

Original research paper

Sero prevalence of Equine Infectious Anemia (EIA) Virus in selected regions in Sudan

Keywords: Equine_ Infectious anaemia _Sero-prevalence_ Horse _ Donkeys_ ELISA _ Sudan

Abstract

Equine infectious anaemia (EIA) is an acute to chronic lentivirus disease affecting members of equidae. In this study, the prevalence of EIA virus antibodies was investigated in 358 sera samples collected randomly from apparently healthy horses and donkeys during 2008-2013. Seven regions in Sudan were investigated, including Khartoum, Nyala, Atbra, Elfashir, Halfa, Madani and Kurdofan.

Results revealed that, the prevalence of EIA virus antibodies in the total samples examined using Indirect ELISA, was 5.58% ($20/358 \times 100$); of which 3.17% ($6/189 \times 100$) horse samples and 8.28% ($14/169 \times 100$) donkey samples. Across the regions sampled; the highest prevalence (11.1%) was detected in donkeys from Halfa. However, all of the sampled donkeys and horses from Atbara were sero-negative to EIA virus

Conclusion: Equine Infectious Anemia Virus was detected in horses and donkeys in selected regions in Sudan.

High sero-prevalence of the disease in horse from Elfashir and donkeys from Nyala was recorded. The highest prevalence in regions examined was detected in Halfa . Statistically, the chi-square analysis showed that; there is an association between infection and location ($P = 0.001$).

Further studies for virus and antibody detection such as PCR and ELISA should be conducted to enable rapid identification

of Equine Infectious Anemia Virus and to monitor the prevalence of the disease in other parts of the country.

1 Introduction

The causative agent of equine infectious anemia (EIA) virus is *alentivirus* in the family *Retroviridae* of subfamily *Orthoretrovirinae*^[40]. The disease is prevalent in all parts of the world and it is of importance for the equine industry^[25]. Infection with the virus is characterized by recurrent febrile episodes with high virus load and anemia. Recovered animals may suffer from recurring clinical (chronic) disease caused by new mutant EIA virus strains, which is characterized by irregular and less frequent viremia, associated with weight loss, dependent edema, progressive non regenerative anemia^{[22]; [23], [39]}. and are often termed as a “swamper”^[24]. The disease then, progresses to an asymptomatic, but, still infectious stage which remains for the rest of the animal's life^[2], and antibody-positive animals older than 6–8 months are identified as virus carriers^[1].

The disease could be transmitted by transferring of virus-infective blood by blood-feeding insects between horses in close proximity. The virus is found free in the plasma or cell associated, principally in monocytes and macrophages of infected animals^[3]. Transmission of EIA by biting flies is purely mechanical, additionally; EIA virus could be readily transmitted iatrogenically through the use of blood-contaminated syringes, needles, or surgical equipment, or by transfusion of infective blood or blood products^[3].

For diagnosis, agar gel immunodiffusion (AGID) tests^[19] and enzyme-linked immunosorbent assays (ELISAs)^[28]^[36] are accurate, reliable tests for the detection of EIA in horses, except for animals in the early stages of infection and foals of infected dams^[35]. AGID test^[4] and ELISA which uses purified viral particles as antigen^[5]^[6] or recombinant EIA virus proteins^{[7],[8],[9]}, are used as screening test and in surveillance studies for EIA virus in recent years^[37; 34].

Although EIA virus has a worldwide distribution^{{1}, {12}}; however, there is no enough information about the disease situation in Sudan. The only study conducted in Khartoum state of Sudan^[14] cannot be considered as a true reflection of the disease in Sudan. It is important to know the situation in other regions of the country; therefore, the aim of this study is to investigate the presence of antibodies against EIA virus in horses and donkeys in selected regions in Sudan.

2 Materials and methods

Samples:

A total of 358 blood samples were randomly collected (apparently healthy or diseased animals) from horses and donkeys from seven regions in Sudan during 2008-2013; including, Khartoum, Nyala, Atbra, Elfashir, Halfa, Madani and

Kurdufan (Table 1). Following collection and blood clot, Sera were separated, clarified by centrifugation and stored at -20°C until they were tested for anti- EIA virus antibodies using ELISA.

Table (1): Region distribution of sampled horses and donkeys in Sudan between 2008 and 2013.

Region	No of horses	No of Donkeys	Total (equidae) samples
Khartoum	89	-	89
Nyala	63	39	102
Atbra	24	30	54
Elfashir	40	-	40
Halfa	-	27	27
Madani	-	18	18
Kurdufan	-	28	28
Total	189	169	368

ELISA test:

EIA virus ID Screen kit (ID Vet Company, France), an indirect ELISA for the detection of p26 (GAG gene) antibodies directed against the EIA virus in horse serum was used. The test was performed according to the manufacturer's instruction.

