

Animal Health Research Institute  
Assiut Regional Laboratory.

## INVESTIGATION ON AN OUTBREAK OF FATAL CHLAMYDIOSIS

(With 11 Figures)

By

**SANAA ABDO-EL SHAMY;**  
**SHAHERA M. R. ABD EL-HASSIB and A. H. BAYOUMI**

(Received at 9/3/2002)

دراسة على وباء الكلاميديا المميت في الرومي

سناء عبده حسن الشامي ، شهيرة محمد رشاد عبد الحسيب ،  
عبد اللطيف بيومي

في دراسة لمزرعتين من مزارع الرومي في محافظة الوادي الجديد وجد بها حالات مصابه بميكروب الكلاميديا المميت. وكانت نسبة الوفيات في القطيع المصاب تتراوح بين 6-11% تقريبا. وقد أظهرت الطيور المصابة أعراض تنفسية في كلا المزرعتين. وقد أظهرت الصفات التشريحية احتقان والتهاب في الأغشية الداخلية وكانت الصفة المميزة للرئة هي وجود احتقان أو مناطق متحجرة داخلها. كما تم أخذ عينات للفحص البكتريولوجي للوقوف على أسباب المرض وتشخيصه. وبالفحص الهستوباثولوجي وجد التهاب رئوي تكاثري وظهور الأجسام الدقيقة المميزة للكلاميديا. أما في الكبد فقد وجدت آفات باثولوجية تتمثل في تركز في خلاي الكبد في 70% من العينات بجانب الاجسام الدقيقة في خلايا كوفر. وقد أوصى باتباع الوسائل الصحية في الرعاية والعلاج ب 20% كلوروتتراسيكلين لمدة 45 يوما على الأقل حتى يمكن تجنب الخسائر الاقتصادية المتزايدة. وقد نصح العاملون بتوخي الحذر من انتقال العدوى بالميكروب واتباع الأساليب الصحية الصحيحة.

### SUMMARY

An epornitic of fatal chlamydiosis among turkey was reported in two farms at El-Wadi El-Gadid Governorate. The mortality rates in the affected farms were 6 and 11 %, respectively. The diseased birds revealed signs of respiratory distress, nasal discharges and conjunctivitis. On postmortem examination hyperemia, inflammation of serous membrane, enlarged spleen, areas of consolidation in the lungs were the prominent features. Samples were taken for etiological and histopathological studies. Proliferative pneumonia and elementary bodies

were characteristic in 70% of the examined livers. Focal granulomatous lesions in addition to the elementary bodies in the kupfer cells were seen. Management, sanitation and medication with 20% chlorotetracyclin in feed for 45 days resulted in reduction of mortality rates. Workers were advised about Chlamydiosis to reduce the risk of human infection.

**Key words:** Turkey, Chlamydiosis.

## INTRODUCTION

Chlamydia psittaci infection in domestic birds is world wide and periodically resulted in serious losses (Newman, 1989). The disease appears concurrently in human whose occupations involved production, handling or processing of infected fowl, veterinarians and laboratory workers (Iron *et al.*, 1951; Harris, 1983; Coul and Sillis, 1998). Chlamydia can be seen either with light microscope using special optics or with specific stains. They stained dark purple with Grimenez, blue with castanede and red with Macciavello (Grimenez, 1964). Page (1968) and Schanchter and Dawsa (1978) reported that chlamydia is a genus of intracellular bacteria composed of two species: *C. trachomatis* and *C. psittaci* and classified on the basis of their virulence patterns. There are 2 morphologically distinct forms of Chlamydia (Monire and Tamura 1967). Matancy (1955) observed that many featur of the inflammatory process in the respiratory system were found to be similar to those occuring in infectious sinusitis. Clinical signs include nasal and eye discharge, depression, anorexia and respiratory distress, yellow diarrhea, elevated body temperature and decrease in egg production (Mohan, 1984 and Schwartz, 1995).

On post-mortem examination, Gala *et al.* (1960); Page *et al.* (1975); Tappe *et al.*, (1989) and Vanrompay *et al.* (1995), showed conjunctivitis, sinusitis, rhinitis, keratitis, pneumonia, airsacculitis, pericarditis, hepatosplenomegaly, enteritis and congestion of kidney, the ovaries and testis.

Histopathologically. ocular lesions included epithelial erosions, fibrin depositis in the conjunctiva, corneal ulceration were reported by Dabarr *et al.* (1986) and Vanrompay *et al.* (1995). Respiratory lesions including bronchopneumonia, fibrinous necrotising airsacculitis were also reported by Beasley *et al.* (1959), Beasley *et al.* (1961) and Tappe *et al.* (1989). Inflammatory lesions in serous membranes including

pericarditis, interstitial nephritis, peritonitis were described by Beasley *et al.* (1959) and Tappe *et al.* (1989). Lesions in the digestive tract including catarthai enteritis, hepatocellular proliferation were mentioned by Page and Grimenez (1984) and Vanrompay *et al.* (1995). Necrosis of the spleen was observed by Page *et al.* (1975).

