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Original article

Serological cross-sectional survey of equine infectious anemia in Saudi Arabia

S. Kasem[1,2], O. Hashim[1], A. Alkarar[1], A. Hodhod[1,3], A. Elias[1], M. Abdallah[1], A. Al-Sahaf[1], A. Al-Doweriej[1], I. Qasim[1], A. S. Abdel-Moneim[4]

[1] Ministry of Environment, Water and Agriculture, 65 King Abdulaziz Road, Riyadh, 11195, Saudi Arabia
[2] Department of Virology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, El Geish Street, 33516, Egypt
[3] Animal Health Research Institute – Virology Department – Damanhur Branch – Egypt
[4] Microbiology Department, Virology Division, College of Medicine, Taif University, Al-Taif, Saudi Arabia

Abstract

The equine infectious anaemia virus (EIAV) is one of the most serious equine diseases worldwide. There is scarce information on the epizootiology of equine infectious anaemia (EIA) in Saudi Arabia. Given the importance of the equine industry in Saudi Arabia, this cross-sectional study aims to provide information about the prevalence of EIAV based on serological surveillance of the equine population in the country. A total of 4728 sera samples were collected (4523 horses and 205 donkeys) between December 2017 and November 2019. All samples were tested using commercially available EIAV ELISA. All tested samples showed negative results for EIAV antibodies with a 95% confidence interval. The results provided evidence that Saudi Arabia's equine populations (horses and donkeys) are currently free of EIAV. The results also suggest the need for continuous monitoring of EIAV and strict regulation when importing horses from other countries.

Keywords: equine infectious anaemia, equine, ELISA, Saudi Arabia, seroprevalence

Introduction

Equine infectious anaemia (EIA), which is also known as 'swamp fever', is a blood-borne infectious disease of the *Equidae* family caused by the equine infectious anaemia virus (EIAV). The disease has almost worldwide distribution and is of considerable importance to the equine industry (OIE 2019). Although the incubation period is typically 1-3 weeks, it may be extended by up to 3 months (Sellon et al. 1994). The disease can progress in a significantly mild form or subclincally without any prominent signs, especially at first exposure. The acute clinical presentation of the disease is characterized by fever, thrombocytopenia, anaemia, rapid weight loss, petechiae in the mucous membranes, oedema and abortion in pregnant mares (Issel and Foil 1984, Sellon et al. 1994). If the acute clinical infection does not result in death, a chronic infection that is characterized by the recurrence of febrile episodes may develop (OIE 2019). The severity and consequences of the disease outcomes are affected by the infecting virus's strain and titre, as well

Correspondence to: A.S. Abdel-Moneim, e-mail: asa@tu.edu.sa, asa@bsu.edu.eg

as the health status of the horse. Although morbidity and mortality rates are variable, mortality rates as high as 80% have been reported (Cook et al. 2013).

EIAV is an enveloped RNA virus of the Lentivirus genus from the *Retroviridae* family (Cook et al. 2013). EIAV is mechanically transmitted by haematophagous insects. The most effective transmitters of the disease are large-biting flies, including several species of Tabanus (horsefly) and Stomoxys calcitrans (stable fly) (Cook et al. 2013). EIAV can also be passed in utero from a mare to her foal (Kemen and Coggins 1972). In addition, blood transfusions, contaminated needles, surgical instruments and dental floats may play a role in the transmission of the disease. There is also evidence of pulmonary epithelial cells becoming infected by EIAV, indicating aerosol transmission (Bolfa et al. 2013).

EIAV was first described in France in 1843, and it is currently found throughout most of the world. In Europe, the disease is common, and it is endemic in Italy and Romania. EIAV cases were also reported in Ireland in 2006. EIAV outbreaks have recently been noted in many other parts of Europe, including Hungary, France, Greece, Belgium, Germany and Great Britain (Mooney et al. 2006, Cruz et al. 2015). The disease has also been reported in North America (Nagarajan and Simard 2007) South America (Oliveira et al. 2017) and Asian countries (Bolfa et al. 2017, Sharav et al. 2017). EIAV disease appears to be absent from a few countries including Iceland and Japan and Spain (Mooney et al. 2006, Cruz et al. 2015, Dong et al. 2014).

There is limited information available on the current prevalence of EIAV in the Gulf region. An isolated serological survey was completed in the Sultanate of Oman in 2011, which confirmed the absence of EIAV in certain horse populations throughout the country using both ELISA and AGID tests (Body et al. 2011). In addition, with only a single study completed in the Eastern and Central parts of Saudi Arabia, nothing is known about the status of EIAV in horses and donkeys across the country (Alnaeem and Hemida 2019). This cross-sectional study aims to provide information on the status of EIAV infections in Saudi Arabia.

