

RICERCHE EFFETTUATE

ARGOMENTI VARI

Almugadam SH, Rattigan K, Achcar F, Contini C, Maritati M, Rugna° G, Dallochio F, Bellini T, Hanau S, Barrett M

Metabolomics analysis of Leishmania promastigotes treated with the 6- aminonicotinamide

6th World Congress on Leishmaniasis : 16th-20th May 2017, Toledo, Spain : abstracts book / [s.l. : s.n., 2017]. - p 184 (C0630) [Nr. Estr. 7775]

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

The pentose phosphate pathway (PPP) is a major provider of reducing equivalents in leishmania and enzymes of the pathway could serve as good drug targets. 6-aminonicotinamide (6AN) is an analogue of nicotinamide and in some species is converted to an analogue of NADP which is able to inhibit the dehydrogenases of the oxidative branch of the PPP. We assayed its effect on *L. mexicana* and *L. infantum* promastigotes, and *L. mexicana* promastigotes and amastigotes. 6AN was only inhibitory of leishmania growth at high concentrations. We went on to investigate how its inhibitory effect was exerted at the level of the metabolome. M379 *L. mexicana* and PCM5 *L. infantum* promastigotes were treated with 7.8 mM 6AN for 24 h. Small metabolites were prepared in quadruplicate for HILIC-UV analysis according to Creek et al (Anal Chem 2011;83:8703-871), IILIC was carried out on Dionex UltMate 3000 RSLC system (Thermo Fisher, Hemel Hempstead, UK) using a ZIC-pHILIC column (Merck Sequant). For MS analysis Orbitrap machines (Thermo Fisher Scientific) were operated in polarity switching mode. Data were analysed with IDEOMv19 and MetaboAnalyst 3.0. 3 Results For *L. mexicana*, glutathione, ribose SP, 6Pgluconate levels and downstream PPP intermediates were similar to controls. For *L. infantum*, it was possible to analyse NAD and NADPH which were found significantly decreased together with the PPP intermediate D-sedoheptulose 7-phosphate. 6AN caused significant depletion in phosphoribosylpyrophosphate (PRPP) and nicotinate (Na). Purine and pyrimidine nucleotides, nucleosides, their derivatives and metabolites related to their synthesis and turnover (ribose and deoxy-ribose) all showed peaks lower in 6AN than in DMSO treated cells. On the contrary, their purine and pyrimidines nucleobase precursors accumulated, indicating that the loss of PRPP was responsible for these changes. 4 Conclusions In mammals 6AN is converted to 6ANAD/P by NAD. glycohydrolase, however in leishmania its toxicity is only seen in millimolar range. Our data indicated that this antimetabolite is capable of depleting cellular PRPP levels by its use in the Preiss-Handler NAD salvage pathway. This in turn diminishes conversion of nucleobases to their nucleoside products. When given in high doses 6AN can kill the parasites, probably due to the depletion of nucleotides required for nucleic acid biosynthesis. It seems that in leishmania a NAD* glycohydrolase similar to the mammal one does not work.

Andreani° NA, Renzi° S, Piovani G, Ajmone Marsan P, Bomba L, Villa° R, Ferrari° M, Dotti° S

Potential neoplastic evolution of Vero cells : in vivo and in vitro characterization

Cytotechnology. - Vol. 69 (2017). - p 741-750. - 44 bib ref [Nr. Estr. 7709]

Vero cell lines are extensively employed in viral vaccine manufacturing. Similarly to all established cells, mutations can occur during Vero cells in vitro amplification which can result in adverse features compromising their biological safety. To evaluate the potential neoplastic evolution of these cells, in vitro transformation test, gene expression analysis and karyotyping were compared among low- (127 and 139 passages) and high-passage (passage 194) cell lines, as well as transformed colonies (TCs). In vivo tumorigenicity was also tested to confirm preliminary in vitro data obtained for low passage lines and TCs. Moreover, Vero cells cultivated in foetal bovine serum-free medium and derived from TCs were analysed to investigate the influence of cultivation methods on tumorigenic evolution. Low-passage Vero developed TCs in soft agar, without showing any tumorigenic evolution

when inoculated in the animal model. Karyotyping showed a hypo-diploid modal chromosome number and rearrangements with no difference among Vero cell line passages and TCs. These abnormalities were reported also in serum-free cultivated Vero. Gene expression revealed that high-passage Vero cells had several under-expressed and a few over-expressed genes compared to low-passage ones. Gene ontology revealed no significant enrichment of pathways related to oncogenic risk. These findings suggest that in vitro high passage, and not culture conditions, induces Vero transformation correlated to karyotype and gene expression alterations. These data, together with previous investigations reporting tumour induction in high-passage Vero cells, suggest the use of low-passage Vero cells or cell lines other than Vero to increase the safety of vaccine manufacturing.

Angelone M, Conti V, Biacca C, Battaglia B, Pecorari L, Piana F, Gnudi G, Leonardi F, Ramoni R, Basini G, Dotti° S, Renzi° S, Ferrari° M, Grolli S

The contribution of adipose tissue-derived mesenchymal stem cells and platelet-rich plasma to the treatment of chronic equine laminitis : a proof of concept

Int J Mol Sci. - Vol. 18 no 10 (2017). - Article no. 2122 (17 p.). - 50 bib ref (ultimo accesso 06/06/2018 <http://www.mdpi.com/1422-0067/18/10/2122>) [Nr. Estr. 7675]

Laminitis, a highly debilitating disease of the foot in ungulates, is characterized by pathological changes of the complex lamellar structures that maintain the appendicular skeleton within the hoof. Laminitis is a multifactorial disease that involves perturbation of the vascular, hematological, and inflammatory homeostasis of the foot. Interestingly, the pathogenesis of the disease resembles what is observed in metabolic syndromes and sepsis-induced organ failure in humans and animals. We hypothesized that local administration of mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP) might contribute to establishing an anti-inflammatory and pro-angiogenic environment, and could stimulate the injured tissue in order to restore its functional integrity. According to this assumption, an experimental protocol based on the local intravenous administration of adipose tissue-derived MSCs (aMSCs) in combination with PRP was developed for the treatment of horses affected by chronic laminitis. Nine horses with severely compromised venograms (showing grade III and IV laminitis) that had been unsuccessfully treated with conventional therapies were enrolled. aMSCs and PRP (15 × 10⁶ cells resuspended in 15 mL of PRP) were injected into the lateral or medial digital vein three times, at one-month intervals. The first administration was performed with allogeneic aMSCs, while for the following administrations, autologous aMSCs were used. There was no adverse short-term reaction to the intravenous injection of aMSCs. In the long term, venograms outlined, in all subjects, a progressive amelioration of the vascularization of the foot. An improvement in the structure and function of the hoof was also observed. No adverse events were reported during the follow-up, and the horses returned to a comfortable quality of life. Although the number of animals enrolled in the study is limited, both clinical observations and venography demonstrated an enhancement in the condition of all horses, suggesting that the regenerative therapies in chronic laminitis could be useful, and are worthy of further investigation

Bagni M, Petrini A, Lavazza° A

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

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) 88 p [Nr. Estr. 7616]

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

Barbieri S, Paoli A, Pietrantonio V, Rubini° S, Bortot A, Behr A, Donato D, Tredese A, Vettore G, Vigolo S, Bano M, Franchi M, Bergamini M

Take another shot as penalty: a funny and competitive new adventure in extreme alcoholic games

10th European Public Health Conference : 1-4 November 2017 Stockolm, Sweden / [s.l. : s.n., 2017]. - 1 p (ultimo accesso <https://eph2017-eupha.ipostersessions.com/Default.aspx?s=gallery>) [Nr. Estr. 7783]

European Public Health Conference (10th : Stockolm, Sweden : 1-4 November 2017)

Bertasio° C, Ricchi° M, Boniotti° MB, Vicari° N, Russo° S, Tilola° M, Bellotti° M, Barbara° B [i.e Bertasi° B]

Quantification of Mycobacterium avium subsp paratuberculosis, Listeria monocytogenes and Francisella tularensis by real-time PCR and two digital PCR systems : methods comparisons