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 was determined using frequency, mean, cross tabulation and the chi-square analysis.

3 Results

The prevalence of EIA virus antibodies in the total sera samples examined using an indirect ELISA was 5.58% ($20/358 \times 100$); of which 3.17% ($6/189 \times 100$) horse samples and 8.28% ($14/169 \times 100$) donkey samples (Tables 2&3). Across the regions sampled; the highest prevalence (11.1%) was detected in donkeys sampled from Halfa. However, all of the sampled donkeys and horses from Atbara were sero-negative to EIA virus.

Statistically, the chi-square analysis showed that; there is an association between infection and location ($P = 0.001$).

Table (2): The prevalence of EIA virus in different regions in Sudan

Region	Total No. of samples	Number positive	%prevalence
Khartoum	89	2	2.2%
Nyala	102	9	8.8%
Atbra	54	0	0%
Elfashir	40	2	5%
Halfa	27	3	11.1%
Madani	18	1	5.6%
Kurdofan	28	3	10.7%

Table (3): The Prevalence of EIA virus in horses and donkeys

Region	Horses			Donkeys		
	+ve	-ve	%prevalence	+ve	-ve	%prevalence
Khartoum	1	61	1.6	1	26	3.7
Nyala	3	60	4.76	6	33	15.3
Atbra	0	24	0	0	30	0
Elfashir	2	38	5	0	0	0
Halfa	0	0	0	3	24	11.1
Madani	0	0	0	1	17	5.5
Kurdufan	0	0	0	3	25	10.7
Total	6	183	3.17	14	155	8.28
Total of samples (horses & donkeys) =358				+ve = 20	% prevalence =5.58	

4 Discussions

In the current study, the prevalence of antibodies against EIA virus was studied in horses and donkeys from different regions in Sudan; including, Khartoum, Nyala, Atbra, Elfashir, Halfa, Madani and Kurdufan.

Using an indirect ELISA, in total of 358 serum samples examined, the prevalence of EIA virus antibodies was 5.58% ($20/358 \times 100$) of which 3.17% ($6/189 \times 100$) horse samples and 8.28% ($14/169 \times 100$) donkeys sample. The highest prevalence of the disease in different regions was detected in Halfa. When comparing species, in different regions examined, horses from Elfasher and donkeys from Nyala showed the highest prevalence 5% & 15.3% respectively. This result is higher than that obtained by Ibrahim^[14] when examined antibodies to EIA virus in donkeys in Khartoum state and that of Getacheus *et al*^[30] who found only one seropositive donkey (0.2%) out 1002 sera sample tested in Central Ethiopia. Similar studies conducted in UAE^[31], Bulgaria^[32] showed the absence of this disease in donkey populations; and in donkey and horse in Turkey^[33]. For us, the result is accepted (15.3% for donkeys and 5% for horses), since donkeys population in Sudan is 7506000^[38] which is 10 times the horses population; distributed all over the country and are used in many different activities, for transport and farming; while horses are restricted in some regions, with large numbers and some activities (racing, transport, and raising horses for racing is an economically important in some regions in Sudan). In our study, antibodies to EIA virus were not detected in all samples collected from Atbara; although Atbara is not far away from Khartoum where the disease was previously detected with a prevalence of 8.7%^[14], which is a bit higher than we found (2.2% in horses and donkeys) in this study. For other regions in Sudan, no previous serological or virological studies were conducted concerning EIA. A wide range of laboratory tests are available that allow more rapid diagnosis of EIA. AGID and ELISA are used by many researchers^{[20]; [27]} for diagnosis of the disease; but they could not detect the disease in the early stage of infection and in foals of infected dams^{[26]; [28]}. ELISA test has been used by different laboratory and field studies^{[29]; [21]}, with high sensitivity and specificity.

Serological evidence of EIA virus infection has been reported in many countries around the world, Serbia 0.17 %^[15], Brazil 0.57 %^[16], Italy 17.5 %^[17]; but the disease was not reported in African countries till 2009^[18], where the disease was reported in Nigeria^[13] and in many neighboring countries such as; Ethiopia^[12] and Central African Republic; Chad, Egypt, Eritrea, Libya and South Sudan^[11].

5 CONCLUSIONS

Equine Infectious Anemia Virus was detected in horses and donkeys in Sudan. High sero-prevalence of the disease in horse from Elfashir and donkeys from Nyala was recorded. The highest prevalence in regions examined was detected in Halfa

.Statistically, there is an association between infection and location ($P = 0.001$).

Further studies for virus and antibody detection such as PCR and ELISA should be conducted to enable rapid identification of Equine Infectious Anemia Virus and to monitor the prevalence of the disease in other parts of the country.

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