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Detection of Chlamydia in Rabbit Using Traditional Methods and Electron Microscopy

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Abstract: The present study was done to investigate the presence of *Chlamydia* in rabbits from some areas at Giza governorate, Egypt. The inclusion bodies of *Chlamydia* were demonstrated in impression smears from rabbit internal organs (liver, lung, spleen and intestine) by Gimsa stain whereas, 77.1% of the examined organs were positive for the presence of *Chlamydia* inclusion bodies while the detection of the *Chlamydia* antigen from suspension of collected organs was detected by complement fixation test (CFT) in 31.4%. *Chlamydia* antibodies were detected by CFT in collected rabbit serum in 27%. The transmission electron microscopy for the presence of *Chlamydia* showing the elementary bodies. In conclusion, these results revealed that rabbit is one of the susceptible animals for *Chlamydia* infection.

Key words: *Chlamydia* · Antibodies · Rabbit · Isolation · CFT Transmission electron microscopy

INTRODUCTION

Chlamydiosis is widely distributed around the world. Its importance is not only related to the economic losses in animal production, but also to risks posed to humans. Family Chlamydiaceae is composed of nine species recognized within the two genera of *Chlamydia* and *Chlamydophila* which are associated with a large variety of diseases in animals, birds and humans including abortion, pneumonia, gastroenteritis, encephalomyelitis, conjunctivitis, arthritis and sexually transmitted diseases. The simplest methods for detecting infected animals rely on the detection of *Chlamydia* antibodies in animal sera, by immunofluorescence, ELISA and the complement fixation test [1,2].

Chlamydophila abortus often affects sheep and goats and occasionally deer, cattle or llamas. This species has also been reported rabbits, horses, guinea pigs, mice, green sea turtles and snakes. *Chlamydophila felis* is normally found in cats. It has also been reported from iguanas [3].

Chlamydiae are obligate intracellular bacteria that are unable to synthesize high-energy compounds (ATP, GTP) essential for metabolism and respiration [4].

Chlamydiae share a common biphasic growth cycle, with the infectious elementary bodies (EBs) gaining entry into and exiting out of host cells and the metabolically active, but noninfectious reticulate bodies (RBs) replicating inside cytoplasmic vacuole (also called inclusions) of the host cells [5,6].

The aim of this study was to investigate the prevalence of antichlamydia antibodies as well as *Chlamydia* antigen in rabbits at different governorates of Egypt.

MATERIALS AND METHODS

This study was conducted on diseased and dead rabbits. Complete clinical examination was performed for all diseased animals, while dead animals were subjected to post mortem examination.

Samples: Samples were collected from seventeen diseased and dead rabbits and included eleven serum samples and thirty five internal organs (5 livers, 6 spleens, 12 lungs and 8 intestines).

Detection of *Chlamydiae* Inclusion Bodies: Inclusion bodies were stected by Giemsa stain according to Busby *et al.* [7]. Each organ was freshly used for making impression smears from the cut surface.

Complement Fixation Test (CFT): Reference antisera for *Chlamydia psittaci*, (Seiken, Denka Seiken Co., LTd, Tokyo, Japan) was usedfor CFT to detect *chlamydia* antigens in the suspected materials.

Reference *Chlamydia* antigen from Denka Seiken Co., LTd, Tokyo, Japan was used for serological detection of antibodies.

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Complement: Freeze dried preparation of preserved guinea pig serum (Welcome) was used for complement fixation technique.

Tissue Preparation for Complement Fixation Test: Tissue samples used for detection of chlamydial antigen were ground in a sterile mortar using sterile sand, then the volume of diluent [PBS] required to make a 10-20% emulsion was added and the suspension was thoroughly mixed. Suspension was centrifuged at 3000 rpm/15min and the supernatant of each sample was collected in sterile tube.

- CFT for detection of antigen in internal organs was carried out according to Edwin and Nathalie [8].
- CF for detection of antibody in serumwas carried out according to the method described by OIE [9].

