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DETECTION OF EQUINE HERPES VIRUS 1, EQUINE HERPES VIRUS 4, AND EQUINE ARTERITIS VIRUS ANTIBODIES IN KYRGYZSTAN BY ELISA

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ABSTRACT

The aim of the present study was to detect the presence of Equine herpes virus 1 (EHV-1), Equine herpes virus 4 (EHV-4), and Equine Arteritis Virus (EAV) antibodies in domestic horses in Kyrgyzstan. In this study, 116 serum samples of unvaccinated domestic horses were collected between November 2012 to February 2013, and tested for equine herpes virus (EHV-1, EHV-4) and EAV specific antibodies by commercially available indirect Enzyme Linked Immunosorbent Assay (ELISA). EHV-1, EHV-4, and EAV specific antibodies were detected as 81.89% (95/116), 96.55% (112/116), and 20.68% (24/116) of tested serum samples were found positive for EHV-1, EHV-4, and EAV antibodies, respectively. 95 horses (81.89%) were positive for EHV-1 plus EHV-4. 20 horses (17.24%) were detected seropositive EHV-1 plus EAV while 22 horses (18.96%) were seropositive for EHV-4 and EAV. In conclusion, EHV-1, EHV-4 and EAV infections appear to be widespread in domestic horses in Kyrgyzstan and may also serve as a risk factor for other species.

Keywords: Equine herpes virus 1; Equine herpes virus 4; Equine arteritis virus; horse; ELISA; Kyrgyzstan.

INTRODUCTION

Equine Herpes virus 1 (EHV-1) and Equine Herpes virus 4 **(**EHV-4) are the member of the subfamily *alphaherpesvirinae* in the family *Herpesviridae* (Van der Meulen et al., 2000). These are important viral pathogens of horses' worldwide (Slater et al., 2006). They are double stranded (ds) DNA viruses with molecular weight of 150 kb (Crabb and Studdert 1995). Capsid has icosahedral symmetry and surrounded by lipid membrane with 12 different glycoproteins (Telford et al., 1998; Roizman et al., 2007).

EHV-1 and EHV-4 may cause a variety of multisystemic infections including conjunctivitis, neurological defects, encephalitis, respiratory, abortion, neonatal death, and reproductive disorders (Harless and Pusterla 2006). The viruses can be transmitted to susceptible species by direct contact, aerosol, nasal secretions and contaminated feed (Garre et al., 2009). EHV-1 and EHV-4 can be diagnosed by using serological tests such as type specific enzyme linked immunosorbent assay (ELISA), serum neutralization and complement fixation (Gilkerson et al., 1999; Van Maanen et al., 2001). Aborted material, blood and nasopharyngeal secretions can be used for virus isolation (Lunn et al., 2009). Primary (equine endothelium, equine testis fibroblast, equine embryonic lung) or permanent cell culture (rabbit kidney, Madine Darby Bovine Kidney) can be used for isolation in routine diagnosis of these infections (Tearle et al., 2003).

Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a contagious viral disease of members of the equidae family (Doll et al., 1957; Balasuriya et al., 2004). EVA is caused by EAV, a small positive-stranded RNA virus in the genus *Arterivirus*, family *Arteriviridae* in the order *Nidovirales* (Cavanagh, 1997; de Vries et al., 1997). There is only one serotype recognized (Campbell 2006). Antibodies to EAV have been reported in different equides such as horses, ponies, donkeys, and zebras (Paweska 1997; Balasuriya and MacLachlan 2004; Balasuriya et al., 2004). Clinical signs of EVA may include fever, depression (Balasuriya et al., 2004), abortion (Doll et al., 1957), loss of appetite, conjunctivitis, oedema, nasal discharge, mild respiratory disease (Chirnside, 1992), and death in neonatal foals (Vaala et al., 1992). It has also been found in faeces and urine during the acute phase of infection (Holyoak et al., 2008).

EHV-1, EHV-4, and EVA are economically important viral diseases of equids. The aim of this study was to describe the seroprevalence rate of EHV-1, EHV-4, and EVA infections in domestic horses in Kyrgyzstan.

MATERIALS AND METHODS

Animals and Sampling

A total of 116 samples were collected from unvaccinated horses (clinically respiratory symptoms) that were randomly selected between November 2012-February 2013 in Kyrgyzstan. All the operations were humane according to the animal welfare. Samples (5 mL) were taken from the jugular vein with sterile plain vacuum tubes (BD, Vacutainer®, USA). Collected samples packed in ice were brought to the laboratory, and centrifuged at 3000 × g for 10 min for obtaining serums. Approximately 1 mL of serum was collected into sterile microfuge tubes and stored at -20℃ until analysis.

ELISA

Antibodies to EHV-1 and EHV-4 in sera were detected using a commercially available ELISA test kits (Svanovir, EHV-1/EHV-4 Ab kit, Svanova Biotech AB, Sweden). The test was performed as per the manufacturer's instructions. The plates were then read spectrophotometrically with a 450 nm filter on an automatic ELISA reader (Rayto RT-2100, Japan). Antibodies to EAV were detected using a commercially available ELISA test kit (ID Vet, France).

Statistical Analysis

Differences between antibody statuses were calculated by using chi-square test (Minitab 14.0 Inc., State College, PA, USA). Difference were considered significant when $P<0.05$.