Materials and Methods

Study Design

The Saudi Ministry of the Environment, Water and Agriculture approved this study. According to the General Authority for Statistics, the total number of equine populations in Saudi Arabia was estimated to be 32,921 in 2015. Furthermore, the number of pure-bred Arabian horses in the country has reached almost 28,000. In these cross-sectional studies, the sampling methodology included multistage random sampling. Saudi Arabia is divided into 13 administrative districts, and each of these regions was included. The administrative regions are divided into five sectors (Western, Southern, Eastern, Northern and Central). Each sector contains two-three administrative regions, as shown in Table 1. For horse samples, although all stables were selected in each sector, the number of samples from each stable was limited to five animals to ensure accurate representation of the population. For the donkey samples, convenience sampling was used to collect blood from both stray donkeys and donkeys on farms. Samples were collected from adult animals using stable systemic random sampling from horses and donkeys from various regions and governorates during two cross-sectional studies, with the first conducted in December 2017 and the second carried out in November 2019. These studies used a total of 4728 samples, including 4523 horses and 205 donkeys.

Sample collection, processing and serological analyses

Ten ml whole blood samples were obtained by jugular venepuncture from each animal. The blood samples were centrifuged, and the serum was separated, identified with a unique code and stored frozen at -20°C until testing. A commercial serological ELISA kit (ID: Screen Equine Infectious Anaemia Double Antigen. Vet Innovative Diagnostics, Montpellier, France) was used to screen antibodies against the core protein (p26) antigen of EIAV. Positive and negative controls were used in order to validate each kit lot used. According to the manufacturer's information, the ELISA kit this study used has published 100% sensitivity and specificity.

Results

Detection of EIAV antibodies by ELISA

Between December 2017 and November 2019, 4728 sera samples were obtained from 4523 horses and 205 donkeys who resided in different areas of all regions in Saudi Arabia. Using ELISA, all serum samples tested were negative for antibodies against EIAV proteins with a 95% confidence interval, as there were no positive or equivocal results (Table 1).

Table 1. Number of collected equine samples from all regions of Saudi Arabia and result of tested samples by ELISA.

Sector	Region	No of equine populations in Saudi Arabia	Horse Samples in 2017	Donkey Samples in 2017	Horse Samples in 2019	Donkey Samples in 2019	Positive Samples	Negative Samples	Total Samples	
									Region	Sector
Eastern	Eastern	8320	398	32	399	44	0	873	873	1250
	Northern Boundaries	2777	189	4	177	7	0	377	377	
Western	Makkah	1885	300	0	316	0	0	616	616	782
	Al-Madinah	518	50	5	55	6	0	116	116	
	Al-Bahaa	32	20	1	25	4	0	50	50	
Central	Riyadh	12121	400	10	344	21	0	775	775	1152
	Al-Qassim	2102	190	0	187	0	0	377	377	
Southern	Jazan	603	205	12	114	15	0	346	346	728
	Najran	784	108	6	98	7	0	219	219	
	Asir	424	93	10	51	9	0	163	163	
Northern	Hail	1166	147	0	150	0	0	297	297	816
	Tabuk	1109	134	0	146	5	0	285	285	
	Jouf	1080	112	0	115	7	0	234	234	
Total		32921	2346	80	2177	125	0	4728	4728	4728

Discussion

For the current study, serum samples were examined by ELISA. Although ELISA is relatively fast and easy to perform, its tendency to achieve false positive results requires confirmation through the recommended AGID test (OIE 2019). Due to the importance of EIAV and the risk of its introduction to disease-free countries, continuous monitoring is required to ensure that these countries remain unaffected by this devastating disease. Based on the results of the samples, it is clear that Saudi Arabia's equine population (horses and donkeys) was seronegative for EIA from 2017-2019. These results are consistent with a previous study conducted in the Eastern and Central parts of Saudi Arabia from 2014-2016 (Alnaeem and Hemida 2019) and with studies conducted in other countries, as well (Ataseven and Arslan 2005, Ghadrdan-Mashhadi et al. 2010).

The AGID is typically performed to confirm positive ELISA results. Nonetheless, a high degree of agreement between the presence of specific EIAV genetic material and antibodies in equids can be reported as negative by the Coggins test and positive by the ELISA assay (Issel et al. 2013); ELISA techniques can detect antibodies directed against the p26 EIAV-capsid before the Coggins test and are generally considered to be more sensitive than the Coggins test (Reis et al. 2012). However, the Coggins test is the gold standard for EIA diagnosis because it is more specific (Piza et al. 2007). Put differently, because ELISA techniques can result in false positives, any positive EIA results ELISA detects must be confirmed using the Coggins test (OIE 2019).

Although no positive cases of EIAV were found in the present study, the results of the study's serological analysis of EIAV are representative of the equine population in Saudi Arabia and can represent the entire population throughout the county. This suggests that the prevalence of EIAV in Saudi Arabia is very low. Therefore, continuous surveillance and monitoring of EIAV should be performed to ensure that the Kingdom of Saudi Arabia remains free of EIAV. In sum, it is clear from the results of the two cross-sectional surveys that Saudi Arabia shows no current evidence of EIA among its equine populations. This may provide increased confidence when exporting horses to other countries, despite judicious biosecurity that includes both pre-export and post-arrival testing of EIAV to prevent EIAV-infected horses from being introduced.

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