INTRODUCTION: Control of neglected tropical disease is on the global public health agenda, therefore accurate diagnosis is a crucial basis for epidemiology and assessment of disease burden. The current diagnostic guidelines should be updated to include new tools for parasitology diagnosis and improved with the platform of digital technology. Transmission of knowledge should be enhanced by using informatics learning material and World Wide Web. One example of the importance of updating guidelines and teaching supports is the current manual on “Basic laboratory methods in human parasitology”, which was edited in 1991 by the World Health Organization (WHO).

The aim of this project is to update the WHO manual by adding new techniques for faecal and urine examination, but also by including practical tutorials available as video clip on DVD and on the WHO YouTube website. MATERIALS AND METHODS: The manual is meant to be a multi-centre project, and it will implicate collaboration of many different groups. The partners involved have been chosen by the WHO, among the most competent and leading groups in the development and validation of parasitological diagnostic methods. The manual will be divided in three main sections: general aspects, basic techniques and a new set of images of parasites that were formerly published in the WHO “Bench Aids for the Diagnosis of Intestinal Parasites”. RESULTS: The first part will deal with microscope care and calibration, safety of the operators and precautions for the safe collection, handling and disposal of specimens. A tutorial for the organization of stool and urine collections in a school setting will also be included in this section especially for Mass Drug Administration programmes. The second part will focus on laboratory work and specifically on the following techniques: Formol-ether concentration, Cellophane thick-smear (Kato-Katz thick smear), McMaster egg counting method, Mini-FLOTAC, Bearmann, Harada-Mori, Scotch test for pinworm, urine sedimentation, urine filtration and reagent strip for haematuria, Circulating Cathodic Antigen and Circulating Anodic Antigen test for Schistosoma. High-resolution images of parasites will be selected for the new edition of the Bench Aids. In the third part of the manual. Under the leadership and coordination of WHO, video tutorials will focus on the processing of samples with different techniques. The tutorials include the process from sample collection to processing and reading in the laboratory. The video clips are shot on the field, in order to develop a training tool suitable for all kind of laboratories and technicians. Parasite images are being collected from the different collaborators and selected for the new electronic edition of the Bench Aids. The video tutorials will be made freely available on the WHO YouTube channel. Electronic versions of the manual will also be prepared.

CONCLUSIONS: This project is meant to be an up-to-date reference guide for health workers and laboratory technicians who intend to apply updated parasitological diagnostic techniques based on standard operating procedures recommended by the WHO. This innovative project will aid to broad dissemination of the manual, even in resource-limited settings. In addition, it will improve coordination and easiness of comparing data from surveys that will use standard diagnostic methods and protocols. A broad dissemination of the manual is envisaged, particularly in resource-limited settings.
Predicting West Nile virus (WNV) circulation and the risk of WNV epidemics is difficult due to complex interactions of multiple factors involved. Surveillance systems that timely detect virus activity in targeted areas, and allow evidence-based risk assessments may therefore be necessary. Since 2009, a system integrating environmental (mosquitoes and birds) and human surveillance has been implemented and progressively improved in the Emilia-Romagna region, Italy. The objective is to increase knowledge of WNV circulation and to reduce the probability of virus transmission via blood, tissue and organ donation. As of 2013, the system has shown highly satisfactory results in terms of early detection capacity (the environmental surveillance component allowed detection of WNV circulation 3–4 weeks before human cases of West Nile neuroinvasive disease (WNND) occurred), sensitivity (capacity to detect virus circulation even at the enzootic level) and area specificity (capacity to indicate the spatial distribution of the risk for WNND). Strong correlations were observed between the vector index values and the number of human WNND cases registered at the province level. Taking into consideration two scenarios of surveillance, the first with environmental surveillance and the second without, the total costs for the period from 2009 to 2013 were reduced when environmental surveillance was considered (EUR 2.093 million for the first scenario vs EUR 2.560 million for the second). Environmental surveillance helped to reduce costs by enabling a more targeted blood unit testing strategy. The inclusion of environmental surveillance also increased the efficiency of detecting infected blood units and further allowed evidence-based adoption of preventative public health measures.
conditions, while for the determination of honeys botanical origin they are not suitable and it is necessary to use other analytical characterisations. To satisfy this interest of honey consumers, many scientific studies, using different instrumentations, have been published, but these methods generally use only one analytical technique at time. In this work 68 honey samples (acacia, chestnut, orange, linden, honeydew, sunflower, rhododendron) were analysed by Raman spectroscopy (RAMAN) and near infrared spectroscopy (NIR). Our goal is to evaluate if the information contained in the combined data provided by these rapid analytical techniques, analysed by Multiple Factor Analysis, may improve the discrimination of the honeys according to their botanical origin, when compared to the single analyses discrimination ability. Multiple Factor Analysis (MFA) is a generalization of Principal Component Analysis (PCA). Its objective is to analyse several sets of variables collected on the same set of samples, to provide a set of common factor scores (often called 'compromise factor scores') and then project each of the original datasets onto the compromise to analyse the communalities and discrepancies. In this work two set of variables (RAMAN and NIR) are used to perform MFA. The final results of work showed that RAMAN and NIR have, individually, a relative good ability to correctly classify honeys according to their botanical origin while the application of MFA algorithm has allowed to greatly improve the correct classification of the honey samples.

