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Equine viral arteritis in breeding and sport horses in central Spain

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ABSTRACT

Equine viral arteritis (EVA) may have a high economic impact on breeding stud farms due to the occurrence of EVA-associated abortion outbreaks and the ability of the virus to persist in carrier stallions. While the consequences of EVA in premises with sport horses are usually less severe, the first confirmed outbreak of EVA in Spain occurred in a riding club in Barcelona, but no data on the seroprevalence of EVA in sport horses have been reported in Spain. Given the importance of both Spanish Purebred (SP) breeding horses and sport horses for Spain's equine industry, the aim of this study was to determine and compare the seroprevalence of EVA in these two horse populations in central Spain. Serum samples from 155 SP breeding horses residing in 16 stud farms and 105 sport horses of different breeds housed in 12 riding clubs, collected between September 2011 and November 2013, were tested using a commercial EVA antibody ELISA test with a 100% sensitivity, and confirmed by seroneutralisation (SN) test. EVA seroprevalence in SP breeding horses was higher 21.1% (95% CI 15.3–26.8%) than that in sport horses (6.7%, 95% CI 1.89–11.45%). However, the primary use (breeding vs. sport) was not significantly associated with seropositivity to Equine Arteritis Virus (EAV), suggesting that different management factors do not affect EVA circulation in these two horse populations.

Equine viral arteritis (EVA) is a contagious disease of equidae caused by a ribonucleic acid (RNA) virus, the equine arteritis virus (EAV) (Bryans et al., 1957; Cavanagh, 1997; Snijder and Meulenberg, 1998). Transmission of EAV can occur via respiratory or venereal routes, although it can also occur vertically in utero and less frequently via fomites (Timoney and McCollum, 1993; Bryans et al., 1957; Golnik, 1992; Vaala et al., 1992; Guthrie et al., 2003; Timoney, 2000). The most typical clinical signs of EVA are fever, anorexia, depression, leukopenia, oedemas, conjunctivitis, urticaria, abortion, and fatal interstitial pneumonia in young foals (Del Piero et al., 1997; Golnik et al., 1981; Timoney, 2000).

EVA can be devastating in breeding stud farms since it can cause abortion outbreaks, with morbidity rates varying between <10% and >50%, resulting in important economic consequences (Coignoul and Cheville, 1984; Timoney and McCollum, 1993; Vaala et al., 1992; Timoney, 2003). Moreover, 10–70% of stallions infected with EAV remain asymptomatic carriers of the virus in the accessory sex glands (Timoney and McCollum, 1993; Timoney, 2000).

Even though the consequences of an EVA outbreak in premises of (non-breeding) sport horses are usually less catastrophic, EVA outbreaks have been reported in countries France and Spain (Miszczak et al., 2012; Monreal et al., 1995). The latter occurred in 1992 in a riding club in Barcelona and it was the first confirmed outbreak of EVA clinical disease in Spain (Monreal et al., 1995).

To date, only one study has been carried out in the central region of Spain that determined antibodies against EAV by seroneutralisation (SN) in 16.8% of Spanish Purebred (SP) breeding horses during 2011–2013 (Cruz et al., 2015a), but there is no information in the literature regarding the seroprevalence of EAV in sport horses in Spain. Here we present the results of a cross-sectional study aimed at determining and comparing the seroprevalence of EVA in breeding SP and sport horses in central Spain.

The central region of Spain (comprising the province of Madrid) was selected as the study area, as it is considered to be representative of the whole of Spain with regard to average SP horse density and climate types (Cruz et al., 2015a, 2015b, 2016; Anon, 2012; Rubel and Kottek,

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2010) and it is the region of Spain where the majority of competitions are held (Anon, 2013). Breeding SP horses registered in the study area in 2012 ($n = 7255$) were considered as the reference population for breeding SP horses, and sport horses registered in the area of Madrid in 2013 ($n = 12{,}608$) were considered as the reference population for sport horses (Anon, 2013). Both populations were stratified by stud farm/riding club, and eligible premises were selected based on their total number of horses, population density and geographical distribution (Cruz et al., 2015a, 2015b, 2016; Anon, 2012).

Between September 2011 and November 2013, serum samples were obtained from 155 SP breeding horses housed in 16 different stud farms as part of a wider study on several equine infectious diseases (Cruz et al., 2015a, 2015b, 2016), and from 105 non-breeding sport horses of several breeds housed in 12 riding clubs in the central region of Spain (province of Madrid). Fig. 1 shows the distribution of stud farms (white dots) and riding clubs (black dots) sampled within the central region of Spain.

On every stud farm, 10 ml whole blood samples were obtained by jugular venipuncture from 100% of the breeding SP stallions and from at least 25% of the breeding SP mares, by selecting those mares that were easiest to handle. On every riding club, the same procedure was applied to at least 25% of the horses older than 2.5 years of age in the premises. For each sampled horse, data on age, sex, and reproductive activity were collected via a questionnaire completed by the stud farm veterinarian.

