Volume 395, April 2016 ISSN: 0737-0806

Journal of Equine Veterinary Science

WWW.J-EVS.COM



Journal of Equine Veterinary Science

Editorial Staff

Journal of Equine Veterinary Science (JEVS) is an international publication designed for the practicing equine veterinarian, equine researcher, and other equine health care specialist. Published monthly, each issue of *JEVS* includes original scientific research, reviews, case reports and studies, short communications, and clinical techniques from leaders in the equine veterinary field, covering such topics as laminitis, reproduction, infectious disease, parasitology, behavior, podology, internal medicine, surgery and nutrition. *JEVS* is also an official publication of the Equine Science Society.

Editor-in-Chief

Edward L. Squires, MS, PhD, ACT (hon), University of Kentucky, Gluck Equine Research Center, USA

Associate Editor

James A. Orsini, DVM, Dipl. ACVS, University of Pennsylvania, Laminitis Institute, New Bolton Center, USA

Editorial Board

Willem Back, DVM, Cert. Pract. KNMvD (Equine Practice), PhD, Dipl. ECVS, Utrecht University, The Netherlands James Belknap, DVM, PhD, Dipl. ACVS, The Ohio State University, USA Hilary M. Clayton, BVMS, PhD, Dipl. ACVSMR & MRCVS, Michigan State University, USA Linda A. Dahlgren, DVM, PhD, Dipl. ACVS, Virginia Polytechnic Institute and State University, USA John Dascanio, VMD, Dipl. ABVP & ACT, Ross University, USA Thomas J. Divers, DVM, Dipl. ACVECC & ACVIM, Cornell University, USA Bernd Driessen, DVM, PhD, Dipl. ACVA & ECVPT, University of Pennsylvania, USA Sue Dyson, VetMB, PhD & FRCVS, Animal Health Trust, Suffolk, United Kingdom Lutz S. Goehring, DVM, MS, PhD, Dipl. ACVIM, Ludwig-Maximilians University of Munich, Germany R. Reid Hanson, DVM, Dipl. ACVECC & ACVS, Auburn University, USA Jerry H. Johnson, DVM, MS, Dipl. ACVS & CVA, Lexington, KY, USA Kevin H. Kline, PhD, University of Illinois, USA Arno E. Lindner, DVM, Dr. Med. Vet., Jüllch, Germany Patrick M. McCue, DVM, PhD, Dipl. ACT, Colorado State University, USA Kenneth H. McKeever, PhD, Dipl. FACSM, Rutgers, The State University of New Jersey, USA Martin K. Nielsen, DVM, PhD, Dipl. EVPC, University of Kentucky, USA Giuseppe Piccione, DVM, University of Messina, Italy Sarah L. Ralston, VMD, PhD, Dipl. ACVN, Rutgers, The State University of New Jersey, USA Chris W.W. Rogers, MAgriSci, PhD, Massey University, New Zealand John Schumacher, DVM, Dipl. ACVIM & AVBP, Auburn University, USA Donald L. Thompson Jr., PhD, Louisiana State University, USA Peter Timoney, MVB, MS, PhD, Dipl. FRCVS, University of Kentucky, USA Dirk K. Vanderwall, DVM, PhD, Dipl. ACT, University of Pennsylvania, USA Nathaniel A. White II, DVM, Dipl. ACVS, Virginia Polytechnic Institute and State University, USA

JEVS is an official publication of the Equine Science Society.

Publication Information: Journal of Equine Veterinary Science (ISSN: 0737-0806) is published monthly by Elsevier Inc., (360 Park Avenue South, New York, NY 10010-1710, USA). Further information on this journal is available from the Publisher or from the Elsevier Customer Service Department nearest you or from this journal's website (http://www.j-evs.com/). Information on other Elsevier products is available through Elsevier's website (http://www.elsevier.com). Periodicals postage paid at New York, NY and additional mailing offices.

USA POSTMASTER: Send change of address to Journal of Equine Veterinary Science, Elsevier Customer Service Department, 3251 Riverport Lane, Maryland Heights, MO 63043, USA. © 2016 Elsevier Inc. All rights reserved.

This journal and the individual contributions contained in it are protected under copyright by Elsevier Inc. No part of this publication may be reprinted or copied either wholly or in part without the permission of the publisher.

YEARLY SUBSCRIPTION RATES: United States and possessions: Individual \$345; Student \$139. All other countries (prices include airspeed delivery): Individual \$378; Student \$171. To receive student/resident rate, orders must be accompanied by name of affiliated institution, date of term and the signature of program/residency coordinator on institution letterhead. Orders will be billed at the individual rate until proof of status is received. Current prices are in effect for back volumes and back issues.

