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Equine viral arteritis: Seroprevalence patterns and risk factors in equids from western Europe

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ABSTRACT

Equine viral arteritis is a notifiable infectious disease with sanitary and economic implications at a global scale. A cross-sectional serosurvey was performed to determine the exposure of equids to the *Alphaarterivirus equid* [Equine Arteritis Virus (EAV)] in three regions in western Europe. Serum samples from 1425 equids (1196 horses, 104 donkeys, and 125 mules/hinnies) from Catalonia (northeastern Spain), Andalusia (southern Spain) and southeastern United Kingdom (UK) were collected during the period 2011–2023. The overall EAV seroprevalence in EAV-unvaccinated equids was 9.7 % (138/1425; 95 %CI: 8.1–11.2 %) using a commercial ELISA test. Seropositivity by study region was higher in Catalonia (15.6 %), followed by Andalusia (8.1 %) and UK (3.3 %). At species level, the prevalence of anti-EAV antibodies was 10.2 % in horses, 7.7 % in donkeys and 6.4 % in mules/hinnies. Among all the variables assessed in the multivariate analyses, only the "study region" was considered a statistically significant risk factor associated with EAV exposure in equids within the study area. The present study constitutes the first large-scale serosurvey of EAV comprising horses, donkeys, and mules/hinnies in Europe, as well as the first detection of EAV seropositivity in mules/hinnies in this continent. Our findings describe a moderate, heterogenous and widespread circulation of EAV in the analysed regions. The circulation of EAV requires the improvement of control measures mainly based on vaccination strategies to effectively reduce the circulation of this virus in equid herds in Europe. Also, the establishment of surveillance programs will be pivotal for the monitoring of EAV in high-risk regions.

1. Introduction

Equine Viral Arteritis (EVA) is a notifiable infectious disease in the European Union caused by the *Alphaarterivirus equid* [Equine Arteritis Virus (EAV)], an enveloped and single-stranded RNA virus of the family Arteriviridae that affects equids worldwide (Balasuriya, 2014). Venereal infection is a major transmission route for EAV, where males act as persistent infected reservoirs infecting mares during the natural or artificial insemination (Del Piero, 2000). Moreover, the airborne transmission has been confirmed as another major infection route that favours the circulation of EAV in herds (Timoney and McCollum, 1993; Cruz et al., 2016).

Equine Arteritis Virus evidence specific tropism for stromal cells (e.g., fibrocytes and tissue macrophages) and CD8+ T and CD21+ B lymphocytes (Carossino et al., 2017). Although EVA predominantly causes subclinical infections, when clinical signs occur (particularly in immunocompromised and young individuals), they can vary widely in severity and can result in mortality (Timoney and McCollum, 1993; Balasuriya, 2014). Clinical signs and lesions can include oedema of the prepuce, scrotum, mammary gland, eyes and/or lower legs, temporary

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sub-infertility in stallions, significant abortion rates (10–70 %), nasal discharge, fever, conjunctivitis, skin rash, depression, enteritis and pneumonia (Timoney and McCollum, 1993; Holyoak et al., 2008; Balasuriya, 2014). Critically, a substantial proportion of infected stallions may become persistent carriers of the virus, for which no treatment is currently available. Previous studies also suggest that genetic factors based on EqCXCL16 susceptible genotypes could favour the presence of long-term EAV carriers (Sarkar et al., 2016; Socha et al., 2020). Therefore, EAV entails economic losses in equids due to the combination of direct (e.g., abortions, denied export for EAV-carrier stallions) and indirect (e.g., cancellation of horse races, reduction of participation in sport events) impacts that this viral disease has in equids worldwide (Timoney and McCollum, 1993).

EAV was first isolated in 1953 in Ohio (United States) (Doll et al., 1957). During the following decades, virus circulation in equine populations was also detected in Europe, with seroprevalence values ranging between 14.4 % and 28.1 % (Moraillon et al., 1978; de Boer et al., 1979). Since then, EAV has remained endemically circulating in southern and central Europe at least since the 1960s. In this continent, an extensive literature review on EAV seroprevalence (Table 1) showed that anti-EAV antibodies ranged from 1.3 % (United Kingdom; hereinafter "UK") to 79.2 % (Bulgaria) (Newton et al., 1999; Chenchev et al., 2011). Moreover, EVA outbreaks have been reported in some European countries, particularly in Spain and UK (Monreal et al., 1995; Wood et al., 1995). Despite these previous studies, there is a lack of up-to-date and large-scale epidemiological information about the exposure to EAV in equid populations in Europe, particularly in the western area. The present study aimed to evaluate the seroprevalence of EAV and identify risk factors associated with exposure to this virus in equine populations from different European regions.

