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Serological Monitoring for *Leptospira* Spp. and Monitoring of Productive and Reproductive Indices on Dairy Farm

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Abstract

Leptospirosis is a zoonosis caused by spirochetes of the genus *Leptospira*. It has a worldwide distribution with greater occurrence in tropical and subtropical countries. It is endemic in Brazil. It affects domestic, wild and production animals. The goal of this study was to assess dairy herd productive and reproductive indexes on a monthly basis by serologically monitoring the infection dynamics on two experimental groups: one with animals with negative results at study onset (G-1) and another with animals tested positive for at least one leptospira serovar (G-2). The serum microscopic agglutination test (MAT) was employed. Animals with titer equal to or greater than 100 IU were considered reactive. Animals were evaluated for productive and reproductive indexes based on data provided by the dairy's IT system. Blood was collected from all animals in both groups once a month for nine months. Analysis showed interference between animals seroreactive to leptospirosis and both milk production and number of pregnancies for G-2 at collection moments 3, 4, 5, 6, 7 and 9 whereas for G-1 the same indexes showed decrease only in the 5th and 9th study months. The most prevalent serovars were Hardjoprajitino 59.5%, Pyrogenes 21.04%, Pomona 11.07%, Wollfi 11.07%, Hardjo 8.78%, Guaricura 6.55%, Copenhageni 5.09%, Icterohaemorrhagiae 1.11%, and Ctg 0.83%. Serovar Hardjoprajitino showed a relationship with herd milk production decrease.

Keywords: leptospirosis, animal production, dairy cattle

1. Introduction

Leptospirosis is a worldwide bacterial zoonosis showing greater occurrence in tropical and subtropical countries. It is transmitted mainly through direct contact with animals or urine but can also be acquired indirectly by ingesting contaminated water or food. The disease is typically occupational affecting particularly farmers, slaughterhouse workers, veterinarians and their co-workers [1].

Leptospirosis brings economic loss to cattle raisers as it causes reproductive disturbances like abortion or infertility. It is considered a reproductive system disease [2].

Clinical signs can be chronic such as abortion, mainly at the pregnancy's middle third, around the fifth month, estrus repetition and stillbirths as well as placental retention not always occurring. It can cause agalactia or decrease in milk production as well as infection of young calves [3].

Cattle are the main reservoirs of serovars Hardjo [4], and others such as Pomona and Grippotyphosa [5]. They are the preferential hosts of serovar Hardjo. Serovar Hardjoprajitino is responsible for decreases in cattle milk production and conception rates [6], and serovar Hardjobovis is associated with reproductive failures [2–7].

The main serovars found in Brazil are Hardjo, Wolffi, Pomona, Grippotyphosa, Icterohaemorrhagiae [3]. There is a prevalence of serovar Hardjoprajitino, also present in commercial vaccines. However, as with other domestic mammals, cattle can be infected by any pathogenic serovar [8]. Despite some degree of agent species selectivity, the disease is not serovar-specific.

Considering the impact of leptospirosis on cattle breeding as well as its effects on human and animal health, the present study was proposed with the goal of evaluating the consequences of leptospiral infection on the pregnancy and milk production rates of a confined dairy cattle herd with respect to the serological response to 16 serovars of *Leptospira* spp., of importance for herbivores, during 9 months, having it associated with productive, referring to milk production, and reproductive, referring to pregnancy rate, indexes as well as monitoring leptospiral infection evolution in two groups set up and kept under similar conditions, one with animals serologically positive for at least one of the evaluated serovars and another, the control group, with animals serologically negative at study onset, the results thereof compared vis-à-vis the studied variables.

2. Material and methods

With owner's consent secured, the study took place in a dairy property the authors were familiar with. These premises were selected due to the permanent availability of veterinary assistance and the authors' good understanding of its zoosanitary practices. The dairy is a fenced property capable of animal self-replacement, located in the central region of the State of São Paulo, Brazil. Its stock counts 750 animals of which about 400 are of high genetic lineage, pure origin Black and White Dutch lactating cows kept in a semi-confinement system and milked three times a day. The production system is completely computerized, allowing data to be obtained on a monthly basis to evaluate the individual and herd productive and reproductive indexes. Milking is carried out with the help of a carousel-type parlor.

Animals are vaccinated against IBR, BVD, brucellosis and leptospirosis one week before dry-off, approximately 60 days before parturition, and receive a second shot 30 days later. Lactating cows are vaccinated between 120 to 128, 270 to 278 and 420 to 428 days of the lactation cycle.

The experimental groups were formed splitting 202 lactating animals in two groups. One group had 50 animals with non-reactive results to anti-leptospiral antibodies (G-1) while the other has 50 sera reactive animals with a microscopic agglutination test (MAT) titer ≥ 100 IU for at least one *Leptospira* serovar (G-2). G-2 was reduced to 39 animals by the end of the study as 11 were discarded by the owner during the experimental period. Both groups were set up with animals picked at the beginning of their lactation cycles affording longer monitoring times within their milk production periods.

G-1 and G-2 Blood samples were collected monthly for 9 months by mammary vein puncture to assess herd infection dynamics. In order to diagnose infection,

MAT were performed employing live antigens from 16 serovars belonging to 10 serogroups: serovar Bratislava (serogroup Australis); Castellonis (Ballum); Canicola (Canicola); Djasiman (Djasiman); Grippotyphosa (Grippotyphosa); Copenhageni, Icterohaemorrhagiae (Icterohaemorrhagiae); Pomona (Pomona); Pyrogenes (Pyrogenes); Tarassovi (Shermani); Guaricura, Hardjobovis, Hardjo CTG, Hardjoprajitono, Mini, Wolffi (Sejroe). The titer cut-off point was 100 IU.

Production indexes, such as the monthly average of milk production in liters, as well as reproductive factors such as interval between birth and conception, conception rate at the first service, conception rate in all services, services per conception, age at first delivery and number of lactations were evaluated from each animal's history obtained from the dairy's database.

The results of this longitudinal observational study were analyzed by evaluating the relationship between infection by *Leptospira* spp. and the productive and reproductive parameters of cows. Regarding milk production, every month the corresponding milk output log10 was computed and used first to compute the area under the curve (AUC) for each animal and then the mean and standard deviation.

