Dept. of Animal Medicine,
Fac. Vet. Med., Assiut University

# SOME CLINICAL, DIAGNOSTIC AND EPIDEMIOLOGICAL STUDIES ON CHLAMYDOPHILA INFECTION IN CAMELS

(With 3 Tables and One Figure)

By

*U. ABD EL-HAKIM*

(Received at 2/3/2008)

## بعض الدراسات الإكلينيكية والتشخيصية والوبائية للإصابة بالكلاميدوفيلا فى الجمال

*اسامه عبد الحكيم*

للتعرف على صورة (أو صور) الإصابة بالكلاميدوفيلا (الاسم الجديد للكلاميديا بعد إعادة التصنيف) فى الجمال و لمعرفة الدور الوبائى الذى يمكن أن تقوم به الجمال فى نقل البكتيريا المسببة لهذه الإصابة إلى حيوانات أخرى تم دراسة هذه الإصابة من الناحية الإكلينيكية والتشخيصية والوبائية فى الجمال والأغنام المحتكة بهذه الجمال. فى هذه الدراسة تم استخدام ٣٠ جمل و٣٠٠ من الأغنام من ٦ مزارع مختلفة تعيش فيها الأغنام مع الجمال (فى المنطقة الغربية من المملكة العربية السعوديه), ٣ من هذه المزارع كانت تعانى من إجهاضات فى الأغنام. الدراسة التشخيصية شملت استخدام نوعين من الاختبارات, اختبار الإليزا واختبار التفاعل التبلمرى المتسلسل. تم إجراء هذين الاختبارين على عينات من السيرم والحليب من الجمال والأغنام ٣ مرات متتالية يفصل بين كل مرة وأخرى شهر. نتائج الفحص الإكلينيكى أوضحت أن الجمال المصابة بالكلاميدوفيلا تعانى فقط من بعض الأعراض التنفسية بينما بعض الأغنام المصابة أجهضت والبعض ظهرت عليه أعراض تنفسية تتراوح بين خفيفة وشديدة بينما لم يتم تسجيل أى حالات إجهاض فى الجمال المصابة. الدراسة التشخيصية أثبتت إصابة الجمال بثلاثة أنواع من الكلاميدوفيلا (كلاميدوفيلا أبورتس – كلاميدوفيلا بيكوريم – كلاميدوفيلا نيمونى) بينما تم التعرف على الكلاميدوفيلا أبورتس والكلاميدوفيلا بيكوريم فقط فى الأغنام المصابة. إختبار الإليزا الذى تم استخدامه لم يتمكن من التفريق بين الأنواع المختلفة للكلاميدوفيلا بينما باستخدام التفاعل التبلمرى المتسلسل تم التعرف على هذه الأنواع الثلاثة والتفريق بينها. البيانات التى تم جمعها والتاريخ المرضى للمزارع التى تم إجراء البحث على بعض حيواناتها ونتائج هذه الدراسة (ومنها التحول السيرولوجى الذى حدث فى ١٦ غنم والتعرف على الحامض النووى للكلاميدوفيلا فى ٢٥ غنم كانت سلبية وذلك بعد إدخال جملين مصابين بالكلاميدوفيلا إلى المزرعة التى لم تكن تعانى من أى حالات إجهاض أو أى صورة من صور الإصابة بالكلاميدوفيلا) تثبت أن الجمال تلعب دور هام فى وبائية هذا المرض. أثبت التفاعل التبلمرى المتسلسل أنه أشد حساسية من اختبار الإليزا حيث أنه أعطى نتائج إيجابية مع عدد ١٨ جمل و١٤٢ غنم بينما

١١ جمل و ١٠٩ غنم فقط كانت إيجابية مع الإليزا وفى نفس الوقت لم يسجل التفاعل التبلمرى المتسلسل أى نتيجة إيجابية مع الحيوانات المحصنة بينما كانت كل الحيوانات المحصنة إيجابية لاختبار الإليزا. من خلال النتائج التى تم الحصول عليها من هذا العمل أستطيع أن أسجل أن الجمال تصاب بالكلاميدوفيلا لكن معظم الجمال المصابة تبدو سليمة ظاهريا لهذا يمكن أن تلعب دورا هاما فى نقل هذه الإصابة إلى الحيوانات المحتكة معها. كما يمكن تسجيل التفاعل التبلمرى المتسلسل كاختبار حساس وسريع ويمكن استخدامه لتشخيص جميع صور الإصابة بالكلاميدوفيلا والتفريق بين أنواعها المختلفة فى الحيوانات المصابة وتزيد الحاجة اليه فى الحيوانات الحاملة للميكروب وتبدو سليمة ظاهريا بينما اختبار الإليزا يمكن استخدامه فى إجراء المسح السيرولوجى فى المزارع التى تعانى من الإجهاضات.

## SUMMARY

To investigate form (or forms) of *Chlamydophila* infection in camels and to determine the role that can be played by camels in transmission of this organism to other animals, this infection was studied from clinical, diagnostic and epidemiological aspects in camels and contact sheep. In this study, 30 camel and 300 sheep from different 6 farms where camels and sheep found together in the same place in Western region of KSA were used, three of these farms suffered from abortion in sheep. Diagnostic study included two assays, enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). These tests performed on serum and milk samples of camels and sheep three successive times one month apart between each two examinations. Results of clinical examination showed that some camels infected with *Chlamydophila* suffered only from respiratory signs while some sheep aborted and other suffered from respiratory signs varied from mild to severe, no abortion recorded in infected camels. Diagnostic study proved infection of camels with three spp. of *Chlamydophila* (*C. abortus* – *C. pecorum* – *C. pneumoniae*) while only *C. abortus* and *C. pecorum* were recorded in sheep. ELISA used in this study couldn't differentiate between different spp. of *Chlamydophila* while PCR could identify and differentiate between three *Chlamydophila* Spp. Collected data and disease history of the used farms with results of this study (seroconversion of 16 sheep and detection of *Chlamydophila* nucleic acid in 25 sheep after introducing of *Chlamydophila* infected newly purchased two camels to the farm without any previous history of abortion and did not suffered from any form of Chlamydophil, all these animals were negative for *Chlamydophila* antibodies and nucleic acid at the beginning of the study) proved that camels plays a very important role in transmission and epidemiology of *Chlamydophila* infection. PCR

assay proved to be more sensitive than ELISA where it gave positive results with 18 camels and 142 sheep while ELISA was positive in 11 camels and 109 sheep only. At the same time, PCR didn't give any positive results with vaccinated sheep while all vaccinated animals were positive with ELISA. From results of this work I can record that camels could infected with *Chlamydophila* but most infected camels appear healthy so they can play a very important role in transmission of this infection to contact animals. Also PCR could be recorded as fast and sensitive technique and can be used for diagnosis of all forms of *Chlamydophila* infection, its importance increase in apparently healthy carrier animals while ELISA could be used in serological screening of animals in farms suffered from abortion.

