Ureaplasma diversum and reproductive disorder in Brazilian cows and heifers; first report

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Abstract

The species Ureaplasma diversum is associated with bovine reproductive illnesses, in particular granular lesions of the vulva and vagina or granular vulvovaginitis (GVV). In Brazil, this pathology is unknown and, until this point in time, the presence of U. diversum in the Brazilian herds has been ignored. With the intention of detecting the microorganism, vulvovaginal mucus of 152 animals located on seven farms in the São Paulo State, Brazil were analyzed. Those animals had evidence of reproductive disorders at the time of the sample collection. The technique used for microorganism detection was bacterial isolation. Statistical analysis assessed: the exposure of studied farms to U. diversum, relative risks for different symptoms, susceptibility of the animals according to age and breed. The frequency of that microorganism in tested animals was 38.8% and this frequency suggests that U. diversum can be related to GVV in Brazilian herds and possibly with other reproductive illnesses. As a result, the U. diversum differential diagnosis could be very important. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Ureaplasma diversum; Granular vulvovaginitis; Bovine; Reproductive disorder

1. Introduction

The Ureaplasma diversum involvement in bovine reproductive disorders has been documented internationally. Granular vulvovaginitis, salpingitis, endometritis, cervicitis, infertility, embryonic loss, abortions, neonatal death, seminal vesiculitis, balanopostitis and epididymitis are among the main problems related to the presence of U. diversum in the

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bovine reproductive tract (Ruhnke et al., 1978; Doig, 1981; Mulira et al., 1992; León et al., 1994; Miller et al., 1994; Rae et al., 1995).

Vulvitis and vaginitis are periodically observed in heifers and in cows. The vaginal infection seems to be more frequent in young females, but has been found in animals of any age. The infection occurs both in the acute and chronic forms, resulting in lesions 1–5 days after contact with the microorganism. Apart from the characteristic signs of the illness, such as hyperemia, presence of granulates in the vulvar and vaginal epithelium, and whether, or not followed by purulent vaginal discharge, the animals revealed high epithelial sensibility and those manifestations frequently involved to endometritis (Doig et al., 1979; Mulira et al., 1989; Gilbert and Oettlé, 1990; Miller et al., 1994; Rae et al., 1995). Those signs have recognized significance for reproductive control in economically functioning farms and can, therefore, cause reduction in different levels of production with obvious economic consequences.

Transmission of the organism has been reported to occur by natural and artificial insemination with contaminated semen (Kirkbride, 1987; Britton et al., 1988; Miller et al., 1994). Embryo transfer is also a potential means of disseminating the organism (Doig et al., 1981; Britton et al., 1988; Miller et al., 1994).

There are no reports documenting the presence of *U. diversum* in Brazilian cattle. The objectives of this study were to determine the frequency of *U. diversum* in vulvovaginal mucus of cattle presented with a history of reproductive disease and to determine the strength of association between clinical symptoms, age and breed with the agent.

### 2. Materials and methods

#### 2.1. Sampling

Vulvovaginal mucuses (n=152) were analyzed, which were chosen from seven herds of farms located in São Paulo State, Brazil. The animals showed evidence of a reproductive disease with symptoms such as hyperemia, granularity in the vulvovaginal epithelium, edema, purulent discharge and/or disorders as abortion and repeat breeding. The animals had not been submitted to treatment with antibiotics in the 15 days prior of the collection.

The breeds represented were Nelore (25), Holstein (96) and Jersey (30), and one animal of unspecified breeding. The animal age could only be accurately determined from 125 animals.

The vulvovaginal mucus was taken by introduction of the swab in the vagina vestibule and friction in the lateral walls, clitorian area and internal walls of vulvar lips. The swab was dipped into transport medium A3XB (Cunha et al., 1987) and maintained under refrigeration (4°C). The laboratory processing was accomplished at the latest 48 h after the collection.

