

Full Length Research Paper

# Serological prevalence of ovine and caprine *chlamydophilosis* in Riyadh region, Saudi Arabia

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The serological prevalence of *Chlamydomphila abortus* was determined in 399 sheep and 171 goats in Riyadh region, Saudi Arabia, using the CHEKIT enzyme-linked immunosorbent assay (ELISA). Sera from 30 (7.52%) sheep and 59 (34.50%) goats were positive for anti-*C. abortus* antibodies. Higher serological prevalence and antibody concentration (percent optical density) was recorded in goats as compared to sheep. In both species, the prevalence of *chlamydomphila* was markedly higher in female than male animals. Statistical analysis showed highly significant species and sex effects on prevalence. Further studies should be undertaken to assess the role of *chlamydomphila* in ovine and caprine abortion in Saudi Arabia, its economic impact and the need for implementing effective control strategies such as vaccination.

**Key words:** *Chlamydomphila abortus*, ELISA, enzootic ovine abortion, caprine abortion, Riyadh and Saudi Arabia.

## INTRODUCTION

*Chlamydomphila* is a major cause of abortion in domestic ruminants. Enzootic ovine abortion, caused by *Chlamydomphila abortus* (formerly *Chlamydia psittaci* serotype 1) is believed to be responsible for 20 to 50% of all spontaneous abortions and stillbirths in sheep worldwide (Aitken, 2000; Cobb, 2009). Most infections in sheep and goats are asymptomatic apart from late term abortion or stillbirth. However, the infection sometimes causes placentitis with necrotic changes in the cotyledons and accumulation of reddish brown exudate in intercotyledonary areas (Jones et al., 1997; OIE, 2008).

The infection is usually transmitted through inhalation of infected barn dust or ingestion of contaminated food and water and although infected animals develop immunity after abortion, they might remain carriers of the organism in their reproductive tract for up to 3 years (Kreplin and Stone, 1988).

Rams may also acquire *chlamydomphila* from infected ewes and may spread the disease to other ewes at the time of breeding (Helms, 2011). The disease is also important from a zoonotic standpoint as it may cause abortion and other complications in pregnant women who come in contact with aborted fetuses and birthing fluids (Helm et al., 1989; Jorgensen, 1997).

In Saudi Arabia, indigenous sheep and goats are estimated at 7.7 and <3.5 million heads, respectively. They are economically the most important farm animals in the Kingdom, serving as major sources of meat, milk and income for a large sector of the population. More than 5 million sheep and goats are also imported annually during the *Hajj* (pilgrimage) season and other religious events (Anonymous, 2001). Only two reports are currently available regarding *chlamydomphila* among these animals in Saudi Arabia. The first report described detection of anti-*C. abortus* antibodies in 36 out of 186 (18.4%) camels in Riyadh region (Hussein et al., 2008) while the second report (Abd El-Razik et al., 2011) gave an overall prevalence of anti-*C. abortus* antibodies in 4.55 and 5.66% of sheep and goats, respectively, in

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Al-Qaseem region (27° 4' N; 43° 28' E). The incidence of the infection among animals in other parts of Saudi Arabia is unknown. Therefore, the following study was undertaken to determine the serological prevalence of *C. abortus* among sheep and goats in Riyadh region (24° 41' N; 46° 42' E) in central Saudi Arabia.

## MATERIALS AND METHODS

### Animals

A total of 399 sheep, comprising 106 ewes and 293 rams of the indigenous *Najdi* breed, aged 0.5 to 3.5 years, and 171 goats, comprising 139 female and 32 male goats of the indigenous *Aardi* breed, aged 2 to 4 years, were randomly screened for *C. abortus* antibodies. All animals were clinically normal at the time of sampling although a few individuals had previous history of abortion of unknown cause. Both flocks were kept indoors and fed on a ration comprising Rhodes grass (*Chloris gayana*), alfalfa (*Medicago sativa*) and commercial concentrate cubes (13% crude protein). They were vaccinated against *Brucella melitensis* using REV-1 vaccine. They were also vaccinated against clostridial diseases, sheep and goat pox, peste des petits ruminants (PPR) and pasteurellosis, and were given anthelmintic medication and coccidiostats as necessary. No vaccination program is used against *chlamydia* in Saudi Arabia.

