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INFECTIOUS BURSAL DISEASE IN DUCKS

(With 3 Tables and 13 Figures)

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مرض الجمبورو في البط

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تم فحص عدد ٢٠٠ عينة سيرم بط ووجد ٢٠% إيجابي باختبار الترسيب. تم عزل ١١ عترة إيجابي باختبار الترسيب وتم تمرير المعزولات في أجنه البيض والزرع النسيجي الخلايا الفيبروبيلاست خلايا الفيرو ووجدت تغيرات وتم التأكد باختبار التعادل لثلاثة معزولات والتأكيد الكيميائي بمقاومة الأثير والكلوروفورم درجة حرارة ٥٦°م واختبار التلازن لكرات الدم من أنواع مختلفة من الحيوانات والطيور. تم عدوى بط وبداري تسمين بأحد العترات ووضع معهم بداري وبط بالترتيب وتم الذبح عند ٤ ، ٧، ١٥ يوم وتم عمل الصفة التشريحية والتأكد من الفيروس في السيرم والاحشاء واختبارات الدم المختلفة والهستوباثولوجي والإختبار الإشعاعي.

SUMMARY

Two hundred serum samples were examined by agar-gel precipitation test and 20% were positive. Eleven isolate were obtained by agar gel pricipitation test. Virus gave cytopathic effect (CPE) in chicken embryo fibroblast and verocell and change in embryonated chicken eggs. Four strains of virus were charcterised by heat stability, chloroform, ether, heam agglutination resistance and three of them were used in neutralization test. One virus; inoculated in chicks group of chicks and duckling put with them and inoculated induckling and chicks put with them. Agargel pricipitation used for expermented serum and organ (bursas, spleens, kidneys, livers) and also immuno fluorscent. Heamatology and histopathology were done.

Key words: *Infectious bursal disease in ducks.*

INTRODUCTION

Natural and experimental infection with infectious bursal disease (IBD) virus has been reported to occure in turkey (Weiseman and Hitchener, 1978) and Quail (Weiseman and Hitchmer 1978; Hamouda et al., 1997; Ahlam and Sabry, 2000) village weaver (Nawathe et al., 1978 and Codon bleu (Nawathe et al., 1978) as well as chicken. The deaths of English sparrows an a form during an out break of IBD were reported (Ed Gar and Cho, 1965) but not confirmed data were presented. IB were isolatd from bigeon (Farghali et al., 2000). IBD virus has been isolated from feaces of healthy ducks and was shown to be serotype 4 (McFerran et al., 1980) but the pathogenicity and antibody response to the virus is not well documented. Yamadi et al. (1982) experiment inoculation of IBDV in ducks, only antibody response were recorded, the level increased after two weeks no post mortem lesion, histopathological chang or trial of reisolation.

Christopher (1982) reported antibody responses to inoculation of ducks with infectious disease (IBD) virus. Eddy, (1990) study the antibody responses of ducks following exprimental inoculation with IBD virus using either the chicken virus serotypes I and turkey isolate (serotype II) the titer was the highest in the first group and the highest titre was at 5 weeks.

Zhouzongan et al. (1998) made trial for isolation of IBDV in ducks, geese and sparrows. Two isolates of IBDV were obtained from ducks and sparrows. IBDV virus caused specific effect in chicken embryo fibroblasts and the isolate was pathogenic to specific pathogen free (SPF) chickens. Analysis of RNA and structural protiens of IBDV by SDS-PaGE indicatied that all IBDVs isolates consisted of ds RNA and there was no significant difference of RNA migration rates in agarose gels among the different. The two isolates were identified as IBDV serotype I. It is suggested that chickens are not the only natural reservoir of IBDV.

MATERIAL and METHODS

1- Virus detection:

25 bursas from duckling were hemogenized and diluted with Mem 1:1 and used for agargel against reference serum (Dr. Attia. virological section professor of virology veterinary medicine Cairo

University). For virus isolation (organ diluted 1:10) in chicken embryo and tissue culture CEFj.

2- Virus Isolation:

This was done in do day-old embryonated chiken egg by chorrio allantoic membrane (CAM) inoculation at ten days as well as in chicken embryo fibroblast cellculture and vero using tissue homogenet as inocula; 3 blind passages were carried out.

3- Agar gel precipitation antibodies:

Precipitating antibodies aginst IBD were detected by immune diffusion test accoroding to the method described by Anon (1977) 1.2% agarose dissolved in phosphate buffer saline (PBs) 8.5% sodium was poured in petridishes. After solidification wells were cut. The known reference serum were put in centre are serrounded by antigen to be tested and the opposite were done by sera. The petridishes were put in inculation in humid chamber and examined during 3 day for the presence of specific lines.

