141
Argentinean agid test for diagnosis of equine infectious anemia: six years of history

M. Bok*[1], G. Asenzo [1], M.M. Vena [1], V. Parreño [1], A. Wigdorovitz [1]
[1] *IncuINTA, Virology Institute, CICVyA, National Institutes of Agricultural Technology, Buenos Aires, Argentina*

Equine infectious anemia (EIA) is a disease of high economic impact on the equine industry worldwide. Since horses are frequent travelers, EIA falls under strict regulatory control programs in many countries. In Argentina the national animal health authority (SENASA) states that all horses imported, moving within the country, or congregating at public assemblies must have a negative EIA report conducted within the previous 2 months. The agent causing EIA is a RNA virus from the *Retroviridae* family and its major capsid protein named p26 is the most immunogenic protein in the viral particle. Thus, the detection of specific antibodies directed to p26 is the aim of most diagnosis tests available in the world. The agar gel immunodifusion (AGID) is the officially accepted method to certify the diagnosis of EIA in Argentina. Since 2009 IncuINTA was working on the scaling up and production of the *KIT AIE IDGA RP26*, an Argentinean AGID test entirely developed in the laboratory containing a recombinant p26 protein to detect EIA antibodies in horses' serum. Until 2015 IncuINTA produced two pilot batches and six commercial batches (one per year) containing from 24000 determinations in 2011 to 39600 determinations in 2015. Since the product was launched in 2011, the sales were increased 109%. Up to date we have placed on the market 170640 determinations. As expected, the number of laboratories buying the *KIT AIE IDGA RP26* was also increasing through time being 26 in 2011 and 36 in 2015. This number of clients represents 17% of the 207 laboratories authorized by SENASA to diagnose EIA in Argentina. These laboratories are located mostly in Buenos Aires, Santa Fe, Entre Ríos, Formosa, La Pampa, Rio Negro, Córdoba, Corrientes, Salta and Tucumán provinces. Until 2009 there was no Argentinean EIA test available in our market being the imported ones very expensive. IncuINTA, which is a R&D laboratory, could scale up, produce and sell the *KIT AIE IDGA RP26* during six consecutive years. After this success, IncuINTA perspective is to increase the number of batches each year to be able to attend the demand of most diagnosis laboratories in the country.

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167
Detection of equine herpes virus in Uruguay

E.R. Castro*[1], J. Murgia [2], A. Caramelli [2], J. Arbiza [3]
[1] *Department Virology Veterinary Laboratories Division (DILAVE) MGAP, Montevideo, Uruguay;* [2] *División Animal Industry, MGAP, Montevideo-Uruguay;* [3] *Section Virology Faculty of Science, UDELAR, Montevideo, Uruguay*

In recent years, an increase in the number of cases of equine neurological disease caused by equine herpes virus 1 (EHV1) neuropathogenic variants, has been observed in numerous countries. The purpose of this study was to detect the presence of the viral genome of EHV1 and equine herpes virus 4 (EHV4) in bronchopulmonary lymph nodes of 47 horses, from various locations of Uruguay, obtained in a slaughterhouse. The genes encoding the glycoprotein H (gH) of EHV1 and B (gB) of EHV4 were amplified by a semi-nested PCR. Of the total samples analyzed, 27% and 6% of lymph nodes contained the gene for gH and gB, respectively. To determine whether the genomes of EHV1 possess the mutation associated with neuropathogenesis (G2254 / D752), the gene for the viral DNA polymerase was amplified and sequenced. One of the five genomes sequenced presented the mutation. The results confirm the presence of EHV1 in our country. Furthermore, there is evidence for the first-time detection of EHV4 and the neuropathogenic variant (G2254 / D752) of EHV1 in Uruguay. This finding provides new insights into the epidemiological situation of EHV-1 and EHV-4 in our country.

