

protein epitopes, the sequence alignment of p26 protein was performed for four major monophyletic groups isolated in North American, China, southern Japan and Ireland respectively. The alignment analysis revealed that the defined linear epitopes were not highly conserved. The corresponding mutants were constructed and expressed in HEK293T cells. The cross-reactivity between MAbs and their respective variant epitopes was analyzed with western blotting and AC-ELISA. The results showed that 9H8 could only recognize EIAV strains isolated from China and Japan, while 1G11 could react with all the four EIAV strains. This result indicated that 1G11 have a broadly recognized capacity to p26 epitope from different EIAV strains and could serve as a useful tool for the development of new methods on the anti-EIAV antibody determination.

Reference

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Evolution of clinical, virological and histological findings of equine infectious anaemia (EIA) in naturally infected mules following immune suppression

G.L. Autorino, C. Eleni, R. Frontoso, F. Rosone, A. Caprioli, C. Cocumelli, G. Manna, A. Mastromattei, R. Nardini, M.T. Scicluna Istituto Zooprofilattico Sperimentale Lazio e Toscana, Rome, Italy

EIA is a disease affecting equidae, including the mule were information relative to the characteristics of the evolution of EIA in this hybrid species is scarce [1] with descriptions regarding a limited number of naturally and experimentally infected animals. Also, extensive studies on the effects of immune suppression exist only for horses and donkeys. This study reports the clinical, virological and histological findings of EIA in ten naturally infected mules that were divided into two groups on the basis of their serological reactivity in the agar gel immunodiffusion test (AGIDT): Group P were mules with a clear positive reaction, while Group N, those with an equivocal or negative in AGIDT as described in a previous paper [2]. On recruitment, none of the mules presented evident EIA clinical signs. Both groups underwent pharmacological immune suppression (IS), using dexamethasone (Rapison®) (0,11 mg/kg bw/die) for 8 to 10 days, to investigate the correlations among these characteristics in view of the risk such animals could represent in the transmission of EIA. Clinical evolution was evaluated by hyperthermia and thrombocytopenia, together with alteration of the general condition of the animal. The total observation period lasted for a minimum of 84 days, divided in 56 days pre-IS and 28 days post-IS. Viral replication was assessed using a quantitative real time PCR, in terms of viral RNA (vRNA) copies in the plasma pre and post-IS and viral DNA (vDNA) loads in the tissues of different organs (brain, lung, heart, spleen, liver and kidney) on slaughter of the animals that were also examined for gross and histological lesions. Mules belonging to both serological groups had fluctuating vRNA loads with intervals of negativity independently from the IS period. vRNA peaks, prevalently occurring post-IS, were usually concomitant to hyperthermia and thrombocytopenia. The major tissue vDNA loads were confirmed by the highest vRNA activity in the same animals, with the spleen presenting the highest levels. No relevant gross lesions were observed, while, microscopically,

tissues lesions were characterised by lymphomonocyte infiltrates and moderate hemosiderosis in the cytoplasm of macrophages and Kupffer cells. From the results of this study, in view of viral peaks these animals presented as a consequence to stress related conditions, it is evident that even this hybrid species in the chronic phase of EIA, may act as potential reservoir of EIAV that is independent from their serological pattern. Moreover, animals with an equivocal serological response will probably go undiagnosed unless tests with higher sensitivity, such as the ELISA, are not routinely employed in the diagnosis of suspect cases and in surveillance programmes.

