Recent biological techniques for diagnosis of Chlamydophila abortus in aborted goats

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Chlamydophila abortus (C. abortus) is one of the most important causative agents of enzootic abortion which has been caused a serious economic problem in domesticated and wild ruminants world wide. This study was aimed to diagnose C. abortus infection in aborted goats in Ras Suder Research Station (South Sinai) - Desert Research Center from 2004-2006. Twenty aborted cases from 130 pregnant nannies were recorded and examined serologically using complement fixation test (CFT). Eighty percent (16/20) of the aborted cases were serologically positive and 20% (4/20) randomly collected from apparently healthy pregnant nannies were also had antibodies against C. abortus. Pathological lesions were detected. Ten aborted fetal samples from serologically positive aborted nannies were subjected to diagnosis using Polymerase Chain Reaction (PCR) showed positive results at 119 bp. According to this result, PCR proved to be feasible, reliable, specific and sensitive diagnostic tool in diagnosis of C. abortus infection.

Chlamydophila abortus (C. abortus) is a Gram-negative intracellular bacterium that was formerly known as Chlamydia psittaci serotype 1 (Everett et al., 1999). It is considered as one of the most economically important pathogens of domesticated and wild animals, which cause abortion, weak neonates, fetal loss and infertility in sheep, goats and cattle in many countries around the world (Nietfeld, 2001; De Garves, et al., 2004; Da Silva et al., 2006). The bacterium is also a zoonotic agent that causes abortion and other clinical symptoms in human (Pospischil et al., 2002; Walder et al., 2003 and 2005). The main source of C. abortus in the environment is placenta and fetal fluids of affected animals, elementary bodies remained infectious for several days (Papp et al., 1994). The route of transmission between animals is mostly fecaloral and the infection is asymptomatic or at the best oligosymptomatic in non-pregnant animals and during pregnancy (Borel, 2008). Few reports suggested inhalation as another route of transmission (Jones and Anderson, 1988). Based on experimental findings, venereal transmission was suggested as a less common route of transmission (Applayard et.al., 1985). Development of clinical signs due to C. abortus infection depends on, the time of infection (Al-Qudah et al., 2004). Sheep and goat infected 5-6 weeks before parturition can develop clinical disease during the current pregnancy (Morgan et al., 1988; Wilsmore et al., 1990). It was found that infected and latently infected sheep and

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goats may shed *C. abortus* in their reproductive tract for up to 3 years post infection (Morgan *et al.*, 1988).

CFT is the most widely accepted serodiagnostic method for diagnosis of chlamydial infection in animals (Kaltenbook *et al.*, 1997; Travnicek *et al.*, 2001) as it gives satisfactory results with ovine, caprine and avian serum samples (Butty and Nicolet, 1987).

Pathological changes observed were subcutaneous petechial haemorrhages in the skin of legs, hips, neck and in the head of chlamydial aborted feti (Studdert, 1968). The necrotic placentitis is the primary pathological lesion of chlamydial infection in sheep and goats (Aitken, 1989).

PCR is a useful tool for the detection of Chlamydophila in biological samples (Laroucou *et al.*, 2001; Wafaa, 2007). PCR makes it possible to process a large number of specimens, is easy to use, and provides rapid results, besides being safer than culturing the microorganism in cell substrates. PCR analysis using fresh or frozen samples in straight forward furthermore, PCR can be adopted for use on formalin fixed and paraffin-embedded samples, the methods permits samples to be kept for retrospective diagnosis from archival material and avoids the zoonotic risk of *C. abortus* (Nieves Ortega *et al.*, 2007).

The purpose of the present study was to diagnose chlamydial infection by PCR, serology (CFT) and pathology.

Materials and methods

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Animals. This study was carried out on 130 pregnant nannies located in Ras Suder Research Station, Desert Research Center; Samples were collected during the period from 2004 -2006.

Samples.

A- Serum samples. Blood samples were collected from 20 aborted nannies (4 weeks post abortion) and 20 blood samples randomly collected from apparently healthy pregnant nannies. Serum samples were separated and submitted to CFT to detect antibodies against *C. abortus*.

