

Freedom from equine infectious anaemia virus infection in Spanish Purebred horses

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ABSTRACT

Introduction: No cases of equine infectious anaemia (EIA) have been reported in Spain since 1983. Factors that could increase the risk of reintroducing equine infectious anaemia virus (EIAV) into Spain include the recent occurrence of the disease in Europe and the absence of compulsory serological testing before importation into Spain.

Aims and objectives: Given the importance of the Spanish Purebred (SP) horse breeding industry in Spain, the aim of this cross-sectional study was to provide evidence of freedom from EIAV in SP stud farms in Central Spain.

Materials and methods: Serum samples from 555 SP horses, collected between September 2011 and November 2013, were tested using a commercially available EIAV ELISA with a published sensitivity of 100 per cent.

Results: All 555 samples were negative for antibody to EIAV, providing evidence of a true EIAV seroprevalence between 0 per cent and 0.53 per cent (95% CIs of the sensitivity and specificity of the ELISA technique used Q10 were 100 per cent and 99.3 per cent, respectively) among the SP breeding population in Central Spain.

Conclusions: These findings should serve to increase confidence when exporting SP horses to other countries.

INTRODUCTION

Equine infectious anaemia (EIA) is an important bloodborne infectious disease of Equidae caused by *equine infectious anaemia virus* (EIAV), an enveloped RNA virus of the *Lentivirus* genus of the *Retroviridae* family which is distributed worldwide (Cook and others 2013). EIAV is transmitted mechanically by haematophagous insects; the most effective are large biting flies such as *Tabanus* several species (horse fly) and *Stomoxys calcitrans* (stable fly) (Issel and Foil 1984, Cook and others 2013). The transmission of the virus depends on the number and species of

vectors, their feeding habits, the population density of horses, the level of viraemia in the host and the amount of blood transferred (Cook and others 2013). Additionally, EIAV may also be passed from a mare to her foal in utero (Stein and Mott 1942, Kemen and Coggins 1972), iatrogenically by blood transfusions or contaminated needles, surgical instruments and dental floats (Williams and others 1981) and there has been recent evidence of the infection of pulmonary epithelial cells by EIAV suggesting an aerosol transmission (More and others 2008a,b, Bolfa and others 2013).

EIA acute clinical disease is characterised by pyrexia, thrombocytopenia, anaemia, rapid weight loss, petechiae in the mucous membranes and oedema and clinically affected pregnant mares may abort. If death does not result from the acute clinical infection, a chronic infection can develop, characterised by the recurrence of febrile episodes. EIAV infection can also be subclinical and affected horses do not show obvious clinical signs (Anon 2013a). The severity of clinical signs is influenced by the strain and dose of the virus and also by the health status of the horse (Cook and others 2013). Morbidity and mortality rates vary and mortality rates as high as 80 per cent have been reported (Cook and others 2013). The infection rate varies with the geographical region; infections are common in humid and swampy regions (Sellon 1993). In premises where the disease has been endemic for many years, up to 70 per cent seroprevalence rates were observed (Tashjian 1984).

In 1978, an outbreak of EIA started in two racecourses in Spain. Both racecourses in Madrid and San Sebastian were quarantined and nearly 900 Thoroughbred racehorses were considered at risk until 1983, when the last outbreak was declared resolved (M Rodríguez, personal communication January

15, 2014) and Spain was declared free from EIAV.

Since the EIA outbreak in Ireland in 2006 (More and others 2008a,b), the disease has affected horses in other European countries such as France, Germany, the UK, Greece and Belgium (OIE World Animal Health Information Database). These outbreaks, along with the absence of compulsory testing for EIAV before importation from these EU member state countries into Spain (Council Directive 2009/156/CE) are considered potentially important risk factors for the reintroduction of EIAV into Spain.

The Spanish Purebred (SP) breed represents 85 per cent of pure bred horses in Spain and nearly 100 per cent of Spanish horses are exported to Europe and America, with this export trade having a monetary value of more than €58 million in 2012 (Anon 2013b). The aim of this cross-sectional study was to provide evidence for freedom from EIAV infection in SP horses in stud farms in Central Spain, thereby enhancing confidence in the export trade of SP horses.