JOURNAL OF EQUINE VETERINARY SCIENCE

Contents: Volume 39S, April 2016	
International Equine Infectious Diseases Conference X	iv
Editorial Preface	
Editorial Preface David W. Horohov	S1
10 th IEIDC Abstracts	
Biosecurity	S2
Diagnostics	S7
Emerging and Re-emerging Diseases	S20
Gastrointestinal	S23
Neurological	S26
Other System Diseases	S33
Parasitology	S45
Reproduction	S56
Respiratory: EHV	S66
Respiratory: EIV	S72
Respiratory: Other	S78
Respiratory: Rhodococcus equi	S83
Respiratory: Streptococcus equi	S88
Working Equids	S98

A complete Guide for Authors for *Journal of Equine Veterinary Science*, and other journal information can be found at http://www.j-evs.com.

Information for Readers

Editorial Correspondence. All editorial correspondence should be addresses to Edward L. Squires, MS, PhD, ACT (hon), University of Kentucky Gluck Equine Research Foundation, 108 Gluck Equine Research Center, Lexington, KY 40546-0099. Telephone: (859) 257-4757, Ext. 81089; Fax: 859-257-8963; E-mail: edward.squires@uky.edu.

Orders, Claims, and Journal Inquiries

Please contact the Elsevier Customer Service Department nearest you:

St. Louis: Elsevier Customer Service Department, 3251 Riverport Lane, Maryland Heights, MO 63043, USA; Telephone: (800) 654-2452 [Toll free within the USA]; (+1) (314) 447-8871 [outside the USA]; Fax: (+1) (314) 447-8029; E-mail: JournalCustomerService-usa@ elsevier.com.

Oxford: Elsevier Customer Service Department, The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK; Telephone: (+44) (1865) 843434; Fax: (+44) (1865) 843970; E-mail: JournalsCustomerServiceEMEA@elsevier.com.

Tokyo: Elsevier Customer Service Department, 4F Higashi-Azabu, 1-Chome Bldg, 1-9-15 Higashi-Azabu, Minato-ku, Tokyo 106-0044, Japan; Telephone: (+81) (3) 5561 5037; Fax: (+81) (3) 5561 5047; E-mail: JournalsCustomerServiceJapan@elsevier.com.

Singapore: Elsevier Customer Service Department, 3 Killiney Road, #08-01 Winsland House I, Singapore 239519; Telephone: (+65) 63490222; Fax: (+65) 67331510; E-mail: JournalsCustomerServiceAPAC@elsevier.com.

Advertising Information

Classified Advertising. Orders and inquiries can be sent to: John Marmero, Elsevier Inc., 360 Park Avenue South, New York, NY 10010. Telephone: (212) 633-3657; Fax: (212) 633-3820; E-mail: j.marmero@elsevier.com.

Display Advertising. Orders and inquiries can be sent to: Joan Coffey, Elsevier, Inc., 360 Park Avenue South, New York, NY 10010. Telephone: (212) 633-3806; Fax: (212) 633-3820; E-mail: j.coffey@elsevier.com.

Reprints. For queries about author offprints, E-mail: authorsupport@elsevier.com. To order 100 or more reprints for educational, commercial, or promotional use, contact the Commercial Reprints Department, Elsevier Inc., 360 Park Avenue South, New York, NY 10010-1710. Fax: (212) 633-3820; E-mail: reprints@elsevier.com. Reprints of single articles available online may be obtained by purchasing Pay-Per-View access on the journal's website, http://www.j-evs.com.

Author Inquiries

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage at www.elsevier. com/wps/locate/issn/0737-0806. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at http://www.elsevier.com/trackarticle. You can also check our Author FAQs at http://www.elsevier.com/authorFAQ and/or contact Customer Support via http://support.elsevier.com.

S[™] The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).

English Language Help Service. Upon request, Elsevier will direct authors to an agent who can check and improve the English of their paper (*before submission*). Please contact authorsupport@elsevier.com for further information.

Funding Body Agreements and Policies. Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies.

© **2016 Elsevier Inc. All rights reserved.** This journal and the individual contributions contained in it are protected under copyright by Elsevier Inc., and the following terms and conditions apply to their use.

Photocopying. Single photocopies of single articles may be made for personal use as allowed by national copyright laws. Permission of the Publisher and payment of a fee is required for all other photocopying, including multiple or systematic copying, copying for advertising or promotional purposes, resale, and all forms of document delivery. Special rates are available for educational institutions that wish to make photocopies for non-profit educational classroom use. For information on how to seek permission visit http://www.elsevier.com/ permissions or call: (+44) 1865 843830 (UK) / (+1) 215 239 3804 (USA).

In the USA, users may clear permissions and make payments through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. Telephone: (978) 750-8400; Fax: (978) 750-4744; and in the UK through the Copyright Licensing Agency Rapid Clearance Service (CLARCS), 90 Tottenham Court Road, London W1P 0LP, UK. Telephone: (+44) 20 7631 5555; Fax: (+44) 20 7631 5500. Other countries may have a local reprographic rights agency for payments.

Derivative Works. Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution. Permission of the Publisher is required for all other derivative works, including compilations and translations. (Please consult http://www.elsevier.com/ permissions).

Electronic Storage or Usage. Permission of the Publisher is required to store or use electronically any material contained in this journal, including any article or part of an article. (Please consult http://www.elsevier.com/permissions). Except as outlined above, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior written permission of the Publisher.

