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## Occurrence of runting and stunting syndrome in broiler chickens in the Sudan

ELMUBARAK (A.K.), KHEIR (S.A.M.), ABUEL GASIM (A.I.). Apparition du syndrome d'amaigrissement et de retard de croissance sur des poulets de chair au Soudan. *Revue Élev. Méd. vét. Pays trop.*, 1990, 43 (3) : 317-322

La première apparition d'une infection à réovirus sur des poulets de chair a été observée au Soudan dans le plus grand élevage industriel de la région. La maladie était caractérisée cliniquement par un retard de croissance, des boiteries, un plumage clairsemé, une dépigmentation des pattes et, de temps à autre, une rétraction de la tête et du cou. En général, un élargissement du proventricule, une atrophie du pancréas et des anomalies osseuses ont été notés. Parmi les changements histopathologiques, on peut citer l'hépatite, la néphrite, la myocardite et la péricardite, l'entérite catarrhale, la névrose du pancréas, l'encéphalomalacie et des altérations de type rachitique. Tous ces symptômes sont décrits en détail. Un réovirus a été isolé à partir des oiseaux malades, puis réisolé sur des poulets infectés expérimentalement. L'infection a été confirmée par les tests d'immunoélectrophorèse et de précipitation sur gel d'agar-agar. *Mots clés* : Poulet - Réovirus - Perte de poids - Symptôme - Diagnostic - Soudan.

### INTRODUCTION

Runting and stunting syndrome have been recognized recently and reported in many parts of the world under different names (6, 8, 11, 9, 16, 17). It has a considerable economic importance for the broiler industry. It is still not clear if the disease is due to one or more agents. However, the possible causative agents include parvovirus-like particles, enterovirus-like particles, rotavirus-like particles, rotavirus, reovirus and *Treponema hyodysentariae* (7). McFERRAN (7) explained that the occurrence of the disease was due to interference between the immune system and a combination of other factors; this explains that the disease had different patterns in various areas. The disease had not been recognized in the Sudan before, and reovirus had never been isolated within this country. This report describes the first isolation of reovirus and the clinical and pathological changes associated with the outbreak of runting and stunting syndrome in the country.

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### History

The Arab-Sudanese Poultry Company (ASPC), the largest poultry industry of the country, is located on the Southern outskirts of Khartoum. It occupies an area of 4,800 acres where farms for egg production, broilers, parent flocks, hatcheries, processing plants and feed mills are located.

On February 26, 1985, several broiler chickens were received in the Poultry Pathology department of the Central Veterinary Research Laboratory. The history indicated a noticeable increase in mortality during the last 5 to 6 weeks in all broiler farms. Losses were estimated to be 15%. The chickens had signs similar to those described for helicopter-like disease. The affected birds exhibited a reduction in growth rate, poor feathering, leg weakness and were unable to stand. Nervous signs and depigmentation of the shanks were also observed. Since these signs were not encountered previously in the diagnosis department, a visit to the farms was made for a closer examination of these flocks and to collect more samples. The chickens were of the Hydrobred breed that was imported from Holland as day-old chickens. They were raised on the floor in closed houses with automatically controlled environment. The ration was balanced; however, during the period from January 9th, 1985 to February 7th, 1985, 10% broiler concentrate was used and in December only 5%.

### MATERIALS AND METHODS

#### Chickens

About 4-6 broiler chickens with clinical signs of the disease from each age group were examined. Apparently unaffected mates of the same age were examined too. Twenty White Hisex birds, one day old, were used for experimental infection. The chicks were hatched locally from parent flocks. Ten chickens were inoculated per os with 0.5 ml of isolated reovirus suspension. The other ten were left as uninoculated

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controls. Each group was kept in a separate cage in a separate room with food and water *ad libitum*. Chickens were necropsied at the end of the 4th week. Intestines, brains, bursae and spleens were taken for virus isolation.

### Virus isolation

Samples from intestines, brains, bursae and spleens were prepared for embryonated egg and cell cultures inoculation according to GEORGIU (4). The cytotoxic effect on cell culture was recorded.

