*Ethiopia, College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit;* [4] *SPANA UK, 14 John Street, London*

*Histoplasma capsulatum var. farciminosum* (HCF), causing Epizootic Lymphangitis (EZL), is endemic in parts of Africa including, Ethiopia, Senegal and Gambia. Despite its high prevalence, impact on animal welfare and socio-economic importance, there is little evidence upon which to build contextually relevant disease control programmes. The performance and availability of diagnostic tests currently used by clinicians is problematic. Methods such as pattern recognition of clinical signs and microscopy lack sensitivity, and other options are either not commercially available or not readily feasible (e.g. culture). This is a significant barrier to further understanding this disease within endemic countries. This study aimed to validate a nested PCR method to confirm the presence of HCF in equine clinical samples. Ethical approval was obtained from the University of Liverpool and the College of Veterinary Medicine and Agriculture. Twenty-nine horses with suspected EZL were included from topographically varied regions of Ethiopia. Clinical examination was recorded and lesion locations drawn onto equine silhouettes. Blood samples and aspirates of pus from unruptured cutaneous nodules were obtained before treatment provided by SPANA. Blood and clinical data were collected from a further 20 horses with no cutaneous EZL lesions. Giemsa stained impression smears of pus and blood were examined microscopically. Aliquots of heat-inactivated pus and blood were inoculated onto Whatman FTA cards and imported to the UK with Defra approved licensing. A nested PCR targeting the ITS region*, was used to identify samples containing HCF, and all PCR products were sequenced. HCF was confirmed in FTA card pus samples from 24 horses, additionally, 23 blood samples were positive from EZL suspected cases. The nested PCR compared favourably over microscopic examination of pus, where characteristic HCF yeast bodies were detected in only 14 of the 24 PCR positive samples examined. All nested PCR amplification products were confirmed as *Histoplasma* spp. by sequencing. Sequencing of cloned PCR amplicons suggested at least two subgroups of HCF based on single nucleotide polymorphisms. These techniques allow the rapid diagnosis of HCF directly from equine clinical samples and offer a useful epidemiological tool. The identification of HCF in blood raises questions about the pathogenesis of HCF in horses and warrants further investigation.

*Jiang, B. *et. al.* (2000) *Journal of clinical microbiology* **38** (1): 241-245.

We gratefully acknowledge funding awarded by SPANA UK, the SPANA Ethiopia team for assisting in sampling, and all participating horse-owners. Additional funds were awarded by the Institute of Infection and Global Health, and an SFAM studentship.

## 128
## A novel molecular diagnostic tool for Equine Arteritis Virus detection and characterization

D. Gaudaire [1], N. Berthet [2], S. Zientara [3], A. Hans [1]

[1] *Anses, Laboratoire de pathologie Equine, Unité Virologie, Goustranville, France;* [2] *CIRMF, Unité Zoonose et Maladies Emergentes, BP769 Franceville, Gabon;* [3] *Anses, Université Paris-Est, Laboratoire de Santé Animale, UMR 1161 Virologie, Maisons-Alfort, France*

Equine arteritis virus (EAV) is a member of the *Arteriviridae* family. EAV infects equidae and can persist in the reproductive tract of stallions only. Stallions persistently infected shed the virus in their semen and spread the virus in the horse population during breeding. Moreover, infection may cause abortion in pregnant mares and the death of young foals. OIE prescribes viral isolation (VI) on cell culture to detect EAV for international trade. However, a recent study showed that the real-time reverse transcription-PCR (RT-qPCR) assay is as sensitive as VI for detecting EAV in semen [1]. The main challenge to EAV surveillance is detecting EAV to prevent costly outbreaks, considering in particular the limited number of viral nucleotides targeted, in some samples. The aim of our study was to increase the sensitivity of the OIE-recommended RT-qPCR method by combining it with an unbiased amplification method using the Phi29 polymerase coupled to a high-density resequencing microarray (RMA) to genotype the viruses detected. Sixty different samples were used in this study. Of the 48 EAV-positive samples, 31 were from semen, 12 were from virus isolation cell culture supernatants and five were tissue samples from the lungs, spleen or liver of one aborted foetus, three young foals and an adult. Our results showed that isothermal amplification polymerase significantly increased the ratio of amplification from a factor ranging from $10^2$ to $10^7$ compared to the OIE-recommended RT-qPCR method [2]. To genotype the viruses detected, we combined the unbiased amplification of nucleic acids with a RMA. The two EAV sequences tiled on the microarray cover a region located in ORF 1 coding for the non-structural protein 9. Surprisingly, the phylogenic tree obtained with the nsp 9 nucleotide sequences retrieved from the microarray was able to separate strains into the NA and EU groups and divided the EU group into subgroups EU-1 and EU-2 [2]. In conclusion, this method can be recommended for the detection of EAV in semen and aborted foetuses, especially when viral load is very low. In addition, this study confirmed and validated the usefulness of the high-density resequencing DNA microarray for both the diagnosis of equine viral diseases and the genotyping of RNA viruses such as equine arteritis virus.

