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Moelerella wisconsensis: first isolation from lungs and spleen of a horse infected with *Streptococcus dysgalactia* subsp. *equisimilis*

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Abstract

Moellerella wisconsensis is a Gram-negative, facultative anaerobic bacillus of *Enterobacteriaceae* family, and it is an uncommon pathogen in domestic animals. To date, five cases were reported including two dogs, two cattle, and a goat. *Streptococcus equisimilis* is the second common bacterial agent after the *S. equi* subsp*. zooepidemicus* in equine pneumonia cases. The present report describes the isolation of *M. wisconses* from lungs and spleen of a 10-year-old Arabian horse (May 08, 2022) at post-mortem examination being co-infected with *S. equisimilis.* Clinical and pathological findings included bilateral nasal discharge, conjunctivitis, sternal recumbency, severe diffuse necrosuppurative rhinitis, multi-focal fibrinopurulent pneumonia and purulent lymphadenitis. Polymerase chain reaction assays showed no viral nucleic acids of equid alphaherpesvirus (EHV) 1, EHV-4, equine arteritis virus and equine papilloma virus. The antibiogram test revealed that the isolate was sensitive to several antibiotics except colistin. Taken together, the present report documents the first isolation of *M. wisconsensis* from lungs and spleen of a horse; hence, experimental studies are needed to clarify the pathogenity and pathogenesis of *M. wisconsensis*.

Introduction

Moellerella wisconsensis is a Gram-negative, facultative anaerobic bacillus of *Enterobacteriaceae* family. The genus *Moellerella* was first documented in stool samples of humans with diarrhea in Wisconsin, United States.[1] Following this report, the bacterium has been reportedly isolated from human clinical specimens in diverse geographical locations.[1-3] The *M. wisconsensis* is also an uncommon pathogen in domestic animals. To date, five case reports are available in the literature review including two dogs (vaginal discharge and urine), two cattle, (gastroenteritis and abortive material), and a goat (pneumonia).[3-5] However, this bacterium is reportedly common in the feces of wild animals including birds, foxes, mustalids, wolves, and raptors.[5-8] Further-more, *M. wiscosensis* was isolated from 0.40% of wild birds and it has been suggested that wild birds might play a role in dissemination of this pathogen. The infrequency of the reports of this microorganism may be due to its mis-diagnosis as *Escherichia coli* or *Klebsiella pneumoniae* subsp. ozaenae.[4] Almost 4 decades after the first detection, the potential role of *M. wisconsensis* in animals infections and its clinical significance have not yet been clearly elucidated.

Equine streptococal infections are well-recognised diseases caused by *Streptococcus equi* subsp. *zooepidemicus, Streptococcus dysgalactia* subsp. *equisimilis,* and *S. equi* subsp*. equi*. The *S. equisimilis* is the second common bacterial agent after *Streptococcus equi* subsp*. zooepidemicus* in equine pneumonia cases.[9] This micro-organism is present in skin and mucous membranes as a commensel; though, it is also responsible for strangles-like disease.[10] It is an opportunistic pathogen for humans and animal species including horses, dogs, and pigs.

The present report describes the isolation of *M. wisconses* from lungs and spleen of a 10-year-old Arabian horse being co-infected with*S. equisimilis.*

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Case Description

A 10-year-old male Arabian horse was brought to the Department of Pathology, Faculty of Veterinary Medicine, Firat University, Elazığ, Türkiye, for post-mortem examinations. According to history, the animal showed lack of appetite, fatigue, high body temperature and nasal discharge for 2 days. Despite the treatment with antibiotics and intravenous fluid administration, the horse showed sternal recumbency and it was found dead in the morning after the second dose antibiotic therapy. The medicinal treatment included intramuscular injection of 500 mg per 200 kg cefazolin (Tum Ekip pharmaceuticals, Istanbul, Türkiye), 1.00 mL per 15.00 kg of Ba-Sülfa (sulfadimidin sodium+trimetophrim; Bavet Pharmaceuticals Istanbul, Türkiye) containing 215.80 mg of sulfadimidine sodium, along with 40.00 mg of trimethoprim per mL. Meloxicam (Bavet Pharmaceuticals) as 0.50 mg kg^{-1} was administered subcutaneously. Lastly, isotonic sodium chloride solution (Polifarma, Istanbul, Türkiye) was administered as a 2,000 mL intravenous infusion. Grossly, body condition of the horse was thin with inadequate body fat stores. The lungs were consolidated, meaty, and contained diffuse petechial hemorrhages together with multi-focal white, round, firm, and pin-point micro-abscess (Fig. 1). Nasal mucosa showed diffuse hyperemia, roughing, and contained mucopurulent exudate. Tracheobronchial lymph nodes were swollen and congested. A Yellow, gel-like material approximately 200 mL, had filled the heart ventricles, atria and large vessels.