MAT serovar positivity at the diverse moments was established by means of descriptive statistics where positivity percentages represented the frequency distribution of occurrences. Association of pregnancy to reactive serovar for the two groups in the different moments was carried out with the Goodman association test for contrast between and within multinomial populations [8, 9], whose significance was designated with the help of lowercase and uppercase Latin letters. With significance indicated by lowercase and uppercase Latin letters. Milk production comparison for reacting serovar for each moment was done by independent samples Student's t-test [10]. Statistical results were discussed at the 5% significance level.

3. Results

In 238 group independent blood samples (67.42%) responded to only one serovar with Hardjoprajitino prevalence. In 29 samples (8.51%) Pomona, in 41 (11.6%) Pyrogenes and Wolffi in 18 (5.09%). When two serovars were found, there was again a predominance of the Harjoprajitino serovar in 96 samples (41.9%), Pyrogenes in 53 (23.1%), Pomona in 20 (8.73%), Wolffi in 16 (6.98%), Hardjobovis in 18 (7.8%) and Guaricura in 13 (5.67%). For three serovars, Hardjoprajitino predominated in 25 samples (25.7%), Pyrogenes in 21 (21.6%), Wolffi in 15 (15.40%), Pomona in 12 (12.37%), Copenhageni in 9 (9.27%), Guaricura in 6 (6.18%) and Hardjobovis in 5 (5.15%). For four serovars, Hardjoprajitino was again prevalent in 18 (22.7%) of the samples, Pyrogenes in 15 (18.9%), Pomona and Wolffi in 9 each (11.3%), Copenhageni in 11 (13.9%), Hardjobovis in 7 (8.86%), Guaricura in 5 (6.32%) and CTG in 3 with (3.79%). For five serovars, Hardjoprajitino predominated in 3 samples (25%), each of Pyrogenes, Copenhageni and Pomona in 2 (16.6%), each of Wolffi, Hardjobovis and Guaricura in 1 sample (8.3%). The most frequent serovars were Hardjoprajitino, Pyrogenes, Pomona and Wolffi. These were also prevalent as co-agglutinants for serovars Copenhageni, Guaricura and Hadjobovis with respect to the same serogroup. The large number of serovars with cross-reactions or co-agglutination is noteworthy.

Figure 1 refers to the 39 animals seroreactive at the first collection (G-2) which remained in the study and shared the same environment with those in G-1.

Figure 2 summarizes the pregnancy results and the positive percentage of serovars at each moment, also in G-2. Pregnancy rates decreases can be observed at moment 3 with 76.9%, moment 4 with 74.3%, moment 5 with 76.9% and moment 8 with 79.4% of pregnancy.

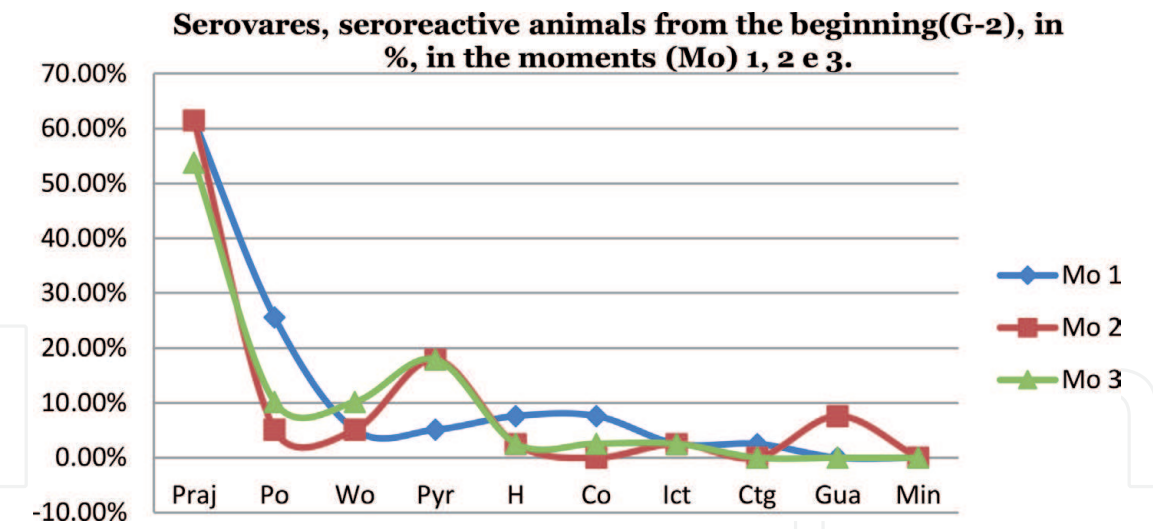


Figure 1. Distribution of results at the beginning of the study (moments 1, 2 and 3) for the G-2 group, according to milk production and pregnancy. Praj = Hardjoprajitino, Po = Pomona, wo = Wolfffi, Pyr = Pyrogenes, H=Hardjo, Co = Copenhageni, Ict = Icterohaemorrhagiae, Ctg = Ctg, Gua = Guaricura, min = mini.

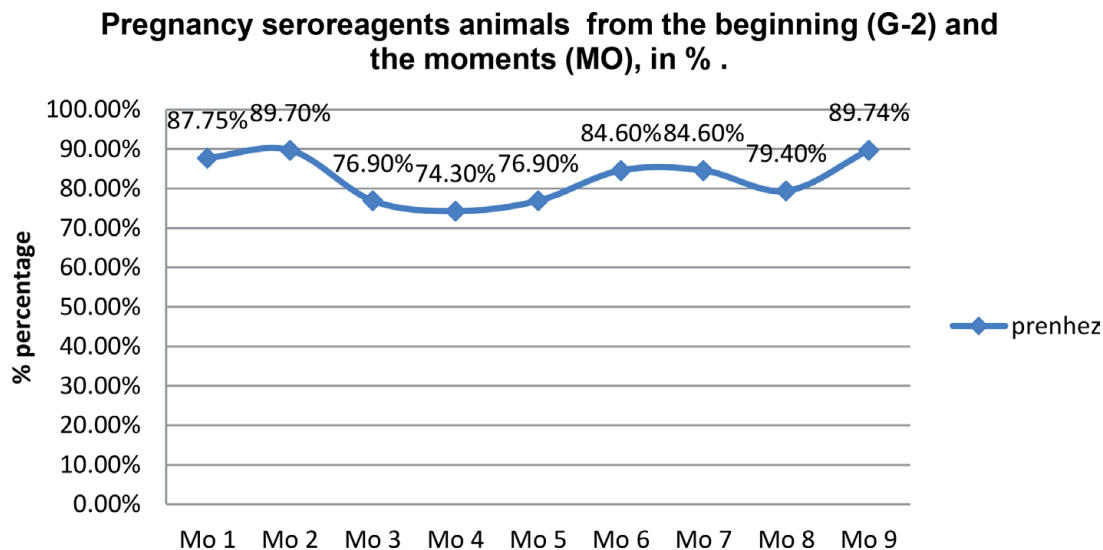


Figure 2. Dynamics of the result of pregnancy rates in the G-2 group, at times (Mo 1) to (Mo 9). G-2 milk production in liters of milk can be seen for different moments in Figure 3.