1. Miszczak, F., et al., Evaluation of two magnetic-bead-based viral nucleic acid purification kits and three real-time reverse transcription-PCR reagent systems in two TaqMan assays for equine arteritis virus detection in semen. J Clin Microbiol, 2011. 49(10): p. 3694-6.
2. Hans, A., et al., Combination of an unbiased amplification method and a resequencing microarray for detecting and genotyping equine arteritis virus. J Clin Microbiol, 2015. 53(1): p. 287-91.

## 191
## Preliminary results on the inclusion of PCR for the diagnosis of equine piroplasmosis (EP)

M.T. Scicluna [1], R. Nardini [1], L.E. Bartolomé Del Pino [1,2], A. Cersini [1], G. Manna [1], G.L. Autorino [1]

[1] *Istituto Zooprofilattico Lazio e Toscana, Rome, Italy;* [2] *Complutense University of Madrid, Spain*

EP is a tick-borne infection, caused by *T. equi* and *B. caballi*. Affected animal presents non-specific signs while introduction of the infection in free areas is due to the movement of inapparent carriers. Laboratory diagnosis of EP is generally requested for the confirmation of clinical suspects and sanitary certification of animals for trade. OIE-prescribed tests are serological methods i.e. ELISA and indirect immunofluorescence (IFAT). Even practitioners mostly request only serological tests for confirmation of suspect

[#] currently: College of Veterinary Medicine, North Carolina State University, USA

cases. In a previous study, real-time (RT) PCRs for each parasite, targeting the rRNA 18s gene (1, 2), were adopted for their optimal performance. This study presents and discusses the results of the use of RT-PCRs in conjunction with ELISA (VMRD, USA) and IFAT (Fuller Laboratories, USA) that were used to analyse 274 horse sera. For comparison of results, PCR was used as reference method since sequencing of derived amplicons confirmed them as specific. The *B. caballi* RT-PCR detected 14 positive samples, none confirmed in ELISA, while the IFAT had a sensitivity (Se) of 50% (7/14) and a specificity (Sp) of 87.7 % (228/260). The *T. equi* detected 137 positive samples with the ELISA showing a Se of 67.2% (92/137) and an Sp of 83.2% (114/137) and the IFAT, a Se of 86.1% (118/137) and an Sp of 81% (111/137). For both parasites, the IFAT presented a limited major sensitivity when compared to the ELISA. Results for *B. caballi* are preliminary as number of samples recruited is limited. In particular, for *T. equi* the serological tests showed a high agreement and a relatively high specificity. An initial evaluation of the correlation between the PCR result and clinical status of the animal was carried out defining as cases those presenting an EP acute form based on temperature >38°C and at least one of the following signs, jaundice, anaemia, and petechial haemorrhages together with a PCR positive result. Of the PCR positive horses for *B. caballi* and *T. equi*, only 28.7% and 19.7% respectively were defined as cases, possibly due to parasite persistence beyond the acute form. Seropositve results of PCR negative samples could be due to sterilization of infected horses, occurring spontaneously or following treatment with a longer persistence of antibodies as usually occurs in most infections. An additional value of the use of quantitative PCR is that treatment efficacy may be monitored especially in view of the side effects this possess. This study demonstrates that the simultaneous use of PCR with serological tests increases the diagnostic probability to define the sanitary state for EP for the purposes stated above and underlines the need for revision of the prescribed diagnostic tests for trade that should include more sensitive methods.

