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Endometritis associated with *Enterococcus casseliflavus* in a mare: A case report

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ABSTRACT

Infectious endometritis is one of the main causes of subfertility/infertility in the mare. In this report, we present the first case of endometritis in mare associated with a strain of *Enterococcus casseliflavus*, an unusual gram-positive bacterium which can also be a zoonotic agent. Furthermore, the isolated strain showed a worrying multidrug-resistant profile. The accurate finding of a successful antimicrobial treatment and consequently, the pregnancy diagnosis indicate the importance to isolate, identify and define the antibiotic resistance profile of bacteria associated with endometritis.

1. Introduction

In mares, the most common indications associated with reproduction are endometritis, retained placenta and placentitis. Endometritis, a major cause of mare infertility, is generally a chronic infection of the uterine endometrium often undiagnosed [1]. Bacterial endometritis represents one of the most important reproductive diseases in mares causing considerable economic loss. *Streptococcus* group C, in particular *Streptococcus zooepidemicus*, and *Escherichia coli* are the most frequently bacterial strains associated with fertility problems [2]. However, other bacterial strains can be cause of uterine infections, for this reason it is important to make a correct diagnosis so as to implement a proper epidemiological approach to the investigation of bacterial causes of endometritis.

The possibility of resolution of an infection and, consequently, the pregnancy rate increase in mares, is the primary objective of veterinarians. Thereby, the knowledge of the appropriate antimicrobial drug is needed to perform an efficacious therapy [3].

The aim of this case report is to describe an unusual case of endometritis caused by a multidrug-resistant *Enterococcus*

casseliflavus (*E. casseliflavus*) strain which was resolved by a successful therapeutic treatment.

2. Case report

An 8 years old Standardbred mare was presented with a reproductive history of infertility. Two years ago (May 2014), it delivered its first foal. Two days after the foaling, the mare was examined due to the presence of swollen vulva and purulent discharge. A peri-vaginal large hematoma, vaginal contusions and retained placenta were diagnosed at the clinical and ultrasonographical examination. Uterine lavages with sterile isotonic saline were performed for three days, until the recovered fluid appeared clean. Benzyl procaine penicillin/dihydrostreptomycin (Gellipen pronto[®], MSD Animal Health Srl, Italy), 22 000 UI/kg IM once a day for 7 days, and flunixin meglumine (Flunixin 5%[®], Vétoquinol Italia Srl, Italy), 1.0 mg/kg IV once a day for 3 days, were administered. Oxytocin (Syntocin[®], Zoetis Italia Srl, Italy), 10 UI I.M. four times daily was administered as well, to stimulate uterine contractions and uterine drainage. Vagina was medicated with Formosulfathiazole paste (Socatil[®], ACME Srl., Italy) for 7 days. Since then, the mare had not conceived, despite repeated inseminations and several empirical treatments, for two subsequent breeding seasons. A Caslick's operation had been performed. Bacteriological and mycological cultures by uterine swabs were negative whereas cytological examination by cytobrush revealed a fair number of PMNs.

On May 2016, at the clinical examination the mare was in good body condition. It was cyclic, but diestrus resulted

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shortened. At transrectal ultrasonography, during the estrus, excessive uterine edema was evident, uterine folds were significantly thickened with hyperechoic borders and hypoechoic centers. Free fluid was detected in some areas.

At the beginning of the estrus, samples were collected by endometrial guarded cytobrush technique. Cytological examination of the sample, stained and examined under microscopy, showed presence of PMNs and absence of bacteria. For the bacteriological examination we processed the uterine brush sample that was inoculated in enrichment broth, Brain Heart Infusion (BHI) medium (Liofilchem srl, Italy), and then, after 24 h of incubation at 37 °C, on different agar plates (blood agar, MacConkey agar, Mannitol salt agar, Sabouraud Dextrose agar from Liofilchem srl, Italy) and incubated for further 24 h at 37 °C.

The isolated bacteria, detected in monoculture, were subjected to MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) (Bruker MALDI Biotyper) analysis and a diagnosis of *E. casseliflavus*-associated endometritis were made.

The sequencing of 16S *rRNA* gene, conducted using genetic analyzer (ABI Prism 3130 Genetic Analyzer), confirmed the MALDI-TOF identification, showing a matching score of 100% with *E. casseliflavus* (ATCC 49996).

Antimicrobial resistance testing, performed by disc diffusion method on Mueller Hinton agar plates, showed a multidrug-resistant profile; precisely it was resistant or intermediate to 18/23 of the tested antibiotics (Table 1). Moreover, the isolated strain was not a biofilm producer as tested by crystal violet method.