### Serological tests

Five ml blood samples were collected by jugular venipuncture from each animal into plain vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, N.J., USA). The samples were allowed to clot at room temperature for 3 h and serum was separated by centrifugation (1,500 g for 15 min) and stored at -20°C. Tests for antibodies against *C. abortus* were performed using an indirect enzyme-linked immunosorbent assay (ELISA) designed to screen IgG antibodies against *chlamydia* in ruminants (CHEKIT-Chlamydia enzyme immunoassay; IDEXX laboratories, Bommeli Diagnostics, AG, Bern, Switzerland). A horseradish peroxidase-labeled monoclonal anti-ruminant IgG conjugate was used, and the test was performed according to manufacturer's procedure in microtiter plates coated with inactivated *C. abortus* antigen. Known positive and negative control sera were included in each test plate. The optical density, corresponding to the degree of color change, was determined at 450 nm using a microtiter plate reader. The percent optical density (%OD) of the samples was expressed according to the following equation:

$$\%OD \text{ of the test sample} = 100 (S - N)/(P - N)$$

Where S, N and P are the OD values of the test serum, negative control serum and positive control serum, respectively. Samples giving %OD of  $\geq 40\%$  were considered positive (Samkange et al., 2010). The test was validated based on the %OD values of positive and negative control sera.

### Statistical analysis

Logistic regression was used to assess the association of species and sex with serological prevalence of *chlamydia* using SAS V9.1 program for Windows. The model was as follow:

$$\text{Logit } P (X) = \beta_0 + \alpha_i (X_1) + \gamma_j (X_2)$$

$$i = 1, 2; j = 1, 2$$

Where:  $\beta_0$ ,  $\alpha_i$  and  $\gamma_j$  were the regression coefficients.  $X_1$  and  $X_2$  were the effects of independent variables of species and sex.

## RESULTS

Table 1 summarizes the results of ELISA tests for anti-*C. abortus* antibodies in sheep and goats. Statistical analysis results, odd ratio (OR) estimates and 95% confidence intervals for the effect of species, sex and sex within species are presented in Tables 2 and 3. Out of 399 sheep tested, 30 animals (20 ewes and 10 rams) were shown to be serologically positive for *chlamydia*, giving an overall prevalence of 7.5%. In goats, serological prevalence was markedly higher - out of 171 goats tested, 59 (57 does and 2 bucks) were shown to be serologically positive, giving an overall prevalence of 34.5%. In addition, about two thirds of all serologically positive sheep had low %OD ranging between 40 to 60%, whereas more than 49% of all serologically positive goats had %OD greater than 100%, including 22 does (38.6%) with %OD exceeding 120%. In both species, serological prevalence in females was around 6-fold that in males (17.24 versus 2.83%, respectively, in sheep and 41.0 versus 6.7%, respectively, in goats). The %OD value, which is directly proportional to the amount of anti-*C. abortus* antibodies in the samples, ranged between 41 to 115% with an overall mean of 65% in sheep and between 41 to 194%, with an overall mean of 93.1% in goats.

Statistical analysis (Tables 2 and 3) revealed highly significant difference in serological prevalence of *C. abortus* antibodies between sheep and goats, with overall serological prevalence in goats exceeding sheep by more than 4-fold ( $P < 0.0001$ ). Highly significant intersex differences were recorded in both species, with considerably higher prevalence in females versus males ( $p = 0.0012$ ) while significant species by sex interaction was recorded in goats ( $P = 0.0018$ ).

## DISCUSSION

This is the first record of anti-*C. abortus* antibodies in sheep and goats in Riyadh region, Saudi Arabia. The only other reference to this infection in small ruminants in Saudi Arabia is a recent report by Abd El-Razik et al. (2011) in which antibodies against *C. abortus* were recorded in sheep and goats in Al-Qaseem. Other infectious causes of abortion among sheep and goats in the Kingdom include *B. melitensis*, which is common throughout the country, with prevalence ranging between 0.5 to 12.3% in sheep and 0.8 to 18.3 in goats in different localities and under different management conditions (Radwan et al., 1983; Bilal et al., 1991). Another common cause of abortion

