Seroprevalence of antibodies to *Chlamydophila abortus* shown in Awassi sheep and local goats in Jordan

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ABSTRACT: A cold complement fixation test (CFT) was used to identify *C. abortus* infection in ewes and does in northern Jordan. Sera from 36 flocks of sheep and 20 flocks of goats were collected randomly. The results showed that 433 (21.8%) out of 1 984 ovine sera, and 82 (11.4%) out of 721 caprine sera, were seropositive for *C. abortus* infection, as indicated by a titre ≥ 1:40. However, all the tested sheep flocks and goat flocks (100%) revealed at least one seropositive animal. There was a strong association ($P < 0.05$) between the rate of *C. abortus* infection and the size of the sheep flock, when larger flocks had higher infection rates. However, in goats, the flock size had no relationship with seropositivity. Age had no significant ($P > 0.05$) impact on *C. abortus* seropositivity. In sheep, there was a significant difference ($P < 0.05$) between the rates of the chlamydial infection in the four studied areas of northern Jordan. The highest infection rate in sheep (31.2%) was recorded in Mafraq area, while the rates in Irbid, Ajloun and Jerash were 18.5%, 11.2% and 13.9%, respectively. In goats, there was no significant difference between the rates of the chlamydial infection in the two areas studied. The rates of goat infections were 10.8% and 11.8% in Ajloun and Jerash areas, respectively.

Keywords: seroprevalence; *Chlamydophila abortus*; sheep; goats

Chlamydophila abortus, formally called *Chlamydia psittaci*, is a non-motile, coccoid, obligate intracellular parasite. It belongs to the family Chlamydiaceae, which was recently reclassified and now comprises nine separate species (Everett et al., 1999). It is one of the most important causes of reproductive failure in sheep and goats (Rodolakis et al., 1998; Aitken, 2000). In sheep the disease is usually manifested as abortion in the last 2 to 3 weeks of gestation, while the goats can abort at any stage of pregnancy, but most abortions are during the last 2 to 3 weeks of gestation (Matthews, 1999; Nietfeld, 2001). *C. abortus* has affinity for placental tissues. In rams and bucks, *C. abortus* can cause orchitis and seminal vesiculitis, resulting in the shedding of the organism in semen (Appleyard et al., 1985). *C. abortus* is zoonotic, and although most human infections are mild and often unnoticed, pregnant women can develop severe, life threatening illness and abort (Jorgensen, 1997). *C. pecorum* is commonly isolated from the digestive tract of ruminants, and it is responsible for a variety of disease syndromes including conjunctivitis and arthritis in sheep and goats (Fukushi and Hirai, 1993; Storz and Kaltenboeck, 1993).

The main source of *C. abortus* in the environment is placentas and foetal fluids of affected animals. During the lambing season, elementary bodies remained infectious for several days (Papp et al., 1994). It was documented that ingestion was the main route of infection (Wilsmore et al., 1986). 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

(Appleyard et al., 1985). Development of clinical signs due to *C. abortus* infection depends on the time of infection. Sheep and goats infected 5–6 weeks before parturition can develop clinical disease during the current pregnancy (Morgan et al., 1988). Animals infected during the last 4 weeks of gestation can develop latent infection and may develop clinical signs in the next gestation (Wilsmore et al., 1990). It was found that infected and latently infected sheep and goats may shed *C. abortus* in their reproductive tract for up to 3 years post infection (Morgan et al., 1988). Lambs and kids born from infected animals are usually weak and die within a few days after birth.

The main infectious diseases that cause abortion in sheep and goats are: *Brucella melitensis* (Darwish and Benkirane, 2001), *Brucella ovis* (Libal and Kirkbride, 1983), *C. abortus* (Aitken et al., 1990), *Salmonella abortus ovis* (Benkirane et al., 1990), *Champylobacter foetus* (Collins and De Lisle, 1985), *Champylobacter jejuni*, *Toxoplasma gondii* (Chanton-Greutmann et al., 2002), *Leptospira pomona* (Ellis, 1994) and *Listeria monocytogenes* (Low and Renton, 1985).

Serological and clinical data from the Ministry of Agriculture in Jordan showed that Chlamydia and *Brucella melitensis* are the predominant causes of abortion in sheep and goats. The Ministry of Agriculture in Jordan has implemented vaccination programs against Brucella in sheep and goats for 15 years, but there was no control program for chlamydiosis. The objective of this study was to investigate the seroprevalence and distribution of *C. abortus* infection in adult sheep and goats in northern Jordan.

