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RECOGNITION OF TWO SPECIES OF GENUS *CHLAMYDIA* DERIVED FROM RUMINANT: *CHLAMYDIA ABORTUS* AND *CHLAMYDIA PECORUM*

By

Ghada, S. A., Armaniouss, W., Samir, A. and Hadia A. A. Mousa

Department of microbiology, Faculty of veterinary medicine, Cairo University, Egypt.

Chlamydia research unit, Animal health research institute, Egypt

ABSTRACT

The Chlamydiae are a diverse group of obligate intracellular Gram-negative bacteria that are known to infect a wide variety of host species. The present study was carried out on 3 species of farm animals (cattle, sheep and goat). The samples were collected from 2 groups of animal. The first group was apparently healthy animals for screening antibodies and antigen against *Chlamydia abortus* and *Chlamydia pecorum* for surveillance study. The second group was animals showing different clinical findings such as respiratory disorders, keratoconjunctivitis, vaginitis, abortion and diarrhea. The samples that collected from cattle, sheep and goats were 40 fecal samples, 30 nasal swabs, 3 ocular swabs, 18 vaginal swabs, 10 samples from internal organs and 65 serum samples. Diagnosis was based on complement fixation test (CFT) for detection Chlamydial antigen in feaces, nasal, ocular and vaginal samples. In addition, it depended on dedication of chlamydial antibodies in serum samples of farm animals (cattle, sheep and goat). In serum samples positive results for antisera of *Chlamydia* spp. were 69 %, 100 % and 78%, respectively. Regarding fecal samples, positive results for antigen of *Chlamydia* spp. were 100%, 55 % and 88%, respectively. Regarding vaginal swabs, positive results for antigen of *Chlamydia* spp. were 75 % in sheep and 67 % in goats. Meanwhile nasal swabs showed positive results for antigen of *Chlamydia* spp. were 86% for cattle and 69% for sheep and in ocular swabs, positive results for antigen of *Chlamydia* spp. were 100% for sheep. *Chlamydia* was isolated on embryonated chicken eggs (ECE) and the yolk sac was stained with Gimenez stain. The results of fecal samples of cattle, sheep and goats revealed the positive results for presence of inclusion bodies of *Chlamydia abortus* and *Chlamydia pecorum* were 100%, 89% and 75%, respectively. Regarding vaginal swabs, positive results for presence of inclusion bodies of *Chlamydia abortus* and *Chlamydia pecorum* were 100% in sheep and 92% in goats.

Regarding nasal swabs, positive results for presence of inclusion bodies of *Chlamydia abortus* and *Chlamydia pecorum* were 78% and 87% for cattle and sheep. Regarding ocular swabs, positive results for presence of inclusion bodies of *Chlamydia abortus* and *Chlamydia pecorum* were 100% in sheep. Finally the percentage of *Chlamydia abortus* and *Chlamydia pecorum* in cattle 68.5%, 31.5%, respectively, in sheep 66%, 34%, respectively and in goats 55%, 45%, respectively. These results were confirmed through staining six random positive samples by toluidine blue stain as a part of procedure of Transmission electron microscope (TEM)). The collected internal organs from animals were stained by Giemsa stain and the positive results included presence of inclusion bodies of *Chlamydia abortus*, and *Chlamydia pecorum* were in 63% in cattle and 50 % in goats. The present study confirmed the work by indirect immunofluorescence test and the positive results for presence of inclusion bodies of *Chlamydia* spp. in 53% of cattle, 60% of sheep and 46% in goats. Transmission Electron Microscope (TEM) confirmed such results also on six random positive samples to confirm the presence of inclusion bodies of *Chlamydia abortus* and *Chlamydia pecorum* in infected yolk sac. The results suggest that, the farm animals (cattle, sheep and goat) may be reservoir of *Chlamydia abortus* and *Chlamydia pecorum* and thus shed the organism from natural orifices.

Key words:

Chlamydia abortus, Chlamydia pecorum, ruminant, {Transmission electron microscope, Immun of fluorescence.

INTRODUCTION

The Chlamydiae are a diverse group of obligate intracellular Gram-negative bacteria that are known to infect a wide variety of host species and are responsible for a wide range of diseases in animals and man. Many of these organisms have been extensively characterized and their zoonotic implications recognized **(Wheelhouse and Longbottom, 2011)**. The Chlamydiae undergo a unique biphasic developmental cycle characterized by two distinct morphological forms, the small extracellular infectious elementary body (EB) (0_3 mm in diameter) and the intracellular non-infectious, metabolically active, reticulate body (RB) (0_5±1_6 mm), as well as by intermediate forms. The developmental cycle starts with the endocytosis of EBs by eukaryotic cells **(Longbottom and Coulter, 2003).**

