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RESEARCH ARTICLE

Epidemiological situation and risk Factors to Infectious Diseases in Dairy Cattle Located in Different Mesoregions of the State of Rio Grande do Sul, Brazil, 2016/2017

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Abstract

The aim of this study was to describe the epidemiology of infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), neosporosis (NEO) and leptospirosis (LEP) from the perspective of the impact these diseases have to cause reproductive problems in dairy cattle herds located in different mesoregions of Rio Grande do Sul State. The herds analysed belong to two cooperatives and an association of producers. The seroprevalences of IBR, BVD, NEO and LEP ranged from 54.4 to 60.3%, 30.0 to 42.5%, 21.8 to 35.0% and 15.8 to 27.5%, respectively. The seroprevalences of IBR show a homogeneous distribution of the disease according to the mesoregions. Nevertheless, IBR was associated with estrus recurrence and abortion in herds located in the northeast and northwest mesoregions. BVD was associated with rearing of estrus in herds located in the southeast, southwest, northeast and northwest mesoregions. NEO was associated with cases of abortion in the herds located in the northeast and northwest mesoregions. LEP was associated with miscarriages in herds located in the northwest mesoregion. It was found that the epidemiological character of the disease cases were distinct in the different mesoregions. In this way, the priorities in the control and prevention programs of these diseases are different between the mesoregions, cooperatives and association of producers. Despite that fact, common control procedures were found amongst the disease cases studied; each one of them has its peculiarity in relation to the epidemiology and consequently control and prevention.

Keywords: Dairy herds reproduction, Bovine reproduction, Reproduction diseases, ELISA, Vaccines

Introduction

The main problems observed in bovine reproduction are associated with the return to estrus, miscarriage, fetal mummification, birth of weak calves and / or calves with congenital defects, premature disposal of breeders among other factors [1]. It is a fact that there is a diversity in the etiology of reproductive problems. Important aspects must be considered in the diagnosis stage, such as the animal/ herd history, age, physiological condition of the cow (dry or suckling), reproductive history (eventual reproductive failures such as abortions, mummified fetuses and / or estrus recurrence), nutritional management and sanitation, and changes in routine management as introduction of external animals without sanitary control. Abortion rates in the herd of up to 1% indicate normality, 2 to 3% are considered as an alert and more than 3% indicate an infectious, environmental or management problem [2].

Infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), neosporosis and leptospirosis [3] are amongst the diseases that can cause reproductive problems in cattle. There are several reports of reproductive problems associated with these diseases in Brazil, this makes it very relevant for the country to control these diseases in dairy cattle herds [4-7]. Nonetheless, the seroprevalence of these diseases may be

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different between the states of Brazil and even in regions of the same State. In the case of herds in Rio Grande do Sul, for instance, NEO has been reported to be widespread among bovine and other animals [8]. In the present study, the seroprevalence of BVD, IBR, leptospirosis and neosporosis varied according to the regions studied [9]. The objective of the research was to evaluate the seroseroprevalence of BVD, IBR, leptospirosis and neosporosis in three distinct populations of dairy cattle located in four different mesoregions of Rio Grande do Sul State and the weight that each of these diseases has on each cattle population.

Material and Methods

Transversal study (seroseroprevalence study) with simple random sampling for herd and animal selection was conducted in four mesoregions of the State of Rio Grande do Sul to estimate the seroseroprevalence of IBR, BVD, NEO and LEP amongst animals and herds. A retrospective observational study was also carried out to evaluate IBR, BVD, NEO and LEP as risk factors for estrus recurrence and abortion observed in animals. The target population or sample universe was composed of all herds of two cooperatives and an association of producers that were in the southeast, southwest, northeast and northwest mesoregions of the State of Rio Grande do Sul.

