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# Molecular detection of *Mycoplasma sp.* in genital tract of healthy domestic donkeys

Detección molecular de *Mycoplasma* sp. en el tracto genital de burros domésticos sanos

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## Abstract

Several *Mycoplasma* species have been identified from reproductive tract of horses being *Mycoplasma equigenitalium* (*M. equigenitalium*) the most prevalent and associated with reproductive failures. However, there are scarce antecedents on its detection in donkey's reproductive tract. A cross-sectional study was conducted with healthy domestic donkeys. DNA from vaginal and preputial swabs from jennies (n=3) and jacks (n=5), that have been PCR negative for *M. equigenitalium* was analyzed by a nested PCR targeting the 16S-23S rRNA intergenic spacer region (ITS). In seven out of eight analyzed specimens, different sized bands between 900 and 400 bp were visualized. This is the first report of *Mycoplasma* sp, detection, besides from *M. equigenitalium*, from healthy domestic donkeys in Argentina.

**Keywords:** *Mycoplasma sp*., vagina, prepuce, jenny, jack, PCR, 16S-23S rRNA ITS

## Resumen

Se han identificado varias especies de *Mycoplasma* en el tracto reproductivo de los caballos, siendo *Mycoplasma equigenitalium* (*M. equigenitalium*) la más prevalente y asociado a fallas reproductivas. Sin embargo, son escasos los antecedentes sobre su detección en el tracto reproductivo de los burros. Se realizó un estudio transversal con burros domésticos sanos. Se analizó el ADN de hisopados vaginales y prepuciales de burras (n=3) y burros (n=5), que habían sido negativos a la PCR para *M. equigenitalium*, mediante una PCR anidada dirigida a la región del espaciador intergénico del ARNr 16S-23S. En siete de las ocho muestras analizadas, se visualizaron bandas de diferente tamaño entre 900 y 400 pb. Este es el primer informe de detección de Mycoplasma sp, aparte de *M. equigenitalium*, en burros domésticos sanos en Argentina.

**Palabras clave**: *Mycoplasma sp*., vagina, prepucio, burra, burro, PCR, ITS 16S-23S ARNr

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## Introduction

*Mycoplasmas* are common inhabitants of respiratory tract and genital mucosa of humans and animals. Although in some species, they are recognized as causes of reproductive failures (Vallely *et al*., 2018: Tamiozzo *et al*., 2021). In horses, several *Mycoplasma* species have been identified from reproductive tract of mares and stallions (Bermudez *et al*., 1987, Spergser *et al*., 2002), being *Mycoplasma equigenitalium* (*M. equigenitalium*) the most prevalent and associated with reproductive failures (Moorthy *et al*., 1977, Kirchhoff, 1978, Kirchhoff *et al*., 1980, Heitmann *et al*., 1979, Bermudez *et al*., 1987, Spergser *et al*., 2002, Nehra *et al*., 2014). Donkeys have a different susceptibility to certain infectious agents and clinical manifestations when compared to horses (Falcão Câmara *et al*., 2020, Burden & Thiemann, 2015). This might be most noticeable in diseases caused by *Mycoplasmas*. In this way, we recently demonstrated the presence of *M. equigenitalium* in adult healthy jennies and jacks without reproductive failures, being one of the few records in the literature about the presence of *Mollicutes* in donkeys (Hui *et al*., 2002; Tamiozzo *et al*., 2022). Considering that *Mycoplasma* isolation is time consuming and requires complex media and further testing for species identification and, the scarce antecedents about the detection of *Mycoplasmas* in the reproductive tract from donkeys, the objective of this study was to detect *Mycoplasma* sp in healthy domestic adult donkeys without reproductive failures.

## Materials and methods

### *Ethics statement*

The study was approved by the Research Ethics Committee of the National University of Río Cuarto, according to the international guidelines of the Council for International Organizations of Medical Sciences (CIOMS).