Bolzoni G, Buffoli E

Post 2015 : cosa accadrà dopo la fine delle quote latte


3Rs and new frontiers in laboratory techniques

Caloni F, Ferrari M, De Angelis I

Toxicology and stem cells: new frontiers


Human and entomological surveillance of Toscana virus in the Emilia-Romagna region, Italy, 2010 to 2012

Toscana virus (TOSV), transmitted by phlebotomine sandflies, is recognised as one of the most important causes of viral meningitis in summer in Mediterranean countries. A surveillance plan based on both human and entomological surveys was started in 2010 in the Emilia-Romagna region, Italy. Clinical samples from patients with neurological manifestations were collected during 2010 to 2012. The surveillance protocol was improved during these years, allowing the detection of 65 human infections. Most of these infections were recorded in hilly areas, where sandflies reach the highest density. Entomological sampling around the homes of the patients resulted in a low number of captured sandflies, while later sampling in a hilly area with high number of human cases (n=21) resulted in a larger number of captured sandflies. Using this approach, 25,653 sandflies were sampled, of which there were 21,157 females, which were sorted into 287 pools. TOSV RNA was detected by real-time PCR in 33 of the pools. The results highlighted the role of Phlebotomus perfiliewi as the main vector of TOSV and a potential link between vector density and virus
Circulation. This integrated system shows that an interdisciplinary approach improves the sensitiveness and effectiveness of health surveillance.

Calzolari M, Defilippo F, Zani G, Colombo M, Dottori M

Characterization of necrophagus entomofauna in a typical agricultural area in Emilia-Romagna region (Northern Italy)


This study presents a checklist of Dipterans and Coleopterans accountable for carrion decay in the Po Valley (Italy), a contribution to the Forensic Entomology knowledge in Italy. Insects colonizing two pig carcasses in an agricultural area in Mezzani municipality (Parma, Northern Italy) were sampled by pitfall traps and original Malaise-like traps, which allowed the sampling of a very relevant number of flying insects. A checklist of 57 taxa was obtained, of which 26 were considered of forensic importance. For the latter the arrival time of adult specimens on the carcasses was recorded, as an important parameter in minimum post mortem interval estimation. Dipterans (6141 specimens) were the most common insects; the fastest specimens to detect and colonize the carcass belonged to the Calliphoridae family, while Fanniidae and Muscidae infested the carrion until completion of the skeletal stage. Coleopterans appeared later (308 adult specimens and 114 larvae were captured). Staphylinidae, Dermentidae, and Histeridae were the most common coleopterans sampled in this study.