Sampling

The sampling calculation was performed according to the formula described by Petrie and Watson (2009) and using the program Epitools® [10]. The parameters used to perform the sampling were: expected seroprevalence in the individuals for BVD, IBR and LEP of 50% and for NEO of 15%. The sample error of 10% for BVD, IBR and LEP and 5% for NEO. The significance level of 95% was used for all diseases as a parameter for the calculation of the samples. To perform the sampling between herds, the parameters used were: expected seroprevalence among individuals for BVD, IBR and LEP of 50% and for neosporosis of 30%. To calculate the sample to estimate the seroprevalence for all diseases, the sample error of 20% and the level of significance of 95% were used as a parameter. The number of animals sampled per herd was obtained by the ratio between the number of animals and the number of herds obtained in the calculation of the sampling. Because the calculations used for the sampling had different parameters, it was used the sampling with greater number of animals and herds to estimate the seroprevalence for all diseases among individuals was simple random.

Diagnosis

Blood samples were collected by venipuncture of the jugular or coccygeal of bovine females older than 24 months of age. 10 mL of blood was collected in individual sterile tubes, without anti-coagulant, through the needle vacuum system (25 mm x 0.8 mm - 21G). Blood samples were centrifuged at 3500 rpm/10 min for serum separation and stored at -20°C for battery testing. The diagnosis of IBR was carried out using the commercial kit infectious IDEXX® Bovine Rhinotracheitis Virus (BHV-1) gB Antibody Test Kit (IDEXX Laboratories, Inc., USA) and for BVD the commercial kit IDEXX® Bovine Diarrhea Virus (BVDV) Antibody Test Kit (IDEXX Laboratories, Inc., USA). In the case of NEO, the diagnosis was carried out using the commercial kit IDEXX® Neospora X2 (IDEXX Laboratories, Inc., USA) at a dilution of 1:100 to determine the number of antibodies as recommended in the manufacturer's manual. For LEP, the screening of the reactive animals was performed by the indirect ELISA test investigating the presence of IgG against the rLipL32 protein according to previously established protocol [11]. Reagent samples were tested by ELISA microscopic agglutination test with live antigens (MAT) according to criteria recommended by the "Human leptospirosis: guidance for diagnosis, surveillance and control" [12].To this end, each serum was diluted 1:50 in PBS buffer for screening and titration.

Obtaining information related to reproductive problems and vaccination scheme of herds

In order to obtain information regarding the reproductive problems observed in the animals selected in the sampling, a questionnaire was applied to all herds sampled. Another point raised with the questionnaire was regarding the scheme of use of vaccines for diseases. The questionnaire was previously tested in other livestock to be finally applied to the herds of the selected sampling.

Statistical analyses

Frequency distributions were performed to evaluate adopting the use of vaccines in cattle. The herds were categorized according to the number of vaccines used in the herd within a year.

The seroprevalence amongst individuals was estimated by the ratio between the number of animals tested and the number of animals with positive results in the laboratory tests. The seroprevalence confidence interval between individuals at a 95% confidence level (95% CI) was estimated according to Sampaio (1998) by the following formula:

$$ICP\ 95\% = P \pm 1.96x$$

With "P" being the seroprevalence found and "n" the number of individuals (cows) sampled.

The seroprevalences that presented confidence intervals that overlap values were considered equal. In the case of not overlapping the confidence interval between seroprevalences, these were considered different.

Bovine infectious rhinotracheitis, bovine viral diarrhea, neosporosis and leptospirosis as a risk factor for reproductive problems

The first step was to identify associations with significance level of $P<0.20$ between serology results for diagnosis of diseases and reproductive problems (estrus recurrence and abortion) by means of a univariate analysis (test of chi-square). The diseases that presented a significance level of $P<0.20$ between serology and reproductive problems were selected for multivariate analysis (multiple logistic regression model). After this step, in the logistic regression models, the variables response or

dependent variables were the reproductive problems (estrus recurrence and abortion) observed by the owners in the selected animals and the explanatory variables or independent variables the results of the serological tests used to diagnose BVD, IBR, NEO and LEP. The diseases that presented the greatest weight on the reproductive problems according to the mesoregions of the State of Rio Grande do Sul were those that presented statistical significance within the logistic regression model ($P<0.05$) and with the highest coefficient (OR).

