

Prevalence of equine viral arteritis in Algeria

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Summary

In order to determine the prevalence of equine viral arteritis in Algeria, 268 sera from non-vaccinated horses were collected from the western and eastern regions. Serological analysis of the sera, which were collected from 2009 to 2011, was performed using the virus neutralisation test, as described by the World Organisation for Animal Health. Overall, 20 sera (7.46%) were seropositive, 152 (56.71%) were negative and 96 sera (35.82%) were cytotoxic. Equine arteritis virus (EAV) seroprevalence was significantly higher in the western region (Tiaret) than in the eastern region (Barika and El-Eulma). Interestingly, more than 20% of the tested horses over 16 years old were seropositive for EAV. However, EAV prevalence did not depend on either horse breed or horse gender. This study is the first to describe the circulation of EAV in the Algerian horse population.

Keywords

Algeria – Equine arteritis virus – Horse – Prevalence – Virus neutralisation test.

Introduction

Equine arteritis virus (EAV) is one of the major viral pathogens of horses (1). It is the causative agent of equine viral arteritis (EVA), and can infect horses, donkeys, mules and zebras (2, 3, 4). The virus is an *Arterivirus* belonging to the *Arteriviridae* family in the order *Nidovirales* (5, 6). It was initially isolated in 1953 from a lung of an aborted fetus on a Standardbred breeding farm near Bucyrus, Ohio, in the United States of America (USA) (7, 8). Based on phylogenetic analysis of ORF5 encoding the major viral envelope glycoprotein 5 (GP5), EAV isolates are classified into two groups: the North American group and the European group (9, 10, 11). The latter can be further divided into two subgroups: European subgroup 1 (EU-1) and European subgroup 2 (EU-2) (12, 13). It has been previously reported that North American and European EAV isolates present 85% of nucleotide identity (2, 11).

Equine viral arteritis is a respiratory and reproductive disease of horses that occurs worldwide (4, 14, 15). The vast majority of EAV infections are subclinical, but acutely infected animals may develop a wide range of clinical

signs, including pyrexia, depression, anorexia, dependent oedema (scrotum, ventral trunk and limbs), stiffness of gait, conjunctivitis, lacrimation and swelling around the eyes (periorbital and supraorbital oedema), respiratory distress and leukopenia (2, 4). The direct consequences of EVA outbreaks are financial losses, mainly due to abortions of pregnant mares, and the death of young foals (16). Following primary EAV infection, up to 70% of the stallions will carry the virus in their reproductive tract, sometimes for years (17, 18). Those carrier stallions will shed the virus in their semen (19).

Several studies have shown that EAV infection has occurred among horses in North and South America, Europe, Australia, Africa and Asia (4, 20, 21, 22, 23). Interestingly, EAV infection prevalence in horses varies between countries and horse breeds (1, 2, 24). Recently, a study has shown that New Zealand is free of active EAV infection (25). Although specific EAV antibodies have been detected in non-vaccinated horses in Morocco (26) and in Tunisia (27), no evidence of the presence of EAV in horses had been reported in Algeria. Vaccination against EAV is not practised and there is no import authorisation for killed and/or

modified live virus vaccines in Algeria. This paper presents the results of the first EAV prevalence study performed on non-vaccinated sport horses in Algeria.

Materials and methods

Sample collection

Serum samples were collected between 2009 and 2011. A total of 268 serum samples were collected from 157 female and 111 male horses. The horses were from different geographic areas of the country and represented the western region of Algeria (from the Chaou Chaoua National Stud Farm, the National Office of Equine and Camel Livestock [*Office national de développement des élevages équin et camélins*], the Equestrian Club and the racetrack located in Tiaret) and from the eastern region of Setif and Batna (from El-Eulma and Barika racetracks). Following blood collection, sera were prepared and kept at -20°C until use. Different breeds were included in the study (e.g. Arabian, Thoroughbred, Barb and mixed-breed). The age of the horses ranged from 1 to 26 years old. None of the horses born and bred in Algeria were vaccinated against equine pathogens.

