SEROPREVALENCE OF EQUINE HERPESVIRUS-1 AND EQUINE HERPESVIRUS-4 INFECTIONS IN HORSE POPULATION IN KELANTAN, MALAYSIA

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ABSTRACT. Equine herpesviruses type-1 (EHV-1) and type-4 (EHV-4) are common pathogens causing respiratory disease, myeloencephalophathy, abortion and neonatal death in horses in all continents. Due to latent infection of EHV, infected horses could become lifelong carriers without any clinical manifestation. In Malaysia, the data on seroprevalence of EHV-1/EHV-4 in horses is limited. Therefore, due to the scarcity of reports on seroprevalence of EHV-1/EHV-4, the objective of this research was to investigate the presence of EHV-1/EHV-4 specific antibodies among the horses in Kelantan, Malaysia. Serum samples from 54 asymptomatic horses collected from various districts in Kelantan from the year 2015 to 2016 with no history of vaccination, were tested for the presence of EHV-1/EHV-4 antibodies using a commercially available enzyme-linked immunosorbent assay (ELISA) assay. The results showed that 1.9% (1/54) of the tested sera was positive for EHV-1 and 85.2% (46/54) were seropositive for EHV-4. This study suggests that both EHV-1 and EHV-4 viruses are present in the horses in Kelantan with EHV-4 infection is more prevalent than EHV-1. This study may contribute to a better understanding of the etiological role and disease management of these herpesviruses in the equine population in Kelantan, Malaysia.

Keywords: Seroprevalence, EHV-1, EHV-4, ELISA, equine herpesvirus

INTRODUCTION

Herpesviruses infections caused by equine herpesvirus type-1 (EHV-1) and type-4 (EHV-4) are endemic in equine populations worldwide (Ataseven *et al.*, 2009). Both EHV-1 and EHV-4 cause respiratory illnesses but EHV-1 also causes neurological disorders, abortion and death in equines (Allen and Bryan, 1986). Such infections have major financial implications for the economy especially in industry involving equines such as agrotourism and sports.

EHV-1 and EHV-4 are closely related viruses that share a high degree of genetic and antigenic similarity (Ma *et al.*, 2013). Prior to 1990, the data from serological surveys were complicated by the extensive antigenic cross-reactivity due to the lack of availability of a type-specific antibody test (Crabb and Studdert, 1993). A specific ELISA test based on the C-terminal portion of glycoprotein G of both viruses has been developed and commercialised which can distinguish between EHV-1 and EHV-4 infections (Crabb *et al.*, 1992; Crabb and Studdert, 1993; Drummer *et al.*, 1995).

Herpesviruses can cause lifelong latent infection where the majority of recovered horses develop antibodies over time but will continuously carry and excrete the virus during the reactivation process induced by stress factors or immunosuppression (Goodman *et al.*, 2012). As equine

recreational activities and endurance race are gaining more popularity in Kelantan, silent virus shedding from latently infected horses carries the risk of infection to other susceptible horses. A report on seroprevalence of EHV-4 in Malaysia was previously published by Kamarudin, (2002). Yet, no published report on the presence of EHV-1 antibody in the horse population in Malaysia is available.

Thus, this study provided serological evidence of the EHV-1 and EHV-4 infections among the horse population in Kelantan using type-specific ELISA. This seroprevalence data is important for the disease management of EHV-1 and EHV-4 in the horse populations in Malaysia.

MATERIALS AND METHOD

Sampling and sample size calculation

This study was designed as a pilot study to determine the seroprevalence of EHV-1/EHV-4 in Malaysia. Based on equine population and accessibility, Kelantan state was chosen. However, the previous prevalence of the disease in the state was not known, therefore based on the prevalence of EHV-1 (9.2%) in a study from neighbouring countries, Ritruechai (2001) was selected. A 5% absolute precision was considered to calculate the number of animals to be sampled from an estimate of 5,000 horses available in Kelantan, n=126 horses were needed for the study. Nonetheless, only 54 serum samples were managed to be retrieved from the archived for this pilot study.

Sample collection

Archived sera from 54 horses with unknown EHV-1/EHV-4 vaccination status were retrieved from the serum collection bank, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. The samples were randomly collected from different stables in various districts of Kelantan between 2015 and 2016. Ethical approval was unnecessary since no experimental trial involving animals was involved and samples were retrieved from the collection bank.