MATERIALS AND METHODS

Selection of study sample

For the purpose of this study, the central region of Spain, comprising the provinces of Madrid, Segovia, Ávila, Cuenca, Guadalajara and Toledo, was chosen. This area has a population of 21,309 SP horses (based on 2012 census data) (Anon 2012), a comparable average density of SP horses with that of Spain (0.39 SP horses/km² in the central area of Spain v 0.35 SP horses/km² in the whole of Spain) and a variety of climate types considered representative of the whole country (Rubel and Kottek 2010, Anon 2012).

The selection of the study population of SP registered horses in the central region of Spain was stratified by stud farms. In order to capture the heterogeneity of the SP breeding stud farms, eligible premises were identified based on the number of horses and the population density on the stud farm, the climate of the area (following the Köppen–Geiger Climate Classification) (Rubel and Kottek 2010) and geographical distribution within the central region of Spain. From these premises, a convenience sample of stud farms was included, with eligible stud farms recruited consecutively until the target sample size was reached. Sample size calculations, based on the population of SP horses in Central Spain, indicated that the minimum number of horses to provide 95% confidence of freedom from EIAV (EIAV seroprevalence of less than 1 per cent) in this area was 297 (WinEpi).

Sampling procedures

On every stud farm, 10 ml whole blood samples were obtained by jugular venepuncture from 100 per cent of the breeding SP stallions and from at least 25 per cent of the breeding SP mares by selecting the mares which were easiest to handle. The inclusion criteria for the

breeding SP stallions and mares were to have actively bred in the past 12 months or to have been in contact with other SP breeding stallions/mares, and to be considered healthy by means of a clinical examination performed by the stud farm veterinary surgeon. Animals were excluded from the study where it was not possible to obtain a blood sample due to the animal presenting a risk for the person in charge of sampling, or where they were resident on the stud farm for less than three weeks. Additionally, where possible, blood samples were obtained from a convenience sample of at least 25 per cent of the SP colts and fillies aged at least eight months, meeting the study inclusion criteria and considered to be healthy by means of a clinical examination performed by the stud farm veterinary surgeon. For each sampled horse, data on age, sex and reproductive activity were also collected via an interview questionnaire completed by the stud farm veterinary surgeon.

Whole blood samples were centrifuged and serum from each animal was separated using Pasteur pipettes, identified with a unique code and stored frozen at -40° C before being thawed and tested.

Serological analyses

A commercial serological screening kit ELISA (ID Screen Equine Infectious Anaemia Double Antigen; ID.Vet Innovative Diagnostics, Montpellier, France) was used to detect antibodies against the core protein (p26) antigen of EIAV. This diagnostic kit uses two fragment antibodies (Fab) on IgG and 10 Fab on IgM which either bind the immunoglobulins to p26 antigen in the microplate or bind a peroxidase antigen used as conjugate. A weak positive serum from an infected horse in the EIAV outbreak in 1978 in Madrid was used in conjunction with positive and negative controls in the kit in order to validate each kit lot used. According to the manufacturer's information, the ELISA kit used in this study has a published sensitivity and specificity of 100 per cent; however, in a study of EIAV seroprevalence in the Sultanate of Oman, the ID Screen Equine Infectious Anaemia Double Antigen ELISA used in this study was reported to have a sensitivity of 100 per cent and a specificity of 99.3 per cent (Body and others 2011). As the Coggins (agar gel immunodiffusion; AGID) test is the OIE standard confirmatory test for EIA, any positive or equivocal samples by ELISA would be tested by Coggins test.

RESULTS

Between September 2011 and November 2013, serum samples were obtained from 555 SP horses residing on 35 different stud farms in the central region of Spain (Fig 1). The proportion of horses sampled in each province of this central region broadly matched that of the 2012 census (Anon 2012), as shown in Fig 2.

