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Case report

PURULENT RENAL PAPILLITIS DUE TO *STREPTOCOCCUS INFANTARIUS SUBSP. INFANTARIUS* IN A HORSE

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A 6-year-old, male Arabian crossbred horse was necropsied after a 10 day history of loss of appetite, debility and weight loss. Gross and histologic examination was consistent with purulent papillitis due to *Streptococcus infantarius subsp. infantarius*. The isolate was sensitive to all the antibiotics tested.

Key words: Horse; equine, *Streptococcus infantarius subsp. infantarius*, pyelitis; early hepatic cirrhosis

INTRODUCTION

The *Streptococcus bovis/Streptococcus equinus complex* (SBSEC) is a group of Gram-positive bacteria consisting of several species inhabiting the animal and human gastrointestinal tract. These microorganisms are commensal, potentially zoonotic and opportunistic pathogens. *Streptococcus infantarius subsp. infantarius* (*Sii*), a member of this complex, was previously classified as *Streptococcus bovis biotype II/2* [1].

CASE PRESENTATION

A 6-year-old, male Arabian crossbred horse was necropsied at the Pathology Department, Veterinary Medical Faculty, Firat University, Turkey. The animal was a carriage horse and used for touristic purposes. Carriage horses were band due to animal rights and welfare issues. Hence, he was donated by the Istanbul Municipality. He was brought from Istanbul to Elazig province 8 months ago to a farm containing 20 horses. The animals were kept outside in surrounded paddocks during the day and

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in the barn at night. The daily ration consisted of crushed barley, dried clover and straw. The horse was healthy and in good body condition until the last 10 days when he showed loss of appetite, debility and weight loss. The horse never received any therapy and was not vaccinated. The horses did not have contact with other animals and people except keepers, veterinarians and wild pigeons. The animal died spontaneously after 10 days of illness.

At necropsy the renal pelvis contained bilaterally a gelatinous, purulent exudate (purulent papillitis) (Fig. 1). The wall of the urinary bladder was thickened containing a small amount of purulent exudate. Calculi formation or urinary obstruction were not present. The liver showed an enhanced lobular pattern and hard consistency.

Representative samples from the major organs were fixed in 10% neutral buffered formalin and then were embedded in paraffin, sectioned at 5 μm thickness, and stained with hematoxylin and eosin (H&E). Selected tissue sections were also stained with Brown-Brenn's (B-B), and Masson's Trichrome.

Tissue samples from the liver, kidneys and lungs were also analyzed microbiologically. For the isolation of bacteria, 5.0% sheep blood was added to the blood agar. Incubation was performed at 37 °C for 24-48 h in an aerobic and 5% CO_2 environment. There was no growth on agar plates from the liver and lung samples. There was growth in isolation from kidney samples only. Gram staining was performed for the suspected colonies, and catalase and oxidase activities were measured.

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, we used the Kirby-Bauer disk diffusion method to assess the antibiotic susceptibility. Bacterial suspensions were prepared according to 0.5 McFarland standards by using sterile 0.9% saline and inoculating in a medium consisting of 5.0% defibrinated horse blood agar and Mueller Hinton fastidious agar (MH-F) containing 20 mg/L β-NAD. Penicillin and vancomycin gradient test strips (bioMérieux, France) were incubated on plates in an atmosphere of 5% CO_2 at 35±1°C for 18±2 h.

According to the manufacturer's instructions using the QIAamp DNA mini Kit (Qiagen, Helden, Germany), bacterial DNA was extracted from the culture suspension.

We performed 16S rRNA gene PCR by using universal primers both 16S rRNA gene PCR with universal primers (primer 16-F [5'-AGGATTAGATACCCTGGTAGTCCA-3'] and primer 16-R [5'-AGGCCCGGGAACGTATTCAC-3']) and sodA PCR with degenerate primers (primer dl [5'-CCITAYICITAYGAYGCIYTIGARCC-3'] and primer d2 [5'-ARRTARTAIGCRTGYTCCCAIACRTC-3']) [2].

The temperature cycling profile included initial denaturation at 95°C for 3 min., followed by 30 cycles at 95°C for 30 seconds, 37°C for 60 seconds, and 72°C for 1 minute. The PCR products were checked by 2.0% agarose gel electrophoresis before purifications of amplicons. Amplicons (500 bp for 16S rRNA and 609 bp for sodA) by Qiaquick PCR purification kit (Qiagen, Hilden, Germany) were sequenced using the Bigdye Terminator V3.1 cycle sequencing kit with an automated DNA sequencing

on ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequence was submitted to blastn program located at NCBI blast server (http://blast.ncbi.nlm.nih.gov). Megablast algorithm and Nuclotide Collection (nr/nt) search set was selected. Returning hits were evaluated for query coverage and e-values.

The liver samples were analyzed for Equine Parvovirus as described earlier. The nucleic acid was extracted by using High Pure nucleic acid kit (Roche, Germany) as described by the manufacturer. At the end of the process, possible DNA was stored at -20 C until it was used.

