

Original research paper**Sero prevalence of Equine Infectious Anemia (EIA) Virus 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 368 blood serum 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 Kurdofoan,. An Indirect ELISA (ID

Vet Company, France) was used for detection of P26 (GAG gene) antibodies directed against the EIA virus in horses and donkeys sera. Results revealed that, the prevalence of EIA virus antibodies in the total samples examined was 5.4% (20/368); of which 3.2% (6/189) horse samples and 8.3% (14/169) donkey's sample. The highest prevalence 11.1% was detected in donkey's samples collected from Halfa. Antibodies were not detected in all samples collected from Atbara. Statistically, there is an association between infection and location ($P = 0.001$). Further studies should be conducted to monitor the prevalence of the disease in other parts of the country.

1 Introduction

Equine infectious anemia (EIA) is a persistent viral infection of equids. The causative agent of equine infectious anemia (EIA) virus is *Alentivirus* in the family *Retroviridae* of subfamily *Orthoretrovirinae*. Infected horses remain viremic carriers for life and yield a positive serological test result. Antibody response usually persists and antibody-positive animals older than 6–8 months are identified as virus carriers^[1]. Infection with EIA virus is characterized by recurrent febrile episodes with high virus load and anemia. After resolution of the primary viremia, most animals developed chronic EIA, which is characterized by irregular and less frequent viremia, associated with weight loss and anemia. The disease then, progresses to an asymptomatic, but, still infectious stage which remains for the rest of the animal's life^[2].

The most important mode of transmission of the disease is 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. 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]. Prior to the development of a reliable ager gel

immunodiffusion (AGID) test also known as Coggins test, sero-diagnosis of EIA was a scientific dilemma^[19]. Currently, 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 for EIA virus. Although the disease has a worldwide distribution^{{1}, {12}}, in Sudan, only one study was conducted in Khartoum state^[14]; there is no enough information about the disease situation in Sudan. The objective of this study was designed to investigate the presence of EIA virus in blood serum of horses and donkeys in different regions in Sudan using ELISA.

2 Materials and methods

Samples:

A total of 368 blood serum samples were randomly collected from apparently healthy 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): Blood serum samples collected from horses and donkeys from seven regions in Sudan during 2008-2013.

Region	Total of samples	Horses	Donkeys
Khartoum	89	89	-
Nyala	102	63	39
Atbra	54	24	30
Elfashir	40	40	-
Halfa	27	-	27
Madani	18	-	18
Kurdufan	28	-	28
Total	368	189	169

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, crosstabulation and the chi-square analysis.

3 Results

The prevalence of EIA virus antibodies in the total serum samples examined using an indirect ELISA was 5.4% (20/368); of which 3.2% (6/189) horse samples and 8.3% (14/169) donkey's sample. The highest prevalence 11.1% was detected in donkey's samples collected from Halfa. No antibodies to EIA virus were detected in all samples collected from Atbara (Tables 2&3; figure 1& 2). Statistically, there is an association between infection and location ($P = 0.001$).

Table (2): The prevalence of EIA virus antibodies in different regions in Sudan as measured by ELISA

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

Table (3): Prevalence of EIA virus antibodies in horses and donkeys as measured by ELISA

Species	Regions													
	Khartoum		Nyala		Atbra		Elfashir		Halfa		Madani		Kurdufan	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
horses	1	61	3	60	-	24	2	38	-	-	-	-	-	-
donkeys	1	26	6	33	-	30	-	-	3	24	1	17	3	25
Total					368				20				5.4%	

