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Histopathology of infectious bursal disease in non-lymphoid organs of chickens

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RÉSUMÉ

OKOYE (J. O. A.). — Histopathologie de la maladie de Gumboro dans les organes non lymphoïdes du poulet. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (3) : 229-234.

Un élevage de poulets de chair a été infecté avec le virus de la maladie de Gumboro (IBD) isolé au Nigeria. Quelques cas de pycnose sur des cellules épithéliales des tubes urinifères et des canalicules ont été observés sur des poulets sacrifiés 1 et 2 jours après l'infection (PI).

Par la suite, seuls les poulets qui sont morts naturellement ont montré des signes de nécrose rénale extrêmement sévère au 5e jour, coïncidant avec les taux de mortalité les plus élevés. On a noté une atteinte épithéliale des canalicules et une infiltration par des cellules mononucléaires à partir du 7e jour et au-delà. La nécrose des hépatocytes n'a été constatée que sur les poulets morts le 6e jour. Des modifications dans les muscles de la cuisse et de la poitrine sont apparues en premier sur les poulets sacrifiés au 3e jour comprenant congestion, œdème, hémorragie et nécrose. Des coupes du cerveau, du poumon et du pancréas des animaux infectés et des témoins n'ont révélé aucune lésion.

Bien qu'il n'y ait aucune preuve histopathologique d'une relation avec les maladies des complexes immuns dans la pathogénie de cette infection, les auteurs suggèrent que la nécrose rénale pourrait être une cause significative de la mortalité dans la bursite infectieuse du poulet.

Mots clés : Maladie de Gumboro - Rein - Foie - Muscle - Histopathologie.

SUMMARY

OKOYE (J. O. A.). — Histopathology of infectious bursal disease in non-lymphoid organs of chickens. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (3) : 229-234.

A flock of broilers was infected with a Nigerian isolate of infectious bursal disease (IBD) virus. Pyknosis of some epithelial cells of the renal tubules and ducts was observed in chickens sacrificed on days 1 and 2 post-infection (PI).

Thereafter, only the chickens that died on their own showed signs of renal necrosis which was most severe on day 5 PI when the highest mortality was recorded. There were casts in the ducts, and infiltration by mononuclear cells was observed from day 7 PI onwards. Necrosis of hepatocytes was seen in only the chickens that died on day 6 PI. Changes in the muscles of the thigh and chest first appeared in chickens sacrificed on day 3 PI and included congestion, oedema, haemorrhage and necrosis.

Sections of the brain, lung, and pancreas of the infected and control chickens did not show any lesion. Although there was no histopathological evidence of immune-complex involvement in the pathogenesis of the disease, it is suggested that renal necrosis could be a significant cause of mortality in IBD.

Key words : Infectious bursal disease - Kidney - Liver - Muscle - Histopathology.

INTRODUCTION

Infectious bursal disease (IBD) was first described by COSGROVE (5). Since then much of the pathology of the disease has not been well understood. Young chickens under 2 weeks of age suffer subclinical form of the disease in absence of maternal antibodies (13, 28). The mechanism of this age-related immunity is yet to be identified. The upper age limit of susceptibility appears to depend on the involution of the bursa of Fabricius (bursa) which contains surface immunoglobin M-bearing B cells which have been reported to be the target cells for IBD virus (IBDV) infection (12, 19). But other workers have shown

that IBDV can replicate in other cells *in vivo* (3, 14, 18). In fact SCHAT *et al.* (26) observed more haemorrhages in the muscles and intestines of embryonally bursectomised chicken than in intact chickens infected with IBDV thereby also questioning the earlier theory of indispensability of the B cells in initiation of IBD (8, 15). Mortality in IBD has also been associated with depletion in complement level (8, 27) and to clotting abnormality (28). The involvement of immune-complex in the pathogenesis of IBD was suggested by LEY *et al.* (16) when they demonstrated immunofluorescence in the glomeruli of infected chickens. In this study histopathological evidence of immune-complexaemia or ARTHUS reaction is examined in some non-lymphoid organs. The microscopic changes in the lymphoid organs have been described in earlier publications (20, 23).

