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Full Length Research Paper

Seroprevalence of rinderpest in non-vaccinated cattle in Unity State, Southern Sudan

A. S. Ali[1]*, S. A. Mamdouh[1] and A. M. Elhussein[2]

[1]Department of Preventive Medicine and Veterinary Public Health, Faculty of Veterinary Medicine, University of Khartoum, Khartoum North, P.O. Box 32, Sudan.
[2]General Administration for Veterinary Research and Laboratories, National Veterinary Laboratory, Soba, Khartoum, P.O. Box 8067, Sudan.

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In the frame work of the Pan African Control of Epizootics (PACE) Program, a cross-sectional serological survey for rinderpest (RP) was conducted among non-vaccinated cattle in Unity State, Sudan. A total of 280 serum samples were collected from cattle of different ages and both sexes in eight sites between June and December 2004. These sera were tested for antibodies against rinderpest virus (RPV) using a competitive enzyme-linked immunosorbent assay (c-ELISA). Only 15.4**% of the sera were positive to the RPV antibodies in the different locations of the state. An obvious and significant ($p<0.05$) variation in the prevalence of antibodies to the virus among various locations was noted. A significantly ($p<0.01$) higher levels of prevalence rates were observed in the age group of 5 to 10 years of cattle compared to other age groups. The results also indicated the prevalence of antibodies to the virus in females is always higher than in the male animals ($p<0.05$). Our data demonstrated the situation of RP in the unity state, the only locus known to harbor the disease in the country at large. This helped by designing a strategic eradication plan leading to declare Sudan as a RP free country in 2005.**

Key words: Rinderpest, c-ELISA, eradication, Unity state, Sudan.

INTRODUCTION

Rinderpest (RP) is one of the oldest recorded plagues of livestock in many parts of the world. It is an acute febrile and contagious viral disease with high mortality affecting domestic and wild bovids. Sheep and goats (Elhaj and Taylor, 1988; Anderson et al., 1990a; Bidjeh et al*.,* 1997), camels (Dhillon et al., 1959) and domestic pigs (Murphy et al*.,* 1999) can also be infected. Game animals are also susceptible to the RP virus (Plowright, 1982).

The most comprehensive descriptions of the clinical pictures of RP were those published by Taylor (1983). The worldwide spread of RP was previously described by Scott (1981). In Africa, the occurrence of the disease outbreaks, in many countries including Sudan, was reported by Vittoz (1965) and the introduction of the cau-

*Corresponding author. E-mail: abumalaz2002@gmail.com. Phone: +249-912924916. Fax: +249-185-312638

sative virus was attributed to cattle imported from Asia for more than one century (Mack, 1970). Many efforts were made to control and completely eradicate the disease in Africa of which the International Rinderpest Joint Project (JP15) that extended from 1962 to 1976 was the largest. The Pan African Rinderpest Campaign (PARC) was also a project launched in 1987 through which a strategic vaccination and disease surveillance programs were employed leading to declare most of the African countries as provisionally free from RP. Another extended project, funded by the European Commission (EC), started 2001 and termed the Pan African Program for Control of Epizootics (PACE) was also employed.

In Sudan, RP was considered as one of the dreadful diseases that caused losses in livestock during the last century. The last officially reported outbreak was that of 1991 in Lagawa town, southern Kordofan State (Rossiter, 1995). Through the three previously mentioned projects (JP15, PARC and PACE), millions of cattle were vaccina-

Figure 1A. Unity State (■), southern Sudan occupies a central location in southern Sudan covering an area of about 170.520 square kilometers. It is located in the western upper Nile area of southern Sudan between latitude 7°- 10.3° and longitude 29° - 31°.

Figure 1B. The eight localities sampled for RP detection in Unity State, southern Sudan: 1 = Abyemnem; 2 = Bentiu; 3 = Nialdiue; 4 = Rubkona; 5 = Thar; 6 = Thoan; 7 = Guity; 8 = Kilo 50.

ted against the disease in Sudan and consequently no outbreaks were recorded for the disease, at least, in the northern regions of the country. However, some loci in the southern Sudan were believed to harbor the virus and determined as surveillance zones. This study was designed and conducted in the framework of PACE project to investigate the situation of RP in Unity State, Southern Sudan, the area that classified by the OIE as a surveillance zone for RP in an attempt to completely eradicate the disease from the country.