2. Material and methods

2.1. Study area and sampling

Between 2011 and 2023, a cross-sectional study was conducted to evaluate the prevalence of antibodies against EAV in 1425 unvaccinated equids from three regions located in western Europe: Andalusia (southern Spain; $n = 779$), Catalonia (northeastern Spain; $n = 437$) and southeastern UK ($n = 209$). Three different equine species were sampled: horses ($n = 1196$), donkeys ($n = 104$) and mules/hinnies ($n = 125$). A minimum sample size of 196 horses (random sampling) in each monitored region was established based on an estimated prevalence of 15 %, according to previous scientific literature (Cruz et al., 2016; Cruz-Lopez et al., 2017), with 95 % Confidence Interval (95 % CI) and an accepted error of 5 % (Thrusfield and Christley, 2018). Regarding equids other than horses, samples from donkeys (*Equus africanus asinus*) and mules/hinnies (*E. africanus* × *ferus*) were also collected using convenience sampling.

Samples were selected from serum banks of equids sampled in epidemiological surveillance programs or submitted for medical check-ups during the study period. Blood samples were obtained by jugular vein puncture using tubes without anticoagulant (Vacutainer®, Becton-Dickinson, USA) that were centrifuged and then stored at −20 °C until laboratory analysis. An epidemiological questionnaire comprising an on-farm interview with the owners was conducted during sampling, which included relevant epidemiological information (12 explanatory variables) for evaluating potential risk factors associated with EAV exposure, which were classified into 1) individual information, 2) herd data, and 3) health and biosecurity features (Table 2).

2.2. Serological analysis

The presence of antibodies against EAV in sera from equids was evaluated using a commercial indirect enzyme linked immunosorbent assay (ELISA), with a monoclonal specific antibody to equine IgG and a complex of structural proteins from the EAV strain VR-176 as coating antigen (Ingezim Arteritis 2.0, R.14.EA2.K.1, Gold Standard Diagnostics, Madrid, Spain). Serological analyses were performed according to the manufacturer's recommendations, which report high sensitivity (98.8 %) and specificity (96.6 %) values.

Table 1
Literature review about serosurveys of Equine Arteritis Virus in Europe chronologically ordered.

Citation	Country	Study period[a]	Species	Seroprevalence[b]	Positive equids/total
Moraillon et al. (1978)	France	1966–1976	Horse	15.2	506/3324
Moraillon et al. (1978)	Other European countries	1966–1976	Horse	28.1	69/245
de Boer et al. (1979)	Netherlands	1963–1966 & 1972–1975	Horse	14.4	80/556
Kolbl et al. (1991)	Austria	1988–1989	Horse	10.9	103/944
Burki et al. (1992)	Austria	1989–1990	Horse	42.5	122/287
Newton et al. (1999)	United Kingdom	1995–1996	Horse	1.3	231/18054
Van Maanen et al. (2005)	Netherlands	1996	Horse	74.8	232/310
Kirmizigül et al. (2007)	Turkey	NA	Horse	8.8	35/400
Turan et al. (2007)	Turkey	NA	Horse	14.3	9/63
Yildirim et al. (2008)	Turkey	NA	Donkey	14.5	11/76
Equine Disease Surveillance (2010)	United Kingdom	2010	Horse	2.8	34/1196
Rola et al. (2011)	Poland	2006–2008	Horse	55.1	97/176
Chenchev et al. (2011)	Bulgaria	NA	Donkey	79.2	152/192
Bulut et al. (2012)	Turkey	NA	Horse	23.4	89/380
Irina-Oana et al. (2012)	Romania	NA	Horse	31.8	129/406
Mangana-Vougiouka et al. (2013)	Greece	2001–2008	Equines	3.3	249/7579
Marenzoni et al. (2013)	Turkey	2004	Horse	16.5	57/346
Cruz et al. (2016)	Spain	2011–2013	Horse	16.8	96/555
Cruz-Lopez et al. (2017)	Spain	2011–2013	Horse	15.0	39/260
Lazić et al. (2017)	Serbia	2013–2014	Horse	15.9	54/340
Gür et al. (2018)	Turkey	2009–2010	Horse	15.0	29/193
Gür et al. (2018)	Turkey	2009–2010	Donkey	8.3	19/227
Gür et al. (2019)	Turkey	NA	Donkey	3.5	53/1532
Bażanów et al. (2021)	Poland	2018	Horse	0.0	0/20
Ince and Sevik (2022)	Turkey	2014–2017	Horse	8.4	22/262
Lazić et al. (2023)	Serbia	2022	Donkey	0.0	0/53
Kaps et al. (2024)	Austria	2001–2021	Horse	14.9	46/308

