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IBR Virus in Sudan: Epidemiological and Seriological Studies

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Abstract: During serosurveillance to detect infectious bovine rhinotraceitis antibodies, 856 serum samples were collected from different states of the Sudan. The overall percentage of positive samples was 47.43% (406 samples) tested by PHA. Of 273 samples tested by ELISA 141 (51.65%) were positive, whereas 69 out of 94 samples tested by SNT 73% were positive. The prevalence was found high in states including Jongeli, West and North Kordofan. In comparison between the three serological tests, SNT is recommended to be the technique of choice followed by ELISA, while PHA is a good technique to define the disease.

Key words: IBR virus, epidemiological serological

INTRODUCTION

Serum-virus neutralization test is the common procedure used for the identification of Infectious Bovine Rhinotracheitis (IBR) virus as well as for the detection of serum antibodies to the virus, but it is time consuming, expensive and require considerable technical expertise[1-3]. Passive haemagglutination (PHA) test on the other hand is considered rapid for detecting IBR antibodies, accurate and economic[2]. Also antibody titre obtained by SNT is relatively low than titre obtained by PHA which is significantly higher[4-6].

IBR antibodies could be detected also by micro-enzyme Linked Immunosorbent Assay (ELISA). The sensitivity of antibody detection by this technique is at least as good as the serum neutralization assay and it could be used as a rapid screen procedure for the disease[7]. One major advantage of ELISA methods over the other two techniques is that this test not only used to test blood sera but also milk samples and tissue fluids for specific antibodies[8].

The aim of this research was to studies the situation of IBR virus antibodies in different Sudan states using three serological tests. Also to compare the ability of these serological tests to detect the virus antibodies in cattle sera.

MATERIALS AND METHODS

Study area: The study was carried out over a period of 2 years 2001-1003 on cattle. The study area is distributed in different Sudan states including Sinnar, Gezira, Blue Nile, South Darfor, West Kordofan, Gedarif, Kassala, White Nile, Red Sea, Khartoum, River Nile, North Kordofan and Jongeli, Fig. 1.

Sera and viruses: Cattle sera were collected form various region of the Sudan. Table 1 shows the locations and numbers of sera samples examined. Los Angeles reference strain (titre $10^{6.5}$) was kindly supplied by Department of Viral Vaccine Production, Central Veterinary Research Laboratory (CVRL).

Passive Haemagglutination test (PHA)

Preparation of sencitized red blood cells: Sheep erythrocytes (RBC) were collected in Alsever's solution, washed three times in Normal Saline (N.S), centrifuged at 1500 rpm for 10 minutes and made into 8% suspension in N.S. An equal volume of 3% formalin and 8% of the RBCs were added and the mixture was incubated at 37°C for 20 h. These formalized e rythrocytes then washed 4 times with DDW and stored at 10% concentration in N.S. at 4°C. Tannic acid was diluted 1/20000 (pH 7.2) and mixed with equal volume of 10% formalized cells. The mixture then incubated at 37°C for 15 min with occasional shaking, washed two times with saline pH 7.2, centrifuged at 1500 rpm for 10 min and 10% suspension was prepared in saline and kept at 4°C till used. Three volumes of the required concentration of South Africa strain were mixed with two volumes of N.S. The mixture was incubated at 37°C for 15 min with occasional shaking, washed

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Table 1: Shows the locations and numbers of sera samples examined by PHA, ELISA and SN tests

Location	Numbers of serum samples by PHA test	Numbers of serum samples by ELISA test	Numbers of serum samples by SN test
Sinnar	47	25	11
Gezira	68	38	14
Blue nile	48	18	11
South darfour	141	21	12
West kordofan	38	12	7
Gedarif	40	15	4
Kassala	57	13	8
White nile	102	24	7
Red sea	52	45	10
Khartoum	83	37	6
River nile	43	25	4
North kordofan	52	Non	non
Jongeli	46	Non	non
Total	856	273	94

twice with serum diluem (1% horse serum in N.S. pH 7.2) and resuspended to have a final concentration of 1%[16].

Procedure of the test: Titrations of tested sera were made in disposable microtitre plate ("V" shaped wells). To each plate eight sera were used, one control positive IBR serum, one control negative serum (new born calf serum) and six test sera. The sera were diluted two fold in 0.025 mL of serum diluent from 1 :2 to 1:1024. Then 0.025 mL of antigen-coated erythrocytes was added to all plate wells. They were covered carefully, shaked well and incubated at room temperature overnight to allow the component to be settled. The result was read the following day and the positive samples were screened from the dilution 1:8 according to[16,7]

Indirect Enzyme Linked Immunosorbent Assay (ELISA): Commercial ELISA kits of Cypress Diagnostic products for sero-diagnosis of IBR disease were used according to the manufecturers direction.