Direct ELISA (Anderson et al., 1983) had always been and still widely used for diagnostic purposes to PR. However, owing to cross-reactions between RPV and peste des petitis ruminants virus (PPRV), competitive enzyme-linked immunosorbent assay (c-ELISA) had been developed and used for that purpose (Libeau et al., 1992; Anderson and Mckay, 1994).

MATERIALS AND METHODS

Study area

The area of this study is the Unity State, southern Sudan (Figure 1A). This state occupies a central location in southern Sudan covering an area of about 170.520 square kilometers. It has an estimated animal population of 1.560.000 which ranks it as one of the most populous state in southern Sudan. Administratively, it is divided into four provinces: RubKona (which harbors Bentiu the state capital), Mayom, Leer and Pariang (Figure 1B). It is located in the western upper Nile area of southern Sudan between latitude 7°- 10.3° and longitude 29° - 31°. The majority of the human population in the state belongs to Neur tribe which is one of the main cattle rearing

tribes in southern Sudan. To the north of the state there are the nomadic pastoral Baggara and Fellata tribes (in south Kordofan) those move south into northern parts of the state for grazing in the dry season.

Cattle population estimates in the study area

According to the official records of the Veterinary Authorities in the country, the cattle population estimates as in 2000 amount to 10,281,382 for the south regions at large and 4,323,048 for the Unity state. The most cattle breed existing in the state is Baggara cattle (zebu cattle) which are characterized by low milk yield (2 liters/ day) with an average of 180- 200 liters in the season and milking period of 90- 100 days.

Blood sampling and processing

The blood sampling was carried out randomly where the study population was divided into exclusive groups known as “strata” by geographical locations such as different localities, and then the strata were divided into units referred to as “clusters” and the clusters were sampled. The areas sampled were Abyemnem, Bentiu, Nialdiue, Rubkona, Thar, Thoan, Guity and Kilo 50 (Figure 1B). A total of 280 samples were collected from non vaccinated cattle from these eight clusters. All the cattle sampled had a confirmed non vaccination history against RP.

Blood samples were taken aseptically from the jugular vein of each animal into plain vacutainer tubes. The blood was left to clot at room temperature for about ten hours and then centrifuged at 1500 rpm for 10 min. The serum was then transferred to sterile tubes labeled with animal number, sex, age and location of sampling. The sera were then packed in ice boxes and transferred to the Central Veterinary Laboratory (Soba, Khartoum) where kept at -20°C before being tested for antibodies against RPV.

Competitive ELISA for detection of antibodies to RPV

The sera were tested for rinderpest virus antibodies using a competitive ELISA (c-ELISA) essentially as described by Afshar et al. (1987) with some modifications. Fifty µl of RPV antigen diluted to pre-titrated concentration (1/100) in PBS was added to all wells of the microtitre ELISA plate (Nunc Maxisorp). The plate was tapped to spread the antigen and incubated for one hour at 37°C on an orbital shaker. The plate was then removed from the incubator and washed three times with PBST 1: 5 (washing buffer) and then blotted and dried. 40 µl of 2% Bovine Serum Albumin (BSA) as a blocking buffer was added to all wells of the plate. Ten µl of test serum was added to test wells (in vertical duplicates). Ten µl of strong positive, weak positive, negative sera and conjugate controls were added in quadruplicates. Fifty µl of monoclonal antibody, diluted in the blocking buffer to a predetermined concentration (1/100), to all wells of the plate were added. The plate was then incubated for one hour at 37°C on orbital shaker before being washed three times and blotted to dry. Then fifty µl of the conjugated antibodies diluted 1:1000 with PBS was added to all wells of the plate and the plate was incubated for one hour at 37°C as before. The plate was then washed three times and blotted to dry also. Then OPD solution as a substrate was prepared, and immediately before use, hydrogen peroxide solution was added. Fifty µl of the substrate/ Chromogen was added to all wells, and the plate was kept at room temperature for 10 - 15 min. The reaction was stopped by adding 10 µl of 1 M Sulfuric acid. The results were read spectophotometrically with an automated ELISA reader (Immunoscan Plus, Flow Laboratories). The optical densities of the samples were measured at 492 nm (OD_{492}). The cut-off end point was determined at three times the mean OD values of the conjugate control well.