### Cell culture

Primary liver cell culture were prepared from 14-day old chickens embryos according to the method previously described by ADAIR *et al.* (1). The cells were plated in disposable plastic tissue culture plates (Sterilin, England). Three plates per sample suspension were used and examined daily for cytotoxic effect.

### Agar gel precipitation test

A known reovirus antigen and positive serum were obtained from Rhône-Mérieux Laboratories (France). Tissue culture harvested from infected cell cultures and serum samples were examined by the agar gel precipitation test according to the method of OLSON (10).

### Counter immunoelectrophoresis (CIE)

The method used by BERG (2) for the infectious bursal disease virus was used for serum samples collected from the infected flocks.

### Bacteriological examination

Bacteriological media such as blood agar and McCONKEY agar were used for primary isolation from livers and spleens of the infected chickens.

### Pathologic examination

Standard procedures were used during necropsy. Gross lesions were recorded and tissues for histopathological examination were kept in 10% formalin. They included livers, spleens, intestines, pancreases, kidneys, lungs, hearts, brains, bursae and bones. Decalcified bones were impregnated with silver nitrate and placed under incandescent light for a few minutes.

## RESULTS

### Necropsy findings

At necropsy, rickets-like changes were prominent in the skeletal system. The keel bone was crooked, the rib curvatures were distorted and the costochondral junction was beaded. The bones were soft or brittle and the joints, especially the hock were enlarged. The head of the femur was very easily broken during disarticulation from the acetabulum. Enlargement of epiphyses and widening of epiphyseal plates were also noticed. Calcification was very poor as illustrated by dipping the split epiphyses in silver nitrate solution and exposing it to incandescent light for several minutes. The brains were yellowish and soft.

The proventriculus was enlarged and the intestines empty, but in some cases they were swollen. The pancreas was atrophic and shreaded in a few birds. Most of the birds examined had little or no fat. Occasionally, peritonitis, hepatitis with focal necrosis and splenitis with whitish nodules were also observed. From these cases *E. coli* and gram positive bacilli were isolated.

### Histopathological examination

The liver revealed moderate to severe hepatitis characterized by proliferation of mononuclear inflammatory cells within the portal areas associated with blood vessels and around dilated and hyperplastic bile ducts.

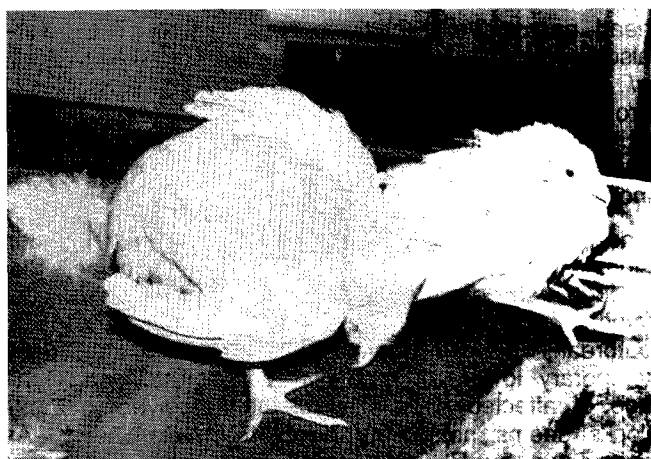
Changes in the kidney included congestion, hemorrhage, lympho-follicular proliferation, glomerular hypercellularity, enlargement of Bowman's capsules, and tubular degeneration and accumulation of homogenous eosinophilic material.

Myocarditis with oedema or mononuclear cell infiltration was detected occasionally as well as pericarditis with fibrinous exudate, granulomatous reaction, lymphoid cells and/or heterophilic infiltration. Acute to subacute catarrhal enteritis characterized by necrosis and sloughing of the epithelium at the tips of the villi, oedema, and mononuclear cell infiltration in the lamina propria were the most important changes in the intestinal tract. Shortening and thickening or fusion of the villi and Goblet cell proliferation were evident. Lymphoid hyperplasia and lymphoid follicle proliferation were common. Occasionally coccidial colonies were detected. Exudative proventriculitis with lymphoid cell infiltration of lamina propria and deep glands, mucus cell proliferation and sloughing of the tips of the villi were seen.