Abortion among ewes due to *Chlamydia* was first described on 1950 in Scotland, it was known as enzootic abortion of ewes **(Schnaffner, 1990)**. Enzootic abortion of ewes (EAE)

caused by *C. abortus* (formally known as ovine strains of *C. psittaci*), is primarily as disease of intensively managed flocks. The disease is economically significant in most sheep-producing countries. In addition, abortion associated with *C.abortus* is best documented in sheep; it has also been reported in other domestic species including cattle, pigs and Goats. Chlamydial infection in cattle and goats often originates from sheep. The source of infection in pigs is less clearly defined **(Schiller *et al.,* 1997).** In ewes and goats, abortion due to *C. abortus* usually occurs during the last trimester of gestation, but it may also take place as early as the fourth month **(Storz, 1971).**

C. pecorum (pathogenic strain) has been considered to play a role in a variety of conditions including pneumonia, conjunctivitis, polyarthritis, intestinal infections, mastitis, metritis, and encephalomyelitis in sheep and goats **(Rodolakis and Mohamad, 2008)**. *C. pecorum* (nonpathogenic strain) isolated from intestinal tract of healthy ruminants **(Mohamad *et al*., 2008).**

Aim of work:

The present study was conducted in order to detect the prevalence of *Chlamydia abortus* and *Chlamydia pecorum* infections among cattle, sheep and goats flocks.

MATERIAL AND METHODS

I.Material :

Samples:

In the present study, 166 samples were collected. It represented sixty-five serum samples (35 from cattle, 7 from sheep and 23 from goats). Forty fecal samples (14 from cattle, 18 from sheep and 8 from goats), thirty nasal swabs (14 from cattle and 16 from sheep), three ocular swabs from sheep, eighteen vaginal swabs (8 from sheep and 10 from goats) and ten samples from internal organs of animals (8 from cattle and 2 from aborted doe).

II.Methods :

The serum samples were inactivated in a water bath at 56ºC for 30 minutes to remove non-specific inhibitors.

1.Complement fixation test according to Edwin and Nathalie (1979):

The cold method was used for detection of *Chlamydia* antigen in the fecal samples, vaginal, nasal and ocular swabs and also was used for detection of antibodies against *Chlamydia* in the serum samples using reference antisera and antigen of *Chlamydia* spp. for CFT which were

purchased from Dienka Sieken co., Itd, 3 - 4 - 2, Nihonbashikayyaba Ocho, chuo, chuo-ku, Tokyo, Japan.

2.*Chlamydia* isolation in Embryonated Chicken Eggs (ECE) according to Pierre and Michel, (1993):

Three fertile eggs (ECE) (7-8 days) were used for each sample.

3.Staining of impression smear of yolk sac which infected by fecal samples, vaginal, nasal and ocular swabs using Gimenez stain according to (Gimenez, 1964):

The dried impression smears from infected yolk sac were fixed gently with heat then covered with filtered working solution of basic carbol fuschin for 1-3 minutes. The smears were then rinsed with tap water until the water ran clear. This was followed by flooding of the smears with 0.8% malachite green for 9-20 seconds. The smears were rinsed with tap water, slipped and read under a light microscope using the oil immersion within 2 hours to 2 days after mounting. Examination of slides were occurred for detection and differentiation between inclusion bodies of *Chlamydia abortus* and *Chlamydia pecorum* as the shape of inclusion bodies are round shape and oval shape for *Chlamydia abortus* and *Chlamydia pecorum* respectively.**(Fukushi and Hirai, 1993).**

4.Staining of impression smear of infected yolk sac using toluidine blue stain according to (Bozzola, J.J. *et al.,*1999):

It was a part of procedure of Transmission Electron Microscope (TEM). (Photo. 3, 4.)

5. Cytological examination of the impression smears of internal organs using Giemsa stain according to Busby *et al.,* (1964):

Impression smears were made from the cut surfaces of the internal organs and air-dried. (Table 10) (Photo. 5).

6.Indirect detection of Chlamydia inclusion bodies in infected impression smear by immunofluorescence according to (lecomate, 1993):

(Table 11) (Photo. 6, 7).

7.Detection of Chlamydia inclusion bodies by Transmission Electron microscope (TEM) according to (Bozzola, J.J., *et al.,* 1999):

(Photo. 8, 9).

RESULTS

Results of complement fixation test (CFT):

Detection of antibodies of *Chlamydia* spp. in serum samples by CFT:

From cattle, 24 out of 35 samples were positive for *Chlamydia* spp. antibodies and its percentage was 77 % from sheep 7 out of 7 samples were positive for *Chlamydia* spp. antibodies so its percentage was 100 % and from goats 18 out of 23 samples were positive for *Chlamydia* spp. antibodies and its percentage was 78%. The titers ranged from 1/8- 1/64 (Table 1).

Table (1): Antibody titre of *Chlamydia* spp. in serum samples of animals detected by CFT.

Type of animal	Titre							
	1/8	1/16	1/32	1/64	+ve	-ve	Total number	% of positive samples for *Chlamydia* spp.
Cattle	3	7	3	14	27	8	35	77%
Sheep	-	1	-	6	7	-	7	100%
Goat	3	5	2	11	21	2	23	91%
Total number	6	13	5	31	55	10	65	
% of positive samples for *Chlamydia* spp.	85%							
% of negative samples for *Chlamydia* spp.	15%							

Detection of antigen of *Chlamydia* spp. in fecal samples by CFT:

Fourteen samples from cattle were collected all of them were positive for *Chlamydia* spp. antigen with percentage of 100 %, from sheep 10 out of 18 samples were positive for *Chlamydia* spp. antigen with percentage of 55% and from goats 7 out of 8 samples were positive for *Chlamydia* spp. antigen with percentage of 88%. The titers ranged from 1/8- 1/64 (Table 2).

