Trop Anim Health Prod (2012) 44:1927–1930
DOI 10.1007/s11250-012-0158-5

ORIGINAL RESEARCH

Subclinical leptospirosis may impair athletic performance in racing horses

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Accepted: 11 April 2012 / Published online: 2 May 2012

Abstract The infection by *Leptospira* in horses, in both its acute disease and subclinical forms, is very common, particularly in endemic regions. Therefore, the objective of this study was to evaluate the effects of subclinical leptospirosis in the athletic performance of racing thoroughbred horses. Athletic performance of 119 racing Thoroughbred horses from Rio de Janeiro, Brazil, was calculated by assigning a point value for the results in racing (performance index (PI)), and serology for leptospirosis was conducted. A total of 85 (71.4 %) horses showed reactive titers (≥100), and of which 52 had high titers (34 with 400 and 18 with ≥800). Although those animals had high titers against *Leptospira*, no clinical signs associated with leptospirosis were observed. Seventeen (89.5 %) out of the 19 horses with substandard performance were seroreactive with high titers, in contrast with 35 % of seroreactivity in horses with good athletic performance ($P<0.0001$). Additionally, seroreactivity to leptospirosis was more often observed in horses with substandard athletic performance in contrast to those with good performance ($P<0.0001$, odds ratio 15.8). The Average PI of this group increased to 133 % after treatment ($P<0.0001$). Leptospirosis may impair performance in racing horses, and antibiotic therapy may improve the performance of affected animals.

Keywords Leptospirosis · Athletic performance · Horse

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Introduction

Leptospirosis is a widespread zoonotic disease caused by spirochetes belonging to the genus *Leptospira* and is largely reported worldwide, particularly in tropical countries (Faine et al. 2000; De Faria et al. 2008). Titers to several serovars have been reported in horses. While in North America (USA), Pomona (Timoney et al. 2011) seems to be predominant, in tropical countries, serovars belonging to the Icterohaemorrhagiae serogroup, such as Copenhageni, tend to be most prevalent (Langoni et al. 2004; Båverud et al. 2009).

The clinical syndrome of leptospirosis in horses is characterized by uveitis (Yan et al. 2010), abortion, stillbirth, prematurely born foals (Timoney et al. 2011), and renal and hepatic dysfunction, and signs that have been commonly observed include hematuria, fever, jaundice, anorexia, and respiratory distress (Yan et al. 2010). Nevertheless, not all infected animals present the acute disease, and subclinical forms are very common, particularly in endemic regions (Langoni et al. 2004; Jung et al. 2010; Houwers et al. 2011).

Poor athletic performance of racehorses is a major problem in the racing industry (Wilsher et al. 2006). Athletic performance may be studied according to a variety of parameters, as number of starts, completion of at least one postoperative start, lifetime earnings, race class level, or earnings per start (Schnabel et al. 2007). There are also more complex means of analysis for assessing performance, including regression models and the calculation of the performance index (PI), as suggested (Martin et al. 2000).

Subclinical forms of various infections may affect one or more systems which are involved in physical exertion and may, therefore, impair athletic performance. Therefore,

laboratory detection of such infections may be required in order to improve both performance and welfare (Richard et al. 2010). In fact, although other subclinical infections, such as babesiosis, have already been associated with reduced athletic performance of horses (De Waal 1992), there are no report regarding to the possible role of subclinical leptospirosis on the athletic performance of racing horses. Therefore, the objective of this study was to evaluate the effects of subclinical leptospirosis in the athletic performance of racing thoroughbred horses.

Material and methods

Animals

From June to October of 2010 (dry season in Brazil), a total of 119 racing Thoroughbred horses were studied, which include 48 fillies, 50 colts, and 21 geldings. Inclusion criteria were age (3–4 years); running on sand track at distances from 1,300 to 1,600 m in Jockey Club Brasileiro in Rio de Janeiro, Brazil; and acceptance of the owners. Additionally, all studied horses were kept, at all time, under the same general conditions (e.g., training and feeding). Although the animals had been foaled and raised in various regions from Brazil, all of them were in the Jockey Club for at least 2 years. Despite the fact that this is an endemic area, none of these animals had been vaccinated against leptospirosis. All horses were tested negative for babesiosis (blood smear staining).