[a] NA: Not Available.
[b] Seroprevalence information derived from ELISA and/or virus neutralization test.

Table 2
Distribution of the seroprevalence of Equine Arteritis Virus in unvaccinated horses by categories and results of the bivariate analysis.

Variable	Categories	No. Pos./ total	Seroprevalence (%)	*P*-value
Study region	Andalusia	50/602	8.3	<0.001
	Catalonia	65/387	16.8	
	United Kingdom	7/207	3.4	
Sex	Female	47/436	10.8	0.228
	Male	53/631	8.4	
Breed	Pure	61/495	11.0	0.468
	Crossbreed	61/579	9.5	
Age	Foal	14/197	7.1	0.012
	Subadult	48/561	8.6	
	Adult	42/297	14.1	
Herd census	Small (1–5)	6/156	3.9	0.153
	Medium (6–20)	18/265	6.8	
	Large (>20)	33/387	8.5	
Cleaning facilities	No	0/35	0	0.099
	Yes	96/981	9.8	
Disinfection facilities	No	3/69	4.4	0.494
	Yes	52/707	7.4	
Shelter (spring–summer)	Indoor	17/260	6.5	0.2908
	Mix	16/173	9.3	
	Outdoor	19/340	5.6	
Shelter (autumn–winter)	Indoor	23/298	7.7	0.191
	Mix	13/154	8.4	
	Outdoor	15/319	4.7	
Water near herd	No	94/676	13.9	<0.001
	Yes	28/520	5.4	
Activity	Sport	20/172	11.6	0.014
	Leisure	21/378	5.6	
	Reproduction	23/183	12.6	
	Work	7/57	12.3	
Body condition	Overweight	1/5	20.0	0.674
	Adequate	98/935	10.5	
	Thin	4/29	13.8	

2.3. Statistical analysis

Seroprevalence was calculated as the ratio of the number of positive animals to the total number of individuals tested, with the exact binomial 95 % CI (Thrusfield and Christley, 2018). Associations between the selected explanatory variables with the EAV exposure (seropositive vs seronegative) were initially evaluated using the Pearson's chi-square test (expected frequencies ≥5) or the Fisher's exact test (expected frequencies <5), as required (Thrusfield and Christley, 2018). Variables with *P*-values ≤0.10 were selected for the multivariate analysis. Collinearity between pairs of variables was tested by Cramer's V coefficient and if a correlation coefficient and the *P*-value were ≥ 0.6 and ≤ 0.05, respectively, the variable with the highest biological plausibility was selected. Then, a Generalized Linear Model (GLM) was run using a binomial error distribution following a backward stepwise strategy, and a logit link function was performed based on the lme4 R-package (Bates et al., 2015). Significance of the fixed effect variables was determined using car R-package (Fox and Weisberg, 2018), and pairwise Tukey post-hoc comparisons were calculated using the emmeans R-package to evaluate the differences among levels of the explanatory variables retained in the multivariate models. All statistical analyses were performed using R software version 4.1.3 (R Core Team, 2024), and significant differences were considered with $P \leq 0.05$ for a double-sided test.

