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With 2 tables in the text.

Infectious Bursal Disease Virus (IBDV) in nine Flocks in Khartoum State, Sudan

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ملخص البحث

عند دراسة تسعة قطعان دواجن تعانى من مرض التهاب جراب فبريشس (قمبورو) بولاية الخرطوم في الفترة بين يونيو الى ديسمبر 2005 سجل المرض فى كل القطعان من سلالات إنتاج البيض الخفيفة (لقهورن البيضاء) و أن المرض منتشر في مناطق مختلفة من الولاية و يصيب الدواجن على مدار السنة. و لقد أوضحت الدراسة أن القطعان التى أصيبت تراوحت أعمارها بين 24−57 يوماً وكانت جميعها محصنة ضد المرض بعترات مختلفة من اللقاح. تراوحت نسبة النفوق بين 9 % و49%. الإصابات تم تشخيصها بولسطة تاريخ الحالة المرضية، العلامات الإكلينيكية والافات المرضيه وتم تأكيد المرض بواسطة التشخيص المصلى للمقايسة المناعية المرتبطة بالانزيم (ELISA)، و في سبع منها عن طريق التغيرات المرضية في الأنسجة و في ست منها عن طريق عزل الفيروس.

Summary

Nine Infectious Bursal Disease Virus (IBDV) suspected infected flocks, in Khartoum, were followed up during the clinical course of the disease over the period of January to December 2005. The investigations showed that the disease occurred in different poultry-producing areas in the State all around the year and caused a mortality that ranged between 9 and 49%. All cases of IBD were reported to the governmental veterinary clinics during this period. The disease occurred in light egg-producing type Leghorn, chickens and no cases were reported in heavy meat broiler chickens. The IBDV vaccine strains were commercially available in the Sudan (D78, Bio-Gumboro and Gumboro 3) including hot and intermediate strain (228E) vaccine. These vaccines were administered according to a variety of vaccination programmes which failed to protect the surveyed flocks from clinical IBDV infection. Diagnosis of the disease was based on clinical signs, gross pathology, histopathology and virus (IBDV) isolation in chicken embryo and was confirmed by serology using ELISA.

Introduction

Infectious bursal disease (IBD), known as Gumboro disease, is an acute highly contagious viral disease (Parkhurst *et al*., 1964). It is of global economic importance (Pitcoviski, 2003). The disease is currently responsible for huge economic loss in poultry industry worldwide (Vander Slufs, 1999).

It causes mortality that ranges from 30-40% (OIE, 2004) in addition to its immuno-suppressive effect. The characteristic gross lesions of the disease include dehydration of the muscles with ecchymotic hemorrhages, enlargement and orange discoloration of kidneys. The bursa of Fabricious shows the main diagnostic lesions in birds that die at the peak of the disease. It becomes enlarged and shows pale yellow discoloration. Intrafollicular hemorrhages may be found. Pin point hemorrhages on the skeletal muscles are usually prominent (OIE, 2004). Clinical signs include ruffled feathers, chicks reluctance to move, anorexia, watery diarrhea, trembling and severe prostration. (Ley *et al*, 1983; OIE, 2004)

The disease has been reported worldwide. It has become a major poultry disease in Pakistan during the last 5 – 7 years (Ahmed and Akhter, 2003). The disease has been reported in Malaysia (Hair-Bejo, 1993), Brazil, Dominican Republic and Venezuela (Alejandro and Pedro, 2004), Madagascar (Rajaonarisan *et a*., 1994), and in the late 1980's, an outbreak of the disease with high mortality was reported in Europe (Chittle *et al*, 1989). In the Sudan, Gumboro was first reported in western Sudan (Shauib *et al*, 1982).

Vaccination against IBDV is one of the significant component of the control (Ahmed and Akhter, 2003). Time of vaccination, type of vaccine, maternal-derived antibody (MDA) in the chicks and pathogenecity of IBDV field challenge are important factors that determine the efficacy of IBD vaccination (Hair-Bejo *et al.*, 2004). In practice, outbreaks are still being recorded despite the use of many vaccination schedules and a various vaccine strains. (Ahmed and Akhter, 2003).

