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Detection of *Mycoplasma equigenitalium* from Genital Tract of Healthy Domestic Donkeys (*Equus africanus asinus*)

Pablo Tamiozzo[1,*], Erika Sticotti[1], Ana Flores Bragulat[2], Carolina Alonso[2], Mauro Marió[1], and Luis Losinno[2]

[1]*Department of Animal Pathology, Faculty of Agronomy and Veterinary Medicine, National University of Río Cuarto, Argentina*
[2]*Equine Production Laboratory, Department of Animal Production, Faculty of Agronomy and Veterinary Medicine, National University of Río Cuarto, Argentina*

[*]Author to whom any correspondence should be addressed; e-mail: ptamiozzo@ayv.unrc.edu.ar

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Abstract

Mycoplasma equigenitalium (*M. equigenitalium*) has been identified in the reproductive tracts of both fertile and infertile mares and stallions. However, there are scarce antecedents on its detection in the reproductive tract of donkeys. A cross-sectional study was conducted with healthy domestic donkeys. Jennies (n=6) and jacks (n=6) were sampled through the collection of vaginal and preputial swabs. The specimens were plated into a commercial *Mycoplasma* medium and analyzed through the use of a species-specific PCR. *M. equigenitalium* was detected by PCR in four specimens (4/12), one from a jack (1/6) and three from jennies (3/6). Moreover, from one of the PCR positive jennies, *M. equigenitalium* could be isolated. Presumably, this is the first report of *M. equigenitalium* detection from healthy domestic donkeys by both culture and PCR methods despite the small number of analyzed specimens. The obtained results are a starting point for the study of *Mycoplasmas* affecting donkeys.

Keywords

Mycoplasma equigenitalium; reproductive tract; vagina; prepuce; jennies; jacks

1. Introduction

Mycoplasmas are common inhabitants of the respiratory and reproductive tract of humans and animals. Although, in some species, *Mycoplasmas* are recognized as causes of reproductive failures [1,2], in horses, *Mycoplasma equigenitalium* (*M. equigenitalium*) has been identified in both fertile and infertile mares and stallions [3–9].

Seldom do veterinarians take into account diseases caused by *Mycoplasmas* in the differential diagnosis of reproductive disorders from horses. Thus, *Mycoplasma* detection tests are almost never requested. That's why there is a lack of information about the presence of *M. equigenitalium* and other *Mycoplasma* species involved in reproductive failures in horses [3–5]. Even rarer is the detection of *Mycoplasma* species in the reproductive tract of fertile or infertile donkeys [10].

Considering the scarcity of the information about the occurrence of *M. equigenitalium* in donkeys and in order to obtain a first approach on its detection in their reproductive tracts, the present study aimed to detect *M. equigenitalium* from specific areas of reproductive tracts from healthy adult domestic donkeys.

2. Materials and Methods

2.1. Ethics Statement

The study was approved by the Research Ethics Committee of the Universidad Nacional de Río Cuarto, according to

the international guidelines of the Council for International Organizations of Medical Sciences (CIOMS).

2.2. Experimental Design, Sample Collection, Processing, and Testing

A cross-sectional study was conducted with resident donkeys that were used for teaching purposes from the Laboratorio de Reproducción Equina, Facultad de Agronomía y Veterinaria (FAV), Universidad Nacional de Río Cuarto (UNRC).

Donkeys were crossbred native from Argentina, with a wide phenotypic variability. Their weights ranged from 180 to 250 kg, and their body scores ranged from 4 to 5 on the Pearson and Ouassat scale [11,12]. The animals were freely grazing mixed grasses and alfalfa pasture with *ad libitum* water and were clinically healthy. All the animals utilized in the current study had a proven fertility; the jennies had at least one foal, and jacks were utilized for breeding during the reproductive season (two years prior to the study). Although donkeys were located in paddocks, separated from other animals, some horses were hosted on the same premises. Horses were not known to have *M. equigenitalium* and were not sampled. Adult (4 to 18 years old) jennies (n=6) and jacks (n=6) were sampled through the collection of vaginal and preputial swabs through the use of sterile Dacron® swabs (Deltalab®, Spain). In jennies, specimens were obtained by swabbing the vaginal wall at the level of the vestibule (caudal vagina) for 10 s avoiding contact with the vulva. In jacks, specimens were obtained by swabbing the prepuce for 10 s avoiding contact with the skin around.

