

STUDY ON INFECTIOUS BURSAL DISEASE (GUMBORO) IN QUAILS

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Eighty migratory quails from EL-Areesh coast were trapped. Out of them, 18 quails (22.5%) were diseased and suffering mainly from digestive disorders. Mortality rate, as well as the characteristic clinical signs, post mortem lesions and histopathological changes were recorded.

Serosurvey of 80 serum samples revealed 5 (6.25%) positive for the presence of IBDV precipitating antibodies. 3 out 12 pooled bursae (25%) were positive to the presence of IBDV precipitinogen. Trials for virus isolation on the CAM of chicken embryos revealed the isolation of 3 IBD viruses from diseased quails. Isolated IBDV viruses were examined by electron microscopy. The disease was reproduced in quails to record the course, the clinical findings, post mortem and histopathological changes. Also seroscreening of serum samples by AGPT were done. The present findings focus the attention to the importance of the quails from the epidemiological point of view as potential source of infection of IBDV to domestic birds that housed with this species or near to this breeding.

INTRODUCTION

The original published description of infectious Bursal disease (IBD) by Cosgrave (1962) defined as acute clinical entity in 2-5 weeks old broilers occurring year around on the Delmarva Peninsula (U.S.A) from 1957 on wards.

Greenfield et al., (1986) reported that Japanese quails were refractory to IBVD infection. They showed no bursal changes and did not form precipitating antibodies. Quail diseases have been reviewed in Spain, (Revilla, 1974) and Japan, (McFerran and McNulty, 1993).

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In Egypt, a few literatures were published on migratory and even domestic quail diseases especially viral diseases. Thus this study was done to investigate the existence of IBDV in migratory quails.

MATERIALS AND METHODS

Quails:

A total of 80 migratory quails were trapped at 12 intervals from Al Areesh coast at the time of southern migration during autumn season of 2006. 18 of the examined quails birds showed clinical ill, while the others were appeared healthy, Table (1).

Samples:

a) **Bursae:** Of each of the 80 quails bursa as collected and stored at -70°C. Bursae were tested for IBVD antigen by agar gel precipitation test AGPT. Bursae were used for isolation.

b) Serum samples:

Sera were collected from quails and used for detection of precipitating IBDV antibodies by AGPT.

Agar gel precipitation test:

Antigen was prepared as described by Hirai and Shimakura, (1972) and the test was done as described by Wood et al., (1979).
Embryonated chicken eggs (ECE):

ECE were kindly obtained from baladi (native) parent stock (private farm) without history of previous vaccination against IBDV.

Virus isolation:

Pooling bursae from live, and freshly dead birds. An organ homogenate comprising 20:80% weight: volume of tryptose phosphate broth treated with antibiotics and centrifuged. The samples were inoculated and propagated in 9-11 day ECE inoculated via the chorioallantoic membrane (CAM) as previously described (Hitchner 1970).

Haemagglutination activity (HA):

The isolates were tested for HA against chicken, rabbit, quails, ducks, mice and sheep RBCs according to Anon, (1971).

Electron microscopy (EM):

3 samples of pool bursal homogenates were filtrated through 0.2 µm membrane filter, processed according to Nobuhiko et al., (1995) and Woolcok et al., (1996)

examined by Selmi electron microscope (Sunny joint stock company, Germany).

Pathogenesis:

Three groups of 14-day-old Japanese quail (*Coturnix coturnix*) inoculated intraocularly by three plates of IBDV, symptoms, PM lesions, histopathology and virus isolation done. Serum samples were collected 7, 14 and 21 days post inoculation for detection of precipitating antibodies against IBDV.

Histopathological examination: Was carried on bursa, spleen, liver and kidney of naturally and experimentally infected quails according to Bancroft and Stevens, (1990).

RESULTS AND DISCUSSION

Clinical findings of the examined quails:

Examination of the 80 migratory quail revealed that 18 (22.5%) diseased birds, while the other 62 birds (77.5%) were apparently healthy table (1). Most of the diseased quails were suffered from digestive disorders with anorexia, depression, diarrhea and cloacal pasting.

