

## Leptospirosis on animal reproduction: IV. Serological findings in mares from six farms in Rio de Janeiro, Brazil (1993-1996)

### Leptospirase em reprodução animal: IV. Achados sorológicos em éguas de seis propriedades no Rio de Janeiro, Brasil (1993-1996)

CORRESPONDENCE TO:  
Walter Lilenbaum  
Departamento de  
Microbiologia e  
Parasitologia  
Universidade Federal  
Fluminense  
Rua Hernani Mello, 101  
24210-130 – Niterói – RJ  
e-mail: mipwalt@vm.uff.br

1 - Departamento de Micro-  
biologia e Parasitologia da  
UFF – RJ

Walter LILENBAUM<sup>1</sup>

#### SUMMARY

From February 1993 until October 1996, 547 blood samples were collected from mares with reproductive problems in six farms of the state of Rio de Janeiro, Brazil, and tested by the microscopic agglutination test (MAT) with live antigens for antibodies to seven leptospira serovars. A total of 235 positive reactions (42.96%) at a minimum serum dilution of 1:100 were observed against one or more serovars, which in comparison to other studies developed in Brazil, may be considered as a high prevalence. On mares with anti-leptospiral antibodies, the predominant serovar was *icterohaemorrhagiae*, reactive on 102 (43.40%) samples, followed by 64 (27.23%) reactions against *bratislava*, and 34 (14.47%) against *pomona*. The high prevalence of antibodies to serovar *icterohaemorrhagiae* observed in this study could have been expected, because in Brazil about 36% of the population of the maintenance host of such strain, *Rattus norvegicus*, are leptospiral carriers, mainly to strains of the *Icterohaemorrhagiae* serogroup.

**UNITERMS:** Leptospirosis; Abortion; Reproduction; Horses.

#### INTRODUCTION

Pathophysiology of reproductive problems and abortion determined by leptospirosis on cows and sows are well studied; however, the knowledge on equine leptospirosis, specially the reproductive pathogenesis of the disease, is still limited.

The association between leptospiral infection and equine disease has been controversial over a number of years, but it is now clear that it is an important cause of equine abortion<sup>3,6</sup>. The leptospiral infection in horses has been associated to uveitis<sup>16</sup>, keratitis<sup>17</sup>, fever and jaundice<sup>19</sup> and, frequently, to abortions<sup>1,5</sup>.

Serological surveys showed that the disease occurs worldwide, but the predominant serovars of *Leptospira interrogans* in different countries may vary<sup>7</sup>. The prevalence of *icterohaemorrhagiae* in horse infection was referred in England<sup>10</sup>, Northern Ireland<sup>6</sup> and Minnesota<sup>16</sup>. The serovar *bratislava* was found in Ontario<sup>11</sup>, Italy<sup>3</sup> and Kentucky<sup>19</sup>. Ellis *et al.*<sup>7</sup> suggested that *bratislava* is host-adapted to the horse, but the clinical significance of the infection with this serovar is still unclear<sup>11</sup>.

In Brazil, in spite of the enormous population of horses, there are few studies on equine leptospirosis. The first study was published in 1957<sup>4</sup>, a serological survey on horses from São Paulo, and observed the predominance of serovar *icterohaemorrhagiae*. In 1960, Freitas *et al.*<sup>8</sup> submitted 34 aborted fetuses to dark field and histological examination and confirmed the occurrence of abortion associated with leptospirosis. In 1968, Santa Rosa *et al.*<sup>15</sup> related the occurrence of antibodies against *pomona* and in 1974 Cordeiro *et al.*<sup>2</sup> carried out a

serological survey in 404 equine blood sera and registered the predominance of serovars *pomona* and *tarassovi*.

The isolation of *Leptospira interrogans* serovar *icterohaemorrhagiae* from the kidney of an aborted foetus was referred in 1981<sup>9</sup> and represents the first isolation of a leptospirae strain from a horse in Brazil. In 1986, Yasuda *et al.*<sup>22</sup> reported the isolation of *Leptospira biflexa* serovar *ranarum* from an aborted equine foetus and discussed its importance in the aetiology of the abortion. The prevalence of *icterohaemorrhagiae* on mares with reproductive problems<sup>12</sup> and in horses without clinical signs in São Paulo<sup>14</sup> was presented in 1993.

The objectives of this study were to determine (1) the seroprevalence of *Leptospira* in mares with reproductive problems in Rio de Janeiro, Brazil and (2) the predominant serovars in those animals.