Demonstration of *Chlamydia* by Transmission Electron Microscopy (TEM): Negative staining of samples for TEM was done according to Hazelton and Gelderblom [10]. Cell suspension from infected tissue were harvested after 3 times freezing thawing then centrifuged for 15 min at 3000 rpm. The supernatant was collected and centrifuged again for 45 min at 13000 rpm. After discarding the supernatant, the pellet was rinsed carefully with distilled water. A droplet of 3% phosphotungstic acid (PTA) was mixed with a droplet of the sample and a copper grid coated with carbon formvar was dipped into the mixture. After drying, the grid was examined with the TEM.

RESULTS

Clinical Findings: Diseased rabbits showed signs of pneumonia, diarrhea, tempany and congestion of eyes. Dead rabbits revealed signs of hepatitis, enteritis, precarditis and pneumonia.

Impression smears from the cut surface of 35 internal organs stained with Giemsa stain, 27 impression smears showed reddish purple to bluish purple intracytoplasmic inclusion of *Chlamydia* (Table 1 and Figures 1, 2 and 3). Tthe percentage of positive results detected in liver, spleen, lung and intestine were 20, 2.8, 8.6 and 5.7, respectively.

Complement Fixation Test (CFT): Resuts for detection of *Chlamydia* antigen in 35 internal organs by using complement fixation is illustrated in Table 2. The percentage of positive results for liver, spleen, lung and intestine were 17.1, 2.8, 8.5 and 8.6, respectively.

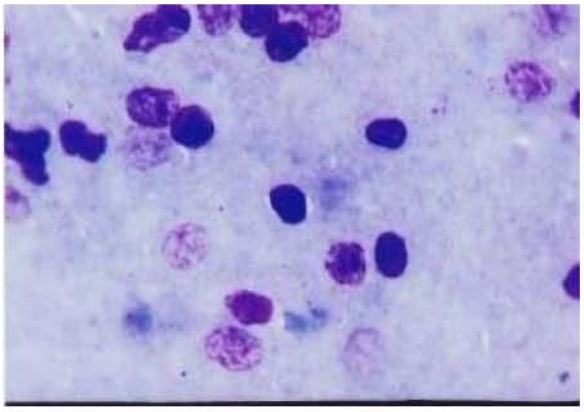

Fig. 1: Impression smear from rabbit liver stained with Geimsa stain and showing Chlamydial inclusion bodies

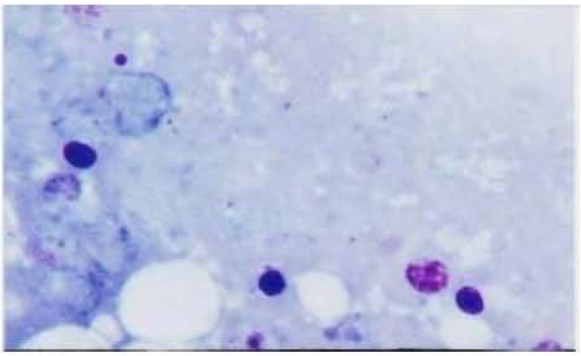

Fig. 2: Impression smear from rabbit lung stained with Geimsa stain and showing Chlamydial inclusion bodies

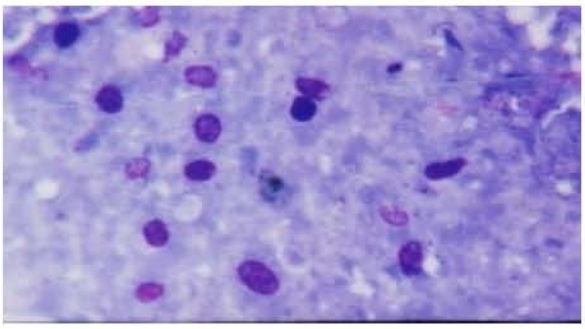

Fig. 3: Impression smear from rabbit spleen stained with Geimsa stain and showing Chlamydial inclusion bodies

Resuts for detection of *Chlamydia* antibody in 11 serum samples by using complement fixation is illustrated in Table 3. A sample was considered to be positive when CF titer is over 1/ 32. The percentage of positive was 27%.