Weather factors influencing the population dynamics of Culex pipiens (Diptera: Culicidae) in the Po Plain Valley, Italy (1997–2011)


The impact of weather variables on Culex pipiens L. (Diptera: Culicidae) population dynamics in the Po Valley, Northern Italy, a densely populated region containing the largest industrial and agricultural areas in Italy, was investigated. Monitoring of mosquitoes was carried out by using CO2-baited traps without light, collecting data weekly from 1700 to 0900 hours during the period May-September, from 1997 to 2011. Daily minimum, average, and maximum relative humidity; daily minimum, maximum, and average temperature; rainfall; and hydroclimatic balance (rainfall-potential evapotranspiration) were obtained from three weather stations within the surveillance zone. The average population dynamic trend over the 15-yr period showed a bell-shaped curve with a major peak in June and a secondary peak at the end of August in the rural areas, whereas bimodality was not evidenced in the urban areas. The correlation analyses showed that the mosquito seasonal population and the population in the period of maximum West Nile virus circulation (August-September) was mostly affected by the relative humidity registered from March to July, particularly in May, and, to a lower extent, also by hydroclimatic balance registered in April-July, and by the rainfall occurred in June-July. In addition, the rate of increase of the population during the spring months influenced the development of the mosquito population of the following months.


Monitoring Hippobosca equina (Diptera: Hippoboscidae) in the regional park Boschi di Carrega: ecological evidences

XXVIII Congresso Nazionale Societa' Italiana di Parassitologia SOIPA: Roma, 24-27 giugno 2014 /
INTRODUCTION: Hippobosca equina is a well known haematophagous fly that infests ruminants in different parts of the world, but is generally considered to be nonpathogenic. However, H. equina, can cause blood loss and serve as vector for pathogenic organisms, phoretically transport lice (Ischnocera) and mites (Acari). High infestation could have negative effects on domestic and wild animals. This study try to clarify some aspects of the ecology of these ectoparasites in the Regional Park Boschi Di Carrega, wich reported previously high prevalence of H. equina in roe deer (Young et al., 1993, J. Wildl. Dis., 29:278-283). MATERIALS AND METHODS: In this study we evaluated the abundance of H. equina in the period ranging from spring-autumn of 2008. We chose 11 different sites based on particular ecological characteristics (i.e. farms, presence of cattle, horses or wild mammals) and on vegetation and hydrological characteristics (presence/absence of creeks). We used blue panels (one trap per site) for the capture of insects (BIOPLANET ColorTRAP B). The data were correlated with thermo-pluviometric reports and relative humidity obtained from the meteorological station of the Park. RESULTS: Of 11 sites all were positive for H. equina presence, with an average number of specimens for trap of 2.11. The highest catches were obtained in sites where the wooded vegetation was most intense and there was a strong presence of wildlife (70% of positive panels and average of specimens for each sampling 11.8) with the greatest number of insect on the trap (n=68) at the end of September. In the period tested an increase in the density occurred with temperatures lower than 25°C and low precipitation. CONCLUSIONS: This study suggests that the density of H. equina would be vulnerable not only to the host but also to the influences of climate, because we observed a density increase in case of low temperature (T<25°C) and precipitation. Moreover, it was demonstrated that the puparia of this species were more resistant to cool rather than warm (T>24°C) temperature extremes (Bennet, 1961, Can J Zoology 39: 379-406). Our results show that understanding the H. equina ecology is useful for the management and control of this ectoparasite.