Statistical analysis

Data were expressed as percentages. The significance of differences between data of different animals localities ages and sexes was determined using chi-square contingency table analyses.

RESULTS

Figure (2) explains the prevalence rates of Abs responses to RPV in different localities of Unity state. The seroconversion findings revealed that only 15.4% of the total sera tested were positive to the RPV- Abs in all locations of the sate. A remarkable difference ($p<0.05$) in the prevalence of Abs was noted among different localities of the state. No Ab responses to the virus were detected in animals of either Bentiu or Thar areas. The highest levels of Ab responses (31.6%) were detected in cattle of Abyemenm area.

Different age groups of cattle showed different prevalence rates of the disease with a significantly ($p <0.01$) higher level of prevalence rate observed in the age group of 5 - 10 years of cattle in all localities of the state (84%), as compared to other age groups. Age group of cattle of more than 15 years old showed the least prevalence rate of antibodies to PRV (2.3%) as depicted in Figure (3).

The prevalence rates of antibodies to PRV obtained in female animals are usually higher ($p<0.05$) than those in male animals. This is true for the whole state and for the eight localities sampled (Table 1).

DISCUSSION

Rinderpest had been endemic and economically important disease in many countries of Africa for the last century. In order to control this devastating viral infection, many projects were implemented by the OIE with coordination with some regional organizations such as the Organization of the African Unity (OAU) and the Interafrican Bureau for Animal Resources (IBAR). The Pan African Program for Control of Epizootics (PACE) is the last to be adopted when started in 2001. The Unity State, southern Sudan, was selected as a surveillance zone based on some factors. Firstly, the area is highly populated of cattle and other domesticated and game animals. Secondly, unity state occupies a central location in southern Sudan and being an area of animal move-ments from various vicinities especially from the northern part where the Baggara and Fellata tribes (from west Kordofan state) usually move south into the state during the dry season. In the frame of PACE program, this study was conducted to assess the prevalence of the disease in non

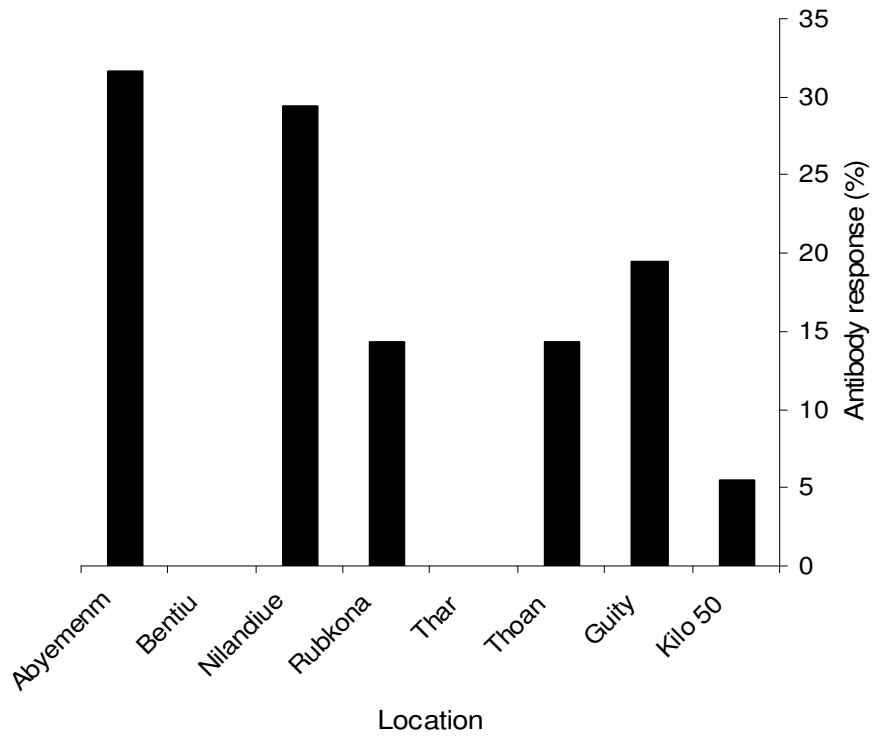

Figure 2. Prevalence rates of antibody responses to Rinderpest virus in different localities of Unity State.