Notice. No responsibility is assumed by the Publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made.

Although all advertising material is expected to conform to ethical (medical) standards, inclusion in this publication does not constitute a guarantee or endorsement of the quality or value of such product or of the claims made of it by its manufacturer.

Journal of Equine Veterinary Science is Abstracted/Indexed by: AGRICOLA; CAB Abstracts; Current Contents/Agriculture, Biology & Environmental Sciences; Elsevier BIOBASE; Focus On: Veterinary Science and Medicine; Global Health (Index Veterinarius, Veterinary Bulletin); Science Citation Index; SCOPUS and others.

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com



International Equine Infectious Diseases Conference X

Organizing Committee Page

International Organizing Committee

David Horohov (Chair) Jenny Evans (International Coordinator) María Barrandeguy (Arg) Ann Cullinane (Ire) Iames Gilkerson (Aus) Alan Guthrie (S. Afr) Julia Kydd (UK) Takashi Kondo (Jpn) Ken Lam (HK) Richard Newton (UK) Martin Nielsen (USA) Klaus Osterrieder (Ger) Nicola Pusterla (USA) Peter Timoney (USA) Andrew Waller (UK) Ulli Wernery (UAE)

Local Organizing Committee

María Barrandeguy (Chair) Cecilia Olguin Perglione (Secretary) Juan Jaworski (Treasurer) Irene Alvarez Gustavo Alvarez Gregorio Daniel Brejov Guillermo Buchanan Pedro Crespo Patricio Digilio Esteban Durante Hugo Forchiniti Jorge Genoud Horacio Houssay Ana Ivanissevich Luis Losinno Florestan Maliandi Octavio Márquez Luciano Miguens Samuel Miño Alejandra Muñoz Jose Ortelli Alejandro Schudel Maria Silvia Tordoya Dante Valenti Cesar Valle Aldana Vissani

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Editorial Preface Editorial Preface



A special thanks to Dr. Maria Barrandeguy, local organizing committee chair, and the local organizing committee for their help. I would also like to thank our many sponsors for their support of this conference.

Dr. David W. Horohov, Chair, IEIDC X International Committee Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA

On behalf of the international and local organizing committees, it is my pleasure to welcome you to the 10th International Equine Infectious Disease Conference (IEIDC X).

This is the latest in a series of meetings focusing on equine infectious diseases which began in 1966 in Stresa, Italy. Other previous meetings included Paris, France -1969 and 1972; Lyon, France - 1976; Lexington, KY - 1987; Cambridge, United Kingdom - 1991; Tokyo, Japan - 1994, United Arab Emirates – 1998, and Lexington, KY - 2012.

This year's conference is in Buenos Aires, Argentina and will once again provide equine scientists and veterinarians from around the world the opportunity to meet and

field.







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

 Zhe Hu, Hao Chang, Man Ge, Yuezhi Lin, Xuefeng Wang, Wei Guo, Xiaojun Wang. Development of antigen captured ELISA for the quantification of EIAV p26 protein, *Appl Microbiol Biotechnol*, 2014, 98 (21):9073-81.

186

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 immunodiffusione 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

[1] Spyrou, V., et. al., (2003) Vet. Microbiol. 95, 49–59.

[2] Scicluna, M.T. et. Al., (2013) Vet. Mic. 165, 123–134.

130

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.

195

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

¹ Facultad de Ciencias Veterinarias UNNE, Corrientes Argentina; ² Laboratorio 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.

198

Equine Infectious Anemia: seroprevalence in the Northeastern region of Argentina

A.G. Espasandin¹, A. Vissani², D.E. Martinez¹, E. Lottero¹, L.P. Carballo¹, C.A. Storani¹, R.A. Jacobo[•], M.F. Cipolini¹

¹ Cátedra de Enfermedades Infecciosas de la Facultad de Ciencias Veterinarias UNNE Corrientes Argentina; ² Instituto 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 coexists, 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 compliment sanitary regulations. It is important, in endemic areas, to carry out studies that evaluate infections dynamics.

094

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

S. Ricotti ^{1,2}, M.I. Garcia², C. Veaute², A. Bailat², E. Lucca³, R.F. Cook⁴, S.J. Cook⁴, A. Soutullo^{*1,2}

¹ Laboratorio 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; ² Laboratorio de Inmunología, Cátedra de Inmunología. Facultad de Bioquímica y Ciencias Biológicas. Universidad Nacional del Litoral. Ciudad Universitaria. Paraje El Pozo. 3000 Santa Fe, Argentina; ³ Cátedra de Infectología. Facultad de Ciencias Veterinarias. Universidad Nacional del Litoral. Padre Kredder 2805. 3080 Esperanza, Argentina; ⁴ Department 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/gp90based 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.

Acknowledgments

The authors thank G. Capello for her helpful assistance, G. Tonarelli and J. Lottersberger for providing peptides, R. Pauli for providing samples from an EIAV-infected horse used as control and I. Alvarez for Western Blot assays. This work was carried out as a cooperative project between Ministerio de la Producción de la Provincia de Santa Fe and Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, and was financially supported by both institutions.