B- Tissue samples for gross examination and histopathological studies. Tissue samples were collected from placenta of aborted nannies and internal organs of aborted foeti (liver, kidneys, heart, brain, lung and spleen). The collected samples were fixed in 10% neutral buffered formalin. The fixed specimens were then, washed, dehydrated and embedded in paraffin wax. The tissues were sectioned at 4-5 μ thickness and stained with haematoxylin and eosin (H&E) for histopatholoical examination and stained with Gemeniz stain as special stain for *C. abortus* (Bancroft *et al.*, 1996).

Antisera. Reference Antisera for *Chlamydia* (*C. abortus CFT* Reagents, "Seiken") were obtained

from Denka Seiken Co[®]., Tokyo, Japan. Antisera were used for detection of chlamydial antibodies in the suspected materials.

Reference *Chlamydial* **antigen.** It was obtained from Denka Seiken Co[®]., Tokyo, Japan. It was used for serological detection of antibodies.

Complement. Freeze dried preparation of preserved guinea pig serum (Welcome^(R)) was used in CFT.

PCR. From 10 serologically positive cases for chlamydiosis, tissue samples (placenta, internal organs of aborted feti as liver, kidneys, lung and brain) were subjected to PCR. After deparaffinizing of the paraffin embedded samples, the process of DNA extraction started.

DNA extraction. The genomic DNA was extracted from samples using Dneasy tissue kit purchased from QIA Gen[®], Basel, Switzerland according to (Venables *et al.*, 1997).

PCR amplification of chlamydial DNA. It was performed on DNA extracted from tissue samples using oligonucleotide primers chla. 2AF and chla. 2Br according to (Sykes *et al.*, 1997).

The expected band length is 119 bp. The amplification condition, the master mix and primers structure are as follows.

Constituent	Initial Conc.	Amount (µl)	Final Conc.	X ⁸
Distilled H ₂ O		13.2		105.6 µl
Buffer	X10	2.0		16.0 µl
DNtps	10 m M	0.4	0.2mM	3.2 µl
Tag polymerase	5 μ /Ml	0.4	2 μ / Ml	3.2 µl
Primere 2AF	20 mM	1.0	1mM	8.0 µl
Primer 2Br	20 mM	1.0	1mM	8.0 µl
Total volume		18.0 µl		
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Primers:

2AF 5- GCTTTTCTAATTTACACC-3

2Br 5- ATAGGGTTGAGACTATCCACT-3 Control: Distilled H_2O as negative control and pure DNA of *C. abortus* as positive control. 2μ l of template added to each tube and 2μ l of distilled H_2O added to tube of negative control.

Thermocycler	adjustment:
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1/95 C	10 minutes				
2/95 C	30 seconds				
3/50 C	30 seconds				
4/72 C	45 seconds				
5/72 C	10 minutes				
6/4 C	pause				
This program w	a wood for 10 avalag				

This program was used for 40 cycles.

Analysis results by electrophoresis on horizontal agrose gel 1.5%.

Results

History of the farm. The rate of abortion was high in the first year in the pregnant nannies and decreased in the following years.

Clinical signs. Abortion was the most prominent symptom in pregnant nannies at late stage of pregnancy at the first year while in the second year stillbirth and weak kids were the prominent signs.

Serological studies. Results of serological analysis were shown in Table (1).

Histopathological findings.

A- Fetal placenta. There was massive necrosis of chorioallantoic villi with sloughing of the trophoblastic cells covering of the villi into the crypts. In addition to inflammatory cells mainly neutrophils and macrophages were seen. Myxomatous degeneration of some chorioallantoic villi was observed (Photo 1). Severe hemorrhages in the intercotyledonary areas were seen. Marked signs of vasculitis were noticed. Chlamydial elementary bodies were detected in the cytoplasm of trophoblasts as red spherical granules in sections stained with Gimenez stain (Photo 2).

B- Aborted fetal organs.

Liver. Showed multiple foci of hepatic cell necrosis associated with diffuse infiltration of mononuclear cells; mostly lymphocytes in hepatic parenchyma (Photo 3). It appeared as bright red granules against blue back ground in section stained with Gimenez stain (Photo 4).

