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RESEARCH ARTICLE

Sero-Survey of Equine Infectious Anemia in the Sultanate of Oman during 2007-2009

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ABSTRACT

Equine infectious anemia (EIA) is a fatal and relapsing infectious disease of equines caused by the lentivirus of Retroviridae family which occurs world-wide. It tends to become an inapparent infection if death does not result from the acute clinical attack. The virus persists in infected animals for life and can be detected by serological tests like enzyme-linked immunosorbent assay (ELISA) and agar gel immunodiffusion (AGID) tests. Keeping in view the importance of EIA, a sero-survey and passive surveillance was designed to establish the status of EIA in Oman. For the current study, ELISA was carried out on 331 random horse serum samples collected from all over Oman and 262 serum samples submitted from race horses. Four (0.67%) out of total 593 serum samples were found positive on ELISA. These samples were further tested by agar gel immunodiffusion (AGID) test for the confirmation and were found negative. Based on the analysis of the samples, it can be assumed that the horse population in the Sultanate was free of the disease during the study period (2007-2009).

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INTRODUCTION

The equine infectious anemia (EIA) is a potentially fatal and relapsing blood borne ailment of family *Equidae* (Higgins and Wright, 1998; Radostits *et al.,* 2007). It is caused by EIA virus of subfamily *Lentiviridae* of *Retroviridae* family which is a close relative of Human Immunodeficiency Virus (HIV) (Montaginer *et al.,* 1984; Nakajima and Sugiura, 1994). EIA in equines may present itself in three clinical entities viz., acute, chronic and inapparent infection. Acute form is usually fatal and affected animal may dies within 2-3 weeks. In recovered animals, EIA virus persists for rest of the life and affected equine may suffer from chronic relapsing disease (swamper) or become an in-apparent carrier (Clabough *et al.*, 1991). The in-apparent carriers are major cause of spread and maintenance of EIA and are usually detected by the serological testing through ELISA and AGID (Issel *et al.*, 1990). In nature the spread of virus is most likely via interrupted feeding of blood sucking horse flies. However, accidental transmission through transfer of blood from an infected horse and use of contaminated needles may spread the disease to healthy equines (Cordes and Issel, 1996). To date, no effective treatment or vaccine is available concerning EIA and stringent implementation of 'test and destroy' policy is the most recommended control method (OIE, 2004).

The serological diagnosis of EIA remained a challenge until AGID test was developed by Coggins and co workers (1972). Although, positive AGID test results are reliable and verifies the presence of EIA, it has its own limitations. It has been reported that horses acquiring the EIA for the first time and in early stage of the disease and foals born to AGID test positive dams can test negative for EIA when tested though AGID (Issel and Cook, 1993). Since late 1980s, ELISA test has been employed for faster and sensitive serological diagnosis of EIA. Moreover, in recent years more sensitive and reliable ELISA to detect antibodies against core p26 antigen has been developed and tested by various workers for the screening of EIA (Soutullo *et al.*, 2001; Paré and Simard, 2004; Piza *et al.*, 2007; Gill *et al.*, 2008; Albayrak and Ozan, 2010). Though ELISA test is more rapid and easier to interpret, the chances of getting false positive results are more (Cullinane *et al.*, 2007; Piza *et al.*, 2007) because it's less specific than AGID. Because of this, OIE recommends that all ELISA positive samples should be confirmed by the AGID before declaring the equines positive for EIA.

A previous study regarding the some viral disease of animals concluded that Oman was free from EIA (Hedger *et al.*, 1980). From 2005 to December 2010, the disease has not been reported in the Middle East region (Anonymous, 2011). However, during 2010 the varying incidence of disease has been reported from 30 countries of Asia, Europe and Americas (Anonymous, 2011). Such is the importance of disease that according to OIE regulations the EIA positive horses can't compete in international events and export of test positive equines is prohibited. Moreover, to acquire the EIA free status, countries are required by OIE to conduct active as well as passive internal and external surveillance to achieve desired confidence in disease freedom (OIE, 2009). Keeping in view the importance of the disease, present survey was undertaken to investigate the serological status of the horses concerning EIA in the Sultanate.

MATERIALS AND METHODS

Total horse population in Oman is 2393 heads (Anonymous, 2005). A total of 331 serum samples were randomly selected through Survey Toolbox Software™ (ACIAR, Australia) from the serum bank (n=2200) of the Veterinary Research Center (Cameron, 1999). These samples were randomly collected from horses of various regions and governorates during 2007-2008 in Oman for the surveillance of equine diseases. Samples size (n=331) for random selection was calculated at 95% confidence interval (CI) and 5% desired absolute precision (DOP) for an expected diseases prevalence of 50% (unknown status) (Thrusfield, 2005). Final sample size was adjusted for finite population and proportional allocation was performed according to the horse population of each area (Table 1). Furthermore, 262 serum samples presented to the center from race horses during the game season 2008-2009 were also used in the current study for EIA screening (Table 2).