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 295 (Poster 154). - 5 bib ref [Nr. Estr. 7600]

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

Bilato° D, Dotti° S, Ghizzardardi° A, Mor° A, Villa° R, Amadori° M

Un metodo basato sulla citometria a flusso per la rilevazione di contaminazioni batteriche delle colture cellulari

Italian Society for Cytometric Cell Analysis (ISCCA) : 8-10 Maggio 2017, Bologna / [s.l. : s.n, 2017]. - 1 p. - 4 bib ref [Nr. Estr. 7689]

Italian Society for Cytometric Cell Analysis (ISCCA) : Bologna : 8-10 Maggio 2017)

Bilato° D, Dotti° S, Renzi° S, Villa° R, Amadori° M

A flow cytometry-based system for detection of bacterial contaminations in cell cultures

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 48 . - 4 bib ref [Nr. Estr. 7611]

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 control of cell cultures for microbial contaminations is mandatory to set up reliable immunological assays and vaccine production procedures. Microbial contaminations derive from multiple sources and include bacteria, fungi (moulds and yeasts), mycoplasma and viruses. Bacterial contaminations are detected by visual inspection of cell cultures and/or bacteriological procedures. The sensitivity of these is often less than optimal, which may give rise to despicable delays in the detection of contaminated cultures and serious downstream losses. In particular, there is evidence that bacteriological media cannot provide suitable growth conditions for some slow-growing bacterial species and intracellular phases of bacteria such as staphylococci [1]. Moreover, several bacterial pathogens undergo mutations in their environment in order to survive and establish an infection. Many stressors are known to affect their size, growth, division and metabolism [2] and novel control procedures are badly needed. Flow cytometry has been used for a long time to detect bacteria [3], yeasts and fungi [4]. On the basis of these findings, we developed a flow cytometry-based detection procedure of bacterial contaminations of cultured cells and tissue culture media. The protocol is

based on two dyes binding to nucleic acids of viable and dead bacterial cells, respectively. Our results showed that it is possible to discriminate the scatter and fluorescence profiles of bacteria from those of nucleoprotein particles released from necrotic and apoptotic cells (non-specific staining). Therefore, a bacterial contamination gate was defined on the basis of both forward scatter and fluorescence, and a threshold number of events in the gate was reckoned following examinations of several uncontaminated cell cultures of different origin, type (fibroblast, epithelial, mesenchymal) and species. Our procedure was shown to detect experimental bacterial contaminations within 4-5 hours of the inoculation. Most important, contaminated cell cultures of our diagnostic laboratory were revealed even before a positive bacteriological test. Each experimental contamination of cell cultures was carried out using one bacterial species at a time. Mycoplasma spp. was not investigated. Owing to the above, this novel and rapid test procedure has a considerable potential for routine applications and is conducive to more reliable, robust sterility controls of cell cultures and also immunological products like vaccines.

Bolzoni° G, Marcolini° A, Ferrini AM, Buffoli° E

Production and validation of an alkaline phosphatase reference material in lyophilized milk

Food Anal Methods. - Vol. 10 (2017). - p 559-564. - 18 bib ref [Nr. Estr. 7301]

The paper describes the characterization and evaluation of a reference material in lyophilized milk to be used for the determination of alkaline phosphatase with the reference method ISO 11816-1:2013. The reference material was produced at three levels of activity, calibrated for the determination of alkaline phosphatase activity in pasteurized (sample "60"), thermized (sample "600"), and raw milk (sample "6000"). The first assignment of the title and the uncertainties were realized through a round of 18 Italian laboratories and the EUR-MMP in 2014. In 2015, a second round was repeated with the participation of 17 expert laboratories of the net of NRLs-MMP. The first titles were confirmed, whereas the second trial succeeded in a good improvement of the final uncertainties (sample "60" = 96.10 ± 2.03 ; sample "600" = 775.91 ± 13.03 ; sample "6000" = 6040.65 ± 126.89 mU/L). The materials were fully characterized according to ISO Guide 35:2006 and proved to be highly cron and temperature stable, so these are excellent candidates to be used for internal instrumental quality control or for the organization of interlaboratory trials for alkaline phosphatase determination in milk.

Bolzoni° G, Marcolini° A, Ferrini AM, Patriarca M, Buffoli° E

Measurement uncertainty for the assigned value of the activity of alkaline phosphatase in lyophilized milk reference materials

EURACHEM workshop "Uncertainty in qualitative and quantitative analysis" : Cyprus 2017 / [s.l. : s.n., 2017]. - 1 p [Nr. Estr. 7715]

EURACHEM workshop : Cyprus : 2017)

Calzolari° M, Bellini R, Defilippo° F, Bonilauri° P, Albieri A, Tamba° M, Dottori° M, Angelini P

Mosquito-based West Nile surveillance in 2016 in Emilia-Romagna Region (Northern Italy)

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 39 [Nr. Estr. 7629]

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

West Nile virus (WNV) circulates between mosquito and wild birds, but can infect humans and

equids as dead end host. Less than 1% of infected humans develop neurologic disease, but circulation of the virus compromises the safety of transplantations and transfusions. The WNV was the target of a multidisciplinary surveillance (addressed to mosquitoes, wild birds, and human cases) in Emilia-Romagna since 2008. The entomological parts of this plan contemplates the fortnightly sampling of mosquitoes in fixed stations, selected in a 120 km² grid. In 2016, 93 traps (77 carbon dioxide-baited traps and 16 gravid traps) were activated from June to September. The collected specimens were sorted in pools (maximum of 200 per pools) according to species, site and date of collection. Pools were submitted to biomolecular analysis, screening also the presence of the related Usutu virus (USUV). In 2016 a number of 247,754 mosquitoes, belonging to 15 species, were sampled (species over 0.1% were *Cx. pipiens* 91.1%, *Ae. caspius* 6.7%, *Ae. albopictus* 1.0%, *Ae. vexans* 1.0%, *An. maculipennis* sl 0.2%). Only *Cx. pipiens* and *Cx. modestus* were considered as vectors and analysed testing 225,666 specimens, of which 205,632 (in 2,049 pools) of the fore, and 47 (in 3 pool) of the last. The WNV was detected in 113 pools, while the USUV was detected in 93 pools, all composed by *Cx. pipiens*. The first WNV-positive pool was sampled on July 12, last pools were sampled on September 28. The first positive result at provincial level, triggered the blood donation-screening in the specific province, which allowed finding nine WNV-infected blood units at regional level. The results obtained demonstrate that the mosquito surveillance, with an adequate trapping effort, is able to define the temporal circulation and spread of WNV. In addition to the health surveillance, these data may be useful to obtain important epidemiological data related to WNV, USUV and possibly other mosquito-borne arboviruses.

Carra° E, Bergamini° F, Calzolari° M, Gennari W, Natalini S, Poglayen G, Varani S, Rugna° G

Molecular characterization of *Leishmania infantum* circulating in humans, dogs, and sand flies in the Emilia-Romagna Region, Northern Italy

6th World Congress on Leishmaniasis : 16th-20th May 2017, Toledo, Spain : abstracts book / [s.l. : s.n., 2017]. - p 999 (Poster C0759) [Nr. Estr. 7771]

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

The unusual increase in the number of visceral leishmaniasis (VL) cases observed since 2012 in central areas of the Emilia-Romagna region (E-R), northern Italy, led health authorities to focus the attention on this re-emergent parasitic disease. With the aim to provide a better knowledge of the parasite population circulating in the region, we investigated the genetic characteristics of *Leishmania infantum* strains from different hosts. Molecular characterization was performed on a panel of 62 *L. infantum* strains obtained from humans, dogs and phlebotomine sand flies between 2013-2016 from different locations around the region, including areas involved in recent VL outbreaks. Isolates obtained from other endemic Italian regions were also included in the study for comparison. Species identification was performed by ITS-1 sequencing, and subsequent subspecies characterization was carried out by k26-PRC typing. Finally, strains were characterized by multilocus microsatellite typing (MLMT), targeting 15 highly polymorphic markers. Genotype data were analyzed by a Bayesian model-based clustering method. 3 Results All strains were identified as *L. infantum* by sequencing of ITS1. Capillary electrophoresis revealed different k26-PCR product sizes; the amplicon of 626bp, typical of MON-1 zymodeme, was the most representative in both canine and extra-region human isolates. Conversely, all the human isolates from the E-R region shared a distinct, never reported amplicon size. Overall, the MLMT detected 48 genotypes, with canine isolates showing a higher level of polymorphism. Bayesian analysis inferred two populations; one grouping canine strains together with extra-regional human strains, named Population A, whereas regional human strains and strains from sand flies were grouped in a second population, the Population B. 4 Conclusions Overall, the study revealed genetic heterogeneity within *L. infantum* population from northern Italy. MLMT proved to be a suitable method for tracking genotypes among different hosts and areas. Despite canine population of the E-R region hosted zoonotic genotypes, recent outbreaks of VL were sustained by a distinct *L. infantum* population, actively circulating in the sand flies and so far undetected in sympatric dogs. Further studies are needed to elucidate the role of wild or domestic animal species in the epidemiology of VL in the region.

