




European Association of Veterinary Laboratory Diagnosticians

This Newsletter:

Foreword

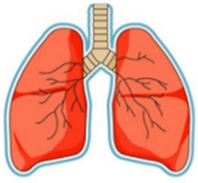
Dear Colleague,
We start this issue with the report of the EAVLD TB WEBINAR organized on 24 of March.
Two scientific works to present a Novel diagnostic approach for the diagnosis of amyloidosis and *Mycobacterium chimaera.*
Finally, a list of important upcoming events and a brief description of a new research project.

Work in progress…
next EAVLD congress will be in 2024






European Association of Veterinary Laboratory Diagnosticians



World TB day WEBINAR Diagnostics and science: a "One Health" perspective

To commemorate the **World Tuberculosis Day, on 24th of March**, the European Association of Veterinary Laboratory Diagnosticians (EAVLD) organized a webinar in collaboration with Jason Sawyer (Head of Immunology and Vaccines TB Research at APHA, UK and board member of EAVLD). Marialaura Corrente, President of EAVLD, and Jason moderated the webinar while several experts in their respective fields spoke about new advances in TB taxonomy, epidemiology, diagnostics, and prevention from a One Health approach.

SPEAKER	AFFILIATION	TITLE
Beatriz Romero Martínez	Deputy Director of the European Union Reference Laboratory (EU-RL) for Bovine Tuberculosis, VISAVET Health Surveillance Centre at the Complutense U of Madrid	The increasing use of molecular diagnostics in the fight against bovine TB
Eamonn Gormley	Professor -School of Veterinary medicine, University College of Dublin Eamonn has worked at UCD since 1998 on tuberculosis in cattle and wildlife	Badgers and bovine TB: the solutions are not black & white
Akinbowale Jenkins	DVM, MsC School of Veterinary medicine, University of Nothingham	A multi-antigenic assessment of cross reactive immune responses in Mycobacterial exposed/infected cattle.
Elena Biasibetti	Researcher at Zooprophylactic Institute, IZS Piemonte, and Valle d'Aosta, Italy	TB Standard Operating Procedures (SOP) for TB histologic diagnosis
Richard Anthony	Senior researcher at the National Institute for Public Health and the environment, Utrecht, the Netherlands. TB reference laboratory	Mycobacterial genotyping, contact tracing and infection control in the human population
Antonio Martinez Murcia	Professor of Microbiology at Miguel Hernández University, Alicante. Director of the genetic PCR solutions™ (GPS™) laboratories Member of EAVLD Board	Genetic diagnosis of Tuberculosis: behind the scenes, a case of the paradigmatic species concept in bacteria
Maria Laura Boschiroli	Senior Researcher. Animal Health Laboratory, National Reference Laboratory for Tuberculosis, Maisons-Alfort, France	It's not always Mycobacterium bovis! Other Mycobacteria and their relevance in the veterinary field
Phil Hogarth	Lead Scientist for Bovine TB at APHA	Use of a DIVA test alongside BCG: making vaccination of cattle against TB feasible

Beatriz Romero Martínez spoke about "**The increasing use of molecular diagnostics in the fight against bovine TB**".
Direct PCR can provide a high diagnostic performance on tissue samples even if always requires verification under the laboratory conditions and get an accreditation system implemented to assure the quality of results. She added that direct PCR on tissue samples could be used to confirm suspected cases as an alternative to culture. Nowadays each country should decide how to implement its use within the eradication/surveillance programs. She concluded that culture is still necessary for epidemiological purposes.