In brief: As were brought to room temperature at least half an hour before used. The serum samples were diluted 1:100. 100 µL of the diluted samples were added to the wells as follows: the threshold control serum in wells A1 and A2, sample 1 in wells B1 and B2....ect and the plate was incubated at room temperature for 1 h. Then the contents of the microplate were emptied, washed three times with washing buffer and let to dry. 100 µL of the conjugate solution diluted 1:50 was added to each well and re-incubated at room temperature for 1 h. Then the plate was washed 3 times. 10 mL of indicator solution was prepared by adding 0.5 mL of the chromogen to 9.5 mL of the substrate solution (Hydrogen peroxide) mixed thoroughly then applied to the plate immediately in volumes of 100 µL per microwell. The plate was then covered and incubated for 10 min at room temperature. The reaction was then stopped by adding 50 µ of phosphoric acid to all wells. The optical densities in microwells were then read using 450 nm filter. The result was calculated by subtracting from each value recorded for the odd columns (which sensitized by IBR virus) the corresponding negative control well in even columns (which sensitized by a lysate of bovine kidney cell line), when the differences in optical densities is equal or greater than the threshold serum the sample must be considered possitive.

Neutralization Test (NT): The method described by Van Oirschot *et al*[8], protocol was followed with some modification. Briefly.

Test sera were first screened for neutralizing activity at a dilution of ¼. After that, serial two-fold dilutions of the positive sera were titrated against 100 $TCID_{50}$/25 µ of Kenya strain of IBR virus in microtitre plate using at least two wells per dilution. The sera were first diluted in PBS then 25 µL of diluted sera were added to 25 µL of the virus and incubated for 24 h in 4°C. Then 100 µL of cell suspension of Bovine Kidney Cell Line (BKCL) were added to the microplate, incubated at 37°C and observed daily for the presence of CPE. Appropiate controls including positive and negative control sera were included in the test. The results were read after 5-7 days and the positive sample was considered at dilution ¼ according to Zyambo *et al*[16].

Statistical analysis: The results were subjected to kappa coefficient analysis using Stata[10] so as to determine the level of agreement between the three diagnostic tests (SNT, ELISA and PHA). A kappa of 1 indicates perfect agreement and no agreement beyond chance gives a kappa of zero. A kappa of at least 0.4-0.5 indicates a moderate level of agreement Martin *et al*[11].

RESULTS

Results of Passive Haemagglutination (PHA) test: The overall percentage of positive sera tested against IBR antibodies was 47.43% (406 of 856) tested sera (the antibodies titre =1/8). The incidence of IBR disease was found high in states including Jongeli 84.78%, Gedarif 77.50%, North Kordofan 76.92% and West Kordofan 73.36%. But it was very low in Kassala 5.08%. shows Table 2. the results of the PHA test in Sudan states.

Serological surveillance using Enzyme Linked Immunosorbent Assay (ELISA): Of 273 sera collected from different states of Sudan (11 states), 141 (51.65%)

Fig. 1: Shows studied areas for IBR disease in Sudan

were found positive to IBR disease by ELISA test. In West Kordofan the disease was observed to have high prevalence rate 83.33%, while River Nile presented the least prevalence 32% Table 3.

Result of serological surveillance using Serum Neutralization Test (SNT): A total of 94 sera, tested by SNT, were selected from those examined by ELISA and PHA test. 69 73.4% sera were found positive to antibodies against IBR disease and 25 26.6% had negative results.

Table 2: Incidence of both IBR positive and negative serum samples by passive haemagglutination test in different Sudan states.

% of -ve sample	No. of -ve sample	% of +ve sample	No. of +ve sample	Locality
63.83	30	36.17	17	Sinnar
44.12	30	55.88	38	Gazira
64.58	31	35.42	17	Blue Nile
24.64	17	75.36	52	West Kordofan
31.76	47	68.24	101	South Darfar
22.50	9	77.50	31	Gedarif
94.92	56	5.08	3	Kassala
85.44	88	14.56	15	White Nile

Table 2: Continue

% of -ve sample	No. of -ve sample	% of +ve sample	No. of +ve sample	Locality
58.82	30	41.18	21	Red Sea
79.01	64	20.99	17	Khartoum
65.91	29	34.09	15	River Nile
				North
23.07	12	76.92	40	Kordofan
15.21	7	84.78	39	Jongoli
52.57	450	47.43	406	Total

Table 3: Incidence of both IBR positive and negative serum samples by ELISA test in different regions.