Serology

Whole blood was collected in Vacutainer® tubes from the jugular vein. Sera were harvested following centrifugation of clotted blood and stored at −20°C prior to batch testing. A microscopic agglutination test (MAT) was employed as recommended (OIE 2008). Samples were screened at a 1:100 dilution using a panel of live antigen strains of *Leptospira interrogans* serovars Australis (Ballico), Bataviae (Swart), Bratislava (Jez bratislava), Canicola (Hond Utrech IV), Copenhageni (M 20), Grippotyphosa (Moskva V), Icterohaemorrhagiae (RGA), Pomona (Pomona), Pyrogenes (Salinem), and Wolffi (3705); and *Leptospira borgpetersenii* serovars Ballum (Mus 127), Hardjo (Hardjobovis), Sejroe (M 84), and Tarassovi (Perepelicin). All strains were grown in a liquid medium (EMJH; for 7–10 days at 28–30°C), free of contamination or autoagglutination. All samples with agglutinating activity at a 1:100 dilution were considered positive and subsequently titrated against reacting antigens, using serial twofold dilutions of serum. The endpoint was the highest tube in which 50 % agglutination was recorded, which was measured by comparison with a control suspension (Faine et al. 2000).

Clinical examination

At the moment of the blood collection, a detailed clinical examination of each horse was conducted, including the following parameters: mental attitude; nutritional status; appetite; presence of ectoparasites; nasal secretions; skin turgor; eyeball retraction; palpation of lymph nodes; measuring of the rectal temperature, pulse, and capillary refill time; auscultation of the digestive tract, heart, and lungs; and a thorough clinical examination of the musculoskeletal system.

Athletic performance

Racing data were obtained based on the studbook of the Brazilian Association of Race Horses. PI was calculated as recommended (Martin et al. 2000) by assigning a point value for the results in racing: first place = three points, second place = two points, and third place = one point. The PI was the mean of the points earned in three races and the sum of total earnings of each horse. Considering the PI of the last 12 races, animals were categorized into two groups: good performance (PI≥8) or substandard performance (PI<8).

Statistics

Nonparametric data were analyzed by the Fischer's exact test, while parametric data were analyzed by Student's *t* test. Data analysis was conducted using the SPSS statistical software (SPSS Inc., Chicago, USA), and results were considered as significant when $P<0.05$ for clinical analysis comparing reactive and nonreactive samples. The odds ratio (OR) was calculated considering a 95 % of confidence interval (CI).

Results

From the 119 samples tested, 85 (71.4 %) showed reactive titers (≥100), all of them against serovar Copenhageni, and of which 52 had high titers (34 with 400 and 18 with ≥800). Weak reactions with low titers were also observed against serovar Icterohaemorrhagiae (from the same serogroup) and were interpreted as cross-reactions. Animals were generally apparently healthy, either seroreactive or seronegative.

Among the 119 horses, 19 (15.9 %) presented with substandard performance (average PI 7.0), while the other 100 (84.1 %) showed good athletic performance (average PI 14.3). When correlated with serology, it was observed that seroreactivity (titers 200 and 400) was observed in 17 out of the 19 horses with substandard athletic performance (89.5 %), in contrast to 35 out of the 100 horses (35 %) with good athletic performance ($P<0.0001$). Additionally, a seroreactive horse was 15.8 times more likely to exhibit

substandard performance than a seronegative one (OR, CI 3.4 to 72.3).

After serodiagnosis, the 17 seroreactive horses that presented insufficient athletic performance were treated with the recommended treatment for leptospirosis (Faine et al. 2000), i.e., a single dose of penicillin combined to streptomycin (Pen & Strep, Lavizoo, São Paulo, Brazil). Three months after that specific treatment, athletic performance was reevaluated, and serological test was reapplied. At that time, all horses were seronegative, and 16 out of 17 (94.1 %; CI 71.3 to 99.8) reached a satisfactory PI (≥8). The average PI of this group increased from 7.6 (before treatment) to 17.9 (after treatment), an increase of 133 % ($P<0.0001$). In the other treated horse, although an improvement of PI could be observed, (PI=0–6), it was still classified as substandard (Fig. 1).