Eamonn Gormley , second speaker gave a speech on "**Badgers & bovine TB. The solutions are not black and white**". Until now badgers have been considered a problem for TB diffusion. Badgers are one of several mammals that can become infected by *M. bovis.* Infected badgers rarely show signs of bTB, with a high proportion of infections resulting in a lengthy period of latency with few obvious lesions at postmortem examination. As such infected and infectious badgers often live with the disease asymptomatically throughout their natural lives, and shed *M. bovis* through their urine, faeces, sputum and discharge from bite-wounds. Transmission of *M. bovis* between cattle and badgers is thought to occur through ingestion of the bacterium at badger latrines. So many intervention have been carried out : vaccination program, culling programme and there are still lots of unanswered questions about the role played by badgers in the spread and maintenance of bovine TB

Akinbowale Jenkins presented a talk with the title "**A multi-antigenic assessment of cross-reactive immune responses in Mycobacterial exposed/infected cattle**" An accurate diagnosis of tuberculosis in cattle may be compromised in areas where there are high rates of exposure to environmental/non tuberculous mycobacteria (NTM). The performance of a diagnostic assay based on a cocktail of antigens was evaluated in animals infected by *M. bovis* and exposed to NTM. the specificity of this test was compared with the tuberculin test and the gamma interferon test. Three of the selected antigens, Rv3615 (ESpC), Rv0287 (esxG) and the ESAT6/CFP10, were immunogenic in the infected cattle, and distinguished the infected cattle from the non-infected NTM exposed animals. The combined data will be useful in future development of novel bTB diagnostic tests.

The first part of the webinar ended with **Elena Biasibetti** who spoke about **"TB Standard Operating Procedures (SOP) for TB histologic diagnosis.** She explained how microscopy can aid in TB diagnosis and how in animal with TB lesions the microscopic analyses is sensible and specific. She reported how an accreditated microscopic procedure comprehensive of TB hematoxylin and eosin; Ziehl Nielsen and immunohistochemistry are used in the framework of eradication program in Italian region.

After a brief coffee break the work went on with **Richard Anthony** who gave an interesting speech on **"Mycobacterial genotyping, contact tracing and infection control in the human population "** highlighting that whole genome sequency (WGS) is important to trace transmission of TB and to delineate outbreaks but it is also important to predict susceptibility of mycobacterium to drug.

Then **Antonio Martinez Murcia** with "Genetic diagnosis of Tuberculosis: behind the scenes, a case of the paradigmatic species concept". He explained different genetic approaches to detect mycobacteria developed in the frame of ARREST-TB project, Horizon 2020 Program. Validation of GPS™ qPCR kit for detection of *M. tuberculosis* complex was achieved according to UNE-EN ISO/IEC 17025 guidelines and labelled CE-IVD. Multi-locus Phylogenetic Analysis (MLPA) indicated the genus *Mycobacterium* is a polyphyletic group composed by five genera (in agreement with full genome relationships) and evidenced that *M. tuberculosis* complex is a single species comprising ecotypes with capacities to colonize different host species (zoonosis).

The event went on with **Maria Laura Boschiroli** who discussed about new TB species with the speech "**It's not always Mycobacterium bovis! Other Mycobacteria and their relevance in the veterinary field**". For livestock animals MOTT give interference with detection of bTB and other major mycobacterioses and furthermore could cause significant or opportunistic infections leading to considerable economic losses.

The webinar ended with **Phil Hogarth** with "**Use of a DIVA test alongside BCG: making vaccination of cattle against TB feasible".** He introduced new data on TB cattle vaccine, describing , its sensibility and specificity and ended with information on the starting point of the new phase to assess the safety of the vaccine giving to the public a new hope to fight against the diseases.

More than 400 people followed it

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Systemic AA-amyloidosis in cats as a natural model to study and compare amyloidogenic processes between animal and human forms

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9 Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Amyloidoses are a group of diseases, affecting humans and animals with chronic inflammation and characterized by localized or systemic deposition of amyloid proteins in organs and tissues. Like the pathogenesis of prion diseases, amyloid fibrils induce a process of "seeding-nucleation," which leads to the conformational change of the normal protein to a β-sheet form that becomes insoluble and accumulates in tissues at the extracellular level. In animals, it has recently been reported that AA- amyloidosis exhibits intra- and inter-species transmissibility. Studies in captive cheetahs have shown a high prevalence of the disease due to horizontal transmission through faeces, whose fibrils administered to mice, induce the disease. In cats, AA-amyloidosis is usually identified only in a few breeds, including Abyssinian and Somali, rarely in domestic short-haired cats.