Chebli H, El_Idrissi AL, Benazzouz M, Eddine Lmimouni BE, Nhammi H, Elabandouni M, Youbi M, Afifi R, Tahiri S, El_Feydi AE, Settaf A, Tinelli C, De_Silvestri A, Bouhout S, Abela_Ridder B, Magnino° S, Brunetti E, Filice C, Tamarozzi F

Human cystic echinococcosis in Morocco : ultrasound screening in the Mid Atlas through an Italian-Moroccan partnership

PLOS Neglect Trop Dis. - Vol. 11 no 3 (2017). - p e0005384 (20 p). - 34 bib ref (ultimo accesso 04/07/2017 <https://doi.org/10.1371/journal.pntd.0005384>) [Nr. Estr. 7583]

Background Cystic echinococcosis (CE) is a neglected parasitic zoonosis with considerable socioeconomic impact on affected pastoral communities. CE is endemic throughout the Mediterranean, including Morocco, where the Mid Atlas is the most prevalent area for both human and animal infection. The highest hospital annual incidence of human CE is recorded in the provinces of Ifrane and El Hajeb. However, hospital-based statistics likely underestimate the real prevalence of infection, as a proportion of cases never reach medical attention or official records. **Methodology/Principal findings** In 2012, a project on clinical management of CE in Morocco was launched with the aims of estimating the prevalence of human abdominal CE in selected rural communes of the above mentioned provinces using ultrasound (US) screening and training local physicians to implement US-based focused assessment and rational clinical management of CE according to the WHO-IWGE Expert Consensus. A total of 5367 people received abdominal US during four campaigns in April-May 2014. During the campaigns, 24 local general practitioners received >24 hours of hands-on training and 143 health education sessions were organized for local communities. We found an overall CE prevalence of 1.9%, with significantly higher values in the rural communes of Ifrane than El Hajeb (2.6% vs 1.3%; $p < 0.001$). CE cysts were predominantly in inactive stage, especially in older age groups. However, active cysts were present also in adults, indicating acquisition of infection at all ages. Province of residence was the only risk factor consistently associated with CE infection. **Conclusions/Significance** Our results show a high prevalence and on-going, likely environmental transmission of CE in the investigated provinces of Morocco, supporting the implementation of control activities in the area by national health authorities and encouraging the acceptance and divulgation of diagnosis and treatment algorithms based on imaging for CE at both national and local level.

Crivelli B, Chlapanidas T, Perteghella S, Lucarelli E, Pascucci L, Brini AT, Ferrero I, Marazzi M, Pessina A, Torre ML, Italian Mesenchymal Stem Cell Group° (GISM) [for Italian Mesenchymal Stem Cell Group, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Ferrari° M]

Mesenchymal stem/stromal cell extracellular vesicles : from active principle to next generation drug delivery system

J Control Release. - Vol. 262 (2017). - p 104-117. - 200 bib ref [Nr. Estr. 7839]

It has been demonstrated that the biological effector of mesenchymal stem/stromal cells (MSCs) is their se-cretome, which is composed of a heterogeneous pool of bioactive molecules, partially enclosed in extracellular vesicles (EVs). Therefore, the MSC secretome (including EVs) has been recently proposed as possible alternative to MSC therapy. The secretome can be considered as a protein-based biotechnological product, it is probably safer compared with living/cycling cells, it presents virtually lower tumorigenic risk, and it can be handled, stored and sterilized as an Active Pharmaceutical/Principle Ingredient (API). EVs retain some structural and technological analogies with synthetic drug delivery systems (DDS), even if their potential clinical application is also limited by the absence of reproducible/scalable isolation methods and Good Manufacturing Practice (GMP)-compliant procedures. Notably, EVs secreted by MSCs preserve some of their parental cell features

such as homing, immunomodulatory and regenerative potential. This review focuses on MSCs and their EVs as APIs, as well as DDS, considering their ability to reach inflamed and damaged tissues and to prolong the release of encapsulated drugs. Special attention is devoted to the illustration of innovative therapeutic approaches in which nanomedicine is successfully combined with stem cell therapy, thus creating a novel class of "next generation drug delivery systems."

Dotti° S, Lombardo° T, Villa° R, Cacciamali° A, Zanotti° C, Andreani° NA, Cinotti° S, Ferrari° M

Transformation and tumorigenicity testing of simian cell lines and evaluation of poliovirus replication

PLoS One. - Vol. 12 no 1 (2017). - p e0169391 (13 p). - 34 bib ref (ultimo accesso 27/02/2017 <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0169391>) [Nr. Estr. 7513]

The key role of cell cultures in different scientific fields is worldwide recognized, both as in vitro research models alternative to laboratory animals and substrates for biological production. However, many safety concerns rise from the use of animal/human cell lines that may be tumorigenic, leading to potential adverse contaminations in cell-derived biologicals. In order to evaluate the suitability of 13 different cell lines for Poliovirus vaccine production, safety and quality, in vitro/in vivo tumorigenicity and Poliovirus propagation properties were evaluated. Our results revealed that non-human primate cell lines CYNOM-K1, FRhK-4, 4MBr-5 and 4647 are free of tumorigenic features and represent highly susceptible substrates for attenuated Sabin Poliovirus strains. In particular, FRhK-4 and 4647 cell lines are characterized by a higher in vitro replication, resulting indicated for the use in large-scale production field.

Dozza B, Lesci IG, Duchi S, Della_Bella E, Martini L, Salamanna F, Falconi M, Cinotti° S, Fini M, Lucarelli E, Donati D

When size matters : differences in demineralized bone matrix particles affect collagen structure, mesenchymal stem cell behavior, and osteogenic potential

J Biomed Mater Res Part A. - Vol. 105A (2017). - p 1019-1033. - 42 bib ref [Nr. Estr. 7530]

Demineralized bone matrix (DBM) is a natural, collagen-based, osteoinductive biomaterial. Nevertheless, there are conflicting reports on the efficacy of this product. The purpose of this study was to evaluate whether DBM collagen structure is affected by particle size and can influence DBM cytocompatibility and osteoinductivity. Sheep cortical bone was ground and particles were divided in three fractions with different sizes, defined as large (L, 1–2 mm), medium (M, 0.5–1 mm), and small (S, <0.5 mm). After demineralization, the chemical–physical analysis clearly showed a particle size-dependent alteration in collagen structure, with DBM-M being altered but not as much as DBM-S. DBM-M displayed a preferable trend in almost all biological characteristics tested, although all DBM particles revealed an optimal cytocompatibility. Subcutaneous implantation of DBM particles into immunocompromised mice resulted in bone induction only for DBM-M. When sheep MSC were seeded onto particles before implantation, all DBM particles were able to induce new bone formation with the best incidence for DBM-M and DBM-S. In conclusion, the collagen alteration in DBM-M is likely the best condition to promote bone induction in vivo. Furthermore, the choice of 0.5–1 mm particles may enable to obtain more efficient and consistent results among different research groups in bone tissue-engineering applications.