Table 1: Results of impression smears for detection of *Chlamydia* inclusion bodies

No. Samples	Liver	Spleen	Lung	Intestine	Total
Negative	2	5	9	6	22
Percentage	5.2	14	26	17.1	62.9
Positive	7	1	3	2	13
Percentage	20	2.8	8.6	5.7	37.1

Total number of samples = 35

Table 2: Resuls of antigen detected by CF in internal organs

No. Samples	Liver	Spleen	Lung	Intestine	Total
Negative	3	5	8	5	21
Percentage	8.6	14	23.5	14	68.4
Positive	6	1	4	3	14
Percentage	17.1	2.8	11.4	8.6	31.3

Total number of samples = 35

Table 3: Results of Antibody titer in rabbit serum detected by CF test

Serum sample	–ve	±ve	+ve	Total
Number	6	2	3	11
Percentage	55	18	27	100

Antibody titer over 1 /32 consider positive

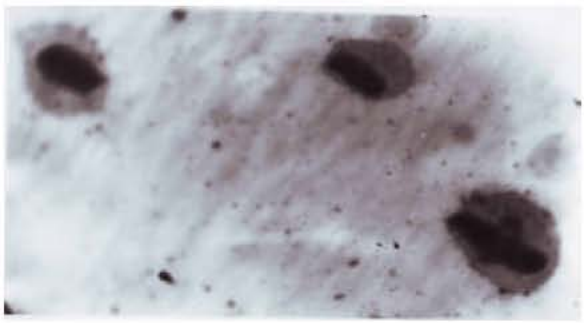

Fig. 4: Transmission electron micrograph of chlamydial particles in tissue from infected rabbits Note the elementary bodies with their dense gene core

Fig. 5: Transmission electron micrograph of chlamydial particles in tissue from infected rabbits

Demonstration of Chlamydia by Transmission Electron Microscopy: Cell suspension from organs were examined with Transmission Electron Microscopy after negative staining. (Photos 4-5). Elementary bodies appeared with their dense gene core. Reticulate bodies were large, fragile and the smaller intermediate bodies appeared with their characteristic condensed nucleoids of nucleic acid.

DISCUSSION

In the present study, impression smears from rabbit organs revealed the the presence of *Chlamydia* inclusion bodies by Giemsa stain. These bodies appeared as reddish purple to bluish purple intracytoplasmic inclusion bodies. Similar bodies of *Chlamydia* were previously shown in impression smears [11].

In the current study, the percentage of positive cases detected in liver, spleen, lung and intestine were 20.0, 2.8, 8.6 and 5.7, respectively. These results agree with those of Pospisil *et al.* [12] who found that after experimental intratesticular inoculation of rabbit by *Chlamydia* , this microorganisms disseminated into internal organs (epididyms, seminal vesicles, kidney, liver, spleen and lung). Also they found that percentage of infection in organs were 40.0, 12.5 and 10.0 % in liver, spleen and lung, respectively.

Detection of *Chlamydia* antigen in internal organs by using complement fixation, is a diagnostic method for *Chlamydia*. In this respect, it was reported that Complement fixation test is a diagnostic method for detecting *Clamydia* antigens in the internal organs [8].

The percentage of positive results detected in liver, spleen, lung and intestine were 17.1, 2.8, 11.4 and 8.6, respectively.

Antibodies against *Chlamydia* in the serum of rabbit were detected by using CFT in 27.0% of the cases and the infected animals showed titer more than 1/32. This result agree with those reported by Pospisil *et al.* [13] who concluded that experimental Chlamydial infection in rabbit released antibodies in the serum and seminal plasma.

Electron microscopy is one of the advanced method which used for identification of chlamydial agents, in our study, after negative staining of tissue suspentions. The developmental form of *Chlamydia* were demonstrated. The elementary bodies (E) with their dense gene core, These form was described by Ward [14] and Vanrompay *et al.* [15] who said that chlamydial elementary bodies (EBs) are small, round or occasionally pear shaped, electron-dense rigid structures approximately 0.2 - 0.3 microns in size, have an electron

dense "black" core of nucleic acid condensed onto chlamydial histone protein.

In conclusion, rabbits can infected naturally with *Chlamydia* from other animals due to bad environmental condition and close housing of large animal and poultry, However, further studies on large number of anima; s are needed for detection of the infective strain or strains which affect rabbits.

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