Table (2): Titre of antigen of *Chlamydia* spp. in fecal samples of animals detected by CFT.

Type of animal	Titre							
	1/8	1/16	1/32	1/64	+ve	-ve	Total number	% of positive sample for *Chlamydia* spp.
Cattle	-	2	5	7	14	-	14	100 %
Sheep	2	2	4	2	10	8	18	55 %
Goat	1	-	5	1	7	1	8	88 %
Total number	3	4	14	10	31	9	40	
% of positive samples for *Chlamydia* spp.	77.5%							
% of negative samples for *Chlamydia* spp.	22.5%							

Detection of antigen of *Chlamydia* spp. in vaginal swabs and placenta samples by CFT:

Eight swabs from sheep were collected 6 out of 8 swabs were positive for *Chlamydia* spp. antigen with percentage of 75% and from goats 8 out of 12 swabs were positive for *Chlamydia* spp. antigen with percentage of 67%. The titers were ranged from 1/8 - 1/64 (Table 3).

Table (3): Titre of antigen of *Chlamydia* spp. in vaginal swabs and placenta samples of animals detected by CFT.

Type of animal	Titre							
	1/8	1/16	1/32	1/64	+ve	-ve	Total number	% of positive samples for *Chlamydia* spp.
Sheep	1	-	2	3	6	2	8	75%
Goat	-	1	-	7	8	4	12	67%
Total number	1	1	2	10	14	6	20	
% of positive samples for *Chlamydia* spp.	70%							
% of negative samples for *Chlamydia* spp.	30%							

Detection of antigen of *Chlamydia* spp. in nasal swabs by CFT:

Fourteen swabs from cattle were collected 12 out of 14 swabs were positive for *Chlamydia* spp antigen with percentage of 86% and from sheep 11 out of 16 swabs were positive for *Chlamydia* spp. antigen and with percentage of 69%. The titers were ranged from 1/8- 1/64 (Table 4).

Table (4): Titre of antigen of *Chlamydia* spp. in nasal swabs of animals detected by CFT.

Type of animal	Titre							
	1/8	1/16	1/32	1/64	+ve	-ve	Total number	% of positive samples for *Chlamydia* spp.
Cattle	1	1	2	8	12	2	14	86%
Sheep	3	1	1	6	11	5	16	69%
Total number	4	2	3	14	23	7	30	
% of positive samples for *Chlamydia* spp.	77%							
% of negative samples for *Chlamydia* spp.	23%							

Detection of antigen of *Chlamydia* spp. in ocular swabs by CFT:

Three swabs were collected from sheep all of them were positive for *Chlamydia* spp antigen so with percentage of 100 %. All samples titers were 1/8. (Table 5).

Table (5): Titre of antigen of *Chlamydia* spp. in ocular swabs of animals detected by CFT.

Type of animal	Titre						
	1/8	1/16	1/32	1/64	+ve	-ve	Total number
Sheep	3	-	-	-	3	-	3
Total number	3	-	-	-	3	-	3
% of positive samples for *Chlamydia* spp.	100%						
% of negative samples for *Chlamydia* spp.	-						

Results of Chlamydia isolation:

Detection of inclusion bodies of *Chlamydia* in Embryonated Chicken Eggs (ECE) infected by fecal samples stained by Gimenez stain.

Fourteen samples were collected from cattle those were positive for inclusion bodies of *Chlamydia* spp. in ECE with percentage of 100 %. In sheep, 16 out of 18 samples were positive for inclusion bodies of *Chlamydia* spp. in ECE representing of 89 % and in goats, 6 out of 8 samples were positive for inclusion bodies of Chlamydia spp. in ECE was representing percentage of 75%.

Table (6): Detection of inclusion bodies of *Chlamydia* spp. in Embryonated Chicken Eggs (ECE) infected by fecal samples stained by Gimenez stain.

Type of animal	+ve	-ve	Total number	% of positive samples for inclusion bodies of *Chlamydia* spp. in ECE
Cattle	**14**	**-**	**14**	**100%**
Sheep	**16**	**2**	**18**	**89%**
Goat	**6**	**2**	**8**	**75%**
Total number	**36**	**4**	**40**	
% of positive samples for inclusion bodies of *Chlamydia* spp. in ECE	**90%**			
% of negative samples for inclusion bodies of *Chlamydia* spp. in ECE	**10%**			

Detection of inclusion bodies of *Chlamydia* in Embryonated Chicken Eggs (ECE) infected by vaginal swabs and placenta samples stained by Gimenez stain:

Eight swabs from sheep were collected all of them were positive for inclusion bodies of *Chlamydia* spp. in ECE with percentage of 100 % and from goats 11 out of 12 swabs were positive for inclusion bodies of Chlamydia spp. in ECE was percentage of 92%.