Favero G, Trapletti V, Bonomini F, Stacchiotti A, Lavazza° A, Rodella LF, Rezzani R

Oral supplementation of melatonin protects against fibromyalgia-related skeletal muscle alterations in reserpine-induced myalgia rats

Int J Mol Sci. - Vol. 18 no 7 (2017). - no 1389 (17 p). - 76 bib ref (ultimo accesso 05/07/2017 <http://www.mdpi.com/1422-0067/18/7/1389>) [Nr. Estr. 7585]

Fibromyalgia is a chronic syndrome characterized by widespread musculoskeletal pain and an extensive array of other symptoms including disordered sleep, fatigue, depression and anxiety. Important factors involved in the pathogenic process of fibromyalgia are inflammation and oxidative stress, suggesting that anti-inflammatory and/or antioxidant supplementation might be effective in the management and modulation of this syndrome. Recent evidence suggests that melatonin may be suitable for this purpose due to its well known anti-inflammatory, antioxidant and analgesic effects. Thus, in the current study, the effects of the oral supplementation of melatonin against fibromyalgia-related skeletal muscle alterations were evaluated. In detail, 90 Sprague Dawley rats were randomly treated with reserpine, to reproduce the pathogenic process of fibromyalgia and thereafter they received melatonin. The animals treated with reserpine showed moderate alterations at hind limb skeletal muscles level and had difficulty in moving, together with significant morphological and ultrastructural alterations and expression of inflammatory and oxidative stress markers in the gastrocnemius muscle. Interestingly, melatonin, dose and/or time dependently, reduced the difficulties in spontaneous motor activity and the musculoskeletal morphostructural, inflammatory, and oxidative stress alterations. This study suggests that melatonin in vivo may be an effective tool in the management of fibromyalgia-related musculoskeletal morphofunctional damage

Ferri F, Brera C, De_Santis B, Fedrizzi° G, Bacci T, Bedogni L, Capanni S, Collini G, Crespi E, Debegnach F, Ferdenzi P, Gargano A, Gattei D, Luberto F, Magnani I, Magnani MR, Mancuso P, Menotta° S, Mozzanica S, Olmi M, Ombrini G, Sala O, Soricelli S, Vicentini M, Rossi PG

Survey on urinary levels of aflatoxins in professionally exposed workers

Toxins. - Vol. 9 no 4 (2017). - no 117 (13 p). - 24 bib ref (ultimo accesso 07/06/2017 <http://www.mdpi.com/2072-6651/9/4/117>) [Nr. Estr. 7544]

Feed mill workers may handle or process maize contaminated with aflatoxins (AFs). This condition may lead to an unacceptable intake of toxins deriving from occupational exposure. This study assessed the serological and urinary levels of AFs in workers exposed to potentially contaminated dusts in two mills. From March to April 2014, blood and urine samples were collected, on Monday and Friday morning of the same working week from 29 exposed workers and 30 non-exposed controls. AFs (M1, G2, G1, B1, B2) and aflatoxicol (AFOH) A were analyzed. Each subject filled in a questionnaire to evaluate potential food-borne exposures to mycotoxins. AFs contamination in environmental dust was measured in both plants. No serum sample was found to be positive. Seventy four percent of urine samples (73.7%) revealed AFM1 presence. AFM1 mean concentration was 0.035 and 0.027 ng/mL in exposed and non-exposed workers, respectively ($p = 0.432$); the concentration was slightly higher in Friday's than in Monday's samples, in exposed workers, 0.040 versus (vs.) 0.031 and non-exposed controls (0.030 vs. 0.024, $p = 0.437$). Environmental AFs contamination ranged from 7.2 to 125.4 $\mu\text{g}/\text{kg}$. The findings of this study reveal the presence of higher AFs concentration in exposed workers than in non-exposed controls, although these differences are to be considered consistent with random fluctuations.

Gaibani P, Scaltriti° E, Benni C, Pongolini° S, Ambretti S, Landini MP, Viale PL, Giannella M, Re MC

Characterization of an IncL/M plasmid carrying blaOXA-48 in a Klebsiella pneumoniae strain

from Italy

New microbiol. - Vol. 40 no 4 (2017). - p 284-285. - 12 bib ref [Nr. Estr. 7760]

Here we report the complete nucleotide sequence of a 49.257-bp IncL/M conjugative plasmid (pRAY) carrying the blaOXA-48 gene collected from a Klebsiella pneumoniae clinical strain isolated in Italy. The genetic environment of pRAY plasmid revealed that the blaOXA-48 gene was located within a Tn1999.2 transposon. The pRAY plasmid differed from blaOXA-48-harboring IncL/M plasmids by genetic context and size. Comparative analysis demonstrated that pRAY plasmid lacked a region of ~15 kb carrying genes encoding proteins involved in pilus assembly and plasmid conjugative apparatus.

Garbarino°C, Capelli° G, Cannistrà° M, Arrigoni° N, Cammi° G, Gibelli° LR, Riccio C, Faggionato° E

Avvelenamenti negli animali : valutare l'esperienza per migliorare l'attività di sorveglianza e di intervento = Animals poisoning : the experience of the Piacenza province

Prax Vet. - Vol. 38 no 3 (2017). - p 9-20. - 24 bib ref [Nr. Estr. 7755]

Da gennaio 2009 fino a fine luglio 2017, sono pervenuti presso la Sezione di Piacenza dell'IZSLER 348 campioni di cui 195 carcasse di animali e 153 sospette esche avvelenate. L'andamento delle positività nel corso degli anni è rimasta pressoché costante. In totale 118 campioni (34%) sono risultati positivi per presenza di sostanze tossiche/nocive, 197 (56%) sono risultati negativi e 33 (10%) non sono stati sottoposti ad analisi tossicologica in quanto il sospetto di avvelenamento non è stato avvalorato. I cani sono la specie maggiormente colpita; seguono i gatti e tra gli animali selvatici le volpi. La prima causa di avvelenamento, è rappresentata dagli insetticidi che nel complesso rappresentano il 43% del totale (nel dettaglio: 20% organofosforici, 20% carbammati, 3% organoclorurati), la seconda causa, sono i rodenticidi ad azione anticoagulante (23%), seguiti da glicole etilenico (17%), stricnina (7%), metaldeide (6%). 113% di campioni conteneva più di una categoria di principi attivi e 11% conteneva materiali nocivi (vetri).

From January 2009 until the end of July 2017, 347 samples arrived at the Piacenza Section of IZSLER. The trend of positivity over the years was almost constant. 118 samples (34%) were positive for the presence of pesticides, 197(56%) were negative and 33 (10%) did not undergo toxicological analysis since the suspected poisoning was not confirmed. Dogs are the most affected species followed by cats and amongst wild animals foxes were the most highly represented. The primary cause of poisoning was by insecticides which represented 43% of the total (in detail: 20% organophosphoric, 20% carbamate, 3% organochlorine), the second cause was by anticoagulant rodenticides (23%), followed by ethylene glycol (17%), stricnine (7%), metaldehyde (6%). 3% of samples contained more than one category of pesticides and 1% contained harmful materials (glass).