Whole blood samples were centrifuged and serum from each animal was stored frozen at −40 °C prior to being thawed and tested. Sera were tested for antibodies against EAV using a commercial indirect ELISA kit (ID Screen® Equine Viral Arteritis Confirmation; ID.Vet Innovative diagnostics, Montpellier, France) with reported sensitivity of 100% and specificity of 96.75% (Anon, 2008). Equivocal and positive samples were then tested by seroneutralisation (OIE reference technique for the serological analysis of EVA) following the OIE procedure (Senne et al., 1985).

Association between the categorical variable “primary use” (breeding vs. sport horse) and the seropositivity to EAV was tested for significance using the Fisher's exact test. To assess the strength and direction of the associations of the horse- and premises-level factors with seropositivity for EAV based on the SN test, mixed-effects logistic regression analysis was used incorporating stud farm as a random effect in order to account for clustering (or non-independence) of horses living in the same premises. Associations were measured as odds ratios (OR) with corresponding 95% confidence intervals (95% CI). The ‘single-variable’ mixed-effects logistic regression model included sex, age and breed as covariates to control for potential confounding effects. Statistical analysis and seroprevalence calculations were performed using Stata 11.1 (StataCorp). Statistical significance was set as $p < 0.05$.

The breeding SP horses included 122 mares (78.7%) and 33 stallions (21.2%), whereas the sport horses included 38 mares (36.2%), 37 geldings (35.2%), and 30 stallions (28.6%). Mean age of the breeding SP horses was 6.7 years ± SD 4.3 years (range 3–23 years). Mean age of the sport horses was 6.3 years ± SD 5.7 years (range 2–25 years). Of the sport horses sampled, none had a history of breeding. While all the breeding horses sampled were SP horses, those within the sport horse group were: 35 (33%) Crossbreed sport horses, 30 (29%) Thoroughbred racehorses, 22 (21%) Warmblood horses, 16 (15%) SP horses, 1 (1%) Connemara pony, and 1 (1%) Friesian horse.

After testing all 260 serum samples for antibodies against EAV by ELISA followed by seroneutralisation in the positive and equivocal samples ($n = 47$), 39 (15.0%) were confirmed to have antibodies against EAV. The seroprevalence of EAV among SP breeding horses in the province of Madrid was 20.6% (95% CI 14.3%–26.9%), whereas the seroprevalence of EAV among sport horses in this region was 6.7% (95% CI 2.0%–11.4%). The characteristics of both groups of horses sampled and the percentages of EAV seropositive horses by SN within each group are summarised in Table 1.

Our results show a higher EVA seroprevalence in the SP breeding horse population compared to that in sport horses of this region ($p = 0.002$). Movements of horses in and out a breeding stud farm are expected to be lower than those in a riding club, as sport horses go to competitions virtually every week. Thus, the source of EAV infection in breeding SP stud farms is likely to be found inside the premises, e.g. the carrier stallions.

Seropositivity to EAV was significantly associated with primary use (Fisher's exact test $p = 0.002$), breed ($p = 0.013$) and age ($p = 0.003$). However, when accounting for clustering of observations at the stud farm level in the mixed-effects logistic regression model, these variables were not retained and only horse age showed a borderline significant association ($p = 0.056$) (Table 2). There was also evidence of significant clustering (lack of independence of observations) at the stud farm level ($\delta = 3.21$, $\rho = 0.94$, LRS < 0.001).

Each horse population was analysed separately by using two mixed-effects logistic regression models. Similarly to a previous study (Cruz et al., 2015b), the model predicting EAV seropositivity in the breeding SP horse population showed significant associations with sex and age ($p = 0.03$ and $p = 0.008$, respectively). Conversely, these variables were not significant in the model predicting EAV seropositivity in sport horses ($p = 0.765$ and $p = 0.636$ respectively).

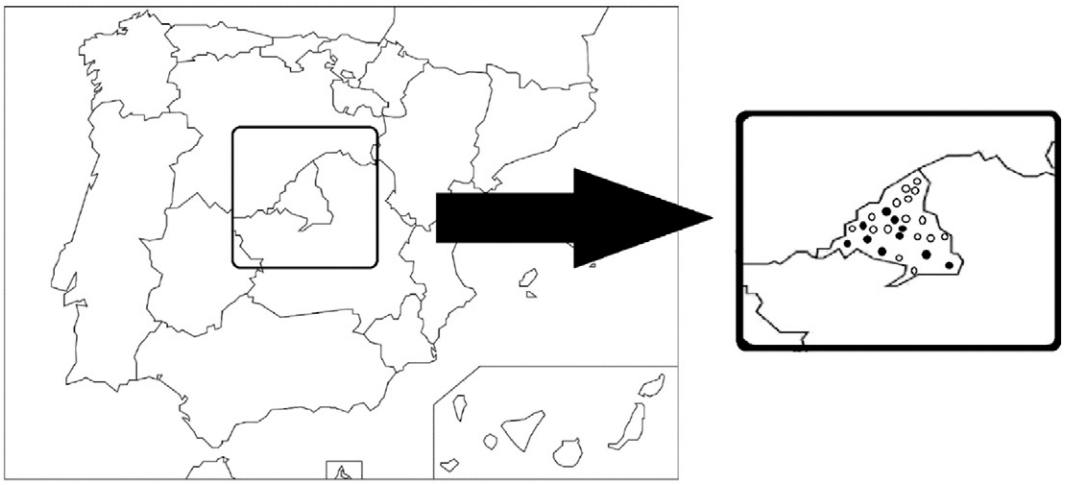

Fig. 1. Distribution of the stud farms (white dots) and riding clubs (black dots) sampled in the province of Madrid.