Preliminary data revealed a high prevalence of AA-amyloidosis (40-85,7%) in some Piedmont cat shelters following diagnostic monitoring performed on necropsy examination, with involvement of liver, kidney, and spleen. The detection by Western blot of amyloid fibrils in bile samples from sick cats suggested a possible horizontal transmission in this species as well.

In the present study, several investigations and analysis were carried out with the aims to define the pathogenetic pattern of systemic AA amyloidosis in cats by searching for amyloid fibrils in organs, tissues and excreta of diseased cats, to isolate and characterize amyloid fibrils from organ and excreta samples and to evaluate a possible route of disease transmission among cats in shelters.

The disease monitoring was performed on the three Piedmont shelters where this high prevalence had been previously observed. Seventy-nine cats that died or were euthanized because they were in great distress were included in this study. A set of data including the state of health, the type of diet, any therapeutic treatments and habits was collected in individual clinical records prepared during periodic follow-up examinations and monitoring of the animals. Different organs and biological fluids were collected from each animal.

The diagnosis of AA-amyloidosis was established on the liver samples by histological analysis with Congo Red (Fig.1). Histological analysis performed on three organs (livers, kidney, and spleen) collected by the 79 cats from the three shelters provided the following results: overall, 38 (48.1%) liver, 46 (58.2%) spleen and 40 (50.6%) kidney samples from cats had amyloid deposits, with 48 animals (60.8%) showing at least one of the three organs affected. Among the affected cats, 35 (72.9%) had three organs involved simultaneously, 6 (12.5%) had two organs, and 7 (14.6%) had only one organ.

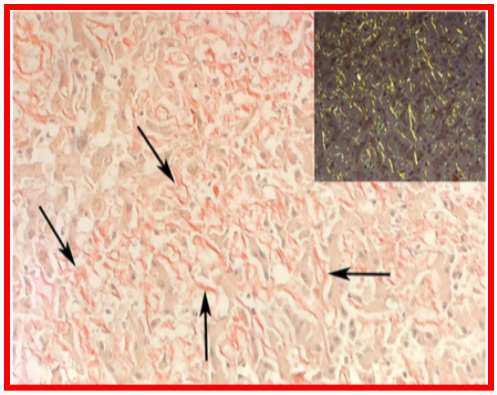

Fig.1: Histological examination of a liver sample with Congo red. A diffuse moderate amount of red stained amyloid (arrows) displaces the hepatocellular cords, sometimes isolating the hepatocytes. Inset: apple-green birefringence under polarized light.

Different analytical methods and protocols have been applied and developed to search the presence of amyloid fibrils in the different organs and excreta of sick cats and then to isolate them in purity, to type them and to determine their structure.

Mass spectrometry analysis conducted on amyloid fibrils isolated and purified from some kidney samples identified SAA protein as their major component. Proteomic and cryo-electron microscopy analysis also showed that the SAA protein extracted from fibrils of kidney of cat with amyloidosis had a cross-β structure characterized by a different fold than human and murine as well as a higher level of stability. This is explained by the different length of the protein sequence and by the presence in it of different aminoacid residues compared to the SAA protein of the other two species. A similarity of 70% was determined between the SAA protein of the cat and that of murine and human; unlike what emerged from the comparison with that of the cheetah which was 99% (Fig.2)

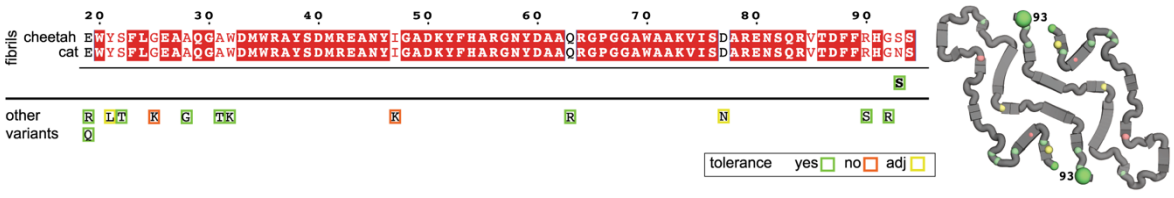

Fig. 2 - Sequence alignment of cat and cheetah amyloid identified in this and a previous study (Zhang *et al.*, 2008). Single residue substitutions are highlighted at the sequence (left) and structure (right) levels. Feline AA amyloid is represented as a gray balloon.

