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Serological Study on Enzootic Abortion of Ewes in Ahvaz, Iran

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Abstract: *Chlamydophila abortus* is one of the major causes of infectious abortion in pregnant sheep (Enzootic Abortion of Ewes) worldwide. Organisms shed through uterine discharges and placentas at lambing time are the main sources of environmental contamination, responsible for transmission to susceptible animals and possibly human. The disease is the most common infectious cause of lamb losses in several countries, resulting in major economic losses to agricultural industries. In the present study, sera from 145 ewes with history of abortion were analyzed for antibodies against *C. abortus* with a commercial ELISA kit (ELISAr, Institut Pourquier, France). According to the result, 13 ewes (13/145, 8.9%), were seropositive in ELISA test. There was not any correlation between infection and ages of seropositive ewes. In conclusion, enzootic abortion of ewes may be prevalent in Ahvaz and further attempts for definitive diagnosis by isolation of its etiologic agents are recommended.

Key words: *Chlamydophila abortus*, serology, ELISA, EAE, Ahvaz, Iran

INTROUDUCTION

Chlamydiae are obligate intracellular bacteria belonging to the bacterial order *Chlamydiales*, which infect epithelial cells and monocyte/macrophages of a wide host range, resulting in a broad spectrum of diseases (Longbottom and Coulter, 2003; Schachter, 1999; Storz, 1988). Chlamydiae undergo a biphasic developmental cycle that consists of 2 distinct morphological forms, the Elementary Body (EB) and the Reticulate Body (RB), which are specially adapted to extracellular and intracellular environments, respectively (Longbottom and Coulter, 2003).

Chlamydophila abortus (formerly *Chlamydia psittaci* serotype, infects the placenta, causing a disease in sheep known as Enzootic Abortion of Ewes (EAE) (Aitken, 2000; Longbottom and Coulter, 2003). It is the most common infectious cause of abortion in lowland flocks intensively managed at lambing time and has a major economic impact on agricultural industries worldwide (Pospischil, 2005; Kerr *et al*., 2005).

The agent is endemic among ruminants and has been isolated from sheep, cattle and goats as well as in association with cases of abortion in other mammals. Women who work with sheep also have suffered sporadic, documented cases of zoonotic abortion due to *C. abortus* (OIE, 2004).

The diagnosis of *C. abortus* infection is complicated by several factors. Isolation of micro-organism requires specialized facilities and personnel experienced in cell culture (Buendia *et al*., 2000). According to the OIE manual the most commonly used method for serodiagnosis of animal chlamydiosis is the Complement Fixation Test (CFT). However, the technique is laborious, of limited sensitivity and often impaired by cross-reactions between chlamydial species. The recently developed serodiagnostic tests are mainly based on the 2 main cross-reactive antigens present in all chlamydial species, lipopolysaccharide and the major outer membrane protein and thus, are not species specific for diagnosing animals infected with EAE. Other more specific, such as those based on specific monoclonal antibodies (Salti-Montesanto *et al*., 1997) and recombinant protein fragments (Markey *et al*., 1996) are developed. Buendia *et al*. (2000) has been evaluated a commercial ELISA kit based on a recombinant antigen for diagnosis of *C. abortus* (Vetoquinol diagnostic, France) and reported its sensitivity and specificity, to be 90.9 and 95.2%, respectively. Vretou *et al*. (2007) reported that *C. abortus* ELISA (Institut Pourquier, France) is more specific and sensitive than CFT for serologic diagnosis of EAE. As there was not any information about EAE in Iran, our aim was to assess the seroprevalence of *C. abortus* in the Ahvaz, the capital of Khuzestan province, Iran, in sheep population.