Virus neutralisation test

Serological analysis of equine sera was performed using the virus neutralisation test (VNT) as described in chapter 2.5.10 of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (28). Sera were inactivated for 30 minutes at 56°C . The test was carried out using sterile 96-well flat-bottomed plates. Sera were diluted from 1/2 to 1/256 in a minimum essential medium without fetal bovine serum, and serial twofold dilutions were incubated for 1 h at 37°C with an equal volume of 30 to 300 median tissue culture infective doses of EAV Bucyrus strain (ATCC VR 796). Then 100 μl of RK-13 cell solution, in a minimum essential medium with 10% fetal bovine serum, at 2.5×10^5 cells/ml, was added to each well. Microplates were incubated for 72 h at 37°C in a humid atmosphere of 5% CO_2 . Controls for cell viability, serum cytotoxicity, viral infectivity, positive serum, and negative serum were included in each assay. The end-point virus neutralisation titre was expressed as the reciprocal of the highest dilution of serum showing no cytopathic effects in any of the wells. In addition, cytotoxic sera were treated using 24 h pre-formed RK13 monolayer cells from which serum/virus mixture was removed after 4 h and replaced by growth medium, as described by Legrand *et al.* (29, 30). Factors that could influence the EVA serological prevalence were studied using the Chi-square test. A p value of less than 1% ($p < 0.01$) was considered significant.

Results

The results obtained showed that the prevalence of EAV in the Algerian horse population tested was 7.46% (20/268). Anti-EVA antibody titres of positive sera ranged from 4 to 96 (Table I). Among the 268 analysed samples, 56.71% (152/268) were negative. Surprisingly, 35.82% (96/268) of the tested sera were cytotoxic for RK-13 cells even after different treatments were applied to reduce sera cytotoxicity.

Table I
Virus neutralisation test results: the number of positive samples from 268 non-vaccinated horses and the distribution of antibody titres

No. of positive sera	Antibody titre
2	4
6	6
3	8
2	12
2	24
1	32
1	48
1	64
2	96
20	

The prevalence of EAV in horses from El-Eulma and Tiaret regions were 4.28% (3/70) and 10.89% (17/156), respectively. None of the 42 horses from Barika region was seropositive for EAV (Table II). Therefore, the prevalence of EAV in horses from the eastern region (Barika and El-Eulma) was significantly lower (Chi-Square test $p < 0.01$) than in the western region (Tiaret) of Algeria.

The prevalence of EAV varied according to the age of the tested horses, as shown in Table III. Indeed, the highest EAV infection rate (21.42%) was seen in horses over 16 years old. Conversely, a prevalence of 4.13% was observed for younger animals (≤ 3 years old).

The results for the different horse breeds are shown in Table IV. The EAV infection rate in each breed studied ranged from 2.27% to 12.12%. No statistically significant difference in seroprevalence of EAV was found between Thoroughbred (Arabian or English) and Barb breeds. Furthermore, no significant difference was observed in EAV infection rate between male and female populations (Table V).

Table II
The prevalence of equine arteritis virus among horses in three different regions of Algeria

Region	Virus neutralisation test		
	No. of tested sera	No. of positive sera	Rate (%)
Barika	42	0	0
El-Eulma	70	3	4.28
Tiaret	156	17	10.89
Total	268	20	7.46

Table III
The prevalence of equine arteritis virus among horses of different ages

Range of ages (years)	Virus neutralisation test		
	No. of tested sera	No. of positive sera	Rate (%)
1 – 3	121	5	4.13
4 – 9	94	6	6.38
10 – 15	25	3	12
16 – 26	28	6	21.42
Total	268	20	7.46

Table IV
The prevalence of equine arteritis virus among horses of different breeds

Breed	Virus neutralisation test		
	No. of tested sera	No. of positive sera	Rate (%)
Arabian Thoroughbred	138	9	6.52
Barb	66	8	12.12
English Thoroughbred	44	1	2.27
Mixed-breed ^(a)	20	2	10
Total	268	20	7.46

a) Arabian–Barb ($n = 13$), English–Arabian ($n = 4$) and English–Barb ($n = 3$).

