjected to a bacteriological examination by the following procedures: (i) direct smear on Blood Agar plates, to be incubated at 37°C in aerobic or anaerobic atmosphere, and (ii) enrichment in liquid Brain Heart Infusion (BHI) medium, followed by biochemical characterization using Biomerieux API Staph and API Coryne test kits. Clinical examination revealed that abscesses, containing yellowish creamy pus, were mostly located in prescapular (39%) and precrural (35%) regions, as well as in supramammary region (6%); their size ranged between 1.5 to 10 cm. Microbiological results revealed, in addition to SAAN (2 samples) and CPS (10 samples), unexpected heterogeneity of bacteria including species commonly isolated in humans like *Trueperella pyogenes*, *Staphylococcus aureus*, *Kocuria kristinae*, *Gemella morbillorum*, *Streptococcus thoraltensis*, *Staphylococcus caprae*, *Prevotella*, *Peptoniphilus asaccharolyticus*. A mixed infection was found in five samples. This must be taken into account towards autogenous vaccine preparation which should be supported by proper bacterial identification procedures. A strong biosafety plan is needed to prevent SAAN and CPS introduction along with infected animals, as well as abscess outbreak in livestock, and to limit environmental contamination due to spontaneous abscess breakage and bacterial spread through direct contact and vector insects.

Filipe J, Curone G, Trevisi E, Amadori° M, Turin L, Moroni P, Vigo D, Addis MF, Riva F

### **Inflammatory response in Holstein Friesian versus a local cattle breed (Rendena) at different time points**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno

S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I COncvegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 39. - 2 bib ref [Nr. Estr. 7608]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

The selective pressure for increased milk production in dairy cows brought about great difficulties in the adaptation of cows to their environment. This normally translates into increased culling rates, reduction of life expectancy and fertility, higher propensity to develop diseases (including mastitis), especially when compared to less selected and lesser producing dairy breeds which are typically characterized by higher resilience. However, not much is known about the biological mechanisms behind the relationship between genetic selection and higher risk of metabolic and infectious diseases (1). It is well known that during the calving period, high-yielding dairy cattle are more susceptible to common environmental stressors. This may have crucial repercussions on disease occurrence and on milk production levels (2). With the aim of investigating the factors associated to this phenomenon, in this study we compared innate immune response patterns of 6 Holstein Friesian (HF) and 4 Rendena cows reared in the same farm and under the same management conditions. Quarter milk samples and blood were collected from all cows at dry-off (Ti), 1 day after calving (T2), 7-10 days after calving (T3) and 30 days after calving (T4). Quarter milk samples were subjected to measurement of the inflammation marker cathelicidin and assessment of different innate immune-related mediators such as lysozyme, CD45, IL-113, TNF- $\alpha$ , PTX3, IL-1R8. Blood samples were used for the analysis of plasma metabolites indicators of systemic inflammation such as haptoglobin, ceruloplasmin, total protein, albumin, total bilirubin, and globulin. HF cows showed a more severe systemic inflammatory response at T2 and T3 in comparison with Rendena cows in terms of haptoglobin, total proteins, globulins and bilirubin. Concerning the milk protein abundance profile, pronounced differences were observed in the colostrum (T2), with significantly higher amounts of protective molecules (immunoglobulins and other immune-related proteins) in Rendena. Moreover, at all time points HF showed higher levels of the inflammation marker cathelicidin in milk. In addition, the expression of innate immune related genes, as well as the CD45/KRT5 expression ratio in milk cells (indicators of leukocyte and epithelial components) were different in HF compared with Rendena. Our results suggest that HF cows develop a systemic and local mammary inflammatory response that could impair the capability of the animal to face the peripartum period and make them more susceptible to disease compared with Rendena cows. Our findings reveal the importance of the autochthonous breeds in the understanding of the immunity mechanisms and indicate that fundamental effector activities of innate immunity in the mammary gland should be included in the breeding programs of HF cows. This kind of integrated approach can be conducive to a substantial reduction of antibiotic usage in dairy farms as a result of greater disease resistance.

Fishbourne E, Ludi AB, Wilsden G, Hamblin P, Statham B, Bin\_Tarif A, Brocchi<sup>o</sup> E, Grazioli<sup>o</sup> S, Dekker E, Eblé P, King DP

**Efficacy of a high potency O1 Manisa foot-and-mouth disease vaccine in cattle against heterologous challenge with a field virus from the O/ME-SA/Ind-2001 lineage collected in North Africa**

Vaccine. - Vol. 35 no 20 ( 2017). - p 2761-2765. - 19 bib ref [Nr. Estr. 7582]

Outbreaks of foot-and-mouth disease (FMD) in North Africa (2013) and the Gulf States (2013) of the Middle East have been caused by a FMD viral lineage (O/ME-SA/Ind-2001) that was before 2013 restricted to the Indian Sub-continent. This study was undertaken to assess the in vivo efficacy of a FMD virus emergency vaccine type O1 Manisa against heterologous challenge with a representative field virus (O/ALG/3/2014) from this emerging lineage. This widely available vaccine was selected since in vitro vaccine-matching results gave inconclusive results as to whether or not it would be protective. Three groups of five cattle were vaccinated with O1 Manisa (homologous potency 6PD50/dose) using study guidelines outlined in the European Pharmacopeia, and challenged at 21 days post-vaccination by tongue inoculation. All animals that were vaccinated with the lowest dose

(1/16) of vaccine developed generalised FMD, defined as vesicular lesions at the feet. One animal vaccinated with a 1/4 dose of the vaccine also developed generalised disease, as did two animals vaccinated with the full dose of vaccine. These results indicate that the heterologous potency of this high potency O1 Manisa vaccine was approximately 3.5 PD50/dose. These data support the use of the O1 Manisa vaccine for FMD control in areas where FMDV is endemic e.g. North Africa, and motivate further studies to evaluate other vaccine candidates (or multivalent combinations) that might be potentially used for emergency purposes in FMD-free settings.

Foglia<sup>o</sup> EA, Pezzoni<sup>o</sup> G, Grazioli<sup>o</sup> S, Bregoli<sup>o</sup> A, Brocchi<sup>o</sup> E

### **Prevalence and characterization of Encephalomyocarditis virus (EMCV) in Italy**

11th Epizone Annual Meeting "Crossing barriers" : 19-21 September 2017, Paris, France : programme / [s.l. : s.n., 2017]. - p 57 (C23). - 4 bib ref [Nr. Estr. 7659]

Annual meeting Epizone (11. : Paris, France : 19-21 September 2017)

Encephalomyocarditis (EMC) is a viral disease caused by a virus belonging to Picomaviridae family, which may induce myocarditis, encephalitis, and reproductive disorders, depending on host species and host aging. It has been demonstrated to be able to affect various mammals, despite swine is the most receptive between farm species. In Italy, the virus was first described in 1986 and, after sporadic detection during the following 10 years, since 1997 the disease occurred endemically in pig farms in northern Italy, causing fatal myocarditis. EMC outbreaks seem to be related to the local rodent population, which works as virus reservoir, causing a typical seasonal trend of the infections in farms and zoos during autumn/winter season. This work aims to give an update on molecular/antigenic evolution of EMCV Italian strains and on prevalence of infection in pig farms. The antigenic profile of 83 EMCV strains isolated between 2013 and 2016 was characterized by an in-house ELISA using a panel of 40 monoclonal antibodies (MAb) identifying seven different antigenic sites, some of them composed by multiple epitopes [1,2]. Only sporadic mutations occurred in two of the three sites involved in neutralization, except for the mutation in the epitope target of one neutralizing MAb, which exhibits low or null reactivity with most samples (77/83), as already observed in isolates collected previously. Occasional mutations were also identified in non-neutralizing sites, but none resulted permanent, substantiating the antigenic stability of the virus. The phylogenetic analysis based on the VP1 coding gene classified all the isolates within a unique lineage (lineage B), comprising also isolates from Belgium and Cyprus [3,4]. The 76 pig strains isolated in Italian farms are organized in few distinguishable clades, with a nucleotide identity ranging from 88.9% to 100%. The analysis included three recent pig isolates from Spain and four strains isolated from primates in an Italian natural park, which resulted to cluster in two further specific clades. To investigate on the diffusion of EMCV infection, a serosurvey was carried out on approximately 20,000 swine sera sampled during 2016 in about 700 different farms, located in Northern Italy. Sera were analysed by an in-house MAb-based competitive ELISA and seroprevalence levels were compared to those recorded in 2010 in a similar sample. The proportion of positive farms increased from 54% in 2010 to 76% in 2016, with a parallel increase of within-herd seroprevalence. Considering the 528 positive farms, the seroprevalence resulted higher than 50% in the 13% of them, between 50% and 20% in another 33%, between 20% and 5% in the 43% of considered farms and lower than 5% in the remaining 11%. Similar levels of seropositivity were detected in farms with and without clinical evidence of encephalomyocarditis, substantiating the subclinical circulation of EMCV.

Foni<sup>o</sup> E, Chiapponi<sup>o</sup> C, Baioni<sup>o</sup> I, Zanni<sup>o</sup> I, Merenda<sup>o</sup> M, Rosignoli<sup>o</sup> C, Kyriakis CS, Luini<sup>o</sup> MV, Mandola ML, Bolzoni<sup>o</sup> L, Nigrelli<sup>o</sup> AD, Faccini<sup>o</sup> S

### **Influenza D in Italy : towards a better understanding of an emerging viral infection in swine**

Sci Rep. - Vol. 7 ( 2017). - Article no. 11660 (7 p). (ultimo accesso 03/11/2017  
<https://www.nature.com/articles/s41598-017-12012-3> ) [Nr. Estr. 7673]

Influenza D virus (IDV), a new member of the Orthomyxoviridae family, was first reported in 2011 in swine in Oklahoma, and consequently found in cattle across North America and Eurasia. To investigate the circulation of IDV among pigs in Italy, in the period between June 2015 and May 2016, biomolecular and virological tests were performed on 845 clinical samples collected from 448 pig farms affected by respiratory distress located in the Po Valley. Serological tests were conducted on 3698 swine sera, including archive sera collected in 2009, as well as samples collected in 2015 from the same region. Viral genome was detected in 21 (2.3%) samples from 9 herds (2%), while virus was successfully isolated from 3 samples. Genetic analysis highlighted that Italian swine IDVs are closely related to the D/swine/Oklahoma/1334/2011 cluster. Sera collected in 2015 showed a high prevalence of IDV antibody titers (11.7%), while archive sera from 2009 showed statistically significant lower positivity rates (0.6%). Our results indicate an increasing epidemiological relevance of the pathogen and the need for in-depth investigations towards understanding its pathogenesis, epidemiology and possible zoonotic potential of this emerging virus.

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

**Applicability of blood sampling through intracavernous venipuncture : the example of *Toxoplasma gondii* in naturally infected red deer (*Cervus elaphus*)**

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 79 [Nr. Estr. 7744]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

Health surveillance of wildlife faces several field and logistic issues. In particular, the difficulty of sampling is one of the most important limitations for monitoring programs in free-ranging animals, especially in case of blood, a very delicate matrix. As blood is frequently collected in the field directly from the major blood vessels by the hunters during the bleeding of carcasses, this sampling method may affect quality or quantity of sera samples. Indeed, sampling protocol has to be adapted to cope with the harsh field conditions to avoid sera alterations such as coagulation, contamination and haemolysis induced, for example, by the delay (several hours) between the death of the animal and the collection of the sample. As these alterations lead to a low sample size/lack of samples and/or to low volume/low quality/suitability of samples, the need is to define alternative sampling methods to solve these diagnostic concerns, mainly in case of zoonosis surveillance. Here, we chose *Toxoplasma gondii* in red deer (*Cervus elaphus*) as a representative host-infection model since in wildlife the protozoan detection is carried through serological tests. Therefore, we investigated the reliability of sera obtained from blood by intracavernous venipuncture (IV), a sampling method developed by Arenas-Montes et al. (2013, Eur. J. Wildl. Res. 59: 299-303), as an alternative to sera from blood of major vessels (MV) in *T. gondii* ELISA test. Overall 142 sera were collected in Stelvio National park (Italian Central Alps) during two culling management plans (2015 and 2016) and obtained by both IV and MV techniques. Samples were tested by a commercial ELISA kit (IDVET, Montpellier, France) and the concordance between results was evaluated through the calculation of the Kappa (K) value. Prevalence of 31% and 26% emerged from MV and IV, respectively, and showed an "Excellent agreement" (K value=0.8450 (95% CI: 0.7478-0.9422)) between the two sampling techniques. The concordance between results emerged from the two techniques supports the use of IV as a useful and valuable alternative for sera field sampling. Moreover, IV technique permits to sample high quality sera from individuals till 8 hours after the shooting and the bleeding of carcasses leading to an increase of the sample size with both a reduction of the time required for sampling and the potential risk of exposure to pathogens. This innovative technique can be thus a useful tool that could be further widely used in wild ungulates health surveillance, especially in case of programs for zoonosis control. Although in this study a valuable concordance between *T. gondii* antibody detection emerged between the two sampling techniques, further analysis should be carried out to investigate the comparison between sera from IV and MV in electrophoresis to evaluate the suitability of this new matrix even for hematologic and biochemical analyses.

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

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

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 19 [Nr. Estr. 7743]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

The complex life cycle of *Toxoplasma gondii* and its broad spectrum of host species rise zoonotic, economic, and conservation issues. Moreover, recent studies supposed the parasite's activity to manipulate intermediate host behaviour leading to neurological symptoms/alterations ("host behaviour manipulation" or "manipulation hypothesis") that promote a longer parasite survival, necessary to complete its life cycle and thus to increase its spread. While in humans, loss of psychomotor performance and concentration, schizophrenia and other mental disorders were related to Toxoplasmosis, in animals several "risky behaviours" that could increase *T. gondii* transmission are reported. Indeed, this parasite is trophically transmitted and behavioural host manipulation may favour its transmission from intermediate to other/s or to definitive hosts. Among *T. gondii* intermediate hosts, wild ungulates can be source of human infection and thus the risk should be evaluated in relation to game meat consumption. Moreover, until now, the infection dynamics and effects of *T. gondii* in these species has received little attention. Focusing on red deer (*Cervus elaphus*) since its intense culling and the increasing consumption of meat, the need is to investigate *T. gondii* in this host species and to evaluate its epidemiology in wild ecosystem. Moreover, no assessments are reported about *T. gondii* associated-behavioural modifications in naturally infected red deer. Therefore, we carried out a serological study composed by two parts. In the first one we investigated (1) the epidemiology, spread and dynamics, of *T. gondii* infection while in the second part we analysed (2) if the parasite may induce behavioural modifications leading to an increase in the culling probability. Overall 376 sera of free-ranging red deer were collected during three culling management plans (2014-2016) from three areas in Stelvio National park (Italian Central Alps) for epidemiological analysis while the behavioural analysis was performed on 53 adult females hunted in 2016. Samples were tested by a commercial ELISA kit (IDVET, Montpellier, France) and results were analysed through Generalized Linear Models. In particular, we used a Binomial and a Gaussian GLMs to investigate factors influencing the probability of being seropositive and the dynamics of the infection, respectively. Moreover, the behavioural hypothesis was tested by a Gaussian GLM that modelled the "days from the beginning of hunting" to evaluate the potential increase in the culling probability induced by *T. gondii*. An overall prevalence (p) of 26% emerged. The seroprevalence increased with age: adults (p=41.5%) were more infected than yearlings (p=19%) and calves (p=5%). Subjects of low anthropised area (p=13%) were less infected than those of the high (p=36.3%) and moderate (p=22.8%) anthropised ones. Subjects of 2014 (p=16%) were less infected than those of 2015 (p=27.8%) and 2016 (p=35.7%). Focusing on seropositive deer (n=98), red deer of 2014 showed serological titres significantly lower than those of both 2015 and 2016. As regard *T. gondii* activity to induce risky behaviour, seropositive adult females (36/53) had a significant higher culling probability than the seronegative ones. The effect of age class and anthropization on the spread of *T. gondii* supports horizontal transmission as the main route. Considering the sporadic presence of lynx (*Lynx lynx*) in Italian Alps, feral and semi-domestic cats are the only definitive hosts responsible for its spread. The difference in prevalence and serological titres between study years leads to a boost event of *T. gondii* spread during 2015 and then a maintenance of the infection during 2016, as the stable prevalence and serological titres between 2015 and 2016 showed. In adult females, the infection seems to favour the increase of the probability to be culled sooner than seronegative individuals. *T. gondii* appears therefore to induce behavioural alterations even in red deer making them more "at risk" to be hunted. The supposed emerged mechanism leads to a *T. gondii* manipulation in this host species that could indeed increase its spread and transmission. Data arisen give evidence to the presence of *T. gondii* in the study area with horizontal transmission as the main route of infection. In this sense, a widespread exposure to infection is supposed and should be considered in relation to the parasite zoonotic potential and, besides, the supposed cyclic introduction-re introduction of the parasite in the study area. Although the emerged relation between the infection and the increase in the culling probability

of adult females could support *T. gondii* host behaviour manipulation, further analyses should be carried out to extend this analysis to other classes and to preyed/found dead/roadkill animals for a wide assessment of the potential alterations induced by *T. gondii*.

Franzo G, Massi° P, Tucciarone CM, Barbieri° I, Tosi° G, Fiorentini° L, Ciccozzi M, Lavazza° A, Cecchinato M, Moreno° A

**Think globally, act locally : phylodynamic reconstruction of infectious bronchitis virus (IBV) QX genotype (GI-19 lineage) reveals different population dynamics and spreading patterns when evaluated on different epidemiological scales**

PLoS One. - Vol. 12 no 9 ( 2017). - p e0184401 (20 p). - 64 bib ref ( ultimo accesso 03/11/2017 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0184401> ) [Nr. Estr. 7668]

Infectious bronchitis virus (IBV) represents one of the poultry industry major threats, particularly in high density producing countries. The emergence and spread of new IBV genotypes have frustrated the various disease control efforts implemented over time. Despite that, few comprehensive and large scale studies have been performed to understand the international and local spreading dynamics of this virus. In the present work, these phenomena were evaluated by implementing a Bayesian phylodynamic approach to reconstruct the epidemiological patterns and population history of the QX genotype (currently renamed GI-19 lineage), the most relevant IBV lineage of the Old-World. Our analysis, based on 807 partial S1 sequences of strains collected from 18 countries between 1993 and 2015, demonstrates that this genotype originated in China well before its first identification. After a prolonged local circulation, it started spreading to other European, Asian and Middle East countries in successive waves, which were mirrored by concomitant fluctuations in viral population size. Interestingly, the within-Europe spread was characterized by a higher estimated migration rate compared with the inter-continental one, potentially reflecting the closer geographic and economic relationships among these countries. Nevertheless, the colonization of new states by the GI-19 lineage appeared to occur mostly by single introduction events in both intra and inter-continental spread, likely because of epidemiological factor and health policy combination which seems to prevent the frequent introduction and mixing of different strains. On the other hand, the within Italy QX circulation reconstruction showed a much more intricate connection network among different locations, evidencing the difficulty in controlling IBV spread especially in highly densely poultry populated areas. The presence of several well supported epidemiological links among distantly related Italian regions testifies that animal transportation and indirect transmission routes rather than local airborne diffusion contribute to the QX success and persistence at local scale. Globally, the spreading dynamics and evolution of the QX genotype were reconstructed from its very origin to nowadays, demonstrating the need of more effective direct control measures, particularly within each country. Unfortunately, the incompleteness of available molecular epidemiology data represents an insurmountable limit which leaves many questions currently unsolved, thus highlighting the compulsoriness of a structured monitoring and data sharing system implementation.

Fregnani° G, Massi° P, Fiorentini° L, Tosi° G, Romboli° C, Bocciero° R, Scaravelli° D, Parigi° M

**Prevalenza dell'infezione da emoparassiti in specie di avifauna selvatica in Emilia Romagna**

Atti della Societa' Italiana di Patologia Aviaria (SIPA) 2017 : LVI Convegno annuale, Il Simposio scientifico : Forlì, 6-7 Aprile 2017 - Parma, 22 Settembre 2017 / [s.l. : s.n., 2017]. - p 87-91. - 12 bib ref [Nr. Estr. 7732]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (56. : Forlì Parma : 6-7 Aprile 2017 22 Settembre 2017)

*Although the presence and the effects of Haemoparasites in feral birds are widely studied in various*

*part of the world, data on their diffusion in Italy are scarce. The aim of this study was to evaluate the prevalence of Haemoproteus/Plasmodium and Leucocytozoon in feral pigeons (Columba livia) and magpies (Pica pica) of the Emilia-Romagna region. A total of 215 spleen collected from culled or dead animals were screened using a nested polymerase chain reaction (nPCR) that amplifies a partial segment of the mitochondrial cytochrome b gene of these parasites. Magpies resulted significantly more parasitized by Leucocytozoon spp than pigeons (70.3% vs 5.1%), whereas both species presented a similar prevalence of Haemoproteus/ Plasmodium spp. (24.3% vs 23%). A small percentage of animals was infected by both parasites (7.5%), mainly represented by magpies. Among pigeons, the prevalence of the parasites was significantly different in the geographical areas investigated. Further genomic studies are necessary in order to evaluate the parasites species and the lineages circulating among this population of feral birds.*

Fusaro A, Monne I, Mulatti P, Zecchin B, Bonfanti L, Ormelli S, Milani A, Cecchetti K, Lemey P, Moreno<sup>o</sup> A, Massi<sup>o</sup> P, Dorotea T, Marangon S, Terregino C

**Genetic diversity of highly pathogenic avian influenza A(H5N8/H5N5) viruses in Italy, 2016-17**

Emerg Infect Dis. - Vol. 23 no 9 ( 2017). - p 1543-1547. - 7 bib ref [Nr. Estr. 7726]

In winter 2016-17, highly pathogenic avian influenza A(H5N8) and A(H5N5) viruses of clade 2.3.4.4 were identified in wild and domestic birds in Italy. We report the occurrence of multiple introductions and describe the identification in Europe of 2 novel genotypes, generated through multiple reassortment events.

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

**Assessing circumstances and causes of dairy cow death in Italian dairy farms through a veterinary practice survey (2013–2014)**

Prev Vet Med. - Vol. 137 ( 2017). - p 105-108. - 21 bib ref [Nr. Estr. 7512]

A questionnaire survey about on farm dairy cow mortality was carried out among veterinary practitioners in Italy between January 2013 and May 2014. The study aimed at investigating the main circumstances of death in dairy cows (euthanasia, emergency slaughter or unassisted death), the primary causes and the risk factors of death. Out of 251 dead cows involved (across 137 farms), 54.6% died assisted and 45.4% were found dead. The main causes of death were metabolic/digestive disorders (22.3%) and mastitis/udder problems (17.1%), while in 14.7% of all cases, reasons of death were unknown. From the univariable generalised linear mixed models, dry cows showed a significantly higher odds to die unassisted compared to lactating cows (OR = 3.2); dry cows also had higher odds of dying from unknown reasons (OR = 11.7). Season was not significantly related to the risk of dying unassisted and for unknown reasons, but during the summer (characterised by hot and muggy weather in Northern Italy) cows died mostly for problems at calving. 54.2% of cows died during the first 30 days in milk (DIM). Half of the multiparous cows that died, died in the first 29.5 DIM, while half of the primiparous cows that died, died in the first 50 DIM. Results pointed out that, especially in dry cows, around calving and during the summer, some failure in management practices and daily inspections may occur. Improvements should be done in monitoring activities and in recognising early symptoms of diseases among stockperson. In addition, in case of diagnosed diseases with poor prognosis, euthanasia procedures should be implemented to prevent cows from dying unassisted.

