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Comparison of the epizootiological and clinical features of caseous lymphadenitis and Morel's disease in goats

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ABSTRACT

Morel's disease and caseous lymphadenitis of sheep and goats are caused by *Staphylococcus aureus* subsp. *anaerobius* and *Corynebacterium pseudotuberculosis*, respectively. Both diseases have similar epizootiology and are characterized by abscess formations in or near major superficial lymph nodes. These similarities in the clinical picture, along with the fact that caseous lymphadenitis is a better known and more common disease result in frequent misdiagnosis of the disease. In this study, the clinical and epizootiological findings of Morel's disease and caseous lymphadenitis were evaluated and compared in two naturally infected goat herds. All animals in both herds were clinically examined by inspection and palpation for the presence of abscesses. Epizootiological data and clinical features, including the localization, number and size of the abscesses, were recorded and compared. The study showed that in the early epizootic stage both diseases equally affect both young and adult goats. The main differences in the clinical picture were related to the smaller average number and size of abscesses per animal in caseous lymphadenitis. However, these differences are insufficient to distinguish individual cases and so isolation and identification of the causative agent is still needed to confirm the diagnosis.

Key words: caseous lymphadenitis, Morel's disease, goats, epizootiology, clinical picture

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Introduction

Morel's disease of sheep and goats is caused by *Staphylococcus aureus* subsp. *anaerobius*. Although both the causative agent and the disease have already been described in the literature (AYNAUD 1922; BAJMOCY et al., 1984; DE LA FUENTE et al., 1985; ALHENDI et al., 1993; DE LA FUENTE et al., 1997; SANZ et al., 2000), data on its occurrence, as well as its epizootiological and clinical appearance, are still scarce. The reason for this probably lies in its clinical picture, which is very similar to that of a better known disease - caseous lymphadenitis (CLA) or pseudotuberculosis of sheep and goats, caused by *Corynebacterium pseudotuberculosis*. Outbreaks of both diseases begin with the introduction of a sick animal into the herd. The most common entry route of both bacteria is through scratches and cuts of the skin. In that way skin injuries, caused by shearing, tagging, docking, or castration, as well as environmental hazards (e.g. splintered wood, metal edges, jutting nails, wire), increase the risk of infection (BAJMOCY et al., 1984; DORELLA et al., 2006). After the colonization and invasion of the site of entrance, the bacteria trigger the immune system. Phagocytes transport phagocytized bacteria to regional lymph nodes. Instead of being killed, they are able to survive, causing the formation of abscess within or in the proximity of the lymph node. The abscess enlarges and becomes encased in a thick fibrous capsule, which protects the microorganisms from the immune system, as well as from the action of antimicrobials (BATEY, 1986; WALKER et al., 1991; PEPIN et al., 1994; RUIZ SANTA QUITERIA et al., 1994). The abscess eventually ruptures, releasing pus that contains millions of bacterial cells, they contaminate the environment and allow the cycle of infection to continue. Both diseases are characterized by abscess formation in or near major superficial lymph nodes, and in cases where there is no isolation of the causative agent, misdiagnosis is expected. The major difference is that, unlike in Morel's disease, in CLA abscesses can also develop internally in organs, such as the lungs, kidneys, liver and spleen (BATEY, 1986; GUIMARAES et al., 2011). Both diseases mainly affect sheep and goats, but infections in other species, including humans, have also been reported. Besides CLA, *C. pseudotuberculosis* causes ulcerative lymphangitis and pigeon fever in horses, and has also been isolated from other species such as: cattle, camels, swine, buffaloes and humans (PEEL et al., 1997; SELIM, 2001; WILLIAMSON, 2001; YERUHAM et al., 2004). *S. aureus* subsp. *anaerobius* is mainly isolated from small ruminants with only a few cases of infection described in humans (CRAWFORD et al., 1994; OVER et al., 2000; FRIEDBERG et al., 2003; PEAKE et al., 2006) and one reported case of isolation from a dog (OLIVEIRA et al., 2006).

