

قسم : أمراض الد واجسن :
كلية : الطب البيطرى - جامعة أسيوط .
رئيس القسم : أ . د . ابراهيم سكر .

مرض التهاب حوصلة فايريس في الرومي :

١ - تقرير مبدئي

صلاح موسى ، عبد اللطيف بيومي

تم اكتشاف الأجسام المناعية الترسيبيه في مصل ومخ بيض
الرومي وكذلك تم عزل ثلاثة عترات للمرض من بدارى الرومى
المصاب . وعند احداث العدوى الصناعية في الرومي بعتره
الفيروس شديده الضراوة والمعزولة من الدجاج لم تحدث
أى تغيرات مرضية ، وذلك بالمقارنة بالآفات المرضية التى
ظهرت بعد العدوى الصناعية باحدى العترات المعزولة
من الرومي .

أظهرت الدراسات السيرولوجيه أن العتره المعزولة تتشابه
مع عتره الدجاج ، ولكن لا تتطابق معها تماما . وتعكس
هذه الدراسات امكانية عدوى الرومى بهذا المرض واحتمال
وجود عترات من الفيروس خاصة بالرومى .

Depts. of Medicine & Poultry diseases and Dept. of Pathology,
Faculty of vet. Med. , Assiut University,
Heads of Dept. Prof. Dr. I. Sokkar and Prof. Dr. M.I. El-Sherry.

**INFECTIOUS BURSAL DISEASE IN TURKEYS:
I - A PRELIMINARY REPORT**
(With 2 Tables and 2 Figures)

By
S. MOUSA and A. BAYOUMI
(Received at 10/11/1982)

SUMMARY

Precipitating antibodies to the Cu-1 strain of infectious bursal disease (IBD) virus were detected in sera and yolk of turkeys. Three IBD virus isolates were recovered from infected turkey poults. Experimental infection with one isolate and with the highly pathogenic Cu-1 strain was conducted. Successful reproduction of the disease was achieved by the isolated virus. Lesions were described and discussed. The isolate was antigenically related to but not identical with the known Cu-1 IBD virus strain.

INTRODUCTION

There is a controversy in the literature about the susceptibility of turkey poults to infectious bursal disease (IBD) virus. Thus naturally occurring antibodies to IBD virus could not be detected in turkeys by some workers (HIROSE and HIRAI, 1976; and NAWATHE *et al.*, 1978). WEISMAN and HITCHNER (1978) reported turkey poults to be refractory to experimental infection with IBD virus. The virus could not be isolated from cloacal swabs and the birds did not respond immunologically. GIAMBRONE *et al.* (1978)* also reported that experimental infection with IBD virus passed subclinical over 6 blind passages in turkey poults. In contrast, MCNULTY *et al.* (1979) isolated IBD virus from naturally infected turkeys.

The present work reports on a naturally occurring IBD infection in turkeys at Elwaady-Elgadeed and observations made during experimentally induced infection.

MATERIAL and METHODS

Flock history: During the period from October 1981 to March 1982, mycoplasmosis and aspergillosis were microbiologically confirmed in two turkey farms at Elwaady-Elgadeed. The first farm included a breeder flock of 2500 laying birds, as well as 4500 poults of 1 - 4 weeks of age and 7500 birds of 4 - 12 weeks of age. In the second farm (distanced about 12 Km) turkey poults are reared until marketed at 5 - 7 months of age. Mortalities recorded during that period amounted to 12% in breeders and 38% in poults. In May 1982, samples for the present investigation were taken when disease condition characterized by watery diarrhoea and ruffling was noticed among turkey poults. A morbidity rate of 15% and mortality of 2.5% were recorded within one month. At autopsy, lesions of nephritis and bursal atrophy or congestion were noticed.

Viruses: A homogenized bursa tissue of chicks infected with the highly pathogenic Cu-1* strain of IBD virus was used as a reference virus.

* : It was kindly supplied by Prof. Dr. I. Gylstorff, Inst. fuer gefluegel-Krankheiten, Muenchen, West Germany.