MATERIALS AND METHODS

Flock history and IBDV

The chickens and the local Nigerian isolate of IBDV used are the same with those already described in the studies of the persistence of the virus and the appearance of precipitins in infected chickens (21). The virus was obtained as 20 p. 100 suspension of bursae of chickens that died of confirmed field outbreaks of IBD in Nsukka area (22). The suspension was found to have a bursal $lesion_{50}$ (BL_{50}) titre of $10^{4.8}$/0.5 ml by method of REED and MUENCH (25). Two hundred chickens were given 0.05 ml of the bacteria-free suspension while 60 that served as control had 0.05 ml of only the sterile phosphate buffered saline (PBS) intra-ocularly.

Histopathology

Five infected and 2 control birds were sacrificed daily for 15 days post-infection (PI). The kidney, liver, muscles of the chest and thigh, pancreas, lung, brain, and proventriculus of the sacrificed and 19 dead chickens were fixed in 10 p. 100 formal saline for a minimum of 24 h. They were processed and embedded in paraffin wax. Thin sections 5µ thick were cut, stained with haematoxylin and eosin (H & E) and examined under light microscope.

RESULTS

Histopathological sections of the kidney of chickens sacrificed on days 1 and 2 PI were congested and haemorrhagic while some epithelial cells of the tubules and ducts were pyknotic and eosinophilic. By day 3 PI oedema and degeneration of the epithelial cells were evident. In birds that were sacrificed on day 4 PI, eosinophilic casts were present in the ducts and tubules. But in those that died of IBD on the same day, there was generalized pyknosis and karyorrhexis of the epithelial cells and more casts in the tubules and ducts. The main renal lesions observed in birds that were sacrificed on day 5 PI were congestion and haemorrhage. Degeneration was not severe. Lesions in birds that died on the same day (Photo 1) were similar — but more severe — to those of the birds that died on day 4 PI. But no casts were seen and there was hyperplasia of the epithelial cells in some ducts and tubules.

On day 6 PI, kidney of sacrificed birds had few degenerative lesions mainly in the ducts, some of which contained casts of desquamated epithelial cells. Heterophils were found around the large collecting ducts. But kidney of birds that died on that day showed severe karyorrhexis and degeneration of the epithelial cells. Hyperplasia of epithelial cells and large eosinophilic casts in the tubules and ducts were also observed (Photo 2). By day 7 PI there was much progress in recovery. Most of the tubules and ducts were normal. But severe focal mononuclear cell infiltration was observed (Photo 3). Lesions on days 8 and 9 PI were similar to those of day 7 PI. On day 12 PI infiltration by mononuclear cells was still evident. Oedema and haemorrhage were seen in few of the renal samples on day 15 PI. The kidney of control birds showed no remarkable changes.

Liver was congested and haemorrhagic on day 1 PI. By day 3 PI oedema and degeneration of some hepatocytes were present. Chickens that died on days 4 and 5 PI had degenerated hepatocytes while birds sacrificed at the same period showed only congestion and haemorrhage which were also present in the dead birds. Karyorrhexis of the hepatocytes was found in birds that died on day 6 PI. Degeneration was last observed on day 8 PI. By day 12 PI only congestion of few portal veins was observed. There was no lesion in the

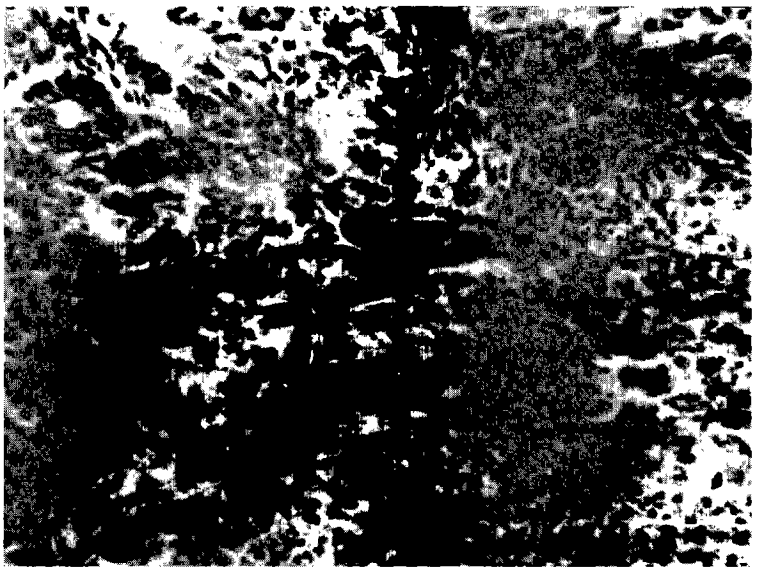

Photo 1. — Kidney of chicken that died of IBD on day 5 PI showing necrosis of the epithelial cells of the tubules and ducts.
H & E × 400