#### MATERIAL AND METHOD

From February 1993 until October 1996, 547 blood samples were collected from mares originated from different farms and with reproductive problems, such as estrous repetition, low conception rates, premature foaling and abortion, about 34 days after the occurrence of the abortion or the estrus repetition. In premature foaling cases, the blood samples were collected 3-5 days *post-partum*, and a second sample, when possible, 30 days after foaling. Sera were obtained by centrifugation and stored at -34°C until being tested as a batch.

Sera were originated from six farms representing all geographic regions of the Rio de Janeiro State, and tested using the microscopic agglutination test (MAT) with live antigens<sup>7</sup> for antibodies to seven leptospira serovars: *andamana* (CH11), *pomona* (Pomona), *canicola* (Hond Utrecht IV), *icterohaemorrhagiae* (RGA), *hardjo* (Hardjoprajtino), *bratislava* (Jez bratislava) and *ballum* (Mus 127). Titres were reported as the reciprocal of the highest dilution in which 50% of the leptospire were agglutinated. Any sample having a titre >100 to any of the seven serovars was considered positive. All samples reactive to more than one antigen at the dilution of 1:100 were subsequently titrated against reacting antigens using serial 2-fold dilutions of serum until the highest titre was obtained in order to determinate the infective serovar.

## RESULTS

Of the 547 mares tested, 235 were positive at a minimum serum dilution of 1:100 against one or more *Leptospira* serovars, representing 42.96%. Titres to more than one serogroup were a common occurrence.

Of the 235 positive mares, 102 (43.40%) reacted against serovar *icterohaemorrhagiae*, followed by 64 (27.23%) against *bratislava*, 34 (14.47%) against *pomona*, 17 (7.24%) against *ballum*, eight (3.40%) against *hardjo*, six (2.56%) against *canicola* and four (1.70%) against *andamana*, a *Leptospira biflexa* serovar (Fig. 1). Reactions to serovar *andamana*, *canicola* and *ballum*, unusual in equine samples, were considered as coagglutinations, and are not included on Fig. 1. With 98 mares, a second serum specimen was obtained, and at least a four fold - increase of titers was observed to serovar *icterohaemorrhagiae* in 63 (64.28%) samples, represented by 29 with 400, 25 with 800 titres, five with 1600 and four samples that reached 3200. To serovar *pomona* nine (9.18%) samples presented titres of 400 (four samples), 800 (four samples) and 1600 (one sample). The titres found with the second sample can be observed on Fig. 2.

## DISCUSSION

As the diagnosis of equine leptospirosis by culture or by demonstrating leptospira in equine tissue is limited<sup>19</sup>, the serological evidences in mares with reproductive problems, specially with a historical of abortions, although not confirmed through statistical methods, suggests the existence of a relationship between leptospirosis and reproduction problems in horses, as it was observed in Northern Ireland<sup>6</sup> and in Kentucky as well<sup>1</sup>.

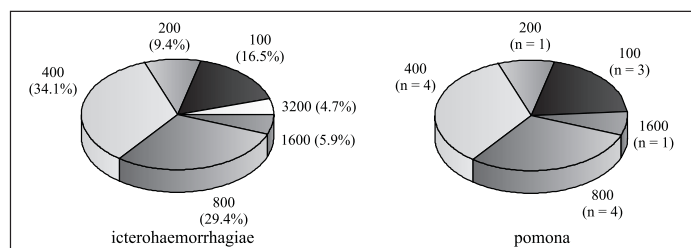


Figure 1

Prevalence of *Leptospira* serovars in mares with reproductive problems from six farms in Rio de Janeiro, Brazil (1993-1996).

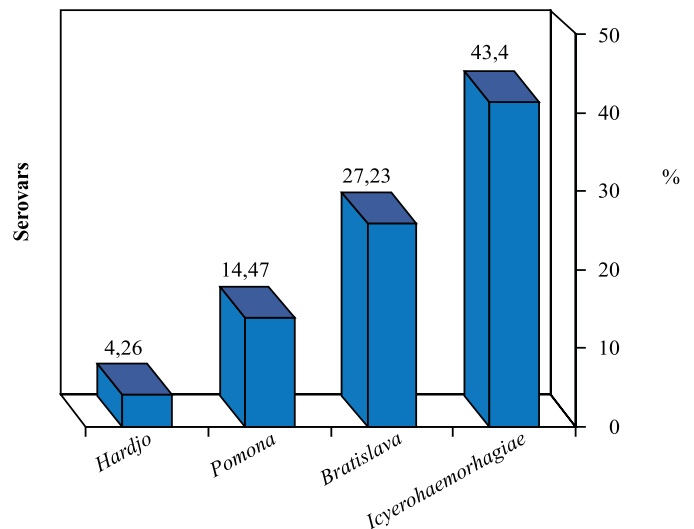


Figure 2

Distribution of titres on the second sample obtained from mares with reproductive problems in Rio de Janeiro, Brazil (1993-1996).

