j.Egypt.vet.med.Assoc 83, no 1. 53 – 58 (2023)

DIAGNOSTIC STUDIES ON CHLAMYDIA INFECTION IN DUCKS

BY

Eman A. Ahmed [1], Mona I. El- Enbaawy [2*] and Amal M. Abdu [3]

[1]Veterinarian in the private sector and master student

[2] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University

[3] Researcher of bacteriology and chlamydia Animal Health Research Institute

[*]Corresponding author

ABSTRACT

Avian chlamydiosis (Ornithosis), is one of the most important neglected diseases in ducks that has a critical zoonotic potential and considered as a public health risk. Chlamydiosis is mostly responsible for poor laying performance and causes severe lesions in multiple organs of ducks. The main causative agent of avian chlamydiosis is considered to be *Chlamydia C. psittaci*, however, other chlamydial species were found to infect ducks as well, such as *C. gallinacea.* In this study, we discuss the incidence of avian chlamydiosis in different aged ducks. One hundred and twenty fecal swabs were collected, purified and injected into embryonating chicken eggs via the yolk sac method and the results were detected by the Gimenez stain. Chlamydial inclusion bodies appeared as bright red cytoplasmic inclusions. Out of the 120 samples, 74 (61.6%) were found to be positive and 46 (38.3%) were negative. This high percentage of infected ducks indicate that ducks may be a highly mobile vector for chlamydial species, posing zoonotic hazard and of economic importance.

Keywords:

Chlamydia, ducks, Zoonosis.

INTRODUCTION

C. psittaci is a type of obligate intracellular Gram-negative bacteria that often infects birds. Less commonly, these bacteria can infect people and cause a disease called psittacosis. Psittacosis in people is most commonly associated with pet birds, like parrots and cockatiels, and poultry, like turkeys and ducks. Economic losses are met due to chlamydiosis in birds commercially raised for meat and egg production, as well as posing a permanent risk for zoonotic transmission to man **(Sachse *et al.,* 2015).** Psitticosis is a respiratory disease that is usually systemic and can be fatal. The disease occurs in birds raised commercially for meat and egg production **(Vorimore *et al*., 2015).**

The main causative agent of psittacosis is *C. psittaci,* however, it may also be caused by other type of chlamydia, *C. gallenacia* **(Sachse, 2014).** These obligate intracellular pathogens have a unique developmental cycle that differs from other bacteria **(Lin *et al.,* 2019).**

Chlamydia spp. have a biphasic developmental cycle of 48-72 hours with four distinct morphological forms, the elementary bodies (EB) and the reticulate bodies (RB), intermediate bodies (IB), and the persistent aberrant bodies (AB) **(Ravichandran *et al.,* 2021).** It is very difficult to culture chlamydia due to the intracellular lifestyle of the pathogen **(Messmer *et al.,* 2000)**. Chlamydial diagnosis is carried out by isolation on the cell culture or egg inoculation **(Condon and Oakey, 2007).** Field studies by **Vorimore *et al.* (2015)** threw light on the widespread occurrence of *C. psittaci* among human linked to duck breeder flocks and their progeny despite no birds showing clinical signs. In Egypt, there have been limited studies about the prevalence of *C. psittaci* in domestic birds, such as pigeons, turkeys, ducks, and chickens **(El-Jakee *et al*., 2017).** Therefore monitoring study of *Chlamedia* spp on ducks has become urgent to clearly identify, in order to take appropriate field management measures to minimize risk for zoonotic transmission to man. This study targeted investigation of chlamydia infection in ducks randomly in Egypt by traditional methods.

MATRERIAL AND METHOD

Samples:

Fecal swabs (120) were collected from diseased and apparently healthy ducks from local markes and small flocks at Qaliubia Governorate, north of Cairo, Egypt. The swabs were dipped into tubes containing phosphate buffered saline and transferred while cold to the laboratory with minimum delay. Table (1) depicts the sample identification concerning the duck ages.

Table (1): Number of collected fecal swabs collected from ducks of different ages.