Jagielski T, Bakula Z, Di_Mauro S, Casciari C, Cambiotti V, Krukowski H, Turchetti B, Ricchi° M, Manuali E, Buzzini P

A comparative study of the in vitro activity of iodopropynyl butylcarbamate and amphotericin B against Prototheca spp. isolates from European dairy herds

J Dairy Sci. - Vol. 100 (2017). - p 7435-7445. - 58 bib ref [Nr. Estr. 7696]

The objective of this study was to assess the in vitro effect of iodopropynyl butylcarbamate (IPBC) and amphotericin B (AMB) on Prototheca zopfii genotype 2 and Prototheca blaschkeae isolates recovered from dairy herds of Belgium, France, Italy, Germany, and Poland. The combination of IPBC with AMB on Prototheca isolates and toxicity of IPBC to the bovine mammary epithelial cells were also evaluated. The in vitro activity of IPBC and AMB against 96 isolates of P. zopfii genotype 2 and 42 isolates of P. blaschkeae was performed. Minimum inhibitory concentrations (MIC) and minimum algicidal concentrations (MAC) of IPBC and AMB were determined. To determine any

synergistic, additive, or antagonistic effect of the combination of IPBC and AMB, 2-dimensional checkerboard combination tests were also performed to calculate fractional inhibitory concentrations. Cytotoxicity analysis of IPBC to the bovine mammary epithelial cell line was performed using a 3-(4,5-dimethyl-2-thiazol-2yl)-2,5- diphenyl tetrazolium bromide (MTT) assay. The MIC for 50 and 90% of isolates (MIC50 and MIC90, respectively) for IPBC were 4 and 8 mg/L versus 0.5 and 1 mg/L for AMB, respectively. The MIC profiles differed between *P. zopfii* genotype 2 and *P. blaschkeae*, with the latter species being more susceptible to both compounds. The MIC50 and MIC90 of IPBC were 4 and 8 mg/L for *P. zopfii* genotype 2 and 1 and 2 mg/L for *P. blaschkeae*, respectively. The MIC50 and MIC90 of AMB were both 1 mg/L for *P. zopfii* genotype 2 and 0.25 and 1 mg/L for *P. blaschkeae*, respectively. Both IPBC and AMB exhibited the ability to kill *Prototheca* spp. The MAC for 90% of isolates of IPBC was twice the MIC90, whereas an 8-fold increase of the MIC90 was algicidal in the case of AMB. Overall, the combined use of IPBC and AMB exhibited an increased algicidal effect, albeit the fractional inhibitory concentration index showed synergistic activity only against 3 *P. zopfii* genotype 2 isolates. For all the remaining isolates (87.5%), this combination produced only an additive effect. The MTT assay results showed both IPBC and AMB, at the concentrations employed in the study, to be nontoxic to the epithelial mammary gland cells (cell viability >90%). Notably, only IPBC at the highest concentration (i.e., 8 mg/L) exerted a slight cytotoxic effect on the cell line tested (mean cell viability: 88.54 ± 3.88 and 90.66 ± 3.0 , after 2 and 4 h of MTT treatment, respectively). The anti-*Prototheca* activity of IPBC was here demonstrated for the first time. In addition, the combined use of IPBC with AMB enhanced each other's effect, creating an additive rather than synergistic interaction. Both agents, used at concentrations corresponding to MIC values against *Prototheca* spp., showed no toxic effect for the mammary epithelial cells. In conclusion, IPBC, used either alone or in combination with AMB, can be considered a promising option in the treatment armamentarium for protothecal mastitis in dairy cows.

Kralik P, Ricchi° M

A basic guide to real time PCR in microbial diagnostics : definitions, parameters, and everything

Front Microbiol. - Vol. 8 (2017). - Article 108 (9 p). - 42 bib ref [Nr. Estr. 7536]

Real time PCR (quantitative PCR, qPCR) is now a well-established method for the detection, quantification, and typing of different microbial agents in the areas of clinical and veterinary diagnostics and food safety. Although the concept of PCR is relatively simple, there are specific issues in qPCR that developers and users of this technology must bear in mind. These include the use of correct terminology and definitions, understanding of the principle of PCR, difficulties with interpretation and presentation of data, the limitations of qPCR in different areas of microbial diagnostics and parameters important for the description of qPCR performance. It is not our intention in this review to describe every single aspect of qPCR design, optimization, and validation; however, it is our hope that this basic guide will help to orient beginners and users of qPCR in the use of this powerful technique.

Maritati M, Michel G, Comar M, Zanotta N, Rugna° G, Hanau S, Govoni M, Bellini T, Mahmoud R, Almuqadam S, Marty P, Contini C

Human asymptomatic Leishmania infantum infection : new insights in patients with chronic rheumatic diseases treated with biological agents and living in rural areas of Northern Italy

6th World Congress on Leishmaniasis : 16th-20th May 2017, Toledo, Spain : abstracts book / [s.l. : s.n., 2017]. - p 1409 (Poster C1020) [Nr. Estr. 7772]

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

Recently, *Leishmania infantum* (Li) began to spread in Italian Northern regions, such as Emilia

Romagna (ER). Aim of our study was 1. to assess the level of Li circulant kDNA in patients affected by chronic rheumatic diseases (CRD), treated with biologic drugs (BD) and as a consequence, more susceptible to the parasite; 2. to correlate the positivity to the area of residence; 3. to study the immune response in this subset of asymptomatic carriers. 2 Methods We retrospectively analyzed PBMC-DNA and sera from both 50 patients affected by CRD, treated with BD for at least 5 years and healthy controls from ER. All the analyzed patients had a CD4/CD8 ratio >2. Each sample was tested with a qPCR for Li kDNA. Cytokines were quantified by a Luminex technology (Bio-Plex, BIORAD Laboratories, Italy). Data analysis was performed with the Chi Square Test, software Stata (v. 13.1) and Graph Pad Prism (v.5). 3 Results Among the 50 CRD patients, 18 (36%) resulted positive for Li PBMC-DNA, with high parasite loads (1 to 136 parasite/ml in 4 patients, 1.000 to 40.000 in 11 patients and over 1.000.000 in 3 patients). Positive patients mainly come from rural areas of ER region with a statistically significant difference (SSD) ($p < 0.0003$) and displayed higher levels of IL-12p70, IFN-g, IL-2ra, TNF-a, IP-10 cytokines (elevated SSD) and IL-10 and IL-13 (low SSD) compared to healthy controls. All the above cytokines were also up-regulated compared to rheumatic patients negative for Li kDNA, although no SSD was reached owing to the small sample size. 4 Conclusions qPCR performed on PBMC detected higher DNA rates compared to those found on whole blood in asymptomatic carriers. This may be due to the fact that PBMC are target cells for Li and BD effect may impair the control of the parasitic infection. The statistically significant IL-10 and IL-13 level values are related to a both natural Treg cells or Th-2 response, which support reactivation of Li in these patients. In contrast, the higher levels of pro-inflammatory cytokines associated to the high CD4+ lymphocyte rate, may strongly amplify the Th-1 response in positive patients with the following disruption of amastigotes and the subsequent protection from the onset of symptoms, despite the detection of high parasite loads. Further studies are needed to increase the sample size and to investigate the kinetics of parasitic infection and immunological response especially in patients under BD in rural areas.

Moreno^o A, Rovida F, Piralla A, Chiapponi^o C, Capra Marzani F, Campanini G, Mojoli F, Girello A, Lelli^o D, Vezzoli^o F, Prati^o P, Foni^o E, Percivalle E, Pavan A, Pozzi M, Gramegna M, Arioli E, Iotti GA, Baldanti F

Severe respiratory infection caused by swine influenza A (H1N1) virus in an immunocompetent man that was not directly exposed to pigs

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 190 (Poster 055). - 7 bib ref [Nr. Estr. 7593]

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

Moriconi M, Rugna^o G, Calzolari^o M, Bellini R, Albieri A, Angelini P, Cagarelli R, Landini MP, Charrel RN, Varani S

Phlebotomine sand fly-borne pathogens in the Mediterranean Basin : human leishmaniasis and phlebovirus infections

PLOS Neglect Trop Dis. - Vol. 11 no 8 (2017). - p e0005660 (19 p). - 119 bib ref (ultimo accesso 03/11/2017 <http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0005660>) [Nr. Estr. 7677]

Pathogens transmitted to humans by phlebotomine sand flies are neglected, as they cause infectious diseases that are not on the priority list of national and international public health systems. However, the infections caused by protozoa of the Leishmania genus and viruses belonging to the Phlebovirus genus (family Phenuiviridae)—the most significant group of viruses transmitted by sand flies—have a relevant role for human pathology. These infections are emerging in the Mediterranean region and will likely spread in forthcoming decades, posing a complex threat to human health. Four species and 2 hybrid strains of Leishmania are pathogenic for humans in the Mediterranean Basin, with an estimated annual incidence of 239,500–393,600 cases of cutaneous leishmaniasis and

1,200–2,000 cases of visceral leishmaniasis. Among the phleboviruses, Toscana virus can cause neuroinvasive infections, while other phleboviruses are responsible for a typical “3-day fever”; the actual incidence of Phlebovirus infections in the Mediterranean area is unknown, although at least 250 million people are exposed. Here, we reviewed the current literature on epidemiology of sand fly–borne infections in the Mediterranean Basin, with a focus on humans. Our analysis indicates the need for increased public health activities directed to determine the disease burden of these infections as well as to improve their surveillance. Among the emerging challenges concerning sand fly–borne pathogens, the relationships between sand fly–borne protozoa and viruses should be considered in future studies, including epidemiological links between *Leishmania* and phleboviruses as well as the conditional capacity for these pathogens to be involved in interactions that may evolve towards increased virulence.