**Table 1.** Results of ELISA test for *Chlamydophilosis* in sheep and goats in Riyadh region (Saudi Arabia).

Species	Sex	Total tested	Total negative	Total positive	Titration (%O.D.)				
					40-60	61-80	81-100	101-120	>120
Sheep	F	116	96	20	14	3	2	1	0
	M	283	273	10	6	1	2	1	0
	Total	399	369	30	20	4	4	2	0
Goats	F	139	82	57	14	7	8	6	22
	M	32	30	2	2	0	0	0	0
	Total	171	112	59	16	7	8	6	22

**Table 2.** Effect of factors in the logistic model.

Factors	Df	$\chi^2$	P value
Species	1	24.4631	<0.0001
Sex	1	10.4349	0.0012
Species (sex)			
Goats	1	0.7609	0.0018
Sheep	1	0.7564	0.3844

**Table 3.** Odd ratio estimates (OR) and 95% Confidence Intervals for the effect of species, sex and sex within species.

Factors	Comparisons	OR	95% CI	
Species	Goats vs. Sheep	4.034	2.321	7.010
Sex	Females vs. males	2.648	1.467	4.782
Species (sex)				
Goats	(Females vs. males)	10.425	2.396	45.370
Sheep	(Females vs..males)	1.422	0.643	3.146

in small ruminants in Saudi Arabia is *T. gondii* with prevalence ranging between 8.5 to >80% in sheep (Hussein et al., 2011) and 8.0 to >60% in goats (Al-mufarrej et al., 2011). In addition, an outbreak of listeriosis was reported in a flock of over 2,000 sheep in the north eastern region of Saudi Arabia. 7.5% of the animals, mostly pregnant ewes, developed septicemic signs and 2.4% died, while none of them aborted (Al-Dughaym et al., 2001). Other abortifacient agents in sheep and goats such as *Salmonella abortus ovis*, *Campylobacteriosis* and *Mycoplasma agalactiae* have not been reported in Saudi Arabia.

The present results indicated considerably higher serological prevalence of *chlamydia* in goats than sheep. Similarly higher prevalence in goats versus sheep was recorded by others (Trávníček et al., 2002; Junior et al., 2010). However, this is not necessarily an indication of higher susceptibility of goats to *Chlamydia* as compared to sheep, since some investigators reported no difference in prevalence of *C. abortus* between these two species (Al-Qudah et al., 2004) while still others reported even higher prevalence in sheep than in goats (Apel et al., 1989; Tsakos et al., 2001; Čisláková et al., 2007).

It should also be pointed out that the number of female goats in the present study was somewhat higher while the number of male goats was markedly lower than that of sheep and the two species were of non-identical age groups; this could partly account for differences in prevalence. Our results also indicated that serological prevalence was higher in female than male animals in both species. Similar findings were reported in other species such as camels (Hussein et al., 2008) and free-ranging yak (Bandyopadhyay et al., 2009). The reason behind this inter-sex difference is unclear but sex may be one of several factors that might affect the prevalence of *chlamydia* such as type of animal production, reproductive management, sanitary procedures, proximity to other farming establishments, animal replacement policy, frequency of abortions, poor nutrition, overcrowding, transport, subclinical diseases and other forms of stress (Junior et al., 2010).

The present findings and those of Abd El-Razik (2011) in Riyadh and Al-Qaseem regions, respectively, underscore the importance of *C. abortus* as a potential cause of abortion in sheep and goats as well as a public health hazard for persons handling these animals in Saudi Arabia. Further studies should, however, be carried out to expand our knowledge regarding the prevalence, distribution and epizootiology of ovine and caprine *chlamydia* among indigenous and imported animals in Saudi Arabia. It is also important to conduct additional tests such as polymerase chain reaction and or to isolate the organism in order to confirm its implication in abortion and other reproductive disturbances in these animals. These studies are necessary for assessing the economic impact of the problem and for developing effective control strategies.

Investigations should also be undertaken to determine if other *Chlamydia* species infect domestic ruminants in the Kingdom, particularly *C. pecorum* which causes a wide spectrum of clinical signs such as polyarthritis, keratoconjunctivitis, pneumonia, enteritis and abortion in sheep, goats and cattle (Anonymous, 2009).

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