

## Prevalence of *Mycoplasma agalactiae* Antibodies in Czech and Jordanian Herds of Small Ruminants

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### Abstract

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We investigated the presence of antibodies against *Mycoplasma agalactiae*, a causal agent of contagious agalactia, in herds of small ruminants. Eighty serum samples collected from 60 sheep and 20 goats in the Czech Republic and 137 samples collected from 78 sheep and 59 goats in Jordan were examined in our laboratory. Only the Jordan animals showed clinical signs of the disease. The sera were analysed for the presence of antibodies by a specific enzyme immunoassay. Of the 137 Jordan sera, eight gave positive reactions, 11 reactions were dubious and 117 were negative. All sera from the Czech animals showed negative reactions. The results provide evidence that, as expected, the Czech Republic is currently free from the mycoplasma agent causing contagious agalactia of sheep and goats and that, in Jordan as well as in many other Arabic countries, this disease is a frequent and serious health problem in herds of small ruminants.

*Sheep, goat, contagious agalactia, serum antibody, Mycoplasma agalactiae, enzyme immunoassay*

*Mycoplasma agalactiae* (*M. agalactiae*) is the causal agent of contagious agalactia, a serious infectious disease of sheep and goats (Lambert et Cabasse 1989; Da Massa et al. 1992). In these small ruminants, the disease is clinically manifested by mastitis, arthritis, pneumonia and keratoconjunctivitis (Lambert 1987; Nicholas 1996; Bergonier et al. 1997; Madanat et al. 2001). Similar clinical and pathological findings also characterise infections caused by mycoplasma species that are grouped in the "mycoides cluster", namely, *Mycoplasma mycoides* subsp. *mycoides* LC, *Mycoplasma capricolum* subsp. *capricolum* and *Mycoplasma mycoides* subsp. *capri* (Da Massa et al. 1992; Nicholas 1996; Bergonier et al. 1997; Pilipčinec et al. 2000 ab; Madanat et al. 2001). A disease caused by *Mycoplasma putrefaciens* (Bergonier et al. 1997) and affecting only goats also has a similar clinical presentation.

Contagious agalactia is most frequently found in the Mediterranean region, the north of Africa and Near and Middle East countries (Al-Zeftawi 1979; Lambert 1987; Erdag 1989; Belaid et al. 1990; Sarris 1996; Rapoport et al. 1999) in which it is responsible for high economic losses. Recently, it has also been diagnosed, though to a lesser degree, in many other parts of the world (Kusiluka et al. 2000; Egwu et al. 2001). The infection can have an asymptomatic course (Bergonier et al. 1997; Lillini et al. 1996; Sanchis et al. 2000) but the animals affected can shed the mycoplasmal agent into the environment for several years and become a source of infection for healthy animals (Lillini et al. 1996). These inapparent infections are difficult to diagnose. The laboratory identification is usually based on the isolation of a mycoplasma or the detection of serum antibodies. On farms, it is sometimes very difficult to introduce and maintain the necessary infection control measures

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that involve a high standard of milking hygiene, separation of infected animals from healthy ones, elimination of infected animals from the herd and, in indicated cases, antibiotic therapy and vaccination (Bergonier et al. 1997). The therapy of contagious agalactia is at present based on administration of tetracycline, macrolide drugs, fluorophenicol, tiamulin and fluoroquinolones. The vaccination strategy involves the use of both attenuated and inactivated vaccines. No transfer of this disease to humans has ever been reported in the relevant literature.

This paper presents the results of an investigation into mycoplasma infections of small ruminants carried out at the Department of Infectious Diseases and Epizootiology of the Veterinary and Pharmaceutical University in Brno by a PhD student from Jordan in cooperation with the departmental research team. The objective of the study was to investigate the prevalence of serum antibodies against contagious agalactia of small ruminants in the Czech Republic and Jordan. In the former Czechoslovakia, the mycoplasma causing contagious agalactia was last detected in sheep imported to the country at the beginning of the 1950s (Koppel 1982). The infection was then managed by killing all the animals and, since then, the Czech Republic has been regarded as free from this disease. One of the objectives of this study was to confirm this assumption. Jordan, on the other hand, is situated in a geographical area in which the occurrence of contagious agalactia of sheep and goats is highly probable but no attention has so far been paid to research on this mycoplasma infection.