Oliveira VA, Favero, Stacchiotti A, Giugno G, Buffoli B, De_Oliveira CS, Lavazza° A, Albanese M, Rodella LF, Pereira ME, Rezzani R

Acute mercury exposition of virgin, pregnant, and lactating rats: Histopathological kidney and liver evaluations

Environ Toxicol. - Vol. 32 no 5 (2017). - p 1500-1512 . - 47 bib ref
<http://onlinelibrary.wiley.com/doi/10.1002/tox.22370/abstract> [Nr. Estr. 7514]

According to the World Health Organization particulate emissions from the combustion of solid fuels caused more than 110,000 premature deaths worldwide in 2010. Log wood combustion is the most prevalent form of residential biomass heating in developed countries, but it is unknown how the type of wood logs used in furnaces influences the chemical composition of the particulate emissions and their toxicological potential. We burned logs of birch, beech and spruce, which are used commonly as firewood in Central and Northern Europe in a modern masonry heater, and compared them to the particulate emissions from an automated pellet boiler fired with softwood pellets. We determined the chemical composition (elements, ions, and carbonaceous compounds) of the particulate emissions with a diameter of less than 1 µm and tested their cytotoxicity, genotoxicity, inflammatory potential, and ability to induce oxidative stress in a human lung epithelial cell line. The chemical composition of the samples differed significantly, especially with regard to the carbonaceous and metal contents. Also the toxic effects in our tested endpoints varied considerably between each of the three log wood combustion samples, as well as between the log wood combustion samples and the pellet combustion sample. The difference in the toxicological potential of the samples in the various endpoints indicates the involvement of different pathways of toxicity depending on the chemical composition. All three emission samples from the log wood combustions were considerably more toxic in all endpoints than the emissions from the pellet combustion.

Pajoro M, Pistone D, Bandi C, Fabbi° M, Scattorin F, Sassera D, Montagna M
Ticks and tick-borne pathogens : avian route over densely populated area in Northern 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 60 [Nr. Estr. 7745]
Convegno Nazionale di Ecopatologia della Fauna (SIEF) (4. : Domodossola : 11-13 Ottobre 2017)

Migratory birds can transport ticks and related tick-borne pathogens (TBPs) of humans and animal importance over long geographic distances. In addition, many avian species are also considered reservoirs of several TBPs. Over the last decades, climate and environmental changes have contributed to drive the range expansion of some tick species to northern latitudes and to higher altitudes. Similarly, ticks and related TBPs are rapidly expanding from wild and natural areas to urban and pen-urban areas increasing human health risks. In our survey, migratory birds were intercepted and captured by nets in the ringing station Fondazione Europea il Nibbio–FEIN (Arosio,

Como, Italy 45°43'54.87" N, 9°12' 40.10"E, 353 m a.s.l.) during their back migration from central Europe. The ringing station is located in a woodland area at the border of a densely populated and infrastructure-rich area in northern Italy. The aim of this study was to investigate the presence of four important zoonoses-causing bacterial agents (*Rickettsia* spp., *Borrelia burgdorferi* sensu lato complex, *Francisella tularensis* and *Coxiella burnetii*) in ticks collected from trapped birds. All the collected ticks, larvae and nymph, were identified as *Ixodes ricinus* and subsequently PCR-screened for the presence of TBPs. Four species of *Borrelia* (*B. afzelii*, *B. garinii*, *B. lusitaniae* and *B. valaisiana*), proved or suspected to cause clinical manifestations of Lyme disease in humans and three species of *Rickettsia* (*R. helvetica*, *R. monacensis* and *R. mendelii*) were detected respectively in 74 (29.5%) and 25 (10%) out of a total of 251 examined ticks. Co-infection with both pathogens was encountered in 7 out of 99 (7.1%) infected ticks. These results confirm the role of birds in tick dispersal and provide an explanation to the observed spreading of ticks and ticks-borne pathogen outside their own elective environments.

Paternoster^o G, Martins SB, Mattivi A, Cagarelli R, Angelini P, Bellini R, Santi^o A, Galletti^o G, Pupella S, Marano G, Copello F, Rushton J, Staerk KDC, Tamba^o M

Economics of one health : costs and benefits of integrated West Nile virus surveillance in Emilia-Romagna

PLoS One. - Vol. 12 no 11 (2017). - p e0188156 (16 p). - 32 bib ref (ultimo 07/12/2017
<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0188156>) [Nr. Estr. 7708]

Since 2013 in Emilia-Romagna, Italy, surveillance information generated in the public health and in the animal health sectors has been shared and used to guide public health interventions to mitigate the risk of West Nile virus (WNV) transmission via blood transfusion. The objective of the current study was to identify and estimate the costs and benefits associated with this One Health surveillance approach, and to compare it to an approach that does not integrate animal health information in blood donations safety policy (uni-sectoral scenario). Costs of human, animal, and entomological surveillance, sharing of information, and triggered interventions were estimated. Benefits were quantified as the averted costs of potential human cases of WNV neuroinvasive disease associated to infected blood transfusion. In the 2009–2015 period, the One Health approach was estimated to represent a cost saving of €160,921 compared to the uni-sectoral scenario. Blood donation screening was the main cost for both scenarios. The One Health approach further allowed savings of €1.21 million in terms of avoided tests on blood units. Benefits of the One Health approach due to shortterm costs of hospitalization and compensation for transfusion-associated disease potentially avoided, were estimated to range from €0 to €2.98 million according to the probability of developing WNV neuroinvasive disease after receiving an infected blood transfusion.

Percivalle E, Sasserà D, Rovida F, Isernia P, Fabbi^o M, Baldanti F, Marone P

Usutu virus antibodies in blood donors and healthy forestry workers in the Lombardy Region, Northern Italy

Vector Borne Zoonotic Dis. - Vol. 17 no 9 (2017). - p 658-661. - 28 bib ref [Nr. Estr. 7658]

Usutu virus (USUV), a member of the genus *Flavivirus*, is known to circulate at low prevalence in Northern Italy, and has been reported to cause overt infection. USUV was first reported in Europe in 2001, but a retrospective study showed that it has been present in Italy at least since 1996. Seroprevalence data for USUV antibodies in sera are being collected in different European countries, showing circulation at low prevalence in human populations. Interestingly, two consecutive studies in Northern Italy indicate a possible increase in the presence of the virus, from 0% to 0.23% seroprevalence in blood donors. In this study, antibodies against USUV were measured in 3 consecutive blood samples collected from October 2014 to December 2015 from 33 forestry workers in the Po river valley, while samples from 200 blood donors from the same

geographical area were tested in parallel. Neutralizing and IgG antibodies were found in six forestry workers (18.1%) and in two blood donors (1%). Our results indicate that USUV circulation in the examined area, part of a highly populated region in Northern Italy, is higher than expected. Healthy subjects exhibit a higher prevalence than what was found in a previous report in an adjoining region (0.23%), while the population at risk shows a much higher prevalence value (18.1%).