Table (7): Detection of inclusion bodies of *Chlamydia* spp. in Embryonated Chicken Eggs (ECE) infected by vaginal swabs and placenta samples stained by Gimenez stain.

Type of animal	+ve	-ve	Total number	% of positive samples for inclusion bodies of *Chlamydia* spp. in ECE
Sheep	**8**	**-**	**8**	**100%**
Goat	**11**	**1**	**12**	**92%**
Total number	**19**	**1**	**20**	
% of positive samples for inclusion bodies of *Chlamydia* spp. in ECE	**95%**			
% of negative samples for inclusion bodies of *Chlamydia* spp. in ECE	**5%**			

Detection of inclusion bodies of *Chlamydia* in Embryonated Chicken Eggs (ECE) infected by nasal swabs stained by Gimenez stain:

Fourteen swabs from cattle were collected 11 out of 14 swabs were positive for inclusion bodies of *Chlamydia* spp. in ECE with percentage of 78% and from sheep 14 out of 16 swabs were positive for inclusion bodies of *Chlamydia* spp. in ECE with percentage of 87%.

Table (8): Detection of inclusion bodies of *Chlamydia* spp. in Embryonated Chicken Eggs (ECE) infected by nasal swabs stained by Gimenez stain.

Type of animal	+ve	-ve	Total number	% of positive samples for inclusion bodies of *Chlamydia* spp. in ECE
Cattle	**11**	**3**	**14**	**78%**
Sheep	**14**	**2**	**16**	**87%**
Total number	**25**	**5**	**30**	
% of positive samples for inclusion bodies of *Chlamydia* spp. in ECE	**83%**			
% of negative samples for inclusion bodies of *Chlamydia* spp. in ECE	**17%**			

Detection of inclusion bodies of *Chlamydia* in Embryonated Chicken Eggs (ECE) infected by ocular swabs stained by Gimenez stain.

Three swabs were collected from sheep all of them were positive for inclusion bodies of *Chlamydia* spp. in ECE with percentage of 100%.

Table (9): Detection of inclusion bodies of *Chlamydia* spp. in Embryonated Chicken Eggs (ECE) infected by ocular swabs stained by Gimenez stain.

Type of animal	+ve	-ve	Total number
Sheep	**3**	**-**	**3**
Total number	**3**	**-**	**3**
% of positive samples for inclusion bodies of *Chlamydia* spp. in ECE	**100%**		
% of negative samples for inclusion bodies of *Chlamydia* spp. in ECE	**-**		

Difference between *Chlamydia abortus* and *Chlamydia pecorum* in different animals:

Examination of slides which stained by Gimenez stain under light microscope using oil immersion lens were occurred for detection and differentiation between inclusion bodies of *Chlamydia abortus* and *Chlamydia pecorum* as the shape of inclusion bodies are round shape and oval shape for *Chlamydia abortus* and *Chlamydia pecorum* respectively**. (Fukushi and Hirai, 1993).**The percentage of *Chlamydia abortus* and *Chlamydia pecorum* in cattle 68.5%, 31.5% respectively, in sheep 66%, 34% respectively and in goat 55%, 45% respectively.

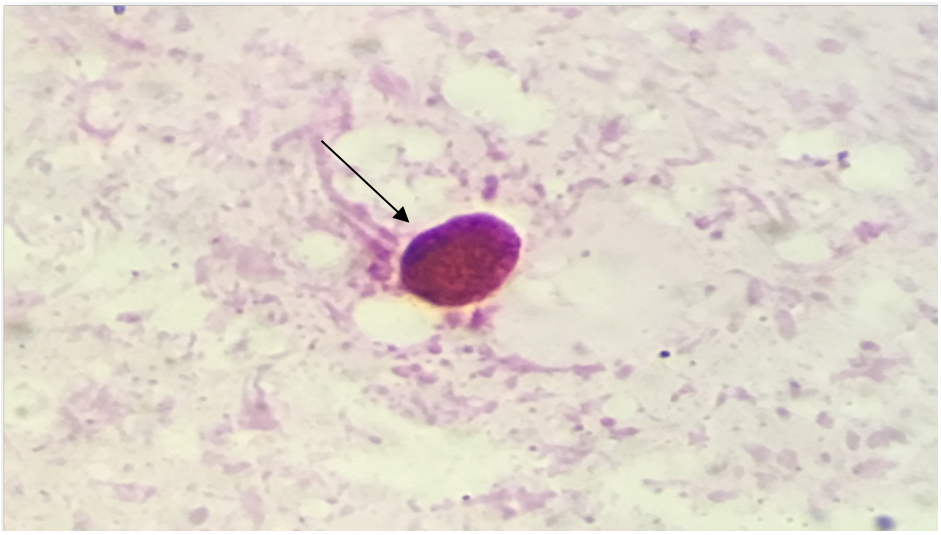

Photo (1): The inclusion bodies of *Chlamydia abortus* (round shape) in infected yolk sac membrane stained with **Gimenez stain. 1200X.**