## References

1. Bhoora R, et al. Vet Parasitol 2010;168:201-211.
2. Kim C, et al. Vet Parasitol 2008;151:158-163.

## 202
## Validation of alphavirus chimeras for diagnosis of Eastern and Venezuelan equine encephalitis

T.L. Sturgill Samayoa*, C.D. Brotherson, K.R. Lake, E.N. Ostlund
USDA, APHIS, VS, STAS, National Veterinary Services Laboratories, National Centers for Animal Health, Ames, IA 50010

Eastern and Venezuelan equine encephalitis cause significant disease in both horses and humans.
Effective equine diagnostics (passive surveillance of cases with compatible clinical syndromes) can serve as advance warning for human illnesses. In Central and South America, there are serious difficulties in obtaining quality reagents for EEE and VEE diagnostics (per Dr. Alfonso Clavijo, Pan American Health Organization (PAHO), personal communication). Utilizing the genome of Sindbis virus (SINV), a relatively nonpathogenic human alphavirus, as a vector, a chimeric SIND/VEE virus expressing all of the structural proteins of the VEE TC-83 vaccine strain has been developed (Paessler 2003, Wang 2007). The nonstructural protein genes and cis-acting RNA genome elements from wild-type SINV strain AR339, and the structural protein genes from either North American EEEV strain FL93- 939, or from the naturally murine-attenuated SA strain BeAr436087, are included in the SIND/EEE chimeras. In collaboration with PAHO, MIDA, and the Instituto GORGAS, the National Veterinary Services Laboratories have acquired these Sindbis/Alphavirus chimeras from Dr. Scott Weaver of the University of Texas Medical Branch to validate proposed diagnostic assays. Validation of diagnostic tests that can be performed at biosafety level-2 will be beneficial for use in both the United States and in other countries. Chimeric virus propagation was performed. Direct comparison of the SIND/EEE chimera to wild-type EEE virus was performed using OIE approved protocols for EEE IgM Enzyme-linked Immunosorbent Assay (ELISA) and Plaque Reduction Neutralization Test (PRNT). Testing was performed utilizing sera from naturally infected horses, determined to be positive by previous diagnostic testing. With the goal of developing inexpensive, easily performed diagnostics, the chimeras were tested without inactivation, using the methods previously mentioned. Antigen at a 1:10 dilution provided consistently valid results with varying concentrations of both conjugate and serum. Additional testing of inactivated chimeras will also be performed. In addition to the increased safety to humans who are conducting the diagnostic testing, proposed use of chimeric strains in diagnostics will improve the ability of North, South and Central American laboratories to easily and inexpensively detect infection in animals, thereby leading to improved prevention of infection in both humans and other equids.

## References

Paessler S1, Fayzulin RZ, Anishchenko M, Greene IP, Weaver SC, Frolov I. (2003) Recombinant sindbis/Venezuelan equine encephalitis virus is highly attenuated and immunogenic. J Virol. Sep;77(17):9278-86.
Wang E1, Petrakova O, Adams AP, Aguilar PV, Kang W, Paessler S, Volk SM, Frolov I, Weaver SC. (2007) Chimeric Sindbis/eastern equine encephalitis vaccine candidates are highly attenuated and immunogenic in mice. Vaccine. Oct 23;25(43):7573-81.

## 097
## Development of a real-time PCR assay for quantification of equine herpesvirus 5 (EHV-5) and studying EHV-5 pathogenesis

L.M. Zarski, E.A. High, R.K. Nelli, S.R. Bolin, K. Williams, G Soboll Hussey*
Dpt. Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, USA

Equine herpesvirus 5 (EHV-5) infection has recently been associated with equine multinodular pulmonary fibrosis in horses. To more completely understand EHV-5 pathogenesis and determine viral and host contributions, further *in vivo* and *in vitro* studies are needed. However, current techniques are unable to rapidly, specifically, and quantitatively characterize EHV-5 infection. The aim of this project was to develop a TaqMan real-time PCR assay to quantify EHV-5 in clinical and cell culture samples, and use this test to describe viral replication over time in primary equine respiratory epithelial cells (EREC)s. Primers and a probe were designed to target gene E11 of EHV-5 for TaqMan real-time PCR. Specificity was verified by testing multiple isolates of EHV-5, as well as DNA from other equine herpesviruses. A plasmid containing the target DNA was generated to create a standard curve and quantify viral copy number. TaqMan real-time PCR was performed on DNA isolated from clinical samples. In addition, ERECs were inoculated with EHV-5, and cells and supernatants were collected daily for 12 days following inoculation and TaqMan real-time PCR was performed to asses EHV-5 infection and replication.