Table 1 summarizes the results respective of the number of animals, pregnancy in percentage and production in liters of milk at time 2 in G-2.

Serovar Hardjoprajitino was detected at the moment 6 in 18 animals (46.1%), Pyrogenes in 13 (33.3%), Pomona in 9 animals (23%) and Hardjo in 6 animals (15.3%), when decreases in milk production and fertility were observed for infection by the Hardjo serovar, G-2.

Figure 4 shows a serovar Hardjoprajitino participation of 69.2% in moment 8, 64.1% in moment 9 and 41% in moment 7, all in G-2. Variation among seroprevalence percentages at those moments can be seen for the various serovars.

Moment 9 (**Figure 4**) had a higher seroprevalence of serovars Hardjoprajitino in 25 animals (64.1%), Pyrogenes in 17 (43.5%), Guaricura in 7 (17.9%), Wolfffi in 4 (10.2%), Copenhageni in 3 (7.6%), Pomona in 2 (5.1%), Hardjo in 1 (2.5%) in G-2.

Figure 5 shows a decrease of pregnancy rates in G-1 at moment 5 which may be related to positivity for serovar Hardjoprajitino. On the other hand, **Figure 6** illustrates G-1 milk production at different times, showing a decrease in milk production at moments 5, 7, 8 and 9 which may be related to calving times. As a matter

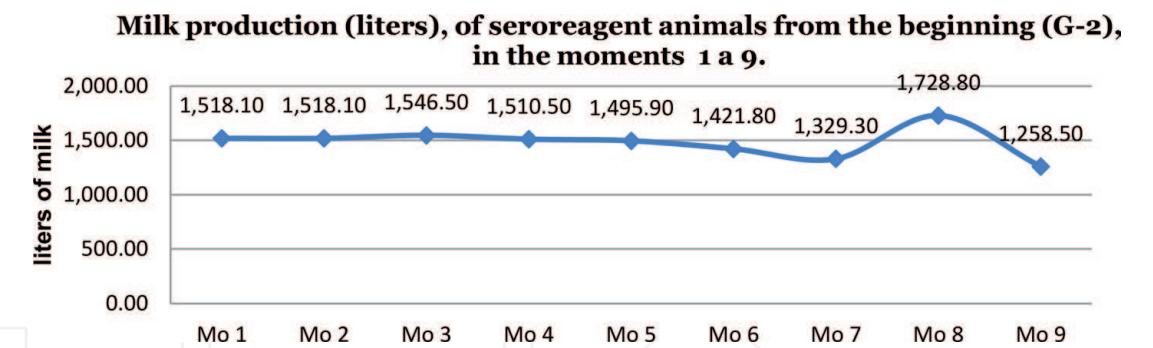


Figure 3.
Dynamics of milk production in liters, in the group of seroreagent animals (G-2), from Mo 1 to Mo 9.

Seroreagents	Animals	(Mo 2)
Serovars	Nº	%
Hardjoprajitino	24	61,5
Pyrogenes	7	17,9
Wollfi	2	5,1
Pomona	2	5,1
Icterohamorrhagiae	1	2,5
Hardjo	1	2,5
Guaricura	3	7,6
Prenhez em %	32	82
Production in liters of milk	39	1.508,3

Table 1.
Productivity of seroreagent animals (G-2), pregnancy and production in liters of milk, at time 2. Results expressed as a percentage.

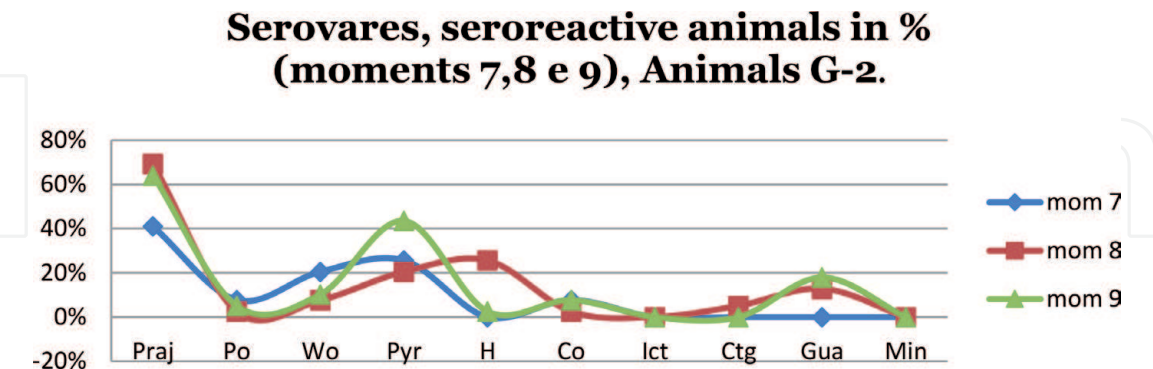


Figure 4.
Kinetics (dynamics) of G-2 antibody titers expression, compared to moments 7, 8 and 9 of observation. Praj = Hardjoprajitino, Po = Pomona, wo = Wolffi, Pyr = Pyrogenes, H = Hardjo, Co = Copenhageni, Ict = Icterohaemorrhagiae, Ctg = Ctg, Gua = Guaricura, min = mini.

of fact, despite attempts to start the experiment with groups as homogeneous as possible, delivery times varied and some animals possibly found themselves in more advanced lactation stages of lactation thus interfering with the group’s overall production.