Table 1: Population of horses and sampling frame adopted for the survey of equine infectious anemia during 2007-2008 in the Sultanate of Oman

Region	Horse Population	Sample Size & Proportional Allocation	Adjusted Final Sample Size & Proportional Allocation
Muscat	997 (41.66)	160	138
Dofar	548 (22.90)	89	72
Al-Batinah	315 (13.16)	50	46
Al-Sharqiyah	226 (9.44)	37	32
Ad-Dhahira	187 (7.81)	31	26
Al-Dakhaliyah	93 (4.01)	16	13
Musandum	24 (1.00)	2	4
Total	2393	385	331

Figures in parenthesis indicate percentage.

Table 2: Distribution of serum samples (n = 262) utilized for surveillance of equine infectious anemia (EIA) during 2008-2009 game season in the Sultanate of Oman

Region/Governorate	Horse serum samples submitted to VRC during game season 2008-2009
Al-Batinah	86
Al-Sharqiyah	76
Muscat	74
Al-Dakhaliyah	24
Ad-Dhahira	2
Total	262

Commercial indirect ELISA (ID Screen® Equine Infectious Anemia Indirect, IDVET, France) was used for screening of serum samples. Testing was performed as per recommendations of the manufacturer. Briefly, samples and wash solution were prepared and 90 µl of dilution buffer was added to each microwell. Pre-designated wells were added 10 µl each of positive, negative and test sera and incubation was performed at room temperature (25°C) for 45 minutes. Afterwards, washing was performed and 100 µl of conjugate was added to each well. Further incubation was performed for 30 minutes at room temperature and washing was performed. Then 100 µl of substrate solution was added to each well and 15 minutes incubation was performed at room temperature in dark. Immediately after incubation, 100 µl of stop solution was added to each well and plates were then read at 450 nm to record the OD values. Interpretation was performed by calculating the S/P percentage as recommended by the manufacturer.

Samples found positive in ELISA test were further confirmed on the commercial Equine Infectious Anemia Virus Antibody Test Kit, AGID (VMRD, Inc., USA). Test was performed as per the recommendations of the manufacturer. Briefly, gel was prepared and 6 circular and 1 central (2.4 mm apart and 5.3 mm in diameter) wells were cut in the agar. EIA p26 antigen (50 µl) was placed in the Central well. Fifty microliters of positive control serum was placed on each side of the samples for the formation of positive control line to facilitate accurate recognition of lines of identity. Remaining wells were filled with 50 µl of test sera and plate was incubated at 22°C for 24 hours. Interpretation was performed as recommended by the manufacturer.

RESULTS

Only 4 (0.67%) out of 593 tested horse serum samples were found positive for EIA virus antibodies upon ELISA. The OD value of the positive sample was 1.52 when read at 450 nm and all the 4 ELISA positive samples yielded higher OD values: 1.68 (Muscat), 1.58 (Muscat), 1.61 (Al-Batinah) and 1.59 (Dofar). As per recommendations of the World Animal Health Organization (OIE) these samples were further tested by AGID and failed to give positive results. Therefore, the ELISA positive sera were marked as false positive.

DISCUSSION

Sampled horse population in Oman was mainly comprised of animals kept by the Royal Court, Royal Oman Police (mainly in Muscat and Dofar Governorates) and private owners (rest of the country). However, during the game season these animals are regularly transferred from one region / governorate to other for participation in equestrian events and annual celebrations. This frequent travelling during hot climatic conditions may result in development of environmental as well as thermal stress in some animals that may influence reproductive hormones, metabolism and immune responses to various diseases (Stull, 1997). Therefore, active as well as passive surveillance was employed in the current study to

maximize the chances of getting valid results (Cameron, 1999; Thrusfield, 2005).

For the current study, serum samples were first quick screened through ELISA and then the positive samples were confirmed by the OIE recommended AGID test. Though ELISA is comparatively quick and easy to perform its tendency to yield false positive results requires the confirmation through recommended AGID test (OIE, 2004; Paré and Simard, 2004; Cullinane *et al.*, 2007; Piza *et al.*, 2007). However, when testing on a larger scale for screening purposes is required within a short period of time, ELISA becomes the test of choice (Issel and Cook, 1993; Paré and Simard, 2004; Piza *et al.*, 2007). Importance of EIA and risk of its introduction in disease free countries requires the continuous surveillance to maintain the OIE certified disease free status regarding this devastating disease. Based upon the results of the samples, it can be safely assumed that horses in the Sultanate were free from EIA during 2007-2009. These results are in accord with a previous study performed by Hedger *et al.* (1980) in Oman and studies conducted elsewhere in the other countries (Ataseven and Arslan, 2005; Gill *et al*., 2008; Kirmizig l *et al.*, 2009; Albayrak and Ozan, 2010; Mashhadi *et al.*, 2010) with the claims of disease free status and warrants the continuous surveillance to acquire the possible EIA free status for Oman.

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