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Detection of IgG and/or IgM antibodies against equine infectious anaemia virus (EIAV) in Nigerian race and polo horses

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Abstract

Equine infectious anaemia (EIA) has aroused a lot of attention over the years. The disease is often fatal in horses and surviving horses remain lifelong carriers; that is why humane destruction of infected horses is highly recommmended. It is caused by the prototype lentivirus of the family retrovirus. A serological screening was carried out in polo and race horses from three selected state capitals in Nigeria. In all, 84 sera samples were collected from race horses from Ilorin in the North Central and Sokoto in the Northwest, and polo horses from Ibadan in the Southwest. They were analyzed for antibodies against the equine infectious anaemia virus (EIAV) by indirect ELISA. Of the 84 samples tested, 2 samples, 1 (1.2%) horse in Ilorin and 1 (1.2%) horse in Ibadan tested positive. It was observed that the positive horses were adult and they showed no fever and symptoms associated with EIA. The positive results were from male and female Arewa breed respectively. In conclusion, EIA is present in certain areas in Nigeria with prevalent of 2.4% among the Arewa breed horses from the population sampled.

Keywords: Antibody, EIA, ELISA, Horse, Nigeria

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Introduction

Equine infectious anaemia (EIA) is a chronic, infectious and potentially fatal viral disease of veterinary medical importance, affecting the immune system of members of the family equidae (Leroux *et al*., 2004). The disease is caused by Equine infectious anaemia virus (EIAV), an RNA virus of the genus *Lentivirus*, subfamily *Orthoretrovirinae* in the family *Retroviridae* (Olsen, 1998). The virus is the simplest and smallest of characterized human and animal lentiviruses (Craigo & Montelaro, 2008). It is closely related to the human immunodeficiency virus (HIV) which causes acquired immunodeficiency syndrome (AIDS). In fact, both viruses share many structural and biochemical features, and EIAV is thought to serve as a useful model for many aspects of HIV research, especially for the discovery of common mechanism of immunological control (Montelaro & Issel, 1990). The viral RNA contains about 8200 bases and three major genes (*gag, pol,* and *env*) encoding viral structural proteins and three minor genes [*tat* (*S1*), *S2,* and *rev* (*S3*)] that encode nonstructural proteins which regulate various aspects of virus replication (Craigo & Montelaro 2008). EIAV also contains two major surface glycoproteins, gp 90 and gp 45; and four major non-glycosylated internal proteins designated as p26, p15, p11 and p9 (Henderson *et al.,* 1987). The major EIAV core antigen is the glycoprotein-specific antigen p26. This protein constitutes about 40% of the total virion protein mass and it is the predominant viral protein employed in commercial diagnostic tests for the detection of EIAV antibodies in animals (Coggins and Norcosis, 1970). Transmission of EIAV is through the bites of haematophagous arthropod vectors in the genera *Tabanus, Stomoxys, Chrysops* and *Hybomitra* (Foil *et al*., 1983) and it is solely mechanical as EIAV does not multiply within the arthropod (Issel & Foil, 1984). It can also be

transmitted by the use of non-sterile needles and blood contaminated surgical instruments (Willians *et al*., 1981). EIAV has been diagnosed in many areas of the world and is considered a worldwide disease of horses (McGuire & Crawford, 1979). Although localized outbreaks of disease can occur, the incidence of EIAV-infected horses is higher in tropical and subtropical climates, presumably due to the longer warm seasons and more abundant populations of insect vectors that may transmit EIA among horses (Craigo & Montelaro, 2008). The EIA has a wide variety of pathological manifestations (McGuire *et al*., 1990) and the rate of infection among equidae varies from 30% to 70%. There are three forms of the disease: acute; chronic; and inapparent (Clabough, 1990). The acute form is the most damaging and the most difficult to diagnose because the signs appear rapidly, and often times, an elevated body temperature is noted (APHIS, 2008). The onset of the disease is so rapid that horses are seronegative because the immune system has had insufficient time to respond to the viral antigens. The chronic form of the disease occurs when the horse develops a recurring clinical syndrome following survival of the acute stage, and is characterized by loss of weight, anaemia, diarrhoea, and dependent oedema (Sellon *et al., 1994*). Moreover, horses that survived the early clinical episodes of the disease are generally able to control virus replication and remain clinically normal (Sellon *et al., 1994)* as inapparent carriers of EIAV (Jae-Hyung *et al*., 2006) for life (Hammond *et al*., 1997) and serve as a source of infection for susceptible animals.