Age	Number of samples
10 days	**20**
2 months	**12**
4 months	**18**
6 months	**10**
1 year	**20**
1.5 years	**20**
2 years	**20**
Total number of samples	**120**

Sample preparation:

Each sample was treated with an antibiotic and antimycotic solution containing streptomycin and candistan (Sigma). The samples were then refrigerated for 24 hours and centrifuged at 3000 rpm for 5 minutes. The supernatant was inoculated into eggs via yolk sac route **(Abdu *et al.*, 2021).**

Isolation and confirmation of Chlamydia:

Specific pathogen free (SPF) 6-7 days embryonated chicken eggs were used to isolate *Chlamydia spp.* according to **Andersen and Tappe (1989).** The yielded yolk sac membranes were used for making impression smears that were exposed to heat fixation then stained by Gimenez stain. The fixed impressions were flooded with Gimenez stain solution for 2 minutes followed by washing tap water. Malachite green was added as counter stain. Inclusion bodies of Chlamydia spp. were detected for confirmation **(Abdu *et al.*, 2021).**

RESULTS AND DISCUSSION

Avian chlamydiosis is one of the most important neglected diseases with critical zoonotic potential**.** According the bird species and ages as well as the type of chlamydial strain, the disease in ducks has been characterized **(Ravichandran *et al.*, 2021)**. Taxonomically, chlamydia belongs to the *Chlamydiaceae* (Order *Chlamydiales*, Phylum *Chlamydiae*) with more than 15 distinct species. Chlamudiae are obligate intracellular, coccoid, Gram-negative bacteria sharing a unique conserved biphasic life cycle in hosts **(Cheong, 2019).**

The number of infected birds with chlamydia is usually missed due to latent infection, nonspecific signs and lack of fast and easy diagnostic methods **(Morais *et al.*, 2013).**

Two methods are employed for the diagnosis of any disease: the first method is the detection of antigen and the second is the detection of antibodies.

The intracellular lifestyle of chlamydiae has a considerable implications in diagnosis, because it cannot be grown on agar plates and need cell culture for isolation. In live birds, Chlamydiosis is usually diagnosed by isolating *C. psittaci* from faeces and cloacal swabs. Cytological stainings like Giemsa, Gimenez, modified Gimenez, Ziehl–Neelsen, and Macchiavello stains of smears from exudates, impressions of tissues, and histological preparations could identify the infectious bodies **(Campbell, 2015).**

In the present study, 120 cloacal swabs collected from ducks of different ages were investigated for presence of chlamydia. Each sample was treated by antibiotic and antimycotic solution **Hulin *et al.* (2015).** The samples were refrigerated for 24 hours then centrifuged at 3000 rpm or 5 minutes. The supernatant was inoculated into specific pathogen-free (SPF) embryonated chicken eggs via yolk sac route **(Andersen, 1991).**

Injection of samples in embryonated chicken eggs resulted in congested dwarfed embryos Fig. (1). By yielding the yolk sac of all embryos and examining them by Gimenez stain, inclusion bodies appeared as small round red dots against bluish green background. The causative agent multiplies in the cytoplasm of eukaryotic cells within membrane-bound vacuoles, termed inclusions, by a unique developmental cycle **(Kuo *et al.*, 2015).**

Huang *et al.* (2001) did not recommend isolation of chlamydia for diagnostic purposes because of its potential hazard to laboratory personnel, time-consuming, and laborious. Meanwhile, **Condon and Oakey (2007)** mentioned that the gold slandard for chlamydial detection is the cell culture or egg inoculation.

In this study, out of 120 fecal swab samples collected from dead, diseased and apparently healthy ducks of different ages, 74 samples (61.6 %) were positive (Table 2).

Hegazy *et al.* (2014) detected high incidence of chlamydia (88%) in ducks by yolk sac inoculation and staining using Gimenez stain for detection of inclusion bodies. This high incidence of chlamydia in ducks indicate that birds are considered highly mobile vectors for shedding chlamydia to humans and animals **(Abdu *et al.*, 2021).** Our results prove the importance of avian chlamydiosis as an important zoonotic infectious disease. This was previously mentioned by **Hogerwerf *et al*. (2017).**

Table (2): Prevalence of chlamydial inclusion bodies in the infected yolk sac impression smears stained with Gimenez stain

Duck age	Number of samples	Positive results	
10 days	20	Number	(%)
2 months	12	14	70 %
4 months	18	8	66.7 %
6 months	10	10	55.56 %
1 year	20	6	60 %
1.5 year	20	10	50 %
2 years	20	14	70 %
Total	120	12	60 %

Fig. (1): Embryo dwarfism and congestion of embryonated chicken egg due to Chlamydia.

In conclusion, Detection of the incidence of avian chlamydiosis in ducks is very important concerning public health as well as economic losses in duck production.

REFERENCES

Abdu, A. M., Osman, K. M., and Ali, H. A. (2021): Detection and PCR-Based Diagnosis of *Chlamydia psittaci* Isolated from Some Avian Species in Egypt. Annals of the Romanian Society for Cell Biology, 25(6), 5009-5022.