Gallina L, Savini F, Lavazza<sup>o</sup> A, Di\_Marco P, Puleio R, Roccaro M, Purpari G, Guercio A, Scagliarini A

**Bovine papillomatosis in Sicily : virus identification, diagnosis and co-infections**

Large Anim Rev. - Vol. 23 no 5 ( 2017). - p 171-174. - 15 bib ref [Nr. Estr. 7728]

**Introduction** - Bovine papillomatosis is an infectious disease distributed worldwide among cattle herds consisting of hyper-proliferative lesions affecting cutaneous tissue and mucosa. The disease, known as papillomas, may occur in different part of the body. Bovine papillomaviruses (BPVs) are non-enveloped double stranded DNA viruses belonging to the Papillomaviridae family, a group of viruses that infect all amniotes., Despite the benign characters of the lesions, in some cases bovine papillomatosis may dramatically reduce the productive performances when the lesions are extensive over the body involving teats and udders. **Aim** - This work was particularly aimed at the identification and characterization of BPVs circulating in Sicily in order to obtain autovaccine against BPVs. **Material and methods** - Thirty-one epithelial lesions, collected from cattle, clinically diagnosed as papillomas, were referred to the Laboratories of Istituto Zooprofilattico Sperimentale of Sicily. Samples were analyzed by electron microscopy, PCR amplification, Rolling Circle Amplification, sequencing, negative staining electronic microscopy and standard histological examinations. **Results and discussion** - The investigation revealed the presence of different BPV types 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, mostly in the form of co-infections. Besides BPV amplification, zoonotic viruses belonging to the genus parapoxvirus within the Poxviridae family were screened and detected in 11 samples. This is the first study performed in Sicily, reporting a wide diversity of BPV genera and types circulating in the territory. **Conclusion** - The data collected showed the necessity of proper diagnosis to produce an effective vaccine while virus characterization is important to know the epidemiological scenario of the region and set appropriate control measures and reliable diagnostic methods for bovine papillomatosis. The presence of co-infecting zoonotic viral agents underlies the need for a better understanding of the possible interactions between the two viruses and suggests the importance of identifying proper measures to prevent the infection to humans.

Garbarino° C, Interisano M, Chiatante A, Marucci G, Merli E, Arrigoni° N, Cammi° G, Ricchi° M, Tonanzi D, Tamba° M, La\_Rosa G, Pozio E

**Trichinella spiralis a new alien parasite in Italy and the increased risk of infection for domestic and wild swine**

Vet Parasitol. - Vol. 246 ( 2017). - p 1-4. - 30 bib ref [Nr. Estr. 7674]

In Europe, *Trichinella spiralis*, the most dangerous species for humans of the genus *Trichinella*, has a patchy distribution with important foci in Eastern countries and Spain. This zoonotic pathogen was apparently not circulating among wild and domestic animals of Italy. In 2016, muscle larvae belonging to this nematode species were detected in a red fox (*Vulpes vulpes*) shot in the Piacenza province (Northern Italy). This parasite may have been introduced into northern Italy from eastern Europe by hunters, by a hunting dog, or by immigrants, who illegally carried infected meat in their personal baggage. In the same year, *T. spiralis* infected sausages illegally introduced by personal baggage into Italy from Romania, were inadequately disposed of in the garbage of a central Italian town. Even though these two episodes may not be connected in time and space, they represent an increased risk of infection for domestic and wild swine, which are highly susceptible to this pathogen. In these animals, *T. spiralis* shows a higher larval burden and a longer survival time than other *Trichinella* species. Since most of the Italian pig production plants are in northern Italy, the circulation of *T. spiralis* should be strictly monitored in wildlife living in these areas.

Gasparrini° S, Alborali° GL, Pitozzi° A, Guarneri° F, Giacomini° E, Baldo° V, Scali° F, Lazzaro° M, Boniotti° MB

**Characterization of *Brachyspira hyodysenteriae* isolates from Italy by multilocus sequence typing and multiple locus variable number tandem repeat analysis**

J Appl Microbiol. - Vol. 123 ( 2017). - p 340-351. - 37 bib ref [Nr. Estr. 7638]

**Aims:** To evaluate and compare the capabilities of multilocus sequence typing (MLST) and multiple locus variable number tandem repeat analysis (MLVA) techniques to characterize *Brachyspira hyodysenteriae* isolates and to investigate the relationship between pleuromutilin resistance and genetic variability. **Methods and Results:** MLST genotyping was performed on 180 *B. hyodysenteriae* isolates, and the results were evaluated considering profiles from 108 other strains previously reported in the database. In total, 37 sequence types were obtained. The MLVA approach completely characterized 172 strains and grouped the isolates into 22 different profiles. The combination of MLST and MLVA showed a slight increase in the discriminatory power, identifying 33 joint profiles. An antibiotic resistance analysis showed a reduction in the susceptibility to pleuromutilins over time, and a weak association between susceptibility to valnemulin and inclusion in clonal complex 4. **Conclusion:** MLST and MLVA are reliable methods for characterizing *B. hyodysenteriae* strains and they have comparable discriminatory power. **Significance and Impact of the Study:** The genotyping of *B. hyodysenteriae* isolates and a database of all the genetic profiles collected during the diagnostic activities could support traditional epidemiological investigations in identifying infection sources and routes of transmission among herds, and in developing more effective control measures.

Giacomelli<sup>o</sup> S, Bianchi<sup>o</sup> A, Archetti<sup>o</sup> I, Rota\_Nodari<sup>o</sup> S, Bertoletti<sup>o</sup> I

### **Prevalence, distribution and physiological effects of *Sarcocystis* on wild red deer in Italian central Alps**

Epidemics6 : Sixth International Conference on Infectious Disease Dynamics : 29 November - 1 December 2017, Sitges (near Barcelona), Spain / [s.l. : s.n., 2017]. - 1 p (Poster P1.122) [Nr. Estr. 7769]

International Conference on Infectious Disease Dynamics. (6th : Sitges (near Barcelona), Spain : 29 November — 1 December 2017)

**Introduction:** Protozoan parasites of the genus *Sarcocystis* (Apicomplexa, Sarcocystidae) have a two-host life cycle with mainly herbivores and omnivores as intermediate hosts and carnivores as definitive hosts. Despite considered mainly apathogenic for intermediate herbivores hosts some studies reports acute and chronic consequences. This study examined the prevalence and the effects of sarcocystosis on reproductive and haematological data from an wild red deer (*Cervus elaphus*) Alpine population. **Methods:** The study was carried out on 202 red deer culled for a population management control plan in the period 31 October 2014- 28 January 2015 in a selected area (25.100 ha) of the Stelvio National Park. All animals were inspected and measured for reproductive data. From the total samples, 172 muscles (oesophagus, diaphragm, heart) were examined through hematoxylin eosin histology for *Sarcocystis* detection and scoring; 61 blood samples were processed for biochemical analysis. **Results:** Prevalence varied from 58.7% (oesophagus) to 67.4% (diaphragm). Calves showed a significant lower prevalence ( $p < 0.001$ ). the exclusion of calves results on 100% prevalence. Histological score significantly increase with the age ( $p < 0.001$ ). An indirect correlation were found between infestation score and corpus luteum diameter ( $p = 0.018$ ), evidence maintained fractionating samples based on the period (16 — 30, November;  $p = 0.003$ ). Haematological data showed significant direct correlation between infestation score and alpha 2 globulin ( $p = 0.001$ ). On a population level, a geographical distribution of positive calves were noticeable. In fact, an increased prevalence is present on the eastern zone ( $p = 0.001$ ). This zone is intensively used for summer pastures by both deer and livestock. **Discussion:** deer are infested continuously and progressively during lifespan. The haematological data showed little immune reaction to infestation (alpha 2 globulin) but the influence on dimensions of corpus luteum suggests a possible delayed conception. Further analysis on distribution of definitive hosts are needed to explain spatial distribution of positive calves.

Giacomelli<sup>o</sup> S, Bianchi<sup>o</sup> A, Salvetti M, Bertoletti<sup>o</sup> I

### **Identification and distribution of Hippoboscidae ectoparasites on wild ungulates in Sondrio**

## Province

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 37 [Nr. Estr. 7742]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

We examined specimens of Hippoboscidae collected on wild ungulates given to the "Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, Sondrio Section". The study of the specimens allowed to detect the presence of three species: *Lipoptena cervi* (Linnaeus, 1758), *Melophagus rupicaprinus* Rondani, 1879, *Melophagus ovinus* (Linnaeus, 1758). A total of 388 *L. cervi*, 65 *M. rupicaprinus* and 10 *M. ovinus* were collected and classified. The largest number of specimens belong to the species *L. cervi*, which was found on red deer (n=10) and roe deer (n=50) but also in chamois (n=7). This shows the great adaptability of *L. cervi* in terms of host species, and its ability to spread and colonize rapidly different areas, exploiting the populations of wild ungulates present in the province of Sondrio. We, then, examined the different distribution of *L. cervi* on his type hosts (red deer) and the most represented host on this study (roe deer). Statistical analysis showed that roe deer have a lower intensity of infestation (ANOVA, n=60, F=4.088, p=0.048) but no effect of age, pathological findings and cause of death on intensity of infestation. *M. ovinus* was detected in one chamois and *M. rupicaprinus* from six chamois and two alpine ibex. We didn't find any other Hippoboscidae of the Genus *Lipoptena* previously described in the same host species collected in our study. Our observations confirm the low specificity of these parasites described by some authors. Low specificity demonstrated from both genus *Lipoptena* (red deer vs roe deer and chamois) and *Melophagus* (chamois vs alpine ibex). As a matter of fact, we found a complete absence of the roe deer type parasites *L. capreoli* (Rondani, 1878). These last result could demonstrate a possible replacement in roe deer from his type parasite. The lower intensity could be due to the residual specificity of the parasite or simpler correlated to the dimension of the host. The knowledge about the distribution of these species are very important because they could reflect relationship and population dynamics of their hosts and because they are potential vectors of transmission of zoonotic bacteria of the genus *Bartonella*.

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

### **Cellule somatiche e carica batterica totale nel latte di massa: potenziali indicatori di benessere in allevamento?**

Le sinergie tra grande distribuzione organizzata, industria, piccole produzioni locale e controllo ufficiale : tutela del consumatore, difficoltà e prospettive : XXVII Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) : Perugia, 13-14-15 Settembre 2017 / [s.l. : s.n., 2017]. - p 30 (Poster P014-49). - 3 bib ref [Nr. Estr. 7642]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) (27. : Perugia : 13-14-15 Settembre 2017)

Il mercato degli alimenti animal welfare friendly è in sensibile crescita e la grande distribuzione organizzata, per soddisfare le esigenze dei consumatori, favorisce i fornitori e gli allevatori attenti al benessere degli animali. Le analisi del latte di massa eseguite di routine, per il pagamento in base alla qualità e per il controllo dei requisiti igienico-sanitari, potrebbero essere un indicatore indiretto e di facile reperibilità per individuare gli allevamenti con problemi di benessere animale. Lo scopo del lavoro è stato di confrontare il punteggio di benessere animale a livello di allevamento ottenuto applicando il protocollo di valutazione del Centro di Referenza Nazionale per il Benessere Animale, con le relative analisi del latte di massa. Sono stati selezionati gli allevamenti di bovine da latte, situati nel nord Italia, valutati dal 2014 al 2016 (punteggio min=41%; punteggio max=90%). Per 278 aziende è stato possibile calcolare la media geometrica della conta delle cellule somatiche (SCC) delle ultime tre rilevazioni nei tre mesi antecedenti la valutazione del benessere (min=75.220 cell/mL; max=591.959 cell/mL). Per 247 aziende è stato possibile calcolare la media geometrica della carica batterica totale (CBT) relativa alle ultime quattro rilevazioni nei due mesi antecedenti la

valutazione del benessere (min=2.711 UFC/mL; max=217.012 UFC/mL). I dati relativi alle SCC e alla CBT sono stati confrontati con il punteggio di benessere utilizzando il coefficiente di correlazione per ranghi Tau di Kendall. Dall'analisi risulta una debole correlazione negativa tra la media geometrica delle SCC e il punteggio di benessere animale (Tau=-0.104; P<0.01) e tra la media geometrica della CBT e il punteggio di benessere animale (Tau=-0.17, P<0.05). Si può affermare, quindi, che a valori crescenti di SCC e CBT corrispondono valori decrescenti di benessere animale, confermando alcuni dati già presenti in letteratura.

Gjerde B, Giacomelli<sup>o</sup> S, Bianchi<sup>o</sup> A, Bertoletti<sup>o</sup> I, Mondani<sup>o</sup> H, Gibelli<sup>o</sup> LR

**Morphological and molecular characterization of four *Sarcocystis* spp., including *Sarcocystis linearis* n. sp., from roe deer (*Capreolus capreolus*) in Italy**

Parasitol Res. - Vol. 116 ( 2017). - p 1317-1338. - 57 bib ref [Nr. Estr. 7535]

Fresh (frozen/thawed) muscle samples from four 2–12-year-old roe deer (*Capreolus capreolus*) from the Sondrio province in north-eastern Italy were examined under a dissecting microscope, and about 180 sarcocysts were isolated and identified to morphological type in wet mounts by light microscopy (LM). Seventy-seven of these sarcocysts were subsequently examined by molecular methods, comprising polymerase chain reaction (PCR) amplification and sequencing of the partial cytochrome c oxidase subunit I gene (*cox1*) of all isolates, as well as PCR amplification, cloning and sequencing of the complete 18S ribosomal RNA (rRNA) gene of two isolates of each species found. By LM, three major sarcocyst types were recognised: spindle-shaped sarcocysts, 0.5–3 mm long, either with no clearly recognisable protrusions (thin-walled) or with finger-like protrusions (thick-walled); and slender, thread-like sarcocysts, 2–3 mm long, with hair-like protrusions. Sequencing of *cox1* revealed that the sarcocysts belonged to four different species. Those with no visible protrusions either belonged to *Sarcocystis gracilis* (n = 24) or to a *Sarcocystis taeniata*-like species (n = 19), whereas those with finger- and hair-like protrusions belonged to *Sarcocystis silva* (n = 27) and *Sarcocystis capreolicanis* (n = 7), respectively. The 19 *cox1* sequences of the *S. taeniata*-like species, comprising five haplotypes, differed from each other at 0–16 of 1038 nucleotide positions (98.5–100% identity). They differed from 25 previous *cox1* sequences of *S. taeniata* from moose and sika deer (with 98.0–100% intraspecific identity), at 33–43 nucleotide positions (95.9–96.8% interspecific identity), and there were 20 fixed nucleotide differences between the two populations. In the phylogenetic analysis based on *cox1* sequences, the two populations formed two separate monophyletic clusters. The *S. taeniata*-like species in roe deer was therefore considered to represent a separate species, which was named *Sarcocystis linearis* n. sp. At the 18S rRNA gene, however, the two species could not be clearly separated from each other. Thus, there was considerable intraspecific sequence variation in the 18S rRNA gene of *S. linearis* (98.1–99.9% identity between 24 sequences), which was similar both in magnitude and nature to the variation previously found in this gene of *S. taeniata*. The new 18S rRNA gene sequences of *S. linearis* shared an identity of 97.9–99.6% with those of *S. taeniata* (overlap between intra- and interspecific identity), and in the phylogenetic tree, sequences of the two species were interspersed. By scanning electron microscopy (SEM), the sarcocysts of *S. linearis* were found to possess regularly spaced, thin and narrow ribbon-like cyst wall protrusions (about 2.8–3.2 µm long, 0.3–0.4 µm wide and about 0.02–0.03 µm thick), terminating in a plate-like structure of the same thickness but with an elliptic outline (about 0.3–0.4 µm wide and 0.7–0.9 µm long). The terminal plates were connected in the middle with the band-like portion of the protrusions like the board of a seesaw (tilting board). The terminal plates of adjacent protrusions were neatly arranged in a hexagonal pattern resembling tiles on a roof. Together, they formed an outer roof-like layer facing the surrounding cytoplasm of the host cell and completely covering the band-like proximal portion of the protrusions, which overlapped and were stacked in three to four layers close to the cyst surface. The sarcocyst morphology of *S. linearis* was consistent with that of an unnamed *Sarcocystis* sp. in roe deer previously found by transmission electron microscopy in several countries, including Italy. A few sarcocysts of *S. gracilis* and *S. silva* were also examined by SEM, confirming the presence of regularly distributed, short knob-like protrusions in *S. gracilis* (as seen in previous SEM studies) and revealing tightly packed, erect 6–7-µm-long villus-like protrusions having regularly distributed round depressions on their surface in *S. silva*. The sequencing of *cox1* of 7, 24 and 27 new isolates of *S. capreolicanis*, *S. gracilis* and *S. silva*, respectively, recovered 7, 11 and 10 new haplotypes from each of the three

species and expanded our knowledge on the intraspecific sequence variation at this marker. Similarly, the study revealed a more extensive intragenomic sequence variation at the 18S rRNA gene of *S. capreolicanis* and *S. silva* than known from previous studies and confirmed a near absence of such variation in the 18S rRNA gene of *S. gracilis*.

Guarda F, Bollo E, Alborali° GL, Giovannini° S, Gradassi° M, Apicella M, Capucchio MT

**Cisti trichilemmali e ipotricosi nel suino sono simili a quelle dell'uomo**

I modelli animali spontanei per lo studio della fisiologia e patologia dell'uomo / Franco Guarda ... [et al.]. - Brescia : Fondazione Iniziative Zooprofilattiche e Zootecniche, 2017. - (Atti delle giornate di studio Fondazione Iniziative Zooprofilattiche e Zootecniche ; 102) p 648-651 [Nr. Estr. 7571]

Hall RN, Capucci° L, Matthaei M, Esposito° S, Kerr PJ, Frese M, Strive T

**An in vivo system for directed experimental evolution of rabbit haemorrhagic disease virus**

PLoS One. - Vol. 12 no 3 ( 2017). - p e0173727 (15 p). - 45 bib ref (ultimo accesso 31/03/2017 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0173727> ) [Nr. Estr. 7534]

The calicivirus Rabbit haemorrhagic disease virus (RHDV) is widely used in Australia as a biocontrol agent to manage wild European rabbit (*Oryctolagus cuniculus*) populations. However, widespread herd immunity limits the effectiveness of the currently used strain, CAPM V-351. To overcome this, we developed an experimental platform for the selection and characterisation of novel RHDV strains. As RHDV does not replicate in cell culture, variant viruses were selected by serially passaging a highly virulent RHDV field isolate in immunologically naïve laboratory rabbits that were passively immunised 18–24 hours post-challenge with a neutralising monoclonal antibody. After seven passages, two amino acid substitutions in the P2 domain of the capsid protein became fixed within the virus population. Furthermore, a synonymous substitution within the coding sequence of the viral polymerase appeared and was also maintained in all subsequent passages. These findings demonstrate proof-of-concept that RHDV evolution can be experimentally manipulated to select for virus variants with altered phenotypes, in this case partial immune escape.

Hocking PM, Harkness A, Veldkamp T, Vinco° LJ

**Do foot pad scores measure turkey welfare?**

Proceedings of the 11th Turkey Science and Production Conference : Chester, UK, March 9th - 10th, 2017 / [s.l. : s.n., 2017]. - p 20-23. - 8 bib ref [Nr. Estr. 7552]

Turkey Science and Production Conference (11th : Chester, UK : March 9th - 10th, 2017)

Iemmi T, Mangia C, Zanin R, Formenti° N, Lanfranchi P, Genchi M, Ferrari N, Kramer LH

**Spread of *Toxoplasma gondii* in the Eurasian magpie and Eurasian kestrel from the Emilian territory**

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 55 [Nr. Estr. 7741]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

The present study is about the investigation of the *Toxoplasma gondii* diffusion in two wild omithic

species, very common in the Italian Northern Regions, the Magpie (*Pica pica*) and the Eurasian (or common) Kestrel (*Falco tinnunculus*), in particular taking in exam healthy birds trapped in the context of a mitigation project of the birdstrike hazard in the pertinences of the "Giuseppe Verdi" airport of Parma . The mitigation hazard project foresaw the capture, the birds marking with numerated rings and the following release in a station located at 18 km from the airport; it was observed a certain homing phenomenon with 22 recapture case on 345 total captured and transferred kestrels, for that birds had been possible to have second blood sample. For both species, the diagnostic research was conducted through serology employing the micro agglutination technique (MAT) considering two dilution level 1:5 (cut-off) and 1:25. Muscular and myocardial sample of magpie (species subject to culling management plan) were molecularly analyzed in RT-PCR (target sequence 529 bp). For the magpie, the research in MAT and RT-PCR were not performed on the same birds group. In the Magpies was observed a prevalence of 43% (n° tested birds=21 ) with the RT-PCR and 47% (n°=16) of which the 29% with high title 1:25 performing the MAT; four Magpies RT PCR positive were genotyped with the PCR-RFLT highlighting the circulation of intermediate types II / III (I sample) and III (3 samples). For Kestrels the study was made for a part of the subjects captured, considering the age class, highlighting a prevalence of 87% (n°=31) for subjects over 1 years of age (17 adults and 14 sub-adult, at least first molting), of 31% (n°=55) for young subjects included in the age class between 45 to 75 days of life and of 45% (e=55) for young birds with an age between 120 to 190 days of life. An interesting data comes from the observation of the antibody titles, for the adults the 50% of positive was with low title 1:5 , in the sub-adults (at the first molt) the 28% and in the young 15 % , in addition it was observed two cases of seroconversion in two young kestrels recaptured after 30 days from the first capture and sampling, both in the second sampling with title 1: 5, based on the observations, we could hypothesis that low titles 1: 5 is indicating newly acquired infections or chronic forms . Data showed the presence of *T.gondii* in the Magpie and Kestrel populations of the study area and in consideration of remark made the two species could to contribute to the maintenance of the parasite in the environment, acting as a concentrator through the predatory activity on micro mammals (and through the consumption of carcass by the magpies) making the parasite available again to their death for saprophytic homeothermic animals (including rodents), moreover, in view of the above, we could take in consideration sedentary carnivorous birds, as Magpie and Kestrel, as sentinel species for the estimation of *T.gondii* oocysts environmental charge and the infection hazard for a specific environment of study.

