Short Communication

Presence of Leptospiral DNA in Semen Suggests Venereal Transmission in Horses

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ABSTRACT

The purpose of the present study was to detect leptospiral DNA by PCR in semen and urine samples of stallions to test for venereal transmission in horses. A total of 10 stallions from four herds were studied, and sampling was conducted in semen and urine for culture and PCR and serum for serology. From the 10 serum samples tested, 6 (60%) were seroreactive. No pure culture was obtained, but leptospiral DNA was detected by PCR in 50% of the semen samples and 30% of urine samples. The present study aimed to detect leptospiral DNA by PCR in semen and urine samples of stallions to test for venereal transmission in horses. Based on these findings, we suggest that there is potential transmission of leptospirosis in horses by sexual transmission.

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1. Introduction

Leptospirosis is an important infectious disease in livestock, determined by the presence of spirochetes belonging to the genus Leptospira sp, and is reported worldwide, particularly in tropical countries [1,2].

Although leptospires are usually reported colonizing the kidneys [3], it has been demonstrated that they can also be present in the genital tract of cattle and small ruminants, leading to reproductive problems [4,5]. Furthermore, the presence of leptospires or their DNA in semen and vaginal fluid of naturally infected cattle, small ruminants, and swine has been demonstrated, indicating a possible transmission of leptospirosis by sexual contact [4-7]. Nevertheless, there is only one report of leptospiral DNA in stallions’ semen [8].

Leptospirosis is known to be strongly associated with impaired reproductive performance in horses [9]. Hence, by analogy to cattle and small ruminants, where leptospiral DNA has been reported in semen [5,6], contaminated stallions’ semen may represent an important source of transmission. The present study aimed to detect leptospiral DNA by PCR in semen and urine samples of stallions to test for venereal transmission in horses.

2. Material and Methods

2.1. Animals

A total of 10 adult (7-18 years old) stallions from different breeds (two Campolina, four Quarter horses, three Mangalarga Marchador, and one Thoroughbred) were studied. Animals were from four herds from Rio de Janeiro, Brazil, and were previously known to be seroreactive for leptospirosis but had no clinical signs compatible with leptospirosis. Additionally, all animals were vaccinated against herpesvirus but not against leptospirosis.

2.2. Samples

Blood, semen and urine samples were collected from 10 animals. Blood samples were collected into evacuated tubes (BD, Vacutainer, Franklin Lakes, NJ, USA) by jugular

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venipuncture. For semen collection, the artificial vagina method, using a Botucatu model (Biotech Botucatu/ME Ltd., Botucatu, SP, Brazil) was used. After semen sampling, furosemide (Intervet, Cotia, SP, Brazil) was administered, 0.5 mg/kg, intravenously, and urine was collected in 50-mL sterile tubes (BD Diagnostics, Franklin Lakes, NJ, USA) and immediately inoculated into culture medium tubes. All samples were chilled and transported to the laboratory.

2.3. Serology

For detection of anti-Leptospira antibodies, a microscopic agglutination test was performed using a panel of live leptospires including 28 serovars representing 11 serogroups, according to the Office International des Epizooties [10]. The infective serovar was that which presented the highest titer. Because animals were kept in an endemic region for leptospirosis, samples were considered reactive when titers reached ≥200.

2.4. Bacteriology

Semen and urine samples were inoculated into liquid Ellinghausen–McCullough media (EMJH) and Fletcher medium tubes (BD Diagnostics). Tubes were incubated at 28°C for 30 weeks and examined weekly with darkfield microscopy [1]. The 5-fluorouracil (Sigma-Aldrich, St. Louis, MO, USA) and filtration (using a 0.22-μm sterile syringe filter; Millipore Corporation, Billerica, MA, USA) were used to maintain cultures.