Table 2 shows all the serovars as percentages, at moment 2 for G-1. The pregnancy rate was 87.7% and the milk production 1,963.8 liters. Comparing these

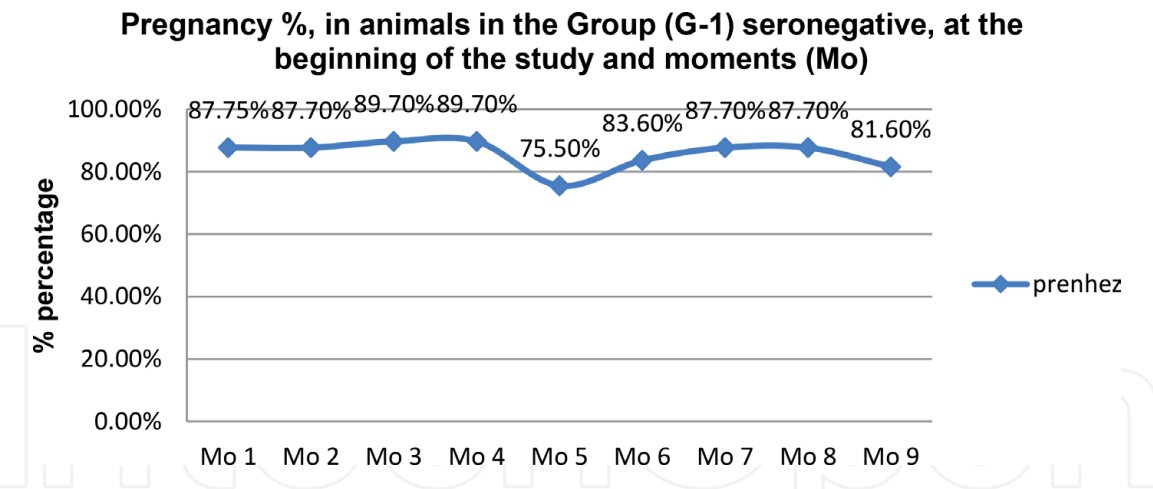


Figure 5.
Dynamics of the pregnancy rate in the G-1 group, from the initial moment Mo 1 to Mo 9.

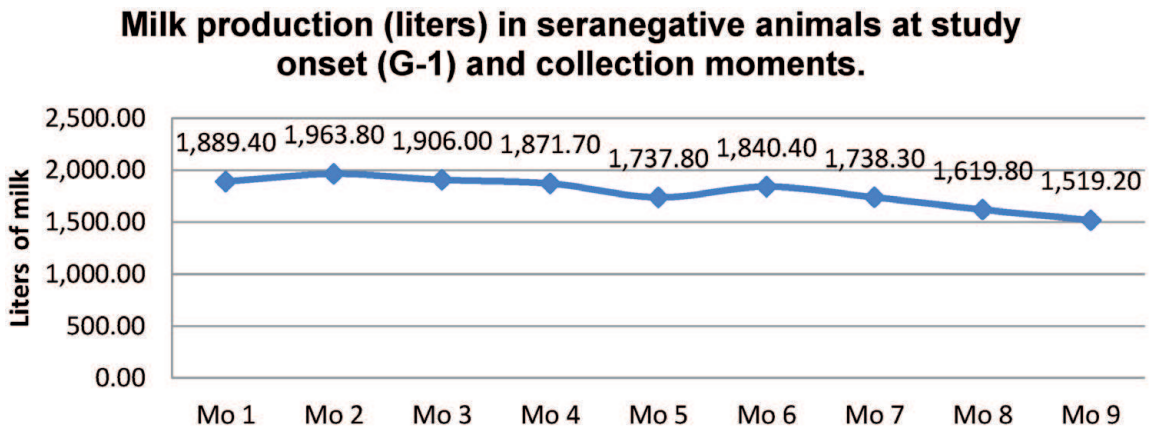


Figure 6.
Dynamics of milk production in liters in the G-1 group, from the initial Mo to Mo 9.

Sororeagents	Animals	(Mo 2)
Serovars	N°	%
Hardjoprajitino	18	36,7
Pyrogenes	4	8,16
Hardjo	1	2,04
Wolffi	1	2,04
Ctg	1	2,04
Icterohaemorrhagiae	1	2,04
Prenhez em %	43	87,7
Produção em litros de leite	49	1.963,8

Table 2.
Productivity of seroreagent animals (G-1), pregnancy and production in liters of milk, at time 2. Results expressed as a percentage.

pregnancy rates and milk production with those for G-2, both pregnancy rate and milk production is higher for G-1 at the study onset probably due to lower infection rates of serovars such as Hardjoprajitino in G-1.

At moment 3, G-1, serovar Hardjoprajitino was found in 21 animals (42.8%), Pyrogenes in 12 (24.4%), Pomona in 9 (18.3%), Wolffi in 7 (14.2%), Copenhageni

in 5 (10.2%) and Icterohaemorrhagiae in 2 (4.08%) with a pregnancy rate of 89.7% and milk production of 1,906.8 liters. Hardjoprajitino remained the most frequent serovar with a slight increase when compared to Mo 2. As mentioned for Mo 2, pregnancy rates and milk production were also higher when compared to the earlier moments, for G-2, where serological response with variable antibody titers for one or more leptospiral serovars were present at study onset. Such observation reinforces the importance especially of the serovar Hardjoprajitino to the productive and reproductive aspects of dairy cattle, the focus of the present study.

Figure 7 illustrates the dynamics of antibodies titres regarding the Hardjoprajitino serovar with 59.1%, 55.1% and 46.9% positivity thus confirming the relevance of this serovar for cattle. The participation of serovar Pyrogenes among the serovars that stand out at different times is noteworthy.

At moment 4 for G-1, serovar Hardjoprajitino was obtained in 29 animals (59.1%), Pyrogenes in 5 (10.2%), Pomona in 4 (8.16%), Hardjo in 4 (8.16%), Wolffi in 3 (6.12%), Guaricura in 2 (4.08%), Copenhageni in 2 (4.08%), Castellonis in 1 (2.04%). The pregnancy rate was 89.7%, and milk production 1,872 liters of milk. There was a slight decrease in milk production but the pregnancy rate was the same as for the previous month (Mo 3).