The size of the horse populations on the 35 participating stud farms varied from 6 to 220 horses (mean 48.1

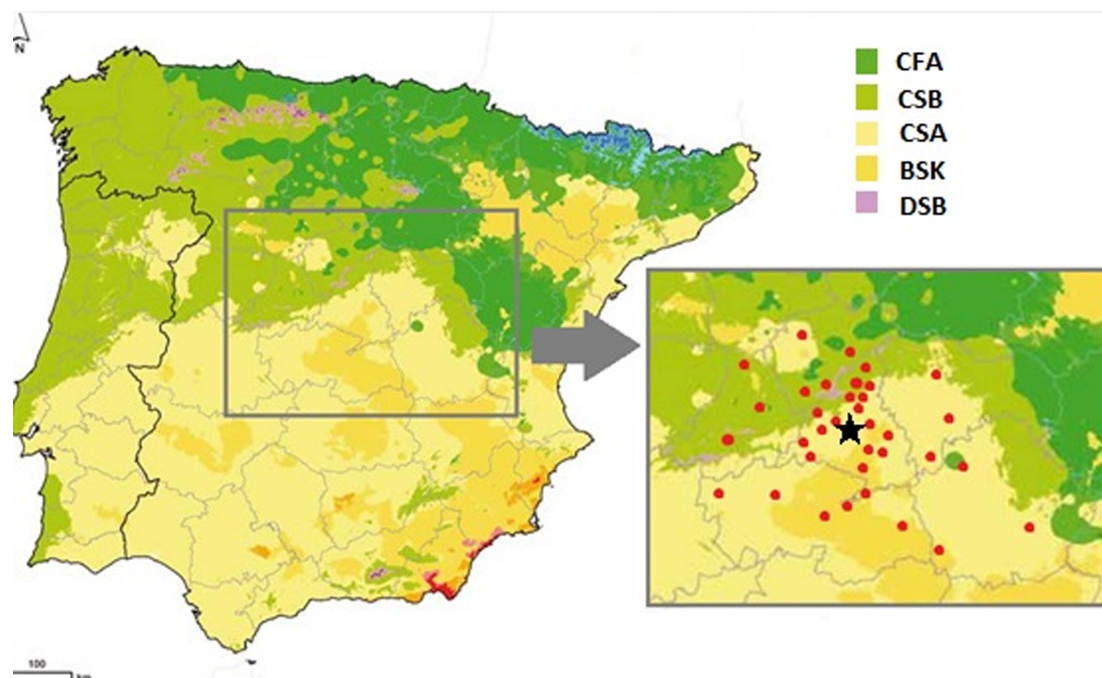


FIG 1: Köppen–Geiger climatic map showing the area of study, its different climate types and the distribution of the Spanish Purebred (SP) stud farms sampled (red points), as well as the location of the equine infectious anemia (EIA) outbreaks in Madrid between 1978 and 1983 (black star). BSK, cold steppe climate; CFA, temperate with a dry season and hot summer climate; CSA, temperate with dry or hot summer climate; CSB, temperate with dry or temperate summer climate; DSB, cold with temperate and dry summer climate.

\pm sd 47.3 horses; median 32 horses, IQR 16–61). The population density on the stud farms ranged from 0.7 to 630 horses/km² (mean 80.4 horses/km² \pm sd 119.8 horses/km²; median 40 horses/km², IQR 12.1–94.4 horses/km²). The locations of the participating stud farms in Central Spain were considered to be largely representative of the overall Spanish climate; 71 per cent of the stud farms had a temperate with dry and hot or dry and temperate summer climate (Csa and Csb,

respectively), these two climates being most commonly present throughout the country (Rubel and Kottek 2010).