While CLA has worldwide distribution, Morel's disease occurs mostly in Africa and Asia (SHIRLAW and ASHWORD, 1962; PEGRAM, 1973; EL SANOUSI et al., 1989; ALHENDI et al., 1993; BEN SAID et al., 2002) and only a few outbreaks have been reported in Europe. Outbreaks were described in France (AYNAUD, 1922), Hungary (BAJMOCY et

al., 1984), Italy (VALENTI and BIELER, 1984), Spain (DE LA FUENTE et al., 1985), Denmark (MOLLER et al., 2000) and Poland (SZALUS JORDANOW et al., 2010). Morel's disease was confirmed in Croatia for the first time in 2004 (HABRUN et al., 2004) and no further cases have been reported since. Due to the intensive traffic of goats between small breeders, it is highly unlikely that the disease was fully eradicated at that time. Presumably it has just been misdiagnosed as the much more prevalent CLA.

The objective of this paper was to describe an outbreak of Morel's disease in Croatia. In addition, the epizootiological and clinical data obtained from two goat herds, naturally infected with *C. pseudotuberculosis* and *S. aureus* subsp. *anaerobius* were compared.

Materials and methods

Epizootiological and clinical examination. This study was conducted on two goat herds (A and B) in which both diseases had already been confirmed by microbiological examination.

Epizootiological data was mainly collected from the archives of the competent veterinary clinics in the given area. The data obtained included herd size, management systems, history of the disease (the introduction, spread pattern and course of the disease), control measures that were taken and information on concurrent diseases, if noted.

All animals in both herds were clinically examined by inspection and palpation for the presence of abscesses. Data on the localization, number and size of the abscesses found during examination were recorded. At the same time, repeated sampling for microbiological examination was performed.

Sampling. Pus and capsule swab samples were collected from subcutaneous abscesses in three crossbred and six Alpine goats from herds A and B, respectively. Briefly, the skin above the lymph node was disinfected with 70 % ethanol; a small incision was made, using a sterile scalpel blade, through which the pus was squeezed into a sterile universal bottle, stored at 4 °C and submitted for bacteriological examination within six hours. In addition, a capsule swab was taken by rubbing a sterile cotton swab against the wall of the abscess after pus evacuation.

Microbiological examination. Direct smears were prepared from fresh pus samples and from capsule swabs, and stained with Gram's stain. Pus and capsule swabs were plated on 5 % sheep blood agar and incubated in aerobic, microaerophilic (incubator with 5 % CO₂) and anaerobic conditions for 24 to 48 hours, at 37 °C. Initial identification of bacterial isolates to the genus level was performed using standard methods (QUINN et al., 1994). Identification of coryneform bacteria to the species level was accomplished by Api Coryne (Bio-Merieux, France). *S. aureus* subsp. *anaerobius* was identified according to colonial morphology, hemolysis, pigment production, ability of growth in aerobic, anaerobic and microaerophilic conditions, production of catalase, clumping factor and

coagulase (DE LA FUENTE et al., 1985). Production of acid from different substrates was explored by Api Staph (Bio-Merieux, France), incubated under microaerophilic conditions for 24 hours at 37 °C.

Results

Epizootiological and clinical data. Both herds consisted of horned animals and both were kept indoors during the winter and grazed during the summer.

Herd A. The owner of a herd A was a long term sheep owner who established a new goat herd 2.5 years prior to this study. Sheep and goats were kept separately but shared the same pasture. At the time of presentation, the sheep flock consisted of 38 animals while the goat herd numbered 60, mainly cross breed goats. No clinical signs of caseous lymphadenitis were noted up to the end of the study in the sheep flock. The first abscess in a goat, at that time considered to be a puncture abscess, was found in one adult animal in June 2012. The second case was reported in December, when the disease started to spread across the herd. In January 2013, 30 % (15/50) of the goats were affected. At that time the owner decided to introduce certain control measures: opening and draining of mature abscesses, limited separation of affected animals (only in the period from when the abscesses opened until the time they closed). Separate milking of affected animals and periodical disinfection of milking area and pens was also practised. Following the introduction of control measures, the prevalence of the disease declined for 3-4 months but then increased again. The second peak of the disease was similar in prevalence and duration and declined again after 2 months. At the time of repeated sampling, only 5 goats had mature abscesses (8.3 %). From the beginning of the outbreak only subcutaneous abscesses were recorded and no internal abscesses or other lesions were found. The disease occurred in adult goats and yearlings, while kids were never affected. The most common observation during the outbreak was one abscess per animal, but cases with up to three abscesses were also noted. The average size of the mature abscesses was 6 x 5 cm (Fig. 1) and they were mainly located on the parotid and mandibular, followed by the subiliac lymph nodes. Abscesses on the superficial cervical lymph nodes were noted only sporadically. The time between the appearance of an abscess and its spontaneous rupture was more than one month. During the observation period of 10 months no relapse of the disease was noticed in affected animals and, according to the archive data, abscess formation was the only manifestation of the disease. The arthritis encephalitis virus was also confirmed in this herd. Approximately 10 % of the goats had clinical manifestations, but no correlation between these two diseases was noted.