Furthermore, Western Blot analysis conducted on the liver and kidney of the animals that were positive revealed the presence of amyloid fibrils not only in these organs, but also in the bile, thus confirming what had been seen preliminarily in this fluid, and in the faeces of these animals (Fig.3). These findings are very important as they further corroborate the hypothesis of horizontal transmission of this disease within the shelters, which would therefore explain its high prevalence.

In fact, the diffusion of amyloid fibrils in the environment through excreta represents a source of contagion for animals that are very often stressed and with serious inflammatory processes also due to the fact that, having a very stable structure, they tend to remain for a long time on surfaces and in the ground if very stringent disinfection procedures are not applied. This would appear to be supported by the fact that the disease is more prevalent in cats that have spent more time in shelters

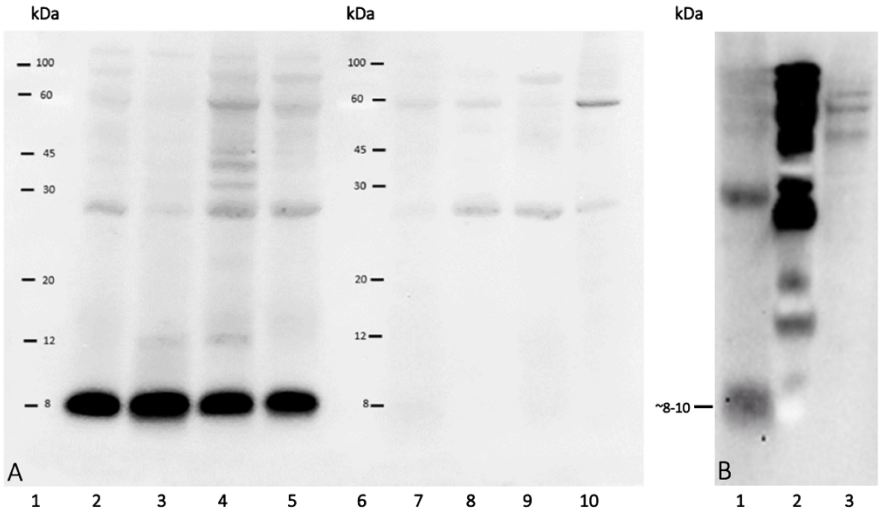

Fig. 3 - Representative Western Blot analysis with anti-SAA1 polyclonal antibody. A) Bile samples: lanes 1 and 6: colored molecular weight markers; lanes 2–5: positive samples; lanes 7-10: negative samples. B) Faeces samples: lane 1: positive sample; lane 2: molecular weight markers; lane 3: negative sample. The bands visible in positive samples have a molecular weight of 8-10 kDa, as well as those found in cheetah faeces, a species in which the transmission of the disease by the fecal-oral route has been proven (Zhang *et al.*, 2008).

In conclusion, the outcome of the present study showed that the amyloidosis studied in cats from the three shelters with high prevalence of this disease is caused by the formation of fibrils of the SAA protein with a cross-β structure, which in the pathologic process becomes aggregated and accumulated in insoluble deposits especially in the liver, kidney, and spleen. The presence, moreover, of this kind of fibrils in the bile and faeces of affected animals suggests a spread within shelters of amyloid fibrils, which, because of their characteristic of persisting for a long time in environments, are the most likely vehicle for horizontal transmission between animals. In view of these outcomes, it is interesting to note that cat shelters may represent a model for investigating the spread of SAA amyloidosis in shelters and farms as well as in wild animals kept in captivity.