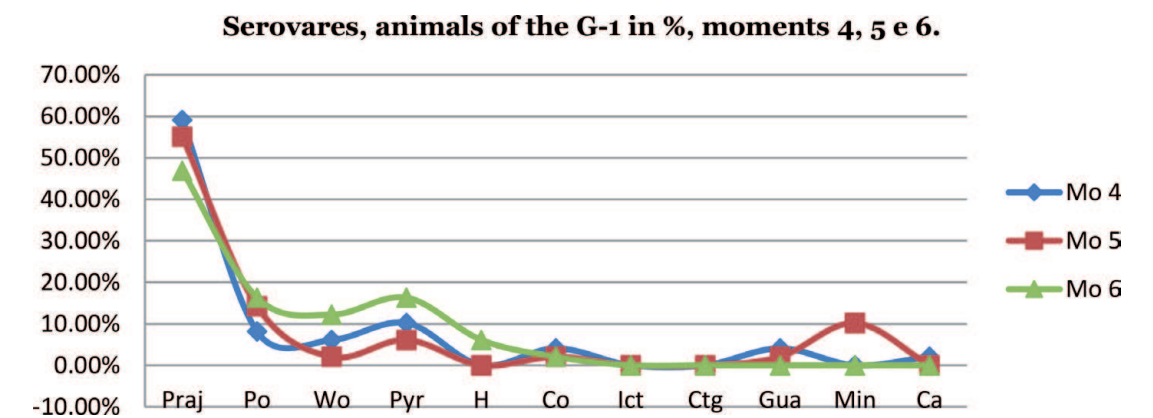


Figure 7.
Kinetics (dynamics) of G-1 antibody titer expression at moments 4, 5 and 6 of observation.
Praj = Hardjoprajitino, Po = Pomona, wo = Wolffi, Pyr = Pyrogenes, H = Hardjo, Co = Copenhageni, Ict = Icterohaemorrhagiae, Ctg = Ctg, Gua = Guaricura, min = mini, Ca = Castellonis.

Sororeagents	Animals	
Serovars	N°	(Mo 5) %
Hardjoprajitino	27	55,1
Pomona	7	14,2
Mini	5	10,2
Pyrogenes	3	6,12
Guaricura	1	2,04
Wolffi	1	2,04
Copenhageni	1	2,04
Prenhez em %	37	75,5
Produção em litros de leite	49	1.737,8

Table 3.
Productivity of seroreagent animals (G-1), pregnancy and production in liters of milk, at time 5. Results expressed as a percentage.

Table 3 summarizes the G-1 results for moment 5. The pregnancy rate was 75.5%, and milk production volume 1,738 liters. There was a decrease in productive and reproductive indexes with a decrease in pregnancy rate and milk production when compared to previous moments.

Reagent serovars in G-1, moment 6, were Hardjoprajitino in 23 animals (46.9%), Pyrogenes in 8 (16.3%), Pomona in 8 (16.3%), Wolffi in 6 (12.24%), Hardjo in 3 (6.12%), and Copenhageni in 1 (2.04%), with a pregnancy rate of 83.6% and a milk production volume of 1,840 liters of milk. There was a decrease in the seroprevalence of Hardjoprajitino from 55.1% to 46.9% and consequently increases in pregnancy rate from 75.5% to 83.6% and in milk production from 1,737.8 to 1,840 liters of milk.

Figure 8 illustrates the dynamics of the response to serovars, in Mo 7, 8 and 9. There are differences among the various moments, seroprevalence oscillating, which is possible since the animals shared the same environment exposing themselves to animal-maintained and environment serovars.

At moment 7 in G-1, serovar Hardjoprajitino can be observed in 7 animals (14.2%), Pyrogenes in 8 (16.3%), Wolffi in 6 (12.2%), Copenhageni in 3 (6.12%), Pomona in 1 (2.04%), with a pregnancy rate of 87.7%, and milk production of 1,738 liters of milk. Despite the lower response to serovar Hardjoprajitino, there was an increase in the pregnancy rate but a decrease in milk production in comparison to moment 6. At this moment there was no response to serovar Hadjobovis. For this same group, in moment 8, serovar Hardjoprajitino was detected in 22 (44.8%), Pyrogenes in 9 (18.3%), both Guaricura and Hardjo in 6 (12.24%), Ctg in 5 (10.2%), Wolffi, Pomona and Icterohaemorrhagiae in 1 (2.04%) each. The pregnancy rate was 89.7% and milk production volume 1,620 liters of milk. There was an increase in the response to serovar Hardjoprajitino, from 16.3% to 44.8% and the serovar Hardjobovis, not found at moment 7, appears in 12.24% which must have influenced the decrease in milk production from 1,738 to 1,620 liters, despite pregnancy rate showing a slight increase from 87.79% to 89.7%.

Table 4 summarizes the most frequent serovars for moment 9, with a pregnancy rate of 81.6% and a milk production volume of 1,519 liters. A decrease in the response to serovar Hardjoprajitino as well as a lack of response to serovar Hardjobovis were observed. There was also a decrease in pregnancy rate and milk production despite the lower response to the serovar Hadjoprajitino, a reduction from 44.8% to 24.4% at moments 8 and 9, and the non-response to serovar Hadjobovis at that moment. Regarding the decrease in milk production also seen in G-1, it should be noted that many animals might have been in an advanced stage,

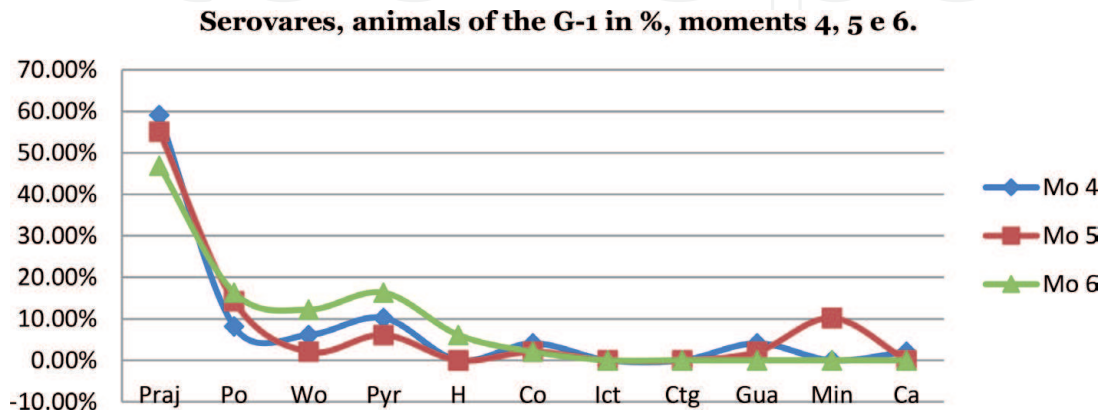


Figure 8.
Kinetics (dynamics) of G-1 antibody titer expression at moments 7, 8 and 9 of observation.
Praj = Hardjoprajitino, Po = Pomona, wo = Wolffi, Pyr = Pyrogenes, H = Hardjo, Co = Copenhageni, Ict = Icterohaemorrhagiae, Ctg = Ctg, Gua = Guaricura, min = mini, Ca = Castellonis.