PCR was applied to the samples as by using the primers targeting the NS gene of a protected gene region of parvoviruses in the PCR reaction. [3]. In order to detect possible viruses and to increase specificity, 1st turn PCR processes were performed with EqPV ak1 and EqPV ak2 primers in Nested PCR feature and 2nd round PCR with EqPV ak3 and EqPV ak4 primers. Then, the PCR samples were run in agarose gel and visualized under UV light.

For scanning electron microscopic (SEM) examination, we fixed the bacterial culture in 2.50% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) and then post-fixed in 1.0% Osmium Tetroxide Solution in the same buffer solution and then dehydrated in graded ethanol solutions and coated with a gold layer (10 nm). The material was examined using SEM.

Histopathologically, the renal pelvis contained fibrinopurulent exudate composed of neutrophils, fibrin, few macrophages and lymphocytes (Fig. 2). In the medulla, there were multifocal interstitial mixed cell infiltrations and scattered necrotic tubules. Round to oval, gram positive microorganisms were present in the interstitial tissue of the medulla (Fig. 3). Neutrophils contained basophilic small particles resembling bacterial fragments. Neutrophilic infiltration and rare fibrinous thrombi were detected in the glomeruli.

Microbiologically, after the incubation period, alpha-hemolytic small, pure gray colonies were present. We observed gram-positive cocci microscopically, and negative catalase and oxidase reactions. The colonies were identified as *Streptococcus infantarius sub. infantarius* (100%) by using Matrix Mediated Laser Desorption Ionization-Flight Time Mass Spectrometry (MALDI-TOF MS) (database v2.0, bioMérieux, France) system.

Roughly 40% of the hepatic parenchyma was replaced by loose fibrous connective tissue and bile duct hyperplasia. Fibrous connective tissue, carpeting around the nodules, consisted of fibroblasts, collagen fibers, arterioles, venules and bile duct proliferations. There was fibrosis around the central veins together with hemosiderin laden macrophages. Veno-occlusive or megalocytic lesions were not present.

We identified the isolate as 100% *Streptococcus infantarius* based on the GenBank and the BLAST server in NCBI. The isolate showed susceptibility to all the antibiotics tested such as vancomycin, rifampicin, ciprofloxacin, trimethoprim/sulfamethoxazole,

norfloxacin, penicillin, tobramycin, clindamycin, linezolid, levofloxacin, chloramphenicol, teicoplanin, tetracycline, gentamicin, erythromycin and micrococcin.

No parvovirus DNA was found in liver tissue by Nested PCR.