Defilippo°F, Pezzoni°G, Pinna°M, Dottori°M, Broccoli°E, Bonilauri°P
Mass-rearing of Trichoplusia ni larvae for implementation of production of proteins expressed by recombinant Baculoviruses
European Congress of Entomology (10th : York, UK : 3-8 August 2014)

A large number of proteins has been successfully expressed in insect cell lines infected with baculovirus. Compared to production in cell culture, insect larvae can produce proteins at reduced cost. In this study the maintenance and propagation of a colony of Trichoplusia ni with two different diets was evaluated: the Diet 1 was a commercial diet; the Diet 2 was a homemade artificial diet. The data concerning the duration of the developmental stages and the larval and pupa survival were recorded. Larvae fed with Diet 2 showed a developmental time shorter than Diet 1 (less 2 days), but the larval and pupa mortality were not different, so both diets were efficient to guarantee the development of T.ni.Two groups of larvae were used to evaluate the heterologous proteins expression. The larvae were infected separately with recombinant baculoviruses expressing respectively the non-structural protein 3 (NS3) of Bovine Viral Diarrhea Virus and the capsid protein of Hepatitis E Virus. One larva produced an amount of antigen corresponding to 2 (NS3) or 3 (ORF2) 165cm² monolayer flasks of infected Sf9 cells, thus proving to be advantageous in terms of costs of production. The preliminary results encourage continuation of the development of this production service.
Dho° G, Grazioli° S, Brocchi° E

**Enhanced sensitivity of FMDV antigen detection ELISA by novel signal amplification systems**


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


**Ready-to-use kits for the detection of antibody to FMDV serotypes SAT1 and SAT2**


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

Eldaghayes I, Dayhum A, Kammon A, Sharif M, Ferrari G, Sumption K, King D, Grazioli° S, Brocchi° E,

**FMD in Libya and the control strategy**


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

Favero G, Stacchiotti A, Lavazza° A, Rodella LF, Re zzani R

**Melatonin promotes beneficial Mitofusin 2 in obese mice kidney**

18th International Microscopy Congress "Microscopy for global challenges: touching atoms, molecules, nanostructures and cells by multidimensional microscopy": 7 - 12 September 2014 Prague, Czech Republic / [s.l.: s.n, 2014]. - 1 p. - 1 bib ref [Nr. Estr. 5826]

International Microscopy Congress (18th: Prague, Czech Republic: 7-12 September 2014)

Obesity is a worldwide health problem which occurs in industrialized countries and influences the duration and quality of life. Currently few data have been reported on the impact of obesity in the kidney and its link with insulin resistance and metabolism [1]. A good translational animal model to study obesity is represented by leptin-deficient homozygous mice (ob/ob), that display hyperphagia, over-weight, hypertension and insulin resistance like human subjects. Considering that mitochondria are favourite target for excessive energy requirement in obesity [2] and that restoration of their proper structure is necessary to renal activity, we tested the efficacy of melatonin, the indoleamine of the pineal gland, in mitochondria in the ob/ob mice kidney. This microscopic study aimed to demonstrate the anti-oxidant role of melatonin in the obese mice kidney, by focusing on proximal tubular epithelium mitochondria morphology and on the renal localization of markers of mitochondrial health and apoptotic signalling. Twenty male mice (3 weeks of age) were organized into four groups containing both C57BL6 lean, as controls, and ob/ob supplemented or not with melatonin in drinking water (100 mg/kg/day) for 8 weeks. Kidneys were extracted and processed for histopathological (H&E and PAS), immunohistochemical and ultrastructural analysis. Body and kidney weights were estimated in all groups. Mitochondria health was evaluated by immunofluorescence of mitofusin 2 (Mf2), a resident protein involved in mitochondria metabolism, and by TEM analysis, the gold technique to visualize mitochondria structure and density. Furthermore renal expression of Bax, a member of Bcl2 family associated to mitochondria-triggered apoptosis, cytochrome c and caspases.
were visualized at a CLSM. Melatonin treatment did not modify body and kidney weights in lean group but significantly reduced body and kidney weights in the obese mice. Moreover melatonin did not affect renal ultrastructure and tubular mitochondria, nor stimulated apoptosis in controls, that showed intense Mf2 signal. By contrast, in ob/ob mice kidney, Mf2 fluorescence disappeared and mitochondria were round, with short peripheral cristae (Figures 1-2). Moreover Bax, cytochrome c, caspases 9 and 3 were visualized in cortical tubules. Remarkably, in ob/ob mice receiving melatonin, Mf2 staining appeared again, even if at tesser grade than in controls (Figure 3), mitochondria were elongated (Figure 4), while apoptotic markers weak. These novel observations suggest that melatonin, by restoring tubular Mf2 signal, influences mitochondria in ob/ob mice kidney and limits the onset and progression toward apoptosis.

