Vet.Med.J.,Giza. Vol.44, No.1.(1996): 37-40.

DETECTION OF CHLAMYDIOSIS IN DOMESTIC DUCKS IN GIZA GOVERNORATE, EGYPT.

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SUMMARY

Cloacal swabs were collected from 156 domestic ducks at Giza Governorate. Chlamydia was isolated in embryonated chicken eggs and by mice inoculation. To demonstrate chlamydia, impression smears were made from yolk sac of inoculated eggs, the liver and spleen of inoculated mice. Complement fixation test (CFT) was applied, using yolk sac of inoculated eggs as an antigen. The prevalence rate of Chlamydiosis in ducks amounted to 69.23%.

INTRODUCTION

Ornithosis is a naturally occurring systemic disease of non-psittacine birds caused by *Chlamydia psittaci* (Family: Chlamydiaceae, Order: Chlamydiales) (Hofstad et al., 1984), Moreover, Chlamydiosis is an infectious disease of birds that can be transmitted to human, where infection is probably acquired by inhalation of aerosol containing elementary bodies of the causative agent found in the droppings of tissues of infected birds (Schaster and Dawson, 1978 and Gary and Christopher, 1982). Calnek (1991) stated that the incidence of Chlamydiosis is highly variable in th avian species. He emphasized, moreover the economic importance of the disease to the producers due to carcases condemnation at slaughter, decrease of egg production and the costs of antibiotic treatment to reduce mortality. Arzey et al. (1990) mentioned that Chlamydiosis in domestic ducks has been a serious economic and occupational health problem in some parts of Europe and United Kingdom because of the public health risks for processing plant workers, especially those engaged in plucking. The present study is a attempt to determine the prevalence rate of Chlamydiosis in ducks on the basis of isolation in embryonated eggs and by mice inoculation.

MATERIAL AND METHODS

I- Samples:

156 cloacal swabs were collected from farms of domestic ducks in Giza. Each was immersed separately in phosphate buffer saline (PBS) containing 0.1 gm/ml of streptomycin sulphate and gentamycin 0.5 mg/ml (Grimes et al., 1987). A 20% suspension was prepared and centrifuged at 3000 rpm/15 minutes. Supernatants were collected and stored at -20°C.

II- Experimental infection:

1- Embryonated chicken eggs (Edwin et al., 1979):

Each sample was inoculated in amounts of 0.2 ml into 3 embryonated eggs (3-5 days old) via yolk

sac route, incubated at 39°C and candled daily. Embryos dying before 72 hours from inoculation were discarded, whereas embryos dying from 3-10 days post inoculation were collected and chilled overnight. The yolk sacs were collected separately for each smaple and impression smears were made and stained with Gimenez stain.

2- Mice inoculation:

Prepared samples were inoculated intraperitoneally (i/p) into 3-4 weeks old mice, according to Busby et al. (1964). Each mouse was inoculated with 0.2 ml of prepared sample and observed daily for 6-7 days after inoculation. The infected mice showed swollen abdomen due to accumulation of fibrinous exudate in the abdominal cavity and died. Impression smears were made from liver and spleen of dead or autopsied mice after 7 days of infection and stained with Giemsa (slow method).

III- Impression smears:

1- Impression smears prepared from the infected yolk sacs were made according to Grimes et al. (1987). They were stained for 10 minutes with Gimenez stain, washed with distilled water, counter-stained for 6-9 seconds with malachite green, washed thoroughly with distilled water, dried, blotted and examined under oil immersion lens for inclusion bodies of Chlamydia.

2- Impression smears made from the liver and spleen of infected mice were stained with Giemsa stain (slow method) after Busby et al. (1964) and examined under oil immersion lens.

IV- Serological test:
Antigen preparation:

Antigen was prepared according to Edwin et al. (1979) from yolk sacs showing inclusion bodies in the impression smears.

Complement fixation test:

Complement fixation test (CFT) was applied according to Ebner and Ayoub (1969) for detection of chlamydia. Two fold dilution for the prepared antigen against reference chlamydia antisera (Denka Sleken Co., Ltd., Tokyo, Japan).

RESULTS

In the present study, 108 smaples out of 156 were confirmed as positive with a prevalence rate of chlamydial infection amounting to 69.23%. Vascular congestion in the yolk sac of embryos dying 3-10 days post inoculation was pathognomonic for chlamydial infection as mentioned by Calnek et al. (1991). Intracytoplasmic inclusion bodies were demonstrated in the impression smears prepared from infected yolk sacs. These inclusion bodies appeared reddish purple in colour against greenish blue background in the Gimenez stained preparations.