The present study was conducted to investigate IBD outbreaks that occurred in Khartoum, Sudan with regard to vaccination practices adopted.

Materials and Methods

Case history:

Nine flocks of young chicks that showed clinical signs and post-mortem gross lesions of infectious bursal disease were reported to two governmental veterinary clinics. Seven affected flocks were in Khartoum and two in Khartoum North. They were keenly observed during the clinical course of the disease. Each flock was visited daily throughout the disease course for data collection and record.

Diagnosis of the disease was based on the clinical signs, post-mortem examination, virus isolation, serology, histopathology and bursa to body weight × 10^{-3} ratio (Eterradossi *et al*, 2004).

Bursa to Body weight ×10^{-3} Ratio:

Bursae of Fabricious of sick or freshly dead birds were removed at the peak of the clinical course and their weight were determined and calculated as a ratio of the body weight multiplied by 10^{-3}.

Histopathology:

Bursae of Fabricious were collected, fixed in 10% formalin, dehydrated in a series of alcohol concentrations, embedded in paraffin wax, sectioned at the thickness of 5 μm and stained with haemotoxylin and eosin (H & E). The histopathological changes were subjectively graded as normal (0), mild (1), mild to moderate (2), moderate (3), moderate to severe (4) and severe (5). This was done according to Hair-Bejo *et al* (2000) as a modified scoring method for previously established method.

IBD virus isolation:

Bursae collected from birds of each flock were suspended in 10% Tryptose Phosphate Broth to which 10 mg/ml of streptomycin sulphate, 1 mg/ml gentamycin sulphate and 1000 IU/ml of penicillin were added. Five 11-day-old, specific-pathogen-free (SPF) embryo-nated eggs were inoculated with 0.1 ml of the suspension via chorioallantoic membrane route according to Paul *et al* (2004).

Serology (ELISA):

Twenty three birds in each flock were bled from wing vein using a one ml syringe. The blood was kept overnight at room temperature and serum was then separated and preserved at -20 C till use. Enzyme Linked Immunosorbent Assay (ELISA) technique was performed according to the manufacturer´s instructions (Bio-Check Company, Holland). Ready antigen coated plate and the ELISA kit reagents that were preserved at 2-4^{o}C, were adjusted to room temperature of 22- 27^{o}C prior to the test.

Sera were diluted by adding 500 μL of the sample diluents to each 1μL of the sample. A volume of 100 μL diluted serum was added into each well and 100 μL of undiluted negative and positive control were added in duplicates. The plate was then incubated at room temperature for 30 minutes after which the contents of wells were discarded and each well was washed four times with 300 μL washing

buffer. Then the plate was rinsed with absorbent paper. Thereafter, 100 µL of the conjugate was added into each well and the plate was incubated at room temperature for 30 minutes. The contents of the wells were then discarded and the plate washed four times with washing buffer (300 µL for each well) and rinsed with absorbent paper. A volume of 100 µL of substrate reagent was added into each well and the plate was incubated at room temperature for 15 minutes.

The absorbance values were measured and recorded at wavelength 405 nm using (ELISA Reader). Antibodies titre was calculated as the ratio of sample absorbance values to the positive control absorbance (S/P) ones.

Results and Discussion

Table 1 and table 2 show that during the year 2005 many outbreaks of infectious bursal disease occurred at different localities in Khartoum wthout any seasonal variation. Among the nine flocks involved in the present study, Bovans breed represent 33.3% followed by Loghman and Hisex (22.2%) and the least were Hiline and Hibrid breeds (11.1%). Variation in susceptibility among poultry breeds was reported in Egypt by Mohamed *et al.* (2002). All outbreaks reported in this study occurred in light breeds (white leghorn) and no outbreak was reported in the heavy broiler chickens which might indicate that light breeds are more susceptible than meat type chickens. This finding agrees with those of Lukert and Saif (1997) and Abdul Ahad (2004). Pathological changes in dead embryo were checked in day 2 to day 4 and in embryos with heads, toes and feathers. Change varied from mild to severe congestion and hemorrhages.