Sera: two hundred and sixteen serum samples were examined against reference antigen (which was kindly prorided by D.M. sabry) Egytic company by agar gel precipitation test.

4- Thermostability test:

Isolates in the form of tissue culture subgeated to 3 cytes of freering and thowing and centrifuged and distributed into tubes (1 ml per tube), incubated in water path at 56 for 5, 10, 15, 30, 60 minutes and samples were chicked for infectivity by tissue culture inoculation.

5- Sinsitivity to ether and chloraform:

Ether sensitivity was carried out according to the method by Andrews and Horstman (1949). Chloroform sensitivity technique was carried out according to the method described by Fieldman and Wong (1961).

6- Heam agglutination activity:

The isolate were tested for HA aclivity against chicken, mice rat, gainea pig. Sheep erethrocytes according to Anon (1977).

7- Neutralization test:

Serial ten fold dilution of antigen and constant amount of titrated reference serum 0.05 ml of vero added to each plate. the plates were incubated at 37°C incubator for 3 days was checked daily for neutralization indexes.

8- Pathogenicity:

Group of duckling were inoculated intra oculaorly with one isolate and chicken put with them and group of chicken in oculated

intraoculorly with the same isolate and duck put with them. The third group were kept as control. Bird sacrified from each group at 4, 7, 15 days after inoculation. Post mortem, heamatology, virus dectection were carried in agar gel pricipitation on serum samples and organs (Bursas, Spleens Kidnys, livers).

Tissucs from the same organ fixed in 40% aqueaus formalin embedded in paraffin sectioned and stand with H and E and imnmnofluorescent.

Fluorescent conjugated anti chicken gama globulin:

For use of inderect fluorescent antibody technique conjugated normal chicken gamma globulin was provided by Animal Health institute (Tumour section) Dokki Cairo.

9- Preparation of slidcdes fixation and staining:

Paraffin slides were prepared from experimentally infected ducks and chickens (bursas, livers splcens and kidneys).

The smears were mounted with hyperimmune sera and inculated in moist chamber at 37°C for 30-45 minetes slides were gently washed 3 times in PBs (pH 7.2) dried and mounted with conjugated gamma globulin and incubated again in moist shamber at 37°C for 30-45 minutes. The proposed slided were washed three times in PBs dried and carried with 3% Evan's blue stain.

RESULTS

Two hundred (200) sera of duckling were examined by Agar gel precipitation test (40) 20% were positive.

Virus isolation:

Eleven from twenty seven were positive for agar gel precipitation test (three of them showing reddness) virus gave cytopathic effect in chicken embry fibroblast and vero round Fig. (4), cell aggregation Fig. (5), cell nesting in two cases and necrotising changes Fig. (8) in the third day. Oedema of eye, ingorgment of blood vesseles of legs, parpoiled heart dwarfed in embryo and four of them were taken and characterised.

Table 1: Sensitivity to Ether and chloroform.

Isolate	Titer of isolate		
	Before	Ether	Chloroferm
GB	4,7,2	5.5	5.5
4	5.5	5.5	5.5
5	5.5	5.5	6.2
11	5.5	6.5	6.5

Termostability:

Virus are table from 1 hour.

Haemagglutination:

Isolates did not haemagglutination activity against chicken, mice, rat, gainea pig and sheep erythrocytes.

Neutralization index: Table 2.

Virus	Neutralization index
4	1-8
5	2-7
GB	2

Pathogenicity:

Diarhea appear induckling and chicken after four days and reddnes in bursa, heamorhage thig, (Fig. 2) liver streaked with heamorhag (Fig. 1), kidney increases. size and that appear (Fig. 3).

Table 3.

Organ Lision	4 day			7 day			15 days		
	Duck	Chicken	Ino./chick	duck	chicken	ino./ chick	duck	chicken	ino./ chick
Liver	3/3	2/4	3/4	1/3	4/4	4/4	1/2	-	4/4
Bursa	-		3/4	1/3	-	-	1/2	1/4	1/4
Kidney	2/3	2/4	1/4	2/3	3/4	¾	2/2	4/4	-
Splean	-	2/4	-	-	1/4	¼	-	1/4	-
Diarria	-	¼	1/4	-	1/4	-	-	-	1/4
Heamorhage in thigh	-	¼	1/4	1/3	3/4	2/4	-	3/4	3/4

Agar gel percipitation of serum 2/4 in inoculated chicken after 7 day and 4/4 after 15 day and chicken incubated with infected ducks showed 2/4 positive for agar gel and 1/4 after 24 days.