Andersen, A. A., and Tappe, J. P. (1989): Genetic, immunologic, and pathologic characterization of avian chlamydial strains. Journal of the American Veterinary Medical Association, 195(11), 1512-1516.

Andersen A.A. (1991): Serotyping of *Chlamydia psittaci* isolates using serovar-specific monoclonal antibodies with the microimmunofluorescence test. J. Clin. Microbiol.; 29 (4):707–711.

Campbell TW. Normal avian cytology. In: Campbell Terry W. (2015): Exotic Animal Hematology and Cytology. 4. Oxford: Wiley-Blackwell; pp. 219-227.

Cheong HC, Lee CYQ, Cheok YY, Tan GMY, Looi CY, and Wong WF. (2019): Chlamydiaceae: diseases in primary hosts and zoonosis. Microorganisms. 7 (5):146.

Condon, K., and Oakey, J. (2007). Detection of Chlamydiaceae DNA in veterinary specimens using a family-specific PCR. Letters in applied microbiology, 45 (2), 121-127.

El-Jakee J, El-Hariri M.D, El-Shabrawy M.A, and Gaber E.S. (2017): Isolation and identification of Chlamydophila in poultry species in Egypt. Int. J. Tech. Res.; 10(5):520–526.

Hegazy AM, El-Sisi MA, Hadia AA and Hala MNT. (2014): Incidence of *Chlamydophila psittaci* in domestic birds in Sharkia governorate, Egypt. Zag. Vet. J. 42: 67-76.

Hogerwerf, L., De Gier, B., Baan, B., and Van Der Hoek, W. (2017): *Chlamydia psittaci* (psittacosis) as a cause of community-acquired pneumonia: a systematic review and meta-analysis. Epidemiology and Infection, 145 (15), 3096-3105.

Huang, J., DeGraves, F. J., GAO, D., Feng, P., Schlapp, T., and Kaltenboeck, B. (2001): Quantitative detection of *Chlamydia spp*. by fluorescent PCR in the LightCycler®. Bio Techniques, 30 (1), 150-157.

Hulin V, Bernard P, Vorimore F, Aaziz R, Cléva D, Robineau J, Durand B, Angelis L, Siarkou VI, and Laroucau K. (2015): Assessment of *Chlamydia psittaci* Shedding and Environmental Contamination as Potential Sources of Worker Exposure throughout the Mule Duck Breeding Process. Appl Environ Microbiol. 28; 82 (5):1504-1518.

Kuo, C. C., Stephens, R. S., Bavoil, P. M., and Kaltenboeck, B. (2015). Chlamydia. Bergey's Manual of Systematics of Archaea and Bacteria, 1-28.

Lin, W., Chen, T., Liao, L., Wang, Z., Xiao, J., Lu, J. and Xie, Q. (2019): A parrot-type *Chlamydia psittaci* strain is in association with egg production drop in laying ducks. Transboundary and emerging diseases, 66 (5), 2002-2010.

Messmer, T., Tully Jr, T. N., Ritchie, B. W., and Moroney, J. F. (2000, January): A tale of discrimination: Differentiation of Chlamydiaceae by polymerase chain reaction. In Seminars in Avian and Exotic Pet Medicine (Vol. 9, No. 1, pp. 36-42). WB Saunders.

Morais, J., Coelho, A. C., and dos Anjos Pires, M. (2013): Psittacosis. In Insights from veterinary medicine. IntechOpen.

Ravichandran, K., Anbazhagan, S., Karthik, K., Angappan, M., and Dhayananth, B. (2021): A comprehensive review on avian chlamydiosis: a neglected zoonotic disease. Tropical animal health and production, 53, 1-17.

Sachse, K., Laroucau, K., Riege, K., Wehner, S., Dilcher, M., Creasy, H.H., Weidmann, M., Myers, G., Vorimore, F., Vicari, N. and Magnino, S. (2014): Evidence for the existence of two new members of the family Chlamydiaceae and proposal of Chlamydia avium sp. Nov. and Chlamydia gallinacea sp. Nov., Systematic and applied microbiology, 37 (2), 79-88.

Sachse, K., Laroucau, K., and Vanrompay, D. (2015): Avian chlamydiosis. Current Clinical Microbiology Reports, 2 (1), 10-21.

Vorimore, F., Thebault, A., Poisson, S., Cleva, D., Robineau, J., De Barbeyrac, B., and Laroucau, K. (2015): *Chlamydia psittaci* in ducks: a hidden health risk for poultry workers. Pathogens and disease, 73 (1), 1-9.