Serological analysis

The commercially available kit Svanovir® EHV1/EHV4 (Svanova Biotech) indirect ELISA was used in detecting the antibodies present in the horse sera. According to the manufacturer, the ELISA assay has a sensitivity and specificity of 100 per cent in detecting the antibody when compared to serum neutralization test. Besides that the ELISA kit is able to differentiate specific EHV-1 and EHV-4 antibodies (Gür and Yapici, 2008; Ataseven *et al.*, 2009; Aharonson-Raz *et al.*, 2014; Yildirim *et al.*, 2015). The ELISA test used in this study was based on recombinant antigens to the C-terminal portion of glycoprotein G of both viruses. In the procedure, serum samples were added to wells coated with non-infectious EHV-1 and EHV-4 antigen, and to wells coated with control antigen in microtiter strips. The antibodies present in the test sample that bound to those EHV-1/EHV-4 antigens in the wells would show positive results. The reaction between the antigen-antibody complex was then measured

spectrophotometrically at 450 nm (ELx808 absorbance reader, BioTek Instruments). Samples were considered positive when the optical density (OD) readings were higher than 0.2, and negative when the OD450 was lower than 0.1. Samples with readings between 0.1 and 0.2 were considered as borderline samples. Each serum sample was tested in triplicates and results were averaged.

Statistical analysis

Significant differences between EHV-1/EHV-4 and the variables (age, breed, sex) were evaluated using the Chi-square (χ^2) with Yates correction. The value of χ^2 more than critical value (7.82) at a *p*-value=0.05 was regarded as significantly different.

RESULTS AND DISCUSSION

The demographic data of horses included in the present study is shown in Table 1. Of the 54 sera tested by ELISA for the presence of EHV-1 or EHV-4 specific antibodies, only one (1.9%) was positive for the EHV-1 antibody, while 46 sera (85.2%) were positive for EHV-4 specific antibodies (Table 1). Among the EHV-1/EHV-4 seropositive samples, one sample was positive for both EHV-1 and EHV-4 antibodies. Four samples were negative for both antibodies and the other four samples were considered borderline as suggested by Crabb *et al.* (1995) in which the OD score was between 0.1 and 0.2. For statistical analysis purposes, the samples with borderline scores were considered positive for EHV-4 antibody. A chi-square test of independence was performed to examine the relation between the variables and seropositivity of EHV-1 and EHV-4. From the Chi-square calculation (χ^2) with Yates correction on the variables (age, breed and sex), the values were higher than critical value (7.82 at p-value=0.05). These results were considered as statistically significant which suggest that the variables were associated with seropositivity of EHV-4/EHV-1.

The current results showed that EHV-4 is more prevalent than EHV-1 with the seropositive rate of 85.2% compared to 1.9% for EHV-1. Although the current data may not be accurate, our findings are in agreement with the results of previous studies with the seropositive rate between 57% to 100% and 1% to 35% for EHV-4 and EHV-1, respectively (Gilkerson *et al.*, 1999; Gür and Yapici, 2008; Ataseven *et al.*, 2009; Aharonson-Raz *et al.*, 2014; Avci *et al.*, 2014).

A previous serological survey conducted by Kamarudin (2002) on the horse population in Malaysia revealed a seroprevalence rate of 60% for EHV-4, which is slightly lower than the present study since our samples were limited only to the horses in Kelantan.

To our knowledge, this is the first study reporting the presence of EHV-1 antibody among horses in Malaysia. Nonetheless, we assumed that most of the domestic horses with seropositive for EHV-1/EHV-4 came from the natural infection as vaccinations for both viruses was not routinely applied in Kelantan, except for the racehorses which were not included in the present study. The vaccination programme was not done by the owners possibly due to lack of awareness of the disease as the disease itself was not commonly reported within the community.

Table 1. Demographic data of horses included in the present study

Variables	No. of tested serum, n (%)	EHV-4 (+), (%)	EHV-1 (+) (%)	χ^2 value, (*p*-value)
Age (years)				
< 3	23 (42.6)	18 (78.3)	0(0)	
4 - 6	19 (35.2)	17 (89.5)	0(0)	
7 - 14	9 (16.7)	8 (88.9)	1(11.1)	
15-20	3 (5.6)	3 (100)	0(0)	42.25 (0.05)
Breed				
Pony	17 (31.5)	15 (88.2)	0(0)	
Crossbreed	25 (46.3)	21 (84)	0(0)	
Thoroughbred	10 (18.5)	8 (80)	1(10)	
Arabian	2 (3.7)	2 (100)	0(0)	42.25 (0.05)
Sex				
Male	11 (20.4)	9 (81.8)	0(0)	
Female	43 (76.4)	37 (86.0)	1(2.3)	1260.25 (0.05)
Total	**54 (100)**	**46 (85.2)**	**1(1.9)**	

Instead, these horses may have developed immunity through repeated natural infections, perhaps originating from the countries where the horses were originally imported from as reported by Smith *et al.*, (2018) in imported horses in the United States.