The present paper describes an outbreak of Chlamydiosis in two turkey farms at El-Wadi- El- Gadid Governarate. Detailed isolation as well as histopathological distribution with illustration were carried out. Trials for treatment were done and attendance were advised to avoid infection.

## **MATERIAL and METHODS**

Two turkey flocks consists of 900 birds suffering from abnormal respiratory signs. Unusual high mortality rate (up to 30%) was reported through one week.

### **Clinical examination:**

Case history and clinical signs of the diseased birds were recorded.

### **Post-mortum examination:**

Dead birds were examined for characteristic gross lesions.

### **Trial for isolation of chlamydia:**

Sample from spleen, liver, lung, fibrinous exudate, air-sac were prepared for chicken-embryo inoculation. 0.5 ml saline suspension of samples, contain 500mg/ml Streptomycin and Kanamycin was inoculated into each embryo of 6 days via yolk sac. Embryos were incubated at 39°C for 12 days postinoculation of suspected material by inoculated interaperetonal in mice (Page, 1980).

### **Histopathological examination:**

The selected tissues were obtained from diseased as well as freshly dead turkey. Specimens from lung, heart, liver, spleen and kidneys were fixed in 10% neutral buffered formalin. The routine histopathological technique was performed and paraffin sections were stained with haematoxylin and eosin. Selected tissue sections were also stained by periodic acid shiff (PAS), Gridley's stain and Geimsa stain.

Some specimens were prepared for semithin sections and stained with Toulidin blue (Bancroft and Stevens, 1982).

**Control by medication:**

Feed grade chlorotetracycline 20% was used for 45 days in infected farms.

**RESULTS**

**Clinical findings:**

Ruffling of feathers, depression, anaroxia cachexia and elevated body temperature were constant features, interminttent gasping, abnormal respiratory signs, ocular and nasal discharge were observed in all diseased birds. Many birds excreted yellow green gelatinous dropping. High morbidity was an outstanding manifestation while mortality rate was 6 and 11% in the two farms.

**Cross findings:**

All dissected birds revealed various degrees of congestion of the internal organs. The air sacs were consistently thickened unilateral or bilateral with yellowish gray discoloration. The exudate could be easily removed and similar exudate was seen in the pericarial sac.

The pulmonary lesions ranged from lung congestion to areas of consolidations, the latter were well-defined and mostly surrounded by hypremic zons. The incised consolidated areas showed dry cut non exudative surface. In most of the examined cases, the liver was enlarged, either dark discolored and congested or with cooked-meat appearance and covered with dirty yellowish gray easily removed exudate, the exudate and the parachyma was friable. Post-mortum examinations revealed inflammation of serous membrane, congested enlarged spleen.

**Microscopic findings:**

All the examined lungs revealed proliferative type of pneumonia. The parabronchi, atria and air capillaries were widest and lined by hypertrophied and hyperplastic epithelial cells. Extensive mononuclear cellular infiltration was prominent and constant feature (Fig1). In some cases, the proliferative changes were associated with exudative inflammatory phenomena. The capillaries were congested, some parabronchi, artia and air capillaries were filled with acidophilic fibrin networks, few number of macrophages and occasionally heterophils were also seen (Fig. 2). In Giemsa stained tissue sections, small oval bodies were occasionally seen, free in the inflammatory exudate (Fig. 3). In

some macrophages, these bodies were arranged in clusters in their cytoplasm and larger sized bodies could be also seen. The large sized bodies stained blue and the smaller forms stained blue to purple. These bodies considered elementary bodies (Fig. 4 and 5).

Histopathological examination of the liver revealed inflammatory reaction along with hepatocytic necrobiotic changes, however, the presence of microphages laden with elementary bodies was the most important pathognomonic feature. The inflammatory reaction was represented by hypermia of sinusoids and mononuclear as well as few heterophilic infiltration (Fig. 6). In addition, activation and mild hyperplastic changes in kupfer cell could be seen. Varied degrees of lymphoid infiltration in portal triate were also observed. Scattered hepatocellular necrosis was seen in all the examined sections, minute focci of coagulative necroses was observed in some cases (Fig. 7). Nearly in all sections, the elementary bodies were found either freely diposited or within mononuclear macrophage cells (Fig. 8). Vassculitis with platelet and fibrin thrombi were also seen (Fig. 9). In samithin sections, the elementary bodies were observed in the Kupfer cell as well as in the necrotic hepatocytes. In the Kupfer cells, the elementary bodies were small in size and oval or rounded in shape. They either occurred in groups composed of numerous minute bodies (minute forms) or as large sized bodies (large forms). The small oval minute bodies stained light blue or purple, while the bodies large sized which occurred either in a manner single or in groups stained blue (Fig. 10) and usually surrounded by a halo zone (Fig. 11).

