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BRIEF REPORT

Mycoplasma maculosum and *Mycoplasma spumans* associated with fertility disorders in dogs from a Bernese Mountain dog kennel

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KEYWORDS
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Antibiotic treatment

Abstract The aim of this short communication is to describe a case of subfertility and other anomalies associated with the presence of *Mycoplasma spumans* and *Mycoplasma maculosum* in a Bernese Mountain Dog kennel. After the arrival of two dogs from abroad, some fertility disorders, such as unsuccessful mating, pregnancy losses and abnormal sperm analysis results, were observed. Two consecutive samplings (vaginal swabs) of three and two bitches with problems, respectively, were performed and *M. spumans* and *M. maculosum* were identified by PCR and sequencing. After treatment for 15 days with doxycycline and 9 days with azithromycin, successful pregnancies were achieved and the results of the sperm analyses were reversed. Considering that no other infectious agents causing subfertility problems were detected and that no management measures or other medication apart from these antibiotics were applied, it was concluded that fertility problems were due to the presence of these two *Mycoplasma* species.

PALABRAS CLAVE
Perros;
Trastornos de fertilidad;
Tracto urogenital;

Mycoplasma maculosum y _Mycoplasma spumans_ asociados con desórdenes de fertilidad en perros de un criadero de boyero de Berna

Resumen El objetivo de esta comunicación es describir un caso de subfertilidad y otras anomalías asociadas a la presencia de *Mycoplasma spumans* y *Mycoplasma maculosum* en un criadero de perros boyero de Berna. Después de la entrada de 2 perros del exterior, comenzaron a observarse algunos trastornos de la fertilidad, como apareamientos infructuosos, pérdidas de

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Mycoplasma maculosum; *Mycoplasma spumans*; Tratamiento antibiótico

preñez y resultados anormales en el análisis espermático. Se realizaron 2 muestreos consecutivos (hisopados vaginales) de 3 y 2 perras con problemas, respectivamente, y se identificó *M. spumans* y *M. maculosum* mediante PCR y secuenciación. Después del tratamiento con doxiciclina y azitromicina durante 15 y 9 días, respectivamente, se lograron obtener preñeces exitosas y se revirtieron los resultados del espermograma. Considerando que no se detectaron otros agentes infecciosos causantes de problemas de subfertilidad y que no se aplicó ninguna medida de manejo ni otra medicación, aparte de los tratamientos antibióticos mencionados, se concluyó que los problemas de fertilidad se debieron a la presencia de estas 2 especies de *Mycoplasma*.

Mollicutes which include the genus *Mycoplasma* are wall-less bacteria that are found in avian, insect, mammalian and reptilian hosts[11]. Until now, more than 15 species have been isolated or detected in dogs[5]. In spite of the fact that *mycoplasmas* can be found in healthy and diseased dogs, some studies have associated the presence of these agents with infertility, respiratory disease, arthritis and colitis[5]. Considering that information about the association of canine *mycoplasmas* with infections in dogs is scarce, this short communication describes a case of fertility disorders and other anomalies attributed to *Mycoplasma spumans* and *Mycoplasma maculosum* in a Bernese Mountain dog kennel.

The Bernese Mountain dog kennel was located in the city of Mar del Plata (Buenos Aires province). It has been operating normally for 21 years, with high rates of pregnancy, producing two to three litters per year. The kennel was considered to be free of brucellosis and leptospirosis because of the lack of antecedents of these diseases and the negative serology of the dogs, which were tested regularly. Dogs were vaccinated with the sextuple vaccine (polyvalent vaccine that confers immunity against canine distemper virus, canine parvovirus, *Leptospira interrogans* serovars *canicola* and *icterohaemorrhagiae*, canine adenovirus type 2 and canine parainfluenza virus) and dewormed regularly 2–4 times a year. In October 2017 a female (Catalina) and a male (Gucci) dogs were imported from Brazil and incorporated into the breeding stock. These dogs were tested only against *Brucella* (immunochromatographic rapid test, FASTest® BRUCELLA canis and blood culture), giving negative results. Thereafter, fertility disorders and other anomalies began to be observed: (i) after two unsuccessful matings between a female named Wish and Gucci, a singleton puppy with tracheal aplasia was born, (ii) Gucci's sperm analysis showed low sperm counts (1.5 mill/ml), without motility, 100% of dead forms and 70% of abnormalities in head, midpiece and tail, reason enough for being excluded from the kennel, (iii) after a male called Egmont mated Catalina, a puppy died of a fatal pneumonia after an unsuccessful treatment with amoxicillin-clavulanic acid. By June 2019, all the bitches still had pregnancy loses. After having ruled out several infectious agents by bacteriology (*Brucella* sp., *Escherichia coli*, *Citrobacter freundii* and beta-hemolytic streptococci) and serology (microscopic agglutination test for *Leptospira* sp.) in all the dogs, the owners sent vaginal swabs from three non-pregnant bitches (Catalina, Clara and Troya) for *Mycoplasma* diagnosis. Once in the laboratory, DNA extraction from vaginal swabs was performed using the Puriprep-S commercial kit (Inbio Highway, Argentina) following the manufacturer's instructions. For *Mycoplasma* spp. detection, a nested PCR targeting the 16S-23S rRNA intergenic spacer region (ITS) was performed under the conditions reported by the authors[13]. Only Catalina rendered a positive result, with two different size bands (330 bp and 210 bp, approximately) observed in the agarose gel. In order to identify *Mycoplasma* spp., both PCR products were purified (Puriprep-GP Kit, Inbio Highway), quantified and sequenced (ABI 3130xl; Applied Biosystems) using the inner primers described by Tang et al.[13] The sequences were curated using the BioEdit software[8] and aligned against the database using nucleotide BLAST (http://www.ncbi.nlm.nih.gov/blast). The 16S-23S rRNA ITS sequence obtained from the 310 bp amplicon (GenBank accession number MW646397) showed 97.44% similarity with the same region of *Mycoplasma maculosum* strains NCTC10168 (LR215037.1), Skotti B (FJ595090.1), PG15 (AF443610.1), ATCC 19327 (AY973564.1), 97.12% with *Mycoplasma leopharyngis* (AY762644.1) and 97.14% with *Mycoplasma* sp. isolate BA019827 (KX863544.1). The 214 bp (GenBank accession number MW646398) sequence showed 98.1% and 97.63 similarity with *M. spumans* (AF538684.1 and AY762642.1) respectively. Based on these results, in July 2019, the 13 male and female adult dogs from the kennel (even Gucci, the male dog) were treated with doxycycline (500 mg/day) for a 15-day period. Two months after the treatment with doxycycline, Gucci's sperm analysis revealed the following parameters: normal sperm counts (52 mill/ml), 61% rapid progressive, 19% slow progressive and 12% *in situ* motility) with 10% of dead forms and 75% of normal forms. Despite the doxycycline treatment, four female dogs (Wish, Catalina, Eureka and Clara) could not get pregnant. For this reason, in August 2019, the owner sent vaginal swabs from two females to the laboratory (Catalina and Eureka). Both samples rendered positive results with one and two bands present in agarose gel, respectively. This time sequencing was not performed. These females were treated with azithromycin (750 mg/day) for a nine-day period starting on

