INCIDENCE OF MYCOPLASMA CANIS IN THE VAGINA IN THREE GROUPS OF BITCHES

TOMASZ JANOWSKI, SŁAWOMIR ZDUŃCZYK, ANDRZEJ JURCZAK, AND PIOTR SOCHA

Department of Obstetrics and Pathology of Reproduction, Faculty of Veterinary Medicine,
University of Warmia and Mazury, 10-719 Olsztyn, Poland
jantom@uwm.edu.pl

Received for publication October 2, 2008

Abstract

The aim of this study was to determine the occurrence of Mycoplasma canis in the vagina in three groups of healthy canine bitches. Two vaginal swab samples were collected from each of the 91 mixed-breed canine bitches. One sample was placed into transport medium, chilled and brought to the laboratory within 24 h. These swabs were cultured on a urea-arginine LYO2 broth, thereafter DNA was extracted and tested by PCR. The second vaginal sample was cultured for aerobic bacteria. Totally, 38 (41.76%) samples were positive. In group I (n = 32) M. canis was detected in 22 (68.7%) bitches, in group II (n = 24) in four (16.7%), and in group III (n = 35) in 12 (34.3%) animals. Mixed infections with other bacteria were common. The mean number of isolates from vaginal samples was 2.1. The results indicate that M. canis is a part of normal vaginal flora in healthy bitches. The differences between groups of bitches in the frequency of incidence of M. canis and other bacteria could result from various environmental conditions.

Key words: bitch, vagina, Mycoplasma canis.

In the genital tract of healthy canine bitches, the physiological bacterial flora composed of various microorganisms, mainly opportunistic pathogens, is present (5, 7, 8, 10). The canine vaginal bacterial flora varies in relation to breed, stage of oestrous cycle, and season (2, 5, 7). There are only few reports about the isolation of Mycoplasma canis from the vagina of healthy bitches and the results are partially conflicting (2, 5, 11). An association of mycoplasmas with infertility in bitches is suggested (1); however, conclusive evidence is lacking. The occurrence of M. canis in the vagina of bitches has not been investigated in Poland as yet.

Material and Methods

The aim of this study was to determine the incidence of M. canis in the vagina in three groups of healthy bitches from animal shelters in different cities in the north-eastern Poland. Two vaginal swab samples were collected from each of 91 mixed-breed bitches. One sample was placed into transport medium (R1 – bioMérieux), chilled, and brought to the laboratory (Laboklin, Bad Kissingen, Germany) within 24 h. These samples were cultured on urea-arginine LYO2 broth (bio-Mérieux); thereafter, DNA was extracted and tested by PCR (3) that targets the RNA polymerase β subunit. For PCR primers, M. canis-1: 5'-GAA TGT TCT TGT CTT CAC G-3 and M. canis-rev1: 5'-TTC ACC CTT AGG AGA AAC ACG A-3 were used. The second vaginal sample was cultured for aerobic bacteria by routine methods in the Department of Microbiology, Faculty of Veterinary Medicine in Olsztyn. The mean numbers of bacteria species per bitch were calculated.

Results

Totally, 38 (41.8%) samples were positive for M. canis. In group I (n = 32) M. canis was detected in 22 (68.7%) bitches, in group II (n = 24) in four (16.7%), and in group III (n = 35) in 12 (34.3%) animals (Fig. 1). Mixed infections with other bacteria were common. There were mainly α-haemolytic streptococci (35.2%), Staphylococcus intermedius (27.5%) β-haemolytic streptococci (18.7%), Lactobacillus sp. (22.0%) and CNS (17.6%). The composition of vaginal bacterial flora varied between groups of bitches (Table 1).