Fleck LE, North EJ, Lee RE, Mulcahy LR, Casadei G, Lewis K
A screen for and validation of prodrug antimicrobials

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

Gatti L, Bucca M
Dieci percorsi FAD : i primi dieci casi

Gatti L, Bucca M
Dieci percorsi FAD : i primi dieci casi

Gatti L, Bucca M
In our country in general the health aspects of coypu Myocastor coypus are evoked as threats for public interests and suggest disturbing scenarios negatively affecting also the management approach of this alloctonous species; but the reality could be different. About the echo-pathological aspects the state of published knowledge is mostly based on a broad survey on causes of death carried out in Argentina, where the species is native and common, integrated with results of a health monitoring plan carried out on animals caught in Modena and comparing the outcomes with those of similar surveys carried out in Louisiana, France and furthermore in other Italian areas. This overview, for conveniente, is taking into account endo- and ecto-parasites, bacteria and viruses, focusing on major aspects of the various investigations carried out on coypu freely living in wild, and integrating them with those of experimental researches carried out by authors from different countries, both in the areas of origin and those of naturalization and farming. The species is receptive to certain infections transmissible to humans and/or cattle, but this needs to verify the practical importance of diagnostic and epidemiological results, in general situations outside of south America and in particular in the Italian situation. The condition of the reservoir was in fact perfectly demonstrated for communicable diseases such as fascioliasis (France) while in other cases (leptosioriasis) such status has been recently proposed (France); of course in Italy these aspects are to prove or considered of minor importance or however, not easily assessable and this leads the attention for this species to a situation of normality, as for any other wildlife. However, this invasive species is relatively recent in our country and this suggests keeping monitored the adaptation process from echo-patological point of view, as populations may develop more interaction with the local fauna and ecosystems. Control plans in Italy produce large amounts of carcasses often disposed of with significant costs: regrettably it is generally overlooked that the depletion of coypu populations in not few areas of the subcontinent of origin and the initial success of its breeding in Europe was initially due not only to the production of fur, but also to the intense use of its meats. Actually, in recent decades, the
duplication of interest has been greatly diminished, not everywhere, almost to cease altogether to be limited to only fur; moreover, due to the sharp depreciation of skins, the interest for breeding vanished and caused the abandonment in the wild of the rodents in many countries, Italy including, where in last decades the species is spread in many regions. In some countries the interest in the use of the meat remains commonly high (Argentina) or is recently promoted (Louisiana), while in others this rodent is used in rural area as both fresh meat and preserves (Germany, France). After World War II, even in Italy, the species has been locally the subject of rural micro-projects based on meat and fur but quickly the production resulted limited to fur. This has left few traces in the requirements for inspections on slaughter activities and meat processing which anyway since 2004 have been replaced by more robust European Regulations. Actually Reg. 853/2004/CE treats rodents freely living in the wild susceptible to be considered <small wild game> hunted for human consumption. From this approach also in Italy the coypu harvested as part of culling plans would be destined for human consumption through an approved game-handling establishment. This possibility to consider coypu also as a resource should not be ignored, avoiding to maintain prospects a priori limited to production of large amount of carcasses to be disposed of with high costs. On the other hand reduction plans producing coypu for meat would require only modest measures in order to rationalize the collection of the carcasses according to the criteria applied to small wild game whose costs would be covered by revenues. These measures may qualify control plans and their operators, would drive game meat of excellent features to the market of ethnic food and culinary curiosity and ultimately may help to consider the coypu in a more technical way.