Fig. 1. Comparison of abscess size in Morel's disease (a) and CLA (b)

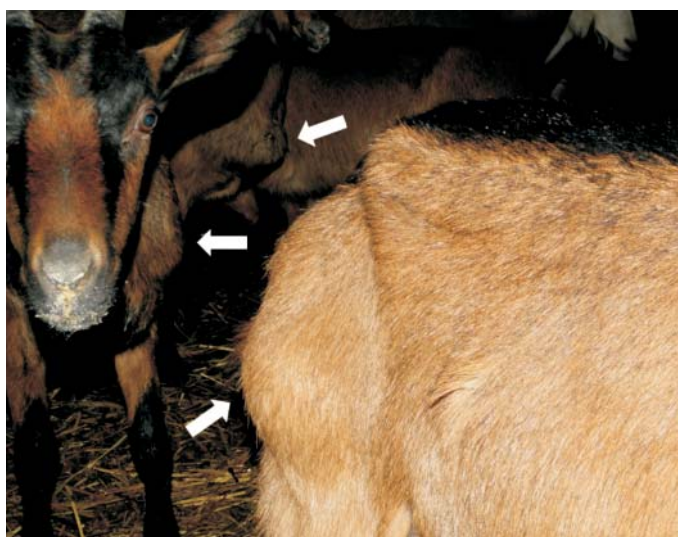


Fig. 2. Different localization, number and size of abscesses in a herd infected by *S. aureus* subsp. *anaerobius*

Herd B. The owner established a goat herd 2 years prior to the study. At the time of presentation, the goat herd consisted of 62 animals. The first abscess, also considered to be a puncture abscess, was noted in one goat in November 2012. This goat was introduced from a herd with no abscess disease history. The second case was reported at the end of March 2013, when the disease started to spread across the herd. No control measures were taken. Three months later prevalence reached 46.7 % (28/60) and was still approximately the same at the time of repeated sampling four months later. Abscesses were located

subcutaneously in the proximity of the superficial lymph nodes. At the beginning of the outbreak no age predisposition was noted, but at the time of repeated sampling mainly yearlings were affected. The most common observation during the outbreak was three abscesses per animal, but cases with only one or more than three were also noted. The average size of the mature abscesses was 11 x 9 cm (Fig. 1) and they were mainly located at or near the cervical superficial and subiliac lymph nodes (Fig. 2). Abscesses of the mandibular, parotid and popliteal lymph nodes were recorded sporadically. The time between the appearance of an abscess and its spontaneous rupture varied, but was generally more than one month. During the observation period of 10 months, no relapse of the disease was noted in the affected animals. No other health issues were reported in this herd.

Microbiological examination.

Herd A. Gram stained smears of pus and capsule swabs (from herd A) revealed pleomorphic gram-positive rod-shaped bacteria (Fig. 3). *C. pseudotuberculosis* was isolated in pure culture from both the pus and capsule swabs of two goats, and in mixed growth with *Trueperella (Arcanobacterium) pyogenes* from a swab taken from the third goat. Growth was obtained under all atmospheric conditions, but was most vigorous on sheep blood agar plates incubated in air supplemented with 5 % CO₂.