MATERIAL AND METHODS

Sampling. A total of 1 984 serum samples were collected from 36 flocks of Awassi sheep during 1–2 weeks of abortion or lambing, from the four northern governorates of Jordan (Mafraq, Irbid, Ajloun and Jerash). The sample was stratified and weighted according to the animal population distribution in the four regions. Additional 721 serum samples were collected from 20 flocks of goats during 1–2 weeks of abortion or lambing from two northern governorates (Ajloun and Jerash), where goat flocks are commonly raised. Sample collection started in December 1998 and continued until August 2001. These sera were tested for the presence of antibodies against *C. abortus*. To evaluate the sensitivity and specificity of the CFT for serological identification of *C. abortus* in aborted sheep, two subgroups of animals were selected randomly. The first subgroup was 100 animals with Ab ≥ 1:40. The second subgroup was 100 animals with Ab titre < 1:40. Paired sera were collected from the two subgroups 3–4 weeks after the first sampling. An increase in the Ab titre in the second serum sample by at least two serial dilutions was considered confirmation of *C. abortus* infection. If the Ab titre in the second serum sample was not increased by at least two serial dilutions, then it was considered that it did not confirm *C. abortus* infection. Sample size was estimated at a 95% confidence with predicted prevalence of 10% (Thrusfield, 1995).

Serology procedure. Animals were bled (while they were in standing position) and serum was separated and stored at –20°C until testing. The complement fixation test was performed according to the protocol published by the Office of International Epizootic (O.I.E., 1996). The Chlamydia (PLT) complement fixation purified antigen, Chlamydia positive and negative control sera, and other reagents were purchased from TCS Microbiology Buckingham, UK. Serum samples with antibody titres ≥ 1:32 were considered positive according to O.I.E. However, for this study serum sample with Ab titre ≥1:40 was considered positive in order to increase the specificity of the CFT. The twofold serial dilution series was started from 1:10 to 1:320.

To test for reliability (repeatability) of the CFT one positive serum (Ab titre ≥ 1:40) and one negative serum (Ab titre ≤ 1:40) were selected randomly and repeated for testing in the second plate. A total of 25 positive and 25 negative sera were retested.

Questionnaire. A questionnaire was constructed by the authors to collect information on herds included in this study. The questionnaire was completed on the day of blood collection. Specific questions included owner's name, date, flock size, type of small ruminant, location and address, number of pregnant animals, number of newly born lambs and kids, number and stage of abortions and stillbirths.

Statistical analysis. Data were coded and entered in SPSS computer statistical program. The association between the seroprevalence of chlamydial infection and flock size, age, type of small ruminant and location were tested using the Chi-square test (χ^2-test). The relationship between the flock size and the seroprevalence rate was tested by simple linear regression.

The weighted rate for the seroprevalence in small ruminants (sheep and goats) was calculated according to the adjusted rate method (Thrusfield, 1995) summarized as follows:

Weighted Rate = ΣWi × Ri

where: Wi = proportion of sheep and goats in the small ruminant population in Jordan
Ri = seroprevalence rate in sheep and goat samples

Standard methods were applied for the calculation of sensitivity, specificity, predictive positive rate and predictive negative rate of the CFT at Ab titre ≥ 1:40.

RESULTS

The sensitivity and specificity of the CFT to identify *C. abortus* infection at Ab titre ≥ 1:40 were 79% and 93.7%, respectively (Table 1). However, the predictive positive and negative rates were 95% and 75%, respectively. Five animals out of 100 positive animals (5%) were positive by the first serum test but failed to show an increase in Ab titre in the second serum sample (paired sera) (Table 1). Those 5 animals (5%) could be persistently infected with *C. abortus* and maintained relatively high Ab titre that did not vary in time.

Out of 50 serum samples repeated by CFT, 45 sera (90%) gave the same Ab titre in the repeated test. However, the 5 sera (10%) that gave different Ab titre in the repeated test, 4 of them gave Ab titre one dilution lower and one serum gave Ab titre one dilution higher than the first test. These results indicate high reliability of CFT as a serological test to identify *C. abortus* infection.

All 56 flocks studied (36 sheep and 20 goat flocks) had a history of abortions and stillbirths. All the flocks of sheep and goats were found to be infected with *C. abortus*. The prevalence of chlamydial infection ranged from 4% to 57% in sheep flocks and 4% to 20% in goat flocks (Tables 2 and 3). The overall seroprevalence of Chlamydia in sheep was 21.8% while the overall seroprevalence in goats was 11.4% (Tables 2 and 3). The seroprevalence of chlamydial infection in sheep was significantly higher ($P < 0.05$) than that in goats. However, the seroprevalence of

Table 1. Sensitivity and specificity of the CFT for *C. abortus* infection in aborted sheep

CFT	*C. abortus*[1]	Not *C. abortus*[1]	Total
Positive (≥ 1:40)	95	5	100
Negative (< 1:40)	25	75	100
Total	120	80	200