The population of SP horses sampled in this study included 237 stallions (42.7 per cent) and 318 mares (57.3 per cent). The mean horse age in the sample population was 7.3 years \pm sd 5.0 years (median six years, IQR 4–10 years). Of the 555 sampled horses, 348 (62.7 per cent) were actively involved in breeding,

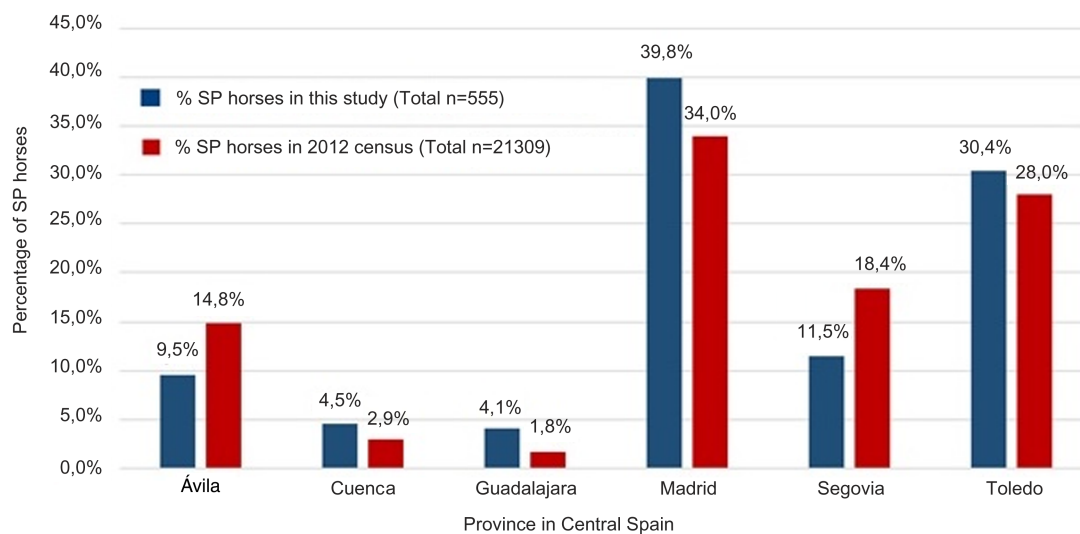


FIG 2: Distribution by province of 555 Spanish Purebred (SP) horses sampled in Central Spain in comparison with the distribution of the general SP breeding population in Central Spain published in the census of 2012 (n=21,309)

whereas 207 (37.3 per cent) were not actively involved in breeding, including 53 colts (26 per cent) and 28 fillies (13 per cent), all less than 3 years of age.

After testing the 555 serum samples for antibodies directed against EIAV proteins by ELISA, all samples were identified as negative. Since there were no positive or equivocal results, no samples were required to be retested by Coggins test. Based on a census-derived population size of 21,309 SP breeding horses registered as of December 31, 2012 in the central area of Spain (Anon 2012), the sample of 555 SP horses serologically screened in this study provided evidence with 95% confidence that the true seroprevalence of EIAV among SP breeding horses in Central Spain was between 0 per cent and 0.53 per cent (WinEpi).

DISCUSSION

The horse population of the central region of Spain that was sampled in this study is considered to represent a high-risk area for the potential introduction of equine infectious diseases, as horses travelling from/to Europe frequently stop in this area and it is the region where the largest number of competitions is held (Anon 2013b). Additionally, the most recent Spanish EIA outbreaks occurred predominantly in the province of Madrid (Fig 1), consequently this region was likely to reflect the highest risk of EIAV persistence since 1983, although no clinical cases of EIA had been detected and reported through passive surveillance in Spain since then (OIE World Animal Health Information Database).

The characteristics of the equine population sampled on the 35 SP stud farms in this study were considered highly representative of the wider SP breeding horse population in Central Spain; the proportions of horses sampled in each province broadly matched those published in the census of 2012 (Fig 2), with the highest percentages of horses sampled in the provinces with a larger SP breeding population (Madrid, followed by Toledo and Segovia) (Anon 2012). The distribution by sex of the sample was also similar to that described in the census of 2012 for the SP breeding population in the central area of Spain (47 per cent stallions and 53 per cent mares) (Anon 2012). Given the excellent representation of the SP breeding horse population in Central Spain in the sample population of this study and also the variety of SP breeding stud farms sampled in terms of size, density of population and climate, EIAV serological results in this study can be considered highly representative of the SP breeding population in Central Spain and could well be representative of the SP breeding population throughout Spain, although further studies using a wider sample population throughout the country would be needed in order to confirm these findings.