Organ showed positive for agar gel pricipitation test bursa of both chick and

after 4 days:

1/4 infected chicken bursa
1/4 infected chick spleen
1/4 infected chicken liver

after 7 days:

1/3 infected ducks bursa chicken put with them ¾ bursa
2/3 infected ducks kidney chicken put with them 2/4 spleen
1/3 infected ducks spleen chicken put with them ¼ liver

after 15 day:
½ infected duck kidney
½ infected duck liver
and chicken put with them 2/4 bursa

Immuno flouroscent:

Bursa spleen was positive at seven days in both infected chicks and duck and chick put with them after 15 day infected chick spleen kidney and ducks and only bursa of chick put with them Fig.. (3 ,7).

Heamatology:

PCV is higher in infected duck at 7 day and slightly higher at 15 day heamoglobin only decreased after 15 days.

PCV increased in infected ducks after 15 days but heamoglobin decreased in infected chicken after 15 days.

Decreases in white blood corpuscle in infected chicken and also red blood corpuscle.

Histopathology:

After 4 days liver showing congestion, fatty change, spleen showed increased in lamina of splenic artery Fig.. (13).

After 7 days bursa showed lymphocitic deplition increase inter follecular lymphocytic Fig.. (8) subcapsular oedema Fig.. (2) in bursas, liver show slightly congestion and kidney showed hyalinization and degeneration changs and lymphocytic depletion in infected bird Fig.. (11).

After 15 day bursa showed no changs except cell wall increased in size but kidney stil show degeneration in distal convaluted tubrule and chicken put with them infected duck showed kidney congestion glomerular cell flattened and liver showed fatty degenerative change Fig. (9, 10) bursa showed lymphocytic depletion of cell 30%, subcapsular oedema, oedema in centre of follicle and spleen showed lymphocytic depletion.

DISCUSION

Serum sample indicate 20% positive this indicate high result did not recorded before. Result for agar gel precipitation test against reference serum eleven isolate, trial for virus isolation from ducks were triald but only McFeran et al., 1980 and Zhou Zongan et al. (1998) succeed to isolate two isolate. Trial for isolation in chicken embryo fibroblast which give cytopathic effect were recoded by the same authors, we use vero cell and characterisation by heat stability.

cholorfern (Fieldman and Wong 1961). Ether (Andrews and Horstmen, 1949) and neutralization test.

Experimental infection was done in duckling and chicken that showed diarhea and heamorhage in thigh, liver streaked with haemorhages, kidney enlargred and bursa reddend 4 and 7 days (Yamada et al., 1982) did not record no pathognomic lesion. Experminental infection used in traocularly, histopathologic lesion was like those in chicks turkeys and quails and pigeon (Fraghli et al., 1989, Fraghli and Sabry 2000, Fraghli et al., 2000) organs were positive for Agar gel pricipition at 1 , 7, 15 days and Fa were positive at 4 – 7 and 15 days and thus agree with pricipitating antibody appear at 7, 15 day in chicken infected wit duck isolate or put with infected duck and this agree with Bastami 1980; Ahlam 1989, while serum did not show antibody until 2 weeks and that did not agree with Yamada 1982, Eddy 1990.

Haemoglobin were decrease in infected chicks and thus agree with Mohamed 1983.

From this result gumboro disease was reported in ducks and play a role in distribution of virus to chicken.

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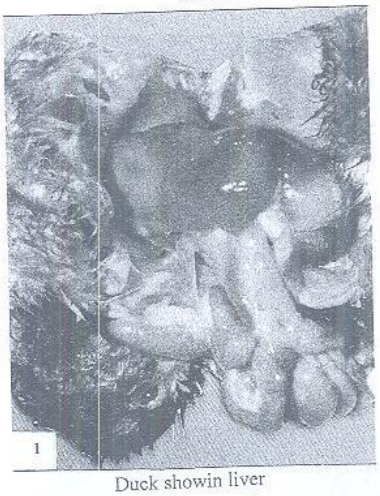
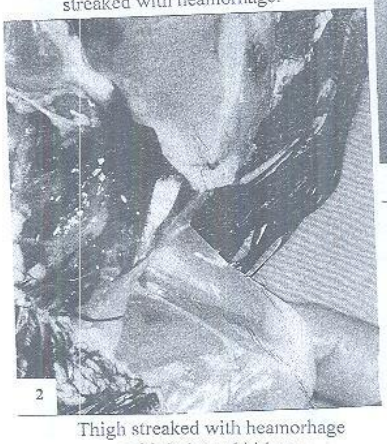


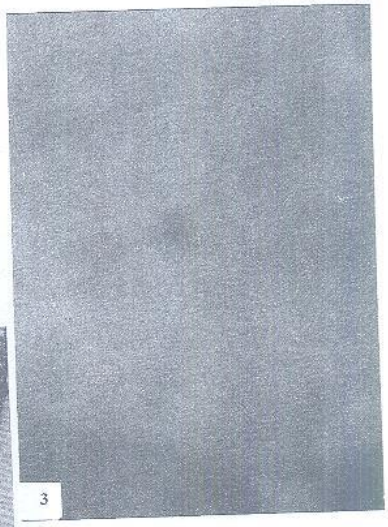
1

Duck showin liver
streaked with heamorhage.