Post mortem findings of the examined quails:

Were varying from Enteritis, nephritis, hemorrhage in muscles to bursitis.

Detection of IBDV antigen (s) in bursal homogenates of examined quails:

Application of AGPT to detect the precipitogen in the tested bursae of the examined quails revealed that 3 pooled bursae were positive out of 12 pools tested (25%) table (1). 1-3 precipitating band(s) were detected within 1-2 days, as reported by Fadly and Nozerian, (1983), the development of 1-3 precipitin lines was discussed by Hirai *et al.*, (1972) who reported that they are attributed to differences in the diffusion rates of IBD viral antigens named (PA1, PA2 PA3).

Serological screening of IBDV precipitating antibodies in serum of examined quails:

Using AGPT, 5 out 80 serum samples were positive (6.25%) for the presence of precipitating antibodies, table (1). This findings were agreed with Farghaly and Sabry, (2000) However, they are higher than that recorded by Abd El Diem (1995). Such difference was suggested to be due to the

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difference in time of the study or the area in which the samples were collected.

Results of IBDV isolation in embryonated chicken eggs:

Table (2) showed the irregular pattern of embryonic mortalities 20-50% during days 2-6 post inoculations via CAM route with gross lesions include cutaneous haemorrhages particularly along feather tract and toe joints, sometimes edematous CAM with foci of hemorrhages, these lesions were similar to those reported by Hitchner, (1970); and Abou Zead, (1999).

Detection of precipitinogen in CAM of chicken embryos post inoculation with IBDV:

No precipitinogen could be detected in all examined CAM homogenates either of dead or survived embryos post inoculation with the 3 field IBDV isolates, this failure may be due to the lack or absence of precipitinogen in the examined CAM agrees the previous reported findings Abou Zead, (1999).

Electron microscopy (EM) examination:

Electron microscopy for bursal homogenates obtained from naturally infected quails, successfully revealed virus

particles with characters of icosahedral, naked with a size of 58.2 ± 2.01 nm with a range of 51-63 nm, Fig. (1), the virus particles were seen occurring in small clusters but sometimes in a single form. According to these morphological grounds the examined virus particles were identified as IBDV as described by Nobuhiko et al., (1995).

Haemagglutination (HA) activity:

No HA activity could be detected for the 3 IBDV isolates with chickens, rabbits, quails, duck, mice and sheep RBCs. The findings agreed with Bastami, (1980).

Course and clinical findings of IBD in experimental quails:

The course was characterized by acute onset, with incubation periods 3 days for G1 and 4 days for G2 and G3 with a course of 6-8 days. Relatively high morbidity rates 100% in G2 and G3 and 80% for G1, with mortality rates 10 in G1, G2 and 30% in G3, during which mortality raise rapidly for the first 4 days then decline sharply returning to normal during the days 5 and 6.

Affected birds showed generalized non specific clinical signs associated with ruffled

feather, watery solid vent (cloacal pasting) depression, water consumption not changed, feed intake is depressed (anorexia), documented depression in financial return from quail groups with IBD compared with uninfected group (4). These signs were similar to those previously reported by Khafagy *et al.*, (1991).

Gross pathology of IBD in experimental quails:

Echymotic hemorrhages in the muscle and fascia of the median aspect of the thigh, in the inguinal region and occasionally in the pectoral area and rarely on the mucosal surface of the proventriculus were seen (Fig.2).

The bursae have variable pathognomic lesions (Fig.3) including enlargement, edematous and covered with gelatinous yellow and/or reddish colour exudates (peribursites) and haemorrhagic bursa included the serosa, mucosa and pelvic surface were observed.

Kidneys were pale and enlarged with ureates. The livers were streaked with haemorrhages and enteritis was observed.

No macroscopic lesions were recorded in the spleen, table (3). Lesions were similar to those previously reported by Khafagy *et al.*, (1991).

The results present in table (4) demonstrated that infected quails shed IBDV 2 days post inoculation and lasts for at least 15 days but not exceeding 17 days, which was agreed with the reported work by Winterfield *et al.*, (1972).

