Detection of *Mycoplasma agalactiae* Antigen in Sheep and Goats by Monoclonal Antibody- Based Sandwich ELISA

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Abstract

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A preliminary epidemiological survey was carried out to find out whether contagious agalactia of sheep and goats was present in herds in the Czech Republic or Jordan. A total of 99 animals were examined for the presence of *Mycoplasma agalactiae* antigen; there were 64 animals from the Czech Republic and 35 from Jordan. All Jordanian animals showed signs of clinical disease. Biological materials for examination included 353 swabs (133 from sheep and 220 from goats) collected from conjunctival, nasal and vaginal mucosae and the external ear canal. A monoclonal antibody-based sandwich ELISA was used to detect the antigen. The results were positive in 11 animals, ambiguous in 10 animals and negative in the rest of them (78). They confirmed our assumption that the Czech Republic is still free from contagious agalactia of sheep and goats but demonstrated that Jordanian herds of small ruminants are infected with *Mycoplasma agalactiae.*

Sheep, goats, contagious agalactia, antigen, Mycoplasma agalactiae, ELISA

Mycoplasma agalactiae (M. agalactiae) is an important pathogen of small ruminants that causes contagious agalactia in sheep and goats (Bergonier et al. 1997). The disease primarily affects the mammary glands, joints, eyes and, to a lesser degree, respiratory tract. It is clinically manifested as mastitis, arthritis, conjunctivitis and pneumonia (Nicholas 1996). However, similar clinical and pathological changes in these animals can also be caused by mycoplasma species (Nicholas 1996; Sarris 1996; Bölske 1994) included into the "*Mycoplasma mycoides* cluster" (*Mycoplasma mycoides* subsp. *mycoides* LC, *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma mycoides* subsp. *capri*). A very similar disease found only in goats is caused by *Mycoplasma putrefaciens* (Bergonier et al. 1997).

Contagious agalactia currently occurs in most of the countries with intensive rearing of sheep and goats. This involves the Mediterranean region and Balkan Peninsula in Europe, western parts of Asia and northern, central and eastern Africa (Al-Zeftawi 1979; Damdinsuren 1989; Erdag 1989; Belaid et al. 1990; Da Massa et al. 1992; Ismail 1993; Sarris 1996; Egwu et al. 1999; Kusiluka et al. 2000; Madanat et al. 2001).

In this preliminary epidemiological survey we examined animals from randomly selected Czech and Jordanian herds for the presence of *M. agalactiae* antigen. In the former Czechoslovakia, the mycoplasma causing contagious agalactia was last detected in sheep imported into the country at the beginning of the 1950s (Koppel 1982). The disease was then managed by culling all the infected animals and, since then, the Czech Republic has been regarded as free from contagious agalactia. Our objective was to confirm this assumption. On the other hand, Jordan is located in the geographical area in which contagious agalactia shows a high prevalence, but no information on mycoplasma infections in Jordanian sheep and goats has so far been available.

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Materials and Methods

Animals examined

The animals from the Czech Republic included a total of 64 sheep and goats from various herds selected on a random basis. There were 32 sheep (Suffolk and Merino Landschaf ewes) and 32 goats (12 adult goats and 10 male and 10 female young animals of the Czechoslovak White Polled breed)

All animals came from farms free of any apparent clinical disease symptoms, except for one sheep herd that had had a history of respiratory disease 12 months prior to examination by our team.

The 35 Jordanian animals came from the Al- Karak district. They comprised 15 Awassi sheep and 20 goats of the local Baladi breed. At the time of sample collection these animals showed a wide range of clinical signs. Non-specific symptoms such as anorexia, depression or somnolence were found in six sheep and four goats. Varying stages of mastitis were diagnosed in 12 animals (3 sheep and 9 goats), seven animals (4 sheep and 3 goats) had arthritis and six animals (2 sheep and 4 goats) suffered from conjunctivitis and respiratory disease.

Sampling

The samples included swabs from conjunctival, nasal and vaginal mucosae and also from the external ear canal. A total of 353 swabs were collected: 133 were from sheep (Czech Republic 106; Jordan 27) and 220 from goats (Czech Republic 140; Jordan 80). The swabs were immediately placed in test tubes containing transport medium for mycoplasma culture. The transport medium for Jordanian samples was supplemented with 1×*g* cefaperazone /ml (90% cefaperazone sodium, Sigma, Aldrich Chemie GmbH, catalogue no. C4292 – 1G). The Czech samples were frozen at -80 °C until use. Because no deep freezer was available in Jordan, the samples were stored at -20 ˚C until transported to the Czech Republic. They were collected by a Jordanian student for his PhD studies carried out at the Department of Infectious Diseases and Veterinary Epidemiology, Veterinary and Pharmaceutical University in Brno, Czech Republic.

All samples of biological material collected in Jordan were transferred under the rules set by the State Veterinary Administration of the Czech Republic.

Detection of *M. agalactiae* antigen

The *M. agalactiae* antigen was detected using a monoclonal antibody-based sandwich ELISA (Mabs – based sandwich ELISA) (Ball et al. 1993; Ball et Finlay 1998). This test was developed and kindly provided by Dr. Hywell J. Ball, Veterinary Sciences Division, Department of Agriculture for Northern Ireland in Belfast, Northern Ireland, United Kingdom.