Seroreagents	Animals	(Mo 9)
Serovars	N°	%
Hardjoprajitino	12	24,4
Pyrogenes	17	34,6
Guaricura	4	8,16
Pomona	5	10,2
Copenhageni	2	4,08
Prenhez em %	40	81,6
Produção em litros de leite	12	1.519

Table 4.
Productivity of seroreagent animals (G-1), pregnancy and production in liters of milk, at the moment 9. Results expressed as a percentage.

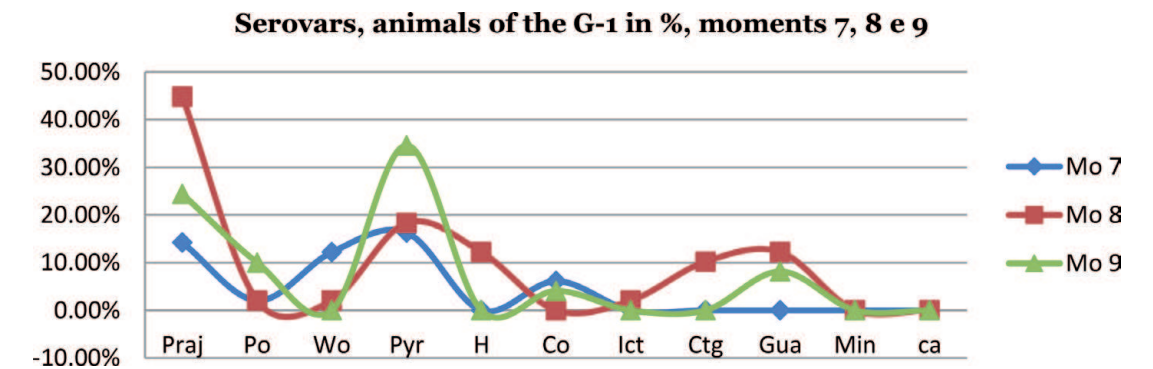


Figure 9.
Average percentage of animals in the G-1, at moments 1 to 9 of observation. Praj = Hardjoprajitino, Pyr = Pyrogenes, Po = Pomona, wo = Wolffii, H = Hardjo, Co = Copenhageni, Ict = Icterohaemorrhagiae, Ctg = Ctg.

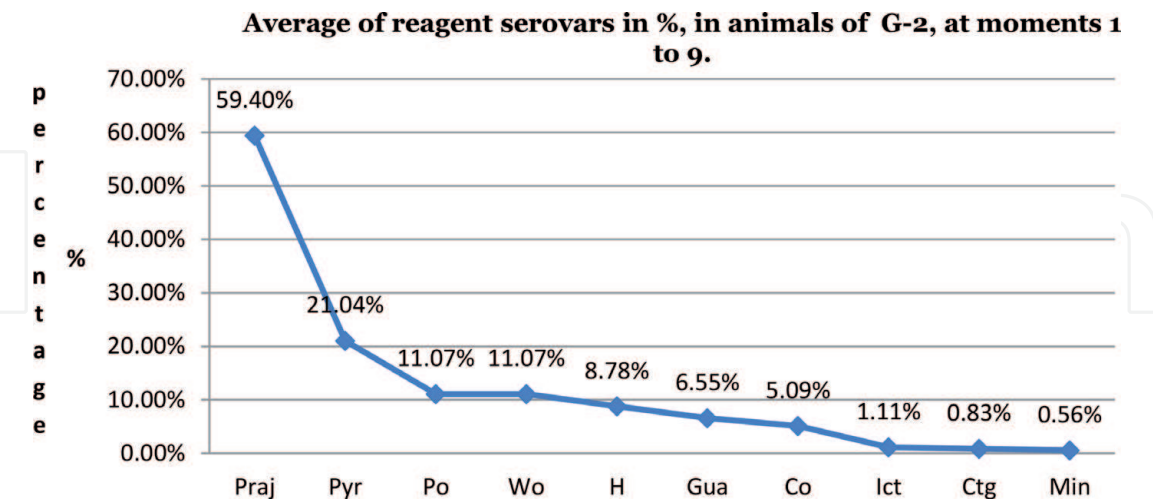


Figure 10.
Average percentage of animals in the G-2, at moments 1 to 9 of observation. Praj = Hardjoprajitino, Pyr = Pyrogenes, Po = Pomona, wo = Wolffii, H = Hardjo, Co = Copenhageni, Ict = Icterohaemorrhagiae, Ctg = Ctg.

near the end of lactation with a consequential decrease in milk production, which was also observed in G-2.

Figure 9 shows the percentage of response to serovars from Mo 1 to Mo 9 with greater prevalence of serovars Hardjoprajitino, Pyrogenes and Pomona. Regarding

the 49 animals in group G-1, **Figure 10** shows the average prevalence of serovars, the decrease in productivity in G-2 at moments 4, 5, 6, 7 and 9 and in G-1 at moments 5 and 9. There was a decrease in the pregnancy rate at moments 3, 4, 5, 6, 8 in group G-2 and at moments 5 and 9 in group G-1.

4. Discussion

Where of response to a single serovar, which occurred in 238 samples, serovar Hardjoprajitino was obtained in 67.4%. Pyrogenes in 41 (11.6%), Pomona in 29 (8.51%) and Wolffi in 18 (5.09%). Hardjo is the serovar most commonly found in cattle, the species considered its primary maintenance host [3]. Serologically identical but genetically distinct types of serovars Hardjo exist: *L. interrogans*, serovar Hardjo, type Hardjoprajitno and *L. borgpetersenii*, serovar Hardjo, type Hardjobovis [11]. The chief cattle infectant serovars are Hardjo, Pomona, Grippyphosa, Icterohaemorrhagiae, Wolffi and Canicola [12]. These were found in the present study at different times with varied percentages both in G-1 and in G-2.

Results show that the serovars are practically the same, seroprevalence varying in both groups with G-2 displaying the greatest differences for most serovars. This shows the importance of environmental contamination and indirect transmission, mainly by water and food. According to Lenharo et al. [13], this serovar is commonly found in wild mammals and these can act as sources of soil contamination and animal infection.