2.3. Samples Processing and Testing

For *M. equigenitalium* isolation, swabs were plated onto *Mycoplasma* Base Medium with selective *Mycoplasma* supplement (Oxoid, Basingstoke, UK). All plates were incubated at 37ºC under 5% CO2 and examined at 96 hrs. For molecular detection, DNA from collected swabs and *Mycoplasma*-compatible colonies was extracted through the use of Puriprep-S Kit (Inbio Highway, Argentina) following the manufacturer's instructions. For species-specific detection of *M. equigenitalium,* DNA specimens were analyzed through the use of a PCR targeting *rpoB* gene as previously described [9]. DNA extraction, amplification, and visualization were carried out in three different rooms. Negative controls were included every five samples, and filter tips were utilized throughout the process. As positive control, lyophilized *M. equigenitalium* DNA from DNA collection (FAV-UNRC) was used.

3. Results and Discussion

M. equigenitalium was detected by PCR in four specimens (4/12), one from a jack (1/6) and three from jennies (3/6). Furthermore, from one of the PCR positive jennies, *M. equigenitalium* could be isolated. Presumably, this is the first report of *M. equigenitalium* detection from healthy adult domestic donkeys by both culture and PCR methods. *Mycoplasma* isolation is time-consuming and requires complex media and further testing for species identification. However, despite the small number of analyzed specimens, *M. equigenitalium* could be isolated through the use of a commercial culture medium. A higher number of specimens rendered PCR-positive results. The lower sensitivity of isolation of *Mycoplasmas* in comparison with molecular methods might be due to a low number of viable *Mycoplasmas* in clinical specimens and/or special requirements for culture [8].

Although, as previously mentioned, *M. equigenitalium* detection in the reproductive tract of fertile and infertile horses has been already informed [3–7], our results were an interesting starting point for the study of *Mycoplasmas* affecting the reproduction of donkeys. In this case, we cannot conclude that *M. equigenitalium* was exclusively in donkeys due to the contact with horses, and the donkeys sampled did not show fertility problems. It is well known that donkeys have a different susceptibility to certain infectious agents and clinical manifestations in comparison with horses. In this regard, some differences, concerning viral diseases of *equids*, have been thoroughly reviewed and highlighted recently [13]. There are few scientific reports of infectious diseases affecting donkeys in general [13–16] and of reproductive or respiratory diseases caused by *Mycoplasmas* in particular. The information about infectious diseases affecting donkeys is scarce, and there is a tendency to extrapolate the knowledge from experimented horse veterinarians to donkeys' medicine [15], which can be erroneous also in diseases caused by *Mycoplasmas*.

Detection of various *Mycoplasma* species may vary according to different sites from the reproductive tract of mares [7], stallions [8], and along the different phases of the estrous cycle [7]. Moreover, several *Mycoplasma* species might be detected from the reproductive tracts of horses [7,8]. Taking this into consideration, further in-depth studies are required in order to associate the occurrence of *Mycoplasmas* species, particularly *M. equigenitalium,* with fertility disorders of donkeys. Nowadays, we are looking for other *Mycoplasma* species in the reproductive tracts of donkeys.

Authors' Contributions

Luis Losinno and Pablo Tamiozzo planned and designed the study. Ana Flores Bragulat and Carolina Alonso collected the specimens, while Pablo Tamiozzo, Erika Sticotti, and Mauro Marió ran the experiments. All authors read and approved the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this paper.

Data Availability

The data supporting the findings of this study are available within the article.

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