Table V
The prevalence of equine arteritis virus among male and female horses

Sex	Virus neutralisation test		
	No. of tested sera	No. of positive sera	Rate (%)
Female	157	13	8.28
Male	111	7	6.31
Total	268	20	7.46

Discussion

The analysis of EAV prevalence in 268 horses from eastern and western regions of Algeria showed that 7.46% of horses had anti-EAV antibodies. Moreover, antibody titres from seropositive horses ranged from 4 to 96, suggesting the circulation of EAV in Algeria. These data are in accordance with studies performed in the Maghreb region (26, 27, 31, 32). Indeed, the first evidence of EAV circulation in the Maghreb area was described in Morocco in 1977 and showed that EAV prevalence was around 23.33% (33). The presence of EAV was then described in Tunisia during the 1990s, with an EAV prevalence range of between 8.75% and 17.23% in the horse population tested (31). Natural infection of horses with EAV results in long-lasting immunity against reinfection (34, 35). The humoral immune response is characterised by the development of both complement-fixing and virus-specific neutralising antibodies (36, 37). Complement-fixing antibodies appear one to two weeks after infection, with a peak at around three weeks, and disappear by eight months post infection, whereas neutralising antibodies are detected within one to two weeks following exposure, with a peak at two to four months, and persist for years thereafter (4, 37, 38, 39, 40).

In the present study, the authors found a very high number of cytotoxic sera. Indeed, 35.82% of horse sera tested were cytotoxic for the RK-13 cells used to perform the VNT. The origin of this cytotoxicity is not known. It has been shown that use of an inactivated equine herpesvirus (EHV) vaccine may induce serum cytotoxicity affecting the EAV neutralisation test (41), but the horses in this study were not vaccinated for EHV. Cytotoxicity can be mistaken for viral cytopathic effect and this has led to increasing difficulties in test interpretation. Use of ELISA-based tests, which are not affected by serum cytotoxicity, could be a useful backup in screening equine sera for EAV-neutralising antibodies (42). But ELISA tests still need to be validated to be used and recognised for international trade. The new and improved competitive ELISA from VMRD (Pullman, WA, USA), developed recently by Chung *et al.* (43), may

be a viable alternative to the serum neutralisation assay and merits additional validation.

The data from the present study show that none of the horses from the Barika region were infected with EAV. Moreover, EAV infection rate in the El-Eulma region (4.28%) was significantly lower than the one recorded in horses in the Tiaret region (10.89%). Therefore, EAV prevalence evaluated using VNT was significantly higher in the western region (Tiaret) than in the eastern region (El-Eulma and Barika). The disparities reported in EAV prevalence may be related to equine population density in each region, but also to trade with neighbouring countries, such as Morocco and Tunisia.

The current study suggests that EAV prevalence in the Algerian horse population varies with age. Indeed, the prevalence in older horses, aged over 16 years, reached 21.42%, which is in full agreement with other studies (1, 26, 32, 44). Moreover, the percentage of seropositive horses has been seen to increase significantly with age (45). According to Balasuriya *et al.* (2, 40), the rise in the EAV-neutralising antibody titre that occurs in the serum of some horses with advancing age is likely a consequence of reinfection during the horse's lifetime. In this study, the high EAV prevalence in horses over 16 years old seems to demonstrate the circulation of EAV in the Algerian horse population during the last 20 years. Conversely, the low percentage of positive horses aged between one and three years indicates that EAV has barely circulated in the studied Algerian regions during the last three years. Moreover, these horses are too young for breeding, thus eliminating the possibility of infection by the venereal route (26, 32). Therefore, antibodies detected in young horses are more likely due to respiratory infection by direct contact with sick horses.