Lucarelli E, Dozza B, Ferrari° M, Duchi S, Lesci IG, Martella E, Teti G, Donati D
Deminerlized bone matrix powder particle size influences mesenchymal stem cells colonization and metabolic activity
Tissue Engineering & Regenerative Medicine International Society, European Chapter Meeting : Genova, Italy : 10–13 June, 2014)

Sequencing of Sylvilagus VDJ genes reveals a new VHa allelic lineage and shows that ancient VH lineages were retained differently in leporids
Antigen recognition by immunoglobulins depends upon initial rearrangements of heavy chain V, D, and J genes. In leporids, a unique system exists for the VH genes usage that exhibit highly divergent lineages: the VHa allotypes, the Lepus sL lineage and the VHn genes. For the European rabbit (Oryctolagus cuniculus), four VHa lineages have been described, the a1, a2, a3 and a4. For hares (Lepus sp.), one VHa lineage was described, the a2L, as well as a more ancient sL lineage. Both genera use the VHn genes in a low frequency of their VDJ rearrangements. To address the hypothesis that the VH specificities could be associated with different environments, we sequenced VDJ genes from a third leporid genus, Sylvilagus. We found a fifth and equally divergent VHa lineage, the a5, and an ancient lineage, the sS, related to the hares' sL, but failed to obtain VHn genes. These results show that the studied leporids employ different VH lineages in the generation of the antibody repertoire, suggesting that the leporid VH genes are subject to strong selective pressure likely imposed by specific pathogens.

Stacchiotti A, Favero G, Giugno L, Lavazza° A, Reiter RJ, Rodella LF, Rezzani R
Mitochondrial and metabolic dysfunction in renal convoluted tubules of obese mice : protective role of melatonin
Obesity is a common and complex health problem, which impacts crucial organs; it is also considered an independent risk factor for chronic kidney disease. Few studies have analyzed the consequence of obesity in the renal proximal convoluted tubules, which are the major tubules involved in reabsorptive processes. For optimal performance of the kidney, energy is primarily provided by mitochondria. Melatonin, an indoleamine and antioxidant, has been identified in mitochondria, and there is considerable evidence regarding its essential role in the prevention of oxidative mitochondrial damage. In this study we evaluated the mechanism(s) of mitochondrial alterations in an animal model of obesity (ob/ob mice) and describe the beneficial effects of melatonin treatment on mitochondrial morphology and dynamics as influenced by mitofusin-2 and the intrinsic apoptotic cascade. Melatonin dissolved in 1% ethanol was added to the drinking water from postnatal week 5–13; the calculated dose of melatonin intake was 100 mg/kg body weight/day. Compared to control mice, obesity-related morphological alterations were apparent in the proximal tubules which contained round mitochondria with irregular, short cristae and cells with elevated apoptotic index. Melatonin supplementation in obese mice changed mitochondria shape and cristae organization of proximal tubules, enhanced mitofusin-2 expression, which in turn modulated the progression of the mitochondria-driven intrinsic apoptotic pathway. These changes possibly aid in reducing renal failure. The melatonin-mediated changes indicate its potential protective use against renal morphological damage and dysfunction associated with obesity and metabolic disease.