#### Materials and Methods

##### Animals examined

Two groups of animals were examined. One comprised sheep and goats from various herds of the Czech Republic. The other included animals from Jordan, particularly the Al-Karak district. The Czech group consisted of 60 sheep (20 Suffolk ewes, 10 Suffolk rams, 30 Merino Landschaft ewes) and 20 Czechoslovakian White Polled goats (10 females and 10 kids of both sexes). No clinical signs indicative of acute disease were observed in animals from the Czech herds, except for one sheep herd that had a history of respiratory disease 12 months prior to examination by our team.

The group from Jordan comprised 63 ewes and 15 rams of the Awassi breed (a total of 78 animals) and 49 female and 10 male goats (a total of 59 animals) belonging to the local Baladi breed. In comparison with the Czech animals, the Jordan sheep and goats showed a wide range of clinical signs. In 15 animals (8 sheep and 5 goats), non-specific clinical symptoms such as anorexia, depression and somnolence were observed. Mastitis of varying degree was found in 22 animals (12 sheep and 10 goats), arthritis was detected in 25 animals (15 sheep and 10 goats), conjunctivitis and keratoconjunctivitis in 15 animals (8 sheep and 7 goats) and respiratory problems accompanied by nasal discharge and occasional cough in 16 animals (7 sheep and 9 goats). Concurrent mastitis and arthritis were observed in 15 animals (10 sheep and 5 goats) and concurrent mastitis and conjunctivitis were found in 14 animals. Seventeen animals (10 sheep and 7 goats) suffered from mastitis, arthritis and conjunctivitis.

##### Sample collection

Blood samples for serological examination were collected from the jugular vein. A total of 80 blood sera were taken from the Czech animals (60 sheep and 20 goats) and 137 samples from the Jordan animals (78 sheep and 59 goats). These animals were examined for clinical signs by the first author in Jordan. Their blood samples were collected and stored, in the form of sera, at -20 °C until they were transported to the Czech Republic. The examination was carried out at the Department of Infectious Diseases and Epizootiology of the Veterinary and Pharmaceutical University in Brno.

##### Serological examination

Specific antibodies against *M. agalactiae* were detected in sheep and goat sera with the use of a CHEKIT-M. agalactiae enzyme immunoassay (EIA) kit (Dr. Bommeli AG 3097 Liebefeld, Berne, Switzerland). Optical density that developed in the assay was measured in a MRX photometer (Dynatech). The procedure was carried out according to the manufacturer's instructions. Microtitre plates were pre-coated with control negative antigen (-Ag) and inactivated positive antigen (+Ag). Dilutions of the serum samples to be tested were incubated in the appropriate wells of the microtitre plate. In samples containing specific antibody against *M. agalactiae*, an antigen-antibody complex developed in the wells pre-coated with +Ag. The unbound material was washed away. Subsequently, a peroxidase-labelled anti-goat IgG conjugate was added, which bound to the sheep or goat antibodies complexed with the *M. agalactiae* antigen. The unbound conjugate was removed by washing and a

chromogen-containing substrate was added to the wells. The intensity of colour that developed (optical density measured at 405 nm; reference wavelength, 492 nm) was directly proportional to the amount of specific *M. agalactiae* antibody present in the sample examined. The net absorbance corresponded to the amount of specific antibodies bound. Its value was calculated by subtracting the value of optical density measured in the wells pre-coated with –Ag from the value read in the +Ag pre-coated wells to which the same sample of serum, either control or tested, was added. The diagnostic relevance of the results was obtained by comparing the net absorbance of the samples examined with that of the positive controls. The resultant value was expressed as per cent. A positive result was defined by a net absorbance value higher than 65 %, a dubious result by values in the range of 40-65 % and a negative result by a value lower than 40 %.