Fig. 1. Gross changes in horse lungs. White pin-point abscesses (arrowheads) and petechial hemorrhages (arrows) in lung cut section from diaphragmatic lobe, (bar = 5.00 cm).

Histopathological findings. Based on Hematoxylin and Eosin staining (H&E), approximately 80.00% of the lung parenchyma was composed of inflammatory infiltration, necrotic debris, fibrin, edema fluid and hemorrhagic foci. The main histopathological lesion was sub-acute to chronic fibrinosuppurative pneumonia characterized by fibrin deposits and neutrophilic infiltrations (Fig. 2A). A significant portion of the neutrophils showed karyorrhexis. The vasculitis characterized by peri-vascular edema and infiltrations were also observed (Fig. 2B). Multi-focal hemorrhages were detected in alveolar lumens and septa. Partial or complete thrombi were formed in the pulmonary, nasal and tracheal mucosae (Fig. 2C). Gram staining showed intra-lesional Gram-positive coccoid microorganisms phagocytized by neutrophils (Fig. 2D) and Gram-negative bacilli. Bronchial and bronchiolar lumens contained necrotic epithelial cells, inflammatory infiltrate, epithelial hyperplasia and edema fluid. Peri-bronchial fibrosis was also present. Pleural suface was effaced by granulation tissue composed of fibroblast and capillaries. Focal splenic micro-abscess and sinus histiocytosis were present in spleen. Nasal turbinates had focal epithelial necrosis and diffuse leukocytic infiltration throughout the mucosa. Bronchial lymph nodes had multi-focally distributed micro-abscess, sub-capsular edema and multi-focally distributed hemosiderin-laden macrophages.

Microbiological analyses. The culture of lungs and spleen on 5.00% sheep blood medium at 37.00 ± 1.00 ˚C yielded rapidly growing colonies. Gram staining of colonies showed Gram-negative bacilli and Gram-positive chain-reproducing bacteria were detected. Subsequently, the bacteria were identified as *M. wisconsensis* with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, database v2.0; bioMérieux, Craponne, France) system. A pure culture of the suspected bacteria was obtained. The MALDI-TOF MS analysis identified the isolate as *Streptococcus dysgalactiae* subsp. *equisimilis*. According to the European Committee on Antimicrobial Susceptibility Testing guidelines, Kirby-Bauer disk diffusion method was used. Bacterial suspensions were prepared according to 0.50 McFarland standards using sterile 0.90 % saline and inoculated in a medium consisting of Mueller Hinton agar. Using the EZ1 tissue kit (Qiagen, Hilden, Germany), bacterial DNA was extracted from the culture suspension. The *16S rRNA* gene polymerase chain reaction (PCR) was performed using universal primers (16-F [5′-AGGATTAGATACCCTGGTAG TCCA-3′] and 16-R [5′-AGGCCCGGGAACGTATTCAC-3′]). The temperature cycling profile included initial denaturation at 95.00 ˚C for 3 min, followed by 30 cycles at 95.00 ˚C for 30 sec, 37.00 ˚C for 60 sec and 72.00 ˚C for 1 min.[11] The PCR products were analyzed by gel electrophoresis before purifications of amplicons. The amplicons from the Qiaquick PCR Purification Kit (Qiagen) were sequenced using the Bigdye Terminator V3.1 cycle sequencing kit with an automated DNA sequencing machine (Applied Biosystems, Foster City, USA). The sequence was submitted to the BLASTN program located at NCBI BLAST server.[12]

