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Seroprevalence of antibodies to *Chlamydophila abortus* in small ruminants in some provinces in Syria

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ABSTRACT

Serological surveys of antibodies to *Chlamydophila abortus* in small ruminants were made in ten provinces located in different parts of Syria from November to December 2009. A total of 808 blood samples were randomly collected from sheep and goats, and subjected to serological analysis using Enzyme linked Immunosorbent Assay. The overall seroprevalence of *C. abortus* among all provinces, research stations and breeders' flocks were 9.3, 8.2 and 10.8%, respectively. The results showed high infection rate in provinces Hama, Daraa, Aleppo and Al-Qamishli (18, 13.5, 11.96 and 11.7%, respectively). The highest percentage of positive results was recorded in Hama (24%), followed by Homs (16%). The prevalence rates in sheep and goat research stations were 8.8 and 6.8%, respectively. The infection rate in research stations ranged between 0 and 19.2%, except in Jidrin station for sheep in Hama (42.5%), where the highest positive reactions were (43.6%), followed by Humeimeh station for goat in Aleppo and Soumakyat station for sheep in Daraa (12.8%). Research stations for sheep, Kaser al-Halabat and Mahasah, were *C. abortus* free. In breeders flocks, high infection rates were recorded in Daraa province (25%), followed by Al-Qamishli (14.8%) and Dayr ez Zawr (12%). High positive reactions were recorded in Aleppo and Dayr ez Zawr (16.7%) and Daraa (13.9%), but the highest rate was in Homs (30.6%). There was significant difference (χ^2 = 37.6, $p<0.01$) between positive and negative results when comparing research stations and breeder flocks. In view of the results, a limited distribution of *C. abortus* was observed in the middle region (Hama and Homs), northern region (Aleppo), eastern region (Al-Raqqa, Al-Qamishli, Al-Hasakah and Dayr-azwr), and the southern region (Daraa), with marked statistically significant difference (χ^2 = 89.0, $p<0.0001$) when the seroprevalence was compared among different provinces.

Keywords: *Chlamydophilia abortus*, seroprevalence, enzyme linked immunosorbent assay, Syria.

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INTRODUCTION

Chlamydophila abortus is one of the most important causes of reproductive failure in sheep and goats (Aikten, 2000; Rodolakis et al., 1998). The agents, *C. abortus* and *C. pecorum* with seven other species, belong to genus Chlamydophila (Everett et al., 1999). *C. abortus* often affects sheep and goats, occasionally cattle, wild animals, reptiles and rodents (OIE Terrestrial Manual, 2008; The Center for Food Security & Public Health, 2005). *C. abortus* leads to abortion, orchitis, seminal vesiculitis and decreased fertility or infertility in the flock (Longbottom and Coulter, 2003; Appleyard et al., 1985). *C. pecorum* causes encephalitis, pneumonia, enteritis, polyarthritis, conjunctivitis and abortion in sheep, goats, cattle and pigs (The Center for Food Security & Public Health, 2005). Infected sheep abort in the last 2 to 5 weeks before parturition (Matthews, 1999), while the goats can abort at any stage of pregnancy (Longbottom and Coulter, 2003; Aikten, 2000; Morgan et al., 1988). Some infected goats may develop a persistent cough, polyarthritis, keratoconjunctivitis or pneumonia (The Center for Food Security & Public Health, 2005). Infection can result in the delivery of healthy lamb, pre mature births, full-term stillborn lambs or weak lamb that generally fails to survive beyond 48 h. Animals that are

Table 1. Number of animals examined in each study area.

Provence	Aleppo	Hama	Homs	Damas	Sweida	Daraa	Kouneitra	Al-Hasakah	Al-Raqqa	Dayr ez Zawr
Public sector	26	100	61	42	47	54	9	33	50	52
Breeders	66	-	121	-	-	20	-	27	50	50

infected in late time of gestation can develop latent infection in the next gestation (Meijer et al., 2004). Infective *C. abortus* are shed at the time of abortion or parturition in the placenta and uterine discharges, which form a source of *C. abortus* contamination for humans (Papp et al., 1994). Infected humans show worsening influenza-like illness symptoms, abortion in pregnant women (Longbottom and Coulter, 2003; Caul and Sillis, 1998), severe pelvic inflammatory disease, and increased vaginal discharge with an occasionally elevated temperature (The Center for Food Security & Public Health, 2005). Economic losses due to *C. abortus* have been reported from most sheep-raising countries (Longbottom and Coulter, 2003; Aikten, 2000), like Syria that have a livestock population estimated in around 19 million sheep and 15 million goats (Annual Statistical Report, 2008). The scarcity of information on *C. abortus* in Syria (Al-Sayed and Rahmoun, 2008) warranted studying antibody finding among public sector and private Awassi sheep breeds in different parts of Syria. This work was conducted in order to have an insight on the seroprevalence and distribution of *C. abortus* in small ruminants, and to specify areas with high infection rate or exposed to a risk of contamination for monitor and control aspects of the disease.