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Validation of a Novel Diagnostic Approach Combining the VersaTREK™ System for Recovery and Real-Time PCR for the Identification of Mycobacterium chimaera in Water Samples

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Mycobacterium chimaera is an emerging pathogen of the *Mycobacterium avium-intracellulare* complex (MAC), well-known opportunistic bacterium responsible for severe lung infection, in people with chronic respiratory diseases, especially in immunocompromised or elderly patients and often associated with endocarditis and vasculitis resulting from cardiac surgery. Its prevalence in the environment is largely unknown in Europe. Since 1995 the diagnostic laboratories of the Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta (IZSPLV) have been involved in the bovine tuberculosis eradication program and in reducing the prevalence of paratuberculosis. These efforts have led to a standardized protocol based on the isolation and molecular characterization of bacterial strains of veterinary interest in the genus *Mycobacterium*. Culture-based methods of detection are the gold standard for diagnosis, however *M. chimaera* can take up to 6–8 weeks to culture on selective solid media, so there is an urgent need for methods that are rapid and reliable that could guarantee better performance and reduce the time for recovery. Several microbiological methods based on liquid media for recovering mycobacteria have demonstrated a reduction in the time to detection (TTD) of targeted bacteria, that in addition to, a rapid and reliable diagnostic tool such as realtime PCR enables the proof of isolation data.

Environmental *M. chimaera* infected simulates at volumes defined in international guidelines were obtained and processed. A suspension (McFarland standard 3) of approximately 9 × 108CFU/mL, was prepared and a logarithmic dilution series was created by adding 1 mL of the undiluted suspension to 9 mL of VersaTREK™ Myco Media (modified Middlebrook 7H9 broth, Thermo Fisher Scientific; mMVT) and phosphate buffered saline (PBS) added with 0.5% Tween 20 (PBS-T, Merck Life Science srl, Milan, Italy). Two sets of eight serial dilutions to 9 CFU/mL were prepared, lettered from "A" to "I", and then each assessed in a culture and molecular assay. Each preparation underwent real-time PCR; inoculates were placed in a VersaTREK™ automated microbial detection system and onto selective Middlebrook 7H11 agar plates. The VersaTREK™ technology is based on the detection of pressure changes due to either the production or the consumption of gas inside the headspace of the medium bottles.

The system continuously monitors changes both in gas production and consumption in the bottles and a “knee shaped” mycobacterial growth curve is generated by a specific algorithm. The instrument gives a positive signal at approximately 1×106 CFU/ mL. The liquid culture was centrifuged and the pellet was re-suspended in PBS and streaked on s7H11 agar plates; growth is assessed weekly and suspected colonies are identified by PCR. Aliquots (0.5 mL) of the suspensions were tested by biomolecular assay. DNA extraction was performed by heat treatment. The real-time PCR protocol was adapted from Zozaya-Valdés et al to detect a 79 bp fragment of the SR1 region, identified as highly specific for *M. chimaera*. The protocol was optimized in 10 µL using iTaq Universal Probes Supermix (Bio-Rad Laboratories srl, Segrate, Italy) with a CFX384 Touch real-time PCR detection system (Bio-Rad Laboratories) conducting 40 cycles at 95 °C for 10 s and at 60 °C for 20 s. Analytical sensitivity of the real-time PCR was measured, starting from extracted DNA brought to a concentration of 100 ng/µL and then diluted in base 10 to a concentration of 1 pg/µL. Assay specificity was tested using DNA extracted from 22 different bacterial species (11 of the genus *Mycobacterium* were derived from international collections -ATCC).

The validation tests showed that real-time PCR detected DNA up to a concentration of 10 ng/µL. A comparison of the isolation tests showed that the PCR method detected DNA in a dilution of 1×102 CFU/mL in the bacterial suspensions, whereas the limit of detection in the VersaTREK™ was <10 CFU/mL. Within less than 3 days, the VersaTREK™ detected an initial bacterial load of 100 CFU. The detection limit did not seem to be influenced by NaOH decontamination or the initial water sample volume; analytical sensitivity was 1.5 × 102 CFU/mL; positivity was determined in under 15 days.