Discussion

The observed occurrence of anti-*Leptospira* agglutinins (71.4 %) in the studied population of horses is high, even when compared to another study conducted in Brazil that reported 66.8 % (Langoni et al. 2004). This finding was not completely unexpected, since Jockey Club Brasileiro in Rio de Janeiro is located in a flat area subject to constant flooding, mainly in the rainy season, which favors the transmission of leptospiral infection. In the present study, all the reactive horses showed titers to serovar Copenhageni. This was also an expected finding, since leptospires of the Icterohaemorrhagiae serogroup are maintained by the brown rat (*Rattus norvegicus*), and the spectrum of leptospiral titers in horses is probably a reflection of exposure to serovars maintained by other animals in the same geographical region (Hathaway et al. 1981). Previous studies in Brazil referred that about 36 % of the *R. norvegicus* population are leptospiral carriers (Lilenbaum 1998), with reports of up to 80.3 % carriage of *L. interrogans* serovar Copenhageni in *R. norvegicus* from urban settings (De Faria et al. 2008).

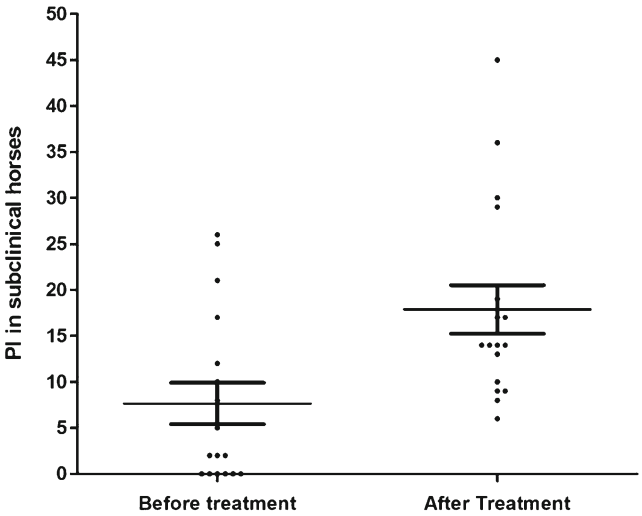

Fig. 1 Performance index in 17 Thoroughbred racehorses of Rio de Janeiro, Brazil, with subclinical leptospirosis before and after treatment with penicillin–streptomycin

Results of the present study also agree with other studies conducted in horses in Brazil (Lilenbaum 1998; Langoni et al. 2004), which indicate either Icterohaemorrhagiae or Copenhageni, i.e., serogroup Icterohaemorrhagiae, as the most frequent agents of leptospirosis in horses.

Although the animals presented high titers anti-*Leptospira*, the animals were generally apparently healthy. No attempt to direct evidence of leptospires (PCR or bacterial culture) was performed. Nevertheless, considering that those animals presented high titers at MAT, that they live in an endemic area with no vaccination program, and that they presented good response with specific antibiotics, we assume that they presented subclinical leptospirosis, what is very often observed in horses (Jung et al. 2010; Houwers et al. 2011).

Suboptimal athletic performance of racehorses is a major problem in the racing industry (Wilsher et al. 2006). In the present study, the majority (89.5 %) of the animals with insufficient athletic performance was seroreactive to leptospirosis, and they showed an average of 133 % improvement of PI after treatment, which are significant findings that reinforce that the subclinical leptospirosis and athletic performance may be related.

A possible bias on this study is the absence of a control-untreated group; since for ethical reasons and due to owners' requests, all the 17 seroreactive horses with substandard athletic performance were treated. Additionally, all horses were weekly examined in the 2 months after treatment. Although all of them showed an improvement in their athletic performance after specific antibiotic therapy, we cannot ignore that it is possible, although not probable for all the horses, that acquired immune responses were responsible for clinical recovery and for improved athletic performance.

We conclude that there is evidence that, even when subclinical, leptospirosis may impair athletic performance in racing horses and that specific antibiotic therapy may improve the athletic performance of affected animals.

Acknowledgments This study was supported by CNPq and FAPERJ, Brazil. WL is a CNPq fellow.

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