MATERIALS AND METHODS

Serological surveys of were performed in small ruminants from November to December 2009 in 10 provinces. The samples were stratified and weighted according to high populations of the Awassi sheep in the northern region (Aleppo), the middle region [Hama and Homs including Palmyra (Tadmur) and Al-Qaryatayn], the eastern region (Al-Raqqa, Al-Qamishli In. Al-Hasakah and Dayr-azwr), and the southern region (Damascus, Sweida, Daraa and Kouneitra). The Bedouins in the northern, eastern regions and Al-badia (the Syrian Desert) still rear most of the Awassi sheep. The western and coastal regions were not sampled because of the small number of the Awassi sheep there. In reality, there are no goats' flocks, except a few owned mainly by the bedouins and some villagers.

Study area

Thirteen research stations (8 for Awassi sheep and 5 for Damascus and Gabali goats) under intensive breed system, belonging to the General Commission for Agricultural Scientific Research (GCSAR), and 26 Awassi sheep flocks under semi-intensive and transhumance conditions, were screened (Table 1).

Sampling

Blood samples were randomly collected from the jugular vein of the animals in the research station [N = 474 (450 female, 24 males)] and breed sector [N = 334 (300 females, 34 males)]. The females were 1 to 6 years old, in the second half of pregnancy, or 21 days after abortion. All sera samples were collected after centrifugation at 1500 RPM for 15 min and stored at -20°C until laboratory testing.

Serology procedure

Blood sera were tested for the presence of antibodies to *Chlamydophila abortus* in the laboratory of general analysis at the Animal Health Directorate (Ministry of Agriculture and Agrarian Reform) using *Enzyme linked Immunosorbent Assay* (Elisa) kit according to manufacturer's instructions (Cypress Diagnostics). Diluted with positive and negative control sera were put into the wells of ELISA plate coated with *C. abortus* antigen and incubated at 37°C for 20 min. The plates were washed with washing solution, and peroxidase conjugated protein G was added to each well. After incubation at 37°C for 20 min, the plates were washed three times. The enzyme substrate was added to each well and incubated for 10 min. The reaction was stopped with stop solution and the plate was read in Elisa reader (Metertech Σ 960) at 450 nm wavelength filter. S/P% percentage for each sample is calculated with following equation:

$$S/P\% = \frac{\text{Corrected OD 450 of the sample (S)}}{\text{Mean corrected OD 450 of the positive control (P)}} \times 100$$

S/P% ≥ 60 samples are being considered *C. abortus* positives. Seroprevalence (proportion of positive results from blood samples) and seropositivity (proportion of positive results from a total number of positive samples) at each site were assigned. Tables and graphics were performed using Microsoft Office Excel 2007. The distribution of positive samples among the different regions was traced with red circled areas on a ready existing country map using Paint program.

Statistical analysis

The chi-square test (χ^2) between proportions was performed using the software package (SAS V7 program for Windows). Analysis of variance using log-linear model was also done to compare prevalence rate between research stations and flocks of breeders in various areas. A significant result was set at $P \leq 0.05$. The odd ratios for *C. abortus* infection in males and females were determined using Proc logistic (to measure the frequency par state and group).

RESULTS AND DISCUSSION

Seroprevalence of anti-*Chlamydophila abortus* among sheep and goat flocks

The results of the present study illustrated in table 2 revealed that anti-*C. abortus* with the ELIZA test was

Table 2. The seroprevalence of anti-*Chlamydophila abortus* according to animal type in different kinds of flocks.