Serovars Bratislava, Djasiman, Hebdomadis, Icterohaemorrhagiae, Pomona and Tarassovi are considered incidental in cattle and indirect transmission is associated with contact with an environment contaminated by leptospirae mainly from wild species or other domestic species [8]. On the other hand, serovars Pomona, Grippyphosa and Icterohaemorrhagiae are frequently identified in incidental infections in cattle and their transmission related to pigs, rodents and wild animals [4, 14]. Bovines can host incidental serovars for an uncertain period [15].

Serovar Hardjoprajitino is responsible for decreases in cattle milk production and conception rates. Also commonly found in pigs, Wolffi is antigenically similar to Hardjo and cause of reproductive disorders and abortions in wild animals and therefore a source of environmental contamination. Serovar Pyrogenes is frequently found in *Rattus norvegicus* and can be considered an incidental contaminant for cattle [6]. Infection by Hardjobovis is frequently observed in cattle in several countries in subclinical forms associated to abortion, while serovar Hardjoprajitno, found in some countries, is characterized as more pathogenic and leading to reductions in milk production and reproductive problems [16].

Serovars Hardjobovis and Hardjoprajitino are adapted to cattle and cause the reproductive and the sudden milk production decrease syndromes. The first is related to serovar Hardjobovis and is characterized by miscarriage, stillbirths, infertility and weak calves. The latter is due to serovar Hardjoprajitino, characterized by udder flaccidity and a sudden decrease in milk production lasting from 2 to 10 days with changes in its consistency and colostrum [17].

Where response was observed for two serovars, the predominance of Hardjoprajitino serovars in 96 of the samples (41.9%), Pyrogenes 53 samples (23.1%), Pomona 20 samples (8.73%), Hardjobovis 18 samples (7.8%), Wolffi 16 samples (6.78%) and Guaricura in 13 samples (5.67%) was noted. The serological response can be influenced by the cross-detection between serovars of the same serogroup. Serovars Pomona, Grippyphosa and Icterohaemorrhagiae are frequently identified in incidental infections in cattle and their transmission is related to pigs, rodents and wild animals [4, 14].

Predominance of Hardjoprajitino serovars with 25 samples (25.7%), Pyrogenes 21 (21.6%), Pomona 12 (12.37%), Wolffi 15 (15.4%), Copenhageni 9 (9.27%), Guaricura 6 (6.18%) and Hardjobovis 5 (5.15%) samples was noted. Cattle is considered maintenance host for serovars Hardjoprajitino and Hardjobovis which are transmitted by urine associated with reproductive failures [2, 7]. Cross-reactions occur between different serogroups, mainly in the acute phase of the disease [17, 18]. The serovar Icterohaemorrhagiae found in the present study falls within the One Health concept mainly due to the presence of rodents [19]. On the other hand, participation of serovar Pyrogenes among the serovars that stand out at different times is highlighted in G-1 for moments 4, 5 and 6. According to Lenharo et al. [20] this serovar is commonly found in wild mammals, which can contaminate the soil and can infect animals.

Pregnancy decreased at moments 3, 4, 5, 6 and 8 in G-2 and at moments 5 and 9 in group G-1. In a study with 25 dairy herds, totaling 500 cows, 32% of the herds were positive for the *Sejroe* serogroup. Of the 500 cows studied, 48 (9.6%) were sera reactive, 38 (7.6%) with 400 IU titers and 10 (2%) ≥ 800 IU. Estrus repetition was the most reported reproductive problem and strongly associated with leptospirosis [21]. Milk production decreased in G-2 at moments 4, 5, 6, 7 and 9 and in G-1 at moments 5 and 9.

Seroprevalence, milk production and pregnancy rate are influenced by environmental contamination from animal urine, particularly regarding serovar Hardjo. This serovar decreases fertility, while Hardjoprajitino is related to milk production, which is in line with the reduction in liters of milk at moment 6 [16]. Increased rainfall contributes to the spread of the agent in both groups. This is a relevant aspect to be considered in zoo-sanitary management in relation to bovine leptospirosis since the environment has an important role in the chain of transmission of the disease [13]. The triad is thus complete: animal, infectious agent and environment plus human involvement which characterizes the idea of One Health since the disease is common to humans and animals.

With regard to the animals in G-2 and the production of liters of milk, there is a decrease at moment 6, in February, moment 7 in March and moment 9 in May. The lower milk production in these months may be related to the greater environmental contamination by lespiras and therefore a reduction in output, possibly influenced by serovar Hardjoprajitino.

Pregnancy rates at Mo 5 were 75.5% in G-1 and 76.9% in G-2. Although figures were close, G-2 saw a slight increase. Milk production decreased in both groups. Preganancy rates and milk production are probably related to infection by serovar Hardjoprajitino. Rainfall increased significantly in October, November, December, January and February possibly favoring cross-contamination between the two groups.

The dog is the natural host of serovar Canicola and the brown rat (*Rattus norvegicus*) of serovars Icterohaemorrhagiae, Copenhageni and Pyrogenes. Serovar Pomona has pigs, cattle and possums as its natural hosts while Grippotyphosa is found in the kidneys of wild animals such as rats, hares, martens and hamsters [22]. These animals can be sources of infection for cattle [3].

Hardjoprajitino is the serovar prevalent in cattle and responsible for decreased milk production and pregnancy rates, a fact observed in the present study. Pomona and Wolffi are adapted to swine and bovine species but Wolffi is frequently found in pigs and can also cause abortion in the final third of gestation, birth of weak fetuses and decreased conception rates [19].

Serovars Hardjoprajitino and Hardjo are the ones most frequently found in cattle and may cause productive and reproductive disorders [17]. Pomona is most commonly found in swine, which is adapted, however, it can infect cattle.

Pomona is most commonly found in swine, to which it is adapted, it may infect cattle. Serovar Pyrogenes is found in the *Rattus norvegicus* species, implying a potential for environmental contamination. Rodent control and site management measures like waste removal and swamp land drainage are biosafety measures for preventing the spread of this serovar. Despite low at the studied moments, the occurrence of serovar Icterohaemorrhagiae should be noted and its adaptation to the rodent species stressed [5, 13].

In order to investigate the effects of rainfall on leptospira infection in cattle, 582 animals were selected and samples from 362 of these collected in the rainy season and from 220 in the dry season. In the rainy season, seropositivity to MAT was 43.6% (158/362) and in the dry season 31.8% (70/220). The Sejroe serogroup predominated (54.8%; n = 125/228), the Javanica serogroup (16.2%; n = 37/228), Icterohaemorrhagiae (7.5%; n = 17/228) and Tarassovi (7.0%; n = 16/228). Seropositivity for incidental serogroups was more frequent in the rainy season (50.0%) than in the dry season (34.3%; $p \leq 0.0001$) [23], reinforcing the environmental aspects of leptospirosis maintenance in cattle herds.

