PREVALENCE OF ANTIBODIES TO INFECTIOUS BOVINE RHINOTRACH-EITIS VIRUS IN SUDANESE CATTLE

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ABCTRACT

A serosurveillance of IBR antibodies in Sudanese cattle was carried out using Passive Haemagglutination Test (PHT). The results indicated that IBR antibodies were widely prevalent in Sudanese cattle ranging from 14% in Northern Kordofan to 72% in Western Kordofan with an overall prevalence of 38%. The prevalence rate was not significantly different ($P > 0.05$) between various age groups being 43%, 45%, 52% and 46% in the age groups less than 1year, 1-2 years, 2 -3 years, and more than 3 years old, respectively. The epidemiological implications of the findings were also discussed.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) caused by bovine herpesvirus- 1 (BHV – 1) is a disease of domestic and wild cattle. The disease in cattle is mainly manifested by fever, dyspnoea, nasal and ocular discharges and loss of condition. BHV- 1 can in additions affect number of systems other than the upper respiratory tract resulting in abortions, encephalitis, conjunctivitis and genital infections (Anon, 1986).

BHV–1 has previously been isolated in the Sudan (Eisa, 1983; Hassan and El Tom, 1985) and a serological survey carried out in 1988 (Hassan and Karrar, 1988) revealed widespread nature of IBR infection in Sudanese cattle.

Several serological methods have been used to detect antibodies to BHV–1 for diagnosis and/or for epidemiological surveys. These include virus neutralization tests, (Gibbs and Rweyemamu, 1977) immunofluores-cence (Wellmans and Leunen, 1973); passive haemagglutination test (PHT) (Kirby *et. al.,* 1974) and most recently enzymelinked immunosor-bent assay (ELISA) (Kramps *et. al.,* 1993). Virus neutralization tests are expensive, cumbersome, and time consuming. On the orher hand, immunoflourescence is subjective and requires specialized equipments while ELISA is expensive and some of its components need to be imported from abroad. PHT was reported by many workers to be specific, sensitive for the detection of IBR antibodies (Whitman and Helerick, 1965; Vengris and Mare, 1971; Schimizu *et al.,* 1972 Zyambo *et al.,* 1973a; 1973b, and Edward *et al*., 1986), technically easier to set up, accurate, and more rapid (Vengris and Mare, 1971).

The purpose of the present communication is to present the results of a survey of IBR antibodies using PHT in Sudanese cattle.

MATERIALS AND METHODS

Test Sera: Serum samples were collected from apparently healthy cattle in Western and Central Sudan (Table 1). The samples were heat inactivat-ed at $56^{\circ}C$ for 30 minutes and stored at – $20^{\circ}C$ until used.

Table (1): Prevalence of IBR Antibodies in Sudanese Cattle as Determined by PHT

State	Total Examined	No. Positive	% +ve
Khartoum	132	79	60
Gezira	243	58	24
Sinnar	48	29	48
N. Kordofan	85	12	14
S. Kordofan	131	33	25
W. Kordofan	99	71	72
Total	**738**	**282**	**38%**

Preparation of BHV – 1 antigen: Confluent monolayers of primary calf kidney cells were inoculated with BHV–1 (Los Angeles strain kindy provided by Dr. Bartha Vet. Res. Institute, Budapest, Hungary). Cells and supernatant fluids were harvested when cytopathic effect was complete usually 48–72 hrs later. Cells and infected fluid were centrifuged at 3000 rpm for 15 minutes to remove debris. Clean infected fluids were then aliquoted and kept at - $70^{\circ}C$ until used. The titer of this virus was $10^{-6.5}$ $TCID_{50}$/ml.