Kind of flock	Animal type	Gender	Total number of samples	Positives	Seroprevalence %
Research station	Sheep	Female	324	27	8.3
		Male	17	3	1.8
	Goat	Female	126	9	7.1
		Male	7	0	0
Big and smallholder farms	Sheep	Female	293	30	10.2
		Male	32	5	15.6
	Goat	Female	7	0	0
		Male	2	0	0

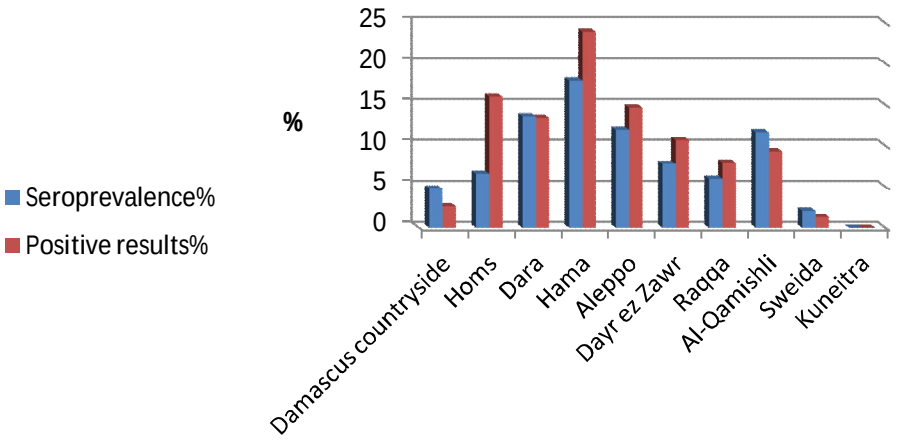

Figure 1. The seroprevalence of *C. abortus* in the screened provinces.

found in 75 out of 808 samples (9.3%).

High infection rate was recorded in Hama, Daraa, Aleppo and Al-Qamishli (18, 13.5, 11.96 and 11.7%, respectively). The highest percentage of positive results (Figure 1) was recorded in Hama (24%) followed by Homs (16%).

Seroprevalence of *Chlamydophila abortus* in the research stations and the Awassi breed flocks

Chlamydophila abortus in the research stations of sheep and goats

Thirty-nine samples out of 474 (8.2%) were seropositive in the research stations of sheep and goats. The seroprevalence rates in sheep and goat stations were 8.8 and 6.8%, respectively. The infection rate ranged between 0.0 and 19.2%, except in Jidrine station (42.5%). The highest positive reaction (43.6%) was in Jidrin station for sheep in Hama, followed by Humeimeh station for goat and Soumakyat station for sheep in Daraa (12.8%). Research station for sheep, Kaser al-Halabat and Mahasah, were *C. abortus* free (Figure 2).

Chlamydophila abortus in private Awassi breed flocks

Antibodies to *C. abortus* were found in 36 out of 334 (10.8%). Figure 3 shows high infection rates in private flocks in Daraa province (25%), followed by Al-Qamishli (14.8%) and Dayr ez Zawr (12%). High positive reactions were recorded in Aleppo and Dayr ez Zawr (16.7%) and Daraa (13.9%), but the highest was Homs (30.6%).

There was a significant difference (χ^2 = 37.6, $p<0.01$) between positive and negative results when comparing research stations and breeder flocks. Serological analysis performed in rams and ewes showed 8 and 57 positive samples out of 49 and 617, respectively. The odds ratio was higher (1.9) in males than in females.

Only 7 samples out of 53 aborted females (13.2%) were found to be positive. No positive results were detected among a very few samples (9) collected from

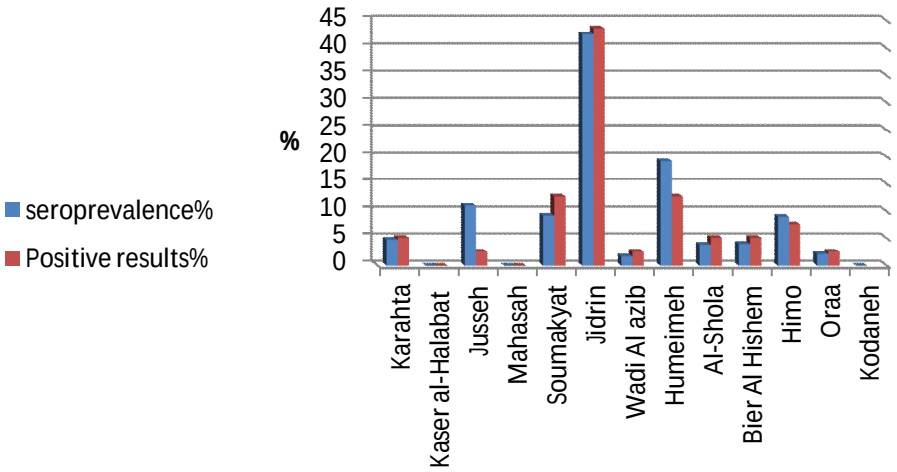

Figure 2. The seroprevalence of *C. abortus* in research stations of sheep and goats.