Results

Using the ELISA method, the presence of *M. agalactiae* antigen was detected in 11 out of 99 animals examined (11%). In ten animals (10%) the results were regarded as ambiguous and in 78 (79%) animals they were negative. All the positive and ambiguous results were obtained with swabs collected from small ruminants in Jordan. Out of 35 animals examined (15 sheep and 20 goats), *M. agalactiae* antigen was detected in two sheep and nine goats and ambiguous results were obtained in two sheep and eight goats. None of the 64 Czech animals (32 sheep and 32 goats) tested positive or showed ambiguous results.

The presence of *M. agalactiae* antigen in swabs collected from different body sites of Jordanian sheep and goats is shown in Table 1.

Table 1
Detection of *Mycoplasma agalactiae* in Jordanian sheep and goats

No. of animals examined	Body site	Results by ELISA Ag		
		+	+/-	-
Sheep	Conjunctiva	1	0	11
n=15	Nasal mucosa	1	1	10
	Vaginal mucosa	0	1	13
	External ear canal	0	1	13
Σ	52	2	3	47
		+	+/-	-
Goats	Conjunctiva	0	3	16
n=20	Nasal mucosa	2	1	16
	Vaginal mucosa	1	0	17
	External ear canal	7	4	9
Σ	76	9	8	59

Discussion

Although the last reports on sheep and goats infected with contagious agalactia date back to the 1950s in the former Czechoslovakia (Koppel 1982), the threat of re-introduction of this highly contagious, devastating disease should always be kept in mind in a country that, like the Czech Republic, is open to animal import and serves as a transit area. This was the reason why we designed a preliminary study to ascertain whether our farms were still free of this disease. This survey is related to our previous study in which small ruminants were examined for specific antibodies against *M. agalactiae* and which gave negative results (Madanat et al. 2002).

In Arabian countries, however, the situation is completely different. Contagious agalactia is a permanent health and economic problem (Belaid et al. 1990). In our previous study we demonstrated the presence of specific antibodies against *M. agalactiae* in Jordanian sheep and goats (Madanat et al. 2002). In this study the demonstration of contagious agalactia in small ruminants in Jordan was completed by the detection of *M. agalactiae* antigen.

The detection of *Mycoplasma* spp. is so far most frequently carried out in culture. However, this method is time consuming and laborious. For the identification of *Mycoplasma* spp., several antigen-capture and sandwich modifications of ELISA are available but they have been used only in research laboratories because of their low sensitivity in comparison with standard culture techniques. In this study we employed for the detection of *M. agalactiae* antigen a specific enzyme-linked immunosorbent assay, the Mabs-based sandwich ELISA (Ball et al. 1993; Ball et Finlay 1998). This technique has particular advantages, since *Mycoplasma* spp., despite well-documented problems with species crossreactivity and interference by nonspecific media components, are largely speciated by serological methods. This Mabs-based sandwich ELISA involves pre-enrichment, a short-term incubation of the sample directly on an ELISA plate, and the use of monoclonal antibodies; the former renders the method more sensitive and the latter facilitates its better reproducibility and increases its specificity. The use of this ELISA method improves laboratory diagnosis of infectious agalactia also in terms of reliability, accuracy and time, which is very important because some infected sheep and goats have shown atypical forms of the disease (Nicolet 1994b); in addition, asymptomatic disease carriers have been reported (Bergonier 1996ab; Lillini et al. 1996; Sanchis et al. 2000) which are difficult to detect by less sensitive laboratory methods.

The results of our study confirmed that Czech herds are currently free from contagious agalactia of sheep and goats and, at the same time, provided the first information on the presence of *M. agalactiae*, a pathogen causing contagious agalactia of sheep and goats, in Jordanian herds.

Průkaz antigenu *Mycoplasma agalactiae* u ovcí a koz pomocí ELISA metody sendvičového typu založené na bázi monoklonálních protilátek

Byla provedena předběžná epizootologická studie s cílem zjistit, zda se v chovech České republiky a Jordánska vyskytuje nakažlivá agalakcie ovcí a koz. Celkem bylo na přítomnost antigenu *Mycoplasma agalactiae* vyšetřeno 99 zvířat, 64 zvířat pocházelo z České republiky a 35 z okresu Al-Karak v Jordánsku. Všechna zvířata jordánského původu vykazovala klinické příznaky probíhajícího onemocnění. Celkem bylo vyšetřeno 353 vzorků výtěrů (od ovcí 133 a 220 od koz), odebraných ze sliznice spojivkové, nosní, vaginální či ze zevního zvukovodu. K detekci antigenu byla použita ELISA metoda sendvičového typu založená na bázi monoklonálních protilátek. Pomocí této metody bylo zjištěno 11 zvířat pozitivních, 10 dubiózních a 78 negativních. Tyto výsledky jsou potvrzením našeho původního předpokladu, že Česká republika je stále dosud nakažlivé agalakcie ovcí a koz prostá, na druhé straně však prokázaly, že stáda ovcí a koz v Jordánsku jsou infikována *Mycoplasma agalactiae.*

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