Three isolates, designated T26, T73, and T193, were recovered from bursae of dead, 4 to 18-week-old turkey poults with bursal lesions suggestive of IBD infection. Bursae reacted positively with Cu-1 antiserum in AGP test. Bursal suspensions caused death of inoculated susceptible chicken embryos with lesions similar to those reported for IBD virus (GIAMBRONE *et al.*, 1976). Infected chorioallantoic membranes (CAM) were homogenized, diluted 1 : 1 in normalsaline, centrifuged and the supernate was stored at -20C. The 50% embryo infective dose $6EID_{50}$ was determined for Cu-Pstrain and T26 isolate by the method of REED and MUENCH (1938) and was found to be $10^{3.7}$ and $10^{3.7}$ EID, respectively.

Hyperimmune sera: Antisera against CU-1 strain and T26 isolate were prepared by injecting 6-month-old chickens with infected CAM extract 4 times intramuscularly over a 3-week period. A virus dose of 10^6 EID₅₀ was used. Chickens were bled 10 days after the last injection and sera collected and stored at -20 °C.

Chicken embryos: 10 to 11 -day-old susceptible white Leghorn chicken embryos were provided by the Faculty of Agriculture, Assiut.

Agar-gel precipitin (AGP) test: The microtechnique was carried out after NANSI (1958).

Virus neutralization (VN) test: This was carried out by the constant-serum virus-dilution method, with tenfold virus dilutions (CUNNINGHAM, 1966). Sera were considered positive to IBD virus antibodies when reduced one log₁₀ unit or more of the titer of Cu-1 IBD virus.

Histopathology: Bursae of experimentally infected birds were fixed in 10% buffered formlin, sectioned and stained with haematoxylin and eosin.

Serological survey of IBD in turkeys:

a- Sera: A total of 1250 serum samples from 2 turkey farms of different ages were tested for the presence of precipitins to the reference strain of IBD virus.

b- Yolk: 1300 infertile eggs from breeders of the first farm were subjected to AGP test, after treatment with chloroform (LUCIO and HITCHNER, 1979), for the presence of precipitins to the reference strain of IBD virus.

Experimental infections:

A total of fifty 5-week-old Bronze turkey poults were divided into three groups. Group A & B, each of 20 birds, were infected intraocularly with 10^5 EID₅₀ / bird of the Cu-1 strain and T26 isolate, respectively. Group C of 10 birds served as uninfected control. All birds were checked for freedom of precipitating and neutralizing antibodies to IBD virus before infection. Each group was kept in a separate unit under strict isolation. Ten birds from each of group A & B and 5 birds from group C were subjected to postmortem examination at 4 and 7 days porinfection (p.i.). Bursae were subjected to histopathological examination and AGP test. Sera of sacrificed birds were examined individually for presence of precipitating antibodies and as one pool for neutralizing antibodies.

RESULTS

Fourty eight of 1250 serum samples (3.8%) and 20 of 1300 yolk samples (1.5%) showed precipitins to the Cu-1 strain of IBD virus. There was no clear correlation between age and number of positive serum samples as shown in table 1.

BURSAL DISEASE IN TURKEYS

Out of 320 examined dead turkey poults from the two farms, 3 bursae were positive with Cu-1 antiserum in AGP test. Chicken embryos inoculated with bursal extracts from dead birds died 4 to 6 days p.i. Embryos were congested and showed icteric or congested livers and congestion of kidneys.

Results of cross-neutralization tests with the Cu-1 reference strain of IBD virus and isolate T26 and their hyperimmune sera are shown in table 2. It is evident that cross-neutralization took place and calculated indexes were higher in the homologous system than in the heterologous.

Results of experimental infections

Birds of group B which were infected with isolate T26 showed marked depression, diarrhoea and inability to move. Morbidity was 60%, while mortality was 20%. One bird died on third day and 3 birds died on the fourth day p.i.

At 4 days p.i. both sacrificed (6) and dead birds (3) showed slightly enlarged and oedematous bursae and congested kidneys. Microscopically, bursae showed slight degeneration in lymphoid follicles with scattered lymphoblasts in the medulla.

At 7 days p.i. bursae of sacrificed birds were markedly atrophied and firm in consistency. Histologically, the lymphoid follicles were greatly reduced in size with relative increase in the interfollicular interstitia. Not only numerical atrophy of lymphocytes but also necrobiotic and degenerative changes in the lymphoid elements were well prominent. Some inflammatory cells could be seen in the interfollicular tissue (Fig. 1).

Serologically, all sacrificed birds showed precipitating and neutralizing antibodies (neutralization index 2.8) 7 days p.i. but not 4 days p.i.