***Key words:*** *Chlamydophila, ELISA, PCR, Camel, Sheep, Abortion*

## INTRODUCTION

The family *Chlamydiaceae* consists of obligate, intracellular gram-negative bacteria that cause a broad range of disease in both humans and animals, which include abortion, pneumonia, gastroenteritis, polyarthritis and conjunctivitis (Twomey *et al.,* 2006; Yang *et al.,* 2006; Harley *et al.,* 2007; Skilton *et al.,* 2007). The family *Chlamydiaceae*, which previously contained the single genus *Chlamydia*, has recently undergone reclassification into two genera, *Chlamydia* and *Chlamydophila*, and nine species (Everett *et al.,* 1999). The most economically important animal pathogen of small ruminants is *Chlamydophila abortus* (previously classified as *Chlamydia psittaci* serotype 1), which causes abortion in sheep and goats (Philips and Clarkson, 1998; Rodolakis *et al.,* 1998; Entrican *et al.,* 2001).

Intracellular bacteria of the order *Chlamydiales* were first associated with diseases of cattle when McNutt and Waller (1940) isolated such organisms from feedlot cattle with sporadic bovine encephalomyelitis. When chicken embryo and cell culture methods for *Chlamydiales* became widely used, around 1955, a number of studies worldwide documented chlamydial agents in many acute diseases of animals. Chlamydial strains from ruminant abortion were identified as serotype 1, biotype 1, immunotype 1 chlamydiae (Perez-Martenez and Storz, 1985). Recently, a reclassification as *Chlamydophila abortus* (*C. abortus*) was proposed (Everett *et al.,* 1999; Schachter *et al.,* 2001). *C. abortus* has also been associated with mastitis (Jee *et al.,* 2004).

Another chlamydial agent has been associated worldwide with clinically severe chlamydial disease manifestations other than abortion (Jee *et al.,* 2004). This chlamydial strain was diagnosed as serotype 2, biotype 2, immunotype 2 chlamydiae (Kaltenboeck *et al.,* 1993) and was classified as a separate chlamydial species (Fukushi and Hirai, 1992). Recently, reclassification of this agent as *Chlamydophila pecorum* was proposed (Everett *et al.,* 1999; Schachter *et al.,* 2001).

Numerous studies confirmed the disease potential of *C. abortus* and *C. pecorum* by experimentally reproducing the acute and severe diseases (Jones, 1997; Jones *et al.,* 1998). Shewen summarized in (1980) the status of our understanding of chlamydial infections in animals, some animals may experience severe or even fatal disease as a result of chlamydial exposure. A well balanced host-parasite relationship represents the common nature of chlamydial infection. The long-lasting inapparent or 'latent' state has been documented in several species: birds, cattle, guinea pigs, sheep and humans. Under circumstances of stress, 'carrier' animals may shed the organisms in large numbers or may in fact lapse into clinical disease.

Ovine enzootic abortion (OEA) resulting from infection of sheep and goats with *C. abortus* is of major economic importance worldwide (Longbottom *et al.,* 2002). *C. abortus* (formerly *Chlamydia psittaci* serotype 1) is the most common infectious bacteria in small ruminants, some studies reported that 39% of examined abortions in sheep and 23% in goats caused by this agent. In newly infected flock, up to 30% of ewes may abort in the last trimester of gestation or give birth to a weak or dead lambs (Gerber *et al.,* 2007). Approximately 71% of aborted cows and 58% of aborted goats had IgG against *C. abortus* in their sera (Wang *et al.,* 2001).

In the United Kingdom, chlamydial abortion accounts for about 50% of all diagnosed abortions, resulting in losses estimated to be in excess of £20 million each year. *C. abortus* can also cause abortion in cattle and represents a significant zoonotic risk to pregnant women (Longbottom *et al.,* 2002).

Over the last 50 years the serological diagnosis of chlamydial infections has been based mainly on complement fixation test (CFT), which lacks both sensitivity and specificity because of cross-reactive antibodies to other gram-negative bacteria, including another common chlamydial pathogen (Longbottom *et al.,* 2002). Jones *et al.,* (1997) used 5 different serological tests for the detection of antibodies against Chlamydophila and suggested that further improvements in Chlamydial

diagnosis must come because none of these tests was sufficiently satisfactory.

Despite improvement in diagnostic techniques, our understanding about the prevalence and pathogenetic significance of these infections has not substantially changed since Shewen's review in 1980. The major impediment has been the cumbersome nature and insensitivity of diagnostic procedures, particularly of the complement fixation test for determination of seroprevalence of chlamydial infection in animals (Kaltenboeck *et al.,* 1997; Jee *et al.,* 2004).

If low-level clinically inapparent infections represent the norm and such infections occasionally aggregate into clinical manifestations. In this case, our detection methods simply would not be sensitive and specific enough to detect such low levels of chlamydial infections. Recently, a highly sensitive PCR method suitable for large-throughput routine detection, quantification, and differentiation of *Chlamydophila* DNA was established (DeGraves *et al.,* 2003a). A 53% prevalence of *C. abortus* and *C. pecorum* infection was detected, supporting the notion of continuous low-level infection (DeGraves *et al.,* 2003b).

ELISA also was used for diagnosis of chlamydia (Salti-montesanto *et al.,* 1997; Buendia *et al.,* 2001) but it couldn't differentiate ovine *C. abortus* and *C. pecorum* (Wang *et al.,* 2001), but was shown to be more sensitive than CFT particularly where abortion had occurred and can be used as screening test for chlamydial abortion (Anderson *et al.,* 1995; Griffiths *et al.,* 1996; Donn *et al.,* 1997; Gerber *et al.,* 2007). However, ELISA was identified as being more sensitive and specific than other serological tests (Buendia *et al.,* 2001; Longbottom *et al.,* 2002; McCauley *et al.,* 2007; Vretou *et al.,* 2007). McCauley *et al.,* (2007) reported 60% and 70% sensitivity of CFT and ELISA respectively in diagnosis of *Chlamydophila* infections, so, they recommended ELISA as an alternative to CFT but they also concluded that the search for more specific and sensitive assay for diagnosis of *Chlamydophila* should be continue.

