

Seroprevalence of Infectious Bursal Disease in Exotic and Backyard Chicken's Sera Collected from Some Parts of Sudan, tested by the Agar -Gel Precipitation and Counterimmunoelectrophoresis Tests

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Abstract : A cross-sectional Seroprevalence study of IBD antibodies in non-vaccinated, apparently healthy exotic and backyard chickens was conducted in four areas of Sudan: Khartoum, Dongola (Northern), Obied (Western) and Kassala (Eastern) States between October 2012 and May 2014. All chickens in the exotic group were 3-10 weeks of age. Chicken in the backyard flocks were 3-12 weeks old. From a total of 664 sera samples collected from exotic chickens in the four areas 273 (41.1%) were found positive tested by AGPT. Regarding backyard flocks 181 samples out of 460 (39.3%) were positive. This indicated a considerably high level in overall Seroprevalence of antibodies in both groups. Sero-reactivity of the unvaccinated apparently healthy flocks in both groups could be explained as due to field exposure which led to subclinical infection.

Detailed results showed that seroprevalence was 46.65% (216/463), 2.6% (3/114), 33.3% (25/75), and 0% (0/12) among exotic breeds sera collected from Khartoum, Northern, Western and Eastern States respectively.

Seroprevalence among backyard flocks was 38.89% (21/54), 0% (0/54), 66.7% (96/144) and 30.769% (64/208) respectively in the four investigated areas.

Both AGPT and CIEFT were comparable and useful in measuring IBD antibodies.

It is recommended to encourage and improve backyard production by small-scale farmers in developing countries. This will produce a good source of income besides availability of proteins thus improving life style and alleviating poverty in these communities.

Key words: IBD, exotic, backyard, Chickens, seroprevalence, AGPT, CIEFT.

Introduction

Infectious bursal disease (IBD) is a highly contagious disease of young chickens with worldwide distribution. It is characterized by severe inflammatory changes in the bursa of fabricius (FB) followed by immunosuppression (Allan, Faragher, and Cullen, 1972; Fadley, Winterfield, and Glander, 1976; Rosenberger and Gelb, 1979). Prevalence of the disease is very high with most flocks suffering from early subclinical infection before three weeks of age or a mild subclinical to severe disease from three to six weeks of age (Lukert and Hetchner, 1984). The disease was reported to impose threat due to mortality, reduced weight gain and condemnation of carcasses because of marked hemorrhage in skeletal muscles, (Van den berg, 2000). Secondary losses due to immunosuppression and vaccination failure to many other diseases are also documented, (Lukert and Safe, 1997).

The virus which was first isolated by Cosgrove in 1957 in the United States of America (Cosgrove, 1962) is a double stranded (ds) RNA virus. It belongs to the Birnaviridae family which together with the Reoviridae family constitutes viruses of RNA double stranded genomes and classified in group 111 by the Baltimore virus classification scheme. Two serotypes of the virus were known: Serotype 1 is pathogenic for chickens, serotype 2 is not pathogenic in chickens and has been isolated from both chickens and turkeys (Chin et al, 1984). Viruses in serotype 1 are further categorized into 4 groups based on their pathogenicity: classical, variant, attenuated and very virulent strains (Lim et al, (1999).

In the Sudan the disease was first reported in 1982 by Shuaib, Salman, Ginawi and Sawi from an outbreak at Elobied town in Northern Kordufan State, (Shuaib et al, 1982). Since then and up-to date outbreaks are reported from many parts of the country.

In this study, surveillance for IBD antibodies was carried out and was meant to include many geographic areas as much as possible. This was to monitor the disease incidence, prevalence and distribution among both exotic and local backyard flocks in order to set a control policy for the disease which has a considerable economic effect on poultry production.

Objectives

To monitor and clarify the incidence of IBD among non-vaccinated, apparently healthy exotic and backyard chickens in Sudan using AGP and CIEF tests.

Materials and Methods

Study area

Includes Khartoum, Dongola (Northern), Obied (Western), and Kassala (Eastern) States:

Khartoum, the national capital of Sudan, located in central Sudan at the confluence of the Blue Nile and the White Nile rivers. Opposite the river in west lies the city of Omdurman the largest city in Sudan. The Sudanese metropolis consists of three cities: Khartoum, Khartoum North (Bahri), and Omdurman. It has a population of estimated more than 3 million inhabitants.

Dongola(Northern region) is one of the 18 States of Sudan. It has an area of 348,765 km² and an estimated population of 833,743. Dongola is the capital of the state. Jebel Uweinat is a mountain range in the area of the Egyptian-Libyan-Sudanese border.

Obeid (Western region) is a city found in North Kordufan, the Sudan. It is located at 13.18 latitude and 30.22 longitudes and it is famous of gum Arabic production and other crops.

