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DISEASE CAUSED BY *MYCOPLASMA MYCOIDES* SUBSPECIES *MYCOIDES* LC IN HUNGARIAN GOAT HERDS

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The occurrence of a goat disease caused by *Mycoplasma mycoides* subsp. *mycoides* LC in Hungary is reported. The disease occurred in two goat herds in the spring of 1999. In one herd 25% of the 4–12 weeks old kids (10 animals) while in the other herd 33% of the 6–12 weeks old kids (20 animals) became affected. The goat kids developed polyarthritis. The most severe lesions developed in the carpal joints. All animals died after 3–8 days of disease. Four dead kids were necropsied. All of them had serofibrinous and purulent polyarthritis, and in two animals bronchopneumonia, fibrinous pleuritis and meningitis were also found. In the articular exudates the presence of mycoplasmas was detected by PCR using a general mycoplasma primer. Mycoplasmas were cultured from the joints of all animals, from the abdominal parenchymal organs of two kids and from the lungs of one animal. The cultured mycoplasmas grew in strikingly large colonies, proved to be glucose positive, arginine negative and phosphatase positive, and liquefied the coagulated serum. They survived incubation at 45 °C for more than 24 h. Based upon their biochemical properties, the results of the immunofluorescence (IF) and growth inhibition tests and the sequence analysis of the PCR product, the cultured strains were identified as *M. mycoides* subsp. *mycoides* LC. Animals purchased in the previous autumn had been introduced to both farms. The disease may have been introduced with asymptomatic carrier animals, as earlier no similar disease had been observed at either farm.

Key words: *Mycoplasma mycoides* subsp. *mycoides* LC, arthritis, goat

Numerous mycoplasma species can cause disease in goats. The most important mycoplasmas pathogenic to goats are *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*), *M. agalactiae, M. mycoides* subsp. *capri* (*Mmc*), *M. mycoides* subsp. *mycoides* LC (*Mmm*LC), *M. capricolum* subsp. *capricolum* (*Mcc*), and *M. putrefaciens*.

M. capricolum subsp. *capripneumoniae* is the causative agent of contagious caprine pleuropneumonia (CCPP). This pathogen causes disease exclusively in goats. The disease occurs in Africa, Asia Minor, Central Asia and India

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(Varga et al., 1999). It is characterised by croupous pneumonia and fibrinous pleuritis. Without treatment, the mortality rate may exceed 60%.

Compared to *Mccp, M. mycoides* subsp. *capri* causes less severe disease which, however, is also restricted to the thoracic organs. Earlier this species was considered the causative agent of CCPP. It is widespread throughout the world, and its occurrence has been reported from Africa, Asia, Europe and Australia (OIE, 1997).

Several mycoplasma species can produce a disease entity accompanied by arthritis. *M. agalactiae* is the causative agent of contagious agalactia in sheep and goats. The disease is endemic along the Mediterranean Sea, in North Africa and in countries of the Near and Middle East (Varga et al., 1999); however, from the Mediterranean region it is sometimes introduced to other countries. This pathogen produces disease in sheep as well as in goats. The most typical changes include interstitial mastitis leading to atrophy of the udder parenchyma and agalactia, arthritis resulting in lameness and limitation of motion, and keratoconjunctivitis leading to blindness. Sometimes abortion may also occur.

The occurrence of *M. capricolum* subsp. *capricolum* has been reported from all continents (Perreau, 1979; OIE, 1997). It affects goats more often than sheep. The most characteristic clinical feature of the disease caused by it is arthritis. In addition, mastitis and occasionally pneumonia may also develop (Cottew, 1985).

M. putrefaciens causes mastitis, agalactia, abortion and arthritis. Until now the disease caused by it has been described in goats only (Da Massa et al., 1987; OIE, 1997).

M. mycoides subsp. *mycoides* has two types which are indistinguishable by serological methods (Cottew and Yeats, 1978). *M. mycoides* subsp. *mycoides* SC producing small colonies (SC) on the medium is the causative agent of contagious bovine pleuropneumonia (CBPP). The variety producing large colonies (LC), *M. mycoides* subsp. *mycoides* LC affects primarily goats and less frequently sheep, causing mastitis, arthritis, pneumonia, pleuritis and, occasionally, encephalitis, peritonitis, abortion and keratoconjunctivitis (Da Massa et al., 1983; East et al., 1983; Ruhnke et al., 1983). It has been recorded on all continents (OIE, 1997).

Contagious caprine pleuropneumonia does not occur in Hungary. Contagious agalactia is present primarily in the Mediterranean countries; however, on one occasion in 1998 it was introduced into an Eastern Hungarian sheep and goat herd, where it caused severe losses eventually requiring the destruction of the herd (Bajmócy et al., 1998).

