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Serological Detection of Equine Herpes Virus (EHV) Type 1 and 4 in Sudan

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Authors' contributions

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

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ABSTRACT

Aims: This study was designed to investigate the presence of EHV 1 & 4 in blood serum of horses and donkeys in different localities in Sudan.
Study Design: A total of 208 blood serum samples were collected from horse and donkeys from different localities in Sudan including; Khartoum, Atbara, Wadmadani and Nyala. With the exception of some horses (cross or foreign breeds), all animals examined were of Indigenous breed's, different ages, and apparently healthy; clinical signs to equine herpes or any disease were not detected.
Place and Duration of Study: The study was undertaken in the Virology lab, Veterinary Research Institute, Ministry of Animal Resources, Fisheries and Range Lands, Khartoum during December

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2012 to February 2013.
Methodology: Blood serum samples obtained from horse and donkeys were investigated in different localities in Sudan using an indirect ELISA (SVANOVIR® EHV1/EHV4-Ab).
Results: EHV 1 was detected in 1.6% of the horse samples from Khartoum, Nyala and Wadmadani, and in 7.3% of the donkeys' sample from Wadmadani and Nyala; while EHV 4 was detected (in all localities) in 58.7%, and 58.5% of the samples collected from horses and donkeys respectively. Mixed infection with both types (1 and 4) was recorded in horse samples from Khartoum and Nyala and in donkeys sample from Wadmadani and Nyala. Statistically, there is an association between infection and location (P –value < 0.001)
Conclusion: Equine herpes virus 1 and 4 was detected for the first time in horses and donkeys in Sudan. High seroprevalence of EHV type 4 (58.7%, in horse and 58.5% in donkey's serum samples) was recorded, compared to 1.6% and 7.3% of type 1 for horses and donkeys respectively. Mixed infection with both types 1 and 4 was recorded in horse samples from Khartoum and Nyala and in donkey's sample from Wadmadani and Nyala. Further studies for virus detection such as PCR should be conducted to enables rapid identification of specific virus causing disease EHV I or EHV 4.

Keywords: EHV 1, 4, antibodies; indirect ELISA; horses; donkeys; Sudan.

1. INTRODUCTION

Equine herpes virus (EHV) is a common virus that occurs in horse populations worldwide [1]. The two most common types that result in serious clinical disease in the horse are EHV-1, which causes respiratory disease in young horses, abortion in pregnant mares and paralysis neurologic disease) in horses of all ages and types, and EHV-4, which usually only causes low-grade respiratory disease but can occasionally cause abortion [2-4]. EHVs are in the family *Alphaherpesviridae* and are enveloped double stranded DNA viruses. There are 5 alpha herpesviruses that infect horses (EHV-1, 2, 3, 4, and 5). The two types, EHV-1 and EHV-4 are closely related alphaherpesviruses of horses with nucleotide sequence identity within individual homologous genes, ranging from 55% to 84%, and amino acid sequence identity from 55% to 96% [5,6]. The two herpes viruses are enzootic in all countries in which large populations of horses are maintained as part of the cultural tradition or agricultural economy. There is no recorded evidence that the two herpesviruses of rhinopneumonitis pose any health risks to humans working with the agents. Infection with EHV-1 is listed by the World organization for animal health (OIE) [2].