Degenerative changes were detected in the acinar tissue of the pancreas. They were minimal or absent around the islets. These included partial or complete disappearance of zymomatic granules and loss of glandular arrangement. The acinar cells were shrunken and basophilic vacuolar changes and edema were prominent around blood vessels. Lymphoid foci or follicles were encountered occasionally.

Degenerative changes detected in the brain were frequent particularly in the cerebellum. Blood vessels were markedly hyperaemic. There was perivascular oedema and occasional cuffing with mononuclear cells. The neurons were degenerated. Perineuronal oedema and vacuolations were also detected. Areas of malacia due to liquefactive necrosis were also seen. Similar degenerations were seen in the spinal cord. Changes in the meninges were exudative.

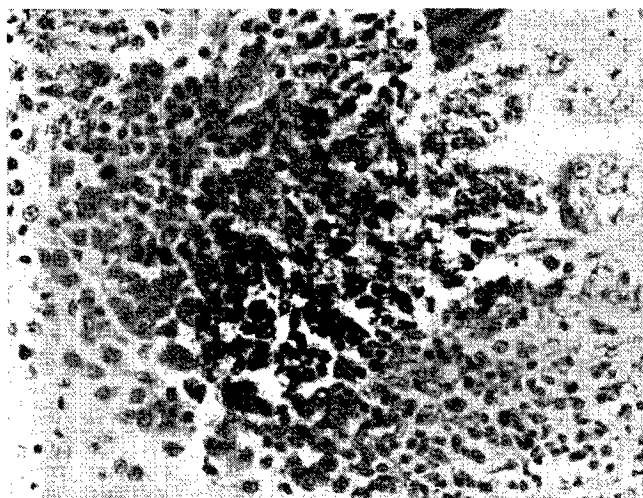
Depending on the age, rickets-like changes, osteoporosis and osteomalacia were detected in the skeletal system. Bone necrosis, spicule of poorly calcified bone, and wide osteoid zones were evident. Metaphyseal cartilage had its hypertrophic zone enlarged and its columnar arrangement disappeared. The bone marrow was replaced by fibrous tissue in some areas. Small hemorrhages could be detected (photos 1, 2, 3, 4a and b).



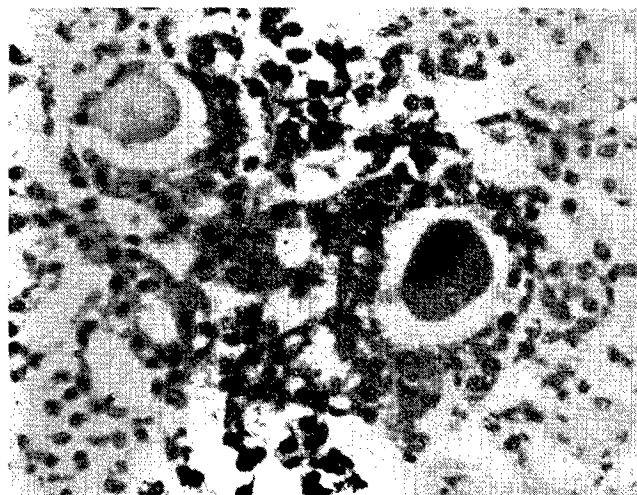
*Photo 1 : Six-week-old chickens. Note extreme variation in size, abnormal feathering, retraction of head, leg weakness and depigmentation of shanks.*

### Antibodies against reovirus

Using Counter Immunoelectrophoresis method (CIE), thirteen out of twenty-four serum samples examined were found to be positive for reoviral antibodies.



