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Investigation of *Mycoplasma agalactiae* in Milk and Conjunctival Swab Samples from Sheep Flocks in West Central, Iran

K.H. Pirali kheirabadi and A. Ebrahimi

Department of Pathobiology, Faculty of Veterinary Medicine,
University of Shahrekord, Shahrekord, Iran

Abstract: In a study to investigate the current status of *Mycoplasma agalactiae* infections in sheep flocks in west central, Iran, a total of 54 and 47 conjunctival swab and milk samples were collected from normal healthy ewes of 26 herds, respectively. Using PCR method. *Mycoplasma agalactiae* detected in 20 out of 101 animals (19.8%) examined, 12 out of 54 (22.2%) of conjunctival swab and 8 out of 47 (17%) milk samples were positive. Samples of 20 (out of 26) herds were positives, that means 77% of sheep herds in this region have at least one ewes infected by *M. agalactiae*. The results confirmed that herds in west central Iran have currently about 20% infection rates for contagious agalactiae of sheep.

Key words: Sheep, milk, *Mycoplasma agalactiae*, Iran

INTRODUCTION

Contagious agalactiae in sheep and goats is a very contagious, acute, sub acute or chronic disease (Greco *et al.*, 2001). Aytu and others reported that the disease could be seen in most European, Mediterranean, Middle East and African countries (Aytu *et al.*, 1990). *Mycoplasma* infection is characterized by mastitis, polyarthritis and keratoconjunctivitis lesions (Aytu *et al.*, 1990; Bergonier *et al.*, 1997). In Spain in a herd of 200 sheep, 20% had uni- or bilateral keratoconjunctivitis and *Mycoplasma agalactiae* was isolated from conjunctival fluids collected from 20 ewes and 3 lambs (Rodriguez *et al.*, 1996).

This infection occurs in animals at every age but advanced pregnant and lactating animals are more susceptible (Aytu *et al.*, 1990; Damassa *et al.*, 1990). It is reported that the incubation period of the disease is 1-2 weeks and recovered animals continue to be carriers for seven months (Aytu *et al.*, 1990). The principal way of diagnosis is agent isolation, however, complement fixation and indirect hemagglutination tests are commonly used (Aytu *et al.*, 1990; Petsaga-Tsimperi and Sarris, 1997; Etheridge *et al.*, 1996). Some researchers (Levisohn *et al.*, 1991; Perez-Gomez *et al.*, 1996; Pepin *et al.*, 2003) suggested ELISA was more successful in the diagnosis of sub clinic mycoplasma infections. Recently PCR has been used to search *M. agalactiae* in sheep milk samples (Tola *et al.*, 1997).

In this study, we investigate the carrier prevalence of *Mycoplasma agalactiae* in milk and conjunctival swab samples of sheep flocks in Shahrekord and Lordegan districts of west central Iran by using a PCR method.

MATERIALS AND METHODS

Chahar Mahal region is a semi-arid area and receives an average annual Rain fall of 400 mm. The study areas were two (out of four) districts (namely Shahrekord and Lordegan). A two-stage random selection procedure was adopted where study flocks were randomly selected from a sampling frame comprising all flocks in each district.

The Shahrekord and Lordegan groups each consisted of 13 flocks (randomly selected from about 260 flocks with a total population of about 600000).

On average, flocks selected to participate in the study constituted about 10% of all herds in the study area. Each of the selected herd was then visited and animals on the day of visit were listed from which 4 apparently healthy ewes were randomly selected for examination and either milk or conjunctival swab sample collection.

During field visits, a structured questionnaire was used to collect animal and herd-level information on knowledge on history of contagious agalactiae and any antibiotic treatment.

Totally 47 (22 from Shahrekord and 25 from Lordegan) milk and 54 (26 and 28, respectively) conjunctival swab samples were collected from two districts. Sheep sampled were in different stages of lactation.

Milk and swab samples were transferred to Razi institute (Hesarak-Tehran) where detection of *M. agalactiae* from conjunctival swabs and milk samples were done by a simple PCR. For preparation of lysates a 1 mL aliquot of prepared sample suspensions was centrifuged, washed in Phosphate-Buffered Saline (PBS), resuspended in 1 mL of water and lysed by being heated at 100 degree Centigrade for 10 min, 16 SrRNA gene PCR was performed using primers 5'-mgso (TGCACCATCTGTCACTCTGTAAACCTC) and 5'-gpo (ACTCCTACGGGAGGAGCAGCAGTA). The PCR amplification products were analyzed by gel electrophoresis on 1% (w/v) agarose gels and visualised after staining with ethidium bromide using a UV transilluminator.