RESULTS

The seroprevalence of EHV-1, EHV-4 and EAV among domestic horses in are shown in Kyrgyzstan is shown in Table 1. The result showed EHV-1, EHV-4, and EAV had seroprevalence rates of 81.89% (n: 95), 96.55% (n: 112) and 20.68% (n: 24) respectively. There was a statistical difference ($p<0.05$) in the seroprevalence of the three viruses amongst the horses studied. Table 2 shows the distribution of positive samples with multiple infections. The distribution showed EHV1 + EHV4 only with 81.89%, EHV1 + EAV anly with 17.24%, EHV4 + EAV only with 18.96%, and EHV1 + EHV4 + EAV having 18.96% seroprevalence rates. There was no statistical difference ($p>0.05$) in the occurrence of the multiple infections with the exception of EHV1 + EHV4 that was statistically different ($p>0.05$) from the others.

Table 1. Seroprevalence of EHV-1, EHV-4, and EAV in 104 horses

Biometric Data	EHV-1	EHV-4	EAV
Examined	116	116	116
Positive	95[b]	112[a]	24[c]
Prevalence (%)	81.89%	96.55%	20.68%

[a, b, c]: values marked with different letters in the same line are statistically significant (P<0.05, chi-square test)

DISCUSSION

Different serological methods can be used for the determination of specific antibodies against EHV-1 and EHV-4 (Studdert et al., 2003) but it is not enough for distinguish two serotypes because of a strong antigenic cross-reactivity. ELISA is commonly using in routine diagnosis preferred other tests for its high sensitivity and its practical advantages (Hartley et al., 2005; Ataseven et al., 2009). In epidemiological studies, detection of specific antibodies against alphaherpesviruses amongst apparently healthy horses is an important indicator of asymptomatic carrier animals in the population (Ataseven et al., 2009).

In this study, EHV-1 specific antibodies were detected in 95/116 (81.89%) of samples while 112/116 (96.55%) of the samples were positive for EHV-4 (Table 1). In previous study, in unvaccinated horses, Crabb and Studdert (1995) detected all of the horses as seropositive for EHV-4 antibodies, while 30% were seropositive for EHV-1 ($P<0.05$). Prevalence studies show that EHV-4 is much more prevalent than EHV1. The same results obtained from this study. In previous studies (Keane et al. 1988, Nordengrahn et al., 1999, Singh et al., 1999), seroprevalence rates for EHV-1 and EHV-4 specific antibodies were reported to be between 8% and 85.2% and over 90%, respectively (Gilkerson et al., 1999; Nordengrahn et al., 1999). There is no information about horse viral infections in Kyrgyzstan. The seroprevalences of EHV-1 and EHV-4 were previously reported to be 23.2% and 78%, respectively, for the sampled horse population in the East Anatolia region of Turkey (Ataseven et al., 2010). Gur and Yapici (2008) detected 3.7% (7/188) seropositivity for EHV-1 while the prevalence of EHV-4 antibody was 56.9% (107/188). This study reveals that the horse population in the Kyrgyzstan consisted of 81.89% EHV-1, 96.55% EHV-4, and 20.68% EAV. Vaccination of horses against these viruses has not been applied in Kyrgyzstan, so this seropositive result indicates natural infections. The reason for this high

Table 2. Seroprevalence of mix infections

Biometric Data	EHV-1 + EHV-4	EHV-1 + EAV	EHV-4+EAV	EHV1+EHV4+ EAV
Examined	116	116	116	116
Positive	95[a]	20[b]	22[b]	22[b]
Prevalence (%)	81.89%	17.24%	18.96%	18.96%

[a, b]: values marked with different letters in the same line are statistically significant (P<0.05, chi-square test)

seroprevalence rate observed in this study could be due to extreme climatic conditions. EHV-1 and EHV-4 infections are not vector-borne but the cold climate is a stressor for horses that can result in reactivation of latent infections (Foote et al., 2003). High seroprevalence rates in this study may also be related to sampled season.

It was observed in this study that most (81.89%) of the horses had mixed infections with EHV-1 and EHV-4. This is similar to the reports from other parts of the world by Whalley et al. (2003); Gur and Yapici (2008) and Ataseven et al. (2010). Other mixed infections observed among the horses were those of EHV-1 and EAV (17.24%), EHV-4 and EAV (18.96%). Borchers et al. (2005) reported mixed infections with viruses of EHV-1, EHV-4 and EAV among Burchell's zebras in the Serengeti National Park (Tanzania).

EAV can be diagnosed by serologically using neutralization, complement fixation, or ELISA (Holyoak et al., 2008, Bulut et al., 2012). ELISA is often used to detect antibodies against EAV. It is reported by serologically in different species in donkeys (Paweska et al., 1996), horses, and zebra (Borchers et al., 2005). It has been reported that EAV usually occurs as subclinical infection in domestic horses and infection induces a long-lasting protective immunity (Borchers et al., 2005). Our results demonstrate a moderate level of exposure to EAV in the Kyrgyzstan horse population. There is no previous publication that reported the presence of horse viral infections in Kyrgyzstan and to the best of our knowledge, this is the first report of EHV-1, EHV-4 and EAV seroprevalence among horses in Kyrgyzstan.

CONCLUSION

Horses have a potential risk to other species. To our best knowledge, this is the first seroprevalence survey of horses infected by EHV-1, EHV-4 and EAV in Kyrgyzstan. These preliminary observations should be followed by a further large-scale survey to establish the extent of EHV-1, EHV-4 and EAV infections in Kyrgyzstan. Also, effective control and preventive measures must be taken to prevent the exposure and spread of these infections.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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