Melatonin limits adaptive ER stress and hepatosteatosis in leptin-deficient mice
Congresso Nazionale della Società Italiana di Anatomia e Istologia (68. : Ancona : 18-20 September 2014)

Non alcoholic fatty disease (NAFLD) impacts on about 30% of the population in industrialized countries, associated to the metabolic syndrome may be reversible or dramatically evolve into cirrhosis or hepatocellular cancer (Wree et al., 2011). Leptin-deficient homozygous mice (oh/ob) represent a well-known animal model to obesity, associated with overweight, liver steatosis and insulin-resistance. Recently ER stress has been reported to contribute to hepatic steatosis and cell damage called lipoapoptosis (Flamment et al., 2010). Melatonin, the main pineal indoleamine, been demonstrated to be useful to limit adipogertesis in many metabolic clinical has conditions (de Luxan-Delgado et al., 2014). Therefore major aims of the present study were: 1.To localize ER stress, energy homeostasis and hypoxia markers in the liver of ob/ob mice receiving or not melatonin in drinking water at 100 mg / kg/ day for 8 weeks; 2.To ch aracterize hepatic steatosis and quantify macrosteatosis in different experimental grps. C57BL6 mice treated or riot with melatonin were used as controls. Remarkably in oh/ob mice receiving melatonin, macrosteatosis, peripoatal GRP78 staining decreased while beta catenin became basolateral into hepato- cytes. Furthermore melatonin limited nuclear CHOP staining, a recognized indext of major sensitivity to apoptosis, but stimulated p62 / SQSTM1 signal, involved in reducing lipogenesis. Moreover by TEM analysis, we visualized in ob/ob mice liver mitochondria that displayed more cristae and strict RER adhesiort after melatonin intake. In conclusion, our morphological analysis suggests that melatonin obese might ameliorate NAFLD by anti-oxidative and ER stress modulatory abilities in mice.

Stoppani° E, Renzi° S, Dotti° S, Villa° R, Ferrari° M, Bassi I, Lucchini F
Valutazione dell'attività antivirale di siRNA nei confronti di differenti biotipi del virus dell'influenza A
Le problematiche di ordine sanitario riguardanti il virus dell’influenza A, che si sono verificate in epoca recente (virus influenzale pandemico H1N1), hanno evidenziato come l’ospite animale possa fungere anche da reservoir nella trasmissione di tale agente patogeno all’uomo. In particolare, il suino riveste un ruolo chiave nell’ambito della potentziale ricombinazione tra biotipi differenti del virus dell’influenza. La comparsa di nuovi biotipi estremamente virulenti in grado di rendere inefficace il protocollo standard di vaccinazione ha portato allo studio di nuovi farmaci/molecole ad azione antivirale ed in questo contesto un particolare interesse è rivestito dal fenomeno noto come RNA interference (RNAi). Recentemente infatti, numerose pubblicazioni hanno dimostrato come gli short interfering RNA (siRNAs), abbiano la capacità di svolgere la funzione di agenti antivirali, inibendo la traduzione di determinate sequenze ribonucleotidiche e quindi la sintesi di proteine essenziali per la replicazione virale. Nel presente studio, è stato dimostrato come l’espressione costitutiva di singole sequenze siRNA in cellule coltivate in vitro, determini l’inibizione della replicazione di differenti biotipi di virus dell’influenza A. A tal fine, sono state impiegate sequenze siRNA disegnate sulla regione della nucleoproteina (NP)conservata nei diversi biotipi. Colture cellulari di MDCK (Madin Darby Canine Kidney) sono state quindi trasfettate stabilmente con un vettore di espressione per i siRNA e sottoposte ad infezione con i seguenti biotipi di virus dell’influenza suina (SIV): A/SW/1521/98, H1N2; A/SW/1523/98, H3N2 e A/SW/1513/1/98, H1N1. Inoltre, è stato utilizzato anche il biotipo di influenza aviaria A/TK/Italy/2676/2000, H7N1. Due siRNA selezionati hanno dimostrato una buona capacità di inibire la replicazione virale in vitro; infatti, in nessun campione cellulare infettato è stata rilevata la presenza di effetto citopatico. Parallelamente, gli stessi risultati sono stati confermati tramite real-time RTPCR. Questa tecnica ha consentito infatti di rilevare una marcata depressione dell’Mrna codificante la NP nei campioni cellulari che esprimono gli specifici siRNA. In sintesi quindi, lo studio svolto ha dimostrato l’efficacia dei siRNA nell’inibire la replicazione in vitro di differenti biotipi virali selezionati dell’influenza A suina ed aviare, suggerendo il prospetto delle indagini in due differenti direttive. La prima volta a valutare l’efficacia di tale strategia nei confronti di virus dell’influenza A umana e di altri biotipi dell’influenza aviaria. La seconda riguardante la valutazione, mediante test in vivo, della reale capacità inibente la replicazione virale in quanto si ritiene che l’impiego di siRNA possa rappresentare una strategia terapeutica innovativa ed efficace da attuare sia in medicina umana che veterinaria.

