SPATIAL ANALYSIS OF PREVALENCE OF ANTI-LEPTOSPIRA AGGLUTININS AND EPIDEMIOLOGIC RISK IN CATTLE IN THE PANTANAL OF CÁCERES-MT, BRAZIL

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Abstract

The seroprevalence, spatial distribution and risk analysis of anti-leptospira agglutinins in cattle were studied in the Pantanal of Mato Grosso, Cáceres, Mato Grosso State (MT), Brazil in 2005. Geo-Technologies and scan statistics to evaluate possible areas of differential risk were used. The objective of this paper is to execute a spatial analysis of anti-leptospira agglutinins prevalence and epidemiological risk in cattle in the Pantanal of Cáceres/MT. The sample size was about 2.5% of cattle by pasture area, based on a population of 79,582 animals, aged over four months. We collected 2,123 blood serum samples from the cattle of four age groups, namely: from 4 to 12 months, from 13 to 24 months, from 25 to 36 months and older than 36 months from both genders. The sera samples were tested by a microscope agglutination technique, against 17 leptospira serovars. About 15% of the male cattle and 85% of female cattle were seropositive with at least one serovar, constituting 57.93% of total of samples. All farms presented seropositive cattle and the average prevalence found in 29 farms was about 61.07%. The risk analysis showed that the infection for pathogenic leptospira at cattle does not occur randomly, showing two areas with the highest relative risk, a primary cluster with RR of 1.45 and a secondary one with RR of 1.38. The primary cluster areas are associated to strong inundation areas whereas the secondary ones to moderate inundation areas, demonstrating that the leptospira bacteria causing leptospirosis can survive longer on strongly inundated areas.

Key words: Geo-Technologies. Spatial distribution. Moisture áreas. Upper Paraguay river basin. Mato Grosso State.

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Resumo

Análise espacial da prevalência de aglutininas anti-leptospira e de risco epidemiológico em bovinos no pantanal de Cáceres-MT, Brasil

A soroprevalência, distribuição espacial e análise de risco de aglutininas anti-leptospira em bovinos foi estudada no Pantanal matogrossense, no município de Cáceres-MT em 2005, utilizando geotecnologias e estatística de varredura para avaliar possíveis áreas de risco diferenciado. Este trabalho propõe realizar a análise espacial da prevalência de aglutininas anti-leptospira e de risco epidemiológico em bovinos no Pantanal de Cáceres/MT. O tamanho da amostra foi de 2,5% dos bovinos por pasto ocupado, baseado em uma população acima de quatro meses de 79.582 animais, sendo coletadas 2.123 amostras de soro sangüíneo de bovinos entre as faixas etárias de 4 a 12 meses, 13 a 24 meses, 25 a 36 meses e mais de 36 meses de ambos os sexos. Os soros foram testados pela técnica de aglutinação microscópica, frente a 17 sorovariedades de leptospiras. Cerca de 15% dos machos e 85% das fêmeas foram sororreagentes a pelo menos uma das sorovariedades, perfazendo 57,93% de todas as amostras. Todas as fazendas apresentaram bovinos sororreagentes e a prevalência média encontrada nas 29 fazendas foi de 61,07%. Na avaliação de risco, demonstrou-se que a infecção por leptospiras patogênicas para os bovinos não ocorre ao acaso, tendo duas áreas com maior risco relativo, um cluster primário com RR de 1,45 e um secundário com RR de 1,38. Sendo que as áreas de cluster primário estão associadas às áreas de inundação forte e as de cluster secundário, às áreas de inundação moderada, demonstrando que a bactéria causadora da leptospirose sobrevive mais tempo em áreas onde a inundação é mais intensa.

Palavras-chave: Geotecnologias. Distribuição espacial. Pantanal Matogrossense. Mato Grosso.

INTRODUCTION

In The Pantanal of Cáceres, the cattle breeding system is extensive and it was developed in large areas with few subdivisions. This fact hampers a more constant and accurate observation on the occurrence of reproductive disorders. In most rural properties the cattle management is done once a year.