Although the double-antigen ELISA technique used to detect antibodies against EIAV in the current study was not the OIE reference technique (Coggins test), when testing on a larger scale for screening purposes is

required within a short period of time, ELISA becomes the test of choice (Paré and Simard 2004, Piza and others 2007). Some authors have demonstrated a high degree of agreement between specific EIAV genetic material presence and antibodies in equids that are reported as negative by Coggins test but positive by ELISA (Issel and others 2013, Scicluna and others 2013); ELISA techniques can detect antibodies directed against the p26 EIAV-capsid before those detectable by the Coggins test and overall are considered more sensitive (Soutullo and others 2001, Reis and others 2012). The reason why the Coggins test is the gold standard technique for EIA diagnosis is that it is more specific (Cullinane and others 2007, Piza and others 2007); that is, ELISA techniques can result in false positives and therefore any positive results to EIA detected by ELISA must be confirmed using the Coggins test (Anon 2013a). In this study, there were no positives to EIAV by ELISA and therefore there was no requirement to confirm any samples by Coggins given the excellent sensitivity of the ELISA used (Body and others 2011).

The lack of EIA cases reported in the central region of Spain during the previous three decades, together with the absence of any EIAV seropositive animals detected in the sample population using a highly sensitive ELISA provided 95% confidence that the true prevalence of EIAV in the SP breeding population in Central Spain was up to 0.53 per cent and could be 0 per cent, confirming freedom from EIAV.

The evidence for freedom from EIAV provided by this study in SP horses in stud farms in Central Spain may offer increased confidence when exporting SP horses to other countries, although judicious biosecurity that includes both pre-export and post-arrival EIA testing should be implemented to prevent the movement of subclinically EIAV-infected horses within Europe.