VersaTREK™ system can expedite mycobacterial growth in a culture. When combined with PCR, it can increase the overall recovery of mycobacteria in environmental samples, making it potentially applicable for microbial control in the hospital settings and also in environments with low levels of viable mycobacteria contamination.

UPCOMING EVENTS

The **XXVI Symposium of the ASSOCIATION OF SPECIALISTS IN VETERINARY LABORATORY DIAGNOSIS (AVEDILA)** will take place at the Congress Center in the city of Elche on November 20th and 21st, 2023.

XXVI SIMPOSIO AVEDILA

The program includes presentations by cutting-edge researchers in veterinary diagnosis from institutions such as the **Veterinary Health Surveillance Center (VISAVET), European Reference Laboratory (EU-RL), Ministry of Agriculture, Fisheries and Food (Laboratories in Algete, Madrid, and Santa Fe, Granada**); the **Centre de Recerca en Sanitat Animal (IRTA-CRESA);** and other members of the **Animal Health Research Network (RISA)** such as **NEIKER** and **SERIDA**. The organizing committee is collaborating with the **Official College of Veterinarians of Alicante**, the **Official College of Veterinarians of Murcia**, the **Valencian Council of Veterinary Colleges**, **Miguel Hernández University**, and the City Councils of Elche and Orihuela. The planned social event is a cultural visit to the neighboring historic city of Orihuela, which will conclude at its **Historic University (16th century)**, currently the Diocesan College of Santo Domingo, where the symposium dinner will take place.

For more details visit the web site https://simposioavedila2023.com/.

UPCOMING EVENTS

5th International Conference of the European College of Veterinary Microbiology (5th ICECVM) that will be held in Bled, Slovenia, on 21-23 September 2023.

The 5th ICECVM is a three-day event dedicated to Veterinary Microbiology, with sessions covering bacteriology, virology, mycology, antibiotic resistance, etc., under the One-Health umbrella. A satellite event consisting of a training school on veterinary clinical bacteriology, organized by the European Network for Optimization of Veterinary Antimicrobial Treatment (**ENOVAT**) will be held on **Wednesday 20th September**. In parallel with the 5th ICECVM scientific conference, we will host sponsored exhibition and workshop events, providing a unique collaborative environment, in one of the most beautiful alpine destinations!

The **XXII Italian National Congress (SIDILV)** will take place in Brescia in the congress Center Paolo VI from 11th to 13st October 2023.

For more details visit the web site
https://sidilv.org/congresso-nazionale-2023/

NOSOBIOSAFE_NOSOCOMIAL INFECTION RISK MONITORING

Genetic Analysis Strategies SL with his registered mark **genetic PCR solutions™** is developing and directing the project **The Environmental Clinical Bio-safety System project: Nosocomial Infection Risk Monitoring**, with the acronym **NOSOBIOSAFE**, wich intends to carry out planned research and critical studies aimed at developing and implementing a new system for rapid monitoring of microbial load, detection of nosocomial pathogens and their genetic profile of virulence and resistance to antibiotics in the air of hospital risk areas. In addition, this system will make it possible to predict outbreaks of new pathogens, even if they have not been previously described.

This research project is perfectly aligned with the challenges of the Strategic Innovation Committee (CEI) proposed in 2022, since the main objective is the prevention of infections caused by nosocomial pathogens and the development of early detection systems for colonizing microorganisms.

The NOSOBIOSAFE project has received a public grant for financial support from the Valencian Innovation Agency (AVI) within the Consolidation of the business value chain program (File No.: INNCAD/2022/23).

EAVLD Members, we need you!

Would you to contribute to our mission?

Do you have news for the EAVLD newsletter?
Do you are a young researcher working across Europe? Tell us your story!
Please send to Marzia Pezzolato, marzia.pezzolato@izsto.it before october, 31 2023.

Do you have ideas or proposals to share with us?
Contact marialaura.corrente@uniba.it

European Association of Veterinary Laboratory Diagnosticians

Newsletter by Marzia Pezzolato