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MATERIALS AND METHODS

The study was based on a sample of 145 sera from ewes with history of abortion up to 2 years before sampling, randomly collected from sheep in different ages (Table 1) of 4 flocks from different parts of Ahvaz between April-June 2005. All the samples were tested for anti-*C. abortus* antibodies using a commercial ELISA kit (ELISAr, Institut Pourquier, France) at the microbiolog laboratory of Veterinary Faculty in Shahid Chamran University of Ahvaz. ELISA kit was used according to the manufacturer's guidelines. Briefly, 20 fold dilutions of all sera (including positive and negative controls) were prepared in dilution buffer and 200 μL added to duplicate wells of 96-well flat-bottomed microtiter plates pre-coated with *C. abortus* antigen. The positive-control serum was added into 2 wells and the negative-control serum into one well. After incubation for 60 min at 37°C, plates were washed 3 times with washing solution. One hundred microliters of an optimum dilution (1/1000) of peroxidase conjugated anti-ruminant IgG antibody is added to all wells and the plates incubated for a further 30 min at 37°C before 3 washing. One hundred microliters of the substrate-chromogen (tetramethylbenzidine and hydrogen peroxide) was added to each well and the plates left at room temperature for 20 min. The reaction was stopped by adding 100 μL of stop solution (0.5M sulfuric acid). Optical density (OD) at 450 nm was measured with a plate reader (Dynatech, Netherlands). Corrected OD values of samples and controls were calculated by subtraction the OD 450 value of the uncoated well from the OD 450 from the coated well and the S/P percentage (corrected OD 450 of the samples/the mean corrected OD 450 of the 2 positive controls × 100) were calculated. Sera with S/P% equal or greater than 60% are considered to be from animals that have been in contact with *C. abortus*.

Seropositivity rates found for *C. abortus* were analysed in relation to age by using the differences between 2 proportions by Fisher's exact test. Values of $p \leq 0.05$ were considered as statistically significant.

Table 1: Age distribution of 145 ewes with history of abortion in Ahvaz

		Age (year)			
		≤3	4	≤5	Total
Flock	A	8	6	16	30
	B	2	12	27	41
	C	2	11	14	27
	D	24	12	11	47
	Total	36	41	68	145

Table 2: Prevalence of anti *C. abortus* antibody in 145 ewes with history of abortion in Ahvaz

		ELISA		
		Positive	Negative	Total
Age (year)	≤3	6	30	36
	4	3	38	41
	≥5	14	64	68
	Total	13	132	145

RESULTS AND DISCUSSION

Thirteen ewes, have been seropositive in ELISA test, so chlamydial abortion rate estimated to be (13/145) 8.9%. All of the positive samples were from flock D and there was not any correlation between infection and ages of seropositive ewes.

Enzootic abortion of ewes has become recognized as a major cause of loss in sheep in Europe, North America and Africa and is the most common infectious cause of lamb loss in the UK, accounting for around 50% of all diagnosed causes of abortions (Aitken, 2000). With the best of our knowledge, so far there has not been any study on serodiagnosis of EAE in Iran, so this study describes the first serologic study of chlamydial abortion in sheep flocks of Ahvaz, southwest of Iran. The serological results of this study indicate that sheep flocks in Ahvaz are exposed to *C. abortus* infection. About 9% of the sheep considered in this study were positive, at S/P% above of 90 in ELISA test. In this study age had no influence on the seroprevalence of *C. abortus* antibodies in ewes (Table 2). This indicates that, under the same circumstances, sheep in all ages have an equal chance of acquiring infection with *C. abortus*; therefore it can be concluded that the disease introduce to this area newly because in previously introduced area, young animals have more chance to acquire the infection. From the present investigation, it was also observed that only ewes from flock D (Elhaeii) were seropositive and infected animals have high anti-*C. abortus* antibodies, these also indicates the newly introduction of the disease to this flock. Donn *et al.* (1997), Apel *et al.* (1989) and Borel *et al.* (2002, 2004) in their studies in Namibia, Scotland and Switzerland also reported that *C. abortus* infection rates may be varying in different geographical regions.

CONCLUSION

The present findings are solely based on observation on 4 flocks kept in semi-intensive system of management, so more investigations involving large sheep population along with attempts for isolation of *C. abortus* should be performed for definitive diagnosis of EAE in Iran. The authors hope that this study will prompt other workers to undertake similar studies in other susceptible populations. In conclusion, this study represents the first investigation carried out on sheep, in

Iran and shows that antibodies to *C. abortus* are present in this species. The isolation of causative agent of EAE should be kept under consideration in Iran.

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