### Results

The CHEKIT-M. agalactiae immunoenzymatic method (Bommeli AG) did not detect specific antibodies against *M. agalactiae* in any of the 80 Czech animals examined (60 sheep and 20 goats). Positive or dubious results were obtained only from the examinations of 137 Jordan animals (78 sheep and 59 goats). Of these, eight (5.8%) had positive, 11 (8.0%) had dubious and 118 (86.1%) had negative results. In the Jordan group, three sheep and five goats showed positive antibody levels and six sheep and five goats had dubious levels. The values of net absorbance in animals with positive or dubious findings (expressed as per cent) are shown in Table 1.

Table 1  
Levels of serum antibodies, expressed as per cent, against *M. agalactiae* in animals with positive or dubious results in the enzyme immunoassay

Animal	Net absorbance value (%)	Result
Sheep No.		
68	95.6	Positive
77	113.3	Positive
113	90.1	Positive
2	46.0	Dubious
49	46.6	Dubious
62	52.8	Dubious
86	51.8	Dubious
99	41.4	Dubious
135	40.5	Dubious
Goats No.		
10	119.9	Positive
45	77.0	Positive
54	81.4	Positive
58	84.3	Positive
126	118.7	Positive
26	49.6	Dubious
55	47.8	Dubious
72	52.8	Dubious
112	45.2	Dubious
118	44.0	Dubious

### Discussion

Contagious agalactia of sheep and goats, which is generally caused by *M. agalactiae* (Da Massa et al. 1992; Lambert et Cabasse 1989), has not been identified on the territory of the Czech Republic for more than 50 years (Koppel 1982). Because the health risks for animals in Czech herds has recently increased due to the development of business links with other European countries and due to the fact that the Czech Republic has become an

important transit area, we believed that a preliminary epidemiological study of this disease was indicated. In the first phase of our investigation, we dealt only with small groups of animals because we expected negative results. These were later confirmed and were in agreement with the findings reported by Nicholas (1995) from Great Britain. This country has been free from the disease for 15 years. Nicholas holds a view that this favourable situation is largely due to the fact that most of the British farmers breed sheep and goats for meat and, therefore, their herds are not at any great risk because, as stated by Lambert (1987), contagious agalactia is a disease primarily affecting dairy animals

A markedly different situation is found in Arabic countries known to have a long tradition of breeding sheep and goats for dairy production; there contagious agalactia of sheep and goats has been a lasting problem in terms of both health and economy (Al-Zeftawi 1979; Erdag 1989; Belaid et al. 1990). We detected specific antibodies against *M. agalactiae* only in the sera of animals from Jordan, which corresponded to the fact that signs of clinical disease were also observed only in these animals. Our results can be regarded as indirect evidence suggesting the presence of contagious agalactia of sheep and goats in Jordan and also provide the first information on the prevalence of this highly contagious disease in the country.

The routine laboratory diagnosis of contagious agalactia is based on cultivation and serological examination. Because culture methods are laborious, costly and time-consuming, we carried out our preliminary examinations with the use of a serological method, i.e., enzyme immunoassay.

Up to now, a number of techniques for detection of serum antibody against *M. agalactiae* have been developed and tested, namely, immunofluorescence methods, agglutination on a slide, test-tube agglutination, growth-inhibition test, immunodiffusion test and others (Lambert 1987; Le Goff et Perreau 1984; Tsaknakis et al. 1992), but the complement-fixation test (CFT) and EIA have been considered to be the most important of all.

CFT was a widely-used method not a long time ago (Perreau et al. 1976) but, because of high variability, it was difficult to interpret the results correctly (Lambert 1987). CFT had another disadvantage because it often gave false positive results or showed cross reactions (Belaid et al. 1990; Le Goff et Perreau 1984). For these reasons, CTF is at present regarded as a test with low sensitivity and specificity (Le Goff et Perreau 1984; Zavagli 1951).

