9. newsletter, May 2014

EAVLD Newsletter

Foreword

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Dear colleague,

In this issue of EAVLD Newsletter you will find a reminder for the next EAVLD Congress 12-15 Oct. 2014 in Pisa. Further there is an important invitation to stand for the EAVLD board.

In this issue, we continue with presentations of different EU reference laboratories, here the EU-RL for ASF.

Finally, there is an article about typing of TSE.

The 3rd Congress of the EAVLD
12-15 October 2014 in Pisa, Italy

We strongly encourage you to attend the EAVLD Congress in Pisa together with European colleagues who share the same professional interests and experiences.

Check the homepage for more information

http://eavld2014.org/

Time for you to be in charge of EAVLD!!

During the General Meeting at the third EAVLD congress in Italy, 2014, a new vice-president and new board members for the EAVLD will have to be elected. During this meeting we will bid farewell to several board members who were already part of the provisional board that founded the EAVLD in 2009. After 5 years of service, their terms are up, and they cannot be re-elected. These board members are: Andrew Soldan, Frederik Widén, Sven Erik Jorsal, Jose Antonio Garcia, and Martin Beer. In addition, Gerard Wellenberg, who was elected for the first time in 2010, will also have to step down after 4 years of service.

In accordance with the constitution of the EAVLD, Miroslaw Polak will become the new president of EAVLD at the upcoming General Meeting. Willie Loeffen will become past-president. The remaining board members may stand for re-election if they wish to do so.

The total number of board members is currently 11, but will be reduced again to 8. The temporary increase to 11 board members was decided on in 2012 to allow for continuity in the board when most of the original founders would all step down at the same time in 2014.

The new vice-president is elected directly by the EAVLD members. Following a two year term as vice president, the elected person automatically will serve for two years as president and another two years as past-president. If you want to become the next vice-president, you will have to stand for election as such.

All other board members are elected with no predetermined position on the board. These positions will be decided on by the board members themselves, after the elections.

This is your opportunity to become more involved with EAVLD! If you consider putting yourself up for election, please contact the secretary of the EAVLD (Gerard Wellenberg or Kirsty Line: **secretary@eavld.org**). Board members will participate in regular board meetings (on average every two months) by teleconference. Besides the secretarial and treasury duties, board members have active roles as web master, editor of the newsletter, coordinator for country representatives, etc. Board members serve for a period of two years, with a possible extension for one other term of two years, dependent on re-election. An exception is the election for the vice-president, as explained before.

How to make use of available EAVLD funds?

EAVLD is a non-profit organisation with a very low annual membership fee. None of the board members are paid anything for their work and most of the money that is spent has to do with developing and maintaining the website. Furthermore, for the EAVLD congress in 2014 we intend to partially sponsor 3 (young) scientists, allowing them to visit the congress. There will also be prizes for best oral and poster presentations. You can read more on this elsewhere in this newsletter, or on the website of the upcoming EAVLD congress in Pisa.

We still have a net accumulation of funds though, which we would like to use for the benefit of our members and EAVLD as a whole. This is, however, not something we, as the board of EAVLD, want to decide on by ourselves. For us it is very important to know what our members are looking for, and how they would like to benefit more from being a member of EAVLD. We challenge you therefore to come up with ideas for activities that fit within the scope and purpose of EAVLD. We are specifically looking for ideas that support an active participation of our members, for the benefit of (all) individuals within EAVLD or the association itself.

Do you have a good idea? Contact the President of EAVLD, or any of the other board members with your proposal! But keep in mind that money is not unlimited. With a yearly income of €4000-€5000, we are thinking of ideas that need funding in the order of a few hundred to a maximum of two-thousand euro's for a really good idea. One-time actions, as well as ongoing activities are possible. And both rough ideas and more detailed plans are welcome. This is another opportunity for you to exercise some control over the activities of EAVLD!

Update required of e-mail addresses of EAVLD members

The e-mail address of our EAVLD members is an important communication tool for both the EAVLD board and EAVLD members. The EAVLD board uses the obtained e-mail addresses of EAVLD members for newsletters, news on congresses, etc., and e-mail addresses are used to get in contact with other members (contact information tool).

At this moment approximately 15 e-mail addresses of our current EAVLD members are not valid anymore, and we can no longer reach them. Therefore, we would like to encourage our members to keep their e-mail addresses up to date.