Jottini S, Cabassi CS, Taddei S, Rosignoli° C, Merenda° M, Flisi S, Cavirani S

**Prevalenza, e antibiotico-sensibilità di *Mannheimia haemolytica* isolata da focolai di malattia respiratoria in allevamenti bovini da latte del Nord Italia** = Prevalence and antimicrobial susceptibility of *Mannheimia haemolytica* isolates from respiratory disease outbreaks in dairy cattle operations of Northern Italy

Large Anim Rev. - Vol. 23 no 6 ( 2017). - p 211-216. - 15 bib ref [Nr. Estr. 7780]

Scopo del presente lavoro e fornire indicazioni circa la prevalenza di *Mannheimia haemolytica* (M.h.), singolarmente o in associazione con altri patogeni, in corso di episodi di Bovine Respiratory Disease (BRD) a carico di vitelli da allevamenti da latte e valutarne la sensibilità agli antibiotici di comune utilizzo nella pratica buiatria. Durante ii periodo 2012-2016 sono stati esaminati 185 focolai di malattia respiratoria acuta che hanno coinvolto altrettanti allevamenti bovini da latte ubicati nell'area padana. Le indagini di laboratorio eseguite su polmoni e tamponi nasali prelevati da vitelli con manifestazioni acute di malattia respiratoria hanno esitato nell'isolamento di 136 stipiti di *Mannheimia haemolytica* con una prevalenza sul totale dei campioni prelevati pan i al 17% e 24% rispettivamente da tamponi nasali e polmoni. Gli stipiti di M.h., sottoposti al saggio di sensibilità agli antibiotici mediante il metodo Kirby-Bauer, hanno mostrato un livello di sensibilità >75% nei confronti di amoxicillina+acido clavulanico, ampicillina, ceftiofur, florfenicolo, trimetoprim+sulfonamidi. Per converso, un livello di resistenza >50% e emerso nei confronti di sulfadiazina e tilosina. In conclusione, M.h. continua a dimostrarsi un patogeno di frequente riscontro in corso di BRD, sia singolarmente che associato ad altri patogeni respiratori. Si ritiene importante mantenere ii monitoraggio circa l'antibiotico sensibilità-resistenza degli stipiti isolati in corso di patologia respiratoria del bovino.

*At present, Mannheimia haemolytica (M.h.) is recognized to be the major bacterial pathogen involved in Bovine Respiratory Disease (BRD) outbreaks. Aim - The study is aimed at providing information on the prevalence of Mannheimia haemolytica, alone or in association with other pathogens, during Bovine Respiratory Disease (BRD) outbreaks involving dairy calves. Mannheimia haemolytica isolates were submitted to assessment of sensitivity to antibiotics widely used in buiatrics. Materials and methods - During 2012-2016, 185 acute respiratory disease outbreaks involving dairy cattle operations located in the Po Valley (Italy) were examined. From calves aged 10-180 days, 715 nasal swabs from animals showing acute respiratory signs and 66 respiratory tracts from died calves belonging to 39 BRD outbreaks were collected and submitted to laboratory investigations at detecting viral and bacterial respiratory pathogens. M. h. isolates were tested for their antimicrobial susceptibility by Kirby-Bauer method. Results and discussion - Results confirm that BRSV is the main viral pathogen involved in BRD. Laboratory investigations on nasal swabs and lung specimens collected from calves with acute clinical signs of disease allowed the isolation of 136 Mannheimia haemolytica strains with a prevalence of 17% and 24% for nasal swabs and lung specimens, respectively. A level of susceptibility >75% to amcodicillin+clavulanic acid, ampicillin, ceftiofur, florfenicol, trimethoprim+sulfonamides was demonstrated. Conversely, a level of resistance >50% to sulfadiazine and tylosin was detected. Our results are in accordance with other surveys carried out in Northern Italy. Conversely, disagreement with European reports regarding antibiotic sensitivity of M.h. was observed. European data regarding amoxicillin-clavulanic acid, third-generation cephalosporins, florfenicol, and fluoroquinolones showed lower resistance than those emerging from our findings. This phenomenon is caused by the extensive use in our cattle operations of antibiotics in general and of the above-mentioned antibiotic classes in particular. Conclusions - To limit the extensive use of antibiotics in BRD, preventive strategies should be carried out by using vaccines. For the control of M.h. - LKT pathogenic effect, monovalent and/or combo vaccines have long been available.*

Kreizinger Z, Erdélyi K, Felde O, Fabbi° M, Sulyok KM, Magyar T, Gyuranecz M  
**Comparison of virulence of Francisella tularensis ssp. holarctica genotypes B.12 and B.FTNF002-00**

BMC Vet Res. - Vol. 13 ( 2017). - no 46 (7 p). - 25 bib ref ( ultimo accesso 28/02/2017  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5301346/> ) [Nr. Estr. 7523]

Two main genetic groups (B.12 and B.FTNF002-00) of Francisella tularensis ssp. holarctica are endemic in Europe. The B.FTNF002-00 group proved to be dominant in Western European countries, while strains of the B.12 group were isolated mainly in Northern, Central and Eastern Europe. The clinical course of tularemia in the European brown hare (Lepus europaeus) also shows distinct patterns according to the geographical area. Acute course of the disease is observed in hares in Western European countries, while signs of sub-acute or chronic infection are more frequently detected in the eastern part of the continent. The aim of the present study was to examine whether there is any difference in the virulence of the strains belonging to the B.FTNF002-00 and B.12 genetic clades. Results Experimental infection of Fischer 344 rats was performed by intra-peritoneal injection of three dilutions of a Hungarian (B.12 genotype) and an Italian (B.FTNF002-00 genotype) F. tularensis ssp. holarctica strain. Moderate difference was observed in the virulence of the two genotypes. Significant differences were observed in total weight loss values and scores of clinical signs between the two genotypes with more rats succumbing to tularemia in groups infected with the B.FTNF002-00 genotype. Conclusions Results of the experimental infection are consistent with previous clinical observations and pathological studies suggesting that F. tularensis ssp. holarctica genotype B.FTNF002-00 has higher pathogenic potential than the B.12 genotype.

Lazzaro° M, Giacomini° E, Scali° F, Salogni° C, Giovannini° S, Pizzoni° G [i.e. Pezzoni], Grazioli° S, Gibelli° L, Nigrelli° A, Alborali° GL

**Encefalomiocardite in un allevamento da riproduzione : forma riproduttiva e mortalita' in**

## **scrofette**

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 179-183. - 7 bib ref [Nr. Estr. 7564]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo 2017)

Le\_Pendu J, Abrantes J, Bertagnoli S, Guitton JS, Le\_Gall\_Reculé G, Lopes AM, Marchandeu S, Alda F, Almeida T, Celio AP, Barcena J, Burmakina G, Blanco E, Calvete C, Cavadini° P, Cooke B, Dalton K, Mateos MD, Deptula W, Eden JS, Wang F, Ferreira CC, Ferreira P, Foronda P, Gonçalves D, Gavier\_Widen D, Hall R, Hukowska\_Szematowicz B, Kerr P, Kovaliski J, Lavazza° A, Mahar J, Malogolovkin A, Marques RM, Marques S, Martin\_Alonso A, Monterroso P, Moreno S, Mutze G, Neimanis A, Niedzwiedzka\_Rystwej P, Peacock D, Parra M, Rocchi M, Rouco C, Ruvoen\_Clouet N, Silva E, Silverio D, Strive T, Thompson G, Tokarz\_Deptula B, Esteves P

### **Proposal for a unified classification system and nomenclature of lagoviruses**

J Gen Virol. - Vol. 98 ( 2017). - p 1658-1666. - 63 bib ref [Nr. Estr. 7639]

Lagoviruses belong to the Caliciviridae family. They were first recognized as highly pathogenic viruses of the European rabbit (*Oryctolagus cuniculus*) and European brown hare (*Lepus europaeus*) that emerged in the 1970–1980s, namely, rabbit haemorrhagic disease virus (RHDV) and European brown hare syndrome virus (EBHSV), according to the host species from which they had been first detected. However, the diversity of lagoviruses has recently expanded to include new related viruses with varying pathogenicity, geographic distribution and host ranges. Together with the frequent recombination observed amongst circulating viruses, there is a clear need to establish precise guidelines for classifying and naming lagovirus strains. Therefore, here we propose a new nomenclature based on phylogenetic relationships. In this new nomenclature, a single species of lagovirus would be recognized and called *Lagovirus europaeus*. The species would be divided into two genogroups that correspond to RHDV- and EBHSV-related viruses, respectively. Genogroups could be subdivided into genotypes, which could themselves be subdivided into phylogenetically well-supported variants. Based on available sequences, pairwise distance cutoffs have been defined, but with the accumulation of new sequences these cutoffs may need to be revised. We propose that an international working group could coordinate the nomenclature of lagoviruses and any proposals for revision.

Lelli° D, Leopardi S, Prosperio° A, Moreno° A, Chiapponi° C, Zecchin B, Priori P, Scaravelli D, Sozzi° E, De\_Benedictis P, Lavazza° A

### **Active and passive surveillance for viral infections in insectivorous bats in Italy reveal a wide variety of mammalian orthoreoviruses**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 98 (Poster 035). - 6 bib ref [Nr. Estr. 7604]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

Lelli° D, Prosperio° A; Chiapponi° C, Debenedictis P, Gibellini AM, Leopardi S, Sozzi° E, Scaravelli D, Moreno° A, Lavazza° A

### **Characterization of a novel Rhabdovirus isolated from insectivorous bat (*Pipistrellus kuhlii*) in Italy**

Infectious diseases of bats symposium : June 29-July 1, 2017 Fort Collins, CO / [s.l. : s.n., 2017]. - p

**Objectives:** Rhabdoviridae is one of the most ecologically diverse families of RNA viruses with clinical importance. Herein we report the isolation and the genome characterization of a novel rhabdovirus detected from a bat collected within a survey implemented in Italy on emerging viruses of bats. **Methods:** A fresh carcass of an adult female of *Pipistrellus kuhlii* spontaneously dead in a wildlife rehabilitation center in Northern Italy was fully necropsied. Tissue samples from different organs (lung, heart, intestine) were subjected to viral isolation on cell culture. Virus identification was performed using negative staining electron microscopy (nsEM) and NGS sequencing. Molecular and phylogenetic analyses were performed. **Results:** An animal reported sensory depression, inappetence, normal body mass and injuries of patagium consistent with a cat bite. The death occurred three days after the admission to the rehabilitation center and no pathological lesions indicative of infectious diseases were observed at necropsy. CPE was observed on VERO cells inoculated with a pool of organs and nsEM performed on cells supernatants revealed characteristic bullet-shaped viral particles referable to rhabdovirus. Tests aimed to exclude rabies and related lyssaviruses resulted negative. The complete genome size was 11,780 nt comprised 5 genes encoding the canonical rhabdovirus structural proteins and an additional transcriptional unit (U1) encoding a small protein (157 aa) located between the G and L genes (3'-N-P-M-G-U1-L-5'). BLAST analysis showed the highest nucleotide identity (65%) to Le Dantec virus (LDV) (human, 1965 Senegal) the prototype strain of the putative genus *Ledantevirus*. The most highly conserved protein L shared 70% and 69% of aa identity with LDV and Keuraliba virus (KEUV) (gerbil, 1968 Senegal) respectively. Phylogenetic tree based on full-genome sequence confirm the belonging of the new isolate to the *ledantevirus* group. **Conclusions:** A novel rhabdovirus was identified from *Pipistrellus kuhlii*, the most common species in urban areas in Italy. This finding represents (beside lyssaviruses) the only bat-borne rhabdovirus isolated in Europe. Specific diagnostic tools for viral detection will be set up for epidemiological investigations aimed to define the viral ecology and diffusion in bats population in Italy, in order also to further characterize and clarify its zoonotic potential.

Lombardi G, Botti I, Pacciarini<sup>o</sup> ML, Boniotti<sup>o</sup> MB, Roncarati G, Dal\_Monte P

**Five-year surveillance of human tuberculosis caused by *Mycobacterium bovis* in Bologna, Italy : an underestimated problem**

Epidemiol Infect. - Vol. 145 ( 2017). - p 3035-3039. - 13 bib ref [Nr. Estr. 7737]

Human tuberculosis (TB) caused by *Mycobacterium bovis* surveillance is affected by a lack of data. The aims of the present study were: (i) to estimate the proportion of human TB caused by *M. bovis* over a period of 5 years in Bologna, Northern Italy, which, like most Western European countries, has been declared bovine TB-free; (ii) to compare the genetic profiles of *M. bovis* strains identified in humans with those circulating in cattle in the last 15 years in Italy. Among 511 TB patients, the proportion of human TB caused by *M. bovis* was 1.76%, significantly associated to extra-pulmonary localization ( $P = 0.004$ ) and to being elderly ( $P < 0.001$ ) and Italy-born ( $P = 0.036$ ). The molecular epidemiology analysis by spoligotyping and Multilocus Variable Tandem Repeat Analysis confirmed that most *M. bovis* strains from Italyborn patients matched those circulating in cattle herds in Italy between 2001 and 2016. Two cases of *Mycobacterium bovis* BCG infection were also characterized. In conclusion, the rate of human TB caused by *M. bovis* was not negligible, highlighting the relevance of molecular typing in evaluating the effectiveness of programmes designed to eradicate TB in cattle in Italy. **Key words.**

Magro G, Biffani S, Minozzi G, Ehrlich R, Monecke S, Luini<sup>o</sup> M, Piccinini R

**Virulence genes of *S. aureus* from dairy cow mastitis and contagiousness risk**

Toxins. - Vol. 9 no 6 ( 2017). - no 195 (12 p). - 41 bib ref ( ultimo accesso 05/12/2017  
<http://www.mdpi.com/2072-6651/9/6/195> ) [Nr. Estr. 7691]

*Staphylococcus aureus* (*S. aureus*) is a major agent of dairy cow intramammary infections: the different prevalences of mastitis reported might be related to a combination of *S. aureus* virulence factors beyond host factors. The present study considered 169 isolates from different Italian dairy herds that were classified into four groups based on the prevalence of *S. aureus* infection at the first testing: low prevalence (LP), medium–low (MLP), medium–high (MHP) and high (HP). We aimed to correlate the presence of virulence genes with the prevalence of intramammary infections in order to develop new strategies for the control of *S. aureus* mastitis. Microarray data were statistically evaluated using binary logistic regression and correspondence analysis to screen the risk factors and the relationship between prevalence group and gene. The analysis showed: (1) 24 genes at significant risk of being detected in all the herds with infection prevalence >5%, including genes belonging to microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), immune evasion and serine proteases; and (2) a significant correlation coefficient between the genes interacting with the host immune response and HP isolates against LP ones. These results support the hypothesis that virulence factors, in addition to cow management, could be related to strain contagiousness, offering new insights into vaccine development.

Mahamat O, Brocchi° E, Grazioli° S, Ben\_Yousef A, Sumption K, Hendriks P, Berkvens D, Saegerman C

**Evaluation de la sensibilite du reseau d'epidemiologie des maladies animales au Tchad pour la surveillance de la fièvre aphteuse**

Epidemiol Santé Anim. - Vol. 72 ( 2017). - p 5-14. - 25 bib ref [Nr. Estr. 7768]

*Early detection of animal diseases is one of the primary aims of epidemiology networks in western and central Africa. A sufficiently high sensitivity is an essential characteristic of an epidemiology network. Foot and-mouth-disease (FMD) is a highly contagious viral disease that affects all artiodactyla. It is included in the list of diseases monitored by the national animal disease epidemiology network (REPIMAT) in Chad. Surveillance of this disease is passive and primarily concerns bovine. Numerous bovine clinical suspicions are raised annually by the epidemiology network, but they are only rarely further investigated in the laboratory. A serological survey was conducted in the cattle population in eight of the nine administrative regions of the country (regions with the highest bovine densities) with the aim to evaluate the sensitivity of REPIMAT for FMD. The samples randomly selected were analysed in the OIE/FAO FMD reference laboratory at IZSLER, Brescia (Italy) with the support of EuFMD (The European Commission for the control of foot-and-mouth disease). Antibodies against non-structural proteins (NSP), indicative of recent or past infection, were analysed by a 3ABC–ELISA. A total of 106 villages/herds and 796 cattle were investigated. The herd-level seroprevalence was 63% (95% CI: 51.9-71.2) and the animal-level seroprevalence was 35.6% (95% CI: 32.2- 39.0). A global significant positive relation was found between the estimated seroprevalence and the number of registered clinical suspicions. However, this relation is weak but heterologous and this could lead to an improvement of the declaration of FMD suspicions in some administrative regions where some correctives measures could be introduced.*

Mancini G, Montarsi F, Calzolari° M, Capelli G, Dottori° M, Ravagnan S, Lelli° D, Chiari° M, Santilli A, Quaglia M, Federici V, Monaco F, Goffredo M, Savini G

**Mosquito species involved in the circulation of West Nile and Usutu viruses in Italy**

Vet Ital. - Vol. 53 no 2 ( 2017). - p 97-110. - 66 bib ref [Nr. Estr. 7624]

Usutu (USUV) and West Nile (WNV) are mosquito-borne Flavivirus emerged in Italy in 1996 and 1998, respectively, and reappeared 10 years later. The aim of this work is to review the Italian mosquito species found positive for WNV and USUV between 2008 and 2014. Moreover, the role of

mosquitoes in promoting the overwintering of these viruses is discussed, as a result of the mosquito collections performed in Molise region between September 2010 and April 2011. Overall 99,000 mosquitoes were collected: 337 and 457 mosquito pools tested positive by real time reverse transcriptase polymerase chain reaction (real time RT-PCR) for WNV and USUV, respectively. West Nile virus was detected in pools of *Culex pip/ens s.L* (329), *Ochlerotatus caspius* (4), *Culex modestus* (2), and *Culex spp.* (2). Positive USUV pools were from *Cx. pip/ens s.L* (435), *Aedes albopictus* (12), *Oc. caspius* (5), *Culex spp.* (2), *Anopheles maculipennis 5.1.* (1), *Culiseta annulata* (1), and *Ochlerotatus detritus* (1). In Molise region, 1,694 mosquitoes were collected, and USUV was identified in *Cx. pip/ens s.L*, *Cs. annulata*, and *Oc. detritus* pools. This paper shows that *Cx. pip/ens s.L* is the mosquito species most involved in the WNV and USUV circulation in Italy, although other species would also support the spread of both the viruses during Winter.

Martelli W, Trogu T, Ferrari N, Formenti<sup>o</sup> N, Viganò R, Pedrotti L, Luzzago C

**Hepatitis E virus in wild ungulates : serological evidence in chamois and red deer in the Alps and genetic assessment of viral variants in Europe**

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 17 [Nr. Estr. 7738]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

In recent years there has been an increasing interest in hepatitis E virus (HEV) because of its zoonotic nature. The different ecological interactions among its numerous host species contribute to make HEV epidemiology unclear and the actual reservoirs and spill-over hosts have to be still defined. Domestic pig and wild boar are considered as the main reservoirs of zoonotic HEV in Europe, while wild ruminants are supposed as spill-over hosts. This study aims to serologically investigate HEV infection in chamois and red deer in order to define their REV seroprevalence and to clarify the epidemiology of this infection in alpine free-living wild ruminants. Moreover a genetic assessment of European 1-1EV wild ungulates sequences retrieved from Genbank has been performed to identify the frequency of genotypes and subtypes, while European HEV sequences isolated from humans, domestic and wild animals have been checked to identify viral mutations associated with treatment failure and virus replication fitness in human. Sampling of sera was carried out from 2013 to 2015 during autumnal hunting seasons and depopulation plans, from three study areas: i) 173 red deer from Stelvio National Park (Valfurva, Sondrio Province, Central Italian Alps), ii) 44 chamois from Orobic Alps, iii) 128 chamois, 81 red deer and 32 roe deer from Lepontine Alps (North-West Italian Alps). Sera were tested by a species-independent immune-enzymatic assay (1-1EV ELISA 4.0v, MP Diagnostics Biomedicals, Singapore). All publicly available European REV sequences from ruminant species (n=7) have been aligned with REV reference and field strains, phylogeny was estimated by the maximum likelihood method and the percentage of nucleotide similarity was calculated. The presence of nucleotide variations have been checked in ORF1 available sequences of ungulates (n=18), humans (n=11) and other species (n=3). Chamois and red deer showed a HEV seroprevalence of 1.16% (2/172; CI 95%:1.03-1.28) and 0.79% (2/254; CI 95%:0- 1.87), respectively. Roe deer resulted REV seronegative. A high level of nucleotide identity (>97%) has been observed among ruminants' genotype 3 sequences (ORF1 or ORF2) and REV reservoirs or human ones isolated in the same country: i) a goat and red deer with a wild boar and swine respectively in Italy; ii) two red deer with a swine and human in Spain; iii) a roe deer and reed deer with swine and wild boar and also a roe deer with human in Hungary. As regard to nucleotide variations, G1634 codon showed substitutions in 16 out of 32 available sequences (6 humans, 6 swine, 2 rabbits, 1 elk and 1 polecat) and V1479 codon in 23/32 sequences (6 humans, 12 swine, 2 rabbits, 1 wild boar, 1 elk and 1 polecat). In 5 humans, 7 swine, 2 rabbits, 1 elk and 1 polecat that mutations occurred together.

Martinelli<sup>o</sup> C, Giovannini<sup>o</sup> S, Salogni<sup>o</sup> C, Ruggeri<sup>o</sup> J, D'Incau<sup>o</sup> M, Pasquali P,

Alborali° GI

**Antimicrobial drug resistance and virulence factors in Escherichia coli from Italian dairy calves with diarrhea during the period 2011-2015**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 226. - 3 bib ref [Nr. Estr. 7628]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

Antimicrobial resistance is a worldwide health problem in veterinary such as in human medicine (1). Calf diarrhea caused by Escherichia coli is one of the most important economic loss in dairy farm. A retrospective study was carried out on antimicrobial resistance profile and virulence genes in E. coli strains isolated during 2011-2015. Seven hundred and forty-six E. coli strains were isolated from carcasses, feces and rectal swabs of calves with diarrhea in farms located in Northern Italy. The isolates were tested for susceptibility to 18 antimicrobials using a Kirby-Bauer disk diffusion assay. Sixty-one of them were also analyzed for virulence genes (LT I, STaP, STb, F4, F5, F6, F41, F18 e STX2e) by specific PCR assays. Fisher's exact test was used to study the statistical relationship between virulence gene coding F5 and antimicrobial resistance. Persisting and high prevalence values of resistant E. coli strains were observed during the study for several antimicrobials: Penicillin and Tiamulin (100%); Doxycycline, Sulfadiazine and Tetracycline (90%); Amoxicillin, Flumequine and Kanamycin (80%); Aminocyclitol; Danofloxacin, Enrofloxacin, Trimethoprim+Sulfamethoxazole (70%) and Gentamicin (60%). The prevalence of resistance to Cefquinome was 40%. Furthermore a significant decreasing resistance trend was observed during the study period for Amoxicillin+Clavulanic acid (from 81% in 2011 to 65% in 2015), Ceftiofur (52% to 32%) and Colistine (53% to 15%). A significant increasing resistance trend was observed for Apramycin (from 30% in 2011 to 55% in 2015). Seven hundred and forty-five out of 746 E. coli strains were resistant to at least three antimicrobials of different classes (1;2;3) and defined MDR (Multidrug-Resistant). Six hundred and fifty-five out of 746 E. coli strains were resistant to 4 antimicrobial classes: Penicillins, Pleuronutlin, Sulphonamides and Tetracyclines. F5 virulence gene was observed in 24 isolates (39%) and, in 14 of them, F5 was associated with STaP and F41. In E. coli F5 positive strains we found a significant difference in antimicrobial resistance for Danofloxacin ( $p < 0.0004$ ), Enrofloxacin ( $p < 0.008$ ) and Flumequine ( $p < 0.0022$ ) than in F5-negative strains. The results of this study suggest that the high prevalence of E. coli MDR strains in dairy calves with diarrhea is an important issue in animal and public health. The use of targeted antimicrobials for strictly necessary periods could significantly reduce the risk of antibiotic resistance also for Critically Important Antimicrobials recommended in international control programs (OIE, 2015; FAO, 2008; WHO, 2012; EMA, 2014; WHO, 2014; ECDC/EFSA/EMA, 2015; EFSA/ECDC 2015).