At the beginning of the subsequent estrus, intrauterine treatment was started with a mucolytic agent, acetylcysteine, (15 mL of a solution at 20%, Equimucil[®], ACME Srl, Italy), which was administered in uterus and flushed out after 1 h. On the basis of the bacteriological investigation, daily intrauterine infusions with Procaine Benzylpenicillin (Depocillina[®], MSD Animal Health Srl, Italy), 4.5 g in 100 mL isotonic sterile saline

during the estrus cycle, 2 days pre-, and 3 days after artificial insemination with cooled semen, were performed. Oxytocin was also given to facilitate uterine emptying. Due to the presence of a concomitant traumatic corneal ulcer, Gentamycin (Agent[®], FATRO SpA, Italy), 7 mg/kg I.V. SID for 7 days, and flunixin meglumine (Flunixin 5%[®], Vétquinol Italia Srl, Italy), 1.0 mg/kg IV SID for 5 days, were also administered. The mare resulted pregnant 14 days after the insemination. Pregnancy was confirmed at 25, 40 and 60 days post-ovulation.

3. Discussion

This study suggests that mares with clinical and, overall, subclinical endometritis require an accurate diagnosis. Sometimes isolation of pathogens is not associated with positive cytological findings or *vice versa*. Furthermore, false negative results can be associated with inadequate sampling. The use of cytobrush to improve the diagnosis of subclinical endometritis in the mare compared to the uterine swab alone is recommended [4,5]. More recently, it was demonstrated that microbiological and cytological results obtained from cytobrush are similar to those obtained from endometrial biopsy [6]. Thus, the combination of cytological and bacteriological examinations of a sample obtained with cytobrush leads to a higher sensitivity in diagnosing endometritis [6].

In our case, a correct diagnosis performing cytobrush technique for both cytological and bacteriological examination was obtained. We also performed the bacterial investigation with a first step in liquid enrichment media and this permitted the growth of a gram-positive bacteria. Instead we obtained no bacterial growth with direct seeding on agar media; this is to emphasize the need in some cases to use a highly nutritious general-purpose growth medium as Brain Heart Infusion Broth. The identification of *E. casseliflavus* by MALDI-TOF MS and 16S *rRNA* sequencing, which are emerging technologies for microbial identification [7,8], was obtained. Furthermore, this strain exhibited an alarming antimicrobial resistance profile (78%). Generally, the multidrug-resistant bacteria have become a serious health public problem, but there are few studies on the development of antibiotic resistance of bacteria in horses [2,3,9].

We noticed that the concomitant local intrauterine and the systemic therapy resulted to be successful. Previously, Dascanio [10] suggested that the systemic antibiotic administration represents a better choice in chronically infected mares. However, it is worth remembering that the use of systemic antimicrobials leads to changes in the intestinal microbiota [11], which has a key role in the maintenance of health and on the pathophysiology of several diseases.

Moreover, successful treatment of persistent mating induced endometritis with a single dose of dexamethasone or with non-steroidal anti-inflammatory drugs administered at the time of insemination has been reported in mares [12]. On the other hand, the treatment with high doses of flunixin meglumine has been associated to a higher incidence of ovulatory failures. However, it was shown that the administration of a therapeutic single dose of flunixin meglumine at the insemination time reduces the number of neutrophils in endometrial cytology samples and does not affect the incidence of ovulation failure [13]. In this case, the treatment with a non-steroidal anti-inflammatory drug may have been effective in modulating post insemination endometritis without detrimental impact on the ovulation.

Table 1

Antibiotic susceptibility testing results.

Antibiotic classes	Tested antibiotics	Doses	S/I/R ^a
Aminoglycosides	Amikacin	30 µg	R
	Gentamycin	10 µg	S
	Kanamycin	30 µg	R
	Neomycin	30 µg	R
	Streptomycin	10 µg	R
Carbapenems	Imipenem	10 µg	R
	Meropenem	10 µg	R
Cephalosporins (third generation)	Ceftiofur	30 µg	R
	Ceftriaxone	30 µg	R
Fluoroquinolones	Ciprofloxacin	5 µg	I
	Enrofloxacin	5 µg	R
	Norfloxacin	10 µg	I
Glycopeptides	Vancomycin	30 µg	S
Lincosamides	Clindamycin	2 µg	R
Macrolides	Erythromycin	15 µg	R
Oxazolidinones	Linezolid	30 µg	S
Penicillins	Amoxicillin-clavulanic acid	30 µg	I
	Ampicillin	10 µg	R
	Penicillin	10 UI	S
Polymyxins	Colistin sulfate	10 µg	R
Rifamycins	Rifampicin	30 µg	R
Sulfonamides	Trimethoprim-sulfamethoxazole	25 µg	R
Tetracyclines	Tetracycline	30 µg	S

^a S: susceptible; I: intermediate; R: resistant.

Enterococci have emerged as increasingly important nosocomial and community-acquired pathogens. The *E. casseliflavus* zoonotic potential has been described in a case of endophthalmitis associated with a human's injury by a horse's tail [14]. And recently, a vancomycin-resistant *E. casseliflavus* was identified in a foal with septic meningitis [15].

To our knowledge, this is the first case of endometritis in a mare associated with a multidrug-resistant *E. casseliflavus* in Italy. Moreover, this report underlines the importance of a correct diagnostic procedure in order to reach an accurate diagnosis and an appropriate therapy.

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Conflict of interest statement

The authors declare that no competing interests exist.

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