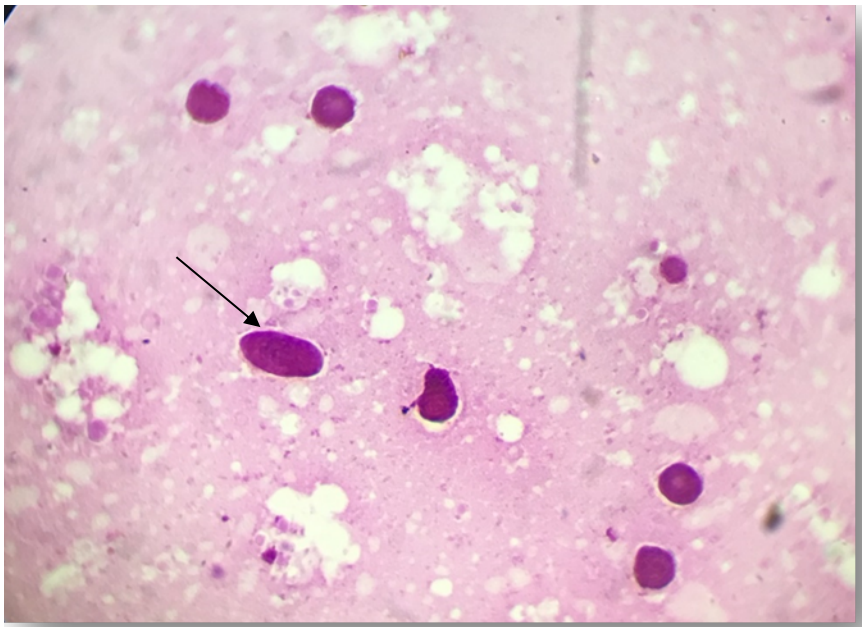

Photo (2): The inclusion bodies of *Chlamydia pecorum* (oval shape) in infected yolk sac membrane stained with **Gimenez stain. 1200X.**

Detection of inclusion bodies of *Chlamydia* spp in infected yolk sac stained by toluidine blue stain (it was a part of procedure of TEM):

Six positive random samples were collected from different farm animals to confirm the presence of inclusion bodies of *Chlamydia* in infected yolk sac. As follow: 2 vaginal swabs from different aborted does, 2 fecal samples from different sheep and 2 fecal samples from different cattle.

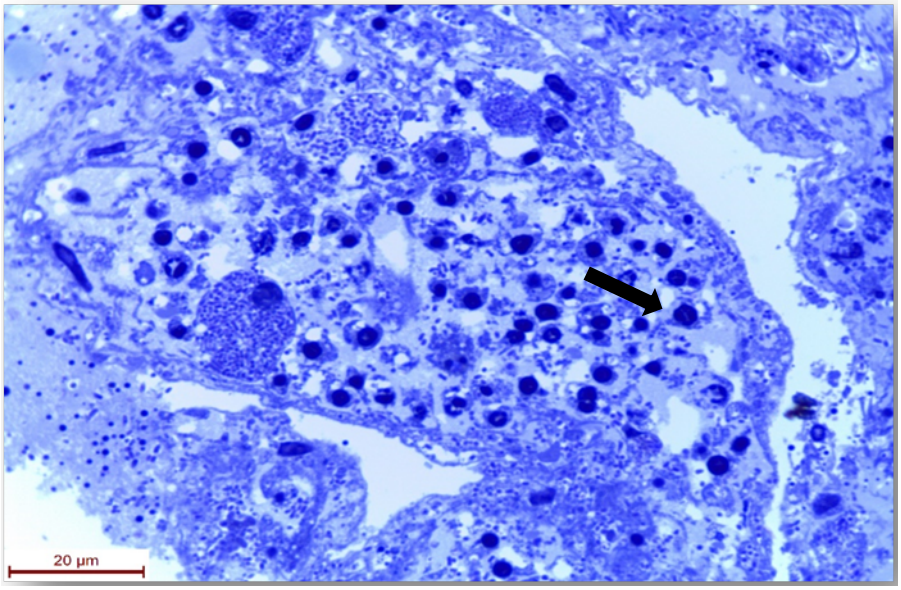

Photo (3): The inclusion bodies of *Chlamydia abortus* (round shape) in infected yolk sac membrane stained with toluidine **blue stain. 1200X.**