The animals were individually examined for observation of the following signs: hyperemia, granulate lesions (granules of 1–2 mm diameter, gray, lightly brown or red, in the ventral area of the vulvar mucosa, adjacent to the clitoris and the lateral walls of the vulva and vagina), edema and purulent vaginal discharge.
2.2. Isolation

The used media and the protocol was established for Ruhnke and Rosendal (1994). The agar plates were incubated for up to 15 days, at 37°C in microaerophilic jar (Genovez et al., 1989), under an atmosphere comprised of 85% N₂, 10% CO₂ and 5% O₂. The broths were incubated up to 5 days at 37°C in aerobic atmosphere. Plates and tubes were observed daily for 15 consecutive days. The *Ureaplasma* sp. growth was characterized by typical colonial morphology of brown-gold colonies on agar. Growth in broth was detected after observing a change of the color, from orange to light pink, due to the alkalization of medium. After the color change a subculture was made for ureaplasmas for growth confirmation. The positive cultures were conserved by freezing (−80°C) for later characterization.

The isolated samples were identified by agar gel double diffusion technique (Ouchterlony, 1949; Lemcke, 1965) using rabbit polyclonal hyperimmune serum, prepared with *U. diversum* sample ATCC 49,783, according to the protocol of A. Blanchard (Oncologie Unité Virale, Institut Pasteur, France/personal communication).

2.3. Statistical analysis

Chi-square test for independent samples, according to Siegel (1975) were used to analyze the effect of farm. Relative risk (RR) (Smith, 1995) was used to study risk measurement (i.e. the association between the agent and symptoms, age and breed). For effect of age, the animal were categorized as heifers (≤2.5 years) and cows (>2.5 years). Confidence Intervals (95%) were developed using the approximation of Katz.

3. Results

From 152 vulvovaginal mucuses samples, 59 (38.8%) were positive for *U. diversum*, whilst 93 (61.2%) samples did not produce any indication of the presence of these microorganisms. All ureaplasma specimens isolated from clinical material had total identity with polyclonal hyperimmune serum produced for *U. diversum* identification by agar gel double diffusion technique.

The different incidence of positive animals on each farm can be observed in the Table 1. For critical χ² rate=12.6, the χ² rate observed was 16.1, indicating that the variation in the number of isolated samples, by farm was statistically significant (P<0.05).

<table>
<thead>
<tr>
<th>Farms</th>
<th>No. of animals</th>
<th>No. of positives (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>9 (36.0)</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10 (50.0)</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>14 (46.7)</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>7 (29.2)</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>14 (56.0)</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>2 (22.2)</td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td>59 (38.8)</td>
</tr>
</tbody>
</table>
Table 2  
Association between the presence of *U. diversum* and GVV symptoms, São Paulo, Brazil, 1997–1998

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No. of positives (%)</th>
<th>RRb</th>
<th>CI/95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulation</td>
<td>54 (91.5)</td>
<td>1.077</td>
<td>0.959–1.209</td>
</tr>
<tr>
<td>Hyperemia</td>
<td>43 (72.8)</td>
<td>1.169</td>
<td>0.936–1.459</td>
</tr>
<tr>
<td>Edema</td>
<td>17 (28.8)</td>
<td>0.864</td>
<td>0.527–1.416</td>
</tr>
<tr>
<td>Purulent vaginal discharge</td>
<td>15 (25.4)</td>
<td>1.488</td>
<td>0.791–2.759</td>
</tr>
<tr>
<td>Abortion</td>
<td>13 (22.0)</td>
<td>0.819</td>
<td>0.456–1.472</td>
</tr>
<tr>
<td>Repeat breeding</td>
<td>10 (16.9)</td>
<td>0.788</td>
<td>0.397–1.564</td>
</tr>
<tr>
<td>Total</td>
<td>59 positives/93 negatives</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a At the moment of collection.  
b Relative risk.

Table 3  
Association between age of bovine females and *U. diversum* isolation, São Paulo, Brazil, 1997–1998

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of animals</th>
<th>No. of positives (%)</th>
<th>RR</th>
<th>CI/95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifers</td>
<td>3</td>
<td>18 (40.0)</td>
<td>1.684</td>
<td>0.990–2.865</td>
</tr>
<tr>
<td>Cows</td>
<td>89</td>
<td>27 (60.0)</td>
<td>0.786</td>
<td>0.601–1.029</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>45 positives/80 negatives</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The relative risk associated with the presence of *U. diversum* and clinical signs or a history of abortion or repeat breeding are presented in Table 2. The relative risk was significantly different for animals showing granulation (1.07), hyperemia (1.16) and purulent vaginal discharge (1.48).