3. Results and discussion

To the best of the authors' knowledge, this study constitutes the first large-scale serosurvey of EAV comprising different equine species in Europe. The overall EAV seroprevalence obtained was 9.7 % (138/1425; 95 %CI: 8.1–11.2 %). Seropositivity was observed in the three study regions, with the highest equid seroprevalence value detected in Catalonia (15.6 %; 68/437), followed by Andalusia (8.1 %; 63/779) and UK (3.3 %; 7/209) ($P < 0.001$) (Fig. 1). The seroprevalence obtained in Catalonia and Andalusia are similar and lower, respectively, than the EAV exposure reported in horses from central Spain during 2011–2013 (15.0–16.8 %) (Cruz et al., 2016; Cruz-Lopez et al., 2017). Regarding UK, the limited exposure of equids to EAV aligns with those previously described in horses from this country in 1995–1996 (1.3 %; Newton et al., 1999) and 2010 (2.8 %; Equine Disease Surveillance, 2010), although this last serosurvey included vaccinated stallions.

By species, the highest seroprevalence was detected in horses (10.2 %; 122/1196), followed by donkeys (7.7 %; 8/104) and mules/hinnies (6.4 %; 8/125) ($P = 0.304$). Other factors, such as the smaller sample sizes for donkeys and mules/hinnies compared to horses, may not provide enough statistical robustness to detect significant differences between groups. The overall seroprevalence obtained in horses is similar to the reported in Austria (10.9 %) (Kolbl et al., 1991). Lower prevalence of anti-EAV antibodies values was detected in horses from Turkey (8.4–8.8 %), Algeria (7.46 %) and UK (1.3 %) (Newton et al., 1999; Kirmizigül et al., 2007; Laabassi et al., 2014; Ince and Sevik, 2022). In contrast, higher EAV seroprevalence was found in horses from Poland (55.1 %; Rola et al., 2011), Serbia (15.9 %; Lazic et al., 2017), and Spain (15.0–16.8 %; Cruz et al., 2016; Cruz-Lopez et al., 2017), among other European countries (Table 1).

Regarding donkeys, the seroprevalence obtained in this study (7.7 %) is lower than that previously reported in several African countries (South Africa, Zimbabwe and Morocco) (12.9–26.7 %) and Europe (14.5 %; Turkey), but higher that those described in UK and Serbia (0.0 %) (Paweska and Barnard, 1993; Paweska et al., 1997; Yildirim et al., 2008; Lazić et al., 2023). About mules/hinnies, the prevalence of anti-EAV antibodies detected in our serosurvey (6.4 %) is higher than those previously described in mules from South Africa (3.5 %) and India (0 %), but lower than the EAV-exposure reported in mules from Morocco (17.9 %) (Paweska et al., 1997). To the best of the authors' knowledge, our study constitutes the first detection of EAV seropositivity in mules/hinnies in Europe, also increasing the scarce epidemiological information about EAV exposure in this hybrid species in the scientific literature.

To avoid methodological bias, statistical analyses were performed on horses, as they constitute the most uniform and representative sampling among countries. The distribution of EAV seroprevalence in horses based on the explanatory variables is shown in Table 2. After data exploration, 5 of 12 explanatory variables were selected from the bivariate analysis ($P \leq 0.10$). The GLM evidenced that the "study region" was a potential risk factor for EAV exposure. Horses from Catalonia showed significantly higher seropositivity (16.8 %) than those from UK (3.4 %; OR: 5.77; $P < 0.001$) and Andalusia (8.3 %; OR: 2.23; $P < 0.001$). Differences in the EAV-seropositivity among the three regions studied aligns with the spatial heterogeneity of virus circulation previously detected between central Spain (15–16.8 %; Cruz et al., 2016; Cruz-Lopez et al., 2017) and UK (1.3 %) (Newton et al., 1999). These variations could be explained by the implementation of different reproductive management practices and programs for detecting EAV-carriers in equid herds from each study region. In this sense, factors related to the expression of some genes (e.g., EqCXCL16) could favour the presence of long-term EAV carriers, as been previously suggested (Sarkar et al., 2016; Socha et al., 2020). Moreover, comparisons among studies should be cautiously interpreted, since several factors, such as differences in the sample size, study design, epidemiological scenario and diagnostic techniques, could also be operating.