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

Andamento della sensibilita’ antibiotica nei confronti di ceppi di Escherichia coli isolati da specie avicole allevate e da avifauna selvatica


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

In the first part of the study a collection of the data on the in vitro sensitivity tests (antibiograms) performed on pathogenic E. coli strains isolated from poultry farms was carried out. During the considered period (from January 2012 to March 2014) there were no significant differences in the susceptibility of the tested strains. A high prevalence of the susceptibility towards aminosidin and colistin was observed. The susceptibility range towards enrofloxacin was between 73% and 79% but an increase of the resistant strains was observed especially in E.coli strains isolated from broiler chicken and meat turkey flocks. In the second part of this study we examined the antibiotic resistance profile of E.coli strains recovered from the gut of wild birds collected in Emilia Romagna region in 2013. Resistance in E.coli isolates was detected on 15 of the 18 tested antibiotics including some antibiotics effective against Gram-negative bacteria.

Diagnostic observations with IZSLER antigen ELISA kits for detection and serotyping of FMDV serotypes O, A, SAT1 and SAT2 in several African countries


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

Zanusso G, Fiorini M, Ferrari S, Meade-White K, Barbieri I, Brocchi E, Ghetti B, Monaco S

Gerstmann-Sträussler-Scheinker disease and “Anchorless Prion Protein” mice share prion conformational properties diverging from sporadic Creutzfeldt-Jakob disease


The role of the GPI-anchor in prion disease pathogenesis is still a challenging issue. In vitro studies have shown that anchorless cellular prion protein (PrPC) undergoes aberrant post-translational processing and metabolism. Moreover, transgenic (Tg) mice overexpressing anchorless PrPC develop a spontaneous neurological disease accompanied with widespread brain PrP amyloid deposition, in the absence of spongiform changes. Generation of PrP forms lacking the GPI and PrP amyloidosis are striking features of human stop codon mutations in the PrP gene (PRNP), associated with PrP cerebral amyloid angiopathy (PrP-CAA) and Gerstmann-Sträussler-Scheinker (GSS) syndrome. More recently, the presence of anchorless PrP species has been also claimed in sporadic Creutzfeldt-Jakob disease (sCJD). Using a highly sensitive protein separation technique and taking advantage of reference maps of synthetic PrP peptides, we investigated brain tissues from scrapie-infected “anchorless PrP” Tg mice and wild type mice to determine the contribution of the GPI-anchor to the molecular mass and isoelectric point of PrP quasispecies under two-dimensional electrophoresis. We also assessed the conformational properties of anchorless and anchored prions under standard and inactivating conditions. These studies were extended to sCJD and GSS. At variance with GSS, characterization of PrP quasispecies in different sCJD subtypes ruled out the presence of anchorless prions. Moreover, under inactivating conditions, mice anchorless prions, but not sCJD prions, generated internal PrP fragments, cleaved at both N and C termini, similar to those found in PrP-CAA and GSS brain tissues. These findings show that anchorless PrPSc generates GSS-like PrP fragments, and suggest a major role for unanchored PrP in amyloidogenesis.