182
Comparative test performance of different serological tests for glanders

R. Raghavan [1], G. Syriac [1], R. Wernery [1], M. Elschner [2], I. Mawhinney [3], U. Wernery [1]
[1] *Central Veterinary Research Laboratory, P.O. Box 597, Dubai, United Arab Emirates;* [2] *Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Institute of Bacterial Infections and Zoonoses, Naumburger Strasse 96a, 07743 Jena, Germany;* [3] *Animal Health and Veterinary Laboratories Agency, Worcester, Hereford and Worcester WR5 2LQ, United Kingdom*

Glanders is a zoonotic disease caused by *Burkholderia mallei*. It is an OIE (Office International des Epizooties) listed disease which may affect a variety of animal species but mainly equids. For international trade of horses, donkeys and mules, the complement fixation test (CFT) is the OIE acknowledged main test (OIE Manual, 2016, in press). However, in recent years researches from different countries have established new serological tests to overcome cross reactions (Neubauer et al., 2005; Naureen et al., 2007; Sprague et al., 2009 & Elschner et al., 2011) which sometimes hamper international trade. Additionally, serological results from donkeys and mules as well as from older equine samples are often difficult to interpret due to anti complementary reactions (Wernery et al., 2012). An outbreak of glanders in Bahrain in 2010/11 (Scholz et al., 2014) provided the opportunity to compare different serological tests on 182 equine sera. The 182 horses tested included 53 horses with clinical glanders signs (Panel A), 43 horses which had direct contact with glanderous horses with no glanders clinical signs (Panel B) and 86 horses with no clinical signs kept in the outbreak area (Panel C). The results of the comparison tests are presented in Table 1.

Table 1 Comparative results of three glanders serological tests on 182 equine sera from Bahrain expressed as positive percentage.

ID	SAMPLE NUMBER	CCPRO CFT %	CVRL cELISA%	FLI WB%
Horse Panel A	53	96.2 (51/53)	98.1 (52/53)	98.1 (52/53)
Horse Panel B	43	90.7 (39/43)	95.3 (41/43)	90.7 (39/43)
Horse Panel C	86	34.9 (30/86)	0 (0/86)	0 (0/86)

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190
Evaluation of the diagnostic performance of equine infectious anaemia (EIA) serological ELISAs as screening tools in control programmes

M.T. Scicluna [1], R. Nardini [1], R. Frontoso [1], I. Ricci [1], C.J. Issel [2], G.L. Autorino [1]
[1] *Istituto Zooprofilattico Sperimentale Lazio e Toscana, Rome, Italy;* [2] *Department of Veterinary Science, Gluck Equine Research Center, Lexington, KY, USA*

EIA is a retroviral disease affecting all equidae and its diagnosis is principally based on the use of serological methods represented by agar gel immunodiffusion test (AGIDT), ELISA and the immunoblot that are used on the basis of the intended purpose. OIE proposes both AGIDT and ELISA as suitable for the demonstration of individual or population freedom from infection. Important characteristic for a serological method to be used as screening test in a control programme is its sensitivity that assures the detection of the highest possible number of cases. Relative to this are different studies reporting on the higher sensitivity of the ELISA compared to the AGIDT (1, 2). As Italy, like in many other countries, has a regulatory control program for EIA, the National Reference Centre for EIA (NRCEIA) conducted a study in which the diagnostic performance of all ELISA serological kits available in the country, as candidate/s for a screening test, was evaluated. Ten official laboratories participated in the study where each examined a sample panel containing negative and positive sera with different levels of positivity, using four commercial and 2 in-house kits. The same kits were also assessed for their precocity by the NRCEIA using a panel of sera from vaccinated animals at different days post-vaccination. All the serum samples used in this study were also tested in AGIDT. The parameters evaluated were: diagnostic sensitivity (DSe) and specificity (DSp), Cohen K, weighted Cohen K, coefficient of variation (CV), accordance and concordance. The results obtained were the following; Dse and DSP for all kits were 100% defining, all tests as accurate. K multiple was equal to 0.76 while the value of K for all laboratories, compared with each other was 0.72. The K values indicate a degree of concordance almost perfect according to the classification of Landis et. al. (3). The CV values obtained for all sera were less than 20%, and for this repeatability and the reproducibility for the kits evaluated was satisfactory. Moreover, accordance and concordance were close to 100% in more than half of the sera. Analysis of these parameters show that all kits employed have a high diagnostic performance and also a higher sensitivity than AGID in terms of analytical sensitivity and precocity. Even if a complete evaluation, according to the OIE standards, is required, all kits resulted suitable candidates as screening tools capable of increasing the efficacy of EIA control programmes.

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