Viral transmission occurs by direct or indirect contact with infectious nasal secretions, aborted fetuses, placentas, or placental fluids [3]. Aborted tissues and placental fluids from infected mares can contain extremely high levels of live virus and represents a major source of infection. Latent infections and latently infected horses represent an infection risk for other horses. Signs of infection become apparent 2–8 days after exposure to virus; but, subclinical infections with EHV-1/4 are common, even in young animals. The severity of respiratory disease varies with the age of the horse and the level of immunity [3], and is most common in weaned foals and yearlings, often in autumn and winter. Abortion usually occurs in late pregnancy (from eight months onwards); but, could happen as early as four months reflecting either recent infection or recrudescence (re-activation) of latent infection in a carrier horse. However, older horses can succumb and are more likely than younger ones to transmit the virus 1 and 4 without showing clinical signs of infection. It is the continual cycling of EHV respiratory disease in young horses and the periodic reactivation of latent EHV in older horses that maintains the risk of EHV abortion in pregnant mares and EHV neurological disease in horses of all types and ages (http://codes.hblb.org.uk/index.php/page/32).[7]. Since there is no previous studies concerning EHV 1 and 4 (rhinopneumonitis) in horses in Sudan, the objective of this study was designed to investigate the presence of EHV 1&4 in blood serum of horses and donkeys in different localities in Sudan using ELISA.

2. MATERIALS AND METHODS

Blood samples were collected from the jugular vein of 208 horses (H) and donkeys (D) from different localities in Sudan (from December 2012 to February 2013) including; Khartoum (H= 52, D=22), Atbara (H= 20, D= 20), Wadmadani

(H= 12, D= 20), and Nyala (H=42, D= 20). With the exception of some horses (cross or foreign breeds), all animals examined were of Indigenous breed's, different ages, and apparently healthy; clinical signs to equine herpes or any disease were not detected. Following collection and blood clot, Sera were separated, centrifuged at 2500 rpm for 10 min. The clarified sera were stored at -20℃ until they were tested for anti- equine herpes antibodies 1&4 using ELISA.

2.1 ELISA Test

SVANOVIR® EHV1/EHV4-Ab (Boehringer Ingelheim Svanova, Box 1545, SE-751 45 Uppsala, Sweden), an indirect ELISA based on type specific recombinant glycoprotein G fusion protein which discriminating antibodies to equine herpes virus type 1 and 4 was used. The test was performed according to the manufacturer's instruction.

2.2 Statistical Analysis

Data was inserted into Microsoft Office Excel (2010) spread sheet program (Microsoft Corporation) to create a database and transferred to Statistical Packages for Social Science (SPSS) version 16, Software. The statistical significance between infection and locality, and infection and species was determined using frequency, mean, and the chi-square analysis.

3. RESULTS

Equine herpes virus (EHV) antibodies to type 1 and 4 were detected in 65.9% (83/126), and 69.5% (57/82) of the total horse and donkey samples examined respectively. Mixed infection with both types (1 and 4) was recorded in horse samples from Khartoum and Nyala and in donkeys sample from Wadmadani and Nyala. Regarding species, EHV 1 was detected in 1.6% of the horse samples and in 7.3% of the donkeys sample; while EHV 4 was detected in 58.7% and 58.5% of the samples collected from horses and donkeys respectively. Statistically, there is an association between infection and location (P –value < 0.001), but not between infection and species (P –value < 0.184). Table 1 shows the distribution of anti- EHV type 1, 4 antibodies in different localities.

