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# 10th IEIDC Abstracts-Diagnostics

## 163
## Viral infections in horses in Argentina: an overview based on laboratory results

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Viral infections are a permanent threat for the equine industry worldwide. In the present work we summarized the information, regarding viral diseases of horses, obtained in our laboratory in the last three years (2013-2015). During this period, 260 tissue samples of abortions from 150 breeding farms; 83 nasopharyngeal swabs from 11 outbreaks of respiratory disease in foals and 808 from horses in the pre-export quarantine; 139 stools from 27 cases of diarrhea in foals; 112 semen samples from Equine Arteritis virus (EAV) seropositive stallions and 8 central nervous system (CNS) samples, were submitted for virological studies. Serum samples were submitted for antibody detection of EAV (n: 13,800) and West Nile Virus (WNV) (N: 5,482) from horses in pre-import/export quarantine and from an EAV surveillance program on registered (Stud book, Fomento Equino and Sociedad Rural Argentina) stallions. The diagnosis approach was done by virus isolation on tissue culture or embrionated eggs, and also by detection of viral genome by PCR. Seroneutralization and MAC ELISA were used for EAV and WNV antibody detection, respectively. The described diagnostic tests were conducted following the recommendations of the World Organization for Animal Health (OIE). Equid herpesvirus 1 (EHV1) was detected in 5% (13/260) of the abortions, and in 5 premises this infection generated multiple fetal losses (abortion storms). All EHV1 were characterized as the non-neuropathogenic ($A_{2254}$) variant. Equid herpesvirus 4 was registered in 36% of the cases of respiratory disease in foals. Influenza virus was not detected in none of the nasopharyngeal swabs analyzed. Diarrhea in young foals due to Rotavirus infection was detected in 48% (13/27) of the cases. EAV was not found in the abortion cases or in the semen samples analyzed. Rabies virus was the cause of neurological disease and death in horses occurred in Salta province, an endemic area of vampire bats (*Desmodus rotundus*). The virus was characterized as antigenic variant 3 (vampire). EAV serology demonstrated that 3% (356/13,800) of the samples analyzed were positive. The EAV positive horses had been either related with the EAV outbreak occurred in Argentina in 2010, or vaccinated, or imported as vaccinated. No "new" EAV positive horses were detected after August 2010. No samples tested positive for WNV were found. The data presented here showed that the horse population in Argentina is exposed to several viral infections; thus, the importance of preventive and control measures as well as the benefits of surveillance programs is emphasized.

## 020
## Detection of Equine Infectious Anemia virus by insulated isothermal RT-PCR (iiRT-PCR) assay using the POCKIT ™ Nucleic acid analyzer

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Equine Infectious Anemia (EIA) is a disease of great concern for the equine industry worldwide. EIA virus (EIAV) infection can result in either an acute or chronic (swamp fever) disease that typically transitions to a life-long, inapparent (asymptomatic) infection. Diagnosis is based on serological testing, being the agar gel immunodiffusion test (AGID) the OIE prescribed test for international trade of horses. To date, the detection of EIAV in the blood by molecular diagnostic assays (e.g. quantitative real-time RT-PCR) has not been implemented for routine diagnosis. Recently, a fluorescent probe hydrolysis-based insulated isothermal PCR (iiPCR) integrated with an optical detection module (POCKIT ™) has been validated for on-site detection of several bacterial and viral infections of veterinary interest in clinical specimens. The aim of this work was to evaluate the performance of an iiRT-PCR targeting the 5′LTR+*gag* gene of EIAV genome using the POCKIT™ platform. Clinical samples included serum, whole blood and buffy coat collected from 165 horses distributed in an endemic (n=53), a sporadic (n=92), and a free (n=20) EIA zone based on previous prevalence studies performed in Argentina. None of the horses included in the study showed clinical signs of disease at the time of sampling. Serum samples were tested by AGID, while whole blood and buffy coat samples were tested by the newly developed EIAV iiRT-PCR and a previously described EIAV real-time PCR (qPCR) assay. The sensitivity of the EIAV iiRT-PCR assay to detect infected horses was assessed on whole blood and buffy coat samples, and compared with the AGID test. A total of 56 and 109 serum samples were AGID positive

and negative, respectively. Regarding the buffy coat samples, iiRT-PCR detected EIAV nucleic acid in 31/56 AGID positive samples, while 108/109 AGID negative samples tested negative by iiRT-PCR. Total agreement was 84.24%. When whole blood samples were evaluated, a total of 16 iiRT-PCR positive and 15 iiRT-PCR negative out of 31 AGID positive samples were obtained. Additionally, EIAV nucleic acid was not detected in 25 AGID negative samples .Total agreement was 73.21% for this sample type. When evaluating the accuracy of EIAV iiRT-PCR and qPCR, 28/165 and 130/165 buffy coat samples tested positive and negative by both assays, respectively. Five iiRT-PCR positive samples were negative by qPCR and two qPCR positive samples were negative by iiRT-PCR. Thus, EIAV iiRT-PCR showed more than 95% agreement with qPCR results. It has been demonstrated that the absence of clinical signs is correlated with very low, frequently undetectable viremia. Therefore, EIAV iiRT-PCR appears to be a promising tool to identify infected horses including those experiencing low infectivity titers in blood. Furthermore, as initial EIAV replication rates are frequently high, recently infected equids pose a considerable transmission risk long before seroconversion. In our study, a horse was determined seronegative by AGID, but was identified as EIAV infected by iiRT-PCR. Thus, EIAV iiRT-PCR could be considered as an alternative diagnostic tool in the implementation of control strategies during an EIA outbreak in the low prevalence area of our country.