the day of mating. After that, Gucci mated successfully with Eureka and Troya and, Egmont mated Catalina, the three bitches became pregnant. In December 2019, Troya and Eureka delivered healthy puppies. Catalina could not deliver because during her pregnancy she suffered from a gastric torsion and needed surgery. After this episode, no reproductive disorders were observed. During 2020, 27 healthy puppies were born.

The success of the antibiotic treatment used, the absence of other pathogens causing infertility, the detection of *Mycoplasmas*, the changes in Gucci's sperm analysis and the recovery from the bitches' previous subfertility after the antibiotic treatments, suggest that *Mycoplasma* species were associated with that subfertility episode in this dog kennel. Although the results of Gucci's sperm analysis improved after treatment with doxycycline, it was only after the treatment with azithromycin that successful pregnancies were achieved. In this regard, azithromycin, doxycycline, enrofloxacin, marbofloxacin, minocycline, orbifloxacin and pradofloxacin have been pointed out as effective against *Mycoplasma*-associated respiratory infections in dogs[9]. However, azithromycin would be the most suitable antibiotic for the treatment of infections caused by *Mycoplasma*[9]. Although *M. canis* is the predominant species identified from the vaginas of fertile and infertile bitches[6,10,12] and *Mycoplasma cynos* had been associated with pneumonia[4,16], we did not find any of those species. *M. spumans* and *M. maculosum* have been identified from the respiratory and genitourinary systems of dogs with and without respiratory infection[1,2,7] and from fertile and infertile dogs[2,6,10]. These antecedents might explain the subfertility episode in this kennel, the fatal pneumonia of Catalina's puppy and even, the tracheal aplasia of Wish's puppy. Further experimental studies are necessary to confirm our results. With regard to *M. leopharyngis*, we did not find any antecedents about its presence in dogs, since together with other six *Mycoplasma* species, it affects felines[3]. Considering the high similarity (98.6%) in the 16S-23S rRNA ITS region between *M. maculosum* and *M. leopharyngis*[14], the detected species could be *M. maculosum*. However, the presence of *M. leopharyngis* in the urogenital tract of dogs cannot be strictly ruled out. The analysis of other target sequences would have allowed the unambiguous identification of *Mycoplasma* species. Taking into account the phylogenetic relatedness and pairwise sequence similarities of the species within the family *Mycoplasmataceae*, a reliable and useful taxonomic tool, analyzing 16S-23S rRNA ITS, RNA polymerase beta subunit (rpoB) and 16S rRNA genes, has been proposed[15]. Regrettably, *rpoB* and *16S rRNA* genes were not addressed in this study. In this way, the analysis of the three-target sequence has been pointed out as a valuable tool for routine analyses[15].

Unfortunately, veterinarians do not often consider *Mycoplasma* in the differential diagnosis of respiratory and reproductive diseases in dogs. For this reason, *Mycoplasma* detection either by culture or molecular-based tests is rarely requested. From a practical point of view, the detection of these agents is important for the implementation of an adequate antibiotic treatment, beyond the identification of the involved species. Due to the lack of cell wall in *Mycoplasma*, they are inherently resistant to β-lactam antibiotics. In this report, prolonged therapy with doxycycline and azithromycin demonstrated to be effective against *Mycoplasma* infections, agreeing with previous data[9]. In spite of the limitation of this study, since *Mycoplasma* culture was not tried, the importance of the diagnosis of these agents was demonstrated.

Conflict of interest

The author declares that he has no conflicts of interest.

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