



## SHORT COMMUNICATION

### Endometritis in Donkeys Associated with *Streptococcus equi* subspecies *zooepidemicus* Infection

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#### ABSTRACT

Endometritis has recently become one of the most important infectious diseases in donkey breeding farms in China. However, so far, no research has been conducted to confirm the causative agents. In the present study, one breeding farm was selected as a research model for isolation and identification of causative agents and epidemiological investigation. *Streptococcus equi* subspecies *zooepidemicus* was isolated from the uterus of symptomatic and asymptomatic donkeys and identified by PCR and MALDI-TOF MS analysis. On the farm, the infection rate was 57.1%, which indicated that endometritis is a severe problem and needs to pay much attention to donkey breeding farms. Moreover, female donkeys without mating experience were all negative which suggested the transmitting route might be artificial insemination or related handlings. This study will lead to a great understanding of the causative agent and epidemiological characteristics for endometritis in intensive donkey breeding farms.

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#### INTRODUCTION

*Streptococcus equi* subspecies *zooepidemicus* (*S. zooepidemicus*), one of the two subspecies of *Streptococcus equi*, is part of the normal bacterial flora in Equidae, where it acts as an opportunistic pathogen that can cause disease in the upper respiratory tract, uterus, and umbilicus (Lindmark *et al.*, 2001). Meanwhile, it can infect a wide range of other mammals such as cows, rabbits, swine and sheep, and occasionally infect humans by contacting with horses or consumption of unpasteurized dairy products (Kuusi *et al.*, 2006). Since *S. zooepidemicus* can cause respiratory diseases, mastitis, septicemia, fibrinous pericarditis and pleuritis in animals (Lindmark *et al.*, 2001; Skive *et al.*, 2017), it has formed a considerable threat to animal welfare and caused significant economic losses in the animal industry worldwide.

Though it has been mentioned that *S. zooepidemicus* can cause endometritis in horses (Lindmark *et al.*, 2001), so far no case has been reported in donkeys. From 2014, the number of intensive donkey breeding farms in China has risen along with the rising consumer demand. Although

this new rearing form improves the economic benefits, it also brings many problems due to the increasing density of donkeys, such as infectious disease. During the last two years, endometritis has become a severe problem in some intensive donkey farms where biosecurity precautions were inadequate. However, so far, no information has been reported for the causative agents and epidemiological investigation. In the present study, we successfully isolated and identified *S. zooepidemicus* associated with endometritis in donkeys under the intensive rearing farm.

#### MATERIALS AND METHODS

**Clinical signs and sample collection:** The purulent vaginal discharge samples were collected from symptomatic donkey mares (Fig.1a) and genital swab samples were collected from female donkeys without clinical signs in one intensive breeding farm which has been obsessed with endometritis from the early of 2019. All collected samples were transported to the laboratory on dry ice and proceed to DNA extraction and bacterial culture in 24 hours.

**DNA extraction and PCR diagnosis:** DNA was directly extracted from the vaginal discharge and swab samples utilizing the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Then PCR assays were performed to detect *S. zooepidemicus* (Cordoni *et al.*, 2015) and screen *Taylorella* spp (Erdman *et al.*, 2011). The PCR products were randomly selected for purification and sequencing to confirm the result.

**Bacterial isolation and identification:** Preliminary bacterial identification was conducted on samples collected from symptomatic donkeys using methylene blue staining. Bacterial isolation and  $\beta$ -hemolytic tests were carried out using 5% blood agar (Hopebio, Qingdao, China). Isolated colonies with  $\beta$ -hemolysis were also examined by Gram staining. Identification of bacterial isolates was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker, Bremen, Germany) (Patel, 2015).

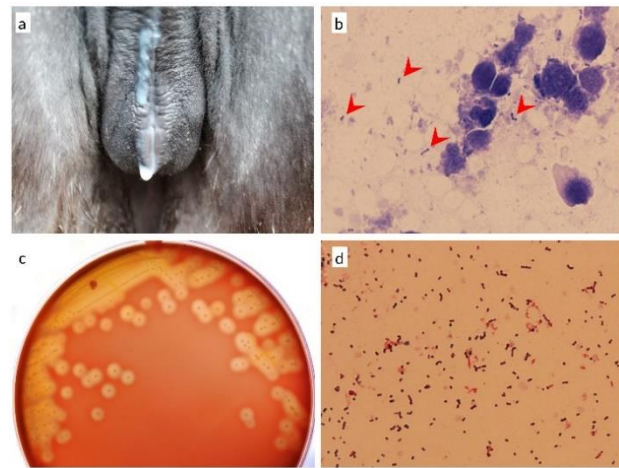
**Antimicrobial sensitivity test and treatment:** The drug sensitivity test was conducted on the isolated bacteria with a slip diffusion method in which antimicrobial agents were selected according to Gram staining results. Then treatment measures were developed and implemented based on the drug sensitivity test.