Table (5) showed that IBDV precipitating antibodies were detected in all 3 groups of experimentally infected quails at 2 days for groups 1, 2 and 3 with a ratio of 10-40% and lasts for 21 days post inoculation. Generally the results in agreement with those reported by Bastami, (1980).

It is noted that diagnosis of IBDV in quails based on precipitating antibodies detect exposure in only 0%, 23.3%, 46.7 % 80% and for groups 1, 2 and 3 at 1, 3, 7, 14 and 21 days post inoculation, table (5) This due to the relative insensitivity of this procedure compared with serum neutralization test and ELISA test, Abou Zead (1999)

Hisopathological lesions of experimentally infected quails with IBDV:

The results showed that quails of all infected groups (1, 2 and 3) had microscopic lesions in the bursae, Fig. (4) showed that IBDV extremely induced an lymphocidal producing necrosis of

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lymphocytes in the medulla of bursa fabricius, inter follicular edema accompanied by heterophilic infiltration as previously documented by Henry et al., (1980), Sharma et al., (1989). Fig. (5) confirms that the lymphoid necrosis of lymphocytes of white pulp of the spleen with heterophilic infiltration which was agreed with Henry et al., (1980).

The liver showed fatty degeneration with haemorrhage Fig. (6) .The kidneys showed degenerative and necrotic changes in the epithelial cells of the proximal convoluted tubules with

hemorrhages and lymphatic infiltration Fig. (7).

In conclusion, the present work adds more confirmatory information on the positive effect of IBDV on performance and immune status of quails and transmission of IBDV though the migratory quails to Egyptian birds should be kept in consideration.

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Table (1): Signs and serological screening of the examined quails.

Code No	No of examined quails	+ve signs		AGPT in serum (+ve)		AGPT for birds homogenate	
		No	%	No	%	No	%
		1	7	-	-	1/7	14.3%
2	5	-	-	-	-	-	-
3	10	3/10	30%	-	-	+ve	100%
4	5	-	-	-	-	-	-
5	5	-	-	-	-	-	-
6	11	2/11	18.2%	1/11	9.1%	-	-
7	7	4/7	57.2%	-	-	+ve	100%
8	8	2/8	25%	-	-	-	-
9	6	-	-	-	-	-	-
10	3	-	-	1/3	33.3%	-	-
11	5	3/5	60%	2/5	40%	-	-
12	8	4/8	50%	-	-	+ve	100%
Total	80	18/80	22.5%	5/80	6.25%	3/12	25%

Table (2): Results of IBD isolation in chicken eggs.

No	Pattern of mortalities (days 0-7)*							Total mortalities	
	a	2	3	4	5	6	7	No	%
1	1	0	1	0	1	0	0	2/4	50%
2	0	0	0	1	1	0	0	2/5	40%
3	0	0	0	0	0	1	0	1/5	20%

(a) non specific deaths in the first day post inoculation were excluded from % mortality calculation.
* five eggs inoculated per sample.

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Table (3): Gross pathology of IBD in experimental quails:

Lesions	No of birds			
	G1	G2	G3	Total
Haemorrhage in muscles	3	2	4	9
Hemorrhage in proventriculus	1	0	1	2
Affected bursae	5	2	6	13
Kidney lesions	2	1	4	7
Liver lesions	0	1	0	1
Spleen	0	0	0	0
Enteritis	1	4	1	6

Table (4): Detection of IBDV antigen (s) by in bursal homogenate of the experimental quails.

Group	Days post inoculation			
	1	2	15	17
G1	0	100%	50%	-
G2	0	100%	40%	-
G3	0	100%	20%	-

Table (5): Detection of precipitating IBDV antibodies in serum of the experimental quails.