2

Thigh streaked with heamorhage
with in larged kidney

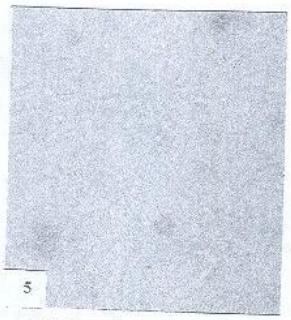


3

Flouroscend in tissue

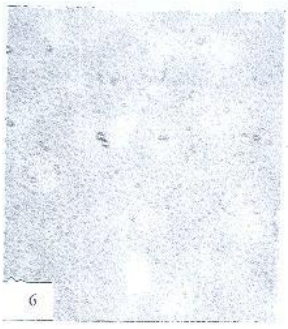
4

Cell showing rounding



5

Cell showing aggregation

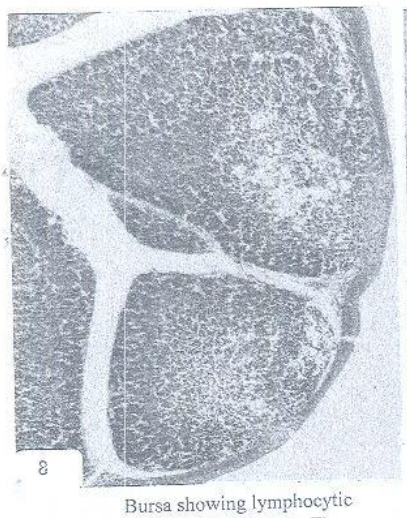


6

Cell showing necrobiticchang

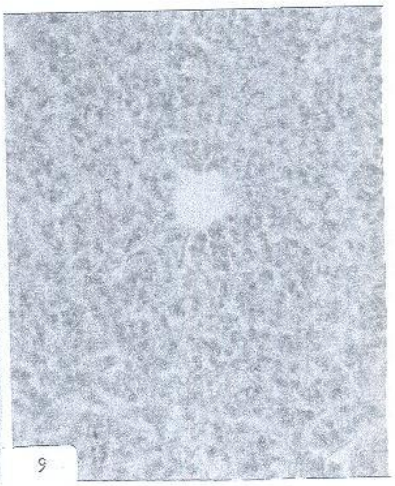
7

Flouroscend antibody



8

Bursa showing lymphocytic depletion (10 x H∞ E)

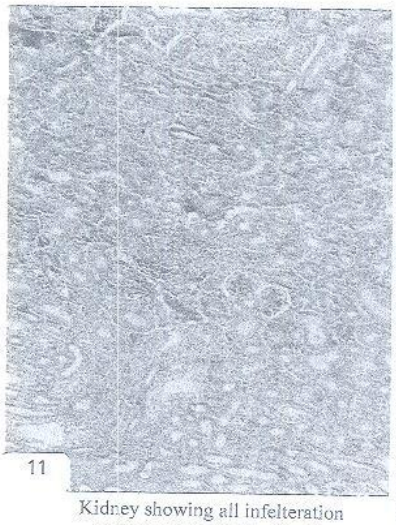


9

Liver showing fatty degeneration (40 x H∞ E)

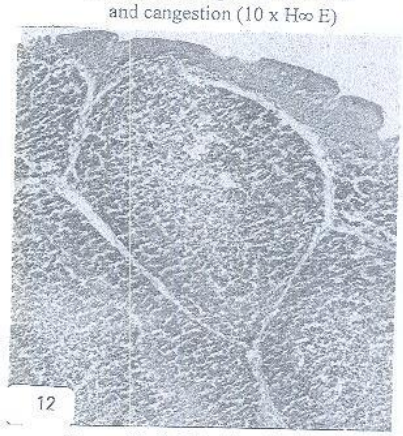
10

Fatty change in liver (10 x H∞E)



11

Kidney showing all infelteration and cangestion (10 x H∞ E)



12

Bursa of infed chicking showing depletion and oedema (10 x H∞E)

13

spleen with thickened artery after 7 day (x H∞E)