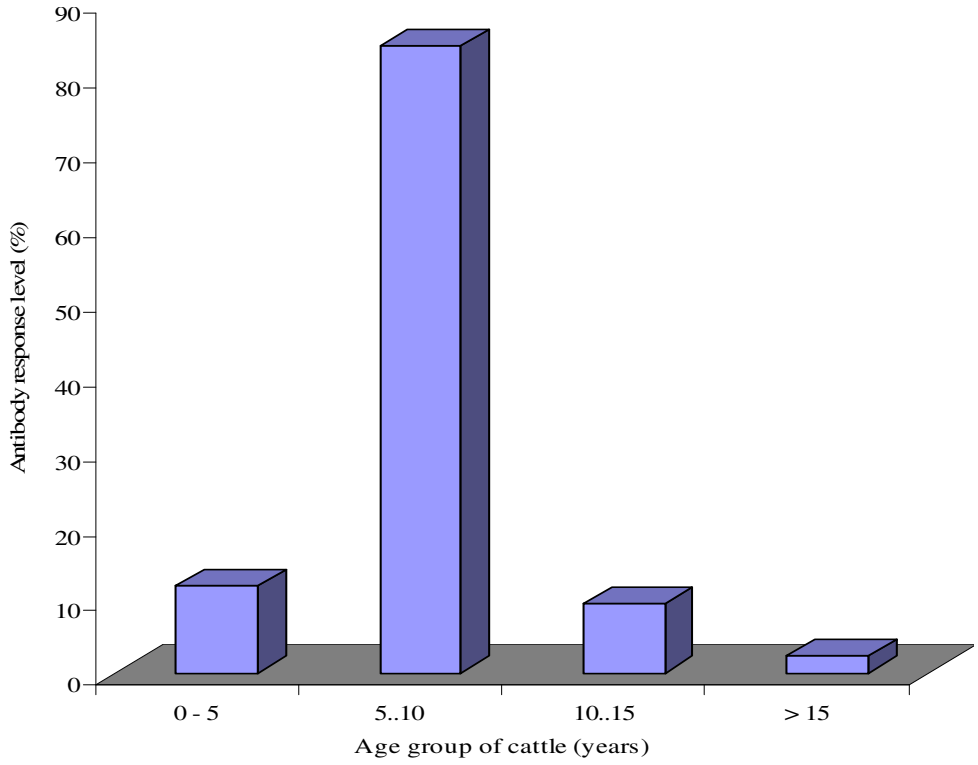

Figure 3. The prevalence rates of antibodies to RPV in different age groups of cattle

Table 1. Prevalence rates of antibodies to rinderpest virus in both sexes of animals in various localities of Unity State.

Locality	Total No. of sera tested			No. of sera positive to PRV		
	Male	Female	Total	Male (%)	Female (%)	Total (%)
Abyemenm	9	10	19	1 (16.7) [a]	5 (83.3)[a]	6 (31.6)[b]
Bentiu	5	11	16	-	-	-
Nilandiue	14	20	34	3 (30)	7 (70)	10 (29.4)
Rubkona	18	31	49	2 (28.6)	5 (71.4)	7 (14.3)
Thar	8	8	16	-	-	-
Thoan	5	9	14	0 (0)	2 (100)	2 (14.3)
Guity	37	40	77	4 (26.7)	11 (73.3)	15 (19.5)
Kilo 50	24	31	55	1 (33.3)	2 (66.7)	3 (5.5)
Total	120	160	280	11 (25.6)	32 (74.4)	43 (15.4)[c]

[a]The percentages of positive male and female animals were calculated from the total positive sera detected in each locality.
[b]The percentage of total positive animals in each locality was calculated from the total sera tested in that locality.
[c]The overall prevalence rate of Abs in the state was calculated as the total number of positive animals out of all sera tested in the state at large (43/280 = 14.4%).