RESULTS

Using the PCR method, the presence of *M. agalactiae* was detected in 20 out of 101 animals examined (19.80%). In 12 animals out of 54 (22.22%) the conjunctival swab samples were positive for *M. agalactiae* and in 8 out of 47 animals (17%) milk samples were positive.

From the positive results that were obtained with swabs, 5 out of 21 (23.8%) were from Shahrekord while for Lordegan the numbers were 7 out of 21 (33.3%), respectively.

In milk samples out of 47 animals tested 22 were from Shahrekord with four (18.18%) positives while for Lordegan the numbers were 25 and four (16%), respectively. Out of 101 animals tested 19 were less than 3 years old with 3 (15.8%) positives and 71 were 3-4 years old with 17 (23.9%) positives, (Table 1).

In tested milk samples 44 (out of 47) were from lactating pregnant ewes with 7 (15.9%) positives, for conjunctival swabs the numbers were 40 out of 54 with 9 (22.5%) positives, respectively, (Table 2).

DISCUSSION

The threat of contagious agalactiae should always be kept in mind in a country that, like the Iran is open to animal import and serves as a transit area. Sheep flocks in Chahar Mahal province of Iran mostly reared as migrating and transient herds. Here is a traditional and tribal life system that the people depending on the season and availability of high quality pastures move their flocks through different provinces of Iran. We designed our study in the time that they were settled in the districts of this province. All ewes in various stages of lactation used in this study were managed as free pasture rearing.

This was the reason why we designed a preliminary study to determine the rate of carrier state in our herds for this disease. In this present investigation there are no significant differences between the different age groups of less than 4 years, although there are no positive samples in age group of above 4 years. This can be accounted for by the fact that ewes within this age group have enough acquired immunity to *M. agalactiae* due to repeated exposure to the organism through earlier ages.

Milk production is known to be a stress factor (Aytu *et al.*, 1990; Damassa *et al.*, 1990). which may probably explain why positive swab samples of pregnant and non pregnant lactating ewes are higher than non lactating ones. Moreover, subsequent (previous) lambing has been shown to increase the chances of infection (Gross *et al.*, 1978).

In our investigation 26 herds were sampled with 20 positives, that means 77% of sheep herds in this region have at least one ewes infected by *M. agalactiae* that may cause clinical contagious agalactiae and rapidly infect most of ewes in that herd.

The predominance of *Mycoplasma agalactiae* in sheep flocks of this province in present study corroborates the observations of Martin *et al.* (2001). These authors have reported at least one positive animal

Table 1: Distribution of *Mycoplasma agalactiae* in conjunctival and milk samples, age groups and districts of Chahar Mahal Province

Districts	Shahrekord		Lordegan		Total	
	Negatives	Positives	Negatives	Positives	Negatives	Positives
Swab samples	21	5 (19.23%)	21	7 (28.57%)	42	12 (22.22%)
Milk samples	18	4 (18.18%)	21	4 (16%)	39	8 (17.02%)
No. <3yrs	5	0 (0%)	8	3 (27.2%)	13	3 (18.75%)
No. =3-4 yrs	24	9 (27.27%)	30	8 (21.05%)	54	17 (23.94%)
No. >4 yrs	7	0 (0.0%)	4	0 (0.0%)	11	0 (0.0%)

Table 2: Distribution of *Mycoplasma agalactiae* in conjunctival and milk samples of pregnant groups of ewes in Chahar Mahal Province

Status	Milk samples		Swab samples		Total	
	Negatives	Positives	Negatives	Positives	Negatives	Positives
Pregnant lactating	38	7 (15.55%)	31	9 (22.50%)	69	16
Non pregnant lactating	0	0 (0.0%)	7	2 (22.22%)	7	2 (22.22%)
Pregnant non lactating	1	1 (50%)	4	0 (0.0%)	5	1 (16.66%)

was detected in 89.4% of the herds. It further shows that the total prevalence of 19.8% carriers in ewes of the province may suggest evidence of previous or recent infection. The results of our study confirmed that Chahar Mahal herds have currently about 20% infection rate for contagious agalactiae of sheep and at the same time, provided the first information on the presence of *M. agalactiae*, a pathogen causing contagious agalactiae of sheep and goats, in West central, Iran.

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