In sheep and goats infected with contagious agalactia through both experimental and natural routes, the detection of antibodies against *M. agalactiae*, using CFT, is possible as early as at the onset of first clinical signs. In adult animals, antibodies are found between day 3 and day 15 after inoculation or infection, in young animals, they are detected from 4 weeks on. These antibodies may persist in the organism from several months to one year or even longer (Zavagli 1951). CFT is a method suitable to supplement clinical and epidemiological findings in the whole herd but is highly unsuitable for the diagnosis of contagious agalactia in individual animals (Lambert 1987; Le Goff et Perreau 1984; Zavagli 1951).

EIA was the method of choice used for serological examination in our study. It was adopted for the diagnosis of contagious agalactia in 1982 and, at that time, served to detect antibodies to *M. agalactiae* and *M. mycoides* subsp. *mycoides* LC. In the following years, trivalent assays for the detection of antibodies against *M. agalactiae*, *M. mycoides* subsp. *mycoides* LC and *M. capricolum* subsp. *capricolum* were developed (Belaid et al. 1990; Calamel et Lambert 1988; Lambert et Cabasse 1989; Lambert et al. 1998; Levisohn et al. 1991; Mega et al. 1993; Schaeren et Nicolet 1982; Tsaknakis et al. 1992). They offered an advantage of high sensitivity and specificity and could be performed in a short time. EIAs are more sensitive than CFT (Belaid et al. 1990; Lambert

et Cabasse 1989; Mega et al. 1993) and are able to detect a very low antibody titres present in latent infections. But even these methods occasionally produce false positive or non-specific reactions. These may result from cross-reactivity between pathogens that have antigens carrying similar epitopes; a "background" reaction can also be involved. A thorough analysis of each stage of the reaction developing in EIA has recently shown that the phenomenon of false positive results can be eliminated by using a conjugate with monoclonal antibodies or with G-protein, without influencing the real, positive results obtained with the conjugate prepared with polyclonal sera (Lambert et al. 1998).

The dynamics of serum antibodies formation detectable by EIA has been studied in sheep and goats experimentally infected with *M. agalactiae*. It was still possible to detect IgG at 13 months after inoculation; IgM appeared earlier but also disappeared earlier (Mega et al. 1993; Bergonier et al. 1997). A similar situation was also found in naturally infected goats. When chronic infections were monitored for several years, it appeared that IgG antibodies persisted in these goats for three or more years (Bergonier et al. 1996; Bergonier, unpublished results).

Enzyme immunoassays are valuable tools for the serological diagnosis of contagious agalactia of sheep and goats and are utilised with advantage for examination of the whole herd (Lambert 1987; Schaeren et Nicolet 1982; Tsaknakis et al. 1992). They are therefore particularly suitable for epidemiological studies (Bergonier et al. 1997; Lambert 1987). If they are used for examination of individual animals, they only stand for a screening method that may facilitate the distribution of animals into three categories: infected animals, those whose health state is dubious and potentially healthy animals.

This study covers only a small part of the issue concerned with contagious agalactia of sheep and goat. Our further objectives are to complete, using the most recent diagnostic methods, the results presented here with more exact and comprehensive data on the isolation and identification of *M. agalactiae* in Jordan animals and to provide more details on the epidemiology of the disease.

### **Prevalence protilátek proti *Mycoplasma agalactiae* v českých a jordánských chovech malých přežvýkavců**

V předkládané práci bylo na přítomnost protilátek proti *Mycoplasma agalactiae* testováno 80 vzorků krevních sér malých přežvýkavců, pocházejících z chovů v České republice (60 ovcí a 20 koz) a 137 vzorků krevních sér ovcí (78) a koz (59) z Jordánska. Klinické příznaky onemocnění byly pozorovány pouze u zvířat jordánského původu. Analýza vzorků sér byla prováděna pomocí imunoenzymatického testu. Z celkového počtu 137 vyšetřovaných jordánských sér bylo zjištěno 8 sér pozitivních, 11 dubiálních a 118 negativních. Česká zvířata vykazovala pouze negativní hodnoty. Zjištěné výsledky potvrdily naše očekávání, že Česká republika je i v současné době prostá původce nakažlivé agalacie ovcí a koz a že v Jordánsku, podobně jako v dalších arabských státech, je tato nákaza poměrně častým zdravotním problémem malých přežvýkavců.

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