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Research Article

An Outbreak of Respiratory Infection in Horses due to Antibiotic Resistant *Streptococcus equi subsp. Zooepidemicus* in Greece

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Abstract

An outbreak of respiratory disease occurred in a herd of 57 horses in Greece in late November 2018. Clinical signs were adequate to respiratory infection. One horse at the age of 7 years that died due to pneumonia and one horse at the age of 9 years that had a ruptured submandibular abscess were sampled for bacteriological analysis by the lung and an abscess sample respectively. From the specimens *S. zooepidemicus* was isolated in pure culture. The *S. zooepidemicus* isolates showed resistance to trimethoprim-sulfamethoxazole, and tetracycline attributed to the carriage sulI, and tetO genes. Pneumonic disease was also confirmed by histopathological examination of lung specimens.

Keywords: *S. zooepidemicus; Horse; Trimethoprim-Sulfamethoxazole; Tetracycline*

Introduction

Streptococcus equi subsp. *zooepidemicus* (*S. zooepidemicus*), is a beta-hemolytic Lancefield group C bacterium, that colonizes the oral cavity, the respiratory tract, and the reproductive tract of horses. Additionally *S. zooepidemicus* is also considered as an opportunistic pathogen in horses isolated from pneumonic infections as well as from strangles [1]. As implied by the specific name "zooepidemicus" this bacterium is not host-adapted, and the spread of resistant isolates represents a public health concern [2]. Based on the knowledge of its predicted susceptibilities, co-trimoxazole and tetracycline are broadly used against *S. zooepidemicus* infections [3]. The use of antimicrobials, however, may result in the selection of resistant microbial isolates. The clinical, laboratory and microscopic findings that are indicative of respiratory infection in horses caused by *S. zooepidemicus* resistant to trimethoprim and sulfamethoxazole and tetracycline are reported in the current study.

Clinical history

An outbreak of respiratory disease occurred in a herd of 57 horses kept inside barns in Edessa region (North West Greece) in late November 2018 and lasted approximately five weeks. Clinical signs were: Decrease in appetite, elevated temperature (38.5 - 39.5°C), serous nasal discharge, lymphadenopathy in the neck region and cough. One horse died due to pneumonia at the age of 7 years along with another 9-years-old horse which had a ruptured submandibular abscess, were sampled for bacteriological analysis by the lung and an abscess sample respectively. The samples were refrigerated and transferred in insulated boxes containing coolants to the laboratory of microbiology and infectious diseases located at the Faculty of Veterinary Medicine of Aristotle University, Thessaloniki within 4 h of collection.

Microbiological examination

Upon arrival to the laboratory, the samples were cultured onto Columbia Agar with Sheep Blood Plus (CASBP, Oxoid Deutschland GmbH, and Wesel, Germany) and incubated at 37°C. After 48 h of incubation, plates were examined for microbial growth. Ten b-hemolytic colonies, 5 per cultured sample, were selected for the following analysis: species identification with polymerase chain reaction (PCR) [4] and antimicrobial susceptibility testing via a disk diffusion method [5]. Furthermore the total DNA of the selected isolates, was analyzed by PCR for the presence of sulI-II and -III [6] and and tetA-B-C and -O resistance genes [7]. The resultant PCR amplicons were sequenced on a genetic analyzer (ABI PRISMH 3100, Applied Biosystems, Foster City, CA) by using Rhodamine-labeled terminators.

Histological examination

Specimens of the lung were fixed in 10% neutral buffered formalin, embedded in paraffin wax, and cut in 5-µm-thick sections for subsequent hematoxylin and eosin staining [8].

Results

Microbiology

After 48 hr of incubation, mucoid colonies with a wide zone of b-hemolysis were obtained in pure culture from the cultured specimens. Ten bacterial isolates (5 per cultures sample), randomly selected from the incubated plates were identified as *S. zooepidemicus* by PCR. Additionally, the isolates showed resistance to trimethoprim-sulfamethoxazole, and tetracycline while they were found sensitive to levofloxacin, vancomycin, erythromycin, clindamycin, linezolid, and chloramphenicol. Finally, all isolates were found to carry sulI, and tetO genes, which encode resistance to co-trimoxazole, and tetracycline, respectively.

Histology

Histological examination of hematoxylin and eosin-stained sections revealed generalized, severe, chronic, broncho-interstitial pneumonia (Figure 1). Alveolar septa were expanded up to 10 times normal by a cellular infiltrate composed of macrophages, neutrophils and lymphocytes, with increased amounts of smooth muscle. Multifocally, there was a severe peribronchiolar and perivascular fibrosis and severe hypertrophy of the vascular wall.