The diagnosis of EIA relies entirely on serological evidence and is commonly done by the agar gel immunodiffusion (AGID) or Coggins test (Coggins *et al*., 1972), which is the mandatory EIAV test in most countries. However, enzyme-linked immunosorbent assays (ELISAs) have also been developed. The AGID [Coggins *et al*., (1972)] and ELISA tests (Suzuki *et al.,* 1982) are accurate and reliable for the detection of EIA in horses, except for animals in the acute stage of infection and foals of infected dams. Since clinical diagnosis is difficult for acute and inapparent infection, the movement of horses across borders poses the risk of causing economic embarrassments and interference with equine sporting events due to EIA. Economic losses could be high as there is currently no treatment or vaccination for this disease. EIA is a reportable disease, although no report of EIA has been made in Nigeria. Nigeria equestrian industries are fast growing and horses now travel for both local and international competitions particularly horse racing, polo tournaments and durbar. It is desirable to know the status of EIA in the country. This study was therefore carried out to screen race and polo horses in three randomly selected state capitals in Nigeria for EIA.

Materials and methods

Sample collection

Eighty-four (84) serum samples used for this study were obtained from race horses from Ilorin and Sokoto, and polo horses from Ibadan. Information about the signalments of each horse was also collected, particularly the age, sex and breed. About 10 ml of blood was collected by jugular venipuncture from each horse into sterile plain vacuteiner tubes and allowed to clot. The sera were then harvested and stored in appropriately labeled eppendorf tubes at -20^{0}C until use. The rectal temperature at the time of sampling was also recorded.

Enzyme linked Immunosorbent Assay (ELISA)

ELISA was performed according to the kit manufacturer's instruction (ID Vet innovative diagnostics, Garosud, Montpellier, France), based on standard indirect ELISA protocol. The ID Vet ELISA kit contained p26 (Gag) recombinant antigen and it detect IgM and IgG antibodies. The ELISA result was obtained by measuring and comparing the absorbance readings, that is the optical density (OD) of the samples against the standards or control, with microplate reader at 450nm.

Validation and Interpretation of Test Result

The test is validated, if: the mean OD value of positive control (OD_{PC}) is greater than 0.350, and the ratio of the mean values of the positive and negative control (OD_{PC} and OD_{NC}) is greater than 3.

Interpretation was done by calculating sample/positive (S/P) percentages of each sample using the formula, $S/P = \{(OD_{sample} - OD_{NC}) / (OD_{PC} - OD_{NC})\} \times 100$, provided by the manufacturer. The antibody of the samples examined in the ELISA was therefore expressed as percentage of S/P ratios of positivity of strong positive standard. Then samples with S/P percentages less than or equal to 50% were considered negative, doubtful if it is less than 60% but greater than 50%, and positive if greater than or equal to 60%.

Results

The mean OD_{PC} was 2.085 and the ratio of the mean OD_{PC} to OD_{NC} was 9.5. S/P percentages for the samples were presented in Tables I. Of the 84 samples tested for EIAV, 2 (1 each from Ilorin and Ibadan) were positive with prevalence of 2.4% of the total population sampled. The respective rectal temperatures of the positive horses from Ilorin and Ibadan were 37.1^{0}C and 38.1^{0}C.

All the 22 (26.2%) sampled horses from Ilorin were male, 11 (13.0%) male and 20 (23.8%) female were from Ibadan and 29 (34.5%) male, and 2 (2.4%) female were from Sokoto. Therefore, of the 84 sampled horses 62 (73.8%) were male and 22(26.2%) were female. The positive horse from Ilorin was a male while that from Ibadan was a female.

The age of the horses was categorized into three: yearlings (1 – 3years), young adult (> 3 – 7years) and adult (>7 – 20 years). All the horses from Ilorin were adults while 5 foals, 10 young adults and 16 adults were from Ibadan, and 5 foals, 24 young adults and 2 adults were from Sokoto. In all, 10(11.9%) foals, 34(40.5%) young adults and 40(47.6%) adult horses were sampled. The age of the two positive horses were 13years (Ilorin) and 11years (Ibadan) respectively.

The breeds of the horses were Arewa (Hausa), Sudanese, Argentine and Talon. The 22 horses from Ilorin were all Arewa while those from Ibadan were 1 Argentine and 30 Arewa, and from Sokoto there were 28 Arewa, 2 Sudanese and 1 talon. Of all the horse's sampled, 80 (95.2%) were Arewa breed, 2 (2.4%) were Sudanese breed, 1 (1.2%) was Argentine breed and 1 (1.2%) are Talon breed. The two positive horses were Arewa breed.