Lung. Displayed aggregations of neutrophils and macrophages in the lumen of alveoli (Photo

5). In addition to the alveolar walls were thickened, pulmonary blood vessels appeared dilated and congested.

Kidney. Showing extensive necrosis of epithelium lining of renal tubules also it, showing hypercellularity of renal glomeruli in addition to necrotic changes of epithelium lining of renal tubules (Photo 6 and 7).

PCR. Ten samples from placenta and aborted feti of aborted nannies which were serologically positive recorded positive results at 119 bp. The positive control showed the expected amplification product at 119 bp as shown in Photo (8).

Table (1): Results of CFT in aborted and apparently healthy pregnant nannies.

Animals	No. of examined	No. of animals with antibodies (CFT)		Titer of serum samples				
	animals	No.	%	1/8	1/16	1/32	1/64	1/128
Aborted nannies	20	16	80	-	-	1	5	10
Apparently healthy nannies	20	4	20	1	3	-	-	-

Positive results $\geq 1/32$

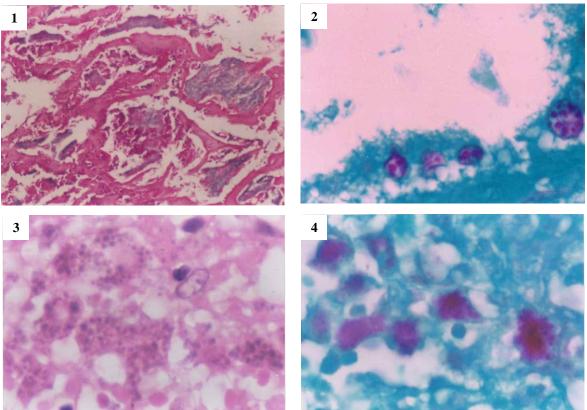


Photo (1): Fetal placenta, showing massive necrosis, myxomatosis of chorioallantoic villi and presence of inflammatory cells and cellular debris within the crypts (H & E, X100).

Photo (2): Fetal placenta, showing presence of chlamydial elementary bodies within the trophoblast cells. (Gimenez stain, X 1000).

Photo (3): Liver of aborted foetus, showing presence of numerous chlamydial elementary bodies within the cytoplasm of hepatic cells (H& E, X 1000).

Photo (4): Liver of aborted foetus, showing presence of intracytoplasmic chlamydial elementary bodies as bright red granules (Gimenez stain, X 1000).

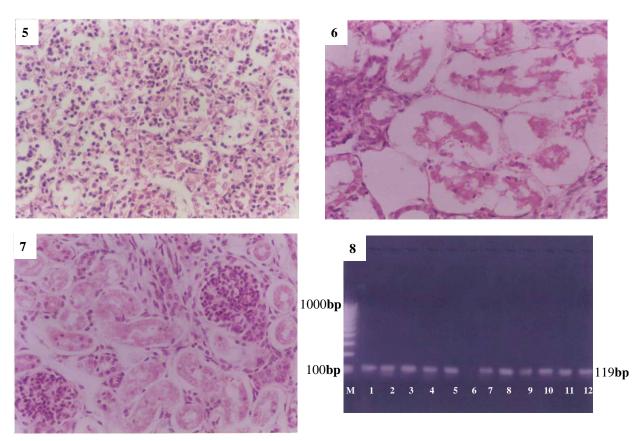


Photo (5): Lung of aborted fetus, showing aggregation of neutrophils and macrophages within the alveoli (H &E, X200).

Photo (6): Kidney, showing extensive necrosis of epithelium lining of renal tubules (H &E, X100). **Photo (7):** Kidney, showing hypercellularity of renal glomereuli in addition to necrobiotic changes of epithilum lining of renal tubules (H &E, X100).

Photo (8): PCR of (*C. abortus*) DNA from formalin-fixed and paraffin embedded tissues of aborted caprine feti (M, 100 bp ladder marker, lane 1-5 and 8-12 specific *C. abortus* PCR product (119bp detected). Lane 6 control negative and lane 7 control positive).