The data did not show any association between the horse breed tested and EAV prevalence, confirming results obtained by Chabchoub *et al.* (27), who showed no significant difference in EAV prevalence between Arabian Thoroughbreds and Barb in Tunisia. Conversely, Ghram

et al. (31) and Moraillon *et al.* (32) showed that the percentage of seropositive horses is higher for Barb and Arabian Barb than for Arabian Thoroughbreds. However, some other studies suggested that EAV infection rates depend on the horse breeds tested. Indeed, a higher EAV infection incidence has been reported in Standardbred horses than in Thoroughbreds (46). Moreover, there was a marked disparity between the prevalence of EAV infection of Standardbred and Thoroughbred horses in the USA (4). Furthermore, a high seropositivity in Austrian Warmblood as well as in mares and stallions of the Hucul horses from Poland was also reported (1, 45). Interestingly, the extensive EVA outbreak in the USA in 2006 and 2007 mainly involved the American Quarter Horse breed and it increased the EAV seroprevalence within this breed (13). The results obtained in the present study also showed that EAV prevalence was very high (50%) in the English-Arabian breed. However, the low number of horses tested ($n = 4$) made it impossible to draw a definitive conclusion.

Finally, the authors did not find any difference in EAV prevalence between male and female horses. These data are in agreement with those described several years ago by Moraillon and Moraillon (47) and Zientara *et al.* (48). ■

The objective of the present study was to investigate the prevalence of EAV in horses from different regions of Algeria. It is the first evidence that EAV is circulating in the eastern and western regions of Algeria. Further studies are necessary to isolate and obtain molecular characterisation of EAV from stallions in Algeria.

Acknowledgements

The authors would like to express their gratitude to all the stud farms, trainers and horse owners who cooperated with this study. They were extremely generous with their time and forthcoming with information, which will be of benefit to the equine industry.

Prévalence de l'artérite virale équine en Algérie

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Résumé

Lors d'une étude visant à déterminer la prévalence de l'artérite virale équine en Algérie, 268 sérums de chevaux non vaccinés des régions orientale et occidentale du pays ont été prélevés en vue d'une analyse sérologique. Les sérums, collectés entre 2009 et 2011, ont été soumis au test de neutralisation virale, conformément aux recommandations de l'Organisation mondiale de la santé animale (OIE). Au total, 20 sérums (7,46 %) ont donné des résultats positifs, 152 sérums (56,71 %) ont donné des résultats négatifs et 96 sérums étaient cytotoxiques (35,82 %). La prévalence sérologique du virus de l'artérite équine était significativement plus élevée dans la région occidentale (Tiaret) que dans la région orientale (Barika et El-Eulma). Fait intéressant, plus de 20 % des chevaux testés âgés de plus de 16 ans étaient séropositifs. En revanche, aucune corrélation n'a été observée entre la prévalence du virus de l'artérite équine et la race ou le sexe des animaux testés. Cette étude constitue la première description de la circulation du virus de l'artérite virale dans la population équine algérienne.

Mots-clés

Algérie – Artérite virale équine – Cheval – Prévalence – Test de neutralisation virale. ■

Prevalencia de la arteritis viral equina en Argelia

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Resumen

Con objeto de determinar la prevalencia de la arteritis viral equina (AVE) en Argelia se obtuvieron 268 muestras séricas de caballos no vacunados de las regiones occidental y oriental del país. Para el análisis serológico de los sueros, extraídos entre 2009 y 2011, se empleó la prueba de neutralización vírica descrita por la Organización Mundial de Sanidad Animal (OIE). En total, 20 sueros (7,46%) resultaron seropositivos, 152 (56,71%) negativos y 96 (35,82%) citotóxicos. La seroprevalencia del virus de la arteritis equina era sensiblemente mayor en la región occidental (Tiaret) que en la oriental (Barika y El-Eulma). Es interesante señalar que más de un 20% de los caballos analizados de más de 16 años de edad eran seropositivos. La prevalencia de la AVE, en cambio, no guardaba relación ni con la raza ni con el sexo del animal. Este estudio es el primero en describir la circulación de la enfermedad en la población caballar de Argelia.

Palabras clave

Argelia – Caballo – Prevalencia – Prueba de neutralización vírica – Virus de la arteritis equina.



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