

*Case report***POLYARTHRITIS IN GOAT-KIDS CAUSED BY
STREPTOCOCCUS EQUI SUBSPECIES *ZOOEPIDEMICUS***

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Infectious arthritides in goats is mainly caused by mycoplasmas and Caprine Arthritis-Encephalitis virus (CAEV). *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) is a member of Lancefield group C beta-hemolytic streptococcus that commonly colonizes the mucous membranes of healthy equids which is also capable to induce different pathological conditions in many animal species. In this paper we described a case of polyarthritis in four Alpine goat-kids caused by *S. zooepidemicus*. Goat-kids originated from a farm where sheep, llamas, shepherd dogs, cows and horses were kept with goats. During the external examination of carcasses, crusts and discrete hyperemia were seen on the earlobes beneath the ear tag, as well as swollen joints. Necropsy findings included severe fibrinous polyarthritis in all four examined kids. Shoulder, knee and hip joints were affected. From all affected joints *S. zooepidemicus* was isolated. Neither *Mycoplasma* spp. nor CAEV genome were detected from the affected joints. Most likely the goat-kids were infected with *S. zooepidemicus* through the lesions on earlobes which were made after the tagging. As a possible source of bacteria, we recognized the horses and dogs. Due to the lack of any signs of illness in other animal species on the farm, as well as negative pathomorphological findings in other organs, we assumed that the isolated strain is highly adapted to goat-kids. Our report is the first described case of polyarthritis in goat-kids caused by *S. zooepidemicus*.

Key words: goat-kids, polyarthritis, *Streptococcus equi* subspecies *zooepidemicus*

INTRODUCTION

Depending on the etiology, arthritis may be either infectious or noninfectious. Infectious arthritis occurs, most frequently, in farmed livestock and horses, especially in young animals where it is commonly a sequel to bacteremia. Causative agents of infectious arthritis in goats are mycoplasma and Lentivirus [1]. A septicemia-arthritis syndrome in goats is caused by *Mycoplasma mycoides* subsp. *mycoides* or *Mycoplasma capricolum* subsp. *capricolum* [1]. The septicemia-arthritis syndrome in goats is an acute febrile disease, associated with a drop in milk yield due to mastitis [1]. Kinde *et al.*

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[2] described an outbreak of arthritis/polyarthritis, clinical mastitis and sudden death in a dairy goat herd where *Mycoplasma agalactiae* was isolated from the joint. Caprine arthritis-encephalitis (CAE) is caused by Lentivirus, the one of few viral agents that has been linked with arthritis in domestic animals [1]. Clinical symptoms of CAE include arthritis as the major or even the only clinical manifestation, with lameness which is often associated with carpal hygromas, weight loss and milk drop [1]. *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*) belongs to Lancefield group C of beta-hemolytic streptococcus [3]. *S. zooepidemicus* is a commensal organism founded on the tonsils, upper respiratory tract, skin and urogenital tract of horses [4]. The specific name „zooepidemicus“ derives from its wide host range [5]. Infectious respiratory disease of dogs [6], stranglers in horses [7], mastitis in goats [8], polyserositis in alpaca [9], rhinitis and meningitis of cats [10], septicemia, and wound infections in lambs, puppies, and greyhounds, septicemias in chickens and dolphins, lymphadenitis in guinea pigs [5], hemorrhagic pneumonia in broilers [11] are examples of its wide range of pathogenicity. Although *S. zooepidemicus* is an opportunistic pathogen for horses, infections in humans are often severe [12]. Human infections with *S. zooepidemicus* usually occur after consumption of unpasteurized products from goat and cow milk, pork meat or close contacts with horses and dogs [3,4,12-14]. Clinical manifestations of human infections with *S. zooepidemicus* include pharyngitis, septicemia, meningitis, purulent arthritis and endocarditis [14].

The aim of this paper was to describe a case of severe fibrinous polyarthritis in goat-kids caused by *S. zooepidemicus*.

CASE PRESENTATION

Four carcasses of Alpine goat-kids (one month old) were submitted to the diagnostic laboratory of Institute of Veterinary Medicine of Serbia for revealing of potential causes of death. Milk samples from two goats were also submitted. The goats originated from a herd of 80 goats which were kept together with 110 sheep, 3 llamas, 1 cow, 1 horse and several shepherd dogs. The farm is located in southeast part of Republic of Serbia, in the Old Mountain region. According to the information given by the owner and local veterinarian, the sudden increase of mortality among goat-kids was observed during the end of the kidding season. In total, 15 goat-kids from the age of two weeks to one month have become ill, whereas only three goat-kids survived. Affected goat-kids were not treated. The disease began with a visible lameness, followed by swelling and painfulness of joints. This led to the inability to move and to struggle to eat despite preserved appetite. The time from the appearance of initial symptoms to the death was between few and 15 days. The barn where the kids were kept was spacious, bright and high with enough clean and dry straw. Animal's diet consisted of high quality meadow hay with addition of different cereals and mineral blocks for licking. Sheep were kept together in the barn with goats. Shepherd dogs and poultry

occasionally stayed in the barn. Goat-kids were not in direct contact with the horse. There were no signs of illness in other animals on the farm.