2.5. PCR

PCR was conducted according to the method described by Hamond et al. [3], using the primers LipL32-45 F (5’-AAG CAT TAC CGCTTG TGG TG-3’) and LipL32-286 R (5’-GAA CTC CCA TTT CAG CGA TT-3’). A molecular weight marker was included for assessing amplicon sizes, and both positive and negative controls were applied. In order to minimize false-negative results, an internal DNA control marker was included for assessing amplicon sizes. Seroreactivity against serovar Bratislava was not surprising, since this serovar has been reported in horses (preferential hosts) for serovar Bratislava was not surprising, since this serovar has been reported in horses and is associated with reproductive failure and important economic hazards, particularly in the region studied [9,11,12]. Furthermore, seroreactivity against incidental serovars such as Icterohaemorrhagiae or Copenhageni was not surprising, since this serovar has been reported in horses [1,3,11], and it was not surprising to find in the present study. Although culturing presented negative results, the most important outcome of this study was evidence of leptospiral DNA in the semen of all of the stallions, all of which were also seroreactive (P < .05). Leptospiral DNA was also detected in 3 of 10 (30%) urine samples from animals that were also seroreactive (P < .05) as shown in Table 1.

4. Discussion

The occurrence of anti-Leptospira agglutinins was expected, as this serological scenario has been reported worldwide, mainly in tropical areas. Leptospirosis is also endemic in Brazil and particularly in Rio de Janeiro, where it has been reported for many species, including horses [2,3,9,12]. Additionally, although those 10 stallions had never been tested, the four studied herds were previously known to present leptospirosis. Seroreactivity for serovar Bratislava was not surprising, since this serovar has been reported in horses (preferential hosts) and is associated with reproductive failure and important economic hazards, particularly in the region studied [9,11,12]. Furthermore, seroreactivity against incidental serovars such as Icterohaemorrhagiae or Copenhageni (members of the same serogroup) has been extensively reported in horses [1,3,11], and it was not surprising to find in the present study. Although culturing presented negative results, the most important outcome of this study was evidence of leptospiral DNA in the semen of stallions regardless of the presence of clinical or reproductive symptoms. An important factor that impairs the isolation of leptospires from clinical samples (e.g., urine or semen) is contamination by other microorganisms. Despite using 5-fluorouracil (Sigma-Aldrich, St. Louis, MO, USA) and filtration (0.22-μm sterile syringe filter; Millipore Corporation), maintenance of cultures to reduce the contamination was not effective.

There is consensus that leptospirosis is transmitted among animals by direct or indirect contact with contaminated urine of carriers [11]. Nevertheless, those findings suggest that, by analogy to ruminants, where it has been discussed [5,6], venereal transmission of leptospirosis may also occur in horses.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Serological, molecular, and bacteriological outcomes in semen and urine samples for the diagnosis of leptospirosis in 10 stallions from Rio de Janeiro, Brazil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>Serology (serovars)</td>
</tr>
<tr>
<td></td>
<td>Bratislava Copenhageni</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
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<td>3</td>
<td>A</td>
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<td>7</td>
<td>C</td>
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<td>8</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>D</td>
</tr>
<tr>
<td>10</td>
<td>D</td>
</tr>
<tr>
<td>Total no. of positive (50%)</td>
<td>3 (30%)</td>
</tr>
</tbody>
</table>

+, positive; -, negative.
A possible limitation of this study is the possibility that the leptospiral DNA detected in semen originated from urine that could have contaminated the urethra. In order to minimize that bias, semen samples were collected prior to the administration of furosemide and after the last voiding of the animal. Nevertheless, despite all efforts to avoid urinary contamination in semen, we cannot ignore the fact that sampling methods used did not unequivocally prove DNA detected in semen was not a result of such contamination. It is noteworthy that independently of the period of permanence (permanent or transitory), leptospires were present in the semen, which may represent a source of the transmission of the disease among horses by sexual contact. It is well known that leptospirosis in horses represents an important disease of the reproductive sphere, as it leads mainly to abortions and the birth of weak foals. Additionally, leptospire-induced abortions in mares usually occur in the last months of gestation and usually result from infections acquired later in pregnancy [13]. Although apparently less common, early embryonic death has been reported in cattle [13] and may also occur in mares, reinforcing the importance of the venereal transmission of the disease.

5. Conclusions

In conclusion, this study demonstrated the presence of leptospiral DNA in the semen of stallions. Based on these findings, we suggest that there is the potential for transmission of leptospirosis in horses by sexual contact.

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References