The low rates of birth and weaning usually observed, frequently are attributed to seasonality in the supply of native pasture, although other factors such as a lack of reproductive and health management, appropriate to the region, are not considered. The Pantanal of Cáceres has an important role in meat production, with the commercialization of calves, steers and cows cullings, and the region can be considered as a “big maternity”. Allied to it, there is just one reference treating the brucellosis prevalence, performed by Tocantins (2000), and no other disease for cattle reproduction was studied in this area, including the Leptospirosis, a very dangerous infectious disease, because it is a zoonosis and responsible for economic losses due to weight loss, mastitis, abortion, stillbirth and mortality of calves (NEGRÃO et al., 1999; AGUIAR et al., 2006).

Ecological conditions influence on the occurrence of this disease, since the environmental contamination and the ability of the microorganism to survive for long periods of wetness, temperature (22°C) and pH (7,0-7,2), are factors which predispose the appearance of it (FAINE et al., 1999; ARAÚJO et al., 2005). Due to its characteristics, the Pantanal of Mato Groso presents ecological conditions favoring the occurrence of bovine Leptospirosis, since the agent survives longer in flooded areas and high temperatures (MADRUGA et al., 1980; PELLEGRIN et al., 1999).

In Brazil Leptospirosis is not subordinated to an organized combat by governmental agencies or private ones directed to animal health. This fact makes it difficult to know the correct extension of Leptospira sp infections in cattle anywhere in the country (ARAÚJO et al., 2005).

The development of a program to adopt appropriate actions for the ecosystem, must be preceded by basic epidemiological studies. Studies aiming to determine the frequencies and spatial distribution of infections caused by Leptospira sp in cattle in this region must obtain useful information for animal health agencies and for the owners of cattle. So it is possible to use Geo-Technology which "[...] *involves a set of techniques that requires health data organization in space, or rather on a geographic space representation. The space data discretization into territorial units, forming polygons, has been one of the best strategies used in epidemiology and especially in the ecological studies*" (BARCELLOS, 2003).

Taking that into account, the purpose of this paper is to execute a spatial analysis of anti-leptospira agglutinins prevalence and epidemiological risk for the cattle in Pantanal de Cáceres-MT. The knowledge obtained can be helpful to the development of future control programs, maximizing the cattle productivity and contribute significantly to increase the profitability for cattle breeders in the region.

MATERIALS AND METHODS

The Pantanal of Mato Grosso, is the worldwide largest floodplain in continuous area (138,183 km), covering part of both Mato Grosso and Mato Grosso do Sul States. Among the 11 Pantanal sub-regions, each one with its distinct ecological, topographical, soil and vegetation characteristics (SILVA; ABDON, 1998), there is the Pantanal of Cáceres, the area under study, named Sector I by *Instituto de Defesa Agropecuária do Estado de Mato Grosso* – INDEA-MT (Governmental Agriculture and Livestock Defense Institute of Mato Grosso State).

During the first visit to the rural properties, in 2004, an update of the cadastre was made, concerning the amount of farms, roads (trails) and positioning of the head offices using a GPS device, model Map 76, Garmin Stamp, which allowed the preparation of a map of the area under study (Figure 1).

To delineate the farm perimeter, initially a cadastral map at 1:500,000 scale was used, produced in 1942 by the Prefecture of Cáceres. Based on the coordinates of the cadastral map, eight topographic maps (SE –21-V-B-I, II, IV e V; SE-21-V-D-I, II, IV e V) were acquired from the *Diretoria de Serviço Geográfico do Exército* – DSG (Geographical Service from the Army) in 1:100,000 scale, to cover the area under study. Each farm perimeter was established and reviewed in the maps, with the support of the owners or their foremen. After validation from the areas of farms, the respective borders were traced, along with information from topographical maps (e.g. road system, hydrography, localities) and formed the base map used for the delineation of the pasture area from properties of Sector I.

The interviews were conducted from August to December 2004/2005 at the farms, using closed questionnaire seeking to obtain information related to the characterization of the property, the profile of the producer, animal demography, health and reproductive management. Other information were obtained from the INDEA-MT database, Local Unit of Execution in Cáceres. There we asked for the authorization to collect cattle blood. Totally 41 farms were visited, 35 had cattle, and from these 35 farms we worked with 29. Based on questionnaires and sketches of the pasture divisions we obtained the cattle size and composition by sex and age.