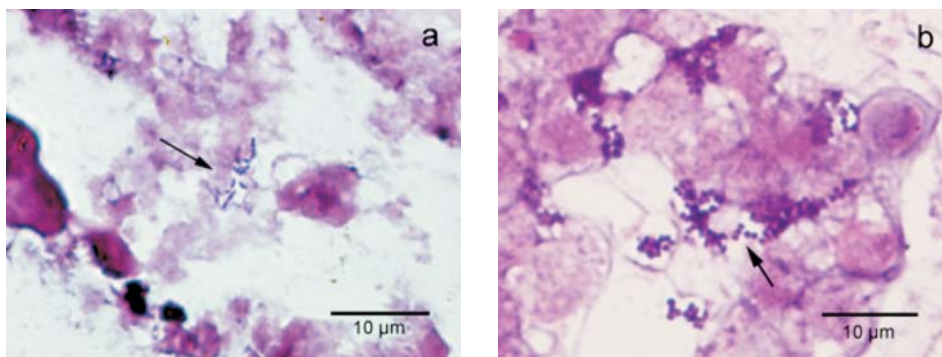


Fig. 3. *Corynebacterium pseudotuberculosis* (a) and *Staphylococcus aureus* subsp. *anaerobius* (b) in the smears of pus from subcutaneous abscesses (Gram stained, ×1000)

Herd B. Gram stained smears of pus and capsule swabs from all except one goat revealed gram-positive cocci in clusters (Fig. 3). *S. aureus* subsp. *anaerobius* was isolated in pure cultures from both the pus and capsule swabs of all five goats, and even from a capsule swab that was negative after Gram staining (it yielded only a single colony on a

sheep blood agar plate). Faint growth was observed after 24 hours of incubation and was seen as hazy hemolysis along the streak lines, before the colonies became visible to the naked eye. After 48 hours white, round, convex colonies of less than 1 mm in diameter with double-zone hemolysis were seen. The growth was obtained on sheep blood agar plates incubated in microaerophilic and anaerobic conditions but not in aerobic incubator. All isolates were catalase and clumping factor negative, coagulase positive, produced acid from glucose, fructose, mannose and saccharose, but not from maltose, lactose, trehalose, mannitol, xylitol, melibiose, raffinose and xylose.

Discussion

Caseous lymphadenitis of small ruminants is well described in the literature, but epizootiological and clinical data concerning Morel's disease in goats, such as the incubation period, appearance and persistence of clinical signs, the course of the disease and changes of prevalence in time are limited. Awareness of the disease is also very limited, at least in Croatia, making the possibility of misdiagnosis with the more common and very well documented CLA highly probable. In order to draw attention to and increase knowledge of the possible differences between these diseases, we investigated the epizootiology and clinics of CLA and Morel's disease in two goat herds, with no previous history of the disease.

Both outbreaks were probably initiated by the introduction of infected animals with abscesses. Six (Herd A) and four months (Herd B) later, the spread of the diseases across the herds was noted. Initial sampling was performed and the presence of CLA and Morel's disease was confirmed by microbiological examination. The observed incubation period of 6 months in a CLA infected herd correlates with the literature data already described of 2 to 6 months (WILLIAMSON, 2001). On the other hand, the previously proposed incubation period of Morel's disease (3 weeks) was significantly shorter (RUIZ SANTA QUITERIA et al., 1994; DE LA FUENTE et al., 1997). Unfortunately, there can be no discussion of the duration of the incubation period in this paper as no microbiological examination was conducted on the goats that were the first to develop abscesses. In addition, both owners constantly increased the herd size by introduction of new animals, making the verification of our data impossible. Different incubation periods of CLA and Morel's disease could explain why these diseases predominate in different age groups. Literature data suggest that CLA is most prevalent in the age group from 1 to 2 years (AL-GAABARY et al., 2009) while Morel's disease occurs mainly in young animals up to 6 months of age (DE LA FUENTE et al., 1997). Our observations indicate that both diseases in this early epizootic stage equally affect both young (<1 year) and adult goats (>1 year) to the same degree. Similar findings were already reported in a goat herd from Poland (SZALUS JORDANOW et al., 2010).

The highest overall prevalence in our study was 30 % (15/50) and 46.7 % (28/60) in CLA and Morel's disease infected herds, respectively. Different data on prevalence rates of CLA in goats, from 0.2 % (MUBARAK et al., 1999) to 100 % (URAL et al., 2008) have already been described in the literature. These variations in the disease frequency could be a result of different management systems, climatic conditions and the enzootic nature of the disease, that is most likely connected with variations in animal immunity and susceptibility (AL-GAABARY et al., 2009). In the CLA infected herd (herd A) we also noticed variations in terms of a temporal decline in disease prevalence. It remains in dispute whether these variations were a result of limited control measures or the partially acquired immunity of the herd.