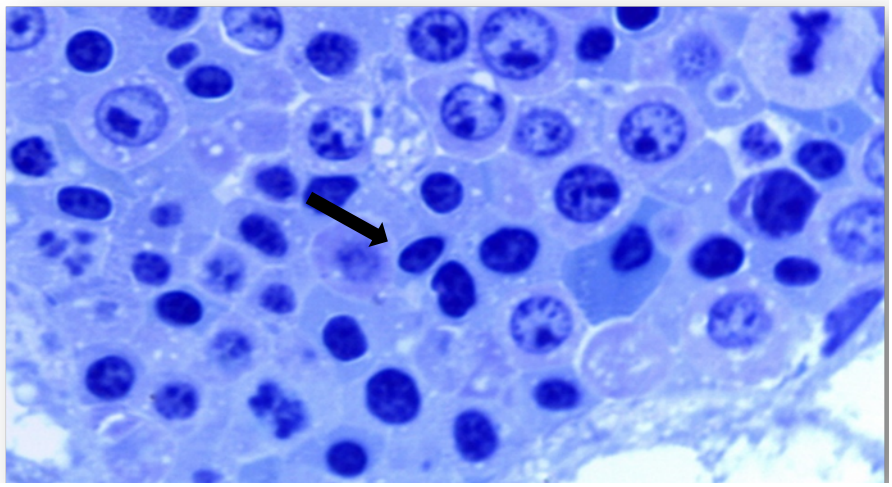

Photo (4): The inclusion bodies of *Chlamydia pecorum* (oval shape) in infected yolk sac membrane stained with toluidine **blue stain. 1200X.**

Detection of inclusion bodies of *Chlamydia* in internal organs of animals stained Giemsa stain:

Eight samples were collected as follow (3 from heart, 1 from lung, 2 from liver,1 from kidney and 1 from spleen) from cattle 5 out of 8 samples were positive for inclusion bodies of *Chlamydia* spp. with percentage of 63% and 2 samples from placenta from aborted does 1 samples were positive with percentage of 50%.

Table (10): Detection of inclusion bodies of *Chlamydia* spp. in internal organs of animals stained by Giemsa stain.

Type of animal	Type of sample	+ve	-ve	Total number	% of positive samples for inclusion bodies of *Chlamydia* spp.
Cattle	Heart Lung Liver Kidney Spleen	2 - 2 - 1	1 1 - 1 -	3 1 2 1 1	63%
Goat	Placenta	1	1	2	50%
Total number		6	4	10	
% of positive samples for inclusion bodies of Chlamydia spp.	60%				
% of negative for inclusion bodies of *Chlamydia* spp. ve samples	40%				

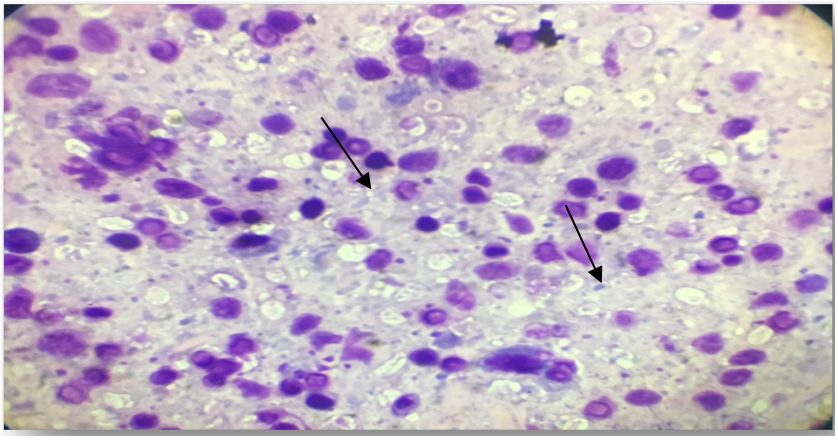

Photo (5): The inclusion bodies of *Chlamydia abotus* (round shape) in liver of infected cattle sac stained with Giemsa stain.

Detection of inclusion bodies of *Chlamydia* in infected impression smear by indirect immunofluorescent test:

Indirect immunofluorescent test was applied on 19 samples from cattle. Ten out of 19 samples were positive for inclusion bodies of *Chlamydia* spp. with percentage of 53%, from sheep 15 out of 25 samples were positive for inclusion bodies of *Chlamydia s*pp. with percentage of 60% and from goat 6 out of 13 were positive for inclusion bodies of *Chlamydia* spp. with percentage of 46%

Table (11): Detection of inclusion bodies of *Chlamydia* spp. in infected impression smear by indirect immunofluorescent test.

Type of animal	+ve	-ve	Total number	% of positive samples for inclusion bodies of *Chlamydia* spp.
cattle	10	9	19	53%
sheep	15	10	25	60%
goat	6	7	13	46%
Total number	31	26	57	
% of positive samples for inclusion bodies of *Chlamydia* spp.	54%			
% of negative samples for inclusion bodies of *Chlamydia* spp.	46%			