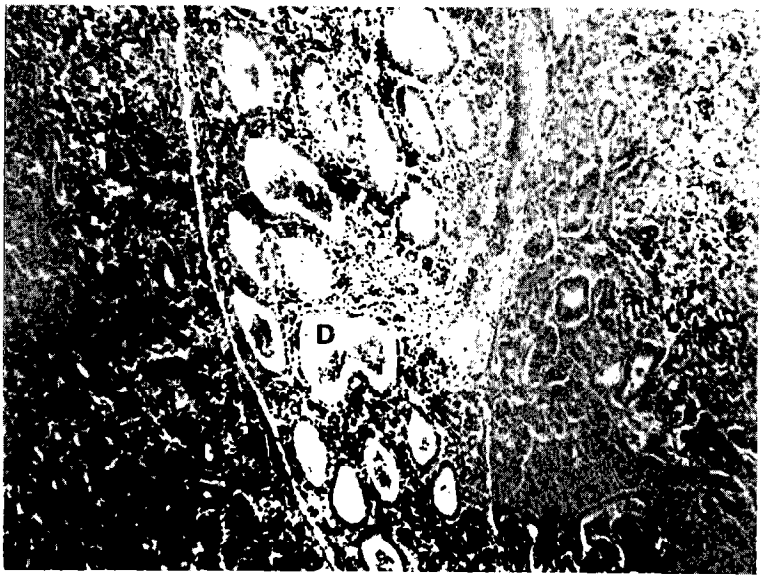

Photo 2. — Kidney of chicken that died of IBD on day 6 PI showing large casts of tissue debris in distented ducts (D).
H & E × 100

liver by day 15 PI. The organ in control birds had no lesion.

Muscle lesions first appeared in birds sacrificed on day 3 PI and comprised congestion, oedema, haemorrhage, and necrosis (Photo 4). In birds that died on day 4 PI, additional lesion was the presence of large mononuclear cells that appeared to be phagocytes. Similar lesions were seen in birds that died on day 6 PI while those that were sacrificed on days 4 and 5 PI showed only congestions and haemorrhage. Oedema, congestion, haemorrhage, necrosis, and infiltration by large mononuclear cells were found on days 7 and 8 PI. The muscle fibres were still necrotic by day 9 PI when the last observation was made. The control birds had no significant muscle lesion.

In birds that were sacrificed on day 6 PI,

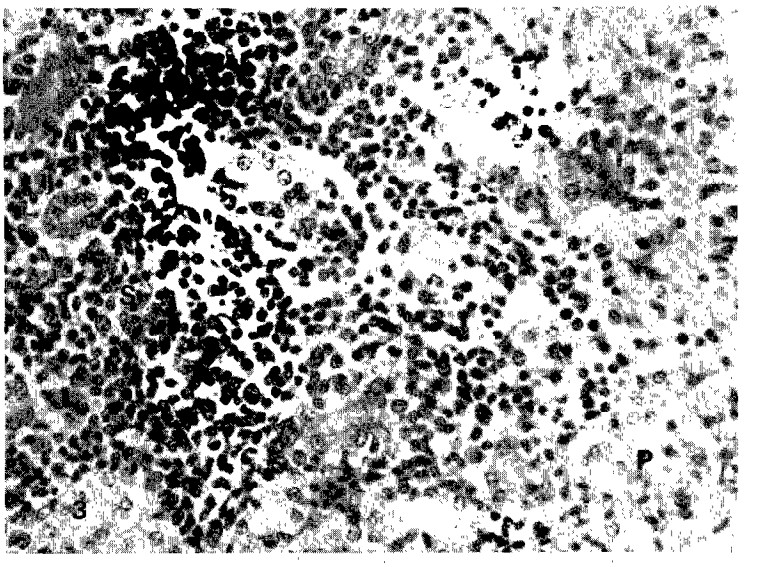

Photo 3. — Kidney of chicken that was sacrificed on day 8 PI showing focal lymphocytic infiltration (S) and degeneration of the epithelial cells (P). H & E × 400.