The prevalence of 45 % in a herd with Morel's disease is similar to data already reported (SZALUS JORDANOW et al., 2010), but outbreaks with a prevalence of 71 % (DE LA FUENTE et al., 1997) as well as 2.2 - 6.5 % have also been described (AL-HARBI, 2011).

Localization of abscesses was determined by clinical examination performed during the repeated sampling and from available archive data. In the CLA infected herd the abscesses were only located in the superficial lymph nodes and no visceral changes were noted at the abattoir. The reason for that may be found in a fact that only very young goats not predisposed to the disease were slaughtered. In addition, visceral forms of the disease are more common in sheep and, as reported in the literature, they appear in a maximum of 20 % of cases in goats (BATEY, 1986). The parotid, mandibular and subiliac lymph nodes were most frequently involved, while abscesses of the cervical superficial lymph nodes were noted sporadically.

Abscesses in the herd affected by Morel's disease were located in the proximity of the superficial lymph nodes, mainly on or near the cervical superficial and subiliac lymph nodes. Changes on the mandibular, parotid and popliteal lymph nodes were noted less frequently. Different data on the localization of the abscesses can be found in the literature (MUBARAK et al., 1999; WILLIAMSON, 2001) and it seems that it depends on the entry site of the bacteria. Therefore predisposing factors, such as injuries from domination fights, scratching shoulders or rear ends against walls, fences or other sharp or hard objects will partially determine the localization of the abscesses (FONTAINE and BAIRD, 2008; GUIMARAES et al., 2011).

The major difference in the clinical picture of CLA and Morel's disease in this study was related to the size and number of abscesses per animal. In the CLA infected herd, a smaller average number of abscesses per animal was recorded. Although cases with up to 3 abscesses were noted, one abscess per animal was the most common finding. The average size of the abscesses was 6 x 5 cm. In contrast, goats with Morel's disease had a larger number of abscesses (commonly three) per animal, with an average size of approximately 11 x 9 cm (Fig. 1). Similar observations have already been reported in a

herd with an enzootic CLA infection that was additionally infected with Morel's disease (SZALUS-JORDANOW et al., 2010).

In conclusion, the clinical picture and epizootiological data of both diseases are very similar, and despite the established differences in the size and number of abscesses, in a case of an outbreak, confirmatory diagnosis of the disease should always be based on microbiological examination.

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SAŽETAK

Morelova bolest i kazeozni limfadenitis su zarazne bolesti koza i ovaca uzrokovane bakterijama *Staphylococcus aureus* subsp. *anaerobius* odnosno *Corynebacterium pseudotuberculosis*. Usprkos različitoj etiologiji, obje bolesti imaju vrlo sličnu epizootiologiju i obje se očituju tvorbom apscesa unutar ili u blizini površinskih limfnih čvorova. Podatci o pojavi Morelove bolesti u Hrvatskoj vrlo su oskudni. Kazeozni limfadenitis javlja se češće i o toj bolesti postoji više pisanih podataka. Stoga su, osobito u slučajevima kada se ne provodi mikrobiološka pretraga, pogrešne dijagnoze vjerojatno vrlo česte na štetu Morelove bolesti. U ovom radu usporedili smo epizootiološke i kliničke nalaze objiju bolesti u dva prirodno zaražena stada koza. Sve životinje klinički su pretražene inspekcijom i palpacijom, te su zabilježeni podatci o lokalizaciji, broju i veličini apscesa. Usporedbom dobivenih podataka utvrdili smo da je učestalost objiju bolesti u ranom stadiju epizootije jednaka u mladim i odraslim koza. Osnovna razlika između kazeoznog limfadenitisa i Morelove bolesti utvrđena je u kliničkoj slici odnosno broju apscesa po životinji i njihovoj prosječnoj veličini. U stadiju u kojem je utvrđen kazeozni limfadenitis utvrđen je manji broj apscesa po životinji kao i manja veličina apscesa. Usprkos utvrđenim razlikama između opisanih bolesti smatramo da na temelju epizootioloških podataka i kliničke pretrage nije moguće objektivno dijagnosticirati bolest već je za njihovo razlikovanje potrebno izdvojiti i identificirati uzročnika.

Ključne riječi: kazeozni limfadenitis, Morelova bolest, koza, epizootiologija, klinički nalaz
