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Original Research

Epidemiological Survey of Equine Pythiosis in the Brazilian Pantanal and Nearby Areas: Results of 76 Cases

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ABSTRACT

A clinical epidemiological study was conducted among 34 rural properties located within the Brazilian Pantanal region and nearby areas between 2007 and 2010. The diagnosis of equine pythiosis was based on antibody detection (by enzyme-linked immunosorbent assay), polymerase chain reaction, histopathological analysis, and cultures positive for *Pythium insidiosum*. The majority of the affected animals (85%) were in the Pantanal biome, which had a higher disease prevalence (0.9%–66.7%) than that of the Cerrado (2.7%–33.3%). The disease was more prevalent in the rainy season (January–March), with an abrupt fall in the number of cases during the drought period (April–September; correlation of $R^2 = 0.77$; $P < .01$). Generally, the average prevalence of equine pythiosis in both regions was 5%, with mortality and lethality rates of 1.3% and 23.1%, respectively, in the Pantanal and 2.3% and 45.5%, respectively, in the Cerrado. However, the treatment with immunotherapy may have underestimated these numbers, especially in the Pantanal. Animals older than 1 year were 8.09 times more affected by the disease than younger animals in the same environment ($P < .05$). A correlation between the anatomical area of the lesion and the type of skin color was also observed. Approximately 73% of the lesions were found in dark-pigmented areas, and animals with a dark coat color were affected more frequently. These findings highlight the importance of hematophagous insects in the epidemiology of pythiosis because these areas are preferred for blood feeding.

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1. Introduction

Pythiosis is a life-threatening infectious disease in mammals and birds caused by the oomycete *Pythium insidiosum* [1,2]. Horses are the animals most frequently infected and typically develop ulcerative granulomas with

abundant serosanguineous discharge [1,3,4]. The *P. insidiosum* organism is essentially aquatic, which explains the prevalence of the disease in people and animals that live in direct contact with stagnant water contaminated with zoospores, such as rice fields in Thailand and the Pantanal biome in Brazil [5,6]. Small skin lesions, such as those caused by mosquito bites, are believed to be the locus of penetration of *P. insidiosum* zoospores [1,7].

Equine pythiosis has been reported worldwide, although the Brazilian Pantanal probably contains the largest concentration of cases caused mainly by the combination of three factors: warm temperatures, flooded lands, and high

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numbers of animals [4,6,8]. Treatment of the disease in horses is based on radical surgical excision and/or immunotherapy. However, the rate of success is variable and depends upon many factors [1,3], which has spurred the research of different treatment options [9–12].

The disease poses significant economic losses for equine breeders in Brazil, but the numbers are unknown as are many aspects of its epidemiology and etiopathology. Therefore, this study reports the epidemiological aspects of 76 cases of *P. insidiosum* equine infection that occurred in the Brazilian Pantanal and nearby areas.

2. Materials and Methods

2.1. Characterization of the Studied Area

Data were obtained from 34 rural properties located within the subregion of Poconé, in the northern area of the Pantanal Matogrossense, Brazil. Twenty-four properties were located in the Pantanal biome, which is characterized by the intercalation of clay and sandy soils commonly associated with swamps, and 10 properties were located in the Cerrado (Brazilian savannah) and ecotones, which are more permeable but seasonally waterlogged soils that resemble the Pantanal.

Seasons in the studied regions are well defined, with the rainy period ranging from October to March and the drought period ranging from April to September (average annual rainfall is between 800 and 1,200 mm). The hot and humid weather is characteristically tropical, with an average annual temperature of 25.5°C and an average minimum temperature of 20°C in the winter and 32°C in the summer.

Climate data including the average temperature and monthly rainfall were obtained from the meteorological station Mestre Padre Bombed and the experimental farm at the Federal University of Mato Grosso.

2.2. Selection of the Study Population

The descriptive observational method was used for the study of the equine population at risk between the years 2007 and 2010. Cross-sectional analyses were used to evaluate the prevalence risk ratio in the herd. Analyses of each case were conducted because of migratory characteristics of the herd. Epidemiological data regarding the sex, age, species (horses or hybrids, which were composed mainly of mules), breed, coat color (bays, buckskins, chestnuts, grays or tobianos), presence of underlying diseases, vaccination, and previous treatment approaches were assessed for each animal enrolled. Clinical data for the site (axial or appendicular) and presentation of the lesions (unifocal or multifocal), and management and outcome of infection were also collected. The temporal distribution of cases was based on the size and aspect of the horse's lesion and body condition and information from the breeders.

2.3. Diagnosis

The presumptive diagnosis was based on the observation of ulcerated, granulomatous skin lesions with abundant

serosanguineous discharge. Mean lesion diameters varied from approximately 7 cm in the first week to 15–20 cm within 3 weeks. Chronic lesions showed draining fistulas containing yellow-gray stony masses referred to as “kunkers” [1] and were associated with weight loss in the animals. Definitive diagnosis was established by a positive result from at least two of the following procedures: indirect enzyme-linked immunosorbent assay (ELISA) [4], histopathological examination of tissue specimens with Grocott silver stain, isolation of the pathogen and subsequent induction of zoosporogenesis [13], and polymerase chain reaction (PCR) assay of the lesions conducted with *P. insidiosum*-specific primers [14].

2.4. Statistical Analysis

Data were analyzed using Epi Info software version 6 (Centers for Disease Control and Prevention, Atlanta, GA) [15]. The chi-square and Fisher exact tests were used to evaluate the association among the studied variables.

3. Results and Discussion

Seventy-six cases of equine pythiosis were diagnosed in the studied area from 2007 to 2010. The triad of flooded soil, abundance of aquatic vegetation, and high temperatures are highly favorable for development and maintenance of the oomycete *P. insidiosum* [1,4,6,7,16]. This environment is characteristic of the Pantanal biome, where 85.5% (65 of 76) of the cases were observed. The remaining 14.5% (11 of 76) of the cases were observed in the Cerrado biome and in transitional areas between the Pantanal and the Cerrado. The presence of naturally flooded areas forming small ponds was associated with disease in animals in the Cerrado region. Equids usually remain for long periods inside flooded areas or ponds, where they eat the grass on the surface, or simply as a behavioral characteristic [4,17], increasing the chance of contact with the pathogen. The spatial distribution showed a predominance of properties located in the Pantanal (70.6%), followed by the Cerrado (20.6%) and ecotones (8.8%). A highly variable disease prevalence was also observed between the biomes. The prevalence in the Pantanal region ranged from 0.9% to 66.7%, whereas in the Cerrado, it ranged from 2.2% to 33.3%. An irregular distribution of the cases was noted in the herds in consecutive years. This was likely to be a consequence of animal migration to different areas and/or rainfall variation. Generally, considering the total of animals exposed, the disease prevalence was approximately 5% in both biomes (Table 1).

Infection by *P. insidiosum* was more prevalent in adult animals (>1 year old). The relative risk of adult animals versus that of animals younger than 1 year old acquiring the disease was 8.07 ($P < .05$; 95% confidence interval [CI], 1.13–57.8, using the chi-square test; Table 2). This finding is in agreement with previous studies which indicate that equine pythiosis is more frequent in adult animals [6,18–21]. Interestingly, equine sarcoid has also been reported in adult animals between 1 and 7 years of age [22,23]. Indeed, the association between pythiosis and sarcoid has been previously reported, showing that ulcerated lesions

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