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Investigations on Nine Flocks Infected with Infectious Bursal Disease Virus (IBDV) in Khartoum State (Sudan)

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Abstract: Nine flocks suspected to be infected with IBD between January to December 2005 were followed up in Khartoum state during the period of the clinical disease course. Our careful investigations showed that the disease occurred in different poultry producing areas all around the year and causing mortality between 9 - 49%. All cases of IBD reported to the governmental veterinary hospitals during this period were occurred in light egg type leghorn and no heavy meat broiler chickens was reported during the period of the survey. The four anti-IBDV vaccine strains commercially available in Sudan market (D78, Bio-Gumboro, Gumboro3) including hot vaccine (228E) and a variety of vaccination schedules performed failed to protect the surveyed flocks from clinical IBD infection. Diagnosis based on clinical signs and postmortem gross lesions was confirmed in all surveyed flocks by serological tests (ELISA), in seven out of nine flocks by histopathological examination and by isolation methods in six out of nine flocks.

Key words: Infectious bursal disease, vaccination practices, bursae tissues

Introduction

Infectious bursal disease (Gumboro disease) is infectious disease of global economic importance (Pitcovski, 2003). The relatively unknown infectious bursal disease virus (IBDV) is currently responsible for huge economic impact on the worldwide poultry industry. (Vandersluis, 1999). It has become a major poultry disease in Pakistan during the last 5 - 7 years (Zaheer and Saeed, 2003). The disease has been reported in Malaysia (Hair - Bejo, 1993), in Brazil, Dominican Republic and Venezuila (Alejandro and Pedro, 2004), in Madagascar (Rajaonarisan *et al*., 1994) and in late 1980's outbreak of the disease with high mortality was reported in Europe (Chettle *et al*., 1989).

In Sudan Gumboro disease was first observed in western Sudan in 1979 and reported by Shauib *et al*. (1982).

The disease is an acute highly contagious viral infection and it infect young susceptible chicks (Parkhurest, 1964). The disease by itself usually causes mortality of 5 - 10% but this rate can reach 30 - 40% (OIE manual, 2004).

Vaccination against IBDV is one of the significant component of the control of the disease, if improved may help in lowering the incidence in poultry (Zaheer *et al*., 2003). Time of vaccination, type of vaccine, maternal derived antibody (MDA) in the chicks and pathogencity of IBDV field challenge are important factors determine on the efficacy of IBD vaccination.(Hair-Bejo *et al*., 2004).

In practice many vaccination schedules and a variety of vaccine strains are used, despite that outbreaks are still recorded (Zaheer and Saeed, 2003).

The present study was conducted to investigate the picture of IBD outbreaks occur in Khartoum state-Sudan. With regard to vaccination practices.

Materials and Methods

Area and chicks flocks: Nine flocks of young chicks showed clinical signs and postmortem gross lesions of infectious bursal disease were reported to 2 governmental veterinary hospitals, seven in Khartoum town and two in Khartoum north town were followed up during the clinical course of the disease. Each flock was visited daily throughout the disease course to fill in a data collection sheet in order to record information and observations.

Diagnosis of infectious bursal disease was based on clinical signs, postmortem gross lesions, bursa to body weight $\times 10^{-3}$ ratio (Eterradossi *et al*., 2004) in addition to serology, histopathology and isolation of the virus.

Post mortem gross lesions: Characteristic gross lesions: Including dehydration of the muscles, with ecchymotic hemorrhage, enlargement orange discoloration of the kidneys, the bursa of Fabricious shows the main diagnostic lesions where in birds that die at the peak of the disease it enlarged and with pale yellow discoloration. Intrafolicular hemorrhage may be found. Pen point hemorrhage on the skeletal muscles (OIE manual, 2004).

Clinical signs: clinical signs observed included are ruffed feathers, chicks reluctant to move., anorexia, watery diarrhea, trembling and severe prostration. (Ley *et al*., 1983; OIE manual, 2004).