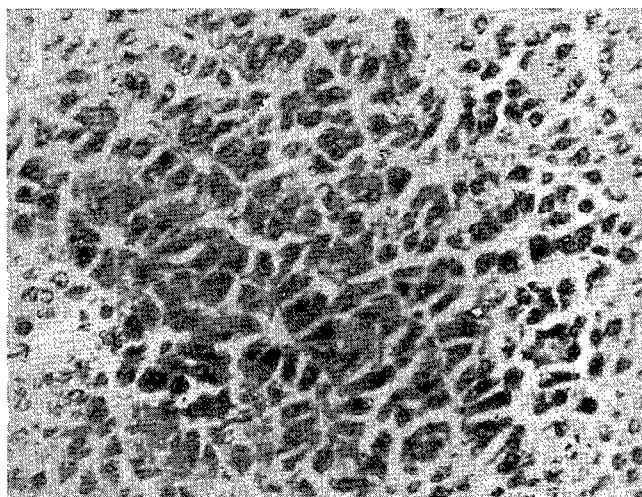
*Photo 2 : Liver. Note proliferation of mononuclear cells within portal area around dilated and hyperplastic bile duct.*



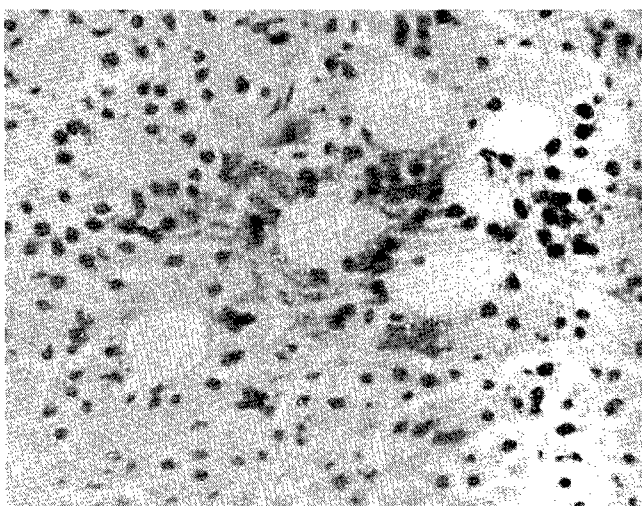
*Photo 3 : Kidney tubules. Note degeneration and accumulation of homogeneous (eosinophilic) material in their lumens.*

### Virus isolation

No evidence of cytotoxic effect was observed in the cell cultures inoculated with spleen, bursa and brain homogenates; only cells inoculated with intestinal homogenate showed syncytial formation indicative of virus replication. The isolated virus was shown to be reovirus.



a)



b)

Photo 4 : Pancreas. Degenerative changes in acinar tissue. (a) Note loss of glandular arrangement. (b) Note cell shrinkage, loss of granules and prominent vacuolar change.

### Experimental chickens

The chickens were weighed in groups weekly for 4 weeks. The infected group was found to be lower in weight compared to the control. The average weight of the infected chickens was 38, 72, 91.2 and 103.1 while that of the control chickens was 51.5, 82, 95.4 and 108.6 grammes at weeks 1, 2, 3 and 4, respectively. The infected groups were uneven in their growth pattern as some were stunted. On *post-mortem* examination no specific lesion could be detected. Reovirus

could be isolated only from the intestinal homogenate. In the control group chicks were normal and no virus was isolated.

### DISCUSSION

The signs and lesions detected in affected chickens are typical of those seen in broiler flocks in Europe (5), United State (11) and Australia (12). Depigmentation of skin and shank is described as of major economic importance (11). However, in this country, it has no commercial significance and thus the depigmentation associated with the disease is not a worry to broiler raisers.

The degenerative pancreatic changes that were seen in this case characterized by vacuolation and loss of zygomatic granules are identical to those already described (13, 14, 15). The percentage of affected birds was very high: however, the atrophy and marked interstitial fibrosis could not be detected in our case. This might be due to the pathogenicity or degree of affinity of our isolate to pancreatic tissue.

In about every case examined pathological degenerative changes were very prominent particularly femoral head necrosis and brittle bone structure. They were also prominent in cases investigated by WANDER *et al.* and VERTOMMEN *et al.* (16, 17). The low level of broiler concentrate for 3 weeks and the advanced age of chickens might have led to increasing these changes. It is obvious that they resulted in the high incidence of lameness observed.