## RESULTS

**Clinical signs and prevalence:** From the March of 2019, one intensive breeding farm located in the middle east of China was obsessed with endometritis. There were 113 female donkeys on the farm, consisting of 84 female donkeys while 29 female donkeys were under two and a half years old without mating experience. Based on clinical observation, 17 of 84 (20.2%) mares exhibited milky and purulent vaginal discharge (Fig.1a) along with repeat breeding. The remaining 67 donkey mares and unbred female donkeys (n=29) did not show any clinical signs of estrous/endometritis.

**Molecular diagnosis:** PCR results showed all 17 symptomatic donkey mares and 31 of 67 (46.3%) donkeys without clinical signs were positive for *S. zooepidemicus* infection. Based on the PCR diagnosis results, the infection rate is 57.1% in donkey mares at this farm. Besides no positive donkey was detected in 29 unbred female donkeys, and no donkey showed positive for *Taylorella* spp. infection.

**Histochemical staining and isolation:** Chains of *streptococcus* were observed microscopically (Fig. 1b) in the result of Methylene blue stain. Meanwhile,  $\beta$ -hemolytic colonies were observed (Fig. 1c) and also Gram-positive as indicated by Gram staining (Fig. 1d) for all 17 samples of purulent vaginal discharge collected from symptomatic donkey mares. For swab samples collected from donkey mares without clinical signs,  $\beta$ -hemolytic colonies were observed in 14 of 67 (20.9%) samples collected from donkeys without clinical signs, and all 14 samples were positive in PCR diagnosis. Meanwhile, no  $\beta$ -hemolytic colonies observed in the samples collected from unbred donkeys.



**Fig. 1:** Clinical signs, isolation, and microscopic examination. (a) Milky and purulent vaginal discharge; (b) Methylene blue staining: Cocci arranged in chains (arrow); (c)  $\beta$ -hemolytic colonies of *S. zooepidemicus* on blood agar; (d) Gram staining: isolated bacteria showed Gram-positive cocci. b and d = Oil lens - 1000 $\times$

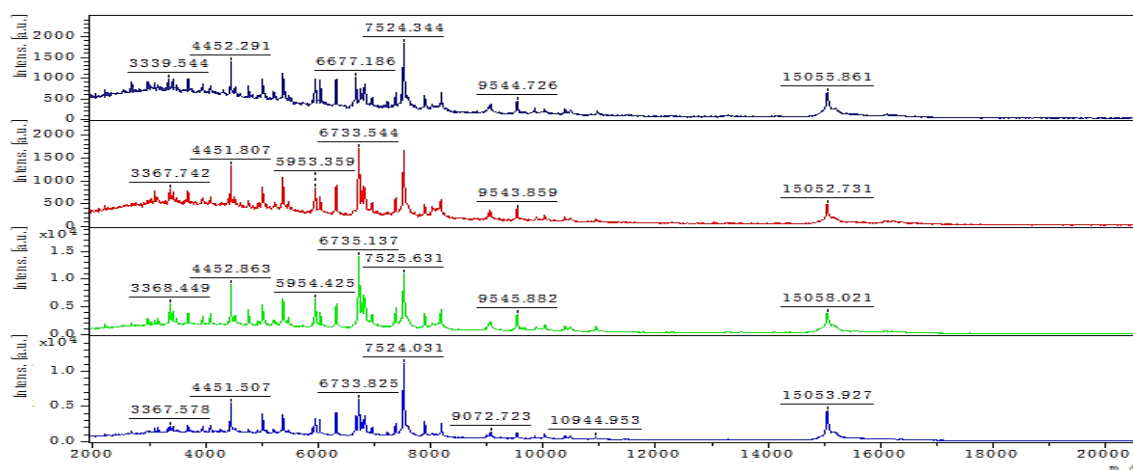
**Bacteria identification:** For each bacterial isolated positive sample, three  $\beta$ -hemolytic colonies were picked up for identification. All of them were identified as *S. zooepidemicus* by MALDI-TOF MS (Fig. 2).