The explanation for this seroprevalence data is directly linked to the latency of EHV-1 and EHV-4 specific antibodies post-infection. It was reported that the EHV-4 antibody was maintained at a high level from age 2 to 9 years (Crabb *et al.*, 1995). EHV-4 antibody is also believed to be regularly boosted via reactivation or reinfection. In this study, the highest percentage of EHV-4 seropositive were found in adults and older horses (7 to 20 years old). Nevertheless, in the case of EHV-1, such antibody maintenance and regular reactivation were not observed. After the abortion, EHV-1 antibodies from the mares could only last up to four years before becoming seronegative (Crabb *et al.*, 1995). It has been shown that after the outbreak of neurological EHV-1 disease, the antibodies persisted only for a year (Van Maanen *et al.*, 2001). A significant reduction in type-specific antibodies has been shown in several horses after a certain period of infection with EHV-1 (Van Maanen *et al.*, 2001). The short-term protection could be due to the reactivation or/and the reinfection episodes which are not common to EHV-4 (Crabb *et al.*, 1995).

Furthermore, other factors such as temperature and season could also possibly contribute to this low prevalence of EHV-1 antibodies. A study on respiratory disease caused by EHV-1 in race horses in Japan showed that EHV-1 infection occurred during winter while EHV-4 infection occurs all-year-round (Matsumura *et al.*, 1992). While other countries usually close the race tracks during winter, in Japan, the races are carried out throughout the year. This could cause the horses in Japan to suffer from stress due to cold weather and hard training during the period which contributed to the reactivation of the latent EHV-1. The seroprevalence of EHV-1 in this study was low (<2%). Although the horses were trained in the hot and humid climate, the weather seemed less likely to cause too much stress compared to training in cold winter. In a study by Gür and Yapıci (2008), the results for EHV-1 seroprevalence in Turkey were also low with only 3.7% (7/188). The sampling was conducted in summer and it was assumed that season could be one of the factors that cause such a low rate of EHV-1 seropositivity. Another study by Aharonson-Raz *et al.* (2014) in Israel also reported the same trend of low EHV-1 seroprevalence with a rate less than 1%. They presumed that the extremely low seroprevalence to EHV-1 was due to the short and mild winter experienced by horses in Israel. However, more well-structured studies based on climate as the risk factor should be done to provide evidence that seasonal variance can strongly influence the seroprevalence rate of EHV-1/EHV-4.

In the seroprevalence study, a positive serum sample indicates the exposure of EHV-1 and EHV-4 infection. ELISA test, however, cannot differentiate between vaccinated and unvaccinated horses or maternal antibodies and true infection. Therefore, we cannot presume that all seronegative EHV-1/EHV-4 horses were never infected with the virus. Ataseven *et al.* (2009) in a parallel study involving molecular detection of PCR, serology, and virus isolation detected EHV-1 and EHV-4 DNA in seronegative samples from unvaccinated animals. Similarly, Vargas-Bermudez *et al.*, (2018) indicated serum sample negative for EHV-1/EHV-4 antibodies, were positive for EHV-1 and EHV-4 DNA. Thus, the low seroprevalence rate of EHV-1 in the present study does not rule out the absence of the EHV-1 virus in the horse for the virus may escape into the environment due to inadequate protective immunity against the reinfections (Pusterla, 2014). Thus, the absence or presence of this virus in the host need to be confirmed using molecular methods such as PCR or quantitative PCR. (This PCR method is for antigen detection while ELISA with 100% sensitivity is for antibody detection).

Effective management can be implemented by the authorities to the horse premises. The procedures include segregation of horse population into the specifically designated area for weanlings, yearlings, new arrivals, and transients, isolation or quarantine of the new arrivals on the premises, subdivision of pregnant mares and foaling group, and stress reduction (Wilson, 1997; Allen, 2002; Allen *et al.*, 2004). Although the results of this small study may not equally represent the true prevalence of EHV-1, it is perhaps the first report on the presence of EHV-1 antibody in Malaysia.

CONCLUSION

The present study suggests that EHV-1/EHV-4 is circulating among horses in Kelantan with higher EHV-4 prevalence compared to EHV-1. These horses serve as potential reservoirs and disseminators of EHV-1/EHV-4 in the environment. Therefore, this study provides immunological evidence of EHV-1 and EHV-4 which will serve as a valuable reference for veterinary authority to control and prevent future outbreaks among the horse population in Kelantan.

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