Martinelli° C, Giovannini° S, Salogni° C, Ruggeri° JV, D'Incau° M, Birbes° L, Terrini° A, Pasquali P, Alborali° GL

**Critical important antimicrobial resistance and virulence factors of Escherichia coli isolated from Italian dairy calves with diarrhea**

5th European Buiatrics Forum : Bilbao, Spain, 04th - 06th October 2017 : program and proceedings / [s.l. : s.n., 2017]. - p 209 (abstract ABSEBF00188). - 2 bib ref [Nr. Estr. 7766]

European Buiatrics Forum (5th : Bilbao, Spain : 04th - 06th October 2017)

OBJECTIVE Antimicrobial resistance is a worldwide health problem and control programs have been defined by different International Sanitary Organizations (OIE, WHO, etc.)<sup>1</sup>. A retrospective study was carried out on some Critically Important Antimicrobials (Cefquinome, Ceftiofur, Colistine, Flumequine, Danofloxacin, Enrofloxacin and Marbofloxacin) in 281 Escherichia coli strains isolated during 2010-2016. METHODS E.coli strains were isolated from calves with diarrhea in farms located in Northern Italy. Susceptibility to antimicrobials was carried out using a Kirby-Bauer disk diffusion

test and virulence genes were identified by specific PCR assays. Chisquare test was used to study the statistical relationship between virulence gene coding F5 and antibiotic resistance. RESULTS Persisting prevalence values of resistant E.coli strains were observed for Quinolones and Fluoroquinolones classes (70%-80%) and for Cephalosporins (3rd and 4th generation) (40%). Instead, a significant decreasing resistance trend was observed for Polymyxins (56% in 2010 to 17% in 2016). The F5 virulence gene was found in 59 isolates (21%) in association with the StaP gene (16 strains), StaP and F41 (43) or F41 (1). E.coli F5 negative strains were characterized by the gene stx2e (3), StaP (3) and StaP associated with STb and LTI (2). In E.coli F5 positive related to negative strains we found a significant difference in antimicrobial resistance for ceftiofur ( $p < 0,014$ ) and danofloxacin ( $p < 0,38$ ). CONCLUSIONS The results confirm the importance of a prudent use of antimicrobials<sup>2</sup> in dairy cattle health management and the application of prevention programs that also take into account the antimicrobial resistance profile, the virulence factors and co-infection in enteric disease.

Martinelli° C, Giovannini° S, Ventura° G, Salogni° C, Boldini° M, Gradassi° M, Pasquali P, Alborali° GL

**Abortion agents diagnostic survey in bovine aborted fetuses in Northern Italy from 2014 to 2016**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 146 (Poster 013). - 3 bib ref [Nr. Estr. 7588]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento, Italy : June 7-10, 2017)

Martinello M, Baratto C, Manzinello C, Piva E, Borin A, Toson M, Granato A, Boniotti° MB, Gallina A, Mutinelli F

**Spring mortality in honey bees in northeastern Italy : detection of pesticides and viruses in dead honey bees and other matrices**

J Apic Res. - Vol. 56 no 3 ( 2017). - p 239-254. - 109 bib ref [Nr. Estr. 7584]

In spring there is often a rise in honey bee mortality incidents. The aim of this study was to investigate the potential correlation, in the reported incidents, between exposure to pesticide treatments and virus infections. Here we summarize the situation in northeastern Italy during spring 2014, evaluated by monitoring 150 active ingredients and three honey bee viruses in dead honey bees and other matrices. At least one active ingredient was found in 72.2% of the 79 dead honey bee samples, with the most abundant (59.4%) being insecticides, mainly belonging to the class of neonicotinoids (41.8%), followed by fungicides (40.6%). Imidacloprid, chlorpyrifos, tau-fluvalinate, and cyprodinil were the most frequently detected active ingredients. Multiple virus infections were monitored, revealing a high prevalence of chronic bee paralysis virus (CBPV) and deformed wing virus (DWV), detected in all samples except one. 71 and 37% of the CBPV- and DWV positive samples, respectively, showed a high number of viral copies per bee ( $>10^7$ ). This work emphasizes the possible relationship between spring mortality in honey bees and pesticide treatments. Honey bee viruses could synergistically exacerbate the negative impact of pesticides on honey bee health, endangering the survival of colonies.

Massi P

**Aggiornamenti sulla bronchite infettiva aviare**

Atti della Societa' Italiana di Patologia Aviare (SIPA) 2017 : LVI Convegno annuale, Il Simposio scientifico : Forli, 6-7 Aprile 2017 - Parma, 22 Settembre 2017 / [s.l. : s.n., 2017]. - p 43-53. - 23 bib

ref [Nr. Estr. 7779]

Convegno annuale Societa' Italiana Patologia Aviaria (SIPA) (56. : Forlì Parma : 6-7 Aprile 2017  
22 Settembre 2017)

Mazzariol S, Di\_Francesco G, Badagliacca R, Di\_Provvido A, Ferri N, Centelleghè C, Panin M, Cozzi B, Zanetti E, Marcer F, Bonsembiante F, Casalone C, Mignone W, Giorda F, Pautasso A, Modesto R, Cocumelli C, Cersini A, Cardeti G, Terracciano G, Petrella A, Troiano P, Rubini° S, Menotta° S, Podestà M, Garibaldi F, Mattiucci S, Cipriani P, Zaccaroni A, Andreini R, Berto D, Fernandez A, De\_Bernaldo Quiros Y, Morell M, Denurra D, Di\_Francesco CE, Pietrolungo G, Di\_Guardo G

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

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 245 (Poster 107). - 7 bib ref [Nr. Estr. 7594]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

Montoya M, Foni° E, Solorzano A, Razzuoli E, Barattelli M, Bilato° D, Cordoba L, Martin\_del\_Burgo MA, Martinez J, Martinez-Orellana P, Chiapponi° C, Perlin DS, del\_Real G, Amadori° M

#### **Expression dynamics of innate immunity in influenza virus-infected swine**

Front Vet Sci. - Vol. 4 ( 2017). - Article 48 (13 p). - 36 bib ref ( ultimo accesso <https://doi.org/10.3389/fvets.2017.00048> ) [Nr. Estr. 7543]

The current circulating swine influenza virus (IV) subtypes in Europe (H1N1, H1N2, and H3N2) are associated with clinical outbreaks of disease. However, we showed that pigs could be susceptible to other IV strains that are able to cross the species barrier. In this work, we extended our investigations into whether different IV strains able to cross the species barrier might give rise to different innate immune responses that could be associated with pathological lesions. For this purpose, we used the same samples collected in a previous study of ours, in which healthy pigs had been infected with a H3N2 Swine IV and four different H3N8 IV strains circulating in different animal species. Pigs had been clinically inspected and four subjects/group were sacrificed at 3, 6, and 21 days post infection. In the present study, all groups but mock exhibited antibody responses to IV nucleoprotein protein. Pulmonary lesions and high-titered viral replication were observed in pigs infected with the swine-adapted virus. Interestingly, pigs infected with avian and seal H3N8 strains also showed moderate lesions and viral replication, whereas equine and canine IVs did not cause overt pathological signs, and replication was barely detectable. Swine IV infection induced interferon (IFN)-alpha and interleukin-6 responses in bronchoalveolar fluids (BALF) at day 3 post infection, as opposed to the other non-swine-adapted virus strains. However, IFN-alpha responses to the swine-adapted virus were not associated with an increase of the local, constitutive expression of IFN-alpha genes. Remarkably, the Equine strain gave rise to a Serum Amyloid A response in BALF despite little if any replication. Each virus strain could be associated with expression of cytokine genes and/or proteins after infection. These responses were observed well beyond the period of virus replication, suggesting a prolonged homeostatic imbalance of the innate immune system.

Moreno° A, Franzo G, Massi° P, Tosi° G, Blanco A, Antilles N, Biarnes M, Majó N, Nofrarías M, Dolz R, Lelli° D, Sozzi° E, Lavazza° A, Cecchinato M

#### **A novel variant of the infectious bronchitis virus resulting from recombination events in Italy and Spain**

Infectious bronchitis is considered to be one of the most devastating diseases in poultry. Control of its spread is typically attempted through biosecurity measures and extensive vaccination. However, the remarkable genetic and antigenic variability of the virus, which originate from both mutations and recombination events, represents an unsolved challenge for this disease. The present study reports on the emergence and spread of recombinant clusters detected in Italy and Spain between 2012 and 2014. A total of 36 Spanish and Italian infectious bronchitis virus (IBV) field strains were investigated and genetically characterized using phylogenetic, molecular, recombination and selection pressure analyses of the complete S1 gene. Based on the partial S1 sequencing, 27 IBV strains originating from Spain and nine from Italy were initially classified as being closely related to the Guandong/Xindadi (XDN) genotype. Phylogenetic analysis of the complete S1 gene revealed that the XDN strains formed a homogeneous clade with the Spanish IBV isolates within the QX genotype, whereas there was higher variability within the Italian strains. Recombination analysis determined that these strains belonged to four groups, which originated from independent recombination events between the QX and 793B IBV genotypes. Our data support the hypothesis of two different scenarios: firstly, in Spain, the large and homogeneous clade probably originated from a single offspring of the recombinant founder, which became dominant and spread throughout the country. Secondly, the nine Italian recombinants, which are characterized by three different recombination patterns, probably represent less fitted strains, because they were less viable with respect to their recombinant parents.

Moreno° A, Lelli° D, De\_Sabato L, Zaccaria G, Boni A, Sozzi° E, Prosperi° A, Lavazza° A, Cella E, Castrucci MR, Cicozzi M, Vaccari G

**Detection and full genome characterization of two beta CoV viruses related to Middle East respiratory syndrome from bats in Italy**

Virology J. - Vol. 14 no 1 ( 2017). - no 239 (11 p). - 31 bib ref (ultimo accesso 15/01/2018 <https://virologyj.biomedcentral.com/articles/10.1186/s12985-017-0907-1> ) [Nr. Estr. 7727]

Background: Middle East respiratory syndrome coronavirus (MERS-CoV), which belongs to beta group of coronavirus, can infect multiple host species and causes severe diseases in humans. Multiple surveillance and phylogenetic studies suggest a bat origin. In this study, we describe the detection and full genome characterization of two CoVs closely related to MERS-CoV from two Italian bats, *Pipistrellus kuhlii* and *Hypsugo savii*. Methods: Pool of viscera were tested by a pan-coronavirus RT-PCR. Virus isolation was attempted by inoculation in different cell lines. Full genome sequencing was performed using the Ion Torrent platform and phylogenetic trees were performed using IQtree software. Similarity plots of CoV clade c genomes were generated by using SSE v1.2. The three dimensional macromolecular structure (3DMMS) of the receptor binding domain (RBD) in the S protein was predicted by sequence-homology method using the protein data bank (PDB). Results: Both samples resulted positive to the pan-coronavirus RT-PCR (IT-batCoVs) and their genome organization showed identical pattern of MERS CoV. Phylogenetic analysis showed a monophyletic group placed in the Beta2c clade formed by MERS-CoV sequences originating from humans and camels and bat-related sequences from Africa, Italy and China. The comparison of the secondary and 3DMMS of the RBD of IT-batCoVs with MERS, HKU4 and HKU5 bat sequences showed two aa deletions located in a region corresponding to the external subdomain of MERS-RBD in IT-batCoV and HKU5 RBDs. Conclusions: This study reported two beta CoVs closely related to MERS that were obtained from two bats belonging to two commonly recorded species in Italy (*P. kuhlii* and *H. savii*). The analysis of the RBD showed similar structure in IT-batCoVs and HKU5 respect to HKU4 sequences. Since the RBD domain of HKU4 but not HKU5 can bind to the human DPP4 receptor for MERS-CoV, it is possible to suggest also for IT-batCoVs the absence of DPP4-binding potential. More surveillance studies are needed to better investigate the potential intermediate hosts that may play a role in the interspecies transmission of known and currently unknown coronaviruses with particular attention to the S protein and the receptor specificity and binding affinity.

Moreno\_Martin A

**Laringotracheite infettiva aviare : diagnosi, caratterizzazione dei ceppi e strategie di controllo**

Atti della Societa' Italiana di Patologia Aviare (SIPA) 2017 : LVI Convegno annuale, Il Simposio scientifico : Forli, 6-7 Aprile 2017 - Parma, 22 Settembre 2017 / [s.l. : s.n., 2017]. - p 33-39. - 16 bib ref [Nr. Estr. 7773]

Convegno annuale Societa' Italiana Patologia Aviare (SIPA) (56. : Forli Parma : 6-7 Aprile 2017 22 Settembre 2017)

Mulatti P, Zecchin B, Monne I, Vieira JT, Dorotea T, Terregino C, Lorenzetto M, Loli\_Piccolomini L, Santi° A, Massi° P, Bonfanti L, Marangon S

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

Avian Dis. - Vol. 61 ( 2017). - p 261-266. - 13 bib ref [Nr. Estr. 7636]

After the H7N7 highly pathogenic (HP) avian influenza (AI) outbreak in 2013, and a single case of H5N8 HPAI in 2014, in April 2016, a H7N7 HPAI virus was detected in northeastern Italy. The case occurred in an organic free-range laying hen farm located in proximity with one of the highest densely populated poultry areas (DPPAs) in Italy. Control measures provided by the Council of the European Union in directive 2005/94/CE were promptly applied, and enhanced surveillance activities were implemented in the DPPAs. On May 16, 2016, a second case was confirmed in a fattening turkey farm within the protection zone of the previous outbreak. Following an epidemiologic inquiry, another turkey farm was considered at risk of transmission and was subjected to preemptive culling. Epidemiologic data and phylogenetic analyses indicated that the virus was likely introduced from wild birds as a low pathogenicity AI strain, through direct contact. The rapid containment of the outbreak proves the level of preparedness of the veterinary public health sector in Italy. Nevertheless, the recurrent introductions from wild birds indicate the need of improving both the biosecurity levels in the DPPA and the surveillance activities in wild birds to quickly detect the presence of AI in the territory.

Nigrelli° AD, Lombardi° G, Faccini° S, Caleffi A, Bardini R, Franzini° G, Rosignoli° C

**Efficacia di un vaccino inattivato bivalente contro Cl. difficile e Cl. perfringens di tipo A**

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 153-154. - 3 bib ref [Nr. Estr. 7561]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo 2017)

Nigrelli° AD, Rosignoli° C, Faccini° S, Franzini° G, D'incau° M, Alborali° L

**Gestione di un focolaio in Leptospira serovar pomona in un sito 1**

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 173-174. - 1 bib ref [Nr. Estr. 7562]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo 2017)

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

**Antibiotico resistenza in ceppi di Actinobacillus pleuropneumoniae e Pasteurella multocida isolati nel suino nel periodo 2002-2016 in Italia**

= Antimicrobial resistance in Actinobacillus pleuropneumoniae and Pasteurella multocida strains isolated during the period 2002-2016 in Italian pigs

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 209-217. - 34 bib ref [Nr. Estr. 7565]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo 2017)

In questo studio è stato valutato il tasso e il trend di resistenza nei confronti di diversi antibiotici di 354 ceppi di *Actinobacillus pleuropneumoniae* e 1058 ceppi di *Pasteurella multocida* isolati da casi di pneumopatie in suini appartenenti ad allevamenti ubicati in Nord Italia, nel periodo 2002-2016. Tutti i ceppi sono stati testati per la loro suscettibilità a 9 antibiotici impiegando il metodo della disco diffusione (Kirby-Bauer): Amoxicillina+Acido Clavulanico (20pg\10pg), Ampicillina (10pg), Cefalexina (30pg), Ceftiofur (30pg), Enrofloxacin (5pg), Florfenicolo (30pg), Lincomicina (2pg), Tetraciclina (30pg) e Trimethoprim-Sulfametossazolo (1,25pg\23,75pg). Con l'impiego di un test chiquadrato per trend lineare è stato valutato l'eventuale trend in aumento o in diminuzione della resistenza nei confronti di ogni antibiotico nel periodo considerato. I ceppi di *A. pleuropneumoniae* hanno evidenziato, nell'intero periodo dello studio, un trend in aumento della resistenza statisticamente significativo nei confronti del Florfenicolo ( $\chi^2 = 8.34 P < 0.01$ ). Al contrario, un trend di diminuzione della resistenza è stato riscontrato nei confronti del Trimethoprim+Sulfametossazolo ( $\chi^2 = 5.65 P < 0.05$ ). Per quanto riguarda invece i ceppi di *P. multocida* è stato rilevato un trend di aumento della resistenza nei confronti sia della Lincomicina ( $\chi^2 = 20.03 P < 0.01$ ) sia del Florfenicolo ( $\chi^2 = 5.67 P < 0.05$ ).

*This study evaluated the rate and the trend of resistance to various antibiotics of 354 strains of Actinobacillus pleuropneumoniae and 1058 strains of Pasteurella multocida from diseased pigs, belonging to herds located in Northern Italy, in the period 2002-2016. The strains were analyzed for their susceptibility to 9 antimicrobials by disk diffusion method (Kirby-Bauer): Amoxicillin+Clavulanic acid (20pg\10pg), Ampicillin (10pg), Cephalexin (30pg), Ceftiofur (30pg), Enrofloxacin (5pg), Florfenicol (30pg), Lincomycin (2pg), Tet-racycline (30pg) e Trimethoprim-sulfamethoxazole (1,25pg\23,75pg). With the use of a linear trend chi-square test was evaluated the increasing or decreasing trend of resistance to each antibiotic in the period considered. The A. pleuropneumoniae bacterial strains showed, over the whole period of the study, a statistically significant increasing trend of resistance to Florfenicol ( $\chi^2 = 8.34 P < 0.01$ ). Conversely, a statistically significant decreasing trend of resistance was observed to Trimethoprim + Sulfamethoxazole ( $\chi^2 = 5.65 P < 0.05$ ). P. multocida strains showed an increasing trend of resistance to Lincomycin ( $\chi^2 = 20.03 P < 0.01$ ) and Florfenicol ( $\chi^2 = 5.67 P < 0.05$ ).*

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

#### **Identification of a recombinant PEDV/SeCoV strains during a molecular surveillance of Italian epidemic waves between 2015 and 2017**

11th Annual Meeting Epizone "Crossing barriers" : 19-21 September 2017, Paris, France : programme and abstracts / [s.l. : s.n., 2017]. - p 136 (Poster P 3 4). - 2 bib cit [Nr. Estr. 7657]

Annual meeting Epizone (11th : Paris, France : 19-21 September 2017)

Introduction: Porcine epidemic diarrhea (PED) is an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea and high mortality among suckling pigs. The PED virus (PEDV) belongs to the Coronaviridae family, characterized by a positive-sense singlestranded RNA genome. Coronaviruses (CoVs) are prone to genetic evolution through accumulation of point mutations and homologous recombination. In the last years, PED had a big economic impact on swine industry in Asia and United States of America (1). In 2014, PEDV has also re-emerged in many European countries; a PEDV strain genetically related to the moderate pathogenic American S-INDEL 0H851 was reported in Belgium, Germany, Netherlands, France, Spain, Slovenia, Austria and Portugal (1). In Italy, strains circulating during 2007-2012 showed a high genetic variability (2). In particular, from 2009 to 2012 a new recombinant coronavirus, called Swine enteric Coronavirus (SeCoV), was present; the genome of this recombinant belongs to another swine Coronavirus, the Transmissible Gastroenteritis Virus (TGEV), but the S-gene derived from PEDV (2). In July 2014, two new Italian cases of PED were detected with high nucleotide identity to PEDV S-INDEL circulating in Europe (2) and at the beginning of 2015 a new severe

epidemic wave occurred. This study reports the detection and genetic characterization of PEDV circulating in Italian epidemic waves during 2015-2017 and the rapid spread of a recently discovered recombinant strain. Materials and Methods: From January 2015 to May 2017, feces or intestine samples of pig enteritis cases were analyzed by Si PEDV Real-Time PCR. Most of the samples came from the North of Italy (i.e. the area with the highest density of pig production) and only few from the rest of the country. To identify PEDV variants, 51 gene sequence was obtained from 489 out of 526 PEDV positive samples coming from 408 farms. For the phylogenetic analysis, 213 complete Si gene sequences were selected, excluding identical and/or partial nucleotide sequences. The tree was constructed by using the neighbor-joining method p-distance model and bootstrap test of 1,000 replicates in MEGA 6. Putative recombination breakpoints were determined using the Recombination Detection Program (RDP) 4.43. Results and discussion: Since January 2015, PEDV rapidly spread in the high-density pig production area in the North of Italy. Between 2015 and 2017, three peaks of outbreaks occurred during winter months. PEDV-reinfection occurred in 24.3% and 19.7% of previously infected farms during the second and third epidemic wave, respectively. The Si phylogenetic tree showed different clusters consistent with the hypothesis that different entry events could have occurred in Italy. Si gene sequencing of 190 samples (89.2%) showed a high sequence similarity with European S-INDEL strains circulating during 2014-2015 (98.5-100% for nucleotide and 97.4-100% for amino acid sequence). The remaining 23 samples (10.8%) shared a high degree of sequence identity (99.4-100% for nucleotide and 99.1-100% for amino acid sequence) with the PEDV strain SLOreBAS-1, identified in Slovenia during 2015 (KY019623). The Si gene of Slovene strain and Italian homologous strains contained a fragment (-400 nt) showing high identity with SeCoV. Recombination analysis detected breakpoints at position 240-636, suggesting the occurrence of a recombination event between PEDV and SeCoV. In Italy, the PEDV/SeCoV recombinant strain was detected for the first time in May 2016, but between January and April 2017 it rapidly spread. The PEDV/SeCoV strain has overcome the S-IN DEL 0H85 1-like strain, representing the 93.7% of the circulating strains. The emergence of new PEDV variants with potentially different pathogenic features is of great concern for swine health. A continuous molecular surveillance is important to allow implementation of efficient control measures.