Results

None of the herds located in the northeastern and northwestern mesoregions which were linked to Cooperative 2 used a vaccine in the previous year. Of the total herds in the study, 15 (17.6%) used the vaccine regularly once a year. These herds were in the southeast, southwest and northwest mesoregions of the state and were linked to Cooperative 2 and producers' association. Among the herds that used at least one dose in the year of the IBR / BVD or LEP vaccine, it was reported that not all the animals selected in the sampling received these vaccines, since they were included in the post-vaccination herds. Based on the results, it was observed that more than 80% of the herds did not use vaccines for IBR, BVD and LEP adequately, considering at least two to three doses per year recommended. No use of vaccine for NEO was observed in the sampled herds.

The highest seroprevalence observed among the diseases were IBR, ranging from 54.4% to 60.3% for the northeastern and northwestern mesoregions of the State of Rio Grande do Sul (Table 1, 2). No difference was observed between the seroprevalences for IBR according to the mesoregions, indicating that the disease was homogeneously distributed among mesoregions, cooperatives and producers' associations.

The second most prevalent disease in the group of herds studied was BVD, with seroprevalences varying from 30.0% to 42.5% (Table 2). The lowest seroprevalence (30.0%) was observed in the cows of the herds of the producers' association located in the northwestern mesoregion of the state, which was different from the seroprevalence amongst cows of Cooperative 2 receiving milk from herds located in the northeast and northwest mesoregions (42.5%).

In the case of NEO, a seroprevalence variation of 21.8% to 35.0% was observed (Table 2). The highest seroprevalence for NEO was in the animals linked to the association of producers located in the northwest mesoregion, and the lowest was in the animals located in herds in the southeast and southwest regions linked to Cooperative 1. Herds of Cooperative 2 located in the northeast and northwest mesoregions showed a seroprevalence of individuals equal to the southeast and southwest mesoregions.

Differently from previous diseases, LEP was the disease with the lowest seroprevalence, ranging from 15.8% to 27.5% (Table 2). The seroprevalence amongst the herds located in the northeastern and northwest mesoregions were similar, with 15.8% and 17.8%, respectively, for the association of producers and Cooperative 2, respectively. However, these seroprevalences were lower and different from those observed in the herds located in the southeast and southwest mesoregions and linked to Cooperative 1, with 27.5%.

Table 1: Frequency distribution of the vaccination scheme adopted in the herds of the cooperatives and association of producers located in the State of Rio Grande do Sul, Brazil

Vaccionation schedule	Mesoregion of RS					
	1		2		3	
	n	%	n	%	n	%
Vaccinate regularly until once in the year	9	42.9	26	53.0	8	47.1
Only LEP or only IBR / BVD	1	4.8	02	4.0	0	0.0
Does not use any more than one year	3	14.3	4	8.2	0	0.0
Does not use any	8	38.1	17	34.7	9	52.9
Total	21	100.0	49	100.0	17	100.0

[1]Southeast / Southwest (Cooperative 1); [2] Northeast / Northwest (Cooperative 2); [3]Northwest (Association of producers LEP - leptospirosis; IBR - infectious bovine rhinotracheitis; BVD - bovine viral diarrhea

Table 2: Seroprevalence of reproduction diseases according to cooperatives and association of producers located in the State of Rio Grande do Sul, Brazil

Disease	Meso-region of the State of Rio Grande do Sul		
	Southeast / Southwest (Cooperative 1)	Northeast / Northwest (Cooperative 2)	Northwest (Association of producers)
IBR	0.592^{cA}(0.542-0.643)	0.603^{cA}(0.558 - 0.648)	0.544^{cA} (0.482-0.605)
BVD	0.395^{bAB}(0.345-0.445)	0.425^{bB}(0.380-0.471)	0.300^{bA} (0.243-0.357)
NEO	0.218^{aA}(0.176 - 0.260)	0.246^{aA}(0.206 - 0.285)	0.350^{bB} (0.292-0.409)
LEP	0.275^{aB}(0.229 - 0.321)	0.178^{aA}(0.143-0.213)	0.158^{Aa}(0.113 -0.203)

95% CI - 95% seroprevalence confidence interval; IBR - infectious bovine rhinotracheitis; BVD - bovine viral diarrhea; NEO - neosporosis; LEP - leptospirosis; Different lowercase and upper case letters mean statistical difference with 95% confidence level between rows and columns respectively.