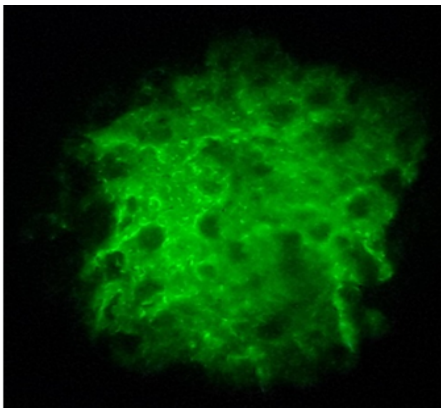

Photo (6): +ve indirect IFT. For inclusion bodies of *Chlamydia* spp

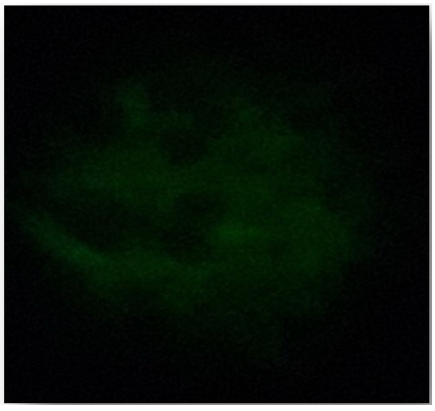

Photo (7): - ve indirect IFT. For inclusion bodies of *Chlamydia* spp

Detection of *Chlamydia i*nclusion bodies by Transmission Electron microscope (TEM):

Six positive random samples that were collected from different farm animals to confirm the presence of inclusion bodies of *Chlamydia* in infected yolk sac and to differentiate between inclusion body of *Chlamydia abortus* (round shape) and *Chlamydia pecorum* (oval shape) **(Borel *et al*., 2010).** It represented 2 vaginal swabs from different aborted does, 2 fecal samples from different sheep and 2 fecal samples from different cattle.

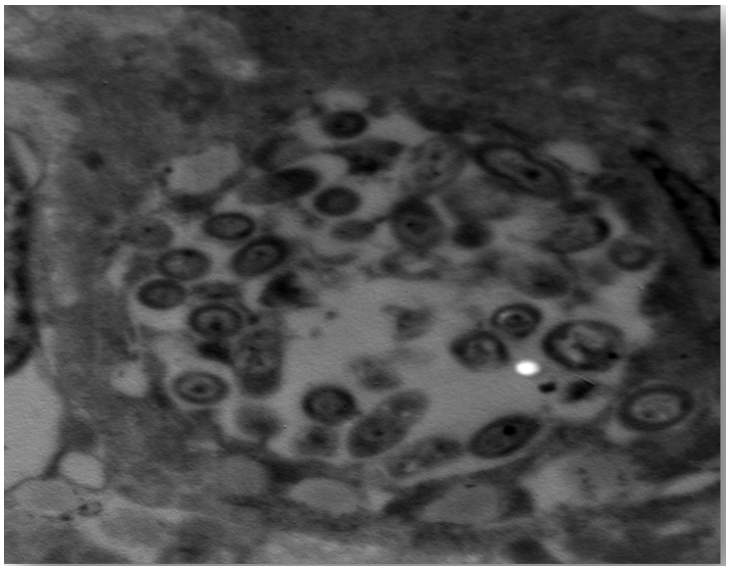

Photo (8): The inclusion bodies of *Chlamydia abortus* (round shape) by **TEM. 6000X**.

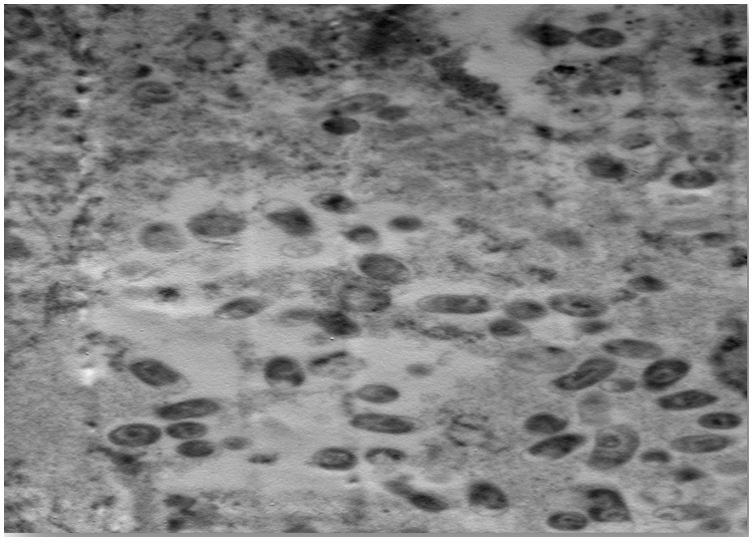

Photo (9): The inclusion bodies of *Chlamydia pecorum* (oval shape) by **TEM. 6000X.**

DISCUSSION

Chlamydiaceae are spread globally and cause acute diseases in humans and animals. They may provoke ocular, pulmonary, genital, articular, and intestinal illness, but very often, they induce persistent, chronic, or subclinical infections. All *Chlamydia* species are potential zoonotic pathogens, although *Chlamydia psittaci* and *Chlamydia abortus* are the most important **(Rodolakis and Mohamad, 2008).**

The disease characterized by abortion, usually towards the end of pregnancy, stillbirths and the birth of weak lambs or kids. The placental membranes instead of being clear and shiny, were opaque, reddened, thick, and often had a leathery appearance and a layer of yellow exudates. The cotyledons, which attach the placenta to the caruncles on the inside the uterus, were thick and rigid instead of being pliable. Sometimes the fetus had a potbelly due to the collection of fluid in the abdomen and enlargement of the liver. **(Palmer,1990).**