In conclusion, our results evidence a moderate, heterogeneous and widespread circulation of EAV in equid populations in western Europe from 2011 to 2023, with a notable seroprevalence in horses from Catalonia (northeastern Spain). To mitigate the spread and persistence of EAV in European equids, it is crucial to enhance control measures, including vaccination programs. The establishment and promotion of

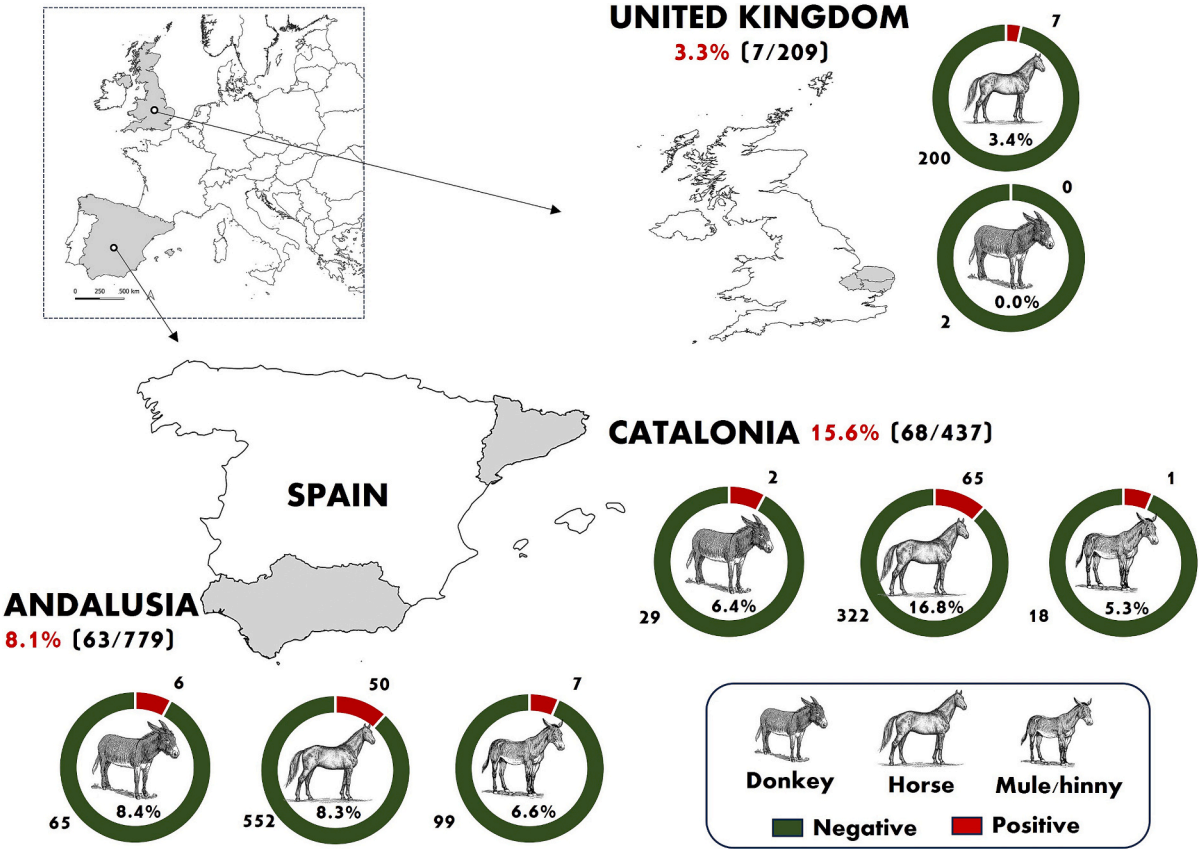

Fig. 1. Seroprevalence of Equine Arteritis Virus by equid species and study region.

surveillance programs for EAV across the continent is also essential. Moreover, raising awareness among equid owners and veterinarians about the importance of early detection and preventive strategies could significantly contribute to control the virus circulation.

CRediT authorship contribution statement

Juan J. Franco: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Moisés Gonzálvez:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **David Cano-Terriza:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Jesús Barbero-Moyano:** Writing – review & editing, Validation, Methodology, Formal analysis. **Eduard Jose-Cunilleras:** Writing – review & editing, Validation, Methodology. **Eduardo Alguacil:** Writing – review & editing, Validation, Methodology. **Jesús García:** Writing – review & editing, Validation, Methodology. **Ignacio García-Bocanegra:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

None of the authors of this study has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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