Parigi° M, Massi° P, Fiorentini° L, Tosi° G, Romboli° C, Vandi L, Bocciero° R, Fregnani° G

#### **Valutazione dell'efficacia di una miscela di acidi organici e fitoterapici nel controllo dell'infezione da Escherichia coli nel tacchino**

Atti della Societa' Italiana di Patologia Aviare (SIPA) 2017 : LVI Convegno annuale, Il Simposio scientifico : Forli, 6-7 Aprile 2017 - Parma, 22 Settembre 2017 / [s.l. : s.n., 2017]. - p 141-145. - 6 bib ref [Nr. Estr. 7731]

Convegno annuale Societa' Italiana Patologia Aviare (SIPA) (56. : Forli Parma : 6-7 Aprile 2017 22 Settembre 2017)

*Colibacillosis is a systemic multifactorial disease caused by E. coli that still represents a major problem in the turkey industry worldwide. The aim of the present study was to test the effects of an enhanced feed acidifier on the replication of an antibiotic resistant E.coli. Ninety-six turkey poults of one day of age were randomly assigned to 4 poultry isolators (24 animals/isolator), fed with 4 different dietary treatments for 30 days and orally challenged with 1,38x10<sup>8</sup> cfu/mL of an antibiotic resistant E. coli strain. At day 20 and day 30, birds were euthanized in order to evaluate the presence of gross lesions of colibacillosis and an aliquot of their intestinal content and liver has been examined by bacteriological analysis. At any interval of the trial, birds of Group 2, treated with the highest dosage of acidifier, presented a lower E.coli population, both in the intestine and in the liver than the other groups and absent or mild lesions of colibacillosis. Thus, since feed acidifiers are included in poultry diets as alternative to antibiotic growth promoters, this E. coli reduction could lead to a reduced environmental contamination and, consequently, limit the horizontally diffusion of the pathogen.*

Parini M, Tosi° G, Coelho M, Buccioni A, Paoli A

### **Efficacy of a specific composition of short- and medium chain 1-monoglycerides in controlling *Clostridium perfringens* induced necrotic enteritis in broiler chickens**

Poultry Science Association Annual Meeting "Immunology, health, and disease II" : July 17-20, 2017 Orlando, Florida / [s.l. : s.n., 2017]. - 2 p. (poster 337) [Nr. Estr. 7734]

Poultry Science Association Annual Meeting : Orlando, Florida : July 17-20, 2017)

Necrotic enteritis (NE), in acute or subclinical forms, is a concern for the poultry industry, particularly in antibiotic-free poultry production. The causative agent of NE is the *Clostridium perfringens*, and one of predisposing factors is the *Eimeria* spp. colonization. The efficacy of a specific composition\* of 1-monoglycerides of short- and medium-chain fatty acids (1-MG), in reducing *C. perfringens* infection and *Eimeria* spp. colonization in broiler chickens was investigated. Ninety female one-day old Ross 308 broiler chicks, not vaccinated against coccidiosis, were randomly housed in poultry isolators and allotted to 3 Treatments. Birds were fed diets supplemented or not with 1-MG (Control diet, C: 0% in all periods: Treatment 1, G1: 0.5% from d 1 to d 10 and 0.25% from d 11 to d 21; Treatment 2, G2: 0.5% from d 1 to d 10 and 0.025% from d 11 to d 21). Each bird was orally infected at d 5 of life with 3,000 sporulated oocysts of *Eimeria acerrulina*, *maxima* and *tend/a*, and at d 11-12 with 10<sup>6</sup> cfu of *C. perfringens*. The *Clostridium* strain belonged to toxin type A, producing a-toxin in vitro and carrying the netB gene. On d 16, 21 and 35 of age 10 birds from each treatment were weighted and sacrificed to collect intestinal samples. A 4-point lesion scoring system according to Johnson and Reid (1970) was applied for each *Eimeria* species. Macroscopic gut lesions due to *C. perfringens* were evaluated according to the procedure of Keyburn et al. (2006). Results showed that *C. perfringens* and *E. tenella* intestinal lesion score in birds of Treatments G1 and G2 were significantly lower than in Control group ( $P < 0.01$  and  $P < 0.001$  respectively, according Chi-squared test, GraphPad Software): no mortality was recorded in treated groups G1 and G2 while the mortality in Control group was 3.3%. Weight gain in treated groups was significantly higher compared with Control group ( $P < 0.001$  - MIXED procedure of SAS). Results suggest that the specific 1-MG composition may prevent NE, decrease *Eimeria* spp. colonization, maintain high-standard weight gain and represent an alternative to enteric antibiotics in broiler feed programs. \*The 1-MG composition was supplied by SILO S.p.A. (Firenze).

Paternoster<sup>o</sup> G, Tomassone L, Tamba<sup>o</sup> M, Chiari<sup>o</sup> M, Lavazza<sup>o</sup> A, Piazzini M, Favretto AR, Balduzzi G, Pautasso A, Vogler BR

### **The degree of one health implementation in the West Nile virus integrated surveillance in Northern Italy, 2016**

Front Public Health. - Vol. 5 ( 2017). - Article no. 236 (10 p). - 17 bib ref [Nr. Estr. 7661]

West Nile virus (WNV) is endemic in the Po valley area, Northern Italy, and within the legal framework of the national plan for the surveillance of human vector-borne diseases, WNV surveillance has over time been implemented. The surveillance plans are based on the transdisciplinary and trans-sectorial collaboration between regional institutions involved in public, animal, and environmental health. This integrated surveillance targets mosquitoes, wild birds, humans, and horses and aims at early detecting the viral circulation and reducing the risk of infection in the human populations. The objective of our study was to assess the degree of One Health (OH) implementation (OH-ness) of the WNV surveillance system in three North Italian regions (Emilia-Romagna, Lombardy, Piedmont) in 2016, following the evaluation protocol developed by the Network for Evaluation of One Health (NEOH). In detail, we (i) described the OH initiative (drivers, outcomes) and its system (boundaries, aim, dimensions, actors, stakeholders) and (ii) scored different aspects of this initiative (i.e., OH-thinking, -planning, -sharing, -learning, transdisciplinarity and leadership), with values from 0 (=no OH approach) to 1 (=perfect OH approach). We obtained a mean score for each aspect evaluated. We reached high scores for OH thinking (0.90) and OH planning (0.89). Lower scores were attributed to OH sharing (0.83), transdisciplinarity and leadership (0.77), and OH learning (0.67), highlighting some critical issues related to communication and learning gaps. The strengths and weaknesses detected by the described quantitative evaluation will be investigated in detail by a qualitative evaluation (process evaluation), aiming to provide a basis for the development of shared recommendations to refine the initiative and conduct it in a more

OH-oriented perspective.

Peacock D, Kovaliski J, Sinclair R, Mutze G, Iannella A, Capucci° L

**RHDV2 overcoming RHDV immunity in wild rabbits (*Oryctolagus cuniculus*) in Australia**

Vet Rec. - Vol. 180 no 11 ( 2017). - no 280 (3 p). - 27 bib ref (ultimo accesso 12/06/2018  
<http://veterinaryrecord.bmj.com/content/180/11/280.2.info> ) [Nr. Estr. 7870]

Pellacini M, Canelli E, Luppi° A

**Mal rossino in suinetti sottoscrofa**

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 25-29. - 3 bib ref [Nr. Estr. 7558]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. :  
Reggio Emilia : 16-17 Marzo 2017)

Piredda I, Palmas B, Noworol M, Pintore P, Pedditzi A, Rebechesu L, Pintore A,  
D'Incau° M, Boniotti° MB, Bertasio° C, Cherchi M, Denurra D, Ponti MN

**First isolation of *Leptospira* spp from a dolphin (*Tursiops truncatus*) in the Mediterranean sea**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians  
(WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 249 (Poster 111). -  
4 bib ref [Nr. Estr. 7595]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD)  
(18th : Sorrento : June 7-10, 2017)

Pistone D, Pajoro M, Novakova E, Vicari° N, Gaiardelli C, Vigano R, Luzzago C,  
Montagna M, Lanfranchi P

**Ticks and bacterial tick-borne pathogens in Piemonte region, Northwest Italy**

Exp Appl Acarol. - Vol. 73 ( 2017). - p 477-491. - 32 bib ref [Nr. Estr. 7912]

A molecular screening for tick-borne pathogens was carried out in engorged and in questing ticks collected in Verbano Cusio Ossola county, Piemonte region, Italy. Engorged ticks were removed from wild and domestic animal hosts. The most abundant and common tick species in the area was *Ixodes ricinus* (192 adults, 907 nymphs). Few individuals of *Ixodes hexagonus* (15) and *Rhipicephalus sanguineus* (7) were found among the ticks removed from domestic animals (46 examined ticks). The presence of *Rickettsia* spp., *Borrelia burgdorferi* sensu lato, *Francisella tularensis* and *Coxiella burnetii* was evaluated by PCR and sequencing in 392 individuals of *I. ricinus* (adult and nymphal stages) and 22 individuals of the two other tick species. Five *Borrelia* species (i.e. *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, *B. valaisiana* and *B. lusitaniae*), proved or suspected to cause clinical manifestations of Lyme disease in humans, showed 10.5 and 2.2% combined prevalence in questing and engorged *I. ricinus*, respectively. In addition, two species of rickettsiae (*R. helvetica* and *R. monacensis*) were identified and reported with 14.5 and 24.8% overall prevalence in questing and in engorged ticks. The prevalence of *F. tularensis* in the ticks collected on two wild ungulate species (*Capreolus capreolus* and *Cervus elaphus*) was 5.7%. This work provided further data and broadened our knowledge on bacterial pathogens present in ticks in Northwest Italy.

Pregel P, Scaglione FE, Ruggeri° J, Chirullo B, Martinelli° N, Drumo R, Ammendola

S, Battistoni A, Corradi A, Ossiprandi MC, Alborali° GL, Pasquali P, Bollo E

**Histopathological findings in attenuated *Salmonella typhimurium* monophasic variant vaccinated piglets**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 269. - 1 bib ref [Nr. Estr. 7627]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

*Salmonella Typhimurium* and its monophasic variant are increasingly responsible of food borne infections in humans. The main source of infection is pork meat. Indeed, the subclinical disease in swine is the cause of the transmission of the infection into the human food chain [1]. Aim of this study was to evaluate the alterations of the intestinal mucosa of pigs immunized with an attenuated vaccine against *S. Typhimurium* monophasic variant AznuABC (mSTAznuAB) and experimentally infected with homologous and heterologous strains of *S. Typhimurium*. A histopathological assessment was performed in order to evaluate the effects of vaccination and infection on different intestinal tracts. Twenty weaned piglets were divided in 4 groups: 5 piglets were vaccinated with mSTAznuAB and infected with virulent *S. Typhimurium* monophasic variant (mST) (group A), 5 piglets were vaccinated with mSTAznuABC and infected with virulent *S. Typhimurium* (ST) (group B), 5 piglets were infected with virulent ST (group C) and 5 piglets were infected with virulent mST (group D). At day 20 after infection, piglets were euthanized and samples of tonsils, ileocecal lymph nodes, spleen, ileum, caecum and colon were collected for histological evaluation. A numerical value based on the degree of lesions was assigned to each examined intestinal tract, considering epithelium, submucosa and Peyer's patch conditions, congestion and lesion patterns.

Haemorrhages, congestion and lymphoid tissues were also scored in tonsils, ileocecal lymph nodes, and spleen. Statistical analysis was then performed by Two-Way Anova for Repeated Measures or by Kruskal-Wallis tests, assuming statistically significant differences when  $p < 0.05$ . Histologically, a diffuse epithelial conglutination was revealed in all the examined intestinal tracts and in all groups, associated to vascular congestion and lymph nodes depletion. Only in one case (group C, ileum) necrosis was revealed. Mucosal lesions were significantly more severe in caecum and colon of groups B and C. Differences were detected neither in lymphocyte amount and distribution, nor in mucosa and submucosa hemorrhages. No differences were revealed in spleen or lymph nodes for each of the examined parameters. In tonsils, a significantly higher congestion, as well as a higher activation of lymphoid follicles, were revealed in group A in comparison to group D. Although mSTAznuABC vaccine is able to reduce immune system colonisation and faecal shedding of homologous and heterologous virulent strains, the pattern of histological lesions was not clearly altered in the examined tissues. Only in group A tonsils appeared more reactive than in unvaccinated group.

Puggioli A, Bonilauri° P Calzolari° M, Lelli° D, Carrieri M, Urbanelli S, Pudar D, Bellini R

**Does *Aedes albopictus* (Diptera: Culicidae) play any role in Usutu virus transmission in Northern Italy? Experimental oral infection and field evidences**

Acta Trop. - Vol. 172 ( 2017). - p 192-196. - 38 bib ref [Nr. Estr. 7548]

This study evaluated the vector competence of *Aedes albopictus* in transmitting USUV after oral infection under laboratory conditions. *Ae. albopictus* showed a low vector competence for USUV, although the positive body sample found with a very high number of viral copies at one week post infection indicates that a replication in the mosquito body can occur, and that USUV can escape the midgut barrier. Field data from an extensive entomological arboviruses surveillance program showed a relevant incidence of *Ae. albopictus* USUV positive pools in the period 2009–2012 while all pools were negative from 2013 on. No conceivable explanation regarding this field evidence was

addressed, suggesting that attention must be paid to the trend of development of this vector-pathogen association, being aware of the potential rapid arbovirus' adaptation to new vectors, to prevent possible new disease's emergence.

Razzuoli E, Mignone G, Lazzara F, Masiello L, Ferraris M, Vencia W, Vivaldi B, Ferrari A, Amadori° M

#### **Impact of cadmium exposure on swine enterocytes**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 43. - 4 bib ref [Nr. Estr. 7610]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

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 and gut is the first target of interaction. However, little is known about Cd interaction with the intestinal tract (1). The aim of our study was to investigate the effects of low and high concentrations of Cd on swine enterocytes in terms of gene expression, cytotoxicity, Cd uptake, as well as of host-pathogen interaction. Swine intestinal IPEC-J2 cells were used as model. These were treated with 2 pM or 20 pM Cd solutions and incubated at 37°C in 5% CO<sub>2</sub> for 1, 3, 6 or 24 hours. The parameters under study were described in previous studies (2-3-4). Each test was performed twice, untreated wells being used as negative control. The statistical significance of differences among the experimental groups were evaluated by one-way ANOVA or Kruskal-Wallis test. The significance threshold was set at  $P < 0.05$ . The ability of IPEC-J2 cells to uptake Cd was investigated first. Our data showed a significant ( $P < 0.001$ ) increase of intracellular Cd after 3 ( $P < 0.001$ ), 6 ( $P < 0.001$ ) or 24 hours ( $P < 0.001$ ) of exposure with respect to 1 hour. This was confirmed for both 2 p.M ( $P < 0.001$ ) and 20 pM ( $P < 0.001$ ) Cd. The absorption of Cd was related to a significant reduction ( $P < 0.0001$ ) of cell viability after treatment with 20 pM Cd. No effects were shown after treatment with 2 pM Cd. Concerning the modulation of gene expression, cells treated with 2 pM Cd for 1, 3, 6 or 24 hours showed a significant increase ( $P < 0.05$ ) of inflammatory gene expression (IL-6, IL-8, MYD88, NFκB1, NFκB-p65, IL-18) at all-time points, respect to untreated wells. These data are in agreement with previous studies (1) and highlight a pro-inflammatory effect of low concentrations of Cd. Treatment with 20 p.M Cd caused up-regulation ( $P < 0.05$ ) of IL-8 after 1 hour of exposure followed by a reduction of IL-8, p38, NFκB1, CD14 and STAT3 gene expression after 3 hours of treatment. At the same time, we observed up-regulation of IL-18, TNF-α, MYD88, JNK, IFN-γ, BD1, BD2, TLR5 and MD2 gene expression. These effects were followed by up-regulation of Type I IFNs and IL-8 gene expression after 6 hours of exposure. 20 pM Cd caused up-regulation of IL-8, JNK, BD1, BD3 and BD4 and down-regulation of p38, NFκB1, MYD88, NFκB-p65 CD14, and TLR4 after 24 hours of treatment. These data support the ability of Cd to modulate inflammatory responses in swine enterocytes. Moreover, the down-regulation of inflammatory responses observed after 3 h of treatment with 20 p.M of Cd, was associated with a significant ( $P < 0.05$ ) reduction of Salmonella typhimurium penetration into IPEC-12 cells. In conclusion, our results indicate that exposure to Cd may modify the basal level of cytokine expression, thereby influencing different compartments of the innate immune response.

Re R, Bertocchi° L, Pilo G, Monteverde VP, Nicolussi PS

#### **Welfare and biosecurity risk assessment in dairy sheep : development of a check list**

Networking : tool for an excellent research: a public veterinary health without borders to face new emergencies : atti del 4° Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria : Roma 6

Aprile 2017 / edited by Marina Bagni, Antonio Petrini, Antonio Lavazza. - [Teramo] : Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, 2017. - (Veterinaria Italiana. Collana di Monografie ; Anno 53, Monografia ; 26) p 83 [Nr. Estr. 7722]

Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria (4 : Roma : 6 Aprile 2017)

Starting from methodology EFSA (Welfare Quality project), the National Reference Centre for Animal Welfare has perfected a new system for the welfare assessment, based both on management-based measures (M8M) and animal-based measures (ABM). Ruminant Welfare Project, funded by the Ministry of Health, has the purpose to Identify the main welfare risks for semi-extensive systems of dairy sheep through the development of a check list. It was decided to identify production categories (lactating sheep, dry sheep, weaned lambs, lambs up to 30 days, rams). Check list includes some macro areas: Area A — management and personnel procedures; Area B - facilities and equipment; Area C animal based measures, Area D bio-security, Area E great risks and alarm systems. Risk threshold (above which there is evidence of adverse effect) has been identified, as well as adverse effects on animal and risk measurement. The check list has been tested in 36 dairy sheep farms of Sardinia and Sicily; the most severe Identified risk factors have been in water availability (absence), Inadequate dimensions of the troughs, structural deficiencies in facilities.

Renzi° M, Santi° A, Procopio° A, Carra° E, Galletti° G, Paternoster° G, Di\_Nardo° S, Sabatino\_Ottavio M, Rugna° G, Tamba° M

**Serological and molecular survey of *Leishmania infantum* in stray cats in the Province of Bologna (Italy)**

6th World Congress on Leishmaniasis : 16th-20th May 2017, Toledo, Spain : abstracts book / [s.l. : s.n., 2017]. - p 1390 (Poster C0668 ) [Nr. Estr. 7758]

World Congress on Leishmaniasis (6th : Toledo, Spain : 16th-20th May 2017)

Background In May 2013, public health authorities of the Province of Bologna expressed a concern regarding a significative upsurge in human cases of visceral leishmaniasis, pointed out since November 2012. They found indeed 14 cases in six months, which represents an over five-fold increase compared with the annual average of 2.6 cases (range: 0-8) from 2008 to 2012 (Varani et al., 2013). Due to the importance of dogs as primary reservoir of *Leishmania* parasites, a canine leishmaniasis (CanL) surveillance program was launched in 2007, with the purpose of monitoring the presence of sandflies and infected dogs in the public kennels of Emilia-Romagna region (Santi et al., 2014). All data collected demonstrated that the yearly prevalence of CanL in this area dropped from 3.1% in 2013 to 1.1% in 2015. Although no specific information is available about the transmission of *Leishmania* spp. to cats, it is fair to assume that cats are susceptible to infection in the areas where *L. infantum* is present In 2015-2016 a survey program on stray cats living in the province of Bologna was implemented in order to obtain accurate informations on the prevalence of the disease in this species. 2 Methods 562 sera samples and 566 conjunctival swabs were collected from 566 stray cats captured for the sterilization program in the urban areas of the Province of Bologna. Sera samples were tested using Immunofluorescence technique (IFAT) according to the laboratory procedures described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OLE, 2014). Materials were provided by the National Reference Centre for Leishmaniasis (C.Re.Na.L., Sicily, Italy). Animals with IFAT titre of 1/80 were considered infected, as reported by Pennisi et al. (2012). Conjunctival swabs were analysed using Real Time PCR (Galletti et al., 2011). 3 Results IFAT was positive (serum titres ranged from 1:80 to 1:320) in 7 of 562 sampled cats (1.24%; IC95% 0.50- 2.55). Only 1 of 566 conjunctival swabs (0.17%; IC95/0 0.004-0.98) tested using RT-PCR was positive, but IFAT was negative. Each cat was clinically examined prior to sample collection and only one of them has been shown to be affected by ocular conjunctivitis. This cat was IFAT positive (1:320) but RT-PCR negative. Our findings confirm the presence of CanL infection in stray cats living in the Province of Bologna. Despite the seroprevalence in dogs and cats living in the same endemic area seems to be similar, generally cats do not show any clinical symptom.

Rigamonti° S, Floriano AM, Scaltriti E, Vecchio\_Nepita, E, Comandatore F, Casadei° G, Longbottom D, Laroucau K, Donati M, Vicari° N, Magnino° S

**Whole genome sequencing of C. avium and C. pecorum isolates from Italy**

4th European Meeting on Animal Chlamydiosis (EMAC) : 2017, Zagreb : book of abstract / [s.l. : s.n., 2017]. - 7667]

European Meeting on Animal Chlamydiosis (EMAC) (4. : Zagreb : 2017)

Few whole genome sequences of chlamydial isolates and strains of veterinary relevance have been published so far. In October 2016 we initiated the PRC2015003 project funded by the Ministry of Health of Italy with the aim of performing whole genome sequencing of chlamydial field isolates grown and stored at the Italian National Reference Laboratory for Animal Chlamydioses. Up to now, whole genome sequences have been obtained from two isolates of the collection: C'. pecorum PV 7855, recovered from a chamois affected with pneumonia, and C. avium PV 4360-2/99, isolated from a pigeon that showed no clinical signs nor lesions at necropsy. The two chlamydial isolates were grown in LLC-MK2 cells and their elementary bodies (EB) were purified according to the sucrose gradient separation protocol. Their genomic DNAs were extracted and libraries were prepared with the Nextera XT DNA Sample Preparation Kit (Illumina) and sequenced with MiSeq System instrument (Illumina). We obtained 4,329,111 paired-end reads for C. pecorum and 1,018,412 paired-end reads for C. avium. Genome sequences were subsequently obtained by applying a bioinformatic pipeline (1) aimed at selective assembly of the intracellular bacterial genomes from mixed reads (intracellular bacteria and host), and finishing was performed through bioinformatic techniques (2, 3). For C'. pecorum we obtained 2 contigs, together spanning —1.11 Mb (N50 = 788,718 bp, GC% = 41.1), while for C. avium we obtained 3 contigs that together reach —1.04 Mb in length (N50 = 1,040,914 bp, GC% = 36.9) The presence of a plasmid was detected in both isolates. Their lengths are 7,674 bp for C. pecorum and 7,640 bp for C. avium. Our results for C. avium PV 4360-2/99 are very similar to the ones published for the only genome of C. avium 10DC88 that has been completely sequenced so far (2 contigs, N50 = 1,041,170 bp, GC% = 36.9) (4). The comparison of the C. pecorum PV 7855 whole genome with the ones of the 12 isolates deposited so far in the NCBI gene bank is under way. Further comparisons will focus on SNPs and differences in the plasticity zone (PZ) and in the cluster of genes that encode polymorphic membrane proteins (PMPs), two genomic regions where nucleotidic variations are frequent. That might lead to identify additional genes and metabolic ways associated to the pathogenesis of chlamydial infections.

