1. Abstract

In daily veterinary medical practice, indications for bacteriological examinations frequently arise. However, interpreting the results of bacteriological cultures can be challenging, especially when analyzing vaginal swabs from female dogs to determine the relationship between isolated bacterial strains and reduced fertility.

The objective of this study was to establish the qualitative composition of the aerobic bacterial flora in the vagina of healthy breeding female dogs with no reproductive issues and those experiencing reproductive difficulties. Additionally, the study aimed to determine the correlation between this flora and selected fertility parameters. Another goal was to investigate the presence of *M. canis* in the vagina of breeding female dogs and its impact on fertility. The study also included an assessment of the prevalence of *M. canis* in mated females and those yet to begin reproductive activity, as well as the percentage of its occurrence in purebred dog breeding facilities.

The study involved 275 female dogs in the anestrus phase, originating from 82 kennels and representing 35 different dog breeds. The dogs were divided into two clinical categories:

• Group I – female dogs with no reproductive difficulties, with no history of reproductive disorders (198 dogs).

• Group II – female dogs with reproductive issues, exhibiting pathological vaginal discharge, signs of infertility, embryo resorption, miscarriages, and varying degrees of neonatal mortality (68 dogs).

Crucially, the interpretation of results was based on clinical and survey-based assessments.

Vaginal swabs were collected from the female dogs and subjected to bacteriological examination for aerobic bacteria. The genetic material of *M. canis* was isolated using the PCR method.

The most frequently isolated aerobic bacterial strains from the vagina of all female dogs were *Streptococcus* spp. (38.91%), *Staphylococcus* spp. (38.55%), *Mycoplasma (M.) canis*

(34.91%), and *Escherichia coli* (32.0%). Less frequently isolated strains included *Bacillus* spp., *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp., and *Citrobacter* spp.

Analysis of the obtained results showed that the bacterial isolates in both groups of female dogs were similar, with no statistically significant differences. The prevalence of *Streptococcus* spp. in the group of reproductively healthy dogs (Group I) was 38.38%, while in the group with reproductive problems (Group II), it was 41.18%. Similarly, minor differences were observed for *Staphylococcus* spp., with a prevalence of 36.36% in reproductively healthy dogs and 42.62% in those with reproductive issues.

However, significant differences were observed in the composition of vaginal bacterial flora between the two groups concerning the number of bacterial strains present. In reproductively healthy female dogs, the presence of two bacterial strains was most commonly detected. The percentage of individuals with monobacterial flora was statistically significantly higher in the group of dogs classified as having reproductive issues. Interestingly, in cases where *M. canis* was identified in problematic female dogs, it was typically accompanied by at least two other bacterial species. *M. canis* was not found in monoculture in any of the examined dogs.

Using the PCR method, *M. canis* was identified in 33.33% of reproductively healthy females and in 41.18% of those with reproductive issues, though this difference was not statistically significant. Across the entire studied population of breeding female dogs, *M. canis* was the third most frequently detected bacterium. The applied research methods did not confirm an association between the presence of *M. canis* and symptoms of vaginal infections or specific reproductive disorders. Notable similarities in vaginal microflora were observed in female dogs regardless of their fertility status.

M. canis was detected in 33.14% (56/169) of mated females and slightly more frequently in those that had not yet been mated, at 37.74% (40/106). This finding confirms that mycoplasmas are transmitted not only during mating and copulation. Their prevalence in breeding facilities was also high, as they were found in 55 out of the 82 examined kennels, representing 67.07%.

The conducted study demonstrated that the vaginal bacterial flora of reproductively healthy and problematic female dogs is similar. No association was found between the presence

of *M. canis* in the vagina and fertility disorders. The results confirmed the hypothesis that *M. canis* is not an obligate pathogen of the female canine reproductive system but rather an opportunistic bacterium that may be part of the natural, non-pathogenic vaginal microflora. It may exert a negative effect only under specific conditions and in the presence of other potentially pathogenic bacteria.

The presented research findings are highly relevant to daily veterinary practice in the context of responsible and justified antibiotic use, in accordance with the "One Health" principle, which aims to combat the growing issue of bacterial antibiotic resistance. The obtained results clearly indicate that the interpretation of bacteriological test results from vaginal swabs in female dogs should be approached with caution. They should always be considered within the broader context of the overall health status of the examined individual. A comprehensive clinical and gynecological examination (including cytology and vaginoscopy) as well as an evaluation of the reproductive history of the female dog are essential before making decisions regarding antibiotic treatment.