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REFERENCES

- Anon (2012) Ministerio de Agricultura, Alimentación y Medio Ambiente. Programa ARCA. www.magrama.gob.es/es/ganaderia/temas/zootecnia/razas-ganaderas/razas/catalogo/autoctona-fomento/equino-caballar/espanola/iframe-ejemplo-arca.aspx. Accessed December 31, 2013
- Anon (2013a) Equine infectious anemia, Chapter 2.5.6. In *OIE manual of diagnostic tests and vaccines for terrestrial animals 2013*, Vol. 2. pp 1–6
- Anon (2013b) Daemon Quest by Deloitte: Estudio del Impacto del Sector Ecuestre en España [Study on the impact of the Equestrian Sector in Spain]. Real Federación Hípica Española (RFHE) www.rfhe.com/estudio-global-de-impacto-econo/. Accessed January 20, 2014
- Body M., Al-Rawahi A., Hussain M., Al-Lamki K., Al-Habsy S., Almaawali M., Alrawahi Q. (2011) Sero-survey of equine infectious anemia in the Sultanate of Oman during 2007–2009. *Pakistan Veterinary Journal* 31, 235–238
- Bolfa P., Nolf M., Cadoré J. L., Catoi C., Archer F., Dolmazon C., Mornex J. F., Leroux C. (2013) Interstitial lung disease associated with Equine Infectious Anemia Virus infection in horses. *Veterinary Research* 44, 113
- Cook R. F., Leroux C., Issel C. J. (2013) Equine infectious anemia and equine infectious anemia virus in 2013: a review. *Veterinary Microbiology* 167, 181–204
- Council Directive 2009/156/CE of 30th November 2009, on animal health conditions governing the movement and importation from third countries of equidae. *Official Journal of the European Union L* 192, 1–24
- Cullinane A., Quinlivan M., Nelly M., Patterson H., Kenna R., Garvey M., Gildea S., Layons P., Flynn M., Galvin P., Neylon M., Jankowska K. (2007) Diagnosis of equine infectious anemia during the 2006 outbreak in Ireland. *The Veterinary Record* 161, 647–652
- Issel C. J., Foil L. D. (1984) Studies on equine infectious anemia virus transmission by insects. *Journal of the American Veterinary Medical Association* 184, 293–297
- Issel C. J., Scicluna M. T., Cook S. J., Cook R. F., Caprioli A., Ricci I., Rosone F., Craigo J. K., Montelaro R. C., Autorino G. L. (2013) Challenges and proposed solutions for more accurate serological diagnosis of equine infectious anaemia. *The Veterinary Record* 172, 210
- Kemen M. J., Coggins L. (1972) Equine infectious anemia: transmission from infected mares to foals. *Journal of the American Veterinary Medical Association* 161, 496–499
- More S. J., Aznar I., Bailey D. C., Larkin J. F., Leadon D. P., Lenihan P., Flaherty B., Fogarty U., Brangan P. (2008a) An outbreak of equine infectious anaemia in Ireland during 2006: investigation methodology, initial source of infection, diagnosis and clinical presentation, modes of transmission and spread in the Meath cluster. *Equine Veterinary Journal* 40, 706–708
- More S. J., Aznar I., Myers T., Leadon D. P., Clegg A. (2008b) An outbreak of equine infectious anaemia in Ireland during 2006: the modes of transmission and spread in the Kildare cluster. *Equine Veterinary Journal* 40, 709–711
- Paré J., Simard C. (2004) Comparison of commercial enzyme-linked immunosorbent assays and agar gel immunodiffusion tests for the serodiagnosis of equine infectious anemia. *Canadian Journal of Veterinary Research* 68, 254–258
- Piza A. S. T., Pereira A. R., Terreran M. T., Mozzer O., Tanuri A., Brandao P. E., Richtzenhain L. J. (2007) Serodiagnosis of equine infectious anemia by agar gel immunodiffusion and ELISA using a recombinant p26 viral protein expressed in *Escherichia Coli* as antigen. *Preventive Veterinary Medicine* 78, 239–245
- Reis J. K., Diniz R. S., Haddad J. P., Ferraz I. B., Carvalho A. F., Kroon E. G., Ferreira P. C., Leite R. C. (2012) Recombinant envelope protein (rgp90) ELISA for equine infectious anemia virus provides comparable results to the agar gel immunodiffusion. *Journal of Virological Methods* 180, 62–67
- Rubel F., Kottke M. (2010) Observed and projected climate shifts 1901–2010 depicted by world maps of the Köppen-Geiger climate classification. *Meteorologische Zeitschrift* 19, 135–141
- Scicluna M. T., Issel C. J., Cook F. R., Manna G., Cersini A., Rosone F., Frontoso R., Caprioli A., Antognetti V., Autorino G. L. (2013) Is a diagnostic system based exclusively on agar gel immunodiffusion adequate for controlling the spread of equine infectious anaemia? *Veterinary Microbiology* 165, 123–134
- Sellon D. C. (1993) Equine infectious anemia. *Veterinary Clinics of North America: Equine Practice* 9, 321–336
- Soutullo A., Verwimp V., Riveros M., Pauli R., Tonarelli G. (2001) Design and validation of an ELISA for equine infectious anemia (EIA) diagnosis using synthetic peptides. *Veterinary Microbiology* 79, 111–121
- Stein C., Mott L. (1942) Studies on congenital transmission of equine infectious anemia. *Veterinary Medicine* 37, 37–77
- Tashjian R. J. (1984) Transmission and clinical evaluation of an equine infectious anemia herd and their offspring over a 13-year period. *Journal of the American Veterinary Medicine Association* 184, 282–288
- Williams D. L., Issel C. J., Steelman C. D., Adams W. V., Benton C. V. (1981) Studies with equine infectious anemia virus: transmission attempts by mosquitoes and survival of virus on vector mouthparts and hypodermic needles, and in mosquito tissue culture. *American Journal of Veterinary Research* 42, 1469–1473