Reproductive failures such as early embryonic loss and consequent estrus repetition are increasingly associated with leptospiral infection. Although these failures are frequently associated with several factors, two studies with cattle revealed a strong association of estrus repetition with seroreactivity for the serogroup Sejroe [12, 21]. Contrary to the results obtained, according to Faine et al. [17] Hardjoprajitino is associated with decreases in milk production.

In the present study a greater participation of the serovar Hardjoprajitino, serogroup Sejroe, was also observed however the correlation between milk production and pregnancy rates in both G-1 and G-2 had no statistical significance with $p > 0.05$. A limiting aspect is the impossibility of comparing the results of both the dynamics of antibodies and those of milk production and pregnancy rate, as in the present study, since no similar research with two groups of animals living under the same environmental and management conditions on the same property can be found in the literature.

Although in the present study there was no statistical association ($p > 0.05$) between milk production and seropositivity in both groups, except for the months of May and August, which may be associated with a drop in temperature, when results were analyzed for each groups separately, G-1 showed a decrease in pregnancy rate at moments 5, 6, 7, 8 and 9 and in milk production at moments 5, 6, 7, 8 and 9, related to January (Mo 5) 161.6 mm (Ciagro – Centro Integrado de Informações Agrometeorológicas) and February (Mo 6) 363.3 mm rainfall. Those were months of high rainfall favoring environmental contamination. In G-2 the pregnancy rate decreased at moments 2, 3, 4, 5, 6, 7 and 8, October (Mo 2) 234.4 mm, November (Mo 3) 135.2 mm, December (Mo 4) 137.8 mm, January (Mo 5) 161.6 mm and February (Mo 6) 363.3 mm, all months with high rainfall. Productivity decreased at moments 4, 5, 6, 7 and 9.

There was no statistical association between pregnancy rate and seropositivity, $p > 0.05$ in either group. There was also no statistical association ($p > 0.05$) between milk production and positivity in either group except in May and August, when there was a decrease in milk production which may be related to food management, temperature drop and health of the mammary gland as a result of probable cases of mastitis. The property carrying out somatic cell counting (SCC) of milk samples from the expansion tank but not from individual animals was a limiting factor.

According to Ellis [4], bovine leptospirosis is most often caused by strains adapted from the serogroup Sejroe. bovine leptospirosis is most often caused by

strains adapted from serogroup *Sejroe*. In these cases, disease acute phase may be subclinical except for infections in lactating cows where agalactia may occur. Clinical cases are less frequent and can represent outbreaks, the disease then characterized by abortions at any time during pregnancy [24, 25], albeit more frequent in the average period of pregnancy [3].

Seropositivity for leptospira and clinical cases of leptospirosis are often associated with environmental risk factors, such as rain and floods [26]. For the *Sejroe* serogroup, specifically the Hardjo genotypes, adapted to cattle, direct animal-to-animal transmission is more common than indirect transmission from environmental contamination. On the other hand, infections by incidental serovars by serogroup Icterohaemorrhagiae or Pomona lead to renal excretion. Transmission in incidental infections is more dependent on the presence of other host species and environmental factors, especially accumulated water [4].

Research on leptospiral DNA in the vaginal secretion of apparently asymptomatic cows reinforces the belief that in addition to environmental contamination infection can occur from female to male through vaginal discharges and secretions during natural mating [27]. This can hamper control programs by maintaining infection and disease endemic in the property.

For the Copenhageni, Pomona, Wolffi and Prajitino serovars, frequency of positive titers greater than 800 IU was significant, with $p < 0.05$, in the comparison of positive reagent greater than negative reagent. Cattle infected with adapted strains, including those related to cases of agent isolation [28] the property, often have low antibody titers [10].

Although leptospires can be detected in the urine of cattle infected with adapted strains [10], leptospiuria is intermittent and not very intense [4, 29]. Serovars Pomona, Grippotyphosa and Icterohaemorrhagiae are frequently identified in incidental infections in cattle and their transmission is related to pigs, rodents and wild animals [4, 14].

Infection transmission by incidental serovars is more dependent on the presence of other host species and environmental factors. A high percentage of isolation of the serovar Hardjo from the genital tract of cows is emphasized, suggesting tropism for that region [16]. Also, according to Ellis [4], as previously mentioned, the genotypes of Hardjo serovars are adapted to cattle and associated with the chronic reproductive form of leptospirosis.

The farm where the present study was developed carries out vaccination against leptospirosis every four months and elevated titers such as 800 IU, 1600 IU and 3200 IU were found. In vaccinated cattle, post-vaccination IgM and IgG titers are low (between 100 and 400) and transient between four to six months after vaccination [3]. This fact reinforces the possibility of the higher titers having been produced in response to infection.

With regard to milk production and pregnancy rates, Ellis [16] demonstrated relationship with serovar Hardjopravii, a result also found in the present study which corroborates the findings of reductions in milk production and pregnancy rates at the moments when Hardjopravii was the most detected serovar. Comparative discussion regarding data from the literature in similar studies is hindered due to the scarcity of research on infection dynamics with different groups of animals. The present study showed that the several serovars are maintained in the two groups of animals (G-1) and (G-2), that seroprevalence is also variable, and that some serovars show greater importance in these groups. It can also be observed that milk production and pregnancy rates decreased at those moments when the frequency of a given serovar, like Hardjobovis, increased.

5. Conclusions

The serovars were practically the same, seroprevalence varying among the animals of the two groups, most of them showing greater variations in G-2, indicating possible environmental contamination and indirect transmission especially through water and food.

Seroprevalence, milk production and pregnancy rates were influenced by the contamination of animals in the environment as well as by the increase in rainfall levels and the possibility of leptospires in the urine of infected animals, considering the two groups G-1 and G-2, and the serovar Hardjoprajitino was the most prevalent, 36% in G-1 and 59.5% in G-2, showing a relationship between positivity and decreases in milk production.

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Ethics committee approval


This study was approved by the Animal Use Ethics Committee (CEUA) of FMVZ-UNESP/Botucatu, SP, process nr. 0154/2019, September 11, 2019.

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