References

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Overview of surveillance of equine infectious anaemia (EIA) in France in 2012

D. Gaudaire¹, G. Amelot¹, F. Lecouturier¹, J. Tapprest¹, A. Hans¹
¹ANSES, Laboratoire de pathologie équine, Goustranville, France

Equine Infectious Anemia virus (EIAV) belongs to the Retroviridae family, genus lentivirus as human immunodeficiency virus (HIV). EIAV infects horses, donkeys and mules and has a worldwide distribution. The virus is responsible of a persistent infection associated with clinical signs such as fever, anorexia and anemia. Non symptomatic horses are contagious and act as a viral reservoir. Consequently, positive horses need to be isolated before euthanize them. In 2012, the French laboratory network approved by the ministry of agriculture to perform the serological diagnosis of equine infectious anemia (EIA), completed 15,691 tests using Agar Gel Immuno-Diffusion (AGID). Twenty seven of these tests were positive for EIA and involved eight horses kept in the Gard and Vaucluse counties in two little towns, approximately 50 kilometers away from each other. The surveillance plan implemented following those cases led to the testing of more than 500 horses in those two counties. Phylogenetic analysis of the isolates collected from the infected equids shows that the cases reported in the Gard and Vaucluse counties in 2012 are independent. Even if these two cases are only a few kilometers away, molecular characterization of viral isolates shows that they are different and do not present a common origin. Those data confirm the information collected during surveys that showed no epidemiological link between the two premises.

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Seropositivity of Equine infectious anemia by 2005 to 2014 in provinces of north-west of Argentina

C.A. Storani¹, R.A. Jacobo¹, M.F. Cipolini¹, D.E. Martinez¹, A.G. Espasandín¹, V. Paine², J.A. Godoy²

¹Facultad de Ciencias Veterinarias UNNE, Corrientes Argentina;
²Laboratorio de Diagnostico Veterinario Privado

Equine infectious anemia (EIA) is a disease caused by a lentivirus specific from equidae family. It had been diagnosed in all continents except in Antarctica. Morbidity and mortality depends on the sensibility of the population and the virus strains. The clinics symptoms of the acute presentation tend to be unspecific and infected horses often recover and remain as chronic carriers. EIA virus (EIAV) infection can result in either an acute or chronic (swamp fever) disease that typically transitions to a life-long,

unapparent (asymptomatic) infection. The virus of EIA is transmitted by blood or contaminated blood derivatives, but in the nature the main way of transmission is by bloodsucking insects. Diagnosis is based on serological testing, being the agar gel immunodiffusion test (AGID) the test prescribed by OIE for international trade of horses. The purpose of this study was to evaluate EIA presentation in northern-west provinces of Argentina, based on results of AGID from serum samples. The results were compiled from a laboratory which belongs to a net of laboratories regulated by the sanitary authorities (SENASA) and is located in Corrientes city. Datum obtained correspond to 56,391 samples obtained from equines of Corrientes (n=48,661), Misiones (n=6,722) and Chaco (n=1,008) provinces, analyzed between 2005 and 2014. A total of 1,954 animals were positives in this period. Percentage of positivity (PP) was determinate, discriminated by province and year. The average PP was determinate by province for the period between 2005 and 2012. In all cases, tendencies shows the decrement of PP from 2005 to 2012 (Misiones, Chaco) or 2014 (Corrientes). In Corrientes province, the rank of PP was 6,03% (2005) to 1,24% (2013); in Misiones was from 4,25% (2005) to 0,8% (2010) and in Chaco was from 20,05% (2008) to 0% (2005 and 2006). The average PP for the period between 2005 and 2012 was 3,77%, 3,35% and 5,91% in Corrientes, Misiones and Chaco, respectively. Since that analysis was made from a non-randomly sampling from serum that arrives to diagnosis in laboratory, this findings cannot be expressed in terms of prevalence. For the same reasons, the PP reached is lower than those reported for equines of the same areas in prevalence studies, because the animals that are frequently controlled are those destined to sports and shows activities and their situation does not reflect what occurs in equines destined to do farm tasks, which are often excluded from controls and where the disease prevalence is higher. This analysis shows, however, a tendency which is in agreement with reported by other authors for the provinces studied, with a higher prevalence in Chaco when compared with Corrientes and Misiones provinces.