Preparation of BHV – 1 Sensitized Sheep Erythrocytes: These were prepared as described by Whitman and Heterick (1965) using the Los Angeles strain of BHV – 1. In brief, sheep erythrocytes were collected in Allseivers solution and washed in normal saline. Then equal volume of 3% formaline in normal saline and 10% of RBCs were mixed and incubated at $37^{\circ}C$ for 20 hours. The RBCs were then washed four times with double distilled water and made up to 10% in normal saline and stored at $4^{\circ}C$. Equal volumes of the 10% formalized RBCs were mixed with 1: 2000 tannic acid pH 7.2 and incubated at $37^{\circ}C$ for 15 minutes. They were then washed with saline: pH 7.2 two times and suspended into 10% in normal saline. For sensitization, 3 volume of the IBR virus were mixed with two volume of normal saline pH 6.4 and one volume of the taint RBC and then incubated at $37^{\circ}C$ for 15 minutes with occasional shaking, washed twice with serum diluent 1% horse serum in normal saline pH 7.2 and resuspended to half final concentraction of 1%.

Preparation of Hyperimmune Serum: BHV–1 hyperimmune sera were prepared in rabbits according to the method used by Ireri *et. al.*, (1989). In brief rabbits received one intramuscular inoculation of 2 ml of clarified supernatant of BHV-1 (described above) in 2 ml Freunds adjuvant followed by 4 intravenous injections of the same virus preparation at weekly intervals. Sera were collected from the rabbits one week after the last inoculation and kept at -20 $^{\circ}$C until required.

Passive Haemagglutination Test: The PHT was carried out as described by Zyambo *et al.*, (1973a).The test was carried in round bottom 96 well disposable microtiter plates and serum was diluted two-fold in 25 μl from 1:2 to 1:1024. To each serum dilution was added 25 μl of Ag coated erythrocytes. Control used for each test was a known IBR positive rabbit antiserum and a known negative IBR antiserum and Ag coated erythrocytes plus diluent to established setting pattern. The end point was taken as the highest dilution of the serum giving reading of PHT comparable to that of the positive control serum. End points were expressed as $\log_2$ titer. Sera having titers of 3 $\log_2$ and above were considered positive (Zyambo *et al.,* 1973b).

Statistical Analysis: The data were subjected to statistical analysis using T test and Chi squire in order to test the variation between different locations and age groups using SPSS programme.

RESULTS

The prevalence of IBR antibodies as determined by PHT in Sudanese cattle are shown in (Table 1). The prevalence varied between 14% in Northern Kordofan and 72% in Western Kordofan with an overall average prevalence rate of 38%. On the other hand no significant ($P > 0.05$) variation was observed in the prevalence of these antibodies among the various age groups (Table 2). The highest prevalence rate was observed in the 2-3 years old group (52%) and the lowest (43%) in the less than one year old group.

Table (2): Prevalence of IBR Antibodies in Various age groups Cattle in Sudan

	Age group (Years)			
State	**< 1**	**1-2**	**2-3**	**> 3**
W. Kordofan	5/9 (55%)	20/31 (64.5%)	10/12 (83.3%)	23/25 (92%)
S. Kordofan	1/5 (20%)	8/29 (27.5%)	5/11 (31.8%)	12/45 (26.6%)
Sinnar	-	0/2 (0%)	5/5 (100%)	21/38 (55.3%)
Gezira	-	6/34 (17.6%)	9/21(43.8%)	11/37 (29.7%)
Total	6/14 (42.8%)	34/96 (44.7%)	31/60 (51.6%)	67/145 (46.2%)

Table (3): FrequencyDistribution of $\log_2$ Titers of IBR Antibodies in Sudanese Cattle

	$\log_2$ Titers						
State	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
Khartoum	29	10	18	8	11	3	79
Gezira	30	15	6	7	-	-	58
Sinnar	3	11	8	6	1	-	29
N. Kordofan	3	5	4	-	-	-	12
S. Kordofan	14	10	5	2	1	1	33
W. Kordofan	21	23	10	8	1	2	71
Total	100	74	57	31	14	6	282
(%)	35.4	26.2	20.2	11	5	2.1	100

