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***CORYNEBACTERIUM RENALE* CYSTITIS IN COW - CASE REPORT -**

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Abstract:

Corynebacterium renale is a common inhabitant of the the vulva, vagina and prepuce of apparently normal cattle, but also an opportunistic pathogen and the cause of cystitis and purulent pyelonephritis in cows. In this paper, we show the isolation of *C. renale* from the urine of cows with clinical cystitis, colonial, microscopic and biochemical characteristics of the isolates, relevant data on virulence factors, clinical manifestations of disease and basic principles of therapy.

Key words: cow, *Corynebacterium renale*, cystitis

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CORYNEBACTERIUM RENALE CYSTITIS KOD KRAVE -PRIKAZ SLUČAJA-

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Kratak sadržaj:

Corynebacterium renale je uobičajeni deo mikrobiota sluzokože vulve, vagine i prepucijuma klinički zdravih goveda, ali i oportunistički patogen i uzročnik *cystitisa* i purulentnog *pyelonephritisa* krava. U ovom radu prikazujemo izolaciju *C. renale* iz urina krave sa kliničkim cistitisom, osnovne kulturelne, mikroskopske i biohemijske karakteristike izolata, relevantne podatke o faktorima virulencije uzročnika, kliničkim manifestacijama bolesti i osnovnim principima terapije.

Ključne reči: krava, *Corynebacterium renale*, *cystitis*

INTRODUCTION

Bovine cystitis is an inflammatory process of the urinary bladder. The causative agents are most commonly bacteria which are an integral part of the microbiota of the genital and gastrointestinal tract, such as: *Escherichia coli*, *Corynebacterium renale* group (*Corynebacterium renale*, *cystidis* and *pilosum*), *Trueperella* (formerly *Arcanobacterium*) *pyogenes*, rarely *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp., *Klebsiella* spp., (Yeruham et al., 2006; The Merck Manual, 2014). Infections are more common in cows, compared to male cattle (Andrews and Williams, 2004). The reasons are related to the anatomical features (short urethra), hormonal status (high levels of estrogen may affect the functional integrity of the epithelium in the urethra and urinary bladder), risks associated with pregnancy or iatrogenic procedures (Stevens et al., 2007)

Corynebacterium renale belong to the *Corynebacterium renale* group, genus *Corynebacterium*, family *Corynebacteriaceae* (Quin et al., 2013). The family contains a large number of ubiquitously widespread species that are commensals on skin and mucous membranes and opportunistic pathogens to humans and animals (Stevens et al., 2007). The main representative of the

species, is a highly contagious human pathogen *Corynebacterium diphtheriae*. In domestic animals, nondiphtherial *Corynebacteria* cause different infections: *Corynebacterium pseudotuberculosis* causes caseous lymphadenitis in sheep and goats; *C. pseudotuberculosis* causes ulcerative dermatitis in cattle, while *C. ulcerans* and *C. bovis* cause mastitis. According to this new classification, the *Corynebacterium renale* group contains three species (previously three types): *C. renale* (type I), *C. pilosum* (type II) and *C. cystitidis* (type III). The *C. renale* is the cause for cystitis and pyelonephritis in cows, ulcerative (enzootic) balanoposthitis in sheep and goats and osteomyelitis in goats (Markey et al., 2013; Quin et al., 2013). *C. cystitidis* causes haemorrhagic inflammation of the bladder in cows with ulceration of the mucous membrane of the bladder, urethritis and pyelonephritis. *C. pilosum* has lower virulence and the infection typically results in a less severe clinical picture or in an uncomplicated cystitis. In natural infections, pyelonephritis develop less frequently than *C. renale* (Hayashi et al., 1985).

CASE REPORT

Sample: Catheterized urine of cows (Fig. 1).

Laboratory examination: An amount of 100 mL of the urine sample was inoculated on 2 plates of Columbia blood agar base supplemented with 5% sheep blood and the MacConkey agar (CM0007, Oxoid, Basingstoke, UK).

Incubation atmosphere: The plates are incubated aerobically (blood and i MacConkey agar) and in 5-10 per cent CO₂ conditions (blood agar).

Incubation temperature: 37°C

Incubation time: 48h.

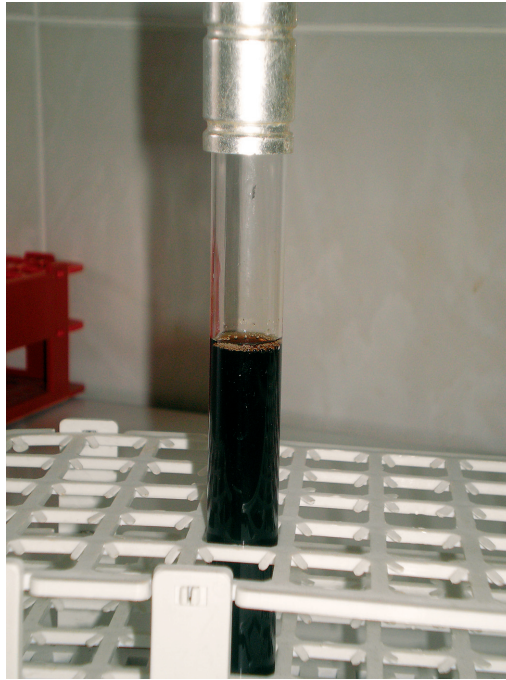


Fig. 1. Blood urine samples

Expected results: The isolation of the following causal agents of cow cystitis: *Escherichia coli*, *Trueperella pyogenes*, *Corynebacterium renale* group (*C renale*, *C cystitidis*, and *C pilosum*), *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*.