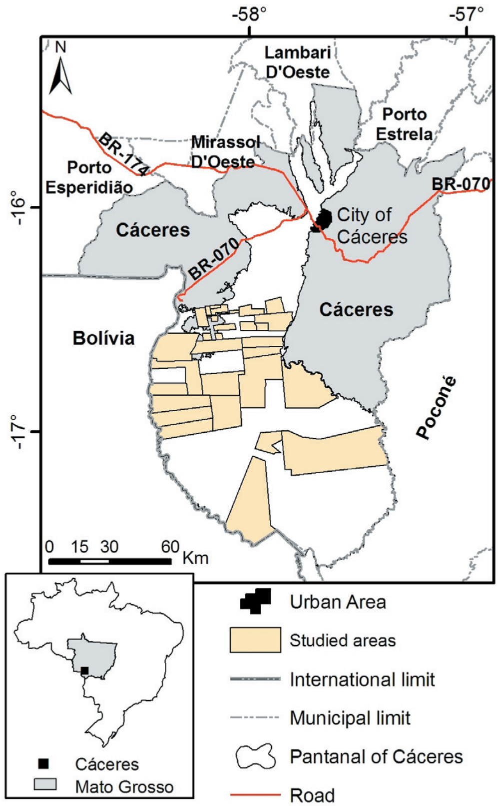

Figure 1 - Location of studied area – Farms of Sector I

The number of cattle serum samples was statistically established according to procedures recommended by the "*Centro Pan-Americano de Zoonoses*" (1979) – (Panamerican Center of Zoonosis), based on the relevant literature to the topic (MADRUGA et al., 1980; VASCONCELLOS et al., 1997; PELLEGRIN et al., 1999; LANGONI et al., 2000; HOMEM et al., 2001; LEITE, 2003). From the values found in these works, we considered the average and worked with the prevalence of expected infection by pathogenic leptospiras to the cattle of 50%, with 95% of confidence level and a standard error of 5%.

$$N = \frac{p(100 - p).z^2}{\frac{p\,.d^2}{100}}$$

Where:
N = Sample size,
p = estimated prevalence,
z = confidence level,
d = standard error expected

The estimated values were:
p = 50%
d = 5%
z = 95%

The N value found was 1,536. To determine the proportion of each flock to be collected, we used a simple rule of three. The population that formed the basis for this calculation (62,185 head) was obtained at INDEA-MT, based on the vaccination campaign against FMD (Foot-and-mouth disease) and update of the cattle population registration, in November-December/2003.

X = 1.536 x 100/62.185 X = 2,5%

Samples from cattle blood was collected from both sexes from the following age groups: 4-12 months, 13-24 months and 25-36 months and older than 36 months.

On farms that containment trunks equipped with guillotine and where the cattle was close to the farmhouse, it was brought to the barn and the animals were placed randomly within the trunks until completing the sufficient amount to achieve the sample value (2.5% of the cattle, by sex and age, in each occupied pasture area). On farms which had no guillotine or the cattle was far away, the collection took place in the pastures near to the troughs of salt, where the animals gather a part of the day (rodeos). In these rodeos with the help of horseback cowboys, the lassos were thrown randomly and the bovines laced fell down and tied with ropes, until completing the amount necessary to achieve the percentage of 2.5% for each pasture area. From the randomly selected and contained animals the identification was made, referring to sex, age, farm and pasture area). After the identification was made, 9.0 ml of blood from the jugulate or caudal vein was obtained. The vials containing the blood samples were taken to the farmhouse, where each sample was centrifuged at 7000 RPM during 10 minutes. The tube supernatant (serum) was transferred to a properly identified micro-tube, then kept in a cool refrigerator, when available at the farm, or in coolers with ice, and subsequently frozen.