Table 1
Distribution of the breeding horses ($n = 155$) and sport horses ($n = 105$) sampled by sex, age, breeding activity and group, and percentage of horses seropositive to EAV by seroneutralisation.

	Sex	Age	Breed
Breeding horses ($n = 155$):	Stallions (21.3%, $n = 33$):	3–5 years (23.2%, $n = 36$):	SP (100%, $n = 155$):
- 20.6% EAV seropositive	- 18.2% EAV seropositive	- 2.8% EAV seropositive	- 20.6% EAV seropositive
	Mares (78.7%, $n = 122$):	6–9 years (46.4%, $n = 72$):	
	- 21.3% EAV seropositive	- 18.1% EAV seropositive	
		10–15 years (22.6%, $n = 35$):	
		- 37.1% EAV seropositive	
		>15 years (7.7%, $n = 12$):	
		- 41.7% EAV seropositive	
Sport horses ($n = 105$):	Stallions (29%, $n = 30$):	2–5 years (30.5%, $n = 32$):	SP (15%, $n = 16$):
- 6.7% EAV seropositive	- 3.3% EAV seropositive	- 6.2% EAV seropositive	- 6.2% EAV seropositive
	Geldings (35%, $n = 37$):	6–9 years (31.4%, $n = 33$):	Thoroughbred (29%, $n = 30$):
	- 5.4% EAV seropositive	- 3.0% EAV seropositive	- 0.0% EAV seropositive
	Mares (36%, $n = 38$):	10–15 years (23.8%, $n = 25$):	Warmblood (21%, $n = 22$):
	- 10.5% EAV seropositive	- 12% EAV seropositive	- 13.6% EAV seropositive
		>15 years (14.3%, $n = 15$):	Crossbreed and other (35%, $n = 37$)
		- 6.7% EAV seropositive	- 8.1% EAV seropositive

Previous studies have shown that the age was a risk factor for seropositivity to EVA (Gimeno Suarep et al., 2011; Rola et al., 2011). The increased risk of EVA seropositivity with age in the population of SP breeding horses in our study may be due to a higher risk of exposure to EAV at least once in a horse's lifespan up to the time of sampling, suggesting the occurrence of periodic infections within the premises (probably spread venereally through carrier stallions). This significant increase in EVA seropositivity with age did not appear in sport horses, suggesting that infection in these horses is more likely to be spread via the respiratory route during a competition or event with concentration of horses (McCollum and Swerczek, 1978).

Among the sport horses, there were differences in EVA seroprevalence over the different breeds (Table 1), as reported previously (Timoney, 2003). For example, EVA seroprevalence has been reported to be higher in Standardbred horses than in Thoroughbreds in the UK (18.5%–24% vs. <3% respectively) (Newton et al., 1999), and similarly in Australia, where the differences were even larger (72% in Standardbred horses vs. 4.5% in Thoroughbreds) (Huntington et al., 1990b). It has also been suggested that certain breeds could be more susceptible to EAV infection (Rola et al., 2011). Breed-specific differences are likely to reflect different management-related factors and owners' attitudes towards infectious disease control in horses of different populations and breeds (Holyoak et al., 2008).

EAV is more prevalent in breeding SP stud farms than in riding clubs in the study region (20.6% vs. 6.7%, respectively); however, seropositivity to EAV was not associated to primary horse use in our study when a mixed effects logistic regression model was carried out. Further studies on EAV in breeding and sport horses living in other parts of Spain are required to further evaluate the role of the factors associated with EAV seropositivity in these populations.

Conflict of interest

The authors are grateful to Id.Vet Innovative diagnostics, Montpellier, France for providing the EVA ELISA kits used in the initial screening of this study.

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Table 2
Output of the mixed-effects multivariable logistic regression model for variables associated with seropositivity for EAV in breeding horses and sport horses in central Spain ($n = 260$; 39 seropositive and 221 seronegative).

Variable	EVA-positive ($n = 39$)	EVA-negative ($n = 221$)	Odds ratio	95% CI	*p*-Value
Horse's age (continuous)	–	–	1.91	1.00–1.27	0.056
Horse's sex					
Stallion	7	56	Reference		
Mare	30	130	2.16	1.16–20.80	0.031
Gelding	2	35	0.97	0.28–44.12	0.331
Primary use					
Sport horse	7	98	Reference		
Breeder	32	123	0.41	0.03–209.60	0.685
Breed					
Spanish Purebred	33	138	Reference		
Thoroughbred	0	30	−0.01	0–∞	0.988
Warmblood	3	19	0.41	0.02–462.98	0.684
Crossbreed	3	34	−0.61	0.01–11.71	0.542

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