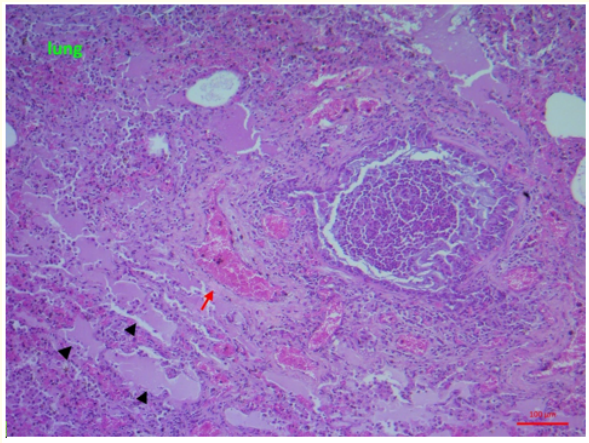

Figure 1*: Histological examination of hematoxylin and eosin-stained sections of lung.*
Alveolar septa expanded up to 10 times normal by a cellular infiltrate composed of macrophages, neutrophils and lymphocytes, with increased amounts of smooth muscle (black arrows). Multifocally, there is severe peribronchiolar and perivascular fibrosis and severe hypertrophy of the vascular wall (red arrow).

Antibiotic	EUCAST break-point (mm)	Isolate.1 Zone (mm)	Isolate.2 Zone (mm)	Isolate.3 Zone (mm)	Isolate.4 Zone (mm)	Isolate.5 Zone (mm)	Isolate.6 Zone (mm)	Isolate.7 Zone (mm)	Isolate.8 Zone (mm)	Isolate.9 Zone (mm)	Isolate.10 Zone (mm)
Trimethoprim-sulfamethox-azole	15	12	0	0	0	10	10	0	0	12	0
Tetracycline	20	10	0	0	12	0	0	0	0	10	0
Levofloxacin	17	22	23	26	21	28	28	26	24	22	26
Vancomycin	13	28	28	24	32	26	34	28	33	28	32
Erythromycin	18	23	28	29	33	27	21	32	27	29	30
Clindamycin	17	32	26	29	31	33	28	33	30	27	32
Linezolid	16	33	32	28	26	34	29	31	34	32	30
Chloramphenicol	19	28	27	22	29	26	27	29	26	28	25

Table: *Antibiotic resistance raw data.*

Zone diameters lower than the breakpoint are marked in red.

Discussion

The importance of *S. zooepidemicus* in veterinary specimens is not easy to determinate because it is difficult to distinguish colonization from primary infection. A recent study established that the isolation of Streptococci from submandibular abscesses is an important finding [9]. The associated infection is mainly based on the elaboration of exposed surface proteins such as SzPSe, the hyaluronic acid capsule, the antiphagocytic SeM protein, and Mac protein [10]. In contrast, in a later report [11]. *S. zooepidemicus* was isolated from mucus membranes of healthy horses. According to results from the present study, *S. zooepidemicus* was the causal agent of the respiratory infections in the examined horses and not a contaminant of the respiratory tract. This statement is supported in 2 ways. First, the clinical examination indicated that the animals were suffering from pneumonic disease, as observed by the clinical signs. Second, the results of microbiologic and histopathologic examination confirmed that the pneumonic disease was associated with *S. zooepidemicus* in pure culture. The generalized, severe, chronic, broncho-interstitial pneumonia was undoubtedly the result of the bacterial infection.

In the present outbreak, it is possible that a single strain of *S. zooepidemicus* appeared to have selective pathogenic potential. This is supported by the identical results of the antibiotic sensitivity test and the resistome analysis that the examined isolates shared. Our study is limited to the point that no investigation for the presence of superantigen genes of *S. zooepidemicus* that is associated to respiratory infections were performed [12]. Although our results indicate a pathogenic potential of the strain of *S. zooepidemicus*, more studies are needed to identify the mechanisms that influence the infection of the respiratory track of the horses.

The global emergence of multiple antibiotic-resistant isolates of *S. zooepidemicus* has been previously reported [13,14]. In the current study, the *S. zooepidemicus* isolates showed resistance to trimethoprim-sulfamethoxazole, and tetracycline attributed to the presence of sulI, and tetO genes, respectively. Because co-trimoxazole, and tetracycline are recommended for the treatment of pneumonic infections in horses resistance, patterns of the type found in the current study are significant in terms of therapy [9]. Moreover, zoonotic transmission of *S. zooepidemicus* through contact with horses is well documented [15]. In respect to this statement the presence of antibiotic resistance of *S. zooepidemicus* clones can be considered as an important issue for public health.

Conclusion

In conclusion antibiotic resistant strains of *S. zooepidemicus* should be considered as potential etiologic agents of pneumonic infection in horses in Greece. Early investigation is important to avoid serious complications and it could be life saving.

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