Group	Days post inoculation			
	1	2	14	21
G1	0	40%	100%	60%
G2	0	10%	60%	40%
G3	0	20%	80%	40%
Total	0	23.3%	80%	46.6%

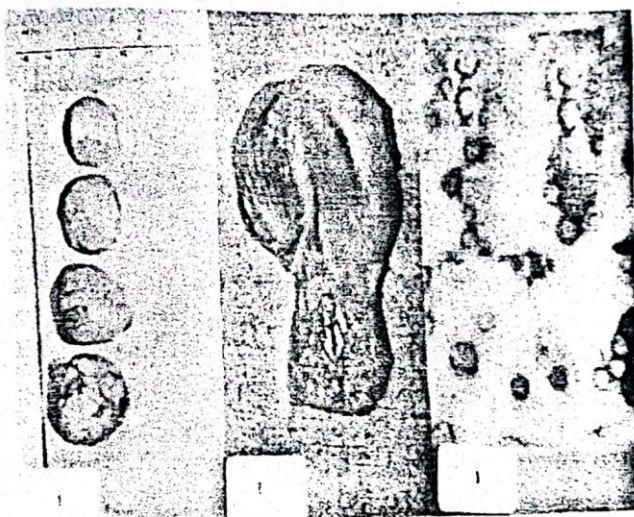


Fig. (1): Bursa of fabricius of experimentally infected quails with IBDV showed the developmental stage of infection (enlargement).

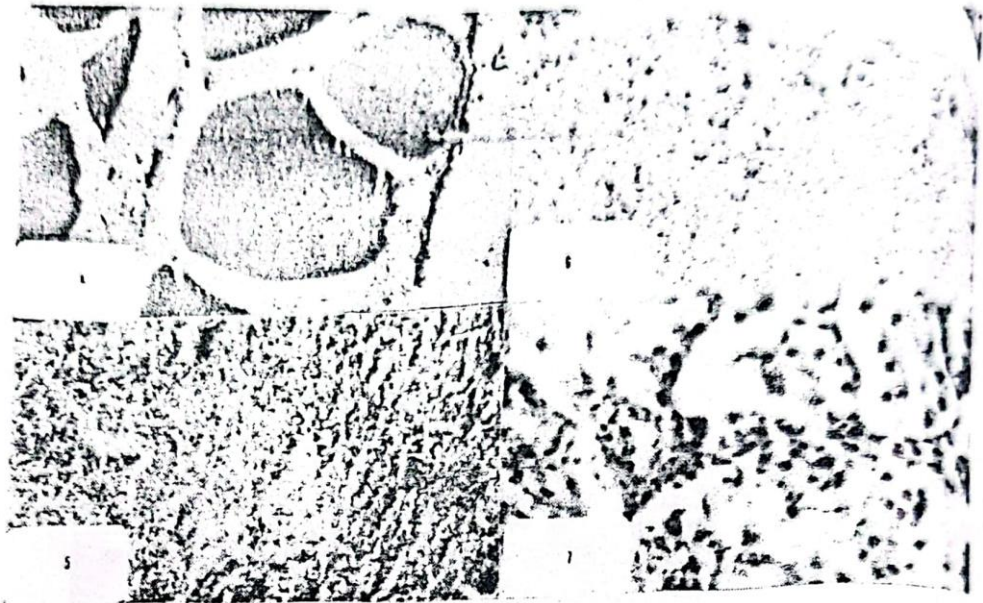


Fig. (2): Hemorrhage in junction between proventriculus and gizzard of experimentally infected quails with IBDV.

Fig. (3): Electron micrograph of filtered bursal homogenate.

Fig. (4): Bursa of fabricius of experimentally infected quails with IBDV showing degeneration of lymphoid follicles with depletion of lymphocytes and interfollicular oedema (H & E x 600).

Fig. (5): Spleen of experimentally infected quails with IBDV showing severe depletion of lymphocytes of white pulp and degenerative changes (H & E x 600).

Fig. (6): Liver of experimentally infected quails with IBDV showing fatty degeneration with hemorrhages (H & E x 600).

Fig. (7): Kidney of experientially infected quails with IBDV showing destruction in the epithelial cells of the renal tubules with hemorrhages and lymphatic infiltration (H & E x 600).

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