Mcc, M. putrefaciens and *Mmm*LC are widespread pathogens. The diseases caused by them have been reported from numerous countries of the world, but there are no data on their occurrence in Hungary.

In 1999, a disease caused by *Mmm*LC and resulting in huge economic losses was diagnosed in two Hungarian goat herds. These outbreaks are reported in this paper.

Materials and methods

The clinical, gross pathological, histopathological and bacteriological examinations, *Mycoplasma* culture, PCR and sequencing were performed as described in an earlier paper (Bajmócy et al., 1998). To identify the mycoplasmas cultured, the isolated strains were filtered and passaged three times to obtain a pure culture. The strains were examined by immunofluorescence (IF) as described by Rosendal and Black (1972). The growth inhibition tests were carried out according to Black (1973). Antibodies produced against the following mycoplasmas were used for the tests: *Mmm*SC (PG1), *Mmm*LC (Y goat), *Mmc* (PG3), *M. agalactiae* (PG2), *Mcc* (Cal Kid), *Mccp* (F38). To differentiate *Mmm*LC and *Mmm*SC, liquefaction of coagulated serum and the heat sensitivity test at 45 °C were performed according to Cottew and Yeats (1978).

Results

Clinical signs and course of the disease

The disease occurred in two Eastern Hungarian goat dairies in the spring of 1999.

Herd A. This herd included 27 female goats of different age, one buck and their progeny. About 100 sheep were kept in the same air space but separated from the goats. The first disease cases occurred at the beginning of March. The joints of the 6–12 weeks old kids became swollen and painful. The carpal joints were affected most severely. Animals showing signs of polyarthritis developed severe lameness and soon became recumbent. The keeper observed dyspnoea in some animals and nervous signs in one kid. The disease invariably led to death in 3–8 days. Therapy was not administered. The disease lasted about 5–6 weeks, during which time 10 kids (25% of the 6–12 weeks old kids) died. Kids younger than 6 weeks and adult goats were not affected. The sheep did not show similar disease signs.

Herd B. This herd included 65 female goats, 2 bucks and their progeny. In January 1999 a major outbreak of abortion occurred, when nearly 20 female goats aborted. The abortions were not investigated. The first disease cases among the kids started at the end of February. The clinical signs were the same as in herd A, with the difference that here the disease occurred also in kids as young as 4 weeks. The disease lasted nearly two months. During that time, 20 kids (35%

of the 4–12 weeks old kids) became affected. Despite antibiotic therapy which was attempted in a few cases, all the diseased animals died.

Gross and histopathological examinations

From both herds, one dead kid and one affected kid slaughtered while showing signs of arthritis were necropsied. Gross pathological examination revealed thickening of the articular capsule, oedematous infiltration of the periarticular tissues, and accumulation of serofibrinous exudate in the articular cavity of the extremital joints. The most severe changes were observed in the carpal joints, but all joints of the extremities were affected in a milder form. In one kid, accumulation of serofibrinous exudate was seen also in the atlanto-occipital joint. In Giemsa-stained smears made from the articular exudate, neutrophilic granulocytes were the dominant cell type. Two animals had bronchopneumonia involving a small area of the lungs, accompanied by fibrinous pleuritis in one kid. Histopathological examination revealed thickening and mononuclear cell infiltration of the alveolar wall, perivascular and peribronchial lymphoid hyperplasia and, here and there, appearance of pus cells and cell debris in the airways. In the kid showing inflammation of the atlanto-occipital joint, the medulla oblongata was covered by fibrinous exudate which was found to contain numerous mononuclear and pus cells on histopathological examination.

Bacteriological examinations

Bacteriological examination of the articular exudates, the abdominal parenchymal organs and the brain yielded negative results. From the lungs of a kid showing gross lesions of bronchopneumonia, *Pasteurella haemolytica* bacteria were cultured in small numbers. Attempts to detect chlamydiae in Stamp-stained impression smears made from the joints and the inflamed pleura were unsuccessful.

Mycoplasma culture

Mycoplasmas were cultured from the joints of all the four goat kids. Mycoplasmas were isolated also from the lungs of one out of the two kids examined from herd A and from the abdominal and thoracic parenchymal organs of both kids examined from herd B. The mycoplasmas grew in strikingly large colonies. The size of the colonies was identical with that of *Mmm*LC and substantially exceeded the size of SC-type colonies. The isolated mycoplasmas proved to be glucose positive, arginine negative and phosphatase negative. Surface film and 'spot' formation could not be observed. The isolates liquefied the coagulated serum. In the immunofluorescence test they gave a positive reaction with *M. mycoides* subsp. *mycoides* (PG1 and Y goat) antibodies and did not react with antibodies to several other *Mycoplasma* species (*M. agalactiae, Mmc, Mcc, Mccp*).