Kassala (Eastern state), traditional region, east-central Sudan. It is bordered on the east by Ethiopia. Rainfall decreases steadily from south to north, with 40 inches (1,000 mm), falling 13 inches (330 mm) at Kassala town. Most of Kassel's population is engaged in agricultural pursuits, and cereals, oilseeds, cotton, and peanuts mills, and soap factories. Cattle and camels are raised in the northern and southern parts of Kassala.

Study Population

Consists of two groups of non vaccinated, apparently healthy chickens : the exotic breed ,3-10

weeks old and the backyard local breed ,3-12 weeks of age.

Sample size

A total of 664 and 460 samples collected from exotic and backyard chickens respectively from the four investigated areas were as follows:

Khartoum State: 463 and 54

Northern State: 114 and 54

Western State: 75 and 144

Eastern State: 12 and 208

The sample size was determined by using the formula described by Thrustfield (1995). Collection was made with consideration of an expected prevalence of 50% and an absolute precision of 5% with 95% confidence level.

Sera Samples collection

Blood was collected from both exotic and backyard breeds in small- scale commercial layer farms and from house-hold farms at different rural settings. Collection was made from the heart and/ or wing vein. All sampled chickens were non vaccinated and apparently healthy.

Methods

Samples were tested by the AGPT and results were compared with that performed by the CIEF test.

Agar Gel Precipitation Test (AGPT)

Preparation of 500 ml buffer for IBD AGPT

Nacl 40.0gm

Phenol crystal 2.5gm

DDW 500ml

3ml of 1 molar NaoH solution was added to make the PH of the buffer 7.4

Preparation of the agar

1.4 gm purified agar was boiled in 100 ml of AGPT buffer for one hour. Then it was dispensed in Petri dishes (10 cm size) in 17 ml quantities per dish and left to cool and solidify.

Test procedure

A seven gel cutter template, 3.5mm in composite diameter was used to cut out wells in the agar. The six outer wells(used for test and control

sera) were 5mm in diameter, spaced 5mm from the inner well which was used for the specific positive antigen.

The inoculated gel was then incubated in a humidified chamber at room temperature for 48 hours. The test was read at an illuminated chamber using a magnifying glass. Clear precipitin lines were recorded as positive result. Results were recorded in table 2.

Counterimmunoelectrophoresis(CIEF) test

Method

For comparison, 150 AGPT- positive sera were retested by the CIEF technique. Glass slides were flooded with 3ml per slide with hot melted agar. Three parallel rows of wells, two outer with eight wells each and one inner of nine wells were pierced on the melted agar. The two outer wells were used for the test samples, positive and negative controls whereas the inner wells were used for the positive specific IBDV antigen,

Preparation of tris Barbitone buffer for IBDV Sera Assay by CIEF

Tris	1.5gm
Barbitone	4.48g
DDW	1000ml
PH was adjusted to 7.2	

Preparation of electrophoresis buffer

75ml tris Barbitone buffer PH 7.2 + 25 ml DDW

These were mixed together and used for agar preparation.

Preparation of the agar

Purified agar	1gm
Electrophoresis buffer solution	100ml
The agar was dissolved by boiling for one hour.	

Procedure of the test

The test was performed according to Ninna (1982). When the agar was melted each microscope slide was flooded with 3.5 ml of agar. After the agar had solidified, three rows of wells were cut. The slides were placed in the electrophoresis chamber. Wicks cut from Whatman no. 1 filter paper were used to connect the agar and the buffer. The anode- oriented wells

were filled with serum while the cathode- oriented wells were filled with standard IBD antigen,

The samples were electrophorized at a constant voltage of 5 volts/cm for 45 minutes at room temperature. Reading was observed against an indirect illumination. A positive reaction was shown by the formation of precipitation lines between the antigen and serum wells. Positive and negative control sera were included.

Results

Of the 664 total number of sera collected from exotic chickens and tested by AGPT, 273 (41.1%) were found positive for IBD antibodies. The total number of sera collected from local backyard chickens from all regions was 460 of which 181 (39.3%) were positive tested by AGPT also (table 1).

“Table 1. Result of Seroprevalence of Exotic and Backyard Samples Collected from the four Investigated Areas

Flock type	Total No, tested	No, positive	%Positive
Exotic	664	273	41.1
Backyard	460	181	39.3

Detailed results according to areas investigated were shown in table 2.and were as follows:

Khartoum State

Exotic : total number tested =463, number positive = 216 (46.65%)

Backyard: total sera tested = 54, total positive = 21 (38.89%)

Northern province:

Exotic: total sera tested = 114, number positive = 3 (2.63%)

Backyard: total: total samples tested = 54 which were all negative

Western region) Obied town):

Exotic: total sera tested = 75 of which 25 were positive (33.3%)

Backyard: total sera testes = 144 with 96 samples positive (66.7%)