At the Zoonosis Laboratory, from "*Escola de Veterinária*" (School of Veterinary Medicine) from UFMG (Federal University of Minas Gerais State), the samples were tested by microscopic serum-agglutination test (Galton et al., 1965, COLE et al., 1973, modified by HERRMANN, 2002). Were used living antigens of 17 serovars of *L. interrogans*. Sera were diluted at 1:100 and, defined as positive when they had a reaction equal to or larger than 50% agglutination. The sero-positive were titrated in a geometric series of dilutions with ratio two. The final titration was presented as the reciprocal of the highest dilution where agglutination occurred.

The collection was made in each occupied pasture area to know the location of each one because the vast majority of farms are large land properties and could present different vegetation and floodplain characteristics, but the results were presented totally for each farm.

The spatial analysis technique was used to describe the spatial distribution pattern of the infection. This technique is based on the construction of indicators used in the preparation of thematic maps. The analyses units were farms coded by numbers.

The indicator used was prevalent morbidity which is represented by a coefficient called Prevalence. In this study, the indicator was named Prevalence of sero-positive cattle (Pp). It measures the frequency of existing disease in a population in a restricted interval of time. The prevalence includes all individuals which were sick in the past and are still sick, as well as the new cases. The sero-positive prevalence (indicator) in the cattle was estimated by dividing the number of sero-positive cattle (S) by the number of heads of cattle collected from each farm (N) multiplied by 100 (CÔRTES, 1983).

The results were inserted into the geographic database of the study, to elaborate a thematic map and spatial analysis in GIS MapInfo version 5.4 (1996).

A cluster consists of a group of occurrences geographically limited in size and concentration that is unlikely to occur by chance (ASSUNÇÃO, 2001). Statistical tests for the detection of the highest risk clusters are divided into two categories: generic and focused. The focused tests characteristically verify the existence of clusters in one or in a few regions defined and delimited before the observation of the event. The generic tests are distinguished by not supposing beforehand a specific location as a possible cluster with increased risk; i.e. they test the null hypothesis that there is no cluster in the studied area against the alternative hypothesis that there is a cluster in the area under study without specifying where this possible cluster could be (BEATO FILHO et al., 2001; PELLEGRINI, 2002).

The test used in this study is classified as generic. Each section of the area under study presents a number of cases and controls for the Bernoulli probability model (KULLDORFF, 1997). This model is based on cases and controls, which can be selected from a population study, or may represent the population as a whole. The expected number of events in any area is equal to è times the population at risk. The constant è is the per capita rate of occurrence of events in the area and, under the null hypothesis, è has the same value in every and any area of the map. At the alternative hypothesis, it is assumed that any area of the map has a value of è, which is larger inside than outside it (BEATO FILHO et al., 2001; PELLEGRINI, 2002).

The method used for risk analysis in this study was proposed by Kulldorff & Nagarwalla (1995) and consists basically in scan statistics. This method prioritizes a region formed by the areas whose centroids are within a circle. When the radius and center of the circle varies, the possible clusters are formed. In each circle the ratio of the maximum likelihood is calculated under the alternative hypothesis that q is larger inside than outside the circle and the maximum likelihood under the hypothesis of constant risk, which does not depend on the possible cluster considered.

This maximum value is the ratio of maximum likelihood test statistics and is called as T. The region associated to this maximum is called the more likely or primary cluster. This distribution and the associated p-value are obtained by simulating the data sets generated under the null hypothesis. The null hypothesis is rejected (a = 0.05) when less than 5% of the simulated values of T are larger than the true observed value of T obtained from the non-simulated data. This method provides not only the most probable clusters, but also the secondary clusters, composed for all areas where the null hypothesis is rejected (PELLEGRINI, 2002).

Significance is evaluated by the Monte Carlo simulation, where the null hypothesis of non-existent clusters is rejected at the 0.05 level, just when the p-value simulated is less than or equal to 0.05 for the most likely cluster. In this sense, simulated the p-values run at the same way as the p-values calculated mathematically. The p-values recorded for the secondary clusters are conservative (KULLDORFF et al., 1997; KULLDORFF et al., 1998).