In the growth inhibition test the strains produced a 2–3 mm inhibition zone with *M. mycoides* subsp. *mycoides* (Y goat) antibodies but did not give an inhibition zone with the other mycoplasma antibodies. The strains survived incubation at 45 °C for more than 24 hours.

On the basis of the above properties the isolated strains were identified as *Mmm*LC. Based upon the results of examinations performed, a disease of goat kids caused by *Mmm*LC was diagnosed in both herds.

PCR

A general mycoplasma primer was used for the PCR. The PCR performed with purified DNA originating from the synovial fluid, an approximately 270 bp PCR product similar in size to the control *M. meleagridis* was obtained, which proved the presence of mycoplasmas in the starting sample materials. The sequence of the amplified DNA segment (the PCR product) was identical with the corresponding segment of *Mmm*LC.

Discussion

This is the first report on the occurrence of a goat disease caused by *Mycoplasma mycoides* subsp. *mycoides* LC in Hungary. The purpose of this paper is to call attention to this disease, since different mycoplasma species including *Mmm*LC can produce considerable losses in goat herds. This is evident also from the two outbreaks reported in this paper; however, foreign reports described goat kid mortality as high as 40–90% (Da Massa et al., 1983; East et al., 1983).

When establishing the differential diagnosis, of the disease entities of non-mycoplasmal origin those bacterial and chlamydia-induced conditions should be excluded which can produce arthritis. Caprine arthritis-encephalitis (CAE) virus causes arthritis just like *Mmm*LC. However, in the two cases reported by us CAE could be ruled out already by the analysis of clinical data.

In addition to *Mmm*LC, further three mycoplasma species (*M. agalactiae, Mcc* and *M. putrefaciens*) can cause arthritis in goats. The biochemical properties important for the differentiation of these four mycoplasma species were reported earlier (Bajmócy et al., 1998).

Growth inhibition tests using monospecific sera and the immunofluorescence test are the most important methods for species identification. Differentiation of the two types of *Mycoplasma mycoides* subsp. *mycoides* was based on colony size, coagulated serum liquefaction, more than 24 h survival during incubation at 45 °C, and the results of sequence analysis.

The use of PCR represented a great diagnostic advantage, as it allowed us to determine the mycoplasmal origin of the disease within 24 h of the arrival of test samples at the institute.

When describing the clinical signs and the course of the disease, we mostly had to rely on the accounts of the goat keepers, as we joined in the investigations only in the last few days of the disease course. Arthritis was the most typical and almost the only clinical sign in both cases. Only a few animals showed dyspnoea and one kid exhibited nervous symptoms. The farmers did not report the occurrence of mastitis, and during our farm visit we did not observe it either. Milk samples were collected from a few female goats the kids of which had died. However, no mycoplasmas could be isolated from these milk samples.

Although goats have been kept on both farms for more than 10 years, no similar disease had occurred before this outbreak. Therefore, we assume that the pathogen may have been introduced by asymptomatic carrier animals. Herd A purchased a buck of unknown origin, whereas herd B purchased a large number of young female goats from several different places in the autumn of 1998. It is known from the literature that infected herds may contain asymptomatic female goats which have an intact udder and produce milk of normal gross appearance. The milk produced by such goats, however, contains large numbers of mycoplasmas. According to some reports, the colostrum may also contain pathogenic mycoplasmas (Da Massa et al., 1983; Ruhnke et al., 1983).

Serious therapeutic attempts were not made in the two herds, as the owners called a veterinarian only in the last few days of the disease course. We called the goat keepers' attention that, if the disease occurs, therapy may be attempted by the use of antibiotics effective against mycoplasmas. However, according to data of the literature antibiotic therapy is unlikely to be very effective, especially against the disease entity accompanied by arthritis (Ruhnke et al., 1983; East et al., 1983). Therefore, in addition to the administration of antibiotics, general biosecurity measures must be taken (newborn kids should be weaned from their dams immediately, they should be given colostrum preheated to 56 °C, the kids should drink pasteurised milk up to 1 month of age, then a full-value milk substitute should be given up to weaning, the affected animals should be removed from the herd, the females carrying the pathogen should be identified and separated from the herd). Successful vaccination trials have also been reported (Bar-Moshe et al., 1984). In herds that are free from the pathogen, the prevention of introduction is the primary task. Infected animals can be identified by serological and bacteriological methods. The veterinary diagnostic laboratories of Hungary should prepare themselves for the performance of such tests, as of the infectious diseases of goats perhaps the disease entities caused by mycoplasmas entail the most substantial economic losses.

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