Table 3: Multiple logistic regression model for risk assessment (OR) for reproductive problems according to diseases and location of dairy cattle herds in the State of Rio Grande do Sul, Brazil

Reproductive problem	Region	IBR	BVD	NEO	LEP
Repetition of heat	South East / South West[1]	NS	4.17 **	NS	NS
	Northeast / Northwest[2]	3.00**	3.40**	NS	NS
	Northwest[3]	NS	NS	NS	NS
Abortion	Southeast / Southwest[1i]	NS	NS	2.43*	NS
	Northeast / Northwest[2]	1.65*	NS	NS	NS
	Northwest[3]	NS	NS	NS	3.70**

BVD - bovine viral diarreria; IBR -bovine infectious rhinotracheitis; LEP - leptospirosis; NEO - neosporosis;[1] Location of Cooperative 1 herds; [2]Location of Cooperativee 2 herds; [3]Location of herds of the producers association; NS - not significant in the chi –square test (P> 0.05); * P <0.05; ** P<0.01

IBR was identified as the risk factor for the recurrence of estrus (OR = 3.00) and miscarriages (OR = 1.65) (Table 3) in animals located in the northeastern and northwest mesoregion belonging to the cooperative 2. However, for these herds, BVD was identified as a risk factor with greater weight (OR = 3.40) for return to estrus in relation to IBR. In these herds of Cooperative 2, NEO and LEP were not considered risk factors for the return to estrus and abortion and BVD for abortion. The BVD was the major risk factor (OR = 4.17) identified in the study of reproductive problems (Table 3). The return to estrus in livestock from the southeastern and southwestern mesoregions associated to Cooperative 1 showed a strong association with BVD. On the other hand, for these herds, NEO was identified as the main risk factor (OR = 2.43) for abortion (Table 3). For these herds of Cooperative 1, the other diseases were not considered as a risk factor for the occurrence of abortions. Regarding the herds located in the northwest mesoregion and belonging to the producer's association, it was observed that LEP was the only risk factor (OR = 3.70) identified and associated with cases of miscarriages (Table 3).

Discussion

The vaccination scheme observed in the herds of cooperatives and producers' association showed a deficiency in the frequency of adoption of the procedures (Table 1). In the 15 (17.5%) herds that vaccinated the animals at least once a year, the deficiency in the adequate adoption of vaccination schemes for IBR, BVD and LEP is also evident, thus promoting a partial immunity in the herds, not preventing reproductive losses related to these diseases, which could occur in different magnitudes. It is likely to infer from the analysis that this partial immunity promoted by the vaccines in the animals may have overestimated the seroprevalence of IBR, BVD and LEP. But, since it was not possible to obtain accurate information from the owners about the vaccination period, all the serology results of the animals selected in the sampling were used to estimate the seroprevalence and risk factors for return to estrus and abortion. Among the control and prevention procedures of IBR, BVD and LEP, the use of vaccines in a proper way, mainly related to the frequency of vaccination, is fundamental to reduce the damages associated with these diseases. Therefore, it is suggested to review the vaccination schedules for each disease for these herds so that the cost/benefit ratio of vaccine adoption is favorable.

Considering above 50.0% a high seroprevalence for IBR and the homogeneous distribution among the mesoregions (table 2), it is suggested the immediate adoption of specific control measures for this disease with the objective of reducing the seroprevalence and the impact on the reproduction of these herds. It is worth mentioning that the etiological agent of IBR is a herpesvirus that induces a decrease in the response of the animal's immune system and may thus interact with other diseases such as mastitis [13]. Considering a percentage of 35 to 52% of unvaccinated herds in these mesoregions, viral circulation is suggested to occur in these mesoregions.

In case of BVD, it was observed that the disease was not distributed homogeneously among the regions, and between cooperatives and producers' associations, as observed in other studies [14]. Southeastern and southwestern mesoregions and Cooperative 1 showed a seroprevalence for BVD intermediate and equal to the seroprevalences of the animals located in herds in the northeast and northwest mesoregions. Although there are herds, both, from the producer association and Cooperative 2 in the northwest mesoregion, the procedures adopted in herds may be different according to the difference in veterinary service or absence of this service [15],allowing this way, in the same middle region, distinct populations of dairy cattle herds have different epidemiological situations for BVD. Like IBR (causing herpesvirus), BVD (causing pestivirus) promotes a decrease in the immune response of the animals and, as a result, increases the chance of interaction with other diseases, as described by Waage (2000) and Wellenberg [16, 17].