All calculations mentioned were made using the SaTScanTM v. 7.0.1. Program (KULLDORFF, 2006), the randomization was done by 9,999 replications in the Monte Carlo test, and the maps originated from these calculations were done in the MapInfo program.

In this software, centroids (center point of each polygons that represent the pastures divisions) were generated. The centroids have their respective latitude and longitude determined by the program and data like the cattle population, and tested sero-positive population, area of the farms, pasture areas, sero-prevalence by farm and cluster scan analysis of higher or lower likelihood were inserted into the geographic database.

RESULTS AND DISCUSSION

The total area of the farms was 494,215,40 ha, from which 428,943,63 ha were pasture, divided into 369 paddocks. In the period of sample collection, only 187 pasture areas were occupied, covering an area of 223.547,97 ha. The cattle population of the farms studied, aged over 4 months is of 79,582 heads, from which 66,069 (83.03%) are females and 13,513 (16.97%) males.

Of the 2,123 samples collected and tested, 1,230 (57.93%) were reactive to at least one serovar of *Leptospira interrogans*. The sero-positive male cattle was 188 (15.29%) and the female 1,042 (84.71%). The infection caused by pathogenic leptospira in cattle is disseminated in the region, being detected in all the 29 farms analyzed (Figure 2). The average serum prevalence found per farm was about 61.07% (CI: 59.22 to 62.92).

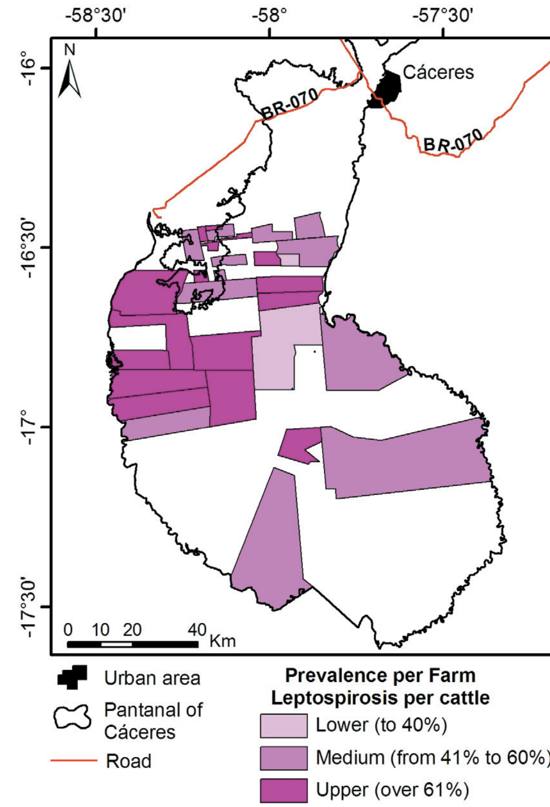

Figure 2 - Seroprevalence per farm from anti-leptospira agglutinins in Cattle in 29 studied properties in Sector I, city of Cáceres-MT, 2005

Results with smaller percentages were found by Pellegrin et al. (1999) in sero-positive cattle in all 28 farms studied in MS, Brazil (38.88%), and by Aguiar et al. (2006) in Monte Negro (53.90%). Higher percentages (97% and 87.5%) were found at Paraiba and Pará States respectively by Homem et al. (2001) and by Leite (2003).Negrão et al. (1999), working in the State of Pará found 66.15%. In countries like Australia and Spain, the leptospirosis prevalence in properties that breed cattle, varies between 72% and 42,8%, respectively (KING, 1991; ALONSO-ANDICOBERRY et al., 2001). Factors related to the epidemiology of this disease, such as cattle density, precipitation index, soil type, breeding system, proximity to other animal species, access to watering points for cattle of different rural properties, explain the differences between the prevalence of values found (ELLIS , 1994).

Analyzing the scan statistic results using Bernoulli's method, it is observed that bovine leptospirosis does not occur randomly in this region. There is a more likely cluster in pasture of eight farms with a relative risk of 1,45 times higher to present bovine leptospirosis compared to the pastures areas of other farms studied (p = 0.0001). There is also a secondary cluster constituted by pasture areas from four other farms that present a relative risk of 1,38 times higher to present cattle leptospirosis than other farms (p = 0.0175) (Figure 3).