Encephalomalacia and the accompanying nervous signs such as torticollis were prominent in older birds. Though described before, these signs did not seem of importance, nor to have the extent seen in the present outbreak. This could be attributed to the isolate, or the temporary low level of broiler concentrate and/or the age of affected birds. The usual diet given to these flocks seems not to be deficient in vitamin E. As speculated by RUFF (15), this might be the consequence of selenium and vitamin E deficiency because of the catarrhal enteritis and the accompanying diarrhoea which are characteristic of this pathology. COLANGO *et al.* (3) indicated the good responsiveness to vitamin E treatment in similar conditions. The isolation of reovirus from the intestines of broiler chickens with typical signs and lesions of infectious stunting syndrome and the reduction of growth rate and size produced in day-old chickens that were inoculated with tissue culture-propagated isolates confirm earlier reports of this syndrome (11, 16).

## CONCLUSION

The broiler industry is a new business in this country and unlike egg production, it started in large farms that have imported day-old or parent broilers from Europe. This outbreak did not seem to be of an internal origin as the farms were isolated the ones from the others and appropriate hygienic procedures were routinely applied. Moreover, the disease has not been observed before in this country. It is speculated, therefore, that the disease originated from imported chicks, from their parents or from the importated vaccines and drugs. The disease has not been reported to the laboratory earlier because mortality was not alarming in the first weeks. The characteristic signs of the disease had escaped notice and the unevenness of

the flocks may have been attributed to overcrowding. This new problem will add more to the complications from the newly appearing disease like infectious bursal disease and infectious bronchitis that were not existing in the country. It is economically costly since hundreds of thousands of birds are kept together in the broiler farms. Once a new disease appears, it is bound to stay as the health programmes are not so strict in quite a large proportion of our poultry industry.

Presence of a concurrent disease with this syndrome has not been reported before by REECE *et al.* (14). Here too, we found *E. coli*, gram positive bacilli and coccidia associated with lesions in pericardium and intestines, respectively. A specific immunosuppression associated with this pathology needs investigation.

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The first outbreak of reovirus infection in broiler chickens was observed in the Sudan in the largest poultry industry of the country. The disease was characterized clinically by growth retardation, lameness, poor feathering, shank depigmentation and occasionally retraction of the head. Grossly there was enlargement of the proventriculus, atrophy of the pancreas and bone abnormalities. Histopathologic changes included hepatitis, nephritis, myocarditis, pericarditis, catarrhal enteritis, pancreatic necrosis, encephalomalacia and ricketic changes. Details were described. Reovirus was isolated from affected birds and reisolated from experimentally infected chicks. Infection was confirmed by immunoelectrophoresis and agar gel precipitation tests. *Key words* : Chick - Reovirus - Runting and stunting syndrome - Diagnosis - Sudan.

ELMUBARAK (A.K.), KHEIR (S.A.M.), ABUELGASIM (A.I.). Aparición del síndrome de adelgazamiento y de retraso de crecimiento en pollos de cría en Sudán. *Revue Elev. Méd. vét. Pays trop.*, 1990, 43 (3) : 317-322

Se observó la primera aparición de una infección por reovirus en pollos de cría en Sudán, en la mayor cría industrial de la región. Se caracterizaba clínicamente la enfermedad por un retraso de crecimiento, cojeras, un plumaje ralo, una depigmentación de las patas y de vez en cuando una retracción de la cabeza y del cuello. En general, se notaron un ensanchamiento del proventrículo, una atrofia del páncreas y anomalías huesosas. Entre las modificaciones histopatológicas, se pueden mencionar la hepatitis, la nefritis, la miocarditis y la pericarditis, la enteritis catarral, la neurosis del páncreas, la encefalomalacia y alteraciones de tipo raquítico. Se describen en detalle todos estos síntomas. Se aisló un reovirus a partir de aves enfermas, luego otra vez en pollos infectados experimentalmente. Se confirmó la infección por pruebas de inmunoelectroforesis y de precipitación sobre gel de agar-agar. *Palabras claves* : Pollo - Reovirus - Adelgazamiento - Síntoma - Diagnóstico - Sudán.

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