Rizzo F, Vicari° N, Braghin S, Ghia C, Belvedere M, David M, Magnino° S, Mandola ML

**Chlamydial infections in poultry : preliminary evidences in Piedmont, Northwestern Italy**

4th European Meeting on Animal Chlamydiosis (EMAC) : Zagreb, 2017 : book of abstracts / [s.l. : s.n., 2017]. - 1 p [Nr. Estr. 7754]

European Meeting on Animal Chlamydiosis (EMAC) (4th : Zagreb : 2017)

Rosignoli° C

**Il botulismo bovino : una patologia sporadica ma potenzialmente devastante per una mandria**

Ruminantia. - Vol. dicembre ( 2017). - 3 p (ultimo accesso 03/04/2018

<http://www.ruminantia.it/il-botulismo-bovino-una-patologia-sporadica-ma-potenzialmente-devastante-per-una-mandria/> ) [Nr. Estr. 7816]

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

**Infezione da virus influenza D nel bovino in Italia** = Influenza D virus infection in cattle in Italy  
Large Anim Rev. - Vol. 23 no 6 ( 2017). - p 123-128. - 22 bib ref [Nr. Estr. 7704]

Un nuovo genere di virus influenzale, provvisoriamente definito Influenzavirus D (IDV), è stato recentemente rilevato prima nel suino e poi nel bovino in diverse parti del mondo. L'obiettivo dello studio è stato quello di indagare sull'epidemiologia di questa nuova infezione negli allevamenti bovini italiani mediante l'applicazione di test molecolari, virologici e sierologici su campioni conferiti al laboratorio diagnostico tra aprile 2014 e ottobre 2016. Sono stati sottoposti ad un test Real-Time RT-PCR per IDV 895 campioni, suddivisi in 744 tamponi nasali e 151 tessuti polmonari, prelevati da bovini con o senza patologia respiratoria (BRDC) provenienti da 574 diversi allevamenti del Nord Italia. Sono risultati positivi a IDV 58 campioni (6,5%). Tra questi, 48 erano stati prelevati da soggetti con BRDC e 10 da bovini senza BRDC. Nel corso dell'indagine sono stati individuati 32 focolai di malattia respiratoria con presenza di positività per IDV. Isolamento virale è stato ottenuto da 3 polmoni e 8 tamponi nasali provenienti da animali di allevamenti diversi. L'analisi genetica ha evidenziato che i ceppi di influenza D italiani sono strettamente correlati tra loro e raggruppano in un cluster riferibile a D/swine/Oldahome/1334/2011. L'indagine sierologica, mediante l'applicazione del test di inibizione dell'emoagglutinazione su 420 sieri prelevati in 42 allevamenti da latte della provincia di Mantova, ha evidenziato la presenza di animali sieropositivi in tutte le unità produttive con una prevalenza globale pari al 92,4%. I dati emersi da questa indagine, non solo forniscono l'evidenza della diffusa circolazione di IDV nei bovini in Italia, ma depongono anche a favore del coinvolgimento di questo virus nell'eziologia del complesso della malattia respiratoria.

*Recently a new genus of influenza virus, tentatively named Influenzavirus D (IDV), was detected in swine and cattle firstly in USA and then in China, Japan, France, Mexico and in Italy. At present it seems that the bovine species is the reservoir of IDV and that this virus plays a role in bovine respiratory disease complex (BRDC). Aim - The aim of this study was to investigate the epidemiology of this new respiratory viral infection in bovine population in Northern Italy, an area with high density of swine and cattle farms, by applying molecular, virological and serological tests on samples submitted to the laboratory for routine diagnostic or surveillance investigations. Materials and method - Between April 2014 and October 2016, 895 samples, 744 nasal swabs and 151 lung tissues, from cattle with or without BRDC, were analyzed by Real-Time RT-PCR and virological methods. The samples originated from 574 different herds. Genetic characterization of IDV isolated strains was performed by NGS sequencing. A serological study, using HI test with the Italian isolate D/swine/Italy/199724/2015, was performed on 420 serum samples randomly selected from 42 farms located in Mantova province, an area where the virus was detected in several herds. Results and discussion - Fifty-eight samples (6.50/0) were found to be positive for IDV with RT-PCR. Among these, 48 samples were taken from cattle with BRDC and 10 from subjects without BRDC. IDV was identified in 32 outbreaks of respiratory disease, 5 in beef fattening unit and 27 in dairy herds. These farms were located in 12 different provinces of Northern Italy. In 20 outbreaks (62.5%) the IDV was the only respiratory virus detected, while in 12 outbreaks (37.5%) the infection was mixed with other major viral respiratory pathogens (BVDV, BRSV, BoHV-1, PI3V, BCoV). Eleven influenza D strains were isolated and their genetic analysis indicated that all clustered with the first detected virus D/swine/Oklahoma/1334/2011. Serology data showed that all the tested farms from Mantova province were positive to IDV, with a global animal prevalence amounted to a value of 92.4%. Conclusion - This study shows evidences of a widespread circulation of influenza D virus in cattle in Italy and provides some data on the involvement of this virus in the etiology of the bovine respiratory disease complex. Many other aspects concerning the field of epidemiology, virology and pathobiology of this new respiratory infection need to be investigated in the future to better delineate the role of Influenzavirus D in BRDC.*

Rossi G, De\_Leo G, Pongolini° S, Natalini S, Zarenghi L, Ricchi° M, Bolzoni° Luca

**The potential role of direct and indirect contacts on infection spread in dairy farm networks**

PLoS Comput Biol. - Vol. 13 no 1 ( 2017). - p e1005301 (19 p). - 51 bib ref ( ultimo accesso 28/02/2017 <http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1005301> ) [Nr. Estr. 7525]

Animals' exchanges are considered the most effective route of between-farm infectious disease

transmission. However, despite being often overlooked, the infection spread due to contaminated equipment, vehicles, or personnel proved to be important for several livestock epidemics. This study investigated the role of indirect contacts in a potential infection spread in the dairy farm network of the Province of Parma (Northern Italy). We built between-farm contact networks using data on cattle exchange (direct contacts), and on-farm visits by veterinarians (indirect contacts). We compared the features of the contact structures by using measures on static and temporal networks. We assessed the disease spreading potential of the direct and indirect network structures in the farm system by using data on the infection state of farms by paratuberculosis. Direct and indirect networks showed non-trivial differences with respect to connectivity, contact distribution, and super-spreaders identification. Furthermore, our analyses on paratuberculosis data suggested that the contributions of direct and indirect contacts on diseases spread are apparent at different spatial scales. Our results highlighted the potential role of indirect contacts in between-farm disease spread and underlined the need for a deeper understanding of these contacts to develop better strategies for prevention of livestock epidemics.

Rossi G, Smith RL, Pongolini<sup>o</sup> S, Bolzoni<sup>o</sup> L

**Modelling farm-to-farm disease transmission through personnel movements : from visits to contacts, and back**

Sci Rep. - Vol. 7 ( 2017). - Article no. 2375 (11 p). - 52 bib ref ( ultimo accesso 05/10/2017 <http://www.nature.com/articles/s41598-017-02567-6> ) [Nr. Estr. 7637]

Infectious diseases in livestock can be transmitted through fomites: objects able to convey infectious agents. Between-farm spread of infections through fomites is mostly due to indirect contacts generated by on-farm visits of personnel that can carry pathogens on their clothes, equipment, or vehicles. However, data on farm visitors are often difficult to obtain because of the heterogeneity of their nature and privacy issues. Thus, models simulating disease spread between farms usually rely on strong assumptions about the contribution of indirect contacts on infection spread. By using data on veterinarian on-farm visits in a dairy farm system, we built a simple simulation model to assess the role of indirect contacts on epidemic dynamics compared to cattle movements (i.e. direct contacts). We showed that including in the simulation model only specific subsets of the information available on indirect contacts could lead to outputs widely different from those obtained with the full-information model. Then, we provided a simple preferential attachment algorithm based on the probability to observe consecutive on-farm visits from the same operator that allows overcoming the information gaps. Our results suggest the importance of detailed data and a deeper understanding of visit dynamics for the prevention and control of livestock diseases.

Rovida F, Piralla A, Marzani FC, Moreno<sup>o</sup> A, Campanini G, Mojoli F, Pozzi M, Girello A, Chiapponi<sup>o</sup> C, Vezzoli<sup>o</sup> F, Prati<sup>o</sup> P, Percivalle E, Pavan A, Gramegna M, Iotti GA, Baldanti F

**Swine influenza A (H1N1) virus (SIV) infection requiring extracorporeal life support in an immunocompetent adult patient with indirect exposure to pigs, Italy, October 2016**

EuroSurveillance. - Vol. 22 no 5 ( 2017). - no 30456 (5 p). - 12 bib ref ( ultimo accesso 28/02/2017 <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=22707> ) [Nr. Estr. 7524]

We describe a case of severe swine influenza A(H1N1) virus infection in an immunocompetent middle-aged man in October 2016 in Italy who had only indirect exposure to pigs. The patient developed a severe acute distress respiratory syndrome which was successfully supported by extracorporeal membrane oxygenation and treated with antiviral therapy. The sole risk factor for influenza was a body mass index > 30 kg/m<sup>2</sup>. After a month of hospitalisation, the patient was discharged in good health.

Rubini° S, Armaroli E, D'incau° M, Tassinari M, Fontana° MC, Merialdi° G, Giacometti F

**Prevalence of *Salmonella Enterica subsp. enterica* in hedgehog (*Erinaceus europaeus*) in Ferrara province (Emilia Romagna, Italy)**

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 41 [Nr. Estr. 7735]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

Hedgehog (*Erinaceus europaeus*) is a small mammal, largely distributed in Europe, adapted to a nocturnal way of life. Hedgehog is omnivorous, and its diet includes invertebrate animals (e.g. worms, snails, spiders, centipede), vertebrate animals (e.g. frogs, toads, snakes, bird eggs), carrion, and vegetables (e.g. mushrooms, berries, melons, acorns). Hedgehogs have a fairly wide range of action, and in a night, they can walk 1-3 km, move in hunting areas that can range up to 30-100 hectares. Generally, specimens living in open environments move more than those who settle in wooded or sheltered areas. During the summer, they could change nest 0-30 times. Hedgehogs are host to a wide variety of bacterial and protozoan pathogens, of which a number have become a matter of concern to public health. In addition to vector-borne agents, hedgehogs are a potential reservoir for enteric bacteria (such as *Salmonella* and *Campylobacter*), and protozoan parasites (*Giardia* and *Cryptosporidium*), which may cause enteritis in humans, livestock, and pets. *Salmonella enterica subsp. enterica* is a microorganism largely distributed all over the world, able to survive for long time in the environment, and, it can cause disease in humans and animals. The primary transmission route to humans is believed to be food-borne (via the fecal-oral route through the ingestion of contaminated water or food), however, (indirect) contact with an animal reservoir can be an alternative source of infection. According to EFSA (European Food Safety Authority) *Salmonella* is the second most common cause of human gastroenteritis in Europe. The role of wildlife as *Salmonella* spp. carriers has been highlighted in several studies on a variety of species, included hedgehogs. Wild animals are relevant to the epidemiology of salmonellosis because of their role as healthy carriers of a broad range of *Salmonella* serotypes as observed by in the United States and in Norway. Additionally, similar strains have been isolated from both humans and wildlife, suggesting that wildlife species could be a reservoirs for *Salmonella* spp. From 2012 to date, 166 hedgehog carcasses have been tested for the presence of *Salmonella* spp.. All the hedgehogs have been collected in the Ferrara province and delivered to a local wildlife rehabilitation center. The animals that died in this center were stored frozen and, about once a week, delivered to the laboratory where a necropsy and the research of *Salmonella* spp. were carried out. In most cases the cause of death was due to traumatic injuries. The research of *Salmonella* spp. has been carried out from the intestine of the animals, according to ISO method 6579:2002/Amd 1:2007 using the selective medium MSR/V (Modified Semisolid Rappaport Vassiliadis). The suspected colonies were confirmed biochemically with API 20 E system and the serotyping of *Salmonella* strains was performed using commercial antisera; polyvalent *Salmonella* O and H antisera were used to obtain a presumptive diagnosis but the definitive antigenic designation was assigned using monovalent antisera following the White-Kauffman-Le Minor serotyping scheme. *Salmonella enterica subsp. enterica* was isolated in thirty-five samples out of the 166 tested (21,1%). Twenty-one strains out of 35 belonged to the serovar *S. Enteritidis* (60%). In the current study, hedgehogs in Ferrara areas harbor a not negligible number of zoonotic agents, and therefore may contribute to the spread and transmission of zoonotic diseases. Although the majority of *Salmonella* spp. infections are foodborne, other sources should not be ignored. Wildlife could play a notable role in maintaining *Salmonella* spp. in the environment, even if, to what extent can hedgehogs maintain the enteric zoonotic agents in natural cycles, and the role of (spill-back from) humans or other bred species, mostly avian species, remains to be investigated.

Rubini° S, Cangini M, Servadei I, Chendi S, Marino G, Barbieri S, Govoni G,

Salogni° C, Montanari° S, Pigozzi S, Milandri A

**Prymnesium parvum causes fish mortalities in brackish water ponds of Emilia Romagna (Italy)**

Toxicol Lett. - Vol. 280 Suppl 1 ( 2017). - p s204-s205 (P-06-01-09) [Nr. Estr. 7688]

Congress of the European Societies of Toxicology (EUROTOX) (53rd : Bratislava, Slovakia : 10th-13th September, 2017)

The Haptophyta *Prymnesium parvum* is a microscopic algal species that can form blooms in brackish waters worldwide, often resulting in fish mortality, making it an important Harmful Algal Bloom (HAB) forming species. It produces more than 15 bioactive compounds with a broad range of biological effects. An episode of fish mortality interested a system of brackish water ponds in Emilia Romagna region (Italy) in March 2017. Several species were affected, e.g. seabass (*Dicentrarchus labrax*), carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), grass carp (*Ctenopharyngodon idella*), pike-perch (*Sander lucioperca*), channel catfish (*Ictalurus punctatus*) and wels catfish (*Silurus glanis*). Ten fishes were collected and examined for necropsy, which revealed hemorrhagic lesions on the skin, base of the fins, gill congestion in all specimens. Viral detection was performed using a pool of viscera ( kidney, brain, heart, spleen) through isolation on fish cell lines (EPC and BF2) and resulted negative. Water was taken from four sites of the area including an adjacent tributary channel, although no mortalities were reported from that point. Samples were observed by inverted microscopy (Utermohl, 1958) to detect the presence of harmful phytoplankton and a maximum abundance of 38,187,072 cells L<sup>-1</sup> of *Prymnesium parvum* was determined in pond waters. Blooms of algae like *Prymnesium* are referred to as ecosystem disruptive algal blooms (EDABs), because of the serious damage they cause. These phenomena are conditioned by many factors, not all fully elucidated. In the last decades few similar episodes have been recorded in Emilia Romagna and need further studies.

Ruggeri° J, Chirullo B, Drumo R, Pesciaroli M, Martinelli° N, Scaglione FE, Pregel P, Ammendola S, Lazzaro° M, Giacomini° E, Ossiprandi MC, Corradi A, Battistoni A, Bollo E, Pasquali P, Alborali° GL

**Applicazione di protocolli vaccinali per il controllo di *Salmonella typhimurium* variante monofasica nell'allevamento suino** = Application of different vaccination protocols to control *Salmonella typhimurium* monophasic variant in pigs

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 223-228. - 11 bib ref [Nr. Estr. 7566]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo, 2017)

L'infezione umana sostenuta da mST è sempre più frequente negli ultimi anni ed il consumo di carne di suino contaminata rappresenta una delle principali fonti di trasmissione. Nell'allevamento suino l'infezione è controllata attraverso l'uso di antibiotici, tuttavia l'incremento di ceppi multiresistenti ha determinato una riduzione dei principi attivi utili per la terapia nell'uomo e negli animali e per tale motivo risulta necessario applicare nuove strategie di controllo. La vaccinazione rappresenta uno strumento efficace soprattutto se associato ad un'ottima gestione manageriale dell'allevamento. Tuttavia, vaccini efficaci e sicuri non sono attualmente disponibili per la profilassi in Italia. L'obiettivo di questo lavoro quello di descrivere i risultati ottenuti dalla vaccinazione con un ceppo attenuato di mST testato in condizioni sperimentali ed i risultati ottenuti dalla vaccinazione con un ceppo inattivato e stabulogeno di mST testato in condizioni di campo. Il protocollo vaccinale ideale caratterizzato dalla vaccinazione delle scrofe, prima del parto, con un vaccino inattivato o stabulogeno garantendo un'adeguata trasmissione di immunoglobuline alle nidiate attraverso il colostro. Successivamente, si completa con la vaccinazione dei suinetti con un vaccino attenuato che meglio attiva l'immunità cellulo-mediata. In questo modo, sia la componente umorale che quella cellulo-mediata sono correttamente stimulate garantendo una protezione completa.

*Human infection caused by *Salmonella Typhimurium* monophasic variant (mST) is increasingly frequent during last years and contaminated pork meats represent one of the most important source*

*of transmission. Control of infection in piggeries is principally based on administration of antibiotics in feed. Unfortunately, increment of multidrug-resistant strains has underlined the necessity to apply new control strategies. Vaccination, associated to good farming management, is effective, however, efficacious and safe vaccines are not available. The aim of project is to show results obtained from different research activities focused on application of a mST attenuated vaccine tested in experimental conditions and on application of a mST bacterins administered in animals of an endemic farms. An ideal vaccination protocol is characterized by vaccination of sows with an autogenous vaccine, before delivery, guaranteeing an adequate transmission of immunoglobulin through colostrums and vaccination of piglets with an attenuated vaccine which better activates cell-mediated immune system. In this way, both humoral and cell-mediated immune systems are correctly activated to protect animals.*

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

#### **Immune parameters discriminate PRRS "stable" from "unstable" farms**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 158 (Poster 025). - 6 bib ref [Nr. Estr. 7590]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento, Italy : June 7-10, 2017)

Ruggeri° J, Foresti F, Pavesi R, Terrini° A, Giudici° F, Padoan D, Corradi A, Ossiprandi MC, Pasquail P, Alborali° GL

#### **Reduction of Salmonella typhimurium monophasic variant shedding by administration of organic acids and phytochemical in pigs**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 316. - 4 bib ref [Nr. Estr. 7625]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

Salmonella Typhimurium (including S. Typhimurium 1,4,[5],12:i-), associated to other enteric pathogens and to other stressful factors, causes the Post Weaning Diarrhea in piglets. This disease is responsible for economic losses, for development of chronic infection and, thus, for the introduction of zoonotic bacteria into the slaughterhouse [1]. It is largely demonstrated the importance of controlling infection in farm for preventing disease in humans [2]. Furthermore, the increment of antibiotic resistance strains determines the necessity to find new control strategies, as administration of vaccines [3] or organic acids [4]. The aim of this trial was to assess the effects of organic acids and phytochemicals on controlling S. Typhimurium 1,4,[5],12:i- shedding. Animals were divided in three groups. In group A (460 piglets), animals were feed with a base diet added of additive (organic acids and phytochemicals) at the concentration of 1kg/t. In group B (460 piglets), additive was added to the water at the final concentration useful to obtain a final pH value equal to 4.5 in relation to water hardness. The last group (460 piglets) was the control group. Microbiological analyses were conducted on feces and environmental swabs to evaluate Salmonella-shedding in a representative number of animals (15 ear-tagged piglets) of each group. The sample timing was: a week before arrival (TO) and at day 0 (Ti), 15 (T2), 22 (T3), 43 (T4) and 57 (T5) after arrival. Serum samples were collected a week before arrival (TO) and at day 0 (Ti), 15 (T2), 43 (T4) and 57 (T5) after arrival to evaluate antibody titers against Salmonella sp. Results demonstrated that environmental swabs were positive before piglets arrival and after cleaning procedures. The shedding evidenced that animals were negative from breeding farm and started to shed Salmonella since day 15 post weaning. In treated animals (groups A and B), the shedding rapidly reduced and difference with control group was statistically significant at 15. The antibody titers reached the peak at the end of the trial, when bacterial load was extremely low especially in treated groups (A and B).

In conclusion, all pens were contaminated with *S. Typhimurium* 1,4,[5],12:i- and animals generally got infected at post weaning, one week after their arrive. Probably, the reason is that site 2 is a re-adapted farm which has severe structural problems. Nevertheless, the shedding rapidly decreased in treated groups (A and B) and bacteria load in feces was statistically different in comparison to control group at T5.