Table 1: History of the flocks infected with IBD

Vacc. sched Days	Vaccine strain	Date at which IBD occurred	Breed	No of birds	Area	Flock No
15 - 42	D78 - D78	May.2005	Hisex	4 000	Kalakla	1
15 - 30	D78 - D78	Feb.2005	Bovans	3 000	Dikheenat	2
14 - 21	D78 - 228E	Oct2005.	Loghman	6 000	Buri	3
14 - 21	Biogumboro-Biogumboro	Apri.2005	Bovans	3 100	Shigailab	4
13 - 19	D78 -D78	Oct.2005	Hibrid	4 000	Hlfaia	5
21 - 28	D78 - D78	Juli2005	Bovans	1 300	Rimaila	6
21 - 28	D78 - D78	Jan.2005	Loghman	4 000	Soba	7
14 - 28	228E - 228E	Jan.2005	Hiline	1 400	Dikheenat	8
15 - 21	Gumboro3 - Gumboro3	March.2005	Hisex	3 500	Kadaro	9

Bursa to Body weight ×10^{-3} Ratio: Bursae of Fabricious of sick or freshly dead birds at the peak of the clinical course were removed and their weight were determined using a sensitive balance and the average weight was calculated as a ratio of the body weight multiplied by 10^{-3}. Part of the bursae were then preserved for histopathology in neutral buffer formalin. And at -20°C in plastic container for viral isolation.

Histopathology: For histopathology, bursae tissues were fixed in 10% buffered formalin. Each bursa was trimmed to the thickness of 5 mm in size, fixed and dehydrated in a series of alcohol concentrations and embedded in paraffin wax using an automatic processor. Sectioning of tissues was done to the thickness of 5 micrometer on a microtome, the bursa tissues was mounted on glass slide, dewaxed and stained with (H and E). Bursa tissues were then examined under microscope using ×4, ×10 and ×40 objectives for histopathologic changes The histopathologic changes were subjectively graded as normal (0), mild (1), mild to moderate (2), moderate (3), moderate to severe (4) and severe (5). This method was done according to Hair-Bejo *et al.* (2004) as a modified scoring method for previously established method.

IBD virus isolation: For viral isolation bursae from each flock collected and a 10% suspension was prepared in tryptose phosphate broth with 10 mg/ml of Streptomycin sulphate, 1 mg/ml Gentamycin sulphate and 1000 IU/ml of Penicillin. Five 11 day old specific pathogen free (SPF) eggs were inoculated with 0.1 ml of the suspension via chorioallantoic membrane route according to Paul *et al.* (2004). Mortality at day one post inoculation was not considered as it might be due to mechanical injury. Pathological changes in dead embryo day 2 to day 4 were checked for. Such change are varied including mild or severe congestion and hemorrhages in the feather, tracts, skin and toes and the congestion of the head.

Serology (ELISA): For serology, twenty three birds in each flock were bled and blood for serum was collected from wing vein using 1 ml syringe and kept overnight at room temperature and serum was then separated in Eppendorff tubes and preserved at -20°C. (Eterradossi *et al.*, 2004; OIE manual, 2004.

Enzyme Linked Immunosorbent Assay (ELISA) Technique was performed as described by the manufacturer, ELISA reader and infectious bursal disease ELISA kit were obtained from Bio-Check company - Holland.

Sera samples that preserved at -20°C, the antigen coated plate that consisted of 96 wells and the ELISA kit reagents that preserved at 2-4°C were adjusted to room temperature of 22- 27°C prior to the test.

Sera samples were diluted by adding 500 µL of the sample diluents (Phosphate buffer with protein stabilizers and sodium azide preservative (0.1% W/V) to each 1µL of the sample prior to the assay using automatic pipette with disposable tips. Hundred µL of diluted sample was added into each well and 100µL of undiluted negative control into well A 01 and well B 01 and 100µL of undiluted positive control into well C 01 and well D 01, the plate was then covered with lid and incubated at room temperature of 22-27°C for 30 minutes after which the contents of wells were aspirated and each well was washed with 300 µL of wash buffer (1 bag of powdered phosphate buffered saline with tween in 1 L of distilled water) for 4 times and wells were inverted and taped firmly on absorbent paper to dry. After which 100 µL of the conjugate reagent (sheep anti-chicken; Alkaline phosphatase in tris buffer with protein stabilizers, inert red dye and sodium azide preservatives (0.1% W/V))was added into each well and the plate was covered with lid and incubated at room temperature of 22-27°C for 30 minutes, the contents of the wells were then aspirated and washed 4 times with wash buffer (300 µL for each well) and the plate was inverted and taped firmly on absorbent paper to dry. Hundred µL of the substrate reagent (Diethanolamine buffer with enzyme co-factor) was added into each well and the plate was covered with lid and incubated at room temperature of 22-27°C for 15 minutes, after which 100 µL of the stop solution (Sodium hydroxide in diethanol-amine buffer) was added into each well to stop the reaction.