non-vaccinated cattle in the state before a strategic and massive vaccination campaign had to be launched Our findings in this study showed that the overall prevalence rate of Abs to PRV in the unity state is generally low (15.4%). This is in contrast to previous results published by Majok et al. (1991) who showed that the prevalence rate of Abs to PRV in Bahr el Ghazal Province of southern Sudan, in the western part of Unity State, as high as 77.4% during the period of their study. This indicates an improved situation due to huge efforts of PARC for vaccinating cattle in southern Sudan from that time had occurred. Despite low rate of Ab responses to RPV in this study, this is still indicative of an active circulation of the rinderpest virus infection in the area. This situation conform with the finding previously published by Rossiter et al. (1987) who indicated that in endemic areas, and in herds recovering from epidemics, the number of clinically affected animals may be very low, however, the virus may still circulate in these areas. The absence of the clinically affected animals in the area can be attributed to the moderate immunity levels that they have as well as to the good grazing conditions and the availability of pasture and drinking water all over the year. The presence of antibodies to RPV in non-vaccinated cattle in the study area could be due to the presence of the virus in sheep and goats grazing with cattle in the same area. Such result was previously confirmed by Sheikh-Eldin et al. (1995) who reported the presence of Abs to RPV in sheep and goats in some provinces of Darfur explaining the endemicity of the area with RP and the disease outbreaks flaring up in the cattle in the area from time to time. This is pretty true as it was established that some RPV strains caused acute disease in sheep and goat, and was capable of being transmitted to contact susceptible calves (Anderson et al., 1990b). The same notion was substantiated later by Ramesh Babu and Rajasekhar (1988) who reported that subclinical RPV infections in small ruminants can act as potential source of the infection to susceptible bovine populations.

We could also suggest that the source of PRV to cattle in the study area can be the game animals which are abundantly present in the area. This can be verified using previous reports of Shanthikumor and Atilola (1990) who stated that game animals are not considered as natural hosts for RPV, but during outbreaks of the disease they play their role in transmitting the virus to susceptible animals. The presence of antibodies to RPV in non vaccinated cattle in Unity state may also be due to the reason that the virus is circulating in wildlife in the area, which are in direct contact with the cattle. That is particularly true during the dry season when all the cattle from Unity state and eastern cattle route (from west Kordofan) are grazing together in an area which is very rich with wildlife from different spices. The infectivity of PRV to wildlife animals were also documented (Kock et al., 1999; Huchzermeyer et al., 2001; Roeder and Taylor, 2002; Frolich et al., 2005; Couacy-Hymann et al., 2005; Kock et al., 2006; Rossiter et al., 2006).

The antibody levels to RPV in Abyemnem area, though low, are explained by the fact that the origin of the animals in that locality is Kordofan State (which moves to the Unity state through the eastern cattle route). They might have been covered by some vaccination regimes in previous times. No antibodies were detected in cattle from Bentiu and Thar areas which are in the middle of the state, while the highest levels of antibodies was found in the southern part of the state (Guity) and other locations in the middle (Niladiue) and northern parts of the state

(Abyemnem). Bentiu is the state capital and is likely to have received more veterinary concerns and had good vaccination practices as well as Thar which is in its vicinity.

The study revealed also the fluctuation of prevalence rates among different age groups of animals with a significantly high rate in age group 5 - 10 years cattle as compared to other groups. This is in disagreement with the previous findings of Silva et al. (1998) who indicated that seropositive bovids over three years of age had approximately four-fold higher chances of being seropositive compared to those that were equal to or less 3 years old. The young animals (0-5 years old) expressing seropositivity to PRV antigens in this study indicates that they are exposed to an active viral infection, and seropositivity is probably not a residue of maternally derived antibodies (MDA) as they are supposed to be weaned. Based on this fact an advice by Provost (1972) and Cheneeau (1985) for vaccination prohibition of calves against RP before 3 - 9 weeks of age was made.

We also observed that female animals always showed higher seropositivity to RPV as compared to male animals. We could not locate similar data in previous reports. However, we can suggest that female animals are more stressed based on their physiological and behavioral components, thus are more likely to develop RP.

The c-ELISA test used in this study was meant to differentiate antibodies due to Peste des petitis ruminants virus (PPRV) and RPV (Libeau et al., 1992), as they sharing some epitopic proteins. The c-ELISA involves the competition between the monoclonal antibodies and serum Abs for specific epitopes.

Conclusion

In conclusion, although the Ab response to PRV in Unity state is low, our data are still suggestive for active virus circulating in the area before and/ or during the period of study. Some recommendations pertaining to intensification of the vaccination projects in the area beside special attention to sheep and goats and wildlife present in the area, as sources of the infection, should be made. These helped by implementing vaccination strategy which led to declare Sudan as RP free zone in 2005.

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