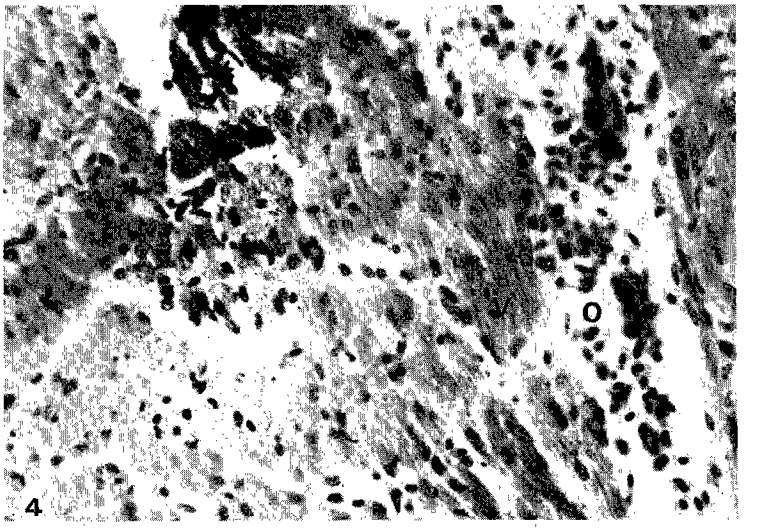

Photo 4. — Thigh muscle of chicken that died of IBD on day 4 PI showing haemorrhage and oedema (O) and necrosis of the muscle fires (V). H & E × 400

the proventricular glands were congested while those that died on that day also had many necrotic glandular cells in the lumina. The organ was normal by day 8 PI. No lesion was observed in the proventriculus of control chickens. Sections of the brain, lung, and pancreas of infected and control chickens showed no lesion.

DISCUSSION

In the kidney there was no sign of neither thickened glomerular basement membrane nor mesangial cell proliferation. Similar observations have been made by LEY *et al.* (17) who also reported that ultrastructural change suggestive of acute immune-complex glomeru-

lonephritis in IBD was rare. Furthermore HENRY *et al.* (11) found atrophic glomeruli in birds examined on days 1 to 5 PI. The infiltration by mononuclear cells has also been described (11, 17). But the generalized and coagulative necrosis of the epithelial cells observed in this study appear to be more severe than already described for the kidney in IBD (4, 11). This could be part of the reason why IBD in Nigeria is commonly associated with abnormally high mortalities of up to 43.8 p. 100 (24), over 50 p. 100 (6) and 11.5 to 33.5 p. 100 (22). HELMOLDT and GARNER (10) and LEY *et al.* (17) recorded tubular necrosis in few of the infected chickens.

Apart from pyknosis in some epithelial cells on days 1 and 2 PI, chickens sacrificed on days 3 to 15 PI showed no evidence of renal necrosis. But all those that died on days 4 to 6 PI had generalized karyorrhexis and many casts in the ducts. Tubular necrosis was most severe in chickens that died on day 5 PI when the highest daily mortality (52.7 p. 100 of the entire mortality) was recorded. These observations tend to suggest that generalized karyorrhexis in the tubules and ducts may be the immediate cause of death in IBD. Previous studies of renal microscopic changes in IBD have been mainly on sacrificed chickens (4, 10, 11, 17). This could be part of the reason why these earlier workers found mild renal changes.

The histopathological lesions in the liver appear to be in agreement with observations of CHO and EDGAR (4). Necrosis of the hepatocytes although present in less than 30 p. 100 of the cells was found in only the chickens that died on day 6 PI. The hepatic changes do not appear to be of much significance in the pathogenesis of IBD.

Arteritis was not observed in the muscles of the chest and thigh. This supports the observation of SCHAT *et al.* (26) who produced muscle lesions in embryonally bursectomised chickens which could not have been able to produce antibodies and immune-complexaemia.