The percentage of reactors was 42.96%. A similar finding was observed in Kentucky, where Williams *et al.*<sup>19</sup> reported 42.2% of positive horses. In England, Hathaway *et al.*<sup>10</sup> reported 34.6% of reactivity and, in Australia, sera from 41.5% of the horses reacted to one or more of the tested serovars<sup>18</sup>. Therefore, it was lower than with those observed in Northern Ireland by Ellias *et al.*<sup>7</sup>, that found antibodies in 89.1% of the tested sera.

In comparison to other studies developed in Brazil, 42.96% of reactors may be considered a high prevalence, since they found positivities of 4.53%<sup>9</sup>, 10.11%<sup>21</sup> and 26.92%<sup>2</sup>. This could be explained by the non-inclusion of serovar *bratislava* as an antigen in those surveys, since in the present study the seroreactivity to this serovar represented 11.70% of the total of samples.

Although equine leptospirosis is a widespread infection, the spectrum of leptospiral titres in horses is probably a reflection of exposure to serotypes maintained by other animals in the same geographical region<sup>10</sup>, consequently may vary significantly by region<sup>19</sup>.

The greatest number of positive horses in this study reacted with serovar *icterohaemorrhagiae*, which is maintained by rats. Ellis *et al.*<sup>7</sup> found that 26% of mares had antibodies to a serovar of *Icterohaemorrhagiae* serogroup in Northern Ireland; Williams *et al.*<sup>19</sup> related in central Kentucky, USA an average of 21.5% of seropositivity to that serovar, and, in Brazil, it was reported the predominance of *icterohaemorrhagiae* on serological surveys accomplished on horses<sup>9,14</sup>. The incidental infections of horses by strains maintained by other hosts are important, and there is a considerable opportunity of transmission between rats and housed horses<sup>6</sup>. So, the high prevalence of antibodies to serovar *icterohaemorrhagiae* in mares from Rio de Janeiro could have been expected, since about 36% of the population of *Rattus norvegicus* are leptospirae carriers in Brazil, with the predominance to strains of *Icterohaemorrhagiae* serogroup<sup>13</sup>.

The serovar *bratislava* was suggested to be host-adapted to the horse<sup>7</sup>, what could explain the high prevalence of antibodies usually observed with this serovar. However, isolations data are still insufficient to support this fact<sup>19</sup>. In this study, 27.23% of positive

mares reacted to *bratislava*, making it the second most prevalent serovar to *icterohaemorrhagiae* in terms of frequency of occurrence. Since host-adapted strains causes little pathogenicity in this host<sup>20</sup>, a higher prevalence of non-adapted and, consequently, more aggressive strains could be expected in those mares with reproductive problems and abortions.

The serovar *pomona* was found in many parts of the world, and is linked to periodic ophthalmia, jaundice and abortion in horses<sup>1,16,17,19</sup>. It is maintained in cattle and swine, and the horse is an incidental host. In Brazil, *pomona* has been recognized as an important

agent in animal leptospirosis, including cattle, swine and horses<sup>8</sup>. In 1968, Santa Rosa *et al.*<sup>15</sup> related *pomona* as the most frequent serovar on equine leptospirosis, with rates of 13.6% of reactivity. In 1974, Cordeiro *et al.*<sup>2</sup> worked with horses of the state of Minas Gerais and also observed predominance of *pomona*, with 26.15% of positivity.

Knowledge of the role and importance of leptospirae infections in horses is necessary in order to allow the understanding of the epidemiology of the disease all over the world, since economic losses due to abortions and low reproductive efficiency on mares infected with leptospirae strains have still not been calculated.

## RESUMO

De fevereiro de 1993 a outubro de 1996, 547 amostras sanguíneas foram colhidas de éguas com problemas reprodutivos de seis propriedades no Estado do Rio de Janeiro, Brasil, e testadas pela técnica da soroaglutinação microscópica (MAT) com antígenos vivos para anticorpos perante sete sorotipos de leptospiras. Um total de 235 reações positivas (42,96%) a um título mínimo de 100 foram observadas perante um ou mais sorotipos de leptospira, o que, em comparação com outros estudos já realizados no Brasil, pode ser considerado uma alta prevalência. Em éguas com anticorpos antileptospiras, o sorotipo predominante foi *icterohaemorrhagiae*, reativo em 102 (43,40%) amostras, seguido de 64 (27,23%) reações positivas para *bratislava* e 34 (14,47%) perante *pomona*.

**UNITERMOS:** Leptospirose; Aborto; Reprodução; Equinos.

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