Prevention and control of OEA can be achieved by application of live vaccine. Results of works of Borel *et al.,* (2005) and Gerber *et al.,* (2007) showed that by using serology, no distinction can be made between vaccinated and naturally infected animals. As a result, confirmation of negative OEA status in vaccinated animals can not be determined by serology. ELISA also couldn't differentiate between different *Chlamydophila* species (Siarkou *et al.,* 2002).

PCR was attempted as recent technique for diagnosis of *Chlamydophila* infections (Ciervo *et al.*, 2003; Ongor *et al.*, 2004; Yang *et al.*, 2006; Gullsby *et al.*, 2007; Branley *et al.*, 2008). PCR has the potential to detect a single organism (Laroucau *et al.*, 2001; Amin, 2003a; Masala *et al.*, 2007). Some study groups reported positivity rates of up to 100% detection of *Chlamydophila pneumoniae* by PCR (Apfalter *et al.*, 2001).

Unfortunately, little is still known about the ability of *C. abortus* to persist in animal (Gerber *et al.*, 2007). The prevalence of *Chlamydophila* infections in camels and the rates of acquisition and transmission have not been studied in detail. Therefore, the present study was designed to study *Chlamydophila* infections in camels and to investigate presence and cause of persistence of *Chlamydophila* species in farms containing camels and sheep. Comparison between ELISA and PCR as a diagnostic tools for *Chlamydophila* infections was an another objective of this study.

## MATERIALS and METHODS

**Animals**

30 camels and 300 contact sheep found in different 6 farms were used in this work (5 of these sheep were vaccinated against *Chlamydophila*), three of these farms had a previous history of abortion but none of the used animals were suffered from abortion before this investigation, After planning and the beginning of the study, 2 newly purchased camels were added to farm (that hadn't history of abortion). These 2 camels were added to the study.

**Serum**

For ELISA, blood was collected from the jugular vein with a 7-ml blood collection tube (Vacutainer tubes with Hemogard closures; Becton Dickinson and Co., Franklin Lakes, N.J.). The serum was separated by centrifugation at 1,300 × *g* for 15 min and stored at –80°C in 2-ml microcentrifuge tubes with screw caps till use.

**Milk**

For DNA extraction, 600 µl of milk was mixed with 600 µl of 6 M guanidine-HCl, 10 mM urea, 10 mM Tris-HCl, and 20% Triton X-100 (vol/vol), pH 4.4, in a 2.0-ml microcentrifuge tube with a screw cap.

**ELISA**

Antigen equivalent to 0.7 µg of EB protein per well, diluted to 100 µl in coating buffer (15 mM $Na_2CO_3$ and 35 mM $NaHCO_3$ [pH

9.6]), was added per well to white C-bottom 96-well microtiter plates (White MaxiSorp; Fisher Scientific Co.). Plates were incubated overnight at 4°C, the coating solution was aspirated, and wells were washed five times with wash buffer (0.1 M Tris-HCl [pH 7.5], 0.15 M NaCl, and 0.1% Tween 20). Wells were blocked by adding 200 µl of assay diluent (0.1 M Tris-HCl [pH 7.5], 0.15 M NaCl, 0.1% Tween 20, and 10% normal rabbit serum) for 1 h at room temperature. The assay diluent was removed, and 100 µl of serum sample diluted 1:100 with the assay diluent was added to each well of the plate except wells A1,A2 and B1,B2 which used for positive and negative controls. Incubation for 1.5 h at room temperature. After five washes, 100 µl of alkaline phosphatase (AP)-conjugated antibodies against immunoglobulin A (IgA), IgG, or IgM (Bethyl Laboratories, Inc., Montgomery, Tex.) per well was added, diluted in assay diluent (IgM, 1:300; IgG, 1:600; and IgA, 1:500), and incubated for 1 h at room temperature. Plates were washed five times, 150 µl of freshly prepared AP substrate buffer solution (BM Chemiluminescence ELISA Substrate AP; Roche Applied Science) per well was added, and the luminescence emitted was read with a microplate reader (Labsystems, A Thermo Bioanalysis Company, Research technology Devision, Helsinki, FINLAND).After 10 min of shaking. Luminescence data were calculated and evaluated as follow:-

1-Calculation of negative control mean (NCX)

$$(NCX) = \frac{A1 + A2}{2}$$

2-Calculation of positive control mean (PCX)

$$(PCX) = \frac{B1 + B2}{2}$$

3-Caculation of blocking % for tested samples

$$\text{Blocking \%} = \frac{NCX - \text{optical density of tested sample}}{NCX} \times 100$$

4-Caculation of blocking % for positive control

$$\text{Blocking \%} = \frac{NCX - PCX}{NCX} \times 100$$

5-Interpritation of the result

- Serum with blocking % less than 45% were considered negative for *Chlamydophila* antibodies.
- Serum with blocking % equal or greater than 45% but less than 55% considered suspected and retested.
- Serum with blocking % of 55% and greater considered positive for *Chlamydophila* antibodies.

ELISA was performed according to Longbottom *et al.*, (2002) and manufacturer's (IDEXX Laboratories,Inc. Switzerland) directions.

**POLYMERASE CHAIN REACTION (PCR)**

**(a) DNA extraction.** Isolation of milk nucleic acid for PCR was performed with a High Pure PCR Template Preparation kit (Roche Applied Science) according to the manufacturer's instructions. 120 µl of proteinase K (20 mg/ml in double distilled $H_2O$) was added to milk samples and samples were incubated for 30 min at 72°C with shaking at 600 rpm. 300µl of isopropanol and 300 µl of chloroform were added to milk samples. After brief agitation, the sample solution was transferred to the DNA-binding glass fiber filter device, except for the lipophilic chloroform bottom phase of the milk samples. Samples were filtered by centrifugation at 3,000 x *g* for 3 min, followed by the addition of 500 µl of inhibitor removal buffer and centrifugation at 3,000 x *g* for 3 min. Samples were washed twice with 500-µl wash buffer and were centrifuged at 3,000 x *g* for 3 min. Traces of wash buffer were removed by centrifugation at 13,000 x *g* for 10 s, and 20 µl of elution buffer (10 mM Tris-HCl [pH 8.4], and 0.01 mM EDTA) prewarmed to 72°C was added to each sample filter inserted into the collection tube. The glass fiber filter devices were incubated for 2 min at 72°C with shaking at 600 rpm, and elution buffer was recovered by centrifugation at 13,000 x *g* for 1 min. After a second elution step with 20 µl of buffer, the eluted DNA stock (typically 35 µl per specimen) was stored at –80°C.