The absorbance values were measured and recorded at wavelength 405nm using ELISA reader. IBD antibodies

Table 2: Results of different diagnostic methods performed to test the IBD infected flocks

Histopa. grade	Bursa to B Wt($\times 10^{-3}$)	viral isolation	PM gross lesions	S/P ratio	Elisa Titre	Mortality %	Age days	Flock No
3.4±0.97	3.254±.125	Possitive	Possitive	5.254±0.521	18520±1245	45	57	1
3.5±1.43	3.581±.257	Negative	Possitive	4.263±0.852	14711±1512	9	40	2
0±0.00	3.547±.352	Possitive	Possitive	6.112±0.652	19735±1966	17	40	3
3.6±1.26	2.947±.852	Possitive	Possitive	6.851±0.657	21618±2145	31	25	4
2.8±1.39	2.454±.154	Possitive	Possitive	5.968±0.745	19950±1724	32	24	5
3.2±0.92	3.145±.252	Possitive	Possitive	6.652±0.521	21212±1841	49	42	6
0±0.00	4.012±.325	Possitive	Possitive	3.592±0.484	9731±1182	30	44	7
2.1±1.10	2.529±.132	Negative	Possitive	5.545±0.364	18816±2241	9	62	8
3±1.41	3.0254±.528	Negative	Possitive	4.752±0.554	12834±1532	19	48	9

titre and sample absorbance to positive absorbance (S/P) ratio of samples were calculated to interpret the results.

Results and Discussion

Data in Table 1 and Table 2 showed that outbreaks of infectious bursal disease during the year 2005 occurred in different areas in Khartoum state and all around the year and no difference observed between seasons. Pathogenicity of IBDV is effected by breed, age, immunization of the challenged birds, dose and route of inoculation and the presence of contaminating agents such as Adenovirus or Infectious anemia virus. (Rosenburger *et al*., 1975) Among the nine flocks involved in the present study Bovans breed represent 33.3% followed by Loghman and Hisex (22.2%), the least were Hiline and Hibrid breeds (11.1%). Variation in susceptibility among poultry breeds was also report by Mohamed *et al*. (2002) in Egypt. All flocks reported in this study occurred in light breeds (white leghorn) and no outbreak was reported in heavy broiler chickens indicating that light breeds are more susceptible than meat type chickens, this finding agree with Lukert and Saif (1991) and Abdul Ahad (2004).

IBDV infections occurred in flocks investigated in the present study, despite vaccination of all these flocks against IBDV twice, using different vaccination schedules and different vaccine strains (Table 1). In three flocks vaccination was done by the owner and the rest (6 flocks) was done by a veterinarian. The infection occurred in these flocks may propose vaccination failure (FAO manual, 1991). However IBDV has a potential for antigenic heterogeneity which result in frequent outbreaks in the field even in chicken flocks vaccinated against IBDV (Hassan *et al*., 1998). In the present study, outbreaks of IBDV were occurred at week 4 (22.2%), at week 6 (33.3%), at week 7 (22.2%) and at week 9 (22.2%). No outbreak occurred during the first three weeks, this finding agree with early report of Skeeles *et al*. (1978), or older than 9 weeks. Usually 5-10% of the birds in the infected flocks die but mortality can reach 30-40% (OIE manual, 2004). Mortality rates (Table 2) reported in this study varied greatly and ranged between 9 to 49%, this variation is also reported by Abdul Ahad (2004); Rahman *et al*. (1996) and recorded in OIE manual (2004).

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