Birds of groups A and C showed no signs of illness or postmortem lesions and were free from any microanatomical changes. Bursae showed no lesions (Fig. 2), precipitinogen could not be detected and the virus could not be reisolated. Their sera showed neither precipitating nor neutralizing antibodies.

DISCUSSION

Precipitating antibodies to the Cu-1 strain of IBD virus were detected in both sera and yolk from two turkey farms in El-Waady El-Gadeed province during increased mortality among young age groups. IBD virus precipitinogen was detected in examined bursae of dead turkey poults. Three isolates were recovered from those bursae, which produced lesions in chicken embryos similar to those described for IBD virus (GIAMPRONE *et al.*, 1976).

Reproduction of the disease in turkey poults, with marked clinical signs and gross and microscopic bursal lesions, was successfully established by intraocular application of one isolate designated T26 and failed with the Cu-1 reference IBD virus strain. This suggests either the specificity of the T26 isolate to turkeys or its adaptation to this species after natural passage. Since experimental IPD virus infection in turkeys with a chicken isolate remained subclinical regardless of passage level (GIAMPRONE *et al.*, 1978), it would appear that isolate T26 is turkey specific. That the T26 isolate is antigenically related to but not identical with the known Cu-1 strain of IBD virus is evidence by the results of cross-VN tests.

Further work is being carried out for characterization of the T26 isolate and the other 2 isolates.

REFERENCES

- Cunningham, C.H. (1966): A laboratory guide in virology. 6th ed. Burgess Publ. Co. Minncapolis, Minn. 77.
- Giambrone, J.J., C.S. Edson, R.H. Page, O.J. Fletcher, B.O. Barger and S.H. Kleven (1976): Effect of infectious bursal agent on the response of chickens to Newcastle disease and Mareks disease vaccination. *Avian Dis.* 20, 534-544.
- Giambrone, J.J., O.J. Fletcher, P.D. Lukert, R.K. Page and C.E. Eidson (1970): Experimental infection of turkeys with infectious bursal disease virus. *Avian Dis.* 22, 451 - 458.
- Hirose, M; and K. Hirai (1976): Precipitating antibody against IBD virus in egg yolk and serum of chickens. *Research Bulletin of the faculty of Agriculture, Cifu University No.* 39, 165 - 170.
- Hucio, R. and S.B. Hitchner (1979): Infectious bursal disease emulsified vacaine: Effect upon neubralizing-antibody levels in the dam. *Avian Dis.* 23, 466 - 478.
- McNulty, M.S.; G.M. Allan; and J.B. McFerran (1979): Isolation of IBDV from turkeys. *Avian Pathol.* 8, 205 - 212.
- Nansi, W. (1958): Slide gel diffusion precipitin test, *Nature*, 181, 1289 - 1291.
- Nawathe, D.R.; O. Onunkwo; and I.M. Smith (1978): Serological evidence of infection with the IBDV in wild and domestic birds in Nigeria. *Vet. Rec.* 102, 444.
- Reed, L.J. and H. Muench (1938): A simple method of estimating 50 percent endpoints. *Am. J. Hyg.* 27, 493 - 497.
- Weisman, J. and S.B. Hitchner (1978): Infectious bursal disease virus infection attempts in turkeys and Coturnix quail. *Avian Dis.* 22, 604 - 609.

BURSAL DISEASE IN TURKEYS

Table 1. Results of examination of turkeys sera for precipitating antibodies to IPD virus (Cu-1 strain).

Farm	Age group	No. of birds	serum samples	Pos. samples	%
I	1 to 4 weeks	4500	60	2	3.3
	4 to 12 weeks	7500	450	18	4.0
	9 to 14 months	2500	250	9	3.6
II	12 to 22 weeks	8500	490	19	3.8
Total		23,000	1,250	48	3.8

Table 2. Cross neutralization between Cu-1 strain of IPD virus and isolate T26.

Virus	Antisera	
	Cu-1	T26
T26	3.2 ⁺	4.1
Cu-1	4.3	2.5

+ log₁₀ serum neutralization index.



Fig. (1): Bursa 7 days p.i. showing atrophy of the follicles. decreased number of lymphocytes, degenerative changes and relative increase in interstitial tissue. H & E (X100).



Fig. (2): Bursa, showing normal structure and size of lymphoid follicles. H & E (X400).