The main histopathological findings in the placenta and aborted foeti of aborted nannies due to chlamydiosis were development of necrosis and inflammatory changes in internal organs. These were parallel with that of Buxton et al., (1990) and (2002); Chanton et al., (2002); Desouky et al., (2004). Such changes could be attributed to embolic dissemination of chlamydial infection from placenta (Buxton et al., 1990) as indicated by the presence of elementary bodies in the liver of aborted foeti. The initial interaction of Chlamydia with the host cells begins with the attachment of elementary bodies to the cells followed by phagocytosis within membrane limited vacuole called inclusion which don't fuse with lysosomes of cells and explain the survival of the organism in the intracellular environment (Escalante et al., 1998).

Regarding the advanced techniques for the diagnosis of *C. abortus*, ten randomly selected tissue samples of placenta and aborted foeti from

serologically positive cases were subjected to PCR using both "2A" and "2B" primers which are specific for identification of *C. abortus* DNA. All the examined tissue samples showed the expected amplification product specific for *C. abortus* (119 bp). These findings were in parallel with that of Ongor *et al.*, (2004); Nieves Ortega *et al.*, (2007); Reitt *et al.*, (2007); Wafaa, (2007); Da Silva *et al.*, (2009) who reported that, PCR is the most adequate technique for the detection of *C. abortus* which is feasible, reliable, specific and sensitive diagnostic tool in diagnosis of *C. abortus* infection.

Conclusion

This study has clearly identified the need for more education and awareness about chlamydophilosis. Effective control of this disease is important not only because of its zoonotic importance, but also because of its adverse impact on animal production.

Oxytetracycline used by intramuscular route 105 and 120 day of pregnancy can prevent abortion but cannot prevent the *Chlamydial* shedding at kidding. Intervention by the government and the private sector through farmer training and awareness campaigns is therefore recommended.

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استخدام التقنيات الحيوية الحديثة في تشخيص ميكروب الكلاميدوفيلا أبورتس في الماعز المجهضة

يعتبر ميكروب الكلاميدوفيلا أبورتس من المسببات الهامة للإجهاض المعدي الذي يسبب خسارة اقتصادية كبيرة في المجترات المستأنسة في العالم . وأجريت هذه الدراسة لتشخيص العدوى بميكروب الكلاميدوفيلا أبورتس في الماعز المجهضة بمحطة بحوث رأس سدر التابعة لمركز بحوث الصحراء . سجل عدد ٢٠ حالة إجهاض من اجمالى ١٣٠ ماعز عشر من عام ٢٠٠٤ إلى عام ٢٠٠٦ . وقد تم فحصها سيرولوجيا باستخدام اختبار التثبيت المتمم وكانت النتائج ايجابية بنسبة ١٣ (٢٠ ماعز عشر من عام ٢٠٠٤ إلى عام ٢٠٠٢ . وقد تم معدم المرولوجيا باستخدام اختبار التثبيت المتمم وكانت النتائج ايجابية بنسبة ١٣ (٢٠ %) من اجمالى ٢٠ حالة وكذلك تم فحص عدد ٢٠ عينة سيرم من الماعز العشر السليمة ظاهريا والمخالطة حيث ظهرت ٢ حالات بنسبة (٢٠ %) بها أجسام مضادة للميكروب . جدير بالذكر أن الأعراض الإكلينيكية والتغييرات الباتولوجية غير كافية لتشخيص عدوى الكلاميديا نظرا لتطابقها مع أمراض أخرى ونتيجة للايجابية والسلبية الوارد حدوثها في الاختبارات السيرولوجية فقد تم عمل اختبار البلمرة المتسلسل لعدد ١٠ أجنة مجهضة الحيوانات ايجابية سيرولوجيا وكانت جميع العينات ايجابية لميكروب الكلاميديا نظرا تطرا يقام من المراض أخرى ونتيجة المراض المراض الإكلينيكية والتغييرات الباتولوجية فقد تم عمل اختبار البلمرة المتسلسل لعدد ١٠ أجنة مجهضة لحيوانات المرابية سيرولوجيا وكانت جميع العينات ايجابية لميكروب الكلاميدوفيلا أبورتس عند وزن جزيئي ١٩ ولهذا يعتبر تفاعل البلمرة المتسلسل أكثر دقة وحساسية.