#### **Isolation of chlamydia:**

The inoculated chicken embryos with suspected infected specimens with chlamydia, revealed vascular congestion of yolk-sac membrane deaths within 5-12 days. Suspected material inoculated intravenoulty in mice lead to deaths within 5-15 days. The infected mice revealed hypermic lung, spleen, enlarged liver with fibrinous exudate in peritoneal thoracic cavities.

## **DISCUSSION**

In the present study an epornitic of fatal chlamydiosis occurred in two farms at El-Wadi El-Gadid Governarate. Clinically, turkeys showed ruffling of feather, depression. Cachexia and elevated body temperature, nasal and ocular discharges, anoraxia, respiratory destress and yellow

diarrhea were constant features. Similar clinical signs were described in chlamydiosis of turkey, pigeons, ducks and chickens (Vanrompay *et al.* 1995; Beasley *et al.*, 1959; Tappe *et al.*, 1989; Page *et al.*, 1975). Nervous manifestation such as mild and violent tremors and unsteady imbalanced gait were reported in ducks (Arzey *et al.*, 1990). In addition, incrustment of feathers of the head with the purelent discharge of nostrile were reported (Arzey *et al.*, 1990). In pigeons, the respiratory distress is accompanied by ratteling sounds. Meyer, (1965) and Anderson *et al.*, (1997) observed that the clinical signs of chlamydiosis vary greatly in severity and depend on the age of the bird and strain of organism. Some birds infected without showing clinical signs, these birds act as carrier and can spread the disease and post-mortum examination of died and severely affected birds revealed varied degrees of air sacculities pericardities. Congestion and consolidation areas could be detected in the lungs. Similar findings were seen in ducks, chicken and pigeons (Page, 1959; Page and Grmics 1984; Dabara *et al.*, 1986, Arzey and Arzy 1990 and Vanrompay *et al.*, 1995).

Proliferative pneumonities was constantly observed through micromorphological investigation of all diseased birds. Pathognomonic elementary bodies could be detected in all investigated bird lungs by using specific stain. These findings were incomplete agreement with those of Beasley *et al.*, 1959; Bbeasley *et al.*, 1961 and Tappe *et al.*, 1989). Hepatocytic necrobiosis changes and presence of the pathognomic elementary bodies were constant features in our material. Such result was also described (Beasley *et al.*, 1959 and Page, 1984).

An out breaks of chlamydiosis had been reported among turkeys in two farms at El-Wadi El-Gadid Governrate. The disease was characterized clinically by respiratory distress and digestive disturbances. Pathognomonic micorphological lesions including proliferative pneumonia, hepatic necrobiosis and the demonstration of elementary bodies by specific stain and in semithin sections stained by Toulidin blue were of great diagnostic value. Treatment with antibiotics greatly reduced the mortality rate. Sanitation and hygenic disposal of dead birds was adopted to reduce the morbidity rate. Workers were adviced to reduce the risk of disease transmission.

## LIST OF FIGURES

- Fig. 1:** Lung showing extensive infiltration with mononuclear cells (H & E) (10X40).
- Fig. 2:** Lung showing fibrin deposition along with multinflammatory cellular reaction. (H & E) (10X40).
- Fig. 3:** Lung showing small oval bodies (Giemsa stain) (10X40).
- Fig. 4:** Showing large sized bodies blue in colour and surrounded by a hallow zone (Giemsa stain) 10X40).
- Fig. 5:** Showing small sized bodies free in the exudate with purple red colour (Giemsa stain) (10X40). .
- Fig. 6:** Liver showing mononuclear and heterophilic cellular reactions..
- Fig. 7:** Liver showing focal areas of necrosis (H&E) (10X100).
- Fig. 8:** Showing intracellular elementary body (Giemsa stain) (10X 100).
- Fig. 9:** Blood vessels showing vassculitis with platlels and fibrin thrombi.(Giemsa strain) (10X40).
- Fig. 10:** Showing large sized elementary bodies in the hepatic (Toulidin blue) (10X 100).
- Fig. 11:** Showing large sized elementary body surrounded by hallow zone (Toulidin blue) (10X 100).