RESULTS

Colonial appearance of isolates: After 24 hours of incubation under aerobic and microaerophilic conditions, a significant increase of very small (up to 1mm), non-transparent (opaque), non-haemolytic colonies were noted on the blood agar. After the incubation for 48 hours, colonies turned a pale yellow color (pigment production). There was no growth on the MacConkey agar.

Microscopic appearance: Gram stained smears reveal small, Gram-positive bacteria, short, slightly curved rods, grouped under different angles (looking like “Chinese characters”).

Biochemical reactions: catalase test, oxidase test, urease test (Christensen urea agar), growth in broth (pH 5.4), fermentation of glucose and xylose (acid production) and aesculin hydrolysis.

Identification criteria for isolates: a pale yellow color of colony, absence of haemolysis, absence of growth on the MacConkey agar, coryneform Gram-positive rods, oxidase negative, catalase positive, strong urease production, growth in broth at pH 5.4, glucose fermentation, non acid production from xylose and negative aesculin hydrolysis.

Identification of the etiological agent: *Corynebacterium renale*.

Relevant data of *Corynebacterium renale* cystitis

Infection: *C. renale* is a common inhabitant of the vulva, vagina and prepuce of apparently normal cattle, and the occurrence of the infections contribute to certain predisposing factors. In most cases, trauma, damage to the integrity of the epithelium of the genito-urinary tract or stress (parturition, lactation peak) contributes to the infection (Quin et al., 2013; Merck Manual, 2014). These infections are therefore common in cows that have already calved as well as immediately after parturition (parturition). The stasis of urine is also an important factor of dispositions, occurring in permanent or temporary obstruction of the urinary tract due to the presence of calculi or pressure of the gravid uterus (Andrews and Williams, 2004). A diet rich in protein contributes to the development of the infection because it increases urine pH, which enhances the expression of the flagella *C. renale* and provides favorable conditions for the proliferation of bacteria (Stevens et al., 2007). The adhesion of *C. renale* bacteria to the epithelial cells of the bovine urinary tract is higher at pH of urine above 7.6 (Andrews and Williams, 2004). Non-sterile catheterization may contribute to the spread of infection from cow to cow.

Virulence factors: All species from the *Corynebacterium renale* group (*Corynebacterium renale*, *cystidis* and *pilosum*) possess fimbriae which enable the attachment to the urogenital mucosa, epithelial cells of the urinary bladder and renal pelvis (Hayashi et al., 1985; Yeruham et al., 2007; Markey et al., 2013; Quin et al., 2013). Only *C. renale* (most isolates) possess an extracellular protein that is referred to as renalin. Renalin reacts with ceramides, the integral part of the sphingomyelin cell wall of the red blood cells of mammals. Therefore, it is referred to as renalin "CAMP like" protein because it produces synergistic haemolysis on blood agar with sphingomyelinase of *Staphylococcus aureus* (beta-haemolysin). It is believed that renalin plays an important role in the lysis of the host cells (Markey et al., 2013). Enzyme urease is an important virulence factor in all three species from the *Corynebacterium renale* group.

The urease quickly (within 1 hour) and vigorously hydrolyze the urea, and the resulting products (such as ammonia) stimulate the mucosal inflammation.

Clinical signs: Hematuria is usually the first symptom of infection, and in uncomplicated cystitis, can be the only, permanent or temporary symptom. The ascending spread of the infection results in the development of purulent pyelonephritis (inflammation of the renal parenchyma and the renal pelvis). Clinical signs of pyelonephritis include fever, anorexia, colic, frequent attempts to urinate, poliuria, pyuria, agitation, decreased milk production and anemia. In chronic infections, due to the inflammatory process, the bladder wall becomes thickened, the ureters expands and fills with purulent exudates. *Corynebacterium* spp. are pyogenic bacteria, and purulent inflammation can affect the kidneys and result in the development of multiple abscesses (The Merck Manuals, 2014). In the urinary sediment, a large number of leukocytes and bacteria has been found (Merck Manual., 2013)

Although *C. renale* is widespread and commonly present in the mucosa of the genital tract, the affected individual should be isolated of the herd, in order to prevent an increase in the number of pathogens in the environment and in the prevention of the spread of infection (Merk Manual., 2013). *C. renale* cystitis is established across Europe and North America, but the prevalence is unknown (Andrews and Williams, 2004).

Therapeutic recommendations:

Corynebacterium renale is sensitive to the majority of antibiotics, such as the penicillins, ampicillin, cephalosporins, quinolones, chloramphenicol, tetracyclines, cefuroxime and trimethoprim. The treatment of choice for pyelonephritis due to *Corynebacterium* spp is penicillin, because penicillin is excreted in the urine (e.g. 10 000-15 000 iu/kg daily for at least 10 days) (Andrews and Williams, 2004) or trimethoprim-sulfadoxine (16 mg combined/kg, IM, for ≥ 3 wk) (Merk Manual, 2014). Treatment should begin as early as possible, before the development of serious tissue damage.

CONCLUSION

This is also the only case of *C. renale* cystitis in cows which was etiologically confirmed in the laboratory for clinical bacteriology of the Scientific Veterinary Institute "Novi Sad" for a period of 10 years. Cow's urine samples are usually very rarely submitted to the laboratory for bacteriological exami-

nation. The assumption is that clinical cystitis in the field is treated empirically. The exact prevalence of *C. bovis* cystitis therefore remains unknown, and research of the etiology of cystitis in cows, to our knowledge, and on our epizootic area has not been implemented.

Clinically, *C. bovis* cystitis is indistinguishable from bladder urinary infections caused by other, mostly Gram-negative bacteria (eg, *Escherichia coli*). For proper treatment, etiological diagnosis is necessary, because in this case penicillin preparations are the drug of choice. The treatment is successful if starts on time, hence preventing the development of purulent pyelonephritis.

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