Whenever your e-mail address changes, please update your member information on the EAVLD website, so we can stay in contact with you. This can be done as follows:

“Log in on the members pages at **www.eavld.org** using your user name and your password (if you have forgotten your log-in details you can renew them). At the top of the page you see an icon (a grey box) with the text “My details”. If you click this icon you can see the details you have given when you applied for membership. Here you can change your details including your e-mail address. Then click “save”.

You will see some spaces in the e-mail address no matter how you write it but it has no importance to you. It is just a protection against spam robots.

If you want to check if you have paid your membership you can check it here. At the top you can read the text “membership valid through...” If the field is green you are OK. If the field is red it is time to pay. For most members it should read “valid through 2014-12-31” if you have paid meaning you have paid for the calendar year. For some of you it reads “valid through 2014-xx-xx” meaning you paid one year ago counting backward from that date. This system is being faced out as it is not correct since you pay per calendar year. Anyway, you can use this as an indication if you have paid or not for 2014.

If you are aware of any colleagues who are still a member of EAVLD, but no longer receive e-mails or the newsletter, please inform them that they may need to update their e-mail address. If an outdated e-mail address is not the cause of such a problem, or if you need any further assistance, please contact either our secretary (**secretary@eavld.org**) or treasurer (**treasurer@eavld.org**)

THE EU REFERENCE LABORATORY FOR AFRICAN SWINE FEVER (ASF): TEN YEARS OF HISTORY

European Union Reference Laboratory (EURL) for African Swine Fever. Centro de Investigación en Sanidad Animal (CISA-INIA), 28130 Valdeolmos, Madrid, Spain

By the EURL team: **Gallardo, C; Fernández-Pinero, J; Pelayo, V; Nieto, R; Soler, A; Fernández-Pacheco, P; Martín, E., Simón, A., Pérez, C.; Robles, A; Arevalo, A.; Sánchez, M; Jiménez-Clavero MA and Arias, M.**

Dear members of EAVLD: It's ten years since we started our mission as EU Reference Laboratory for African Swine Fever (ASF EURL), to ensure the quality of the diagnosis and surveillance of this devastating disease of great economic and sanitary impact for the EU pig sector, whose export trade account more than 3.1 million tons product weight annually.

The EURL for ASF is located inside the Biosafety 3 Level CISA-INIA Laboratory, that is composed by 40 laboratories of BSL3 and 2 BSL4 (Agri) laboratories and 19 BSL3 animal rooms for in vivo experiments). Although probably known by many of you, because of the close collaboration maintained in these years, in this occasion we would like to give a brief account of our history along these ten years.

Since the beginning, the EURL adopted a specific and clear objective to build a working-group composed by the National Reference Laboratories (NRLs) of the Member States (MS) and some other NRLs collaborators, to closely collaborate with the EURL-team, with a relationship always leaned on confidence. Along with its duties for the Member States (Directive 2002/60/EC, Annex V), the EURL was settled down on three main pillars to achieve these goals: i) Building Capacities in ASF NRLs, giving support to NRLs of the EU MS, and affected neighboring countries when required, strengthening contacts and collaboration, and harmonization of the ASF diagnosis, through the organization of interlaboratory comparison tests; it also included intensive training and follow-up activities both at the EURL and on site at the NRL ii) gathering and collecting data and information of the disease's likelihood of crossing EU-borders, increasing knowledge on its epidemiology in the affected areas of sub-Saharan Africa and Europe iii) Conducting Research and Development works as a priority directed towards identified gaps concerning diagnosis and control.

When started, in 2003, a NRL network was set up at that time with 12 reference laboratories within the EU. Ten years later, this network comprises 42 laboratories from 35 countries and continues expanding horizons to reinforce national and regional ASF diagnostic capacities worldwide.

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Fig 1. EURL CISA Team and ASF Researchers and technicians collaborators.

Usually the detection of a disease such as ASF spends 1-4 months in the field, but just 4-6 hours are needed to accomplish a full and reliable laboratory diagnosis. Consequently, improving education and training to enable the efficient recognition of the clinical picture of this disease in the field is of key importance. For this reason education and training activities to spread knowledge and preparedness over the key actors in ASF surveillance have been and important goal for the NRLs EU MS and worldwide. In this regard we have organized a remarkable number of training courses on “ASF diagnosis and control” in many different countries of wide territories of the world where this disease constitutes a threat for animal health, including Africa -Kenya (2006, 2007, 2008), Tanzania (2008), Uganda (2009), Nigeria (2009) , Ruanda (2010), Burundi (2010/11), South Africa (2011)-; Asia -China (2010), Kazakhstan (2013)-, and Eastern Europe -Russia (2009,2012), Ukraine (2011), Armenia (2012), Belarus (2012,2013)-. All these training courses have been possible thanks to the collaboration of different Governmental Institutions of the countries involved and Research Institutes collaborating with the EURL, an agreement between our Institute (INIA) and the International Livestock Research Institute (ILRI) and the great support of the European Commission, through the EU projects, the TAIEX Agency, and the new initiative Better Training for Safer Food (BTSF). In addition, EURL ASF experts participate in International workshops and meetings promoted by FAO in Europe and Africa, FAO/OIE (Global Platform for ASF) EFSA, and GARA (Global Research Alliance).