Table (4): Average Titer ($\log_2$) of IBR Antibodies in Various Age Groups of Cattle in Sudan

	Age group (Years)				
State	**< 1**	**1-2**	**2-3**	**> 3**	**Overall Average Titer**
W. Kordofan	4.1	3.8	4.0	4.1	4.0
S. Kordofan	3	4.8	4.7	4.4	4.5
Sinnar	5	-	4.0	4.7	4.6
Gezira	-	3.9	3.4	4.4	3.9
Overall	4	4.3	4	4.4	4.3

The titers of IBR antibodies ranged between $3\log_2$ and $8\log_2$ with most (82%) being in the range of $3\log_2$ -$5\log_2$ (Table 3). However, the average $\log_2$ titers did not vary significantly among age group or among states as shown in (Table 4).

DISCUSSION

An earlier report on the prevalence to BHV-1 virus in Sudanese cattle (Hassan and Karrar, 1988) indicated prevalence rates that ranged between 12% in nomadic cattle in Western and Eastern Sudan to 38% in resident cattle in Central Sudan with an overall prevalence of 21%. These authors also stated that the titers obtained; using virus neutralization test; were low and mostly lying between 4 and 10 (Hassan and Karrar, 1988).

The present study indicated higher prevalence rates ranging between 14% in Northern Kordofan State and 72% in Western Kordofan State with an overall prevalence of 38%. These differences between the two studies may indicate inherent difference in the methods used for the detection of antibodies to IBR virus as PHT have a higher degree of sensitivity, specificity and reproducibility (Shimizu *et al.,* 1972; Vengris and Mare, 1971). Zyambo *et al.,* (1973) also reported that higher titers were consistently observed using PHT than when using neutralization test for detection of IBR antibodies. Otherwise the present results may also represent a real increase in the prevalence of IBR infection in Sudanese cattle. Similar high prevalence rate of IBR antibodies were reported from cattle in Australia (Zyambo *et al.,* 1973b) and in goats in Zimbabwe (Mushi *et al.,* 1989).

The variation in prevalence rate of IBR antibodies noted in the present study between different states may be explained on basis of differences in management and/or ecological differences. For example, the higher prevalence rates in Khartoum (60%) and Western Kordofan (72%) states may reflect ease of BHV-1 transmission between animals in the crowded dairy farms in Khartoum and in animals congregated around waterholes during the dry season in Western Kordofan. The overall increase in the prevalence rate noted here over the previous study (Hassan and Karrar, 1988) may also be attributed to the fact that recent years have witnessed a substantial increase in cattle population allowing for more animals to come in contact with each other while competing for grazing land. It also remains to be determined if a more virulent strain(s) of BHV-1 virus has been introduced from Europe and other places where IBR is prevalent. Such strain(s) will be more apt to be transmitted among animals (Edward, 1988).

No significant differences between prevalence of antibodies and antibody titer to BHV-1 virus were noted among various age groups in the same or different states. These findings may indicate that antibodies detected in the $<$ 1 year age group may not be merely passively acquired antibodies but rather due to active transmission in this group. Although higher antibodies titers (7 $\log_2$, 8 $\log_2$) were mostly noted in animals from Khartoum State where crossbred animals mostly adult were sampled (data not shown), however, most of the animals (82%) had antibodies titers in the 3 $\log_2$-5 $\log_2$ range.

Finally, the present study revealed that BHV-1 infection is highly prevalent in Sudanese cattle. In addition to its proved economic importance as a cause of substantial losses due to abortions, reduced production, infertility…ect., the present performance indicators of the global rinderpest eradication program (GREP) (overseen by FAO) reguire that definitive diagnosis should be carried out for stomatitis enteritis conditions (SEC) to prove that a country is free of rinderpest infection. Hence capabilities to diagnose IBR and other SEC infection are badly needed for the Sudan to safely proceed along the prescribed OIE pathway for freedom from rinderpest infection.

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