Probably this result is a combination of several factors, such as the flooding area which, in the participating properties of the primary cluster is very significant because the region has a series of ebbs (*Vazante*) and streams (*Corixo*) during flood, and during the dry period, the pasture areas are near those areas which do not dry completely. The region where is the secondary cluster is located, has quite intensive *Vazantes* there are quite intense ebbs, such as "Inhatium" and "Chico Correa".

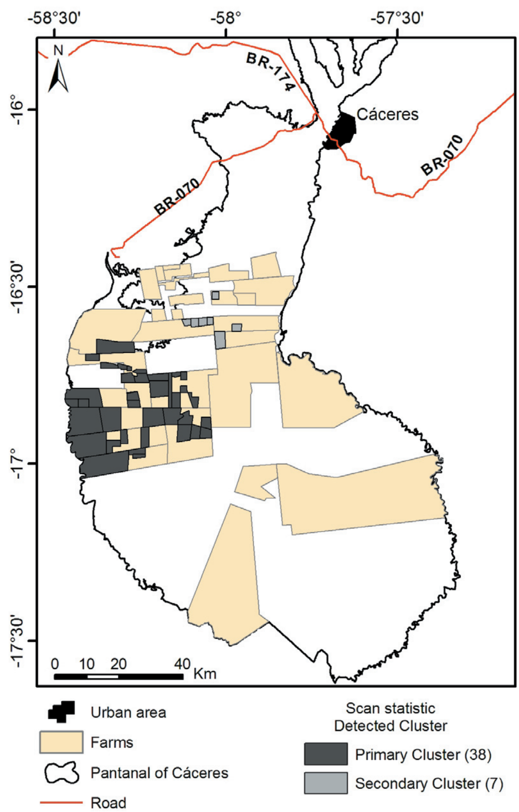

Figure 3 - Scan Statistic in 29 studied farms in Sector I, district of Cáceres-MT-Brazil, 2005, highlighting pastures areas with a higher risk to present cattle leptospirosis

Analyzing the location of pasture areas from the participating farms in the clusters (Figure 4), they are found in floodable accumulation areas that were ranked in the frame of PROJETO RADAMBRASIL (BRASIL, 1982) according to the degree of moisture in average inundation (moderately inundated) and strong (strongly inundated). Neves et. al. (2009) identified via satellite image processing and field validation, that on the moderately inundated areas, the flooding is partial, with a variation of the average height of water (between fifty centimeters and 1 meter) depending on the year and place; and strongly inundated areas remain all year long covered by water (Figure 4).

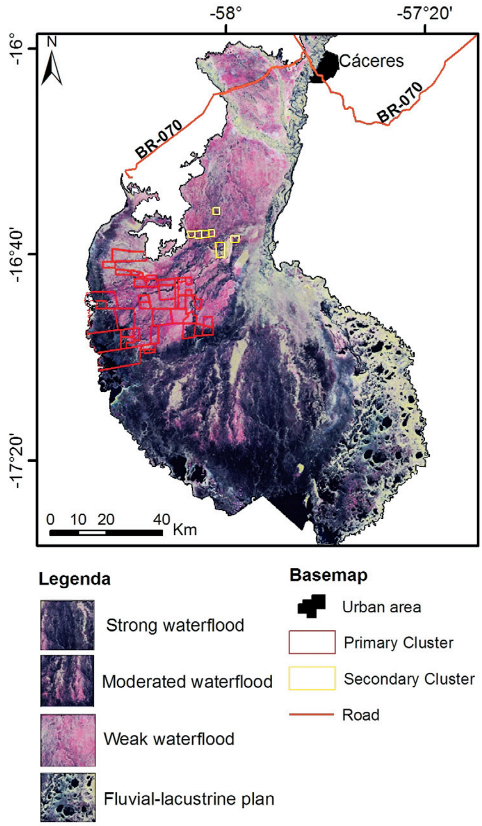

Figure 4 - Pasture distribution of the participating farms of primary and secondary clusters in areas of flood accumulation in the Pantanal of Cáceres/MT

This fact may provide a longer survival period of spirochetes in areas prone to water-logging and flooding, increasing the possibility of another bovine getting contact with the agent. During the dry season, the cattle is all put together and sent to the flooded areas due to water availability. This causes the increase of cattle density in these areas, facilitating its contact with the infectious agent, which has a higher survival in these regions.