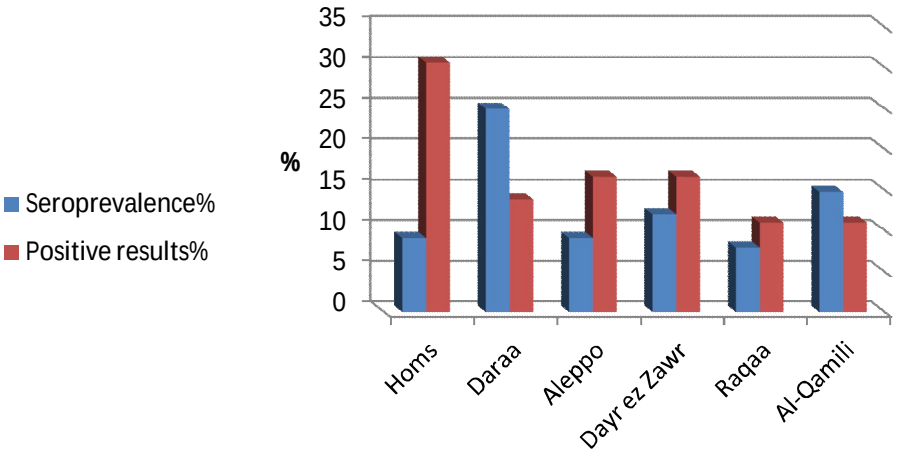

Figure 3. The seroprevalence of *C. abortus* in private Awassi breed flocks.

goats bred in the Awassi sheep breed, and therefore no comparison was made with goat research stations. The distribution of positive samples among the different regions is shown in Figure 4 with the higher seroprevalence (in red circled areas) being noticed in the center and north-east of Syria.

Analysis of variance using linear log model (CATMOD Procedure) showed statistically significant difference (χ^2 = 89.0, $p < 0.0001$) when comparing the seroprevalence among different provinces. The overall seroprevalence of *C. abortus* among all provinces (9.3%) is considered high compared to that observed by Yin et al. (2014) in Belgian ruminants (varying between 0.24 and 4.23%), but below compared to that found by (Al-Qudah et al., 2004) in the Awassi sheep and goats in Jordan (21.8 and 11.4%, respectively), and also low compared to 18.7% found in Tibetan sheep in Gansu province in Northwest China (Qin et al., 2014).

The percentage of *C. abortus* positive from aborting sheep was 13.2%, which is consistant with the study of Al-Dabagh et al. (2014) in which a serprevalence of 11.2% in aborting sheep in Nineveh governorate, Iraq was reported. Otlu et al. (2007) found 5.4% *C. abortus* positive samples within the aborting sheep in Kars province, but Duman and Durak (1998) obtained 20% positive ones among aborting sheep in Konya district in Turkey.

The seroprevalence of *C. abortus* in goat research station: Humiemeh, Karahta, Kodanah and Ora was lower than that obtained in a previous screening study conducted in 2007 in the same stations by Al-Sayed and Rahmoun (2008) which were 27.4, 16.4, 14.3 and 3.7%, respectively, which may be related to the improvement of animal husbandry and farm management in the high cited stations during the period 2007-2009, and to the success of implemented prevention and control programs of the disease.