Mice intraperitonealy (i/p) inoculated with the infectious material succumbed to the disease showing at autopsy accumulation of fibrinous exudate in the abdominal cavity, and enlargement of liver and spleen. Impression smears made from liver and spleen of mice succumbed to infection 6-7 days post inoculation were Giemsa stained (slow method) to demonstrate intracytoplasmic reddish purple inclusion bodies (Fig. 1). The complement fixation test (CFT) was applied to confirm chlamydial etiology. The antigen was prepared from the infected embryonated eggs, while the antiserum is a chlamydial reference antiserum procured from Denka Sleken Co. Ltd.,

Tokyo, Japan. The complement fixation test evidenced a titre ranging from 1/4 to 1/128 for 108 samples at a prevalence rate of 69.23% (Table 1).

Table (1): Results of complement fixation test.

Total no. of samples	Titre							
	-ve	1/4	1/8	1/16	1/32	1/64	1/128	Total no. of +ve samples
156	48	9	13	5	17	35	29	108
Percent*	30.76	5.76	8.33	3.20	10.89	22.43	18.58	69.23

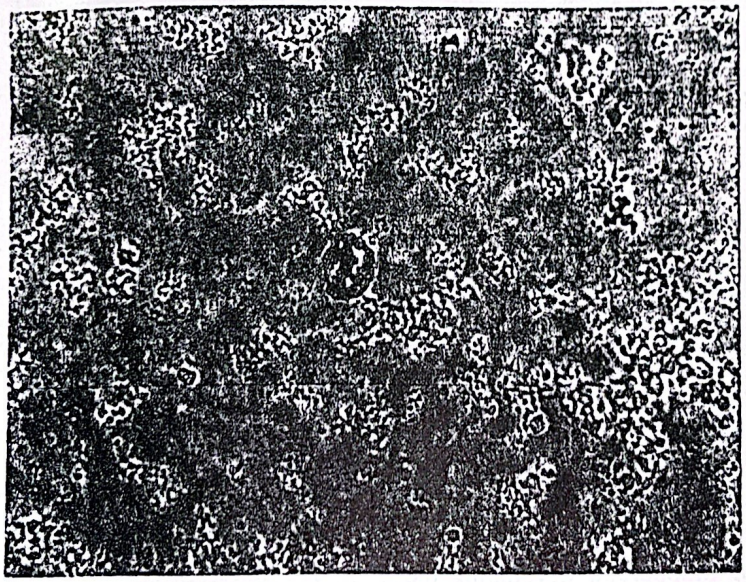

Fig. (1): Impression smear from the liver of an infected mouse showing cytoplasmic chlamydial inclusion bodies (Giemsa stain X 100).

DISCUSSION

Chlamydiosis is a zoonotic disease transmitted between animals, birds and human causing public health hazards (Schaster and Dawson, 1978 and Gary and Christopher, 1982). Moreover, Chlamydiosis causes economic losses in meat and egg production, particularly in ducks and turkeys (Calnek et al., 1991). These authors estimated the incidence of morbidity in ducks between 10% and 80%, a finding that agrees with the results of this study which revealed a prevalence rate of chlamydial infection of 69.23%. Arzey et al. (1990) blamed Chlamydiosis in domestic ducks in the causation of serious economic and occupational health problems in some parts of Europe and United Kingdom. The results of the present study are based on the demonstration of chlamydia by yolk sac inoculation of embryonated chick embryos (Edwin et al., 1978 and Calnek et al., 1991), by mice inoculation (Busby et al., 1964) as well as the presence of intracytoplasmic inclusion bodies of chlamydia in impression smears of infected yolk sac and liver and spleen using cytochemical methods (Busby et al., 1964 and Grimez et al., 1964). Moreover, the complement fixation test was the serodiagnostic tool to confirm the chlamydial etiology, where the antigen was prepared from the infected yolk sacs and matched against reference antiserum applying the method described by Ebner and Ayoub (1969). Table (1) illustrates a prevalence rate of 69.23% of chlamydial infection in ducks, assessing the role of these birds in transmitting the disease to other species as well as to man. Such a high prevalence rate is, moreover, an indication of the bad hygienic conditions under which these birds were allotted. As a rule, the infections in birds are latent or inapparent, developing the disease when their resistance is diminished due to stress factors such as crowding, concurrent infections, unhygienic conditions and other factors. Therefore, alleviating these stress factors and mass prophylactic treatment with tetracyclines are control measures to be considered in poultry farms.

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