## REFERENCES

- Andersen, A. A., Grimes, J. E. and Wyrick, P. B. (1997):* Chlamydiosis (Psittacosis ornithosis). In diseases of poultry, 10th ed. W. Calnek, ed. Iowa state University press Ames I.A . pp. 333-349.
- Arzey, G. G. and Arzey, K. E. (1990):* Chlamydiosis in layer chickens. Aust. Vet. J. 67:461.
- Arzey, K. E., Arzey, G. G. and Reece, R. L. (1990):* Chlamydiosis in commercial ducks. Aust. Vet. J. 67:333-334.
- Bancroft, J. D. and Stevens, A. (1982):* Theory and practice of histological technique. Sed. Ed. Charchill living Stone. Edinbrogh London Melbourne and N.Y.
- Beasley, J. N., Davis, D. E. and Grumbles, L. C. (1959):* Preliminary studies on the histopathology of experimental ornithosis in turkeys. Am. J. Vet. Res., 20:341-349.
- Beasley, J. N., Moore, R. W. and Watkins, J. R. (1961):* The histopathologic characteristics of disease producing inflammation of the air sacs in turkeys: A comparative study of

- pleuropneumonia-like organisms and ornithosis in pure and mixed infections. *Am. J. Vet. Res.*, 22:85-92.
- Coul, E. O. and Sillis, M. (1998):* Zoonoses Biology, Clinical Partice and Public Health Control. Oxford N.Y., Tokyo, Oxford unit press in Palmer S. R. Soulsby L. and Simpson D. I. H., N.Y.
- Dabarr, P. C., Scott, M. D. O'Rourke and Coulter, R. J. (1986):* Isolation of chlamydia psittaci from commercial broiler chickens. *Aust. Vet. J. Vol. 63 NO. II.*
- Gale, C., Sanger, V. L. and Pomeroy, B. S. (1960):* The gross and microscopic pathology of an ornithosis virus of low virulence for turkeys. *Am. J. Vet. Res.* 21:491-497.
- Gimenez, D. F., (1964).* Staining rickettsiae in yolk sac cultures. *Stain Technol* 39:135-140.
- Harris, J. W., (1983):* Zoonotic human chlamydiosis of avian origin : a review with particular reference to epidemiology and control. *World's Poult. Sci. J.* 39:87-98.
- Iron, J. V., Sullivan, T. D. and Bowen, J. (1951):* Outbreak of Psittacosis (Ornithosis) from working with turkeys or chickens. *Am. J. Pub. Health*, 41:931-937.
- Newman, J. A., (1989):* DVM Chlamydia spp. infection in turkey flocks in Minnesota. Reports from the symposion on avain chlamydiosis, *Javma*, Vol. 193, No. 11.
- Manire, G. P. and Tamura, A. (1967):* Preparation and chemical composition of the cell walls of mature infectious dense forms of meningopneumntis organisms. *J. Bacteriol* 94:1179-1183.
- Matancy, G. F., Pomeroy, B. S. and Osborn, O. H. (1995):* Studies on egg transmission of the agent of infection sinusitis of turkeys. *Proc. Book, AVMA*, (1955): 310-315.
- Meyer, K. F., (1965):* Ornithosis. In H.E. Biester and L.H. Schwarte (eds.). *Diseases of Poultry*, 5th ed. Iowa State University Press, Ames, IA, pp. 675-770.
- Mohan, R., (1984):* Epidemiologic and laboratory observations of chlamydia psittaci infection in pet birds. *J. Am. Vet. Med. Assoc.* 184:1372-1374.
- Page, L. A., (1959):* Experimental ornithosis in turkeys. *Avian Dis.* 3(1959): 51-66.
- Page, L. A., (1968):* Proposal for the recognition of two species in the genus chlamydia Jones, rake, and Stearns, 1945. *Int. J. Syst Bacteriol.* 18:51-66.



- Page, L. A., Derieux, W. T. and Dymirc, C. D., (1975):* An Epornitic of fatal Chlamydiosis (Ornithosis) in south caroline turkeys. JAVMA. 166, 2, 175-178.
- Page, L. A. (1980):* Isolation and identificaion of avian pathogens edite hitchnes SB *et al.*, American association avian pathologists, Texas, A.&M university, pp. 43.
- Page, L. A. and Grimes, J. E. (1984):* Avian chiamydiosis (Ornithosis). In M.S. Hofstad, H. J. Barnes, B. W. Calnek, W. M. Reid, and H. W. Yoder, Jr (eds.). Diseases of Poultry, 8th ed. iowa state University Press, Ames, IA. pp. 283-308.
- Schachter, J. and Dausson, C. R. (1978):* Human chlamydial infections. pp. 1-17, PSG publish, Co. Inc., Littleton, Mass.
- Schwartz, L. D., (1995):* Chlamydiosis. In Grower's reference on gamebrd heath. Avicon, Inc. Okimos, M. I. pp. 50-51.
- Tappe, J. P., Andersen, A. A. and Cheville, N. F. (1989):* respiratory and pericardial lesions in turkeys infected with avian or mammalian strains of chlamyia psittaci. Vet. Pathol. 26:386-395.
- Vanrompay, D., Ducatelle, R. and Haebrouck F., (1995).* Pathology of experimental chlamydiosis in turkeys. Vlaams Diserenceskundig Tigischrift 64(1): 19-24.