% of +ve sample	No. of -ve sample	% of -ve sample	No. of +ve sample	Locality
36.00	9	64.00	16	Sinnar
55.26	21	44.74	17	Gazira
44.44	8	55.56	10	Blue Nile
16.67	2	83.33	10	West Kordofan
52.38	11	47.62	10	South Darfor
60.00	9	40.00	6	Gedarif
53.85	7	46.15	16	Kassala
33.33	8	66.67	16	White Nile
57.78	26	42.22	19	Red Sea
37.84	14	62.16	23	Khartoum
68.00	17	32.00	8	River Nile
48.35%	132	51.65%	141	Total

Table 4: Results of PHA, ELISA and SNT for detection of BHV-1

	Positive		Negative	
Technique	No.	%	No.	%
PHA	46	48.94	48	51.06
ELISA	59	62.77	45	37.23
SNT	69	73.4	25	26.6

Table 5: The percentage of agreement between the three diagnostic tests:

Techniques	Percentage of agreement between the two tests
PHA/ELISA	58.51%
PHA/SNT	58.51%
ELISA/SNT	80.85%

The comparison between the serological tests: A total of 94 sera were tested using the three serological tests. The comparison between them was as follows:

Results of PHA, ELISA and SNT for detection of BHV-1: Table 4 shows that 46 48.94% sera were positive for IBR antibodies using PHA test, while 59 62.77% and 69 73.4% of the tested samples were positive by ELISA and SNT technique respectively.

The agreement between the three diagnostic tests applied on collected samples: The three tests were compared to detect antibody to BHV-1. Of the 94 sera samples tested by SNT & ELISA, 76 showed agreement (55 positive and 21 negative). Using ELISA & PHA test 55 samples showed agreement (33 positive and 22 negative), while using SNT and PHA 55 samples showed agreement (38 positive, 17 negative).

Kappa statistic was used to explore the level of agreement between PHA & ELISA the results revealed 58.51% agreement between the two tests, while PHA and ELISA and ELISA and SNT revealed 58.51% and 80.85% revel of agreement respectively as shown in Table 5.

DISCUSSION

In the present research serological tests, including SNT, PHA and ELISA used for the identification of IBR antibodies were compared. The serum neutralization test was used as the most common procedure to determine antibody level, it was also extensively used in differentiation of IBR isolates from other bovine herpesviruses[1,3]. The percentage of the positive samples using SNT were 73% (69 of 94) while 26.6% (25 of 94) were negative.

Also 856 sera were tested using PHA test. This test was recorded to be more rapid, simple, cheap, less time consuming than SNT and the titre obtained was higher than SNT[8,21] suggested that PHA could become available procedure in diagnostic laboratories where large numbers of sera are tested for IBR antibodies. Using PHA test the percentage of positive samples was 47.43% (406 of the 856) and the incidence of antibodies were found high in states including Jongeli 84.78%, Gedarif 77.50%, North Kordofan 76.92%, West Kordofan 73.36% and low in Kassala 5.08%.

During this study 273 sera were tested using ELISA. This test was stated to be sensitive in detecting IBR antibodies[12,13].

On basis of ELISA technique 51.65% was found positive (141 of 273) and the prevalence rate was found higher in West Kordofan 83.33% and low in River Nile 32%. This results showed the high prevalence of the disease in the west, south Sudan and Gedarif states, this may indicates the endemic nature of the disease, and this is because in western Sudan there is shortage in water supply so that the high population of animals accumulate together and share one water site. In Gedarif the high population of animals facilitates the transmission of the disease, in contrast to River Nile state that presented low population of animals. Hassan and Karrar [14] agreed with our findings when they studied the disease incidence in Sudan and found that the percentage of BHV-1 neutralizing antibodies were higher in western Sudan. Also 97 out of 452 (21%) had positive antibodies to BHV-1. This percentage is low compared with those reported in this research, this is because most of the serum samples were collected during January and the disease incidence was mentioned by Sozan[13] to have higher infection rate in winter season. The probability of

more new strains of IBR virus that entered the area during this period of time may be encountered.

In comparative studies 94 serum samples were taken from the results of PHA test, which had high positive titres beside the negative results, and tested by ELISA and SNT. The SNT was proved to be more sensitive in detecting 69 (73.4%) IBR positive serum samples compared with ELISA and PHA, which detected 59 (62.77%) and 46 (48.94%) seropositive samples, respectively. When the 94 sera samples were tested by SNT and ELISA 76 samples (80.85%) revealed agreement between the two tests with 55 positive and 21 negative samples. Using SNT and PHA tests 55 (58.51%) showed agreement, 38 positive and 17 negative, while the agreement between PHA and ELISA was 55 (58.51%) with 38 positive and 22 negative.

Results presented here proved that SNT test is the technique of choice to detect IBR antibodies. Also the ELISA used in this research had similar results compared to SNT and could be recommended as a good diagnostic test. This finding is in full agreement with Edwards et al[12]. In this study similar results were obtained using ELISA and SNT as confirmed also by[b,d]. PHA test is used for rapid detection of antibodies, so it is recommended to use this test for routine serosurvaillance in view of its speed, simplicity and cheapness.

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