Independent of isolation frequencies, the relative risk rate was greater ($P<0.05$) for heifers than for cows as shown in Table 3.

Three breeds were studied: Nelore, Holstein and Jersey. Table 4 shows the frequency of isolated samples, which were observed in the breeds Holstein, Nelore and Jersey. The relative risk analysis revealed that the breed Nelore is more susceptible ($P<0.05$) than Jersey, which is then again more susceptible than Holstein.

4. Discussion

The *U. diversum* frequency found in the 152 vulvovaginals mucuses analyzed by isolation technique (38.8%) produced similar results as previously reported in Kenya (Mulira et al., 1989), Costa Rica (León et al., 1994) and France (Le Grand et al., 1995).
In the analysis on animal exposure by farm, rate $\chi^2 = 16.1$ ($P > 0.05$), for isolation’s frequency varying between 15.8 and 56.0% of positive animals, it is observed that the farms differ with regard to incidence of the microorganism. One hypothesis is that factors such as handling, breed, applied reproduction techniques, existence of enzootic zones and different infection phases, like in other infectious diseases, are probably contributing to the frequency of *U. diversum*.

With regard to the clinical symptoms, the statistical analysis revealed high association between *U. diversum* and purulent vaginal discharge. Infected animals revealed greater purulent vaginal discharge than not infected animals which indicates a probable association between the presence of the agent and uterine infections. Eaglesome et al. (1992) and Miller et al. (1994) reported that chronic cases of GVV, by *U. diversum*, if not diagnosed can evolve to endometritis which can result in abortion or infertility.

In agreement with previous data (Ruhnke et al., 1978; Pilaszek and Truszczynski, 1988; Mulira et al., 1989), then was a high frequency of isolations in animals with presence of granulation (91.5%). A justification for these rates could be the fact that granulate lesions are related to acute infection cases, and as a result the microorganism would be present in larger numbers thereby facilitating the detection.

The agent may be associated with early embryonic death and abortion in the first trimester (Ruhnke and Rosendal, 1994). Maxie (1986) reports that *U. diversum* is one of the most important agents involved in abortion in Ontario, Canada, where the microorganism was responsible for 10% of the cases for 10 years. In this study, the reporter of abortion and repeat breeding, revealed low relative risks with regard to being directly related to *U. diversum*.

Age and breed were analyzed according to the animal’s susceptibility to the microorganism. Cows presented higher frequency (60%) of *U. diversum* detection than heifers (40%). These data are compatible with an epidemic inquiries completed by Huffman et al. (1985) in California, USA, when the prevalence of the microorganism in genital tracts of females of several ages was studied. Young calves, 1-month of age, had a 21% prevalence; breeding age heifers 79% and mature cows had a 45% prevalence. In spite of a higher frequency of detection in cows compared with heifers relative risks were greater for heifers (1.68) compared with cows (0.78). In this case, it was observed that then was a protective effect of exposure of cows to the microorganism (RR < 1).

It was observed that cows may be exposed to *U. diversum* to a greater extent than heifers, which can result in greater susceptibility to infection. Cows may be exposed to a greater extent than heifers, possibly due to the longer potential time for contact with the microorganism.

In the analysis of breed, Holsteins had a lesser infection with the microorganism. This is in agreement with León et al. (1995) who reported the Holstein breed had a lesser rate of infection with the microorganism. The author suggested a persistent immuno-mediated resistance in Holsteins that did not exist in other breeds.

5. Conclusion

The statistical analysis revealed that even though the study was undertaken in the same geographical area, the farms had different rates of infection with *U. diversum*. Purulent vaginal discharge was the symptom most closely related to infection, suggesting an involvement with uterine infection. Cows had a high isolation’s frequency but the risk of
heifers to infection was greater. The Holstein breed was numerically more resistant to the microorganism.

As the GVV associated to *U. diversum* is not known in Brazil, the present data can contribute to a greater understanding of this disease.

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**References**