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Equine Infectious Anemia: seroprevalence in the Northeastern region of Argentina

A.G. Espasandín¹, A. Vissani², D.E. Martínez¹, E. Lottero¹, L.P. Carballo¹, C.A. Storani¹, R.A. Jacobo^{*}, M.F. Cipolini¹

¹Cátedra de Enfermedades Infecciosas de la Facultad de Ciencias Veterinarias UNNE Corrientes Argentina; ²Instituto de Virología, CICVyA, INTA-Castelar, 1712 Castelar, Buenos Aires, Argentina

Equine Infectious Anemia (EIA) also known as “swamp fever” is a life threatening disease which affects members of the Equidae family and has worldwide distribution. Definitive diagnosis is made with serologic testing; the agar gel immunodiffusion test (AGID) is the prescribed test by OIE. The goal of the present work was to determinate the prevalence of EIA infection among work horses in three cattle farms situated in the Northwest of Corrientes. A sample of whole blood was obtained from all the horses (n=212), and the AGID test was carried out. One hundred and nineteen horses were found infected (AGID positive). Regarding the prevalence of infection in each farm, it was 69% (82 out of 119) in San Luis del Palmar, 44% (34 out of 78) in Empedrado and 0% (no AGID positive animals were found among a total of 15 horses) in Santa Lucia farm. The range of infection was from 0 to 69%. The region environment has the same characteristic in the three premises, high humidity, warm temperatures through the year and high density of arthropods, members of Tabanidae family,

well known as mechanical vectors for the EIA virus. It is a remarkable fact that in an endemic region herds can coexist, ones being infected with high prevalence and others being EIA free. This sanitary status, however, is more frequent in sports animals because they are more controlled in order to complement sanitary regulations. It is important, in endemic areas, to carry out studies that evaluate infections dynamics.

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Serologically silent, occult equine infectious anemia virus (EIAV) infections in horses

S. Ricotti^{1,2}, M.I. García², C. Veaute², A. Bailat², E. Lucca³, R.F. Cook⁴, S.J. Cook⁴, A. Soutullo^{*1,2}

¹Laboratorio de Diagnóstico e Investigaciones Agropecuarias. Sub-Dirección de Ganadería y Sanidad Animal. Ministerio de la Producción de la Provincia de Santa Fe. Boulevard Pellegrini 3100, 3000 Santa Fe, Argentina; ²Laboratorio de Inmunología, Cátedra de Inmunología. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Ciudad Universitaria. Paraje El Pozo. 3000 Santa Fe, Argentina; ³Cátedra de Infectología. Facultad de Ciencias Veterinarias. Universidad Nacional del Litoral. Padre Kredder 2805. 3080 Esperanza, Argentina; ⁴Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546, USA

Samples from 59 clinically normal horses stabled at five separate farms in the Santa Fe Province of Argentina were analyzed to compare molecular and serological based techniques for the diagnosis of Equine Infectious Anemia Virus (EIAV). Of these 26 (44.1%) were positive in official AGID tests and/or gp45/gp90-based ELISA. Surprisingly, 18 of the 33 seronegative horses produced positive results in a PCR directed against viral sequences encoding gp45 (PCR+ve/AGID-ve) and only one of these seroconverted during a subsequent two year observation period. The fact that nucleic acid sequences were amplifiable in 7 of the 18 animals with EIAV gag gene specific primers recommended by the OIE and 2 of these 7 horses, produced positive reactions with oligonucleotide primers directed predominantly against the 5' untranslated region of the viral genome suggest these results were not an artifact produced by the original PCR-based test. Furthermore sufficient quantities of serum were available from 8 of these horses to confirm their negative serological status in sensitive Western Blot tests using purified EIAV particles as antigen. Studies involving 7 of the PCR+ve/AGID-ve horses to measure lymphocyte proliferation in the presence of PHA showed no significant differences between this group and control animals. In addition, lymphocytes from 2 of these 7 horses responded to peptides derived from gp90 and gp45. Together these results demonstrate that apparently clinically normal horses with no gross signs of immunodeficiency in terms of T_{helper}-cell function can remain seronegative for at least 24 months while harboring EIAV specific nucleic acid sequences.

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