The obtained results showed that the infection of the vagina in healthy bitches with M. canis is common. Our findings are similar to those of Bjurström and Linde-Forsberg (2), who frequently isolated M. canis from the vagina of bitches. In contrary, Watts et al. (11) found no mycoplasmas in the vagina and uterus of normal bitches. The reason of these differences could be the variation in composition of canine vaginal bacterial flora and methodological problems in the detection and identification of canine mycoplasmas. In our study, the mycoplasmas were detected using molecular based test. The advantages of PCR testing for bacterial identification are many and well known (3, 4).
Fig. 1. Incidence of *M. canis* in the vagina in three groups of bitches.

Totally, the mean number of bacteria species per bitch was 2.1 (range from 1.9 to 2.3; Fig. 2).

![Fig. 2. The mean numbers of bacteria species per bitch.](image)

**Table 1**

Other bacteria isolated from the vagina in three groups of bitches (% of bitches)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Group</th>
<th>Group</th>
<th>Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>n = 91</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>-</td>
<td>20.8</td>
<td>-</td>
<td>5.5</td>
</tr>
<tr>
<td>Staphylococcus intermedius</td>
<td>12.5</td>
<td>29.1</td>
<td>40.0</td>
<td>27.5</td>
</tr>
<tr>
<td>Streptococcus canis</td>
<td>-</td>
<td>-</td>
<td>14.3</td>
<td>5.5</td>
</tr>
<tr>
<td>Haemolytic <em>Escherichia coli</em></td>
<td>21.8</td>
<td>-</td>
<td>-</td>
<td>7.7</td>
</tr>
<tr>
<td>Non-haemolytic <em>Escherichia coli</em></td>
<td>-</td>
<td>25.0</td>
<td>14.3</td>
<td>12.1</td>
</tr>
<tr>
<td>α-haemolytic streptococci</td>
<td>37.5</td>
<td>41.6</td>
<td>28.6</td>
<td>35.2</td>
</tr>
<tr>
<td>β-haemolytic streptococci</td>
<td>21.8</td>
<td>-</td>
<td>28.6</td>
<td>18.7</td>
</tr>
<tr>
<td><em>Lactobacillus</em> sp.</td>
<td>9.3</td>
<td>37.5</td>
<td>22.8</td>
<td>22.0</td>
</tr>
<tr>
<td>CNS</td>
<td>-</td>
<td>16.7</td>
<td>34.2</td>
<td>17.6</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>6.2</td>
<td>-</td>
<td>5.7</td>
<td>4.4</td>
</tr>
<tr>
<td>Other species</td>
<td>15.6</td>
<td>8.3</td>
<td>14.3</td>
<td>13.3</td>
</tr>
</tbody>
</table>
Discussion

The other species of bacteria in the vagina of healthy bitches were similar to those previously isolated from the genital tract (6, 8). In another study, *Pasteurella multocida*, β-haemolytic streptococci and *E. coli* were the most frequently isolated species of bacteria (2).

The differences between groups of bitches in the frequency of incidence of *M. canis* and other microorganisms as well as in the number of bacteria species per bitch could result from various environmental conditions. Between-group variations in the frequency of the isolation of the most common bacterial strains were also described by Bjurström and Linde-Forsberg (2).

The pathogenicity of mycoplasmas for the genital tract in bitches is unclear. Experimental infection with *M. canis* induced purulent endometritis (7). However, mycoplasmas were isolated in similar proportion from bitches with genital diseases and from healthy animals (5). Ström and Linde-Försberg (10) reported the appearance of mycoplasmas in the vagina of bitches during and after antibiotic treatment. *M. canis* has been also isolated from male dogs with genital diseases (5). Generally, the information about mycoplasmas and their role as pathogens in canine reproductive tract diseases is still very limited and, as mentioned above, conclusive evidence is still lacking. This is due to a combination of factors including the inability to detect these microorganisms and lack of research considering their prevalence and pathogenicity.

Our results indicate that *M. canis* is a part of normal vaginal flora in healthy bitches. This is the first report in Poland about the incidence of *M. canis* in the vagina of bitches and more research is needed to establish whether *M. canis* is linked to genital tract diseases.

References