Razzuoli E, Amadori^o M, Lazzara F, Bilato^o D, Ferraris M, Vito G, Bozzetta E, Vencia W, Ferrari A

Salmonella serovar-specific modulation of innate immune responses in jejunal epithelial cells

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 267 (Poster 127). - 7 bib ref [Nr. Estr. 7597]

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

Razzuoli E, Amadori^o M, Lazzara F, Bilato^o D, Ferraris M, Vito G, Ferrari A

Salmonella serovar-specific interaction with jejunal epithelial cells

Vet Microbiol. - Vol. 207 (2017). - p 219-225. - 43 bib ref ultimo accesso 06/06/2018
<https://www.sciencedirect.com/science/article/pii/S0378113517300172?via%3Dihub> [Nr. Estr. 7621]

Gut is often a receptacle for many different pathogens in feed and/or the environment, such as *Salmonella* spp. The current knowledge about pathogenicity of *Salmonella* is restricted to few serotypes, whereas other important ones like *S. Coeln*, *S. Thompson*, *S. Veneziana*, have not been investigated yet in human and animal models. Therefore, the aim of our work was to verify the ability of widespread environmental *Salmonella* strains to penetrate and modulate innate immunity in pig intestinal IPEC-J2 cells. Our results outline the different ability of *Salmonella* strains to modulate innate immunity; the expression of the IFN- β gene was increased by *S. Typhimurium*, *S. Ablogame* and *S. Diarizonae* 2, that also caused an inflammatory response in terms of Interleukin (IL)-1 β and/or IL-8 gene expression. In particular, IL-8 gene expression and protein release were significantly modulated by 5 *Salmonella* strains out of 7. Interestingly, *S. Typhimurium*, *S. Coeln* and *S. Thompson* strains, characterized by a peculiar ability to penetrate into IPEC-J2 cells, up-regulated both IL-8 and TNF- α gene expression. Accordingly, blocking IL-8 was shown to decrease the penetration of *S. Typhimurium*. On the contrary, *S. Diarizonae* strain 1, showing lesser invasion of IPEC-J2 cells, down-regulated the p38-MAPK pathway, and it did not induce an inflammatory response. Our results confirm that IPEC-J2 cells are a useful model to evaluate host-gut pathogen interaction and indicate IL-8 and TNF- α as possible predictive markers of invasiveness of *Salmonella* strains in enterocytes.

Ricchi^o M, Mazzarelli A, Piscini A, Di_Caro A, Cannas A, Leo^o S, Russo^o S, Arrigoni^o N

Exploring MALDI-TOF MS approach for a rapid identification of *Mycobacterium avium* ssp. paratuberculosis field isolates

J Appl Microbiol. - Vol. 122 no 3 (2017). - p 568-577. - 26 bib ref (ultimo accesso 08/06/2017
<https://onlinelibrary.wiley.com/doi/10.1111/jam.13357/full>) [Nr. Estr. 7549]

Aims: The aim of the study was to explore the suitability of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) for a rapid and correct identification of *Mycobacterium avium* ssp. paratuberculosis (MAP) field isolates. Methods and Results: MALDI-TOF MS approach is becoming one of the most popular tests for the identification of

intact bacterial cells which has been shown to be fast and reliable. For this purpose, 36 MAP field isolates were analysed through MALDI-TOF MS and the spectra compared with two different databases: one provided by the vendor of the system employed (Biotyper ver. 3.0; Bruker Daltonics) and a homemade database containing spectra from both tuberculous and nontuberculous Mycobacteria. Moreover, principal component analysis procedure was employed to confirm the ability of MALDI-TOF MS to discriminate between very closely related subspecies. Our results suggest MAP can be differentiated from other Mycobacterium species, both when the species are very close (*M. intracellulare*) and when belonging to different subspecies (*M. avium* ssp. *avium* and *M. avium* ssp. *silvaticum*). Conclusions: The procedure applied is fast, easy to perform, and achieves an earlier accurate species identification of MAP and nontuberculous Mycobacteria in comparison to other procedures. Significance and Impact of the Study: The gold standard test for the diagnosis of paratuberculosis is still isolation of MAP by cultural methods, but additional assays, such as qPCR and subculturing for determination of mycobactin dependency are required to confirm its identification. We have provided here evidence pertaining to the usefulness of MALDI-TOF MS approach for a rapid identification of this mycobacterium among other members of *M. avium* complex.

Ricchi^o M, Mazzarelli A, Piscini A, Di_Caro A, Cannas A, Leo^o S, Russo^o S, Arrigoni^o N

Identification of Mycobacterium avium subsp. paratuberculosis field isolates by MALDI-TOF MS

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 310 (Poster 166). - 1 bib ref [Nr. Estr. 7602]

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

Roman-Sosa G, Karger A, Kraatz F, Aebischer A, Wernike K, Maksimov P, Lillig CH, Reimann I, Brocchi^o I, Keller M, Beer M

The amino terminal subdomain of glycoprotein Gc of Schmallenberg virus : disulfide bonding and structural determinants of neutralization

J Gen Virol. - Vol. 98 (2017). - p 1259-1273. - 47 bib ref [Nr. Estr. 7695]

Orthobunyaviruses are enveloped viruses that can cause human and animal diseases. A novel and major member is the Schmallenberg virus (SBV), the etiological agent of an emerging disease of ruminants that has been spreading all over Europe since 2011. The glycoproteins Gn and Gc of orthobunyaviruses mediate the viral entry, and specifically Gc is a major target for the humoral immune response. For example, the N terminal subdomain of the SBV glycoprotein Gc is targeted by neutralizing monoclonal antibodies that recognize conformational epitopes. Here, we determined the structural features of the N terminus of Gc, and analysed its interaction with monoclonal antibodies. We were able to demonstrate that one of two N-glycosylation sites is essential for secretion and interaction with a subset of Gc-specific monoclonal antibodies. Furthermore, four disulfide bonds (S-S) were identified and the deletion of the third S-S blocked reactivity with another subset of mAbs with virus-neutralizing and non-neutralizing activity. The mutagenesis of the N-glycosylation sites and the disulfide bonds strongly indicated the independent folding of two subdomains within the SBV Gc N terminus. Further, the epitopes recognized by a panel of mAbs could be grouped into two clusters, as revealed by fine mapping using chimeric proteins. Combining the disulfide bonding and epitope mapping allowed us to generate a structural model of the SBV Gc Nterminus. This novel information about the role and structure of the amino terminal region of SBV Gc is of general relevance for the design of antivirals and vaccines against this virus.

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

Authors' reply: Two severe human cases due to swine influenza A (H1N1)v in October 2016 in Europe were chronologic coincident yet distinct events

EuroSurveillance. - Vol. 22 no 10 (2017). - no 30480 (2 p). - 4 bib ref (ultimo accesso 04/04/2017 <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=22736>) [Nr. Estr. 7531]

Rugna° G, Carra° E, Corpus° F, Calzolari° M, Salvatore D, Bellini R, Di_Francesco A, Franceschini E, Bruno A, Poglayen G, Varani S, Vitale F, Merialdi° G

Distinct Leishmania infantum strains circulate in humans and dogs in the Emilia–Romagna Region, Northeastern Italy

Vector Borne Zoonotic Dis. - Vol. 17 no 6 (2017). - p 409-415. - 37 bib ref [Nr. Estr. 7537]

Human leishmaniasis is an emerging problem in Italy and is on the increase in the Emilia–Romagna region, northeastern part of the country. Nevertheless, studies dealing with the molecular characterization of Leishmania spp. circulating in these areas are limited. In the present work, we explored the genetic polymorphism of Leishmania isolates from 28 cases of canine leishmaniasis and three cases of human visceral leishmaniasis (VL), which occurred in 2013–2014 in the Emilia–Romagna region. The characterization was carried out in comparison with nine human isolates of Leishmania from other VL endemic Italian regions and two reference strains. Nucleic acid from 31 Leishmania-positive phlebotomine sandfly pools, sampled in 2012–2013 in the Emilia–Romagna region, were also evaluated. DNA amplification and sequencing of the ribosomal internal transcribed spacer-1 and of a repetitive nuclear region on chromosome 31 were carried out for genotyping. Two size polymorphic targets were also analyzed by PCR, the cpb E/F-gene and the k26-gene. Altogether, the analysis showed the circulation of different Leishmania infantum genotypes in the Emilia–Romagna region: two genotypes found in dogs from public kennels were similar to VL isolates from other Italian regions, whereas a third genotype was detected in VL cases of the Emilia–Romagna region and in all but one of the sandfly pools. The combined molecular tools applied in this study can constitute a helpful support for parasite tracking (e.g., in outbreak investigations) and for a better understanding of the epidemiological evolution of leishmaniasis in northeastern Italy.