A significant amount of work has also been carried out over these years, most done in close collaboration with the NRLs of the countries, the OIE WRL (Madrid, Spain), ILRI (Nairobi, Kenya) and FAO (Rome and Regional Offices) . The main goal of these collaborations was to improve knowledge about the different aspects of this complex disease, -epidemiology, virus evolution disease dynamics, and adaptation to the host tolerance, etc-, in affected areas of sub-Saharan Africa and Europe. These studies also included diagnostic investigations in the affected regions, pathological, clinical and molecular characterization and transmission studies of circulating isolates, including the transmission of ASF virus from carrier pigs -those surviving to infection-, and in vivo experiments pursuing a safe, reliable and efficient vaccine against ASF. A Part of these studies have been also achieved thank to the active

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participation of the EURL CISA Team in six EU projects, funded by EU DG Research (EC), involving total or partially ASF matters: CT97-3441 (1997-2001); QLK2-00486 (1999-2003) ; CT-02216; (2001-2005), - EPIZONE CT-016236 -FOOD (2006-2011); ASFRISK - KBBE -211691 (2008-2011) and ASFORCE KBBE.1.3-02 (2012 -2015). These investigations have renewed our knowledge on this disease and leaded a number of forefront technological and scientific developments extending the range of techniques to be used in diagnostic laboratories under different scenarios, with new PCR systems, serological confirmatory and penside tests.

Fig 2. A view of the CISA-INIA BSL3 High Security Laboratory.

Field studies have been always made in close collaboration with our epidemiologists at CISA and the OIE WRL (UCM, Madrid) recognizing the importance of this discipline to enlighten diagnosis findings in order to improve disease prevention and control.

Currently, the EURL keeps actively involved in leading research on ASF in Europe, strengthening collaborations and supporting the NRLs with the aim to achieve a high degree of harmonization of ASF diagnosis, with special attention to the NRLs in high risk areas, in a view to protect EU-borders, by providing scientific and technical advice for their Laboratory Contingency Plans.

Moreover, we would like to highlight that we have always wanted to convey the view that ASF diagnosis must be linked to a differential diagnosis of classical swine fever (CSF), because of their great clinical similarities. Therefore, always the two EURL laboratories, CSF and ASF organize their annual meetings jointly, performing also joint workshops regularly. Today, ASF fighting must be considered as one of the top priorities in animal health, due to the threat this disease represents for livestock in the whole Europe and worldwide. We would like to give our deepest thanks to you all NRLs for your active and great collaboration in achieving preparedness along EU, and worldwide. We will be right here, working alongside the NRLs, giving support in all their needs.

Read more about the European Reference Laboratory at the website
www.asf-referencelab.info

TYPING TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHY POSITIVE CASES IN SMALL RUMINANTS

Miroslaw Polak, NVRI, Pulawy, Poland

Based on Regulations (EC) No 253/2006 and (EC) No 1053/2003 amending Regulation (EC) No 999/2001 all Member States (MS) have to perform discriminatory testing when analyzing confirmed transmissible spongiform encephalopathy (TSE) positive samples from small ruminants which are not classified as atypical scrapie.

This requirement is a follow-up of a conclusion from the meeting of STEG members (strain typing expert group) of 28 January 2005, when the first case of BSE in a small ruminant (goat) under natural conditions was confirmed in France.

Due to the putative zoonotic potential of BSE, causing vCJD in humans, and based on the assumption that an epidemic of BSE in sheep could be harder to contain than in cattle (due to the widespread peripheral distribution of infectivity of scrapie in sheep and the possible masking of the presence of BSE agent in sheep) which would make food chain protection more difficult, it was decided that extended monitoring of small ruminants would be launched to assess the real threat from BSE, if it is present in sheep and goats at EU level.

This change in surveillance (higher number of animals tested) mostly of caprine animals was introduced by Commission Regulation (EC) No 214/2005 of 9 February 2005 amending Annex III of Regulation (EC) No 999/2001 of the European Parliament and of the Council as regarding monitoring of transmissible spongiform encephalopathies in caprine animals.