Salogni° C, Accini A, Borella° L, Alborali° GL

### **Setticemia da *Aeromonas salmonicida* ssp *salmonicida* in Persico spigola (*Morone chrysops* x *morone saxatilis*) d'allevamento**

Atti del XXIII Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) : Lecce 5-6 Ottobre 2017 / [s.l. : s.n., 2017]. - p 22 (O6) [Nr. Estr. SA7670]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (23. : Lecce : 5-6 Ottobre 2017)

Nel presente lavoro viene descritto un focolaio d'infezione da *Aeromonas salmonicida* ssp *salmonicida* in persico spigola noto anche come hybrid striped bass (*M. chrysops* x *M. saxatilis*). L'episodio si è verificato tra febbraio e maggio del 2017, in una piscicoltura alimentata da acque risorgive alla temperatura di 14-15°C del Nord Italia e caratterizzato da una mortalità giornaliera media del 0,5% circa, protrattasi ininterrottamente per circa tre mesi, raggiungendo un valore cumulativo di quasi il 50%. L'intera partita di pesci (circa 120.000 soggetti), del peso medio di 300 g, era stata introdotta da un altro allevamento alimentato da acqua di fiume circa 2 mesi prima l'inizio dell'episodio di mortalità. In una prima fase la sintomatologia era caratterizzata da apatia, dispnea, grave inappetenza, melanosi cutanea sulla quale spiccava una punteggiatura bianca che veniva riferita ad Ittioftiriosi. Successivamente, nell'arco di circa un mese, l'episodio evolveva clinicamente con comparsa di emorragie ed ulcere cutanee diffuse, unitamente ad un aumento di mortalità. Circa dieci pesci, tra quelli con lesioni più evidenti, venivano quindi campionati e inviati al laboratorio di analisi per essere sottoposti ad accertamenti anatomopatologici, parassitologici e culturali. L'esame autoptico evidenziava emorragie ed ulcere cutanee di varia gravità ed estensione, lesioni oculari (esoftalmo ed oftalmite) e branchiali (iper mucosita ed emorragie), emorragie viscerali, congestione e aumento di volume della milza, distensione della vescica natatoria per accumulo di gas. Gli esami parassitologici (a fresco su impronta e striscio d'organo: cute, branchie ed intestino) rilevavano la presenza di una lieve infestazione protozoaria da *Ichthyophthirius multifiliis* da cute e branchie. L'esame colturale, eseguito su terreni di primo isolamento (agar sangue e TSA), permetteva l'isolamento di colonie in purezza da tutti i visceri in tutti i soggetti analizzati (forma setticemica). Queste erano caratterizzate da un color bianco-crema, diametro massimo dopo 48-72 ore d'incubazione di 2-3 millimetri, aspetto liscio, piatto e rotondo con margini netti e da un alone di emolisi. Su TSA è stato possibile valutare la presenza di una pigmentazione bruna a partire da 48h di incubazione. La colorazione di Gram evidenziava dei coccobacilli Gram negativi. La tipizzazione del germe isolato come 6 stata eseguita tramite caratterizzazione biochimica (macrometodo, sistemi miniaturizzati API20NE (V8.0), API20E (V5.0), metagenomica (analisi del gene 16S rRNA) e sierologica (agglutinazione rapida). La fenotipizzazione con macrometodo similmente al sistema miniaturizzato API20NE ha consentito una chiara e precisa identificazione del microrganismo in oggetto come *A. salmonicida* ssp. *salmonicida*, dimostrandosi più specifico° rispetto all'API 20E che lo ha invece tipizzato come *A. salmonicida* ssp. *masoucida/achromogenes* (% ID 64,1). L'esame sierologico, che ha previsto l'utilizzo di due distinti antisieri per *A. salmonicida* ssp. *salmonicida* (Mono AS — Bionor, Siero Policlonale 2999/1976 - IZSLER) in test di agglutinazione rapida su vetrino, ha evidenziato in entrambi i casi una agglutinazione specifica. L'indagine genetica indipendentemente dalla banca dati utilizzata (Microseq e gene bank BLAST) pur identificando *Aeromonas salmonicida* non hanno permesso di differenziare la sottospecie. Il microrganismo isolato è stata quindi sottoposto a saggio di sensibilità antibiotica con metodica Kirby-Bauer. Sulla base di quest'ultima, il trattamento terapeutico iniziato con mangime antibiotato con ossitetraciclina, è stato sostituito dapprima con sulfamidico potenziato e quindi con flumequina senza dare tuttavia risultati apprezzabili. Tale inefficacia è stata imputata alla pressoché totale inappetenza dei pesci. Solo il trasferimento dello stock ittico in acque più calde (18-20 °C) ha determinato uno spontaneo miglioramento della sintomatologia e rapida diminuzione della mortalità fino alla completa risoluzione del focolaio. Sebbene l'infezione da *A. salmonicida* ssp. *salmonicida* sia stata già

descritta nel persico spigola, è tuttavia rara ed i lavori scientifici a tal riguardo sono pochi. Il presente studio va quindi ad arricchire tale casistica e rappresenta la prima segnalazione della patologia per tale specie sul territorio nazionale.

Salogni° C, Gibelli° LR, Pigoli° C, Lavazza° A, Prosperi° A, Villa° R, Ruggeri° J, Alborali° GL

**Indagine istopatologica ed eziologica su lesioni papillomatose rilevate in una popolazione selvatica di Barbo italico (*Barbus plebejus* Bonaparte, 1839) e Barbo europeo (*Barbus barbus* Linnaeus, 1758) nel fiume Adda**

Atti del XXIII Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) : Lecce 5-6 Ottobre 2017 / [s.l. : s.n., 2017]. - p 68 ( P25 ) [Nr. Estr. 7669]

Convegno Nazionale Società Italiana di Patologia Ittica (SIPI) (23. : Lecce : 5-6 Ottobre 2017)

Le lesioni proliferative cutanee a carattere neoplastico sono descritte con una certa frequenza nei pesci ed osservate sia in soggetti selvatici che allevati, sia in acque dolci che salate. Esse sono anche denominate "Idiopathic Epidermal Proliferation (IEP)" in merito alla loro poco chiara eziologia. Tra le IEP troviamo le lesioni iperplastiche e papillomatose, descritte in numerosi ciprinidi tra cui il barbo europeo. La loro eziologia è stata di volta in volta riferita all'azione di agenti infettivi virali (herpesvirus, adenovirus, retrovirus ecc.) o ad altri fattori quali l'inquinamento o i traumatismi; in tutti i casi risulta tuttavia difficile associare univocamente la patologia ad un preciso agente eziologico. L'incidenza delle lesioni, nei casi dove è stata indicata, ha subito un sensibile incremento quando una popolazione ittica è stata sottoposta a eventi particolarmente stressanti. Lo scopo di questo lavoro è quello di indagare su lesioni proliferative cutanee "papilloma-like" rilevate in una popolazione naturale di barbo nel fiume Adda. Quest'ultima è stata individuata da pescatori sportivi che frequentano assiduamente tale Fiume tra i comuni di Olginate e Brivio (LC), a valle del Lago di Lecco e a monte della diga di Robbiate. I primi pesci affetti sono stati catturati tramite amo e lenza a partire dal mese di Gennaio del 2016. Successivamente si è assistito ad un progressivo incremento di incidenza, sebbene con fasi alterne, tale da far sospettare una possibile contagiosità. È tuttavia impossibile quantificare con precisione l'entità dei soggetti colpiti, non essendo mai stati effettuati campionamenti mirati a tale scopo (es.: reti, elettropesca). Fluttuazioni sono state osservate durante l'anno, probabilmente connesse alla migrazione dei pesci per motivi riproduttivi o alimentari. Nel tratto di Adda segnalato sono presenti numerose specie di pesci tra cui ciprinidi come il barbo: 1) autoctono barbo italico (*B. plebejus*), il barbo europeo (*B. barbus*) e probabilmente i loro ibridi. Nessun'altra specie oltre al barbo è stata segnalata come interessata dal problema. Nel mese di marzo del 2017 tre barbi sono stati campionati e sottoposti ad esame necroscopico, esame istologico, esame parassitologico a fresco da cute, branchie ed intestino; esame batteriologico colturale su terreni agarizzati (agar sangue e TSA) da cervello, fegato, rene, milza e cute; esame virologico da cute e pool di visceri (rene, milza, cervello e cuore) tramite infezione di linee cellulari (EPC and BF2), esame al microscopio elettronico a trasmissione (colorazione negativa a goccia), ELISA (attività retroscrittasi) e diagnostica molecolare (PCR per papillomavirus). L'esame necroscopico ha evidenziato lesioni proliferative cutanee con superficie irregolare, digitiforme e a volte mammellonata, localizzate prevalentemente lungo i fianchi o alle pinne. Le dimensioni erano variabili da 5 a 70 mm. L'esame parassitologico ha evidenziato la presenza di *Piscicola geometra* da cute. L'esame istologico, eseguito con colorazione ematossilina-eosina, ha evidenziato delle lesioni cutanee papillomatose, caratterizzate da iperplasia delle cellule epidermiche con proiezioni digitiformi multiple supportate da connettivo fibrovascolare; al centro delle lesioni papillomatose erano presenti delle cellule mucose. Gli esami batteriologici e virologici hanno dato esito negativo. Sebbene siano stati eseguiti numerosi esami l'eziologia della malattia rimane tuttora ignota. Sono stati tuttavia individuati alcuni fattori ambientali che possono avere agito da importanti stressogeni sulla popolazione ittica affetta, tra i quali si segnala il basso livello idrico e la presenza di una intensa attività predatoria esercitata dall'avifauna.

Salogni° C, Gibelli° LR, Pigoli° C, Lavazza° A, Ruggeri° J, Alborali° GL

**First detection of papillomatous lesions affecting a wild population of Italic barbel (*Barbus plebejus* Bonaparte, 1839) and European barbel (*Barbus barbus* Linnaeus, 1758) in Northern Italy**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 268. - 3 bib ref [Nr. Estr. 7626]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

Skin tumors are recorded in many fish species. They are observed in wild and bred animals and are reported as Idiopathic Epidermal Proliferation (IEP) [3] related to the detection of the unclear etiology. Epidermal hyperplasia and papilloma were described in many cyprinids as the European barbel [2]. The hyperplasia and papilloma were associated to different virus infections and other factors as pollution and mechanical stimulation [1]. Particularly, lesion incidence may increase in a stressed fish-population. The aim of the present work is to describe a case of skin proliferative lesions, like "papilloma", occurred in a wild barbel population in the Adda River. The affected population was detected by a local sportive fisherman that attended the river in the tract between Olginate and Brivio countries. This place is sited between the Lecco Lake and Robbiate dump. The affected fishes were firstly detected in January 2016 and increasing thereafter, but it was impossible to quantify the percentage. Variations were observed during the year, probably connected to fish migration. Different cyprinid species are present in the river, in particular, there are two different species of barbel: the Italic barbel (*Barbus plebejus*) and the imported European barbel (*Barbus barbus*) and, probably, their hybrids. No other fishes, except for barbels, were affected and none notification of the disease was reported in Italy before. In march 2017, three barbels were sampled and processed for different exams: necropsy; histopathology; parasitological examination of skin, gills and gut scrubs by direct light microscopy observation; bacterial examination of brain, liver, kidney and spleen by cultural detection on solid medium; and viral detection of skin and a pool of internal viscera (kidney, brain, heart and spleen) through isolation on fish cell lines (EPC and BF2) followed by confirmation with transmission electronic microscopy. During necropsy, proliferative papilloma-like lesions were observed and they were mainly localized on the fins and hips of the fishes. Their size varied from 5 to 70 mm. Parasitological examination evidenced a weak infestation only of the skin with *Piscicola geometra*. Cultural detection using solid medium did not evidence bacterial growth. Viral investigations were negative, no cytopathic effect has been detected on cell monolayer and no viral particles have been observed by electronic microscopy. Histopathological investigation of the skin lesions showed hyperplasia and multiple finger-like projections of thickened epidermal cells supported by fibro-vascular connective tissue (papillomatous lesions). Mucous cells were also present in the center of papillomatous folds. Although many investigations were performed, the etiology of the case was unresolved. Moreover some environmental elements were identified as predisposing factors responsible for the onset of the disease.

Salvadori C, Lazzerini C, Formenti° N, Trogu T, Lanfranchi P, Rossi L, Obber F, Citterio C, Poli A

**Trombiculosis in Northern chamois (*Rupicapra r. rupicapra*) from Western Italian Alps : prevalence and pathological findings**

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 33 [Nr. Estr. 7739]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

The Trombiculidae family includes more than three thousand species with a worldwide distribution. Of the seven stages in the life cycle of Trombiculidae, only larvae (chiggers) may infest a wide range

of terrestrial vertebrates including mammals, birds and reptiles. In wild ruminants, trombiculosis has been recently reported in white-tailed and sika deer and the Alpine or Northern chamois. Aims of this study were to further investigate the epidemiology of trombiculosis in the Alpine chamois in Western Italian Alps and to describe the related histopathological changes. Superficial scraping and skin samples were obtained from 191 alpine chamois during the hunting season 2015 (September-December). Sixty chamois originated from Lombardy Region (Lecco province) and 131 from Piedmont Region (Verbano-Cusio-Ossola province). Superficial skin scraping was performed on lesions bearing areas, or election site of infection in asymptomatic subjects. Clarification with 10% potassium hydrate solution was followed by morphological identification of mites at 10X magnification. Skin biopsies were also collected from affected and un-affected areas. Specimens were fixed into 10% neutral buffered formalin and routinely processed for histology. Five-micrometer thick sections were stained with hematoxylin and eosin (HE), Toluidine Blue (TB), PAS (periodic Acid-Schiff) and Goldner trichrome staining. Immunohistochemistry labelling on skin sections was performed and number of eosinophils, mast cells, CD3, CD79a, CD68-positive cells were counted on eight 10,000 p.m2 random fields of each biopsy using a semiautomatic analysis system (LASV 4.3, Leica, Germany). Eosinophils were counted on HE sections. Mast cells were counted on TB stained slides. Forty-one (41/191; 21.5%) chamois scored skin scraping positive for presence of Trombiculidae larvae. Of them, 15 were from Lombardy and 25 from Piedmont. Histologically, trombiculid mites were localized on the surface of the epidermis, over the keratin layer. Generally, a low number of mites (1-2 mites at 10X HPF) were detected, except for a pinnae skin biopsy where mites were more numerous (4-6 mites at 10X HPF). Focally extensive or diffuse slight to moderate parakeratotic hyperkeratosis was observed. Rarely slight crusting was also detected. Multifocal erosions were observed only in the fore-mentioned pinnae skin specimen. No alopecia was evident in histological sections. In the superficial dermis, an eosinophilic stylostome with a diaphanous not stained center was associated with mites. Stylostome was surrounded by inflammatory infiltrates and necrotic debris and associated with erosion/necrosis of epidermis and slight exocytosis of degenerated neutrophils. Inflammatory infiltrates were composed mainly by macrophages and T-lymphocytes. Degenerated neutrophils, eosinophils and B-lymphocytes were also present. Mast cells were rarely observed. Inflammatory cells were also moderately diffused in the surrounding superficial dermis. Based on data, trombiculosis is frequent in chamois in the Western Italian Alps, with similar prevalence in Lombardy and Piedmont regions (19% and 25%, respectively). Macroscopical lesions were moderate with involvement of body regions in contact with ground, especially muzzle (pinnae, and areas around eyes and mouth) and limbs, characterized by thin skin where stylostome is easily formed. Infested chamois, showed a normal body condition, except for a 2-year-old female whose body mass was lower than in peers. Histologically, trombiculosis in alpine chamois causes a focal moderate to severe dermatitis with epidermal necrosis, crusts and hyperkeratosis. Lesions were like those described in white-tailed deer or other wildlife. Inflammatory infiltrates are suggestive of granulomatous reaction facing on stylostome, formed to mite saliva and necrotic debris of host epidermal and dermal tissue. However, a wide difference of immune response was detected in affected chamois that could reflect the development of a hypersensitivity reaction to the mites, as described in cats. Finally, chiggers may have a potential zoonotic role as suggested by frequent co-occurrence of infection in humans and animals and their vector role for *Rickettsia* spp., *Bartonella* spp., and other pathogens. In chamois, the role of trombiculosis as vector of other diseases is unknown and further investigations are warranted.

Salvioli° M, Pasquali S, Lavazza° A, Zanoni° M, Guberti V, Chiari° M, Gilioli G

**EBHS in European brown hares (*Lepus europaeus*) : disease dynamics and control**

Hystrix Ital J Mamm. - Vol. 28 no 2 ( 2017). - p 202-207. - 28 bib ref [Nr. Estr. 7746]

Brown hares have undergone a substantial population decline in Europe during recent decades, caused by, among other factors, the occurrence of European Brown Hare Syndrome (EBHS). To improve our knowledge regarding EBHS epidemiology, we developed a mathematical model that takes into consideration both brown hare biology and the infection dynamics of the EBHS virus (EBHSV). The model consists of eight ordinary differential equations simulating the spread of the virus in a closed hare population. Simulations showed that EBHSV's transmission has complex dynamics, which are strongly affected by the hare density. In particular, a density threshold of 7

individuals/km<sup>2</sup> was identified, determining two opposite epidemiological patterns: the extinction of the EBHSV below the threshold and its endemic stability when the hare population density is above the threshold, with a seroprevalence proportional to the population density. The model was validated using serological data collected in different areas in the province of Brescia (Northern Italy). The results suggested that the maintenance of the endemic circulating viral level through density control mechanisms is the best strategy for reducing EBHS's impact.

Sandri GP, Alborali° GL, Corradi A, Cantoni AM, Giovanardi D

**Un episodio particolarmente grave di encefalomiocardite (EMCV) in suinetti di svezzamento (lo strano caso dei suinetti morti all'improvviso in svezzamento)**

Atti Convegno SIPAS. - Vol. 43 ( 2017). - p 21-23. - 1 bib ref [Nr. Estr. 7560]

Meeting Annuale della Societa' Italiana di Patologia ed Allevamento dei Suini (SIPAS) (43. : Reggio Emilia : 16-17 Marzo 2017)

Scaravelli° D, Tosi° G

**Parasitological ecology of the vole *Microtus savii* : bacteriological aspects**

3. Convegno Nazionale sui piccoli mammiferi "I piccoli mammiferi fra gestione e conservazione" : 8-9 novembre 2017, Colle di Val d'Elsa (SI) : riassunti / [s.l. : s.n., 2017]. - p 44 [Nr. Estr. 7733]

Convegno Nazionale sui piccoli mammiferi (3. : Colle di Val d'Elsa (SI) : 8-9 novembre 2017)

*Microtus savii* (de Selys-Longchamps, 1838) is one of the most widespread mammal on Italian territory and surprisingly few information is available on its parasitological niche. In the main frame of a study on the zoonotic potential of wildlife species coming close or living in urban environment, it was studied the presence of zoonotic bacteria in feces of the Cricetidae. Collection of feces was done in trap were only living *Microtus savii* were caught in Imola and Forli surroundings. Feces were analyzed at Istituto Zooprofilattico sperimentale della Lombardia e dell'Emilia Romagna at Forli, following the standard bacteriological methods, using Agar Globuli and Hektoen Enteric Agar culture media. The petri dishes are then aerobically incubated at 37 ° C for 24-48 hours. The colonies obtained were tested by Gram staining and subjected to catalase test (with 3% peroxide) and identification by BBL Enterotube 110 (Bekton Dickinson) miniaturized system. The colonies identified were then stored in the Institute's strains collection. On 30 samples analyzed, 10 have not shown the growth of any potentially zoonotic bacteria. Among others there was the presence on one case each of *Acinetobacter baumannii*, *Acinetobacter haemoliticus*, *Aerococcus viridans*, *Citrobacter koseri*, *Clavibacter michiganensis*, *Coomebacterium accolens*, *Coryne bacterium macginleyi*, *Escherichia colt*, *Edwardsiella tarda*, *Enterococcus faecalis*, *Staphylococcus xylosus* and *Yersinia enterocolitica*. Also 2 cases each with *Acinetobacter lwoffii*, *Acintobacter baumannii*, *Staphylococcus lentus* and 3 cases of presences of *Bacillus* spp. In this scenario *Microtus savii* can be considered a potentially good reservoir for a series of zoonotic bacteria that in human can be responsible of a variety of intestinal disturbs. Study funded by the Ministry of Health in the research project PRC 2013/019 "Wildlife disturbance, synanthropy and possible zoonotic risk".

Scaravelli° D, Tosi° G, Priori P, Massi° P, Dottori° M

**Mapping ticks and tick borne diseases in the transition area between mediterranean and continental zones in Italy**

Final conference on neglected vectors and vector-borne diseases (Eurnegvec) with management committee and working group meetings of the COST Action TD1303 : Chania, Greece, 11-13 September 2017 : abstract book / [s.l. : s.n., 2017]. - p 68 [Nr. Estr. 7729]

Conference on neglected Vectors and Vector-Borne diseases (Eurnegvec) with MC and WG meetings of the COST Action TD1303 : Chania, Greece : 11-13 September 2017)

One of the most interesting scenario where is important to achieve knowledge about tick distribution and prevalence of the different TBD is represented by the border between the Mediterranean and Continental biogeographic belt. This is also important in an era where the global change represents a serious threat when diseases climate related are involved. To try to modelling the possible relationship between landscape, climate and tick presences, a project to map by GIS platforms the ticks distribution in Romagna was started. Romagna region shares parts of its territory both to Mediterranean climate belt and to continental zone and it can represent an important area where analyze the relationship between landscape parameters, ticks and TBDs presence. All the information on ticks distribution and on *Borrelia*, *Babesia*, *Ehrlichia* and *Anaplasma* are under collection, with the more accurate position by coordinates. The different layers of topography, climate, vegetation cover, presence of ungulates and human distribution will be connected with the presence of ticks. During the collection of information was discovered that locally rare species are underestimated as just locally abundant (e.g. *Hyalomma marginatum*) or related to less distributed wild hosts (*Ixodes vespertilionis*, *Carios vespertilionis*). Also will be given new attention to the sampling by dragging in sub-Mediterranean and sub-mountain habitats as well as less known hosts as horses and sheep wild grazing or the carnivores and the rodents. All the new samples will be checked by PCR for the presence of TBDs. The preliminary atlas of ticks of the region will be presented.

Scaravelli° D, Tosi° G, Priori P, Massi° P, Dottori° M

#### **Exploring small mammal and reptile ticks borne diseases in Romagna Region in Italy**

Final conference on neglected vectors and vector-borne diseases (Eurnegvec) with management committee and working group meetings of the COST Action TD1303 : Chania, Greece, 11-13 September 2017 : abstract book / [s.l. : s.n., 2017]. - p 67 [Nr. Estr. 7730]

Conference on neglected Vectors and Vector-Borne diseases (Eurnegvec) with MC and WG meetings of the COST Action TD1303 : Chania, Greece : 11-13 September 2017)

Given that the role of less known hosts for ticks is totally underestimate locally, we started a project with the aim to study the composition of tick community in small mammals and reptiles in Romagna. This region, of 6380 square km, cover the last part of the Po Plain in northern Italy and it is delimited to the south by the Apennine ridge and to the east from the Adriatic Sea. The most common species studied here for ticks presences are the ungulates (*Capreolus capreolus*, *Cervus elaphus*, *Sus scrota*) and *Vulpes vulpes* among carnivores. We are collecting new information about the ticks in small mammals and reptiles. *Erinaceus europaeus* can represent a very good host model for ticks ecology seeing as how it is present and widespread in the wild and also common in anthropogenic habitats. The tick community present a large number of species here dominated by number by *Pholeoixodes hexagonus*. Despite the repeated findings of bacterial zoonoses including *E. coli* and various *Salmonella* (*S. typhimurium*, *veneziana*, *napoli*, *enteritidis*), in few cases we could isolate *Rickettsia* sp. but *Borrelia* sp. was not isolated in the sample at present checked despite it sampled in hedgehog collected in areas with the illness also expressed in humans. Rodents are well known as an important basin for TBDs and we are actually investigating the role of *Myodes glareolus* and *Apodemus sylvaticus* in different forested areas to evaluate their role as potential reservoir for *Borrelia* and *Anaplasma*. Also the locally most widespread vole species, *Microtus savii*, is under study for the first time. This species is very common in all the agricultural areas in Italy and it reaches also pen-urban areas, but its mainly subterranean habitus seems to favor fleas as main ectoparasites with a very poor presence of ticks. Ticks in reptiles, especially lizards, seem to increase but just anecdotally information are historically available. The data actually available are related to few hot spot area in the Apennine where *Ixodes ricinus* nymphs appear to be more common on *Lacerta bilineata* than on *Podarcis muralis*. The ongoing sampling is significantly increasing the knowledge of all these species.