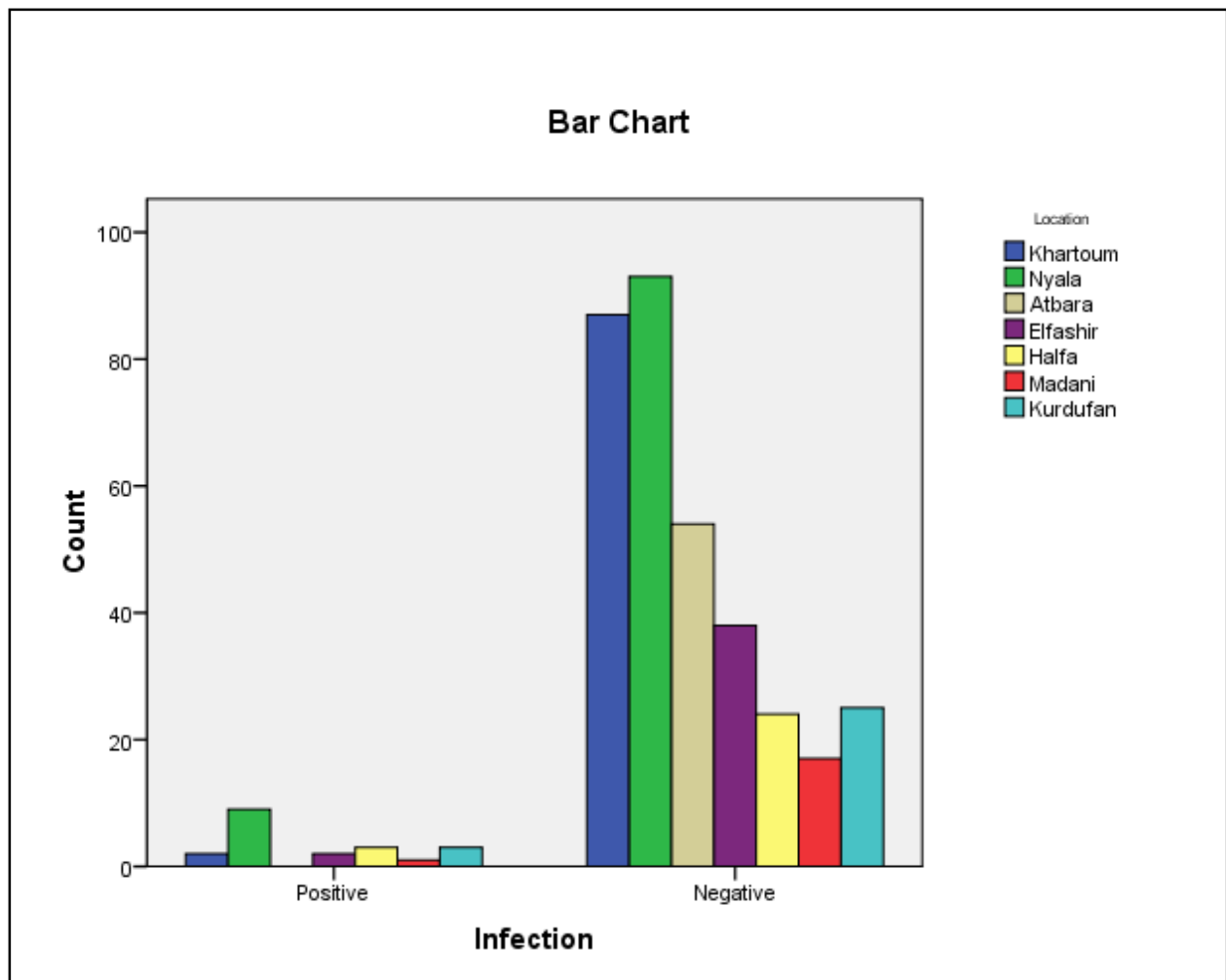


Figure 1: Sero prevalence of EIA virus in different regions in Sudan

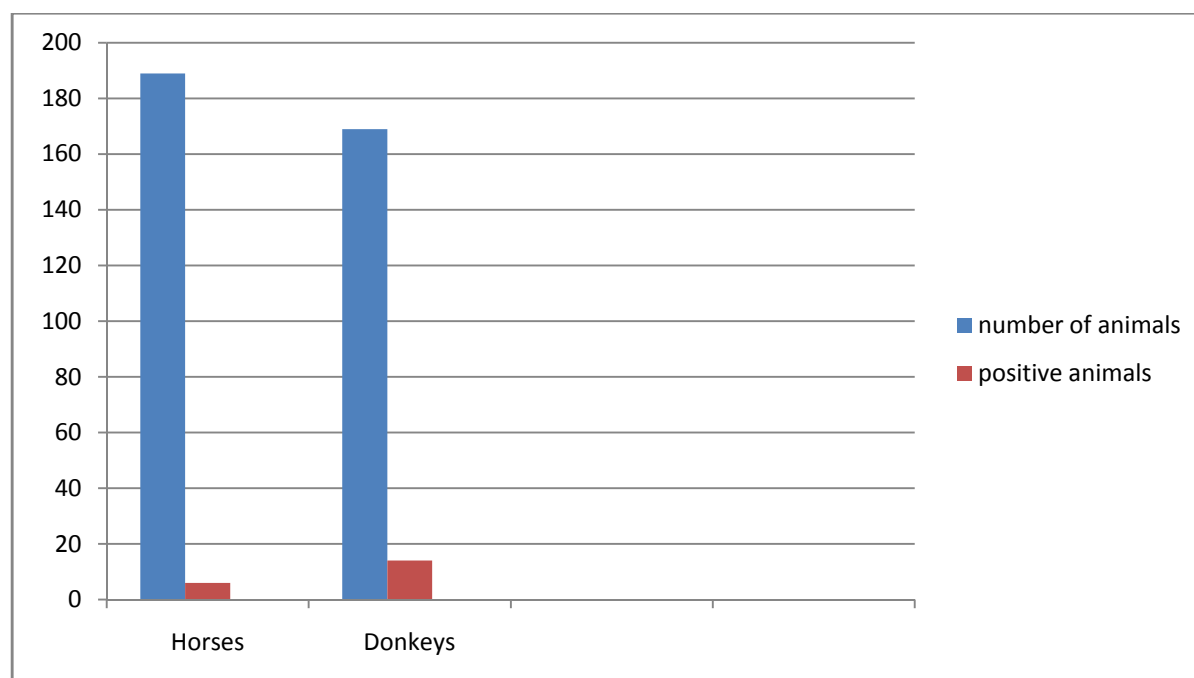


Figure 2: Prevalence of EIA antibodies in horses and donkeys as examined by ELISA test

4 Discussions

Equine infectious anemia (EIA) is a retroviral disease caused by EIA virus, which is a macrophage-tropic lentivirus; chronically and debilitating disease of all equidae, including horses, mules, and donkeys^{[10];[20]}. Clinically, EIA is a persistent infection with recurrent cycles of viremia and episodic fever, anemia, edema and loss of weight. Anemia, thrombocytopenia, hypergammaglobinemia and elevated liver enzymes are common hematological and biochemical findings in clinical disease^[21]. Affected equines often recover and remain carriers throughout their lives. Recovered animals may suffer from recurring clinical (chronic) disease caused by new mutant EIA virus strains with the signs of fever (105-108°F), petechial hemorrhages, depression, weight loss, dependent edema, progressive non regenerative anemia^{[22]; [23]}, and are often termed as a “swamper”^[24].

The disease is prevalent in all parts of the world and it is of importance for the equine industry^{[25]; [26]}. Several serological tests have been used for diagnosis of the infection, AGID and ELISA are the most sensitive tests and they have been used by many researchers^{[20]; [27]}. They are accurate and reliable tests, they could detect the disease in horses, except for animals in the early stages of infection and foals of infected dams^{[26], [28]}.

In the current study, the prevalence of antibodies against EIA was studied in horses and donkeys from different regions in Sudan using ELISA test. In total of 368 serum samples examined, the prevalence of EIA virus antibodies was 5.4% (20/368); of which 3.2% (6/189) horse samples and 8.3% (14/169) donkey's sample. The highest prevalence 11.1% was detected in donkey's samples collected from Halfa. This result is higher than that obtained by Ibrahim Gehan^[14] in donkeys in Khartoum state and Getachew *et al*^[30] who found only one seropositive donkey (0.2%) out of 1002 sera samples tested in Central Ethiopia; similar studies conducted in UAE^[31], Bulgaria^[32] and Turkey^[33] showed the absence of this disease in donkey populations. Antibodies to EIA virus were not detected in all samples collected from Atbara. No previous serological studies were conducted in different regions in Sudan except in Khartoum state with a prevalence of 8.7%^[14], which is a bit higher than we found (2.2%) in this study. The commercial ELISA (ID Vet Company, France) used in current study detects the antibodies against EIA virus p26 (GAG gene) in affected equine sera. 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 Ethiopia^[12] and in Nigeria^[13]. In conclusion, further studies for virus detection and characterization should be conducted in other parts of the country.

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