After necropsy, samples of altered tissues were taken for bacteriological and molecular tests. Goat milk samples were also subjected to bacteriological and molecular investigations. For bacteriological examinations, samples were streaked on Columbia blood agar with 5% sheep blood, MacConkey agar and BHI broth (HiMedia, India). The plates were incubated under aerobic and anaerobic conditions at 37 °C in the course of 24-72 hours. After 24 hours plates were examined for the presence of bacterial colonies while the broth medium was prepared for Gram staining and microscopic examination, and the broth was sieved on blood agar. Commercial tests were used in order to determine the biochemical activity (bioMerieux, France; HiMedia, India). For serotyping we used commercial rapid latex agglutination slide test (Micro Strep, Microgen Bioproducts, UK). As a confirmatory test in the process of identification of culture BBL Crystal G/P ID (Becton Dickinson, USA) kit was used. Molecular methods (PCR and *real time* PCR) were used for the detection of *Mycoplasma* spp. and Caprine arthritis-encephalitis virus (CAEV) genomes. For DNA and RNA extraction, commercial kits were used (QIAamp DNA mini kit, Qiagen, Germany; Gene JET Viral DNA and RNA purification kit, Thermo scientific, Lithuania). For the detection of genome of *Mycoplasma* spp. and CAEV, commercial kits (TopTaq Mastermix kit – Qiagen, Germany; Absolute qPCR ROX mix, Thermo Scientific, Lithuania) and thermal protocols and primers described by Johnson et al. [15] and Kuhar et al. [16] were used.

RESULTS AND DISCUSSION

During the external examination of carcasses, swollen joints (Fig 1) were observed. Discrete hyperemia and crusts (Fig 2a,b) were seen on the earlobes beneath the ear tag. Necropsy findings included severe fibrinous polyarthritis in all examined kids (Fig 3). There were no pathomorphological alterations on other examined organs. Shoulder, knee and hip joints were affected. The articular surfaces were, partially or completely, covered with sheets of white to yellow fibrin.

From the affected joints, bacterial growth was noticed on blood agar after 24 hours in aerobic conditions. Bacterial colonies were round, convex, 1.5 mm in diameter, surrounded with a narrow zone of beta hemolysis (Fig 4). Growth of colonies in pure culture was observed from all samples taken from the affected joints. There was no growth on MacConkey agar, while weak growth occurred after 48 hours on blood agar incubated in anaerobic conditions. Microscopically, gram positive cocci in short chains, rarely in duplets were seen. Catalase and oxidase tests were negative. CAMP test was also negative. Commercial kit for serotyping revealed that isolated streptococci belonged to Lancefield's group C. Based on the determined morphological, cultural and physiological characteristics as well as biochemical activity and serotyping,

bacterial culture was identified as *S. zooepidemicus*. This identification was confirmed by BBL Crystal GP/ID. No bacterial growth was observed from milk samples. Neither *Mycoplasma* spp. nor CAEV genome were detected neither from milk nor joints.



Figure 1. Swollen joint - edema of the affected knee joint. Note marked difference between healthy (white arrow) and affected joint (black arrow)

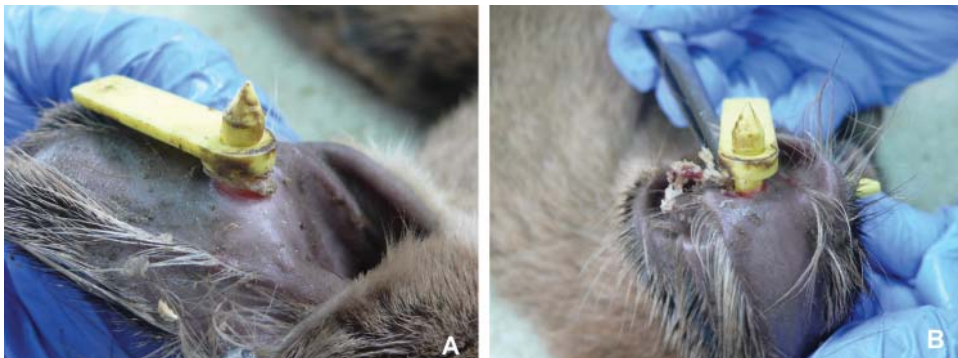


Figure 2. a) Crust beneath ear tag, b) Hyperemia of the edges of ear skin after removing of crust



Figure 3. Fibrinous polyarthritis in different affected joints. **a)** Opened knee joint from Figure 1: Fibrinous exudate covers the articular surfaces. **b)** Abundant fibrinous mass is detached from articular surfaces of knee joint. **c)** and **d)** Hip joints with extensive fibrinous inflammatory exudate