The diagnostic methods used to detect BSE in cattle and scrapie in small ruminants have evolved significantly over time. First it was only histopathology, allowing the detection of final stages of incubation period and the clinical phase of a TSE by the presence of vacuolar lesions in grey matter of neuropil of the brainstem.

Then immunohistochemistry (IHC) was introduced allowing the detection of pathological form of prion protein (PrP^{Sc}) resistant to proteolysis (PrP^{res}) and its distribution in analyzed material. The presence of prion protein resistant to proteolysis in a tested sample is regarded as the confirmation of TSE positive status. This change in testing technique had significant consequences due to the increased diagnostic sensitivity of the method employed. The presence of PrP^{res} precedes the formation of vacuolar lesions and therefore it is possible

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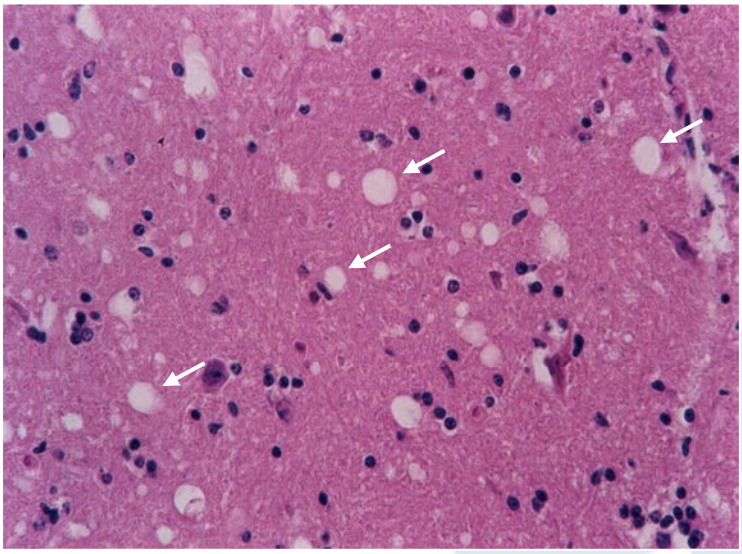

Fig. 1. Histopathology of a positive case (arrows indicate vacuoles)

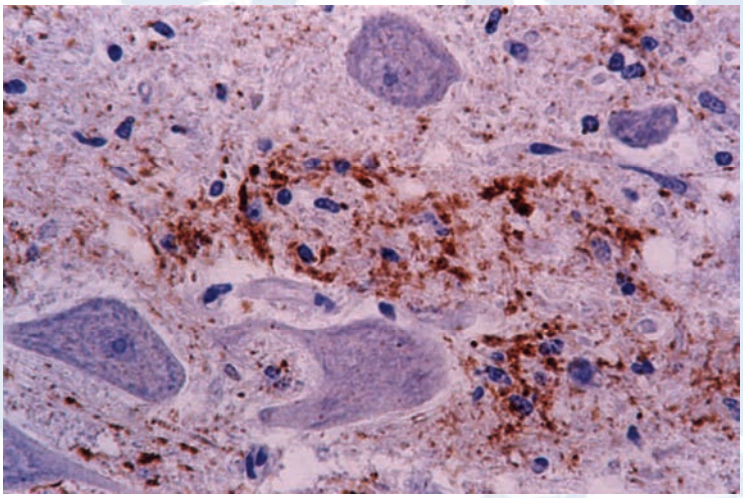

Fig. 2. Immunohistochemistry of a positive case (PrPres is stained brown)

to diagnose a case of TSE in an apparently healthy ruminant, which does not show any neurological signs of disease. However at this stage it was still not possible to distinguish scrapie from BSE and there was not any diagnostic possibility to exclude BSE in small ruminants using histopathology and classical IHC when a positive case was diagnosed and confirmed.

The development of discriminatory methods for BSE and scrapie was also hampered by the lack of positive reference material from small ruminants inoculated with BSE. So far, only two natural BSE cases in small ruminants (goats) have been diagnosed, in France and in UK.

Then, in 2001 Jeffrey et al. described the use of so-called differential or discriminatory immunohistochemistry (dIHC) to distinguish scrapie-affected sheep from BSE experimentally inoculated sheep.

The study showed that it was possible to demonstrate differences in the immunolabelling patterns of disease-specific PrP using different antibodies.