## 024
## Equine arteritis virus antibody cELISA, a well-validated alternative to the World Organization for Animal Health (OIE)-prescribed virus neutralization test

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Equine arteritis virus (EAV) is the cause of equine viral arteritis, a systemic viral disease of equids that is characterized by signs of respiratory disease, abortion, and infrequently, death in young foals. OIE defines a horse as seropositive if its EAV virus neutralization (VN) antibody titer is ≥1:4. The VN test can take up to 72 hours to complete and requires certain laboratory facilities, equipment, and technical expertise to perform. Non-viral cytotoxicity of sera from some horses vaccinated with certain equine herpesvirus-1 vaccines can interfere with interpretation of the test. Inter-laboratory variation in VN results due to variables such as reference virus used, type and cell passage history has been reported. For these reasons an alternative serologic test is desirable, but none of the previously reported ELISA tests (Table 1) have shown equivalent sensitivity and specificity compared to the VN test. With the aim of producing a simpler and faster alternative to the OIE-prescribed test, a cELISA was developed using EAV gp5-specific non-neutralizing monoclonal antibody (MAb) 17B7. This cELISA was validated with diverse sera (n=2469) against the VN test. It had a diagnostic sensitivity of 95.5% and a diagnostic specificity of 99.8% (JVDI. 25:182-8). The MAb-17B7 cELISA was further validated in three EAV-testing laboratories including one OIE-reference and two AAVLD-accredited laboratories: These confirmed a test sensitivity of 99.5% and a specificity of 98.2% (JVDI 25:727-35). As part of test validation, the following five additional analyses were satisfactorily performed according to the OIE-recommended validation protocol: 1. the primary assay was calibrated with the OIE approved reference serum panel for EVA, 2. repeatability of the assay was evaluated within and between runs, 3. analytical specificity was evaluated using sera specific for selected equine viruses, 4. analytical sensitivity was evaluated with sera collected from horses vaccinated with the modified live virus vaccine against EVA (Arvac®, Zoetis Animal Health), and 5. Duration of the positive cELISA antibody was evaluated following EVA vaccination. The analytical sensitivity of the new cELISA was comparable to the VN test in that it detected EAV-specific antibody as early as 6 days post-vaccination. The duration of EAV-specific antibody detection by cELISA was over six years post-vaccination. Based on the data obtained, significant correlation was demonstrated between the VN test and cELISA results ($r^2$=0.79, P<0.0001). The cELISA was further improved using EAV purified by anion-exchange membrane chromatography (JVDI accepted). This enhanced cELISA was validated using diverse sera (n=3255) at the Maxwell H. Gluck Equine Research Center. The relative sensitivity and specificity of this assay against a group of field sera (n=1851) was 99.6% (95% CI 99.4-100.0) and 98.7% (95% CI 98.3, 99.6), respectively, compared to the VN test (manuscript submitted). This rapid, highly sensitive and specific cELISA compares very favorably to the VN test. It is USDA licensed and should facilitate screening of horses intended for international movement.

**Table 1**
Diagnostic performance of previous EAV ELISAs

| Assay format, number sera tested | Diagnostic sensitivity | Diagnostic specificity | Reference |
|---|---|---|---|
| Indirect ELISA (gp5+N+M), 187 | 92.3, 57.1% | 100% | J Virol Methods 76:127-37 |
| Indirect ELISA (peptide, N and replicase), 200 | 99% | 71% | J Virol Methods 73:175-83 |
| Indirect ELISA (WV), 839 | 87.5% | 98.9% | J Vet Med Sci 60:1043-5 |
| Indirect ELISA (gp5), 1500 | 99.6% | 90.1% | J Virol Methods 54:1-13 |
| Indirect ELISA (WV), 46 | 96% | 26% | Equin Vet J 40(2):182-3 |
| Competitive blocking ELISA (M), 100 | 86% | 100% | CIMID. 26:251-60 |
| Indirect ELISA (gp5), 800 | 96.75% | 95.8% | J Virol Methods 90:167-83 |
| Competitive blocking ELISA (gp5), 675 | 90.7% | 99% | CJVR;64:38-43 |

## 045
## A high-performance multiplex immunoassay for serodiagnosis of flavivirus-associated neurological diseases in horses

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