Figure 4. Growth of *Streptococcus equi* subspecies *zooepidemicus* on Columbia blood agar. Note presence of zone of beta hemolysis around colonies

Available data regarding infectious arthritis in goats are scarce. Nevertheless, only mycoplasma and CAEV are considered as causative agents [1,2]. However, in this case, both were excluded by molecular methods. Bearing in mind the fact that beside the lesions on earlobes no other wounds or lesions on other parts of body were found during necropsy, we assumed that those lesions were the entrance for *S. zooepidemicus* infection. Cutaneous lesion as most likely portal of entry for *S. zooepidemicus* followed by arthritis is described once although in human patient [17]. Hematogenous bacterial infections in young animals typically cause polyarthritis [1]. Though *S. zooepidemicus* could also be related to mastitis in goats [8], in the described case, milk was not the source of infection, respecting the negative bacteriological results and the fact that there were no cases of mastitis on the farm among other animals. Since there were no signs of illness in other animal species on the farm, as well as negative pathomorphological findings in other organs, along with the negative bacteriological results from milk samples, the source and the route of infection for goat-kids were unclear. As *S. zooepidemicus* is considered as opportunistic commensal of horses [12], the horse from the farm remained as a possible source of bacteria for goat-kids though less likely because goat-kids were not in contact with the horse. Another possible source of *S. zooepidemicus* for goat-kids could have been dogs [6] which occasionally stayed in the barn with goats. In an attempt to clarify the source of the bacterium, we requested from the owner to forward samples from horse and dogs for additional examinations. However, we did not get those samples. Since there were no symptoms in other animal species, it was assumed that the isolated strain of *S. zooepidemicus* could be highly adapted for joints of goat-kids. It is not clear why certain microorganisms localize more in the synovial membrane during bacteremia than in other structures, but experimental evidence suggests that it is not by chance [1]. Experimental studies indicate that organisms which cause arthritis possess collagen binding components which allow them easier adherence to joint collagen [1]. Members of a family of collagen-binding microbial surface components recognizing adhesive matrix molecules from Gram-positive bacteria are well known virulence factors [18]. *S. zooepidemicus* poses the collagen binding protein that allows the attachment to the host tissue [19,20]. To our knowledge, there is no available data about cases of arthritis in goats caused by *S. zooepidemicus*. In spite of that, there are several reports that *S. zooepidemicus* is detected as cause of arthritis in humans [17,21,22].

Although *S. zooepidemicus* induces wide range of pathological conditions in different animal species, our report represents the first described case of arthritis in goat-kids caused by this bacterium. Due to the progressive course of the disease followed by fatal outcome, infection with *S. zooepidemicus* should be considered in cases of severe, progressive polyarthritis in goats. Special attention to outbreaks with this pathogen should be given with respect to his zoonotic potential.

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Authors' contributions

CD participated in case design, performed necropsy and carried out literature research. RO performed bacteriological examination. MV performed molecular tests. JN and KB performed necropsy and took photography during the necropsy. All authors helped to draft manuscript and have read and approved the final version of manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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POLIARTRITIS JARADI UZROKOVAN SA *STREPTOCOCCUS EQUI* SUBSPECIES *ZOOEPIDEMICUS*

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Infektivni artritis koza su uglavnom izazvani mikoplazmama i kozijim arteritis-encefalitis virusom (CAEV). *Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*) je beta-hemolitički streptokok iz Lansfildove grupe C koji uglavnom kolonizuje sluznice zdravih konja ali je takođe spososan da dovede do različitih patoloških stanja kod

mnogih životinjskih vrsta. U ovom radu je opisan slučaj poliartritisa kod četiri jareta Alpske rase uzrokovanog sa *S. zooepidemicus*. Jarad potiču sa farme gde se, pored koza, drže i ovce, lame, pastirski psi, krava i konj. Spoljašnjim pregledom leševa, uočavaju se kraste i blaga hiperemija na ušnim školjkama ispod ušne markice kao i otok zglobova. Obdukcijom je otkriven težak fibrinozni poliartritis kod sva četiri obdukovana jareta. Zahvaćeni su bili zglobovi ramena, lakta i kuka. Iz svih zahvaćenih zglobova izolovan je *S. zooepidemicus*. Prisustvo genoma *Mycoplasma* spp. i CAEV nije dokazano. Jarad su se najverovatnije inficirala sa *S. zooepidemicus* preko lezija na ušima koje su nastale prilikom postavljanja ušnih markica. Smatramo da su konj i psi mogući izvor infekcije. Imajući u vidu izostanak simptoma oboljenja kod drugih vrsta na farmi, kao i negativan patomorfološki nalaz u drugim organima, pretpostavljamo da je izolovani soj visoko adaptiran za jarad. Naš nalaz predstavlja prvi opisani slučaj poliartritisa jaradi izazvanog sa *S. zooepidemicus*.