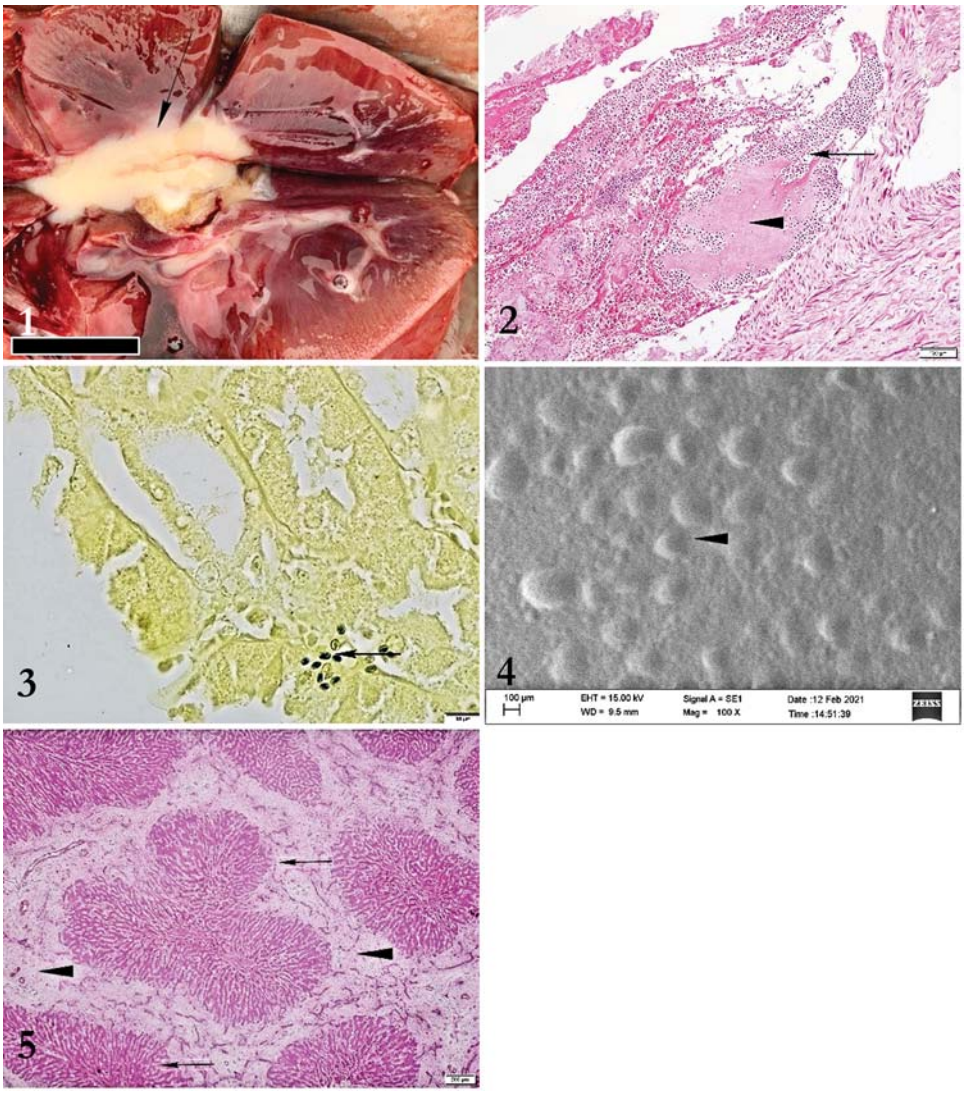

Figure 1. Fibrinopurulent exudate accumulation (arrow) in the renal pelvis of the cut surfaces (Bar:5 cm).

Figure 2. Fibrilar material (arrow head) and purulent exudate containing neutrophils (arrow), macrophages and lymphocytes.

Figure 3. Gram positive microorganism (arrow) in the renal parenchyma.

Figure 4. Scanning electronmicroscopy appearance of the microorganisms (arrow head).

Figure 5. Early cirrhotic changes characterized by regenerative nodules (arrows) and fibrosis (arrow heads)

The feeds given to horses were fresh and good quality, not moldy. In the region, only *Senecio vernalis* and *Heliotropium dolosum* and *Heliotropium circinatum* are the plants containing pyrrolizidine alkaloids, but these plants were not present in the shelter where the animals were kept. Since the DNA of the agent in question was not found in the PCR analysis for Equine parvovirus, we could not find the cause of hepatic fibrosis.

An earlier study in horses showed the increased number of *S. infantarius ssp. coli* in the large intestine might be responsible for the laminitis in oligofructose-induced laminitis model [4]. However, other than the above study, there is no knowledge about *Sii* infection in horses. Hence, we report *Sii* infection for the first time in the horse. However, the members of SBSEC are sporadically isolated in animal infections such as endocarditis, septicemia, and bacteremia; in calf [5], sea otter [6], pigeon [7], mink [8], cat [9] and turkey [10]. Urinary infections due to SBSEC are also unknown in animals, however, bacteriuria due to SBSEC is well-established in humans of underlying urinary disease with approximately (1.0%) positive urine cultures to *S. bovis*. Most of the cases (75.0%) had urinary tract infection signs; the remaining 25.0% had non-specific high fever [11].

Just as cirrhotic changes are not caused by equine parvovirus, microscopic changes are not compatible with pyrrolizidine alkaloid poisoning or cardiac cirrhosis. However, hepatic microscopic changes might be due to aflatoxicosis.

In humans, *Sii* infections are closely associated with intestinal malignant tumors, however, no neoplasia is present in this report. In a retrospective study of humans; the association of colonic and noncolonic tumors with *S. infantarius* was 11.0% (3 of 28) and 57.0% (16 of 28), respectively [12]. The theories for SBSEC-intestinal malign tumor interrelation include; 1. Pro-inflammatory activity of SBSEC, 2. The intestinal tumor microenvironment is suitable for the growth of Sii , 3.both are operative [13].

Taken together collectively, this report demonstrated that Sii can cause renal lesions in horses, however the agent is very sensitive to antibiotics. Since the agent had been detected in fermented milk products before, its pathogenicity was questioned for many years. In the presented case, the cause-lesion relationship was clearly demonstrated. Parallel to our case, Sii was also recognized as an uncommon pathogen in humans. Further as stated in a previous study, the agent is not quite innocent and can cause laminitis lesions in horses if it is found in the digestive system. The presented information might highlight our knowledge in Sii infection in horses, however, the gap of knowledge should be filled such as incidence, pathogenesis, contamination and potential reservoirs of Sii.

Authors' contributions

BO, ST, ZY, OT carried out the molecular microbiologic and virologic studies, participated in the sequence alignment. CAI, BK, HE and YE participated in histopathologic studies. HE, YE and CAI made electron microscopic analysis. YE

participated in its design and coordination and wrote to draft the manuscript. BK conducted necropsy and submitted the manuscript. All authors read and approved the final 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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PURULENTNI RENALNI PAPILITIS KOD JEDNOG KONJA USLED PRISUSTVA *STREPTOCOCCUS INFANTARIUS SUBSP. INFANTARIUS*

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Pastuv Arapske rase, starosti 6 godina, je obdukovan nakon desetodnevnog gubitka apetita, slabosti i gubitka telesne težine. Patoanatomski i patohistološki nalaz odgovarao je nalazu purulentnog papilitisa usled prisustva *Streptococcus infantarius subsp. infantarius*. Izolovani mikroorganizam je bio rezistentan na sve testirane antibiotike.