**(b) Primer design.** Primer sets specific for each of the three species (*C abortus, C pecorum* and *C pneumoniae*) were designed based on the DNA sequences published by Greco *et al.*, (2005) and Liu *et al.*, (2007). These primers supplied to Ministry of Agriculture, KSA from the Johns Hopkins Genetic Core Laboratory. Data of oligonucleotide primers used in this study are summarized in Table (1)

**Table 1:** Sequences and specifications of used olignucleotide primers.

| PRIMER | SEQUENCES 5`- 3` | SPECIFICATION | LOCATION |
|---|---|---|---|
| CpA | GGC GTA TTT GGG CAT CCG AGT AAC G | *C.abortus* | 738 – 762 |
| CpPE | TCA AAT CCA GCG GGT ATT AAC CGC CT | *C.pecorum* | 1117-1142 |
| CpPN | GGT CTC AAC CCC ATC CGT GTC GG | *C.pneumoniae* | 1172-1194 |

**( c ) PCR.** PCR mixture overlaid with 1 drop of mineral oil. The final mixture contained 25 pmol of each primer, 0.25 mM deoxynucleosides triphosphates (dNTPs), PCR buffer, and 2 U of AmpliTaq Gold DNA

polymerase (Perkin-Elmer, Branchburg, N.J.). 2.5 mM $MgCl_2$ was used as recommended by the manufacturer of the polymerase (Perkin-Elmer). PCR was used with a DNA thermal cycler (480; Perkin-Elmer Cetus, Norwalk, Conn.). Cycling times were 75 s at 95°C (to activate a small fraction of the heat-activated DNA polymerase), followed by 60 cycles of denaturation at 94°C for 45 s, annealing beginning at 62°C and ending at 52°C for 45 s, and extension at 72°C for 1 min. The annealing temperature was lowered 1°C every four cycles until it reached 52°C; this annealing temperature was kept until the end of the cycling process. Progressive release of the heat-activated DNA polymerase occurred during the thermal cycling process. The DNA polymerase was gradually activated at each cycle during denaturation to extend its activity to 60 cycles of DNA amplification.

**(d) Electrophoresis**: PCR products (20 µl) were separated by electrophoresis in 1.2% polyacrylamide gels (7 by 10 cm) at 80 mA for 30 min with Tris-borate-EDTA buffer (pH 8.3) and visualized with ethidium bromide (0.5 µg/ml). Jee *et al.*, (2004).

PCR performed according to Degraves *et al.*, (2003a); Degraves *et al.*, (2003b); Ongor *et al.*, (2004)

## RESULTS

**Clinical study**

Camel:

a- Rise of body temperature was reported in 3 camels (infected with *C. pneumoniae*).
b- Nasal discharge only was reported in 6 camels (infected with *C. pecorum*)
c- Rise of body temperature, nasal discharge and cough were reported in 2 camels (infected with *C. pneumoniae*)
d- 7 infected camels were clinically normal.

Sheep:

a- Abortion was recorded in 21 sheep (infected with *C. abortus* and/or *C. pecorum*).
b- Sight respiratory signs in the form of nasal discharge and/or weak cough were recorded in 49 infected sheep (infected with *C. abortus* and/or *C. pecorum*).
c- Severe respiratory signs in the form of strong cough and pneumonia were recorded in 39 infected sheep (infected with *C. abortus* and/or *C. pecorum*).
d- 4 infected sheep were died from sever pneumonia during performing this study.

**ELISA**

a- First: 9 camels and 97 sheep were positive for *Chlamydophila* antibodies.

b- Second: 11 camels and 97 sheep were positive for *Chlamydophila* antibodies

c- Third: 11 camels and 109 sheep (seroconversion of 16 sheep from farm without previous history of abortion was recorded after contact with new purchased two camels) were positive for *Chlamydophila* antibodies.4 sheep were died from severe pneumonia therefore, they excluded from 3rd ELISA.

d- All sheep with a history of previous vaccination against *Chlamydophila* (5 sheep) were positive with ELISA.

**PCR**

a- First: nucleic acid of *Chlamydophila* was detected in 16 camel and 121 sheep

b- Second: nucleic acid of *Chlamydophila* was detected in 18 camel (Two newly purchased camels were added to the farm and examined) and 135 sheep (14 of them were from farm without previous history of abortion and gave negative result with 1st PCR but gave positive results after contact with positive newly purchased camels).

c- Third: nucleic acid of *Chlamydophila* was detected in 18 camel and 142 sheep (number of positive sheep increased by increase the time of contact with positive newly purchased camels), 25 of these positive sheep were from farm without previous history of abortion. 4 sheep were died from severe pneumonia therefore, they excluded from 3rd PCR.

d- The nucleic acid detected positive camels were belong three *Chlamydophila* species (*C. abortus, C. pecorum and C. pneumoniae*)

Results of laboratory investigation are summarized in Tables (2), (3) and results of PCR are shown in Figure (1).

**Table 2:** Results of laboratory investigation:

| | ELISA | | | | | | PCR | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | | 2nd | | 3rd | | 1st | | 2nd | | 3rd | |
| | + | - | + | - | + | - | + | - | + | - | + | - |
| Camel | 9 | 21 | 11 | 21 | 11 | 21 | 16 | 14 | 18 | 14 | 18 | 14 |
| Sheep | 97 | 203 | 97 | 203 | 109 | 187 | 121 | 179 | 135 | 165 | 142 | 154 |

**Table 3:** different *Chlamydophila* Species recorded with PCR

| | PCR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1st | | | 2nd | | | 3rd | | |
| | *C.ab* | *C.pe* | *C.pn* | *C.ab* | *C.pe* | *C.pn* | *C.ab* | *C.pe* | *C.pn* |
| Camel | 4 | 9 | 3 | 6 | 9 | 3 | 6 | 9 | 3 |
| Sheep | 39 | 82 | - | 53 | 82 | - | 60 | 82 | - |

*C.ab= Chlamydophila abortus C.pe=Chlamydophila pecorum C.pn=Chlamydophila pneumoniae*