Salogni° C

L'African turquoise killifish, *Nothobranchius furzeri* (Jubb, 1971), come modello ottimale per lo studio dei processi di invecchiamento nell'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 240-247. - 58 bib ref [Nr. Estr. 7570]

Stacchiotti A, Garcia_Gomez R, Lavazza° A, Monsalve M, Bonomini F

Morphological characterization of a dietary challenged Sirtuin 1 heterozygous mice

Ital J Anat Embryol. - Vol. 122 no 1 (suppl) (2017). - no 205 (1 p). - 2 bib ref [Nr. Estr. 7724]

Congresso Società Italiana di Anatomia e Istologia (71. : Taormina : 20-22 September 2017)

Sirtuin 1 (SIRT1), a member of the silencing information regulator 2 enzymes called sirtuins, is

emerging as a master-regulator of metabolic functions like energy balance, mitochondrial health, browning of white adipose tissue, lipolysis. To best characterize its role in obesity, we analysed metabolic and morphological changes induced in SIRT1 heterozygous mice (HET) [1] by a high fat diet (5.4 Kcal/g from fat-lard TD03584-Envigo) in comparison with C57BL6/J mice. Male C57BL6/J (WT) and HET mice received a standard maintenance diet (SD) (2.9 Kcal/g) or a high fat (HF) diet for 16 weeks from 12 to 28 weeks of age. Hepatic and epididymal white adipose depot (eWAT) reactions to the obesogenic diet were focused on hypoxia, inflammation, and endoplasmic reticulum stress. At euthanasia, blood was collected and the liver and eWAT removed for morphological analysis. WT HF and HET HF groups positively correlated with glucose intolerance, hepatomegaly and adipogenesis when compared with SD groups. Remarkably hepatosteatosis, fibrosis and inflammation were exacerbated in HET HF. Oxidative damage and abnormal lipogenesis were confirmed by elevated 4HNE and SREBP1 expressions. Hepatic mitochondria revealed myelinic figures and abnormal ER-mitochondria juxtapositions in HET HF [2]. eWAT adipocytes showed reduced perilipin but strong TNF-alpha and GRP78 signals in crown-like structures. In conclusion, HET HF mice might represent an intriguing animal model to best understand the complex pathogenesis of obesity and related disorders.

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Hild allelic variants explain Salmonella derby different replication efficiency in human cells

Abstract of papers presented at the 2017 Meeting on microbial pathogenesis & host response : September 12-September 16, 2017 / [s.l. : Cold Spring Harbor Laboratory, 2017]. - p 186 [Nr. Estr. 7690]

Meeting on microbial pathogenesis & host response : September 12-September 16, 2017)

Salmonella enterica serotypes can be largely restricted to a single host or exhibit a wider host range. Multi-host virulence is usually attributed to specific genes while the role of Single-Nucleotide-Polymorphisms (SNPs) is not yet well understood. Data collected since 2012 from our Regional Enter-Net Reference Center for Enteropathogens surveillance show that, among all Salmonella isolates, Derby serotype is more prevalent in swine (28.2%) while it is only rarely isolated in human (2.6%). S. Derby isolates (115 human and 277 swine origin respectively) were genotyped by Pulsed-Field Gel Electrophoresis (PFGE). The two most prevalent profiles in swine are: PFGE_A, significantly less isolated in human than in swine (1 human vs 27 swine isolates) and PFGE_B, proportionally isolated in human as well as in swine (15 human vs 26 swine isolates). Starting from these epidemiological data, we aimed to identify genetic determinants responsible for host-range broadening in PFGE_B isolates. SNPs-based phylogenetic analysis showed that PFGE_A isolates cluster separately from PFGE_B, suggesting different evolutionary paths. 39 isolates belonging to PFGE_A, PFGE_B and closely related profiles were tested for virulence by adhesion, invasion and replication assays in human INT-407 cells. The replication efficiency of PFGE_A isolates is up to 4 logs lower (low replication, LR) than that of PFGE_B isolates (high replication, HR) while there is no differences in adhesion efficiency. Deletion of chromosomal and plasmidic genes exclusive of HR isolates showed no decrease in replication efficiency. We thus focused on nonsynonymous SNPs which discriminate HR from LR isolates. We found two allelic variants of *hilD*, an *araC*-like transcriptional regulator of Salmonella Pathogenic Islands (SPIs) 1, 2, 4 and other virulence genes. The two alleles differ by only one amino acid, with Tyr for LR isolates and Cys for HR isolates at position 291. The *hilD* allele of a HR isolate was replaced by recombination with the LR variant: the obtained mutant shows the same replication efficiency of LR isolates, as well as *hilD*-knockout mutant. Accordingly, a LR isolate with the FIR *hilD* allelic variant shows the same replication efficiency of HR isolates. Here we report, to our knowledge for the first time, that virulence of Salmonella in human cells may be determined by just one SNP in the SPIs transcriptional regulator *hilD*.

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

6th World Congress on Leishmaniasis : 16th-20th May 2017, Toledo, Spain : abstracts book / [s.l. : s.n., 2017]. - p 257 (C0782) [Nr. Estr. 7774]

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

Human leishmaniasis is on increase in the Mediterranean Europe. Nevertheless, the exact prevalence of cutaneous leishmaniasis (CL) is largely unknown as underdiagnosis and underreporting are common. In this study, we evaluated epidemiological, clinical and molecular features of CL cases occurring in the Bologna province, north-eastern Italy. **2 Methods** We performed a retrospective study on CL cases diagnosed in the Bologna province (Emilia-Romagna region, north-eastern Italy) between January 2013 and October 2016. Species identification was carried out by hsp70 amplification and sequencing, while strain characterization was performed by multilocus microsatellite typing (MLMT) targeting 15 highly polymorphic markers. MLMT genotypes were compared with a collection of Italian *L. infantum* strains. **3 Results** During 2013-2016, 36 cases of CL were identified in the Bologna province with an average incidence of 1.00/100,000, with a 4- up to 12-fold increase as compared to previous years. Twenty one out of 36 (58%) CL cases presented as single, typical lesions. Molecular identification of *Leishmania* species by hsp70-based PCR showed signatures of both *L. infantum* and *L. donovani*. MLMT provided preliminary evidence of genetic diversity between strains from CL from the Bologna's province and those commonly circulating in Italy. Further molecular evaluation of strain characteristics is ongoing. **4 Conclusions** We report an increased number of cases of CL in a focal area of north-eastern Italy in 2013-2016. Our preliminary results suggest that an unusual *Leishmania* strain circulates among humans in the Bologna province, north-eastern Italy. This study highlights the importance of CL surveillance in endemic areas and emphasize the need of molecular laboratory surveillance for CL.

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Litter moisture assessment and foot pad dermatitis scoring as animal welfare indicators in commercial turkey production

Abstracts of 9th "Hafez" International Symposium on turkey production : meeting of the Working Group 10 (Turkey) of WPSA : Berlin, Germany, 18th - 20th May 2017 / organized and published by H.M. Hafez. - [Berlin : Institute of Poultry Diseases, Free University Berlin, 2017]. - 2 p. [Nr. Estr. 7770]

"Hafez" International Symposium on turkey production (9th : Berlin, Germany : 18th - 20th May 2017)

A survey was conducted, within the ERA-NET project TURKEYWELFARE, in order to identify major factors that may have an effect on litter quality and the incidence and severity of foot pad dermatitis (FPD) in commercial turkey farms. Prevalence and severity of FPD was assessed by scoring the birds at the processing plant on a 5 point scale. A formula was subsequently applied to the scoring results to obtain a value in order to compare results between single farms. One of the sub-objectives of the trial was to compare five different methods for the evaluation of litter moisture and their correlation with the incidence of FPD. A protocol was developed to identify specific litter assessment and collection points in order to achieve an overall assessment of litter quality throughout the barn. For each device mean and standard deviation of wetness measures per collection point were assessed. The reliability and overall consistency between the five instruments used to measure wetness were high ($\alpha = 0.72$). Measurement of 3 collection points was identified as being sufficient to provide a reliable overall assessment of litter moisture within a barn.