The higher odds ratio of the infection in males

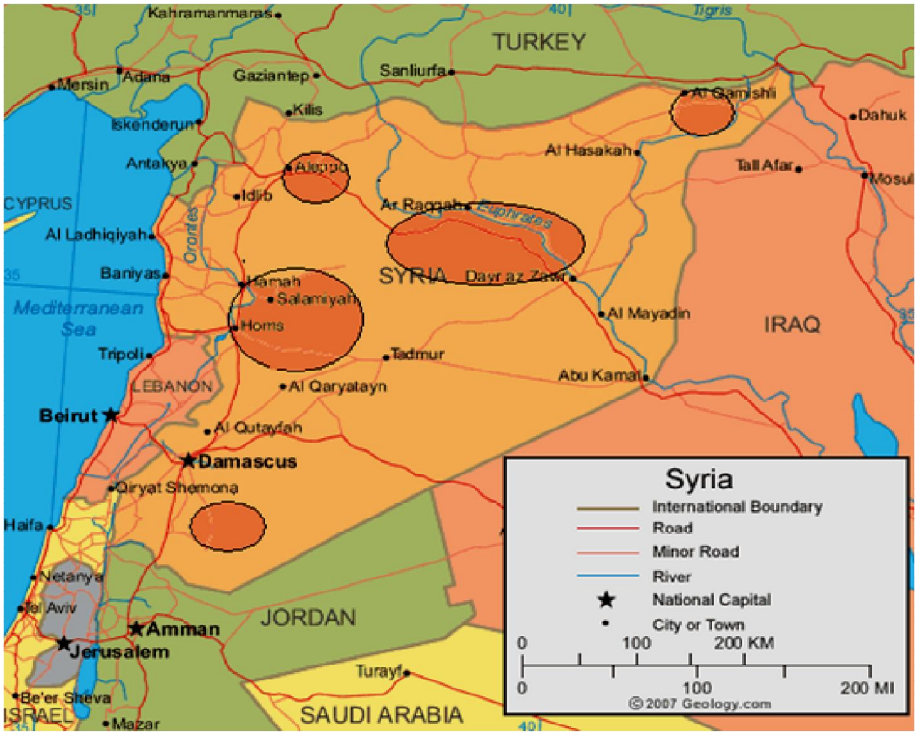

Figure 4. Distribution of positive samples throughout different regions of Syria.

compared to females is in concordance with Qin et al. (2014) who obtained higher *C. abortus* seroprevalence in males than in females 21.5% vs. 17.4%, in Tibetan sheep.

Based on the results, exposure to infection in some research stations like Himo station in Al-Hasakah (Al-Qamishli) and Soumakyat in Daraa is considered high in some regions with higher *C. abortus* seroprevalence, which is seen as animal health threat and requires therefore an adequate surveillance.

The significant difference ($p < 0.01$) in *C. abortus* seroprevalence when comparing research stations and breeder flocks may be explained by the intensive breeding system in research stations, the high standards of maintenance and feeding. In fact, the prevalence rate of *C. abortus* in small ruminants has been reduced in the last 10 years in public sectors as a result of good management of farm and the preventive treatment with Oxytetracycline before mating and during pregnancy. According to Rodolakis et al. (1980), two injections of Terramycin/L.A. at 10 or 15 days interval during the fourth month of pregnancy at the rate of 20 mg/kg are sufficient to limit the number of abortions. Abortion in an infected flock can be prevented by administrating 80 mg/head/day of chlortetracycline. However, some sheep and goat research stations need better practice of animal husbandry and farm management to reduce the infection rate with *C. abortus*. In breeders' flocks the animals are kept out-door in open shelter, barn type, and thus in possible contact with various sources of infestation. These farms have especially poor control of cats, rodents and wild reservoirs; this may increase the chance of environment, food and water contamination (Hotea et al., 2011). *C. abortus* is recognized as a major cause of abortion and lamb loss throughout the world, in particular where flocks are closely congregated, especially in the intensively managed farms (Nielfeld, 2001). An association was observed ($P < 0.05$) between Chlamydia seropositivity and flock size groups (Al-Qudah et al., 2004). The larger the flock size, the higher the prevalence of Chlamydial infection. The geographic differences in the prevalence may be related with differences in living environment and husbandry practices (Qin et al., 2014). Wild animals are found in Syrian Desert and marginal areas, and that according to local herders the source of introduction. People there have also low awareness of disease prevention and control.

The season has also, according to Qin et al. (2014), a significant risk factor for *C. abortus* prevalence. The *C. abotus* elementary bodies can remain infective in the enviroment for only several days in typical spring weather (Center for Food Security & Public Health, 2005). Abortion that occurred in spring leads to high seroprevalence in summer (Qin et al., 2014). As time went on, the antibody level reached the lowest in winter (Qin et al., 2014). Many seropositive animals in one tested herd could have an effect on high level of seroprevalence in evaluated region. Though, high seroprevalence in some region may be connected with high level of infection at herd level. Moreover, the

nomadic management in middle and eastern regions of Syria constitutes a barrier for a successful control of diseases due to animal movements and screening difficulties (Fensterbank, 1986). It should be noted here that the recorded seroprevalence concerns some breeds chosen randomly in various areas of Syria and not the whole sheep population in the country.

CONCLUSION

The results showed limited distribution of *C. abortus* in sheep and goats in the middle region, northern region, eastern region, and the southern region. However, the severity of the infection was different among regions which may be due to environmental diversity, level of health awareness, and the management of animal farms.

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Disclosure

This work has been conducted under consideration of the ethical regulations of the country regarding animals care, health and welfare.

Competing interests

The authors declare that they have no conflict of interests.

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