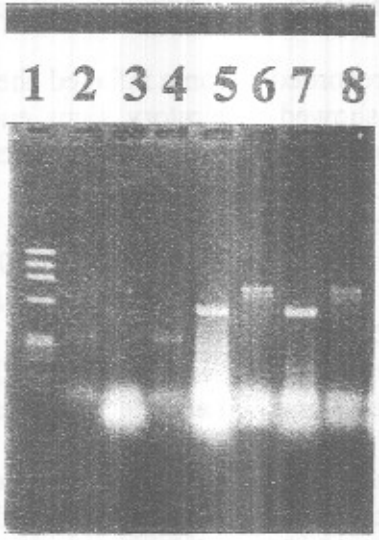


**Fig. 1:** Agarose gel electrophoresis analysis of amplified PCR products in camels and sheep.
Lane1 (DNA size marker). Lanes 2,4 (*C.Pneumoniae* in camels). Lane 3 (negative result). Lane 5 (*C.Abortus* in sheep). Lane 6 (*C.Pecorum* in sheep). Lane 7 (*C.Abortus* in camels). Lane 8 (*C.pecorum* in camels).

## DISCUSSION

Large number of camels examined in the present study and gave positive result with ELISA and/or PCR was apparently healthy without any abnormal clinical signs. Monthly PCR testing revealed persistent recurring infection with *C. pecorum* and *C. abortus* despite the absence of any clinical illness (Jaegr *et al.*, 2007; Miyashita *et al.*, 2007; Reinhold *et al.*, 2007). Both carriers and ill animals can shed the bacterium from many sites, including via nasal and faecal secretions (Jager *et al.*, 2007). Inapparent infection with *Chlamydophila* was recorded (Griffiths *et al.*, 1996; Reinhold *et al.*, 2007).

No abortion recorded in any camels examined in this study even these animals that proved to have *C. abortus* and/or *C. pecorum*. Some of these infected camels showed only respiratory signs. Twomey *et al.*, (2006) studied role of Chlamydia species in upper respiratory tract infections and detected *C. abortus* and *C. pecorum* as a main cause of bovine upper respiratory tract disease outbreak.Some infected camels showed only rise of body temperature. Reinhold *et al.*, (2007) recorded only higher temperature in *Chlamydophila* infected calves. Tibary *et al.*, (2006) investigated the causes of reproductive losses in camels and coudn't record any infection with *Chlamydophila* as a cause of abortion in camels.

Abortion was recorded in some infected sheep in this work while other infected sheep showed respiratory signs end fatally in 4 sheep. *C. abortus* caused abortion in sheep and considered one of the most important cause of abortion in sheep (Tsakos *et al.*, 2001; Longbottom *et al.*, 2002; Ongor *et al.*, 2004; Bagadonas *et al.*, 2007; Masala *et al.*, 2007; Michalopolou *et al.*, 2007; Reitt *et al.*, 2007). Respiratory signs and pneumonia had been recorded in some *Chlamydophila* infected animals (Jager *et al.*, 2007).

In this study I used ELISA and PCR for detection of *Chlamydophila* in camels and contact sheep. McCauley and co workers (2007) recommended ELISA as an alternative to CFT but they added that the search for more specific assay should be continue. PCR has gained increasing importance as a tool for directly demonstrating the presence of *Chlamydophila* in a clinical samples (Michalopolou *et al.*, 2007; Ortega *et al.*, 2007). Oktem *et al.*, (2007) reported that utilization of Just one diagnostic technique such as serological tests or PCR-based detection methods during *Chlamydophila* outbreaks can result in some of the positive samples being missed. After obtaining of results of this work I can add that PCR alone is enough for sensitive diagnosis of *Chlamydophila* as all positive samples with ELISA was positive with PCR in addition to samples give positive results with PCR while it were negative with ELISA.

In this study milk samples were used for detection of *Chlamydophila* nucleic acid in PCR assay and proved to be good sample for diagnosis. PCR can potentially be used for different clinical samples (Messmer *et al.*, 1997; Madico *et al.*, 2000) and Ongor *et al.*, (2004) used milk for isolation of *Chlamydophila* nucleic acid.

In the present investigation, highly sensitive PCR to study the prevalence of *Chlamydophila* sp. infection in camels was used. The

results of this investigation have the potential to shift the focus from *Chlamydophila* infection as a rare, severe disease to *Chlamydophila* infection as a pervasive, low-level infection in camels without apparent disease (silent epidemic) or with only a subtle expression of disease, one that impacts herd health and fertility but is difficult to recognize in individual animals. The same observation was recorded by Reinhold *et al.,* (2007) in cattle.

PCR used in this investigation could differentiate between *C. abortus*, *C. pecorum* and *C. pneumoniae.* Molecular amplification techniques based on genomic sequences have been used for the differentiation of *Chlamyophila* species (Messmer *et al.,* 1997; Madico *et al.,* 2000).

Results of PCR assay was obtained within few hours in this work. PCR technique is fast, simple, inexpensive, and easy to perform and requires minimal sample manipulation, which may reduce the chances for contamination (Madico *et al.,* 2000)

In the present work, ELISA failed to detect any of inapparent infection. Classic methods for the detection of *Chlamydophila* agents and of antibodies against these agents have indicated that these methods demonstrated acute *Chlamydophila* - induced diseases with generally high, *Chlamydophila* seroprevalence. However, it was impossible to consistently detect low levels of these organisms and its antibodies by these classic methods (Jee *et al.,* 2004).

The prevalence of *C. pecorum* in camels in this study was more than C. abortus and *C. Pneumoniae*. Jee *et al.,* (2004) in their study recorded the same observation in calves (except for *C. pneumoniae* which not recorded in calves) and added that the prevalence of *C. pecorum* was approximately five times as high as that of *C. abortus*

18 out of 32 examined camels (56.25%) were positive for 3 different *Chlamydophila* species. Study of Bagdonas *et al.,* (2007) in Lithuania revealed that 54.5% of animals were positive for *Chlamydophila*.

The results obtained from this study confirm the previous observation of Borel *et al.,* (2005) and Gerber *et al.,* (2007) that ELISA can not be used to distinguish between animals vaccinated with the live attenuated vaccine and naturally infected animals.