**Antimicrobial sensitivity test and treatment:** The results of the drug sensitivity test showed that penicillin and cefotifuran were the most sensitive to the isolated *S. zooepidemicus* strains. Considering economic costs, penicillin was selected for treatment. During the heat of the infected donkey mares, uterine lavage was performed daily with a mixture of 500IU/mL penicillin (Keda, Fuzhou, China) and 100 $\mu$ g/mL streptomycin (Yuze, Yantai, China) for 3 to 5 consecutive days until the uterus washes showed PCR negative. After one round treatment, 12 of 17 (70.6%) symptomatic and 28 of 31 (90.3%) asymptomatic donkey mares were cured.

## DISCUSSION

Along with the increasing consumer demand, intensive donkey farming has been popularized in China since 2014, which was thought to bring good benefits to the donkey industry. However, it only brought benefits of fattening donkeys, but caused substantial economic loss to the donkey breeding farms due to the lack of biosecurity measures and making donkey mares and foals susceptible to contagious infectious disease. During the last two years, many donkey breeding farms have suffered from endometritis which caused infertility along with economic losses. However, most veterinarians and techniques thought the endometritis was caused by *Taylorella* spp, but no research was conducted to confirm the causative agent. In the present study, one breeding farm was selected as a research model for isolation and identification of causative agent and epidemiological investigation. Our results showed no mare was infected with *Taylorella* spp, and the endometritis was proved to be associated with *S. zooepidemicus* infection.

*S. zooepidemicus* is an opportunistic pathogen of a broad range of animal species including humans and commonly found on mucus membranes of the respiratory



**Fig. 2:** Identification of the isolated *S. zooepidemicus* strains by MALDI-TOF mass spectra. The score value ranged from 2.096 to 2.447.

tract and reproductive tract of horses (Skive *et al.*, 2017; Fonseca *et al.*, 2020). When condition permit, it can cause infection in the respiratory and reproductive system. Since *S. zooepidemicus* was found able to hide deep within the tissue of horse endometrium for prolonged periods of time (Rasmussen *et al.*, 2013) and able to invade and survive inside epithelial cells which is important for its pathogenesis (Skive *et al.*, 2017), it frequently causes infectious endometritis along with infertility in horse mares (Overbeck *et al.*, 2011; Petersen *et al.*, 2015). In the intensive donkey farm of this study, no *S. zooepidemicus* infection was detected in unbred female donkeys under two and a half years old, which suggested the transmitting route of *S. zooepidemicus* to uterus might be via artificial insemination or other breeding related handlings. Meanwhile, we hypothesized that the improper operations during artificial insemination caused transient inflammatory response or injury in the lower reproductive tract, which can promote the occurrence of *S. zooepidemicus* infection. Further studies and more investigations need to be carried out to confirm the deduction.

In addition, PCR assay was found to be better than bacterial isolation for *S. zooepidemicus* detection during screening the asymptomatic infection. For treatment, although beta-lactam antibiotics such as penicillin were sensitive to the isolated *S. zooepidemicus* strain and can be used for rinsing uterus to cure the endometritis in this study, we strongly suggest it is better to perform drug sensitivity test before treatment in terms of avoiding the misuse of antibiotics and the emergence of drug-resistant strains.

**Conclusion:** In intensive donkey breeding farms in China, endometritis has recently become one of the most important infectious diseases which caused infertility leading to economic losses. The present study revealed that the endometritis was associated with *S. zooepidemicus* infection. Meanwhile, the high infection rate indicated the endometritis has become a severe problem and needs to pay enough attention. Moreover, it suggested the transmitting route of *S. zooepidemicus* might be artificial insemination or related handlings. It is hoped that this study will lead to a great understanding of the causative agent and

epidemiological characteristics for endometritis in intensive donkey breeding farms in China and may contribute to the prevention and control of this disease.

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**Authors contribution:** JZ and JD conceived the idea and designed the study. NG, YD, ZX, SS, JS, MH, XM, RH, WP, CF, and WZ executed the experiment. NG, FS, AK, and JD were involved in data analysis and write up.

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