Sonnenburg J, Ryser-Degiorgis MP, Kuiken T, Ferroglio E, Ulrich RG, Conraths FJ, Gortázar C, Staubach C and APHAEA project partners [for Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna Chiari° M, Lavazza° A)

**Harmonizing methods for wildlife abundance estimation and pathogen detection in Europe : a questionnaire survey on three selected host-pathogen combinations**

BMC Vet Res. - Vol. 13 ( 2017). - no 53 (10 p). - 21 bib ref ( ultimo accesso 04/04/2017  
<http://bmcvetres.biomedcentral.com/articles/10.1186/s12917-016-0935-x> ) [Nr. Estr. 7532]

Background: The need for wildlife health surveillance as part of disease control in wildlife, domestic animals and humans on the global level is widely recognized. However, the objectives, methods and intensity of existing wildlife health surveillance programs vary greatly among European countries, resulting in a patchwork of data that are difficult to merge and compare. This survey aimed at evaluating the need and potential for data harmonization in wildlife health in Europe. The specific objective was to collect information on methods currently used to estimate host abundance and pathogen prevalence. Questionnaires were designed to gather detailed information for three host-pathogen combinations: (1) wild boar and Aujeszky's disease virus, (2) red fox and *Echinococcus multilocularis*, and (3) common vole and *Francisella tularensis*. Results: We received a total of 70 responses from 19 European countries. Regarding host abundance, hunting bags are currently the most widely accessible data source for widely distributed mid-sized and larger mammals such as red fox and wild boar, but we observed large differences in hunting strategies among countries as well as among different regions within countries. For small rodents, trapping is the method of choice, but practical applications vary among study sites. Laboratory procedures are already largely harmonized but information on the sampled animals is not systematically collected. Conclusions: The answers revealed that a large amount of information is available for the selected host-pathogen pairs and that in theory methods are already largely harmonized. However, the comparability of the data remains strongly compromised by local differences in the way, the methods are applied in practice. While these issues may easily be overcome for prevalence estimation, there is an urgent need to develop tools for the routine collection of host abundance data in a harmonized way. Wildlife health experts are encouraged to apply the harmonized APHAEA protocols in epidemiological studies in wildlife and to increase cooperation.

Tamba° M, Caminiti° A, Prosperi° A, Desprès P, Lelli° D, Galletti° G, Moreno° A, Paternoster° G, Santi° A, Licata E, Lecollinet S, Gelmini° L, Rugna° G, Procopio° A, Lavazza° A

**Accuracy estimation of an indirect ELISA for the detection of West Nile Virus antibodies in wild birds using a latent class model**

J Virol Methods. - Vol. 248 ( 2017). - p 202-206. - 33 bib ref [Nr. Estr. 7698]

West Nile virus (WNV) and Usutu virus (USUV), genus *Flavivirus*, are members of the Japanese encephalitis virus antigenic complex, and are maintained primarily in an enzootic cycle between mosquitoes and birds. WNV is zoonotic, and poses a threat to public health, especially in relation to blood transfusion. Serosurveillance of wild birds is suitable for early detection of WNV circulation, although concerns remain to be addressed as regards i) the type of test used, whether ELISA, virus neutralization test (VNT), plaque reduction neutralization test (PRNT), ii) the reagents (antigens, revealing antibodies), iii) the different bird species involved, and iv) potential cross-reactions with other *Flaviviruses*, such as USUV. The authors developed an indirect IgG ELISA with panavian specificity using EDIII protein as antigen and a monoclonal antibody (mAb 1A3) with broad reactivity for avian IgG. A total of 140 serum samples were collected from juvenile European magpies (*Pica pica*) in areas where both WNV and USUV were co-circulating. The samples were then tested using this in-house ELISA and VNT in parallel. Estimation of test accuracy was performed using different Bayesian two latent class models. At a cut-off set at an optical density percentage (OD%) of 15, the ELISA showed a posterior median of diagnostic sensitivity (DSe) of 88% (95%PCI: 73–99%) and a diagnostic specificity (DSp) of 86% (95%PCI: 68–99%). At this cut-off, ELISA and VNT (cut-off 1/10) performances were comparable: DSe = 91% (95%PCI: 79–99%), and DSp = 77% (95%PCI:

59–98%). With the cut-off increased to 30 OD%, the ELISA DSe dropped to 78% (95%PCI: 52–99%), and the DSp rose to 94% (95%PCI: 83–100%). In field conditions, the cut-off that yields the best accuracy for the ELISA appears to correspond to 15 OD%. In areas where other Flaviviruses are circulating, however, it might be appropriate to raise the cut-off to 30 OD% in order to achieve higher specificity and reduce the detection of seropositive birds infected by other Flaviviruses, such as USUV.

Trevisi E, Minuti A, Mezzetti M, Soares JFF, Federica R, Bani P, Amadori<sup>o</sup> M

### **Monensin affects the innate immune response in the rumen**

71° Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET), XVII Convegno S.I.C.V, XV Convegno S.I.R.A, XIV Convegno AIPVet, XII Giornata Studio So.Fi.Vet, IV Convegno RNIV : I Convegno ANIV 28 Giugno - 1 Luglio 2017, Napoli / [s.l. : s.n., 2017]. - p 49. - 2 bib ref [Nr. Estr. 7612]

Convegno Nazionale della Societa' Italiana delle Scienze Veterinarie (SISVET) : 71 Convegno SICV : 17 Convegno SIRA : 15 Convegno AIPVet : 14 Convegno So.Fi.Vet : 12 Convegno RNIV : 4 Convegno ANIV : 1 : Napoli : 28 Giugno - 1 Luglio 2017)

Monensin is a ionophor with a selecting capacity against Gram- rumen bacteria. In the overall, it increases the production of the rumen propionic acid and fosters liver gluconeogenesis. Previously, we observed that epithelial cells of the bovine forestomachs can recognize and respond differently to changes of rumen milieu and that cows show distinct tendencies to leukocyte infiltration into rumen fluids, in accordance to specific diets and dangerous fermentations (1). Considering the positive metabolic effects Monensin offered as rumen bolus during the transition period, we investigated its effects on rumen fermentations and innate immune responses of the forestomachs. On the whole, 43 cows (13 heifers and 30 multiparous) were allocated to two homogeneous groups: Kexx, which received 32.4 g of monensin by Controlled Release Capsule (Kexxtone®, Elanco Animal Health, UK) 21 days before expected calving, and Ctrl (control). Cows were frequently monitored during the transition period for health status, milk yield and quality, inflammo-metabolic profile (2). Moreover, individual rumen samples were collected with an oro-gastric probe at 30 days in milk (DIM), 6 hours after feed distribution. pH was measured immediately after withdrawal and rumen samples were collected with or without a cryoprotectant (fetal bovine serum and dimethyl sulfoxide). Samples were frozen at -80°C for flow cytometry and molecular assays (CD45= total leukocytes; IL-A24=granulocytes and monocytes; CD3=T lymphocytes; sIgM=B lymphocytes; IGCL=Immunoglobulin light chain) and at -20°C for volatile fatty acids (VFA) and ammonia. Rumen data were analyzed with ANOVA (SAS Inst. Inc., Cary, NC), considering the fixed effect of treatment and parity. As opposed to parity (not significant), treatment led to significant differences. Kexx cows at 30 DIM showed a similar pH (around 6.45), and lower concentration of ammonia (58.7 vs 93.3 mg/L of Ctrl; P<0.05), a higher concentration of propionic acid (24.5 vs 22.4 nno1/100 mol of Ctrl; P<0.10, tendency) and a reduced acetic:propionic ratio (2.5 vs 2.8 of Ctrl; P<0.10, tendency). Moreover, Kexx vs Ctrl cows showed a lower number of B lymphocytes (1.66 vs 2.50%; P<0.01) and numerically lower amounts of immunoglobulins (IgM and total Ig) and T lymphocytes. The reduced prevalence of T lymphocytes was more marked in heifers receiving monensin (P<0.10, tendency, vs Ctrl). On the whole, the activity of the innate immune system was more pronounced in the rumen of Ctrl cows, suggesting that monensin could have contributed to stabilize the rumen milieu and to attenuate the inflammatory responses that commonly occur in forestomachs around calving. These data were supported by clinical inspections (the significant lower incidences of diseases observed in the first two months of lactation) and metabolic data (lower NEFA and BOHB concentrations after calving) observed in the same Kexx cows (2). Our data confirm that nutritional and physiological changes in dairy cows can modify the innate immune response of forestomachs and that the evaluation of the rumen fluid can help evaluate animal health and welfare.

Trevisi P, Latorre R, Priori D, Luise D, Archetti<sup>o</sup> I, Mazzoni M, D'Inca R, Bosi P

### **Effect of feed supplementation with live yeast on the intestinal transcriptome profile of weaning pigs orally challenged with Escherichia coli F4**

Animal. - Vol. 11 no 1 ( 2017). - p 33-44. - 31 bib ref [Nr. Estr. 7295]

The ability of live yeasts to modulate pig intestinal cell signals in response to infection with Escherichia coli F4ac (ETEC) has not been studied in-depth. The aim of this trial was to evaluate the effect of Saccharomyces cerevisiae CNCM I-4407 (Sc), supplied at different times, on the transcriptome profile of the jejunal mucosa of pigs 24 h after infection with ETEC. In total, 20 piglets selected to be ETEC-susceptible were weaned at 24 days of age (day 0) and allotted by litter to one of following groups: control (CO), CO + colistin (AB), CO+5x10<sup>10</sup> colony-forming unit (CFU) Sc/kg feed, from day 0 (PR) and CO+5x10<sup>10</sup> CFU Sc/kg feed from day 7 (CM). On day 7, the pigs were orally challenged with ETEC and were slaughtered 24 h later after blood sampling for haptoglobin (Hp) and C-reactive protein (CRP) determination. The jejunal mucosa was sampled (1) for morphometry; (2) for quantification of proliferation, apoptosis and zonula occludens (ZO-1); (3) to carry out the microarray analysis. A functional analysis was carried out using Gene Set Enrichment Analysis. The normalized enrichment score (NES) was calculated for each gene set, and statistical significance was defined when the False Discovery Rate % was <25 and P-values of NES were <0.05. The blood concentration of CRP and Hp, and the score for ZO-1 integrity on the jejunal villi did not differ between groups. The intestinal crypts were deeper in the AB (P=0.05) and the yeast groups (P<0.05) than in the CO group. Antibiotic treatment increased the number of mitotic cells in intestinal villi as compared with the control group (P<0.05). The PR group tended to increase the mitotic cells in villi and crypts and tended to reduce the cells in apoptosis as compared with the CM group. The transcriptome profiles of the AB and PR groups were similar. In both groups, the gene sets involved in mitosis and in mitochondria development ranked the highest, whereas in the CO group, the gene sets related to cell junction and anion channels were affected. In the CM group, the gene sets linked to the metabolic process, and transcription ranked the highest; a gene set linked with a negative effect on growth was also affected. In conclusion, the constant supplementation in the feed with the strain of yeast tested was effective in counteracting the detrimental effect of ETEC infection in susceptible pigs limits the early activation of the gene sets related to the impairment of the jejunal mucosa.

Vecchio D, Bertocchi<sup>o</sup> L, Galiero G, Romano F, Corrado F, Noschese R, De\_Carlo E

#### **The Mediterranean Italian buffalo : research for supply chain sustainability**

Networking : tool for an excellent research: a public veterinary health without borders to face new emergencies : atti 4<sup>o</sup> Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria : Roma 6 Aprile 2017 / edited by Marina Bagni, Antonio Petrini, Antonio Lavazza. - [Teramo] : Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, 2017. - (Veterinaria Italiana. Collana di Monografie ; Anno 53, Monografia ; 26) p 40-43. - 5 bib ref [Nr. Estr. 7605]

Convegno Nazionale sulla Ricerca in Sanità Pubblica Veterinaria (4 : Roma : 6 Aprile 2017)

The report describes the activities developed by the CReNBuF to support the buffalo industry through three research projects. These activities focus on biosecurity, welfare and farm products, the results of each projects have been integrated in order to provide practical tools for use by farmers company veterinarians and the NHS. The results obtained can also be applied to other sectors and constitute a working model for the integration of different public bodies, various skills and livestock rearing.

Vecchio D, Tamaio JE, Bertocchi<sup>o</sup> L, De\_Carlo E

#### **Development of a new approach to evaluate buffalo welfare, the Ruminant Welfare<sup>®</sup> project**

Rev Colomb Cienc Pecu. - Vol. 30 Supl ( 2017). - p 231-233. - 4 bib ref [Nr. Estr. 7792]

Animal welfare is a growing, compelling and urgent topic because of the interest that it generates among the citizens and consumers. The goal to be fulfilled is to define systems and protocols for assessing animal welfare that should be impartial, reproducible and science-based on risk analysis. On these premises, the Italian Ministry of Health has signed an agreement with the IZSLER, through the CReNBA and IZSM through the CReNBuf on development and management of a system to evaluation of welfare and biosecurity in buffaloes breeding system. The checklist development was based on the Risk Assessment Methodology as suggested by EFSA. Building on the CReNBA's work developed on the welfare dairy cow, this method is based on the analysis of two data groups: The assessment of the hazards (non-ABMs) occurring as a result of environmental conditions; the assessment of the risks, with the concerned adverse effects (ABMs), run by animals living in those environments. The system developed consists in 83 observations, listed in a multiple-choice checklist divided in five macroareas: Farm management and personnel, Facilities and equipment, Animal based measures, Biosecurity and Alarm systems. The result of each area also provides an indication of the burden and importance of each of these on the final calculation of the animal welfare value. These checks represent a functional and smart instrument to allow assign a numerical animal welfare index to each farm. In addition, by the data collected in each area, to supply at the Veterinarians and breeders the tools to improve farm management and structures, respecting the farm's sustainability, and is preparatory to given to the development of a Ministerial trademark for animal welfare, giving answers to consumers and add value to the correct activities of the farmers.

Velarde R, Cavadini° P, Neimanis A, Cabezón O, Chiari° M, Gaffuri° A, Lavín S, Grilli G, Gavier\_Widén D, Lavazza° A, Capucci° L

**Spillover events of infection of brown hares (*Lepus europaeus*) with rabbit haemorrhagic disease type 2 virus (RHDV2) caused sporadic cases of an European brown hare syndrome-like disease in Italy and Spain**

Transbound Emerg Dis. - Vol. 64 ( 2017). - p1750-1761. - 46 bib ref [Nr. Estr. 7567]

Rabbit haemorrhagic disease virus (RHDV) is a lagovirus that can cause fatal hepatitis (rabbit haemorrhagic disease, RHD) with mortality of 80–90% in farmed and wild rabbits. Since 1986, RHDV has caused outbreaks in rabbits (*Oryctolagus cuniculus*) in Europe, but never in European brown hares (*Lepus europaeus*, EBH). In 2010, a new RHDV-related virus, called RHDV2, emerged in Europe, causing extended epidemics because it largely overcame the immunity to RHDV present in most rabbit populations. RHDV2 also was identified in Cape hare (*Lepus capensis* subsp. *mediterraneus*) and in Italian hare (*Lepus corsicanus*). Here, we describe two distinct incidents of RHDV2 infection in EBH that occurred in Italy (2012) and Spain (2014). The two RHDV2 strains caused macroscopic and microscopic lesions similar to European brown hare syndrome (EBHS) in hares, and they were genetically related to other RHDV2 strains in Europe. EBHs are common in Europe, often sharing habitat with rabbits. They likely have been exposed to high levels of RHDV2 during outbreaks in rabbits in recent years, yet only two incidents of RHDV2 in EBHs have been found in Italy and Spain, suggesting that EBHs are not a primary host. Instead, they may act as spillover hosts in situations when infection pressure is high and barriers between rabbits and hares are limited, resulting in occasional infections causing EBHS-like lesions. The serological survey of stocked hare sera taken from Italian and Spanish hare populations provided an understanding of naturally occurring RHDV2 infection in the field confirming its sporadic occurrence in EBH. Our findings increase the knowledge on distribution, host range and epidemiology of RHDV2.

Veldkamp T, Hocking PM, Vinco° LJ

**Less foot pad lesions by nutritional adjustments**

Proceedings of the 11th Turkey Science and Production Conference : Chester, UK, March 9th - 10th, 2017 / [s.l. : s.n., 2017]. - p 24-27. - 6 bib ref [Nr. Estr. 7551]

Veldkamp T, Hocking PM, Vinco<sup>o</sup> LJ

**Effect of crude protein concentration and dietary electrolyte balance on litter quality, foot pad dermatitis, growth performance and processing yields in two medium heavy turkey hybrids**

Br Poult Sci. - Vol. 58 no 5 ( 2017). - p 557-568. - 36 bib ref [Nr. Estr. 7764]

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

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**Whole-genome sequencing and typing of Italian isolates of Francisella tularensis subsp. holarctica**

18th International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD), June 7-10, 2017 : Sorrento, Italy : abstract book / [s.l. : s.n., 2017]. - p 327 (Poster 183). - 4 bib ref [Nr. Estr. 7603]

International Symposium of the World Association of Veterinary Laboratory Diagnosticians (WAVLD) (18th : Sorrento : June 7-10, 2017)

Viganò R, Rotelli L, Formenti<sup>o</sup> N, Cerutti MC, Citterio C, Lanfranchi P

**Alpine galliformes, domestic birds and introductions for hunting : sanitary and management risk factors**

Atti del IV Congresso Nazionale di Ecopatologia della Fauna SIEF : 11-13 Ottobre 2017, Domodossola / a cura di Stancampiano L. ... [et al.]. - [s.l. : SIEF, 2017]. - p 56 [Nr. Estr. 7740]

Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

Biodiversity in natural ecosystems is the essential condition for their existence. This concept has to be related to at least three interdependent contexts of diversity: natural ecosystem diversity (regarding environments in which life is present), specific diversity (defined "species richness" in a specific area) and genetic diversity (variety of genes of a species). Since each ecosystem is the result of the interaction of various species, both plants and animals that live in, it is sufficient that one of these species is missing to compromise the dynamic balance of the whole ecosystem. In the Alps, the modification of natural habitats due to the abandon of traditional activities and to the

development of tourist infrastructure, above all those for winter sports; the extinction of native species; the introduction of foreign species; the appearance of new parasite strains and, finally, climate changes are source of extreme danger to biodiversity conservation. Conservation and management of threatened species have an essential role in the biodiversity maintenance; for this reason, during last years, veterinary medicine has enlarged its knowledge beyond traditional clinical and zootechnical fields. Because of their high biological and conservational value, alpine Galliformes are considered as the target species of this concept. In the Italian Alps, both hunted species, such as alpine rock partridge (*Alectoris graeca saxatilis*) and white ptarmigan (*Lagopus muta*); and protected species, such as capercaillie (*Tetrao urogallus*), are in the red list of IUCN and considered vulnerable; while black grouse (*Lyrurus tetrix*) and hazel hen (*Tetrastes bonasia*), although not threatened, need precautionary measures of management. Notwithstanding, shared guidelines for the management of hunting activity, agroforestry and tourism are still missing. Moreover, attention is seldom paid to the health status of these populations, including possible interaction with rural poultry and or farmed wild birds that are released for hunting purposes. Genetic interactions have been largely demonstrated between rock partridge and red-legged partridge (*Alectoris rufa*), not only in Maritime Alps where the two species are innately present, but also where red-legged partridge is not native (Western Alps), as evidence of illegal introductions for hunting. Moreover, other studies and photographic evidences have documented the introduction of some chukar partridge (*Alectoris chukar*), very similar to rock partridge, but very unsuitable for the alpine territory. Furthermore, releases of grey partridge (*Perdix perdix*), quail (*Coturnix coturnix*), and pheasant (*Phasianus colchicus*) for hunting dogs training are still common not only in low-altitude areas less suitable for alpine Galliformes but also in their typical environments. In addition, the presence of domestic avifauna in the alpine pastures should be considered, that could contaminate the soil with gastrointestinal parasites, such as *Heterakis* spp and *Raillietina* spp, found in rock partridge and black grouse population. It is proven that alpine Galliformes evolved together with some gut parasites, such as *Ascaridia compar*, *Aonchotheca caudinflata* and some *Coccidia*, reported in alpine, Scandinavian, Icelandic and Scottish populations; but it is necessary to pay attention to new helminth strains and to the interaction between host and parasite. In this respect, it should be noticed as an example the completely lack of gut parasites in some meta-populations of white ptarmigan in the Alps, in particular Western Alps. This imbalance, probably linked to critical events influencing population dynamic, underlines white ptarmigan vulnerability. Climate changes on Alps had caused temperate and bit snowy winters and hot and wet summers, negatively affecting alpine Galliformes survival and the evolution of their environment, also determining higher survival of infectious parasitic stages in the soil. In fact, UV rays are less able to penetrate the expanding forest cover, thus losing their role in limiting the vitality of helminth eggs; while the lower thermal excursion and the fewer number of days with under 0 °C temperature promote eggs survival also during winter season. This situation may therefore determine an increase of gut protozoa and helminths in relation to the habitat of the species and to the climate conditions. Stress is finally another important factor able to influence alpine Galliformes health status. Winter and summer tourism in suitable area for the species is demonstrated to have a negative effect on these populations in relation to the use of their habitat and to the health and immune status of hosts. For all the reasons above, cooperation between wildlife managers, wildlife conservation experts and veterinarians is needed, to develop a risk profiling model and draw situation based guidelines for both rural poultry raising and famed game birds release in alpine areas important for the conservation of Alpine Galliformes and for biodiversity in general.

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#### **Identifying on-farm factors affecting foot pad lesions**

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Turkey Science and Production Conference (11th : Chester, UK : March 9th - 10th, 2017)

Zehender G, Veo C, Ebranati E, Carta V, Rovida F, Percivalle E, Moreno° A, Lelli° D, Calzolari° M, Lavazza° A, Chiapponi° C, Baioni° L, Capelli G, Ravagnan S,

Da\_Rold G, Lavezzo E, Palu' G, Baldanti F, Barzon L, Galli M

**Reconstructing the recent West Nile virus lineage 2 epidemic in Europe and Italy using discrete and continuous phylogeography**

PLoS One. - Vol. 12 no 7 ( 2017). - p e0179679 (16 p). - 44 bib ref ( ultimo accesso 05/12/2017 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0179679> ) [Nr. Estr. 7699]

West Nile virus lineage 2 (WNV-2) was mainly confined to sub-Saharan Africa until the early 2000s, when it was identified for the first time in Central Europe causing outbreaks of human and animal infection. The aim of this study was to reconstruct the origin and dispersion of WNV-2 in Central Europe and Italy on a phylodynamic and phylogeographical basis. To this aim, discrete and continuous space phylogeographical models were applied to a total of 33 newly characterised full-length viral genomes obtained from mosquitoes, birds and humans in Northern Italy in the years 2013–2015 aligned with 64 complete sequences isolated mainly in Europe. The European isolates segregated into two highly significant clades: a small one including three sequences and a large clade including the majority of isolates obtained in Central Europe since 2004. Discrete phylogeographical analysis showed that the most probable location of the root of the largest European clade was in Hungary a mean 12.78 years ago. The European clade bifurcated into two highly supported subclades: one including most of the Central/East European isolates and the other encompassing all of the isolates obtained in Greece. The continuous space phylogeographical analysis of the Italian clade showed that WNV-2 entered Italy in about 2008, probably by crossing the Adriatic sea and reaching a central area of the Po Valley. The epidemic then spread simultaneously eastward, to reach the region of the Po delta in 2013, and westward to the border area between Lombardy and Piedmont in 2014; later, the western strain changed direction southward, and reached the central area of the Po valley once again in 2015. Over a period of about seven years, the virus spread all over an area of northern Italy by following the Po river and its main tributaries.