Results of this work indicated that PCR is more sensitive and specific than ELISA. Wang *et al.,* (2001) reported that only 22.7% from aborted cows and 33.3% from aborted sheep that gave positive result with PCR was positive with ELISA. PCR assay provides a simple,

sensitive, rapid and reliable means for the detection of the *Chlamydophila* (Madico *et al.*, 2000; Amin, 2003b) and is considered to be more suitable for the detection of *Chlamydophila* (Yang *et al.*, 2006; Reitt *et al.*, 2007). PCR is a useful method for sensitive and early detection and identification of *Chlamydophila*. During endemic infections, the sensitivity of diagnostic tests and rapid diagnosis is particularly important (Oktem *et al.*, 2007; Ortega *et al.*, 2007).

PCR used in this investigation could differentiate between different species of *Chlamydophila* (*C. abortus*, *C. pecorum* and *C. pneumoniae*) while ELISA failed to differentiate between them. Wang *et al.*, (2001) recorded certain degree of cross reactivity between *C. abortus* and *C. pecorum* and therefore they could not differentiate between them by ELISA.

Seroconversion was recorded in 12 sheep (from farm without previous history of abortion) by the 3rd ELISA and after introducing of newly purchased two camels to the farm. These two camels proved to be infected by *Chlamydophila* by 2nd ELISA and 2nd PCR. At the same time 2nd and 3rd PCR detect nucleic acid of *Chlamydophila* in 17 and 24 sheep respectively in this farm. The same observation has been recorded by Twomey *et al.*, (2006) in cattle who added that adult cows were the most likely source of infection to calves after contact with them. This result also clearly proved that PCR is a suitable technique for diagnosis of early detection of *Chlamydophila* infection. PCR is useful method for early detection and identification of *Chlamydophila* (Oktem *et al.*, 2007)

Results of the present investigation proved that camels infected with *C. abortus* and/or *C. pecorum* (even in absence of any clinical signs) are the source of infection of contact sheep. Epidemiological observations and laboratory testing of Twomey *et al.*, (2006) indicated that adult dairy cows were the most likely source of infection with *Chlamydophila* species to calves. Results of Salwa and co workers (2007) suggested that there is some transmission of *Chlamydophila* species occurring between domestic and wild ruminant.

From results of this study, I can conclude that camels could be infected with different species of *Chlamydophila* but most infected camels were apparently healthy, so, they can play a very important role in transmission of infection to contact sheep and persistence of infection for a long time. Also, results of this work proved that *C. abortus* and *C. pecorum* which causes abortion in small and large ruminants couldn't cause abortion in camels. PCR seems to be more sensitive than ELISA in diagnosis of *Chlamydophila* infections and has the priority to

differentiate between different species of *Chlamydophila* as well as differentiate between infected and vaccinated animals while ELISA failed in both differentiations. Therefore, these results recommend using of PCR in diagnosis of any forms of *Chlamydophila* infections specially in silent or latent infection while ELISA can be used in serological screening in areas suffered from abortions.

## REFERENCES

*Amin, A.S. (2003a):* Application of touchdown enzyme time release (TETR)-PCR for diagnosis of *Chlamydophila abortus* infection. Res. Vet. Sci., 74 (3): 213-217.

*Amin, A.S. (2003b):* Comparison of polymerase chain reaction and cell culture for detection of *Chlamydophila* species in the semen of bulls, buffalo-bulls and rams. Vet. J., 166 (1): 86-92.

*Anderson, I.; Herring, A.; Jones, G.; Low, J. and Greig, A. (1995):* Development and evaluation of an indirect ELISA to detect antibodies to abortion strains of *Chlamydia Psittaci* in sheep sera. Vet. Microbiol., 43 (1): 1-12.

*Apfalter, P.; Blasi, F.; Boman, J.; Gaydos, C.; Kundi, M.; Maass, M.; Makristathis, A.; Meijer, A.; Nadrchal, R.; Persson, K.; Rotter, M.; Tong, C.; Stanek, G. and Hirschl, A. (2001):* Multicenter Comparison Trial of DNA Extraction Methods and PCR Assays for Detection of *Chlamydia pneumoniae* in Endarterectomy Specimens. J. Clin. Microbiol., 39 (2): 519-524.

*Bagdonas, J.; Petkevicius, S.; Russo, P.; Pepin, M. and Salomskas, A. (2007):* Prevalence and epidemiological features of ovine enzootic abortion in Lithuania. Pol. J. Vet. Sci., 10 (4): 239-244.

*Borel, N.; Sachse, K.; Rassbach, A.; Bruckner, L.; Vretou, E.; Psarrou, E.; Pospischil, A. (2005):* Ovine enzootic abortion (OEA): Antibody response in vaccinated sheep compared to naturally infected sheep. Vet. Res. Commun. 29: 151–156.

*Branley, J.; Roy, B.; Dwyer, D. and Sorrell, T. (2008):* Real time PCR detection and quantification of *Chlamydophila psittaci* in human and avian specimens from a veterinary clinic cluster. Eur. J. Microbiol. Infect. Dis., 5 (2): 243-249.

*Buendia, A.J.; Cuello, F.; Del Rio, L.; Gallego, M.C.; Caro, M.R. and Salinas, J. (2001):* Field evaluation of a new commercially available ELISA based on a recombinant antigen for diagnosis

of *Chlamydophila abortus* (*Chlamydia psittaci* serotype1) infection. Vet. Microbiol., 78 (3): 229-239.

*Ciervo, A.; Petrucca, A. and Cassone, A. (2003):* Identification and quantification of *Chlamydia pneumoniae* in human atherosclerotic plaques by LightCycler real-time PCR. Mol. Cell Probes, 17 (2-3): 107-111.

*DeGraves, F.D.; Gao, D. and Kaltenboeck, B. (2003a):* High-sensitivity quantitative PCR platform. BioTechniques 34: 106-115.

*DeGraves, F.; Gao, D.; Hehnen, H.; Schlapp, T. and Kaltenboeck, B. (2003b):* Quantitative detection of *Chlamydia psittaci* and *C. pecorum* by high-sensitivity real-time PCR reveals high prevalence of vaginal infection in cattle. J. Clin. Microbiol. 41: 1726-1729.

*Donn, A.; Jones, G.; Ruiu, A.; Ladu, M.; Marchell, J. and Stancanelli, A. (1997):* Serological diagnosis of chlamydial abortion in sheep and goats: comparison of the complement fixation test and an enzyme linked immunosorbent assay employing solubilised proteins as antigen. Vet. Microbiol., 59 (1): 27-36.

*Entrican, G.; Buxton, D. and Longbottom, D. (2001):* Chlamydial infection in sheep: immune control versus fetal pathology. J. R. Soc. Med. 94: 273-277.