Although the investigated flocks were vaccinated against IBDV twice using different vaccination schedules and different vaccine strains (Table 1), the disease occurred in them. In three flocks vaccination was done by the owner and in the rest (6 flocks) was done by a veterinarian. The occurrence of infection in vaccinated flocks raises many questions regarding the quality of vaccines used or the improper handling, reconstitution and use of the vaccine that lead to vaccination failure (FAO, 1991). In addition, IBDV has a potential for antigenic heterogeneity which results in frequent outbreaks in the field even in chicken flocks vaccinated against IBD (Hassan *et al*, 1998). In the present study, outbreaks of IBD occurred at week 4 (22.2%), week

Table 1: History of the flocks infected with IBDV in Khartoum State.

Flock No	Location	No. of birds	Breed	Date of outbreak	Vaccine strain used	Vaccination schedule (day)
1	Al Kalakla	4000	Hisex	May, 2005	D78-D78	15 to 42
2	Al Dikheenat	3000	Bovans	Feb., 2005	D78-D78	15 to 30
3	Al Dikheenat	1400	Hiline	Jan., 2005	228E-228E	14 to 28
4	Buri	6000	Loghman	Oct., 2005	D78-288E	14 to 21
5	AlShigalab	3100	Bovans	April, 005	BioG-BioG	14 to 21
6	Al Halfaia	4000	Hibrid	Oct., 2005	D78-D78	13 to 19
7	Al Rimala	1300	Bovans	Jul., 2005	D78-D78	21 to 28
8	Soba	4000	Loghman	Jan.,2005	D78-D78	21 to 28
9	Al Kadaro	3500	Hisex	Mar., 2005	G3-G3	15 to 21

Bio-G = Bio Gumboro, G3 = Gumboro 3.

Table 2: Results of different diagnostic methods performed to test IBDV infected flocks.

Fl. No	Age (day)	Mortality rate (%)	Antibodies titre (Ab titre)	S/P ratio	Gross lesion	Virus isolation	B/Bwt ratio	Histopath.
1	57	45%	18520±1245	5.254±0.521	P +ve	P +ve	3.254±.125	3.4±0.97
2	40	09%	14711±1512	4.263±0.852	P +ve	N -ve	3.581±.257	3.5±1.43
3	40	17%	19735±1966	6.112±0.652	P +ve	P +ve	3.547±.352	0 ±0.00
4	25	31%	21618±2145	6.851±0.657	P +ve	P +ve	2.947±.852	3.6±1.26
5	24	32%	19950±1724	5.968±0.745	P +ve	P +ve	2.454.154	2.8±1.39
6	42	49%	21212±1841	6.652±0521	P +ve	P +ve	3.145±.252	3.2±0.92
7	44	30%	9731±1182	3.592±0.484	P +ve	P +ve	4.012±.325	0±0.00
8	62	09%	18816±2241	5.545±0.364	P +ve	N -ve	2.529±.132	2.1±1.10
9	48	19%	12834±1532	4.752±0.554	P +ve	N -ve	3.0254±.528	3±1.41

Fl= Flock; B/Bt ratio = Bursa to b.wt. 10-3 ratio; Histopath= Histopathological change grade; P +ve=positive; N –v= Negative

6 (33.3%), week 7 (22.2%) and week 9 (22.2%) and no outbreak was reported in the first three weeks.

These findings agree with thatof Skeeles *et al*. (1978). On the other hand, usually 5 – 10% of the birds in the infected flocks die but mortality rate can reach as high as 30 - 40% (OIE, 2004). In this investigation, mortality rate varied greatly and ranged between 9 and 49% (Table 2) and such a variation has previously been reported (Abdul Ahad, 2004; Rahman *et al*, 1996; OIE, 2004).

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