Table 1: Percentage positive by ELISA from sampled horses in 3 state capitals

Sera sample No	Ilorin	Sokoto	Ibadan
1	1.85	2.06	1.41
2	158.30*	1.47	8.76
3	3.99	0.40	4.63
4	-3.24	-6.16	0.19
5	1.63	0.19	-0.83
6	12.03	5.55	1.05
7	14.39	1.63	2.92
8	5.22	2.06	-0.46
9	4.58	1.15	2.12
10	3.83	4.47	2.54
11	4.21	1.79	-0.72
12	0.65	0.19	1.42
13	-1.84	1.47	-6.16
14	5.17	3.35	0.83
15	1.96	0.34	2.49
16	0.76	0.13	-0.03
17	4.37	6.88	-0.51
18	-0.40	0.03	0.62
19	2.01	3.56	5.65
20	2.33	1.79	2.75
21	3.29	1.90	1.63
22	3.29	3.78	0.04
23		-1.69	-1.31
24		-3.03	1.21
25		-2.81	4.21
26		0.56	1.85
27		-1.42	2.12
28		3.29	173.90*
29		3.56	-1.96
30		-0.40	3.40
31		2.38	6.62

S/P% * indicate the positive sample

Discussion

The results of this study showed that horses can be seropositive for EIAV without manifestation of overt clinical signs such as anaemia, petechial haemorrhage on the mucous membrane, dependent oedema and weight loss, which are associated with chronic form of EIA infection (Sellon *et al.,* 1994; Hammond *et al.,* 1997). Fever with increased rectal temperature ($\geq$38.5^{0}C) was not seen in the positive horses. This is not surprising because fever with rectal temperature greater than 38.5^{0}C is not a constant finding in the chronic and asymptomatic stage of the disease and febrile episode is associated with high level of viraemia (APHIS, 2008). Craigo & Montelaro (2008) had also reported that horses with chronic EIA can be seropositive with variable viraemic levels which are highest during the periodic febrile episodes. It is probable that these two horses whose sera were positive for EIAV antibodies must have been infected, recovered and become inapparently carriers. Craigo & Montelaro (2008) reported that the highest percentages of EIAV-infected horses in the field are in fact inapparent carriers, and they maintain high levels of EIAV-specific antibodies but viraemia is usually undetectable.

Geographical location of these areas (Ilorin and Ibadan) with tropical savanna climate may favour the growth of hematophagous insect populations (Craigo & Montelaro, 2008) and so it is likely that insect vector burdens will be higher in these areas than those with Sahel climate like Sokoto. Moreso the distance between horses and the long time of

exposure to insect bites are also factors that contribute to the rate of insect transmission (Barros & Foil 2007). However, the role of insect vectors was not determined in this study.

Sex susceptibility to EIA has been controversial among researchers and this had been attributed to the purpose of keeping the horses. Silva *et al*. (1999) and de Bessa (2010) reported that males presented greater risk of infection than females. This present study does not reflect such differences as the ratio of male to female is one between the two positive horses. This is in accordance with the report of Borges *et al.* (2013), that observed that EIAV sero-prevalence was not statistically different between the sexes.

In this study, the seropositivity of horses to EIAV were in adult (> 7years). This is in consistent with the report of de Bessa (2010) which indicates that seropositivity may be higher in adult than the foals and this depend on the serological screening methods used. This may be due to fact that most infected foals most of the time do not survive the acute phase of the disease. Silva *et al*. (1999) and Borges *et al*. (2013) also reported that the highest levels of sero-positivity were in older animals (>8 years) consistent with longer exposure to potential transmission factors. The two positive samples accounting for 2.4% of total number of horses sampled better reflected the age distribution of EIA rather than the sex distribution in this study. The positive horses were also Arewa breed reflecting the preponderance of this breed among the horses kept in the areas sampled, which corresponds to 95.2% of the total sampled horses.

In conclusion, this study is the first to report the detect EIAV antibodies in Nigeria to the best of our knowledge. Since there is no vaccination of horses for EIA in Nigeria, possibly because there is no vaccine for EIA, presence of antibodies in horses could only result from exposure to the field virus. The positive horses for EIAV antibodies in the two state capitals suggested that possibly EIAV is present in those areas. This study therefore serves as baseline information on the status of EIA in race and polo horses in the selected area in Nigeria. The study also provides initial information for further investigations. Moreso, screening for EIAV has to be encouraged particularly as criteria for participation in any equine sport or games as this will help control the transmission of the virus among horses.

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