A similar approach based on the use of two separate antibodies directed at two epitopes of PrP was implemented a year later by Stack et al. (2002) but this time using a Western immunoblotting technique.

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This approach, known as molecular typing, is based on the assumption that strain information is supposed to be closely associated with the structural features of PrPres.

The method allowed clear distinction between classical and atypical scrapie in small ruminants, between classical and atypical BSE in cattle and between BSE in sheep and classical scrapie in sheep.

Currently, any TSE case which is confirmed in small ruminant, except atypical scrapie cases, should be subjected to primary molecular testing at the national level (by National Reference Laboratory – NRL) to determine whether it is classical scrapie or BSE-like case (BSE-sheep).

At the moment the EU TSE Reference Laboratory (EURL) recommends 7 discriminatory western immunoblotting methods which can be used by NRLs to perform such studies and these tests include:

- The AHVLA Prionics-based Hybrid Western blot Method
- The ANSES Discriminatory Western blot Method
- The Bio-Rad Discriminatory Test (based on the CEA Discriminatory Western blot Method)
- The FLI Discriminatory Western blot Method
- The CIDC-Lelystad Discriminatory Western blot Method
- The ISS Discriminatory Western blot Method
- The AHVLA Bio-Rad TeSeE-based Hybrid Western blotting Method.

Any NRL performing such tests must first show its competence by successful participation in proficiency testing organized each year by EURL.

An example of such a study is shown in fig. 3. The analyzed samples are loaded in duplicates on two separate gels. Incubation is done with two different antibodies (core antibody and N-terminal antibody) and the interpretation depends on the signal strength and the migration pattern (molecular weight) of PrPres.

The analyzed samples are always compared with BSE and scrapie positive controls which must be loaded on each gel.

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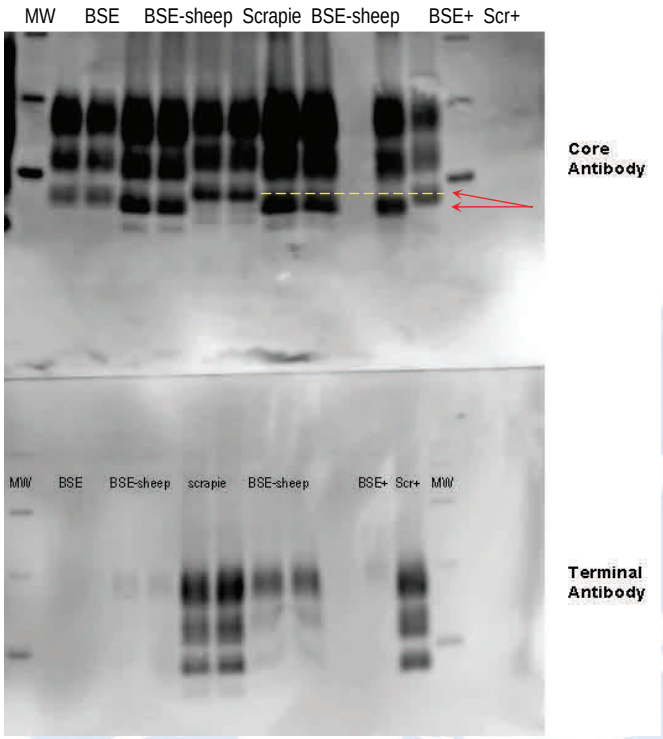

Fig. 3. Primary molecular testing to distinguish BSE from scrapie in small ruminants using a Western immunoblotting technique (MW: molecular weight standard; BSE: positive classical BSE case in cattle; BSE-sheep: experimental BSE in sheep; Scrapie: positive classical scrapie case in a small ruminant; BSE+: BSE positive control; Scr+: scrapie positive control).

All samples except for positive controls were loaded in duplicate.

Tab. 1. Possible result interpretations for this testing are the following:

Sample	Signal with core Ab / Migration pattern-gel location of the lowest band	Signal with terminal Ab
Classical BSE in cattle	Yes/ Lower compared to Scr+ (red arrows in Fig. 3)	No
Classical ovine scrapie	Yes/ Higher compared to BSE+ (red arrows in Fig. 3)	Yes
BSE-sheep	Yes/ Lower compared to Scr+ (yellow dotted line in Fig. 3)	Yes, but much reduced signal
Negative	No	No

Any sample from a small ruminant that gives a banding profile not consistent with classical scrapie using the Western immunoblotting technique should be referred to the EURL for further analysis and investigation if required.

So far only two such cases of BSE in small ruminants (both in goats from France and UK) were diagnosed and confirmed in EU.