### *Experimental design, sample collection, processing and testing*

The study was conducted with specimens collected from healthy adult domestic donkeys from the Equine Production Laboratory, Department of Animal Production, Faculty of Agronomy and Veterinary Medicine, National University of Río Cuarto. Donkeys were cross bred 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 (Pearson & Ouassat 1996; 2000). The animals were freely grazing mixed grasses and alfalfa pasture with ad libitum water and were clinically healthy. All the animals used in this study had a proven fertility, the jennies have had at least one foal and jacks were used for breeding during the reproductive season (two years before 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 *Mycoplasma* sp. and were not sampled. DNA from vaginal and preputial swabs (Deltalab®, Spain) from six jennies and six jacks were previously tested by PCR looking for *M. equigenitalium* and these results were already reported (Tamiozzo *et al*., 2022). For this study, the remaining eight DNA specimens (five obtained from jacks and three from jennies, all of them negatives to a PCR and culture for *M. equigenitalium*) stored at -20ºC, were analyzed for *Mycoplasma* sp detection. For this purpose, a nested PCR targeting 16S-23S rRNA intergenic spacer region (ITS) was performed under the conditions reported by Tang *et al*., 2000. PCR products were electrophoresed on a 1.2% agarose gel stained with SYBR ™ Green I (Thermofisher scientific, Argentina).

## Results and Discussion

Seven out of eight specimens rendered positive PCR results for *Mycoplasma* sp. Four of the PCR products obtained from jacks' specimens showed two different sized band (between 900 and 400 bp approximately), the remained one was negative. Regarding the specimens collected from jennies, two PCR products showed two different sized bands (between 800 and 400 bp approximately) and the third a unique band (600 bp approximately). Despite of the low number of analyzed specimens, our results showed that *Mycoplasma* sp are abundant in the reproductive tract of healthy adult domestic donkeys. Several species of *Mollicutes* have been identified in the reproductive tract of horses (Bermudez, 1987; Spergser *et al*., 2002, Hui *et al*., 2002). In this case, the different sized bands and even the double band observed in some PCR products, strongly suggests the presence of different species of *Mycoplasmas* and/or *Acholeplasmas*, because of the inter-species variation among *Mollicutes* of the 16S-23S rRNA ITS (Volokhov *et al*., 2006). Taking into account that *M. equigenitalium* and *Mycoplasma subdolum* are the most prevalent species identified in horses (Berdumez 1987, Spergser *et al*., 2002) and that, in this case, specimens PCR positive to *M. equigenitalium* were excluded of the analysis, other species of *Mycoplasmas* and/or *Acholeplasmas* might have been

detected. A limitation of this study was not having identified the *Mycoplasma* species found. However, the results are a starting point to recognize different *Mollicutes* species in the reproductive tract of donkeys and also for a better understanding of the pathogenic role of some species affecting donkeys in future studies. The abundance of *Mycoplasmas* in the reproductive tract of adult donkeys might be considered obvious, because of the antecedents in horses. However, a study conducted in stallions and mares from Denmark did not find *Mycoplasmas* neither by culture nor PCR (Baczynska *et al.,* 2007). In this way, it seems that stallions can inoculate *Mycoplasmas* to mares, by natural breeding, but not by artificial insemination (Spergser *et al*., 2000; Baczynska *et al*., 2007). Added to this, there is the fact that different *Mycoplasma* species may be found in different sites of mares and stallion's reproductive tracts (Bermudez *et al*., 1987; Spergser *et al*., 2002) and along the different phases of the estrous cycle (Bermudez et al., 1987). Therefore, we consider that further studies in both, horses and donkeys should be conducted. Considering that the impact of different infectious diseases on donkeys is largely unknown (Burden & Thiemann, 2015), and that they have a different susceptibility to certain infectious agents and clinical manifestations when compared to horses (Burden & Thiemann, 2015), scientific community should be careful not to extrapolate knowledge of diseases from horses to donkeys. We are convinced that further studies are necessary to obtain a better understanding of Mycoplasmal diseases in both horses and donkeys.

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