[1]confirmed diagnosis by paired sera
Sensitivity = 95/120 = 0.79
Specificity = 75/80 = 0.938
Predictive positive value = 95/100 = 0.95
Predictive negative value = 75/100 = 0.75

Table 2. Seroprevalence of Chlamydia infection in ewes according to flock size in northern Jordan (1998–2001)

Size of flock (head/flock)	Number of flocks	Number tested	Seropositives (No.)[1]	Seropositives (%)	Range of seropositive (%)
100–199	13	500	69	13.8	5–21
200–299	12	620	105	16.9	4–31
300–600	11	864	259	30*	13–57
Total	36	1 984	433	21.8	4–57

[1]antibody titer ≥ 1: 40 by CFT

*significant difference $P < 0.05$

chlamydial infection at the flock unit was not significantly different ($P > 0.05$), since all the tested sheep and goat flocks were found to be infected with *C. abortus*.

In sheep flocks, there was an association ($P < 0.05$) between Chlamydia seropositivity and flock size groups. The larger the flock size, the higher the prevalence of chlamydial infection (Table 2). The highest prevalence of chlamydial infection was 30% in the largest flock size group that had over 300 animals. The lowest prevalence of chlamydial infection was 12.8% in the smallest flock size group that had less than 200 animals. There was a significant ($P < 0.05$) linear regression between the flock size and the prevalence of chlamydial infection in sheep. The linear regression equation is

$$Y = 7.91 + 0.039\ X$$

where: Y = the predicted prevalence (%) of chlamydial infection
7.91 = the intercept
0.039 = the regression coefficient (slope)
X = the number of animals in the sheep flock

However, in goat flocks there was no significant correlation ($P > 0.05$) between the rate of *C. abortus* infections and flock size (Table 3).

The seroprevalence of *C. abortus* infection in ewes is not associated ($P > 0.05$) with the three age groups (Table 4). The seroprevalence rate ranged from 19.8% in ewes over 4 years of age to 23.5% in ewes 1–2 years old. In goat flocks there was no correlation ($P > 0.05$) between the seroprevalence of chlamydial infection and the three age groups (Table 4). The seroprevalence rate ranged from 10.16% in does 2–4 years of age to 14.6 in does 1–2 years old. However, the seroprevalence rate of *C. abortus* in sheep was significantly higher ($P < 0.05$) than in goats (Table 4).

In sheep flocks, there was a significant difference ($P < 0.05$) between the seroprevalence of chlamydial infection and the location (Table 5). The highest prevalence rate (31.2%) of chlamydial infection was observed in Mafraq area, while the lowest rate (11.2%) was observed in Ajloun area. In goat flocks, there was no significant difference ($P > 0.05$) between the seroprevalence of *C. abortus* infection and

Table 3. Seroprevalence of Chlamydia infection in does according to flock size in northern Jordan (1998–2001)

Size of the flock	Number of flocks	Number tested	Seropositives (No.)[1]	Seropositives (%)	Range of seropositive (%)
40–69	7	146	17	11.7	4–16
70–99	7	295	30	10.2	7–20
100–130	6	280	35	12.5**	9–19
Total	20	721	82	11.4	4–20

[1]antibody titer ≥ 1:40 by CFT
**not significant $P > 0.05$

Table 4. Seroprevalence of Chlamydia infection in ewes and does in northern Jordan (1998–2001)

Age groups (Years)	Number tested		Seropositives (No.)[1]		Seropositives (%)	
	Sheep	Goat	Sheep	Goat	Sheep	Goat
1–2	638	178	150	26	23.5**	14.6**
2–4	790	305	175	31	22.2	10.2
> 4	546	238	108	25	19.8	10.5
Total	1 984	721	433	82	21.8*	11.4

[1]antibody titer ≥ 1: 40 by CFT
*significant difference ($P < 0.05$) between sheep and goat
**not significant *($P > 0.05$)* within age groups

Table 5. Seroprevalence of Chlamydia infection in ewes and does according to the areas in northern Jordan (1998–2001)

Area	Number tested		Seropositive (No.)		Seropositive (%)	
	Sheep	Goat	Sheep	Goat	Sheep	Goat
Mafraq	808	–	252	–	31.2*	–
Irbid	540	–	100	–	18.5	–
Ajloun	268	341	30	37	11.2	10.8
Jerash	368	380	51	45	13.9	11.8**
Total	1 984	721	433	82	21.8	11.4

*significant difference ($P < 0.05$) between areas by chi-square test
**not significant ($P > 0.05$) between the areas by chi-square test

the area where the flocks were located (Table 5). The prevalence of chlamydial infection was significantly higher ($P < 0.05$) in sheep (21.8%) than in goats (11.4%) when the location was ignored (Table 5). However, when prevalence rates of chlamydial infection in sheep and goats were stratified by location there was no significant difference ($P > 0.05$). In Ajloun area, the rates of *C. abortus* infection were 11.2% and 10.8% in sheep and goats, respectively. Similar results were observed in Jerash area, where the rates of chlamydial infection were 13.9% and 11.8% in sheep and goats, respectively.