Chlamydia pecorum sp. is proposed as the fourth species of the genus *Chlamydia.* Based on the results of a genetic analysis of *Chlamydia* strains that were isolated from cattle and sheep that had various diseases, including sporadic encephalitis, infectious polyarthritis, pneumonia, conjunctivitis, diarrhea, mastitis and abortion. **(Fukushi and Hirai, 1992) and (Philips and Clareskon, 1998).**

In the present study CFT was used to detect antibodies of *Chlamydia* in serum samples and it was found that in cattle 27 out of 35 (77%) were positive , in sheep 7 out of 7 (100%) were positive and in goat 21 out of 23 were positive as shown in (Table 1). Also CFT was applied for to detect Chlamydial antigen in fecal samples of cattle 14 out of 14 (100%) were positive, in sheep 10 out of 18 (55%) were positive and in goats 7 out of 8 (88%) were positive as shown in (Table 2). CFT was used also in detection of chlamydial antigen in vaginal swabs of sheep 6 out of 8 (75%) were positive and in goats 8 out of 12 (67%) were positive as shown in (Table 3). The same test was used also in detection of chlamydial antigen in nasal swabs of cattle 12 out of 14 (86%) were positive and in sheep 11 out of 16 (69%) were positive as shown in (Table 4). CFT was used also in detection of chlamydial antigen in ocular swabs of sheep 3 out of 3 (100%) as shown in (Table 5). These results are nearly similar to that obtained by **(Blewett *et al.*, 1982) and (Appleyared *et al.*, 1983)** who used C.F.T. for detection chlamydial antibodies and antigen. Such results are partial in agreement with **those of Sahar, 2006.** He applied CFT on 36 serum samples from ewe and found 16 out of 36

(44.4%) were positive and found that also sheep excreted chlamydial antigen in their feces with different titers in 36 out of 41 (92.69%) by CFT and the excretion of chlamydial antigen in aborted goats were 18 out of 19 (94.73%) by CFT.

ECE was used for chlamydial isolation of collected samples. Impression smear from infected yolk sac and stained by Gimenez stain. In this study found that in fecal samples of cattle 14 out of 14 (100%) were positive, in fecal samples of sheep 16 out of 18 (89%) were positive, in fecal samples of goats 6 out of 8 (75 %) were positive, in vaginal swabs of sheep 8 out of 8 (100 %) were positive. In vaginal swabs of goat 11 out of 12 (92 %) were positive, in nasal swabs of cattle 11 out of 14 (78 %) were positive, in nasal swabs of sheep 14 out of 16 (87%) were positive and in ocular swabs of sheep 3 out of 3 (100 %) were positive. The percentage of *Chlamydia abortus* and *Chlamydia pecorum* in cattle 68.5 %, 31.5% respectively, in sheep 66%, 34% respectively and in goat 55%, 45% respectively. It was in agreement with those of **(Rajeev and Purohit, 2001).** They used ECE for chlamydial isolation from genitalia of healthy exotic and crossbred service rams; however, our results were higher than those of **(Chanton *et al*., 2002).** They found that, the *Chlamydia abortus* was the most commonly involved agent in the etiology of caprine and ovine abortion (sheep 39%, goats 23%) and **(Sahar 2006)** who isolated chlamydia from fecal samples of sheep and goat and found that 51.2% were positive in case of ewes and 42.1% were positive in case of goats.

Zhaocai *et al.,* 2015 isolated *Chlamydia abortus* from vaginal swabs of yak cows by inoculated it in ECE and found that 23.81% of swabs were positive. This result disagree with ours. In the present study mixed infection between *Chlamydia abortus* and *Chlamydia pecorum* in cattle, sheep and goats and it was agree with **(Wilson, *et al*., 2008).**

Impression smears from internal organs of cattle were stained by Giemsa stain five out of eight were positive (63%). Regarding stained impression smears from aborted does one out of two (50%) was positive. Such result agreed with those of **(Appleyared *et al.,* 1983 and Hadia *et al.,* 1998)** who detected chlamydial inclusion bodies by staining impression smear of liver, spleen and placenta by Giemsa stain.

In the present studies the results were confirmed by using indirect immunofluorescent test and it was found that in cattle 10 out of 19 (53%) were positive, in sheep 15 out of 25 (60%) were positive and in goats 6 out of 13 (46%) were positive. Such results agree with those of **(Griffiths *et al.,* 1992).** They used indirect immunofluorescent test to detect chlamydial

inclusion bodies in sheep flocks and disagreed with **(Mariam 2005)** who applied immunofluorescent test on samples that collected from ewes and found that 26% were positive samples.

In the present study, the results were confirmed using transmission electron microscope. However, the results agree with those of Wilkat *et al.* 2014. However, they used TEM to detect Chlamydia abortus **(Popov and Martinov, 1982).** These authors proved that at electron microscopy is better than light microscopy for identifying elementary bodies and initial bodies in ultra-thin sections of body tissues. It also agree with those of **Borel N *et al*., (2010)** who differentiate between *C. pecorum* and *C. abortus* by TEM.

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