4. DISCUSSION

Equine herpesvirus-1 (EHV-1) infection is ubiquitous in most horse populations throughout the world, and causes disease in horses and extensive economic losses through frequent outbreaks of respiratory disease, abortion, neonatal foal death, and myeloencephalopath, and EHV-4 which usually only causes low-grade respiratory disease but can occasionally cause abortion [2,8,9]. Infections caused by EHV-1 are particularly common in young performance horses, and typically result in establishment of latent infection within the 1st weeks or months of life [10] with subsequent viral reactivation causing clinical disease and viral shedding during periods of stress. For this, rapid diagnostic methods are therefore useful for managing the disease [2]. Equine herpesvirus (rhinopneumonitis) is difficult to clinically differentiate from equine influenza, equine viral arteritis, African horse sickness or other equine respiratory infections solely on the basis of clinical signs [4,11]. Definitive diagnosis is determined by PCR or virus isolation from samples obtained via nasopharyngeal swab and citrated blood sample (buffy coat) early in the course of the infection. Serum antibody levels to EHV-1/4 may be determined by virus neutralisation (VN), complement fixation (CF) tests or ELISA [12,13]. The CF and VN tests detect antibodies that are cross-reactive between EHV-1 and EHV-4 [2]. In the present study, an indirect ELISA based on type specific recombinant glycoprotein G fusion protein which discriminating antibody to equine herpes virus type 1 and 4 was used. Equine herpes virus antibodies to type 1 and 4 (EHV 1, 4) were detected in 65.9% and 69.5% of the total horse and donkeys serum samples examined respectively. When comparing the two types, antibodies to EHV-1 alone was recorded in 1.6% of the horse samples, and in 7.3% of the donkey's sample; while EHV- 4 was detected in 58.7% and 58.5% of the serum samples of horses and donkeys respectively. Mixed infection with both types (1 and 4) was recorded in 5.6% of horse's sample (from Khartoum and Nyala), and 3.7% of the donkey's samples (from Wadmadani and Nyala). High seroprevalence to type 4 was recorded in samples collected from horses in Nyala (85.7%) and donkeys in Khartoum (90.9%); compared to 16.7% and 35% for type 1 samples collected from horses and donkeys in Wadmadani respectively. In this study, the disease passes in a silent cycle, no respiratory signs or aborted cases were

Table 1. Anti- equine herpes virus type 1 and 4 antibodies in horses and donkeys sera from different localities in Sudan as measured by ELISA

Animal spp. location	Horses					Donkeys				
	No. of samples	EHV1 +ve(%)	EHV4 +ve(%)	Mix. EHV1,4 +ve(%)	No. of neg. (%)	No. of samples	EHV1 +ve(%)	EHV4 +ve(%)	Mix. EHV1,4 +ve(%)	No. of neg .(%)
Khartoum	52	0 (0.0%)	32(61.5%)	4(7.7%)	16(30.8%)	22	0(0.0%)	20(90.9%)	0(0.0%)	2(9.1%)
Atbara	20	0(0.0%)	2(10%)	0(0.0%)	18(90%)	20	0(0.0%)	6(30%)	0(0.0%)	14(70%)
Wad Madani	12	2(16.7%)	4(33.3%)	0(0.0%)	6(50%)	20	6(30%)	7(35%)	2(10%)	5(25%)
Nyala	42	0(0.0%)	36(85.8%)	3(7.1%)	3 (7.1%)	20	0(0.0%)	15(75%)	1(5%)	4(20%)
Total	126	2(1.6%)	74(58.7%)	7(5.6%)	43(34.1%)	82	6(7.3%)	48(58.5%)	3(3.7%)	**25(30.5%)**

recorded. In epidemiological studies in Australia, both EHV-1 and EHV-4 were found to be circulating within equine populations in a silent cycle of infection, although EHV-1 and EHV-4 have been detected in South Africa there have no previous large scale epidemiological studies of these viruses in South African horses [14]. Virus isolation and serological field studies have demonstrated that EHV1 and EHV4 infections show wide dissemination in many countries such as Canada [15], New Zealand [16], the USA [17], Japan [18] and China [19]. The ELISA test is useful in disease surveillance, confirmation of clinical cases, monitoring of population or individual animal freedom from an infection prior to movement and monitoring of immune status in individual animals or populations post vaccination [2]. For managing the disease, rapid and sensitive diagnostic methods such as PCR assays and virus isolation, particularly for the detection of viraemia are therefore useful. The real-time PCR assays, could allow for more sensitive detection, greater specificity, simultaneous testing for EHV-1 and EHV-4 and quantification of viral load, [2,20-23].

5. CONCLUSIONS

Equine herpes virus 1 and 4 was detected for the first time in horses and donkeys in Sudan. Further studies for virus detection such as PCR should be conducted to enables rapid identification of specific virus causing disease EHV I or EHV 4.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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