DISCUSSION

Many authors have mentioned an antigenic cross-reaction of *C. abortus* with *C. pecorum*, but cross reactivity has not been translated into figures in Ab titre by CFT (Griffiths et al., 1996). Recently, Rodolakis proposed that Ab titre between 1:10 to 1:40 by CFT was not specific to *C. abortus* and might be related to intestinal infection with *C. pecorum* (Rodolakis, 2001). This is a realistic proposal since *C. pecorum* present in the intestinal tract typically causes no significant pathological changes and induces minimal circulating antibody titres compared to *C. abortus*, which causes more serious pathological changes and correspondingly higher titres. Using a cut point of 1:40 in this study, the specificity of the test reached 95% and the sensitivity was 79% at the animal level. However, if applied at a flock level, that is to say we considered the flock to be infected if at least one animal was is considered infected if at least one animal is seropositive, then the sensitivity would be close to 100%. We strongly believe that CFT, which has been used extensively to diagnose enzootic ovine abortion, is still a gold standard for serological diagnosis at Ab titre ≥ 1:40. Other serological tests like ELISA and IFA have similar problems of false positive and false negative results (Griffiths, 1996).

According to the annual reports of the Ministry of Agriculture (MOA) of Jordan, for the years 1986 to 1994, the seroprevalence of chlamydial infection in sheep and goats was almost constant and ranged from 7–10%. In contrast, our study indicates that the seroprevalence rate of chlamydial infection is 21.8% in ewes and 11.4% in does. The likelihood of false positive results due to vaccination in this study is low because there is no vaccination program against *C. abortus* in Jordan. The estimated number of sheep and goat populations in Jordan is about 3 millions, 2 millions of them (67%) are sheep and one million (33%) are goats (Department of Statistics Annual Report, The Livestock Statistics, 2000). The weighted rate, according to the sheep and goat population distribution, of chlamydial infection in adult female sheep and goats is 14.8%. This rate is significantly ($P < 0.05$) higher than the highest rate recorded by the Ministry of Agriculture (10%) in 1994. This observed difference between the rates of seroprevalence of *C. abortus* infection in this study and what was recorded by the Ministry of Agriculture may be due to increased prevalence of infection, or differences in study design and target populations. In this study, sera were collected from adult female sheep and goats, 1–2 weeks after lambing or abortion in northern Jordan. In addition, the sample distribution of sheep and goats in this study

is quite similar to the sheep and goat population distribution (weighted stratified sample).

The results showed a significant correlation ($P < 0.05$) between chlamydial infection and the flock size in sheep but not in goats. This might be due to the wide range of flock size in sheep while the range of flock size in goats was narrow (Table 2).

Age was not a factor associated ($P > 0.05$) with chlamydial infection. One might expect a higher rate of chlamydial infection in older sheep and goats as a result of cumulative risk. However, since Chlamydia cause abortion and farmers cull animals prone to abortions, there is a balance between the newly infected animals and culled infected animals.

There is a significant difference ($P < 0.05$) between chlamydial infection in sheep and areas of northern Jordan (Table 5). The Mafraq area has the highest rate of chlamydial infection in sheep. Mafraq area extends to the borders of three neighbour countries (Syria, Iraq, and Saudi Arabia). Sheep movement across the borders of these four countries is very common. It reflects a nomadic management of sheep where Bedouins are allowed to cross the borders of these four countries. Therefore, the rate of Chlamydia infection in Mafraq area is almost a double of that found in the interior areas of Irbid, Jerash and Ajloun.

Goat management differs from sheep management in Jordan. Goat flocks are usually owned by villagers, while sheep flocks are owned mainly by Bedouins and some villagers. Goat flocks are concentrated in two adjacent areas of northern Jordan, Jerash and Ajloun. They are hilly and have relatively better rangeland than the Mafraq area; therefore, goat flocks are not transported seasonally. Since the ecological conditions of Jerash and Ajloun are quite similar and goat flock management in these two areas is almost identical, it is not surprising that this study found no significant difference ($P > 0.05$) between chlamydial infection rates in sheep and goats in Ajloun and Jerash areas.

It could be concluded from this study that *C. abortus* infection is highly endemic in small ruminants in Jordan. The *C. abortus* infection rate in sheep in Mafraq area is almost a double of the rate of the other areas in northern Jordan. In the other areas where both species are raised, the infection rate is equal in sheep and goats. Although the impact of *C. abortus* infection on sheep and goats needs more investigations, this study suggests that a control program should be adopted by the Ministry of Agriculture.

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