*Everett, K.D.; Bush, R.M. and Andersen, A.A. (1999):* Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int. J. Syst. Bacteriol. 49: 415-440.

*Fukushi, H. and Hirai, K. (1992):* Proposal of *Chlamydia pecorum* sp. nov. for *Chlamydia* strains derived from ruminants. Int. J. Syst. Bacteriol. 42: 306-308.

*Gerber, A.; Thoma, R.; Vretou, E.; Psarrou, E.; Kaiser, C.; Doherr, M.; Zimmermann, D.; Polkinghorne, A.; Pospischil, A. and Borel, N. (2007):* Ovine Enzootic Abortion (OEA): a comparison of antibody responses in vaccinated and naturally-infected swiss sheep over a two year period. BMC Vet. Res., 3: 24-33.

*Greco, G.; Totaro, M.; Madio, A.; Tarsitano, E.; Fasanella, A.; Lucifora, G. and Buonavoglia, D. (2005):* Detection of *Chlamydophila abortus* in sheep and goat flocks in southern

Italy by PCR using four different primer sets. Vet Res Commun., Suppl 1: 107-115.

*Griffiths, P.C.; Plater, J.M.; Horigan, M.W.; Rose, M.P.; Venables, C. and Dawson, M. (1996):* Serological diagnosis of ovine enzootic abortion by comparative inclusion immunofluorescence assay, recombinant lipopolysaccharide enzyme linkes immunosorbent assay and complement fixation test. J. Clin. Microbiol., 34 (6): 1512 – 1518.

*Gullsby, K.; Storm, M. and Bondeson, K. (2007):* Simultaneous detection of *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* using molecular beacons in a duplex real-time PCR. J. Clin. Microbiol., 45 (3) 354-366.

*Harley, R.; Herring, A.; Egan, K.; Howard, B.; Gruffydd-Jones, T.; Azuma, Y.; Shirai, M. and Helps, C. (2007):* Molecular characterization of 12 *Chlamydophila felis* polymorphic membrane protein genes. Vet. Microbiol., 124 (3-4): 230-238.

*Jager, J.; Liebler-Tenorio, E.; Kirschvink, N.; Sachse, K. and Reinhold, P. (2007):* A clinically silent respiratory infection with *Chlamydophila* spp. In calves is associated with airway obstruction and pulmonary inflammation. Vet. Res., 38 (5): 711-728.

*Jee, J.; Degraves, F.; Kim, T. and Kaltenboeck, B. (2004):* High Prevalence of Natural *Chlamydophila* Species Infection in Calves. J. Clin. Microbiol., 42 (12): 5664-5672.

*Jones, G.E. (1997):* Chlamydia disease-more than just abortion. Vet. J., 153: 249 – 251.

*Jones, G.E.; Low, J.C.; Machll, J. and Armstrong, K. (1997):* Comparison of five tests for the detection of antibodies against chlamydial (enzootic) abortion of ewes. Vet. Rec., 14 (7): 164-168.

*Jones, G.; Donn, A.; Machell, J.; Biolatti, B. and Appino, S. (1998):* Experimental infections of the genital tract of cattle with *Chlamydia psittaci* and *Chlamydia pecorum*, p. 446-449. *In* R. S. Stephens (ed.), Chlamydial infections. Proceedings of the Ninth International Symposium on Human Chlamydial Infection. International Chlamydia Symposium, San Francisco.

*Kaltenboeck, B.; Kousoulas, K. and Storz, J. (1993):* Structures of and allelic diversity and relationships among the major outer membrane protein (*ompA*) genes of the four chlamydial species. J. Bacteriol.175: 487-502.

*Kaltenboeck, B.; Heard, D.; DeGraves, F. and Schmeer, N. (1997):* Use of synthetic antigens improves detection by enzyme-linked immunosorbent assay of antibodies against abortigenic *Chlamydia psittaci* in ruminants. J. Clin. Microbiol. 35:2293-2298.

*Laroucau, K.; Souriau, A. and Rodolakis, A. (2001):* Improved sensitivity of PCR for *Chlamydophila* using pmp genes. Vet. Microbiol., 82 (2): 155-164.

*Liu, X.; Zhang, Y.; Liang, R.; Hou, J. and Liu, J. (2007):* Characterization of the 3`exonuclease of *Chlamydophila pneumoniae* endonuclease IV on double-stranded DNA and the RNA strand of RNA/DNA hybrid. Biochem. Biophys. Res. Commun., 12 (4): 375-382.

*Longbottom, D.; Fairley, S.; Chapman, S.; Psdarrou, E.; Vretou, E. and Livingstone, M. (2002):* Serological diagnosis of ovine enzootic abortion by enzyme linked immunosorbent assay with a recombinant protein fragment of the polymorphic outer membrane protein POMP90 of *Chlamydophila abortus*. J. Clin. Microbiol., 40 (11): 4235-4243.

*Madico, G.; Quinn, T.; Boman, J. and Gaydos, C. (2000):* Touchdown enzyme time release-PCR for detection and identification of *Chlamydia rtachomatis, C. pneumoniae* and *C. psittaci* using 16S and 16S-23S spacer rRNA genes. J. Clin. Microbio., 38 (3): 1085-1093.

*Masala, G.; Porcu, R.; Daga, C.; Denti, S.; Canu, G.; Patta, C. and Tola,S (2007):* Detection of pathogens in ovine and caprine abortion samples from Sardinia, Italy, by PCR. J. Vet. Diagn. Invest., 19 (1): 96-98.

*McCauley, L.; Lancaster, M.; Young, P.; Butler, K. and Ainsworth, C. (2007):* Comparison of ELISA and CFT assays for *Chlamydophila abortus* antibodies in ovine sera. Aust. Vet. J., 85 (8): 325-328.

*McNutt, S.H. and Waller, E.F. (1940):* Sporadic bovine encephalomyelitis. Cornell Vet. 30:437-448.

*Messmer, T.; Skelton, S.; Moroney, J.; Daugharty, H. and Fields, B. (1997):* Application of a nested, multiplex PCR to psittacosis outbreaks. J. Clin. Microbiol. 35: 2043-2046

*Michalopolou, E.; Leigh, A. and Cordoba, L. (2007):* Detection of the genome of *Chlamydophila abortus* in samples taken from the uteri of 304 sheep at an abattoir. Vet. Rec., 161 (5): 153-155.