Eastern region (Kassala town)

Exotic: total samples tested = 12 which were all negative

Backyard: of 208 sera tested 64 were positive (30.769%)

“Table 2: Detailed Result of Samples Collected from the Four Investigated Areas

Area	Exotic			Backyard		
	Total samples tested	Total positive	%+ve	Total samples tested	Total positive	%+ve
Khartoum	463	216	46.65	54	21	38.89
Northern	114	3	2.6	54	0	0
Western	75	25	33.3	144	96	66.7
Eastern	12	0	0	208	64	30.769

CIEF results

150 sera samples which were all positive by the AGPT were retested by CIEF test. Three samples were recorded negative by CIEF test (0.2%). This indicated that AGPT is slightly more sensitive; Moreover, it is simple, cheap and easy to perform. Compared to CIEF test. However, CIEFT is more rapid giving results in 45- 60 minutes.

Discussion

The first appearance of IBD dated back to 1982. The virus had ever since been implicated in considerable economic losses in different parts of the country. Attention had to be paid to the potential hazard IBDV poses to the poultry industry due to the variation of the clinical picture which ranges from subclinical to highly virulent types of the disease.

In this study, from a total of 664 sera collected from exotic breeds from the four investigated regions in the Sudan, 273 samples were found positive by AGPT (41.1%). Regarding surveillance of backyard local breeds, the positivity was 39.3% (181/460). This result showed that seroprevalence was slightly higher in the exotic group indicating that they might be more susceptible to infection by the virus. Similar studies were conducted in two region in Ethiopia with even higher seroprevalence rates exceeding 90% among cross breeds compared to that recorded in local breeds (Zelege et al,2005a; Tadese et al 2014, Zinidu et al,2015). The most striking fact was the detection of such considerable antibody levels in non vaccinated apparently healthy flocks. This showed that chickens were susceptible to IBDV infection and might show subclinical infection and immunosuppression due to field exposure especially at early ages.

The highest incidence of antibody levels among the exotic group was recorded in Khartoum State (46.65%) followed by the Western region

(33.3), then Northern State (2.6%), and lastly the Eastern State where all tested chickens were negative for IBD antibodies.

Regarding result among the backyard groups incidences were 66.7, 38.89, 30.769% and 0% in the Western, Khartoum, the Eastern and the Northern States respectively in descending order.

Seroprevalence among backyard breeds was significant in three of the investigated areas because farming of such types of flocks is widespread especially among rural small holder farming communities at the outskirts of towns. The negative and low antibody levels among both flocks recorded at the Northern State might be due to the fact that no IBD vaccination was practiced and no field IBDV is circulating there.

Moreover, it could be seen that seropositivity among Khartoum and Obied exotic and backyard flocks was higher compared to that in the two remaining regions, The reason for that might be due to vaccination practiced by neighbouring large government farms and possibility of vaccine virus spread. Besides, the relatively considerable seroreactivity among backyard flocks compared to exotic ones in Kassala region might be because farmers prefer rearing local chickens with low cost of production. This might explain the higher number of backyard breed examined compared to that of the exotic one.

A comparison was made between the AGPT and CIEFT used for assay of the samples. Results confirmed that both tests were useful and reliable in determining precipitating antibodies in chicken's sera. However, the CIEF test was more rapid detecting antibodies (or antigens) in 45- 60 minutes. Yet, the AGPT was found slightly more sensitive where 3 out of the 150 AGPT-positive samples were found negative by the CIEF test. Moreover, the AGPT is simple, easy to perform, and cheap. It was reported that the AGPT has been used not only for quantification of IBD antibodies but also to differentiate wild type IBDV directly from infected tissues collected from the field (Snyder, Yancy and Savage, 1992). However, our result was in disagreement with an earlier finding reported by Ninna (1982) who found that CIEF test was more sensitive than AGPT as it detected three positive sera compared to only one by AGPT.

Local backyard chicken farming constitutes an economic resource for small- scale farmers as a source of income. It is recommended to encourage this type of farming because it is not very expensive to run, as low input of chicken feeding is usually required. In addition, this type of farming will provide people in developing countries with proteins helping to improve the life style and alleviate poverty.

Moreover, farmers have to be provided by knowledge and advice about how to follow the right correct methods of keeping their flocks healthy and good producers. Advice to vaccinate flocks is strongly recommended.

Conclusions and Recommendations

The study revealed that IBDV is circulating in most parts of Sudan resulting in an important socio-economic impact manifested in subclinical infection among both exotic and local backyard non vaccinated flocks. This may lead to immune suppression which adversely affects response to vaccination to many other pathogens. Vaccination, especially at early ages, is recommended to prevent this problem. Both AGPT and CIEF are good tools for detection of IBD antibody and antigen, with AGPT being more reliable for detection of the virus especially in laboratories with limited diagnostic facilities.

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