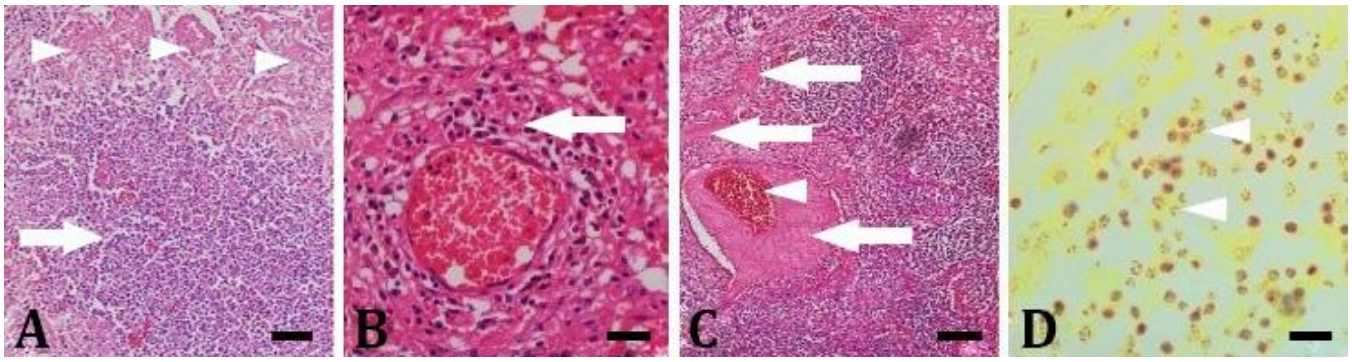

Fig. 1. A) Intra-alveolar fibrin accumulations (arrowheads) and abscess formation (arrow), (H&E staining; bar = 50.00 µm), **B)** Peri-vascular lymphocytic infiltration (arrow) and fibrosis, (H&E staining; bar = 20.00 µm), **C)** Thrombus formation in capillaries (arrows) and a vein with canalization (arrowhead), (H&E staining; bar = 50.00 µm), and **D)** Gram-positive coccoid microorganisms in neutrophils (Gram staining; bar = 10.00 µm).

MegaBLAST algorithm and nucleotide collection (nr/nt) search set were selected. Returning hits were evaluated for query coverage and e-values. The result of antibiogram revealed that the isolate was sensitive to the antibiotics including amoxicillin clavulanate, gentamicin, amikacin, ciprofloxacin, imipenem, meropenem, piperacillin-tazobactam, ceftriaxone, ceftazidime, cefepime and trimethoprim-sulfamethoxazole. However, it was resistant to colistin.

Virological assesment. Lungs, liver, spleen and nasal samples were analyzed for equid alphaherpesvirus (EHV) 1, EHV-4, equine arteritis virus (EAV) and equine papilloma virus nucleic acids by PCR. Extractions were performed for the presence of viral nucleic acid in the tissue samples. For this purpose, GF-1 Nucleic Acid Extraction Kits (Vivantis, Shah Alam, Malaysia) was used as described by the manufacturer. After extraction, reverse transcriptase enzyme was used to convert the RNA genome (for EAV infection) into DNA. For this purpose, the first strand cDNA synthesis kit (Thermo scientific, USA) was used, and the genome was translated into complementary DNA. For primers and PCR optimization, conditions were the same as earlier studies.[13-15] The products formed after PCR were run on agarose gel. At the end of the study, the nucleic acid of the viral agents was not found in any of the samples.

Discussion

The *S. equisimilis* is responsible for equine respiratory disease causing strangles-like disease and also respiratory diseases in pigs and humans.[9,10,16] However, *M. wisconsenses* isolated for the first time from a horse in the present report. Similar to the clinical background, *M. wisconsis* was reportedly isolated from a sputum sample in a human with bronchitis.[2]

Since both *S. equisimilis* and *M. wiscosensis* are opportunistic pathogens, their co-isolation in the present case suggests possible immunosuppression in the horse.

Taken together, *M. wisconsensis* might be regarded as a rare pathogen for horses. However, this findings must be taken with suspicion until being proven by epidemiological and experimental studies.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this study.

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