*Miyashita, N.; Obase, Y.; Fukuda, M.; Shouji, H.; Yoshida, K.; Ouchi, K. and Oka, M. (2007):* Evaluation of the diagnostic usefulness of real-time PCR for detection of *Chlamydophila pneumoniae* in acute respiratory infections. J. Infect. Chemother., 13 (3): 183-187.

*Oktem, I.; Ellidokuz, H.; Sevinc, C.; Kilinc, O.; Aksakoglu, G.; Sayiner, A.; Ucan, E.; Sezgin, S.; Ozdemir, H. and Yuksel, B. (2007):* PCR and serology were effective for identifying *Chlamydophila pneumoniae* in a lower respiratory infection outbreak among military recruits. Jpn. J. Infect. Dis., 60 (2-3): 97-101.

*Ongor, H.; Cetinkaya, B.; Acik, M.; Karahan, M. and Bulut, H. (2004):* Detection of *Chlamydophila abortus* in ovine milk by immunomagnetic separation polymerase chain reaction. J. Vet. Med. B Infect. Dis., 51 (1): 43-45.

*Ortega, N.; Navarro, J.; Nicolas, L.; Buendia, A.; Caro, M.; Del Rio, L.; Martinez, C. Cuello, F.; Salinas, J. and Gallego, M. (2007):* Evaluation of *Chlamydophila abortus* DNA extraction protocols for polymerase chain reaction diagnosis in paraffin-embedded tissues. J. Vet. Diagn., 19 (4): 421-425.

*Perez-Martinez, J.A. and Storz, J. (1985):* Antigenic diversity of *Chlamydia psittaci*of mammalian origin determined by micro immuno - fluorescence. Infect. Immun. 50:905-910.

*Philips, H. and Clarkson, M. (1998):* Experimental infection of pregnant ewes with *Chlamydia pecorum*. Infect. Immun. 66: 2818-2821.

*Reinhold, P.; Jaeger, J.; Liebler-Tenorio, E.; Berndt, A.; Bachmann, R.; Schubert, E.; Melzer, F.; Elschner, M. and Sachse, K. (2007):* Impact of latent infections with *Chlamydophila* species in young cattle. Vet. J., 172 (2): 324-331.

*Reitt, K.; Hilbe, M.; Voegtlin, A.; Corboz, L.; Haessig, M and Pospischil, A. (2007):* Aetiology of bovine abortion in Switzerland from 1986 to 1995: a retrospective study with emphasis on detection of *Neospora caninum* and *Toxoplasma gondii* by PCR. J. Vet. Med. A Physiol. Pathol Clin. Med., 54 (1): 15-22.

*Rodolakis, A.; Salinas, J. and Papp, J. (1998):* Recent advances on ovine chlamydial abortion. Vet. Res. 29:275-288.

*Salti-Montesanto, V.; Tsoli, E.; Papavassiliou, P.; Psarrou, E.; Markey, B.K.; Jones, G.E. and Vretou, E. (1997):* Diagnosis of ovine enzootic abortion, using a competitive ELISA based on monoclonal antibodies against variable segments 1 and 2 of the

major outer membrane protein of *Chlamydia psittaci* serotype 1. Am. J. Vet. Res. 58:228–235.

*Salwa, A.; Anusz, K.; Arent, Z.; Paparocka, G. and Kita, J. (2007):* Seroprevalence of selected viral and bacterial pathogens in free ranging European bison from the Bialowieza Primeval Forest. Pol. J. Vet. Sci., 10 (1): 19-23.

*Schachter, J.; Stephens, R.S.; Timms, P.; Kuo, C.; Bavoil, P.M.; Birkelund, S.; Boman, J.; Caldwell, H.; Campbell, L.A.; Chernesky, M.; Christiansen, G.; Clarke, I.; Gaydos, C.; Grayston, J.; Hackstadt, T.; Hsia, R.; Kaltenboeck, B.; Leinonnen, M.; Ocjius, D.; McClarty, G.; Orfila, J.; Peeling, R.; Puolakkain, M.; Quinn, T.; Rank, R.; Raulston, J.; Ridgeway, G.; Saikku, P.; Stamm, W.; Taylor-Robinson, D.; Wang, S. and Wyrick, P. (2001):* Radical changes to chlamydial taxonomy are not necessary just yet. Int. J. Syst. Evol. Microbiol. 51: 249 – 260.

*Shewen, P.E. (1980):* Chlamydial infection in animals: a review. Can. Vet. J. 21:2-11.

*Siarkou, V.; Lambropoulos, A.; Chrisafi, S.; Kotsis, A. and Papadopoulos, O. (2002):* Subspecies variation in Greek strains of *Chlamydophila abortus*. Vet. Microbiol., 85 (2): 145-157.

*Skilton, R.J.; Cutcliffe, L.T.; Pickett, M.A.; Lambden, P.R.; Fane, B.A. and Clarke, I.N. (2007):* Intracellular parasitism of chlamydiae: specific infectivity of chalmydophage Chp2 in *Chlamydophila abortus*. J.Bacteriol., 189 (13): 4957-4959.

*Tibary, A.; Fite, C.; Anouassi, A. and Sghiri, A. (2006):* Infectious causes of reproductive loss in camelids. Theriogenology. 66 (3): 633-647.

*Tsakos, P.; Siarkou, V.; Guscetti, F.; Chowdhury, H.; Papaioannou, N.; Vretou, E. and Papadopoulos, O. (2001):* Experimental infection of pregnant ewes with enteric and abortion-source *Chlamydophila abortus*. Vet. Microbiol. (82):285–291.

*Twomey, D.; Griffiths, P.; Horigan, M.; Hignett, B. and Martin, T. (2006):* An investigation into the role of *Chlamydophila* spp. in bovine upper respiratory tract disease. Vet. J., 171 (3): 574-576.

*Vretou, E.; Radouani, F.; Psarrou, E.; Kritikos, I.; Xylouri, E. and Mangana, O. (2007):* Evaluation of two commercial assays for the detection of *Chlamydophila abortus* antibodies. Vet. Microbiol., 123 (1-3): 153-161.

*Wang, F.; Shieh, H. and Liao, Y. (2001):* Prevalence of *Chlamydophila abortus* infection in domesticated ruminants in Taiwan. J. Vet. Med. Sci., 63 (11): 1215-1220.

*Yang, J.; Liu, X.; Hao, Y.; He, C. and Zhao, D. (2006):* Development of a rapid real-time PCR assay for detection and quantification of four familiar species of *chlamydiaceae*. J. Clin. Virol., 36 (1): 79-81.