Those participating farms showed high prevalence of primary and secondary clusters, indicating that this factor may also explain the presence of clusters. The same scan statistic using Bernoulli's method was used by Olea Popelka et al. (2003, 2005) when they studied the tuberculosis prevalence and presence of clusters in strains of *Mycobacterium bovis* in cattle and badgers in four geographical areas in Ireland.

The pastures where the primary and secondary risk areas are covered with Savanna vegetation, predominantly grassy-woody formation that occurs in low areas, periodically flooded, constituting a specific physiognomy in the field, whose management is made by fire and grazing (IBGE, 1991). The vegetation is used as food for cattle, constituting a factor for its remaining in a wetland environment. When relating the primary risk clusters to the vegetation mapping made by Probio (SILVA, 2007) the predominant vegetation in those pasture areas were identified as: Wooded savanna, Grassy-woody Savanna, Savana/Pioneer Formations and Vegetation with fluvial or lacustrine influence (Figure 5).

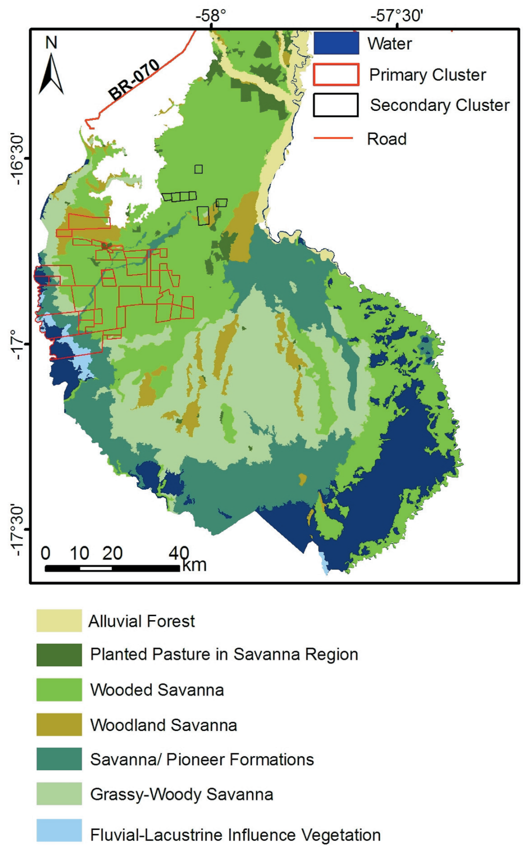

Figure 5 - Pasture divisions showing higher risk of cattle leptospirosis and its vegetation cover

Modified from Silva (2007)

CONCLUSIONS

Results indicate that there is a cattle wide anti-Leptospira agglutinins prevalence, with a spatial distribution at Sector I in the Pantanal of Cáceres (MT, Brazil), considering that all the studied farms were positive for the agent and the they totalize up to 70.73% of the properties from this Sector.

The primary cluster areas, which present a higher probability of epidemiological risk, are located in strong inundated areas and the secondary cluster ones are related to the moderately inundated areas, showing that the bacteria that causes leptospirosis survives longer in areas where flooding is more intense, contributing to the maintenance of this disease in the region;

The permanence of the cattle during the dry season in strongly inundated areas occurs due to the availability of food and water, contributing to the densification of cattle in this region, allowing a higher contact with the leptospirosis agent;

The spatial scan statistic, which uses Geo-Technology tools, performed satisfactorily for the implementation of epidemiological studies conducted in the Pantanal of Cáceres, generating information which can support the development and implementation of disease combat and control programs in the area under study.

It is suggested to carry out a study to evaluate the correlation between floodable accumulation areas and relative epidemiological risk for the investigated disease.

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