The observations in this experiment have not shown any histopathological evidence of immune-complexaemia in the pathogenesis of IBD. Changes in the dead and sacrificed were of different types in the kidney and liver while they were similar but more severe in dead than in sacrificed chickens in the lymphoid organs (20, 23). However, microscopic changes in acute immune-complex glomerulonephritis could be subtle (2). This may be the case in IBD which has short duration. Consequently, immunofluorescence has been found to be a more sensitive method of detecting acute glomerulonephritis (7).

Earlier observations (21) support the report that the clinical signs of IBD seem to depend on the rate and amount of viral production in B lymphocytes (1). The rate of virus detection has been found to be the same irrespective of age and bursectomy in chickens (8, 26). But these workers did not determine the titre of the virus in the organs of the groups at various periods.

RESUMEN

OKOYE (J. O. A.). — Histopatologia de la enfermedad de Gumboro en los órganos no linfoideos del pollo. *Rev. Elev. Méd. vét. Pays trop.*, 1985, **38** (3) : 229-234.

Se han infectado pollos con el virus de la enfermedad de Gumboro aislado en Nigeria. Se observaron algunos casos de picnosis en células epiteliales de los tubos uriníferos y de los canalillos en pollos sacrificados 1 y 2 días después de la infección.

Luego, sólo los pollos muertos naturalmente mostraron síntomas de necrosis renal sumamente graves al 5° día, coincidiendo con las tasas de mortalidad más elevadas. Se notaron un ataque epitelial de los canalillos y una infiltración por células mononucleares a partir del 7° día y después. No se observó la necrosis de los hepatocitos más que en los pollos muertos al 6° día.

Modificaciones en los músculos del muslo y del pecho aparecieron primero en los pollos sacrificados al 3° día, es decir congestión, edema, hemorragía y necrosis. Cortes del cerebro, del pulmón y del páncreas de los animales infectados y de los testigos no mostraron ninguna lesión.

Aunque no existe ninguna prueba histopatologica de una relación con las enfermedades de los complejos inmunos en la patogenia de dicha infección, los autores sugieren que la necrosis renal podría ser una causa significativa de la mortalidad en la enfermedad de Gumboro.

Palabras claves : Enfermedad de Gumboro - Riñon - Hígado - Músculo - Histopatologia.

REFERENCES

1. BECHT (H.). Infectious bursal disease virus. *Curr. top. Microbiol. Immun.*, 1980, **90** : 107-121.
2. BENACERRAF (B.), POTTER (J. L.), McCLUSKLEY (R. T.), MILLER (F.). The pathologic effects of intravenously administered soluble antigen-antibody complexes. II. Acute glomerulonephritis in rats. *J. exp. Med.*, 1960, **111** : 195-200.
3. CHEVILLE (N. F.). Studies on the pathogenesis of

Gumboro disease in the bursa of Fabricius, spleen, and thymus of chickens. *Am. J. Path.*, 1967, **51** : 527-551.

4. CHO (Y.), EDGAR (S. A.). Characterization of infectious bursal disease. *Poult. Sci.*, 1972, **51** : 60-69.
5. COSGROVE (A. S.). An apparently new disease of chickens-avian nephrosis. *Avian Dis.*, 1962, **6** : 385-389.
6. Delegation of the Federal Republic of Nigeria. Gumboro disease in the Federal Republic of Nigeria. *Bull. Off. int. Epizoot.*, 1977, **88** : 291-292.
7. DE WARDENER (H. E.). The kidney : an outline of normal and abnormal structure and function. New York, Churchill Livingston, 1973.
8. FADLY (A. M.), NAZERIAN (K.). Pathogenesis of infectious bursal disease in chickens infected with virus at various ages. *Avian Dis.*, 1983, **27** : 714-723.
9. FADLY (A. M.), WINTERFIELD (R. W.), OLANDER (H. J.). Role of the bursa of Fabricius in the pathogenicity of inclution body hepatitis and infectious bursal disease viruses. *Avian Dis.*, 1976, **20** : 467-477.
10. HELMBOLDT (C. F.), GARNER (E.). Experimentally induced Gumboro disease (IBA). *Avian Dis.*, 1964, **8** : 561-575.
11. HENRY (C. W.), BREWER (R. N.), EDGAR (S. A.), GRAY (B. W.). Studies on infectious bursal disease in chickens. 2. Scoring microscopic lesions in bursa of Fabricius, thymus, spleen, and kidney in gnotobiotic and battery reared White Leghorns experimentally infected with infectious bursal disease virus. *Poult. Sci.*, 1980, **59** : 1006-1017.
12. HIRAI (K.), FUNAKOSHI (T.), NAKAI (T.), SHIMAKURA (S.). Sequential changes in the number of surface immunoglobin-bearing B lymphocytes in infectious bursal disease virus-infected chickens. *Avian Dis.*, 1981, **25** : 484-496.
13. HITCHNER (S. B.). Persistence of parental infectious bursal disease antibody and its effects on susceptibility of young chickens. *Avian Dis.*, 1971, **15** : 894-900.
14. KAUFER (I.). Electron-microscope studies of the pathogenesis of infectious bursal disease after intrabursal application of the causal virus. *Avian Dis.*, 1976, **20** : 483-495.
15. KAUFER (I.), WEISS (E.). Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens. *Infect. Immun.*, 1980, **27** : 364-367.
16. LEY (D. H.), YAMAMOTO (R.), BICKFORD (A. A.). Immune-complex involvement in the pathogenesis of infectious bursal disease virus in chickens. *Avian Dis.*, 1979, **23** : 219-224.
17. LEY (D. H.), YAMAMOTO (R.), BICKFORD (A. A.). The pathogenesis of infectious bursal disease : serologic, histopathologic, and clinical chemical observations. *Avian Dis.*, 1983, **27** : 1060-1085.
18. MULLER (R.), KAUFER (I.), REINACHER (M.), WEISS (E.). Immunofluorescent studies of early virus propagation after oral infection with infectious bursal disease virus (IBVD). *Zentbl. VetMed. Reihe B.*, 1979, **26** (2) : 345-352.
19. NAKAI (T.), HIRAI (K.). *In vitro* infection of fractionated chicken lymphocytes by infectious bursal disease virus. *Avian Dis.*, 1981, **25** : 831-838.
20. OKOYE (J. O. A.). The histopathogenesis of infectious bursal disease in the thymus, spleen and caecal tonsil of chickens. *Trop. Vet.*, 1984, **2** (4) : 225-232.
21. OKOYE (J. O. A.). Persistence of infectious bursal disease virus and the appearance of precipitins in infected chickens. *Trop. Vet.*, 1984, **2** (2) : 91-102.
22. OKOYE (J. O. A.), UZOUKWU (M.). Characterization of Nigerian strains of infectious bursal disease virus of chickens. Clinico-pathological manifestations of naturally occurring field outbreaks. *Bull. anim. Hlth Prod. Afr.*, 1982, **30** : 193-197.
23. OKOYE (J. O. A.), UZOUKWU (M.). Histopathogenesis of infectious bursal disease in the bursa of Fabricius. *Trop. Vet.*, 1984, **2** (2) : 91-102.
24. ONUNKWO (O.). An outbreak of infectious bursal disease of chickens in Nigeria. *Vet. Rec.*, 1975, **97** : 433.
25. REED (L. J.), MUENCH (H.). A simple method of estimating 50 p. 100 end-points. *Am. J. Hyg.*, 1938, **27** : 493-497.
26. SCHAT (K. A.), LUCIO (B.), CARLISLE (J. C.). Pathogenesis of infectious bursal disease in embryonally bursectomised chickens. *Avian Dis.*, 1981, **25** : 996-1004.
27. SKEELES (J. K.), LUKERT (P. D.), DEBUYSSCHER (E. V.), FLETCHER (O. J.), BROWN (J.). Infectious bursal disease viral infections. II. The relationship of age, complement levels, virus-neutralizing antibody, clotting and lesions. *Avian Dis.*, 1979, **23** : 107-117.
28. SKEELES (J. K.), SLAVIK (M.), BEASLEY (J. N.), BROWN (A. H.), MEINECKE (C. F.), MARUCA (S.), WELCH (S.). An age-related coagulation disorder associated with experimental infection with infectious bursal disease virus. *Am. J. vet. Res.*, 1980, **41** : 1458-1461.