The results show that the NEO was homogeneously distributed among the southeast, southwest, northeast and northwest mesoregions. As previously observed for BVD, herds located in the same mesoregion linked to the producer association and the cooperative may have had different procedures oriented by technical assistance or lack of veterinary assistance aiming at the control and prevention of NEO, as observed for other species evaluated in the study conducted by Souza Neto et al. (2008). In addition, it is worth noting that the characteristics of the environment and the vectors of the disease may also be influencing the seroprevalence of NEO [18].

Differently from the IBR, the LEP did not present homogeneous distribution among the mesoregions of the State of Rio Grande do Sul. The highest seroprevalence identified in the Southeast and Southwest mesoregions may be associated with differences in veterinary care or absence of ST, as observed by Lilenbaum and Souza (2003), Martins and Lilenbaum (2017) and consequently sanitary herd management, besides the environmental characteristics [19].

The results of studies of the seroprevalence among the mesoregions show that for BVD, NEO and LEP are no differences relative to epidemiological situation of these diseases according to the mesoregions. In the IBR case, no difference was observed in the epidemiological situation between the mesoregions. However, it was also observed that within the same mesoregion, there may be different epidemiological situations according to the linkage of the herds (cooperatives and producers' association). The association of producers showed the lowest seroprevalence among individuals for IBR, BVD and LEP. Despite, for these herds the highest seroprevalence for NEO was observed. It is suggested that factors related to veterinary technical assistance and the environment may have been determinant for these different epidemiological situations [20, 21].

The results of the seroprevalence and risk factor studies should be analysed together. First, the seroprevalence study shows that all diseases are present in the herds of all mesoregions. Some diseases homogeneously distributed amongst mesoregions and others showed different seroprevalences. The lowest seroprevalence found (15.8%) was for LEP in the livestock herds located in the northwest mesoregion. However, for these herds, the main risk factor for cow abortion was LEP. The results show that although LEP was the lowest seroprevalence among the diseases surveyed, for the herds of the producers' association in the northwest mesoregion was the most important disease amongst the studied ones. Even if IBR was identified with higher seroprevalence, this was not considered the most important for these herds but due to the negative impact caused in the reproduction of the herds, should not have its importance neglected. IBR was the main problem in herds located in the mesoregions of northeast and northwest and linked to cooperative 2, causing both, recurrence of heat and abortions. Nevertheless, BVD also has relevance in the occurrence of return to estrus for these herds. BVD and NEO are diseases with intermediate seroprevalence that should be a priority as they cause reproductive problems in herds of south and southeast mesoregions in the field of Cooperative 1. In these herds, specifically, BVD has been associated with return to estrus and NEO associated with abortion.

The data analysis methodology carried out in the study allowed us to define the weight or magnitude, based on the risk estimate that each disease exerts on the reproductive problems observed in the herds. It was also possible to observe different epidemiological situations amongst the dairy herds linked to different cooperatives and association of producers in the same mesoregion in relation to the diseases surveyed. Nonetheless, it was also observed a difference in the epidemiological situation between the mesoregions studied, based on the seroprevalence of the diseases and their weight within the mesoregions, as observed in other studies (Chaves et al., 2010, Nicolino et al., 2014, 2015; Correa et al., 2016). Possible reasons for different sanitary situations between herds of the same mesoregion and different mesoregions were the link that these herds had with cooperatives and producers' associations. Veterinary technical assistance, which is essential for the reduction of reproductive problems caused by these diseases, may have been different between producer cooperatives and associations. However, the environmental conditions of each mesoregion may also have influenced the impact of diseases on reproductive problems. Studies of the same nature should be carried out later in order to verify if the actions directed to the control and prevention of these diseases are promoting the reduction in the seroprevalence as well as the impact of these in the herds according to the mesoregions and links with the cooperatives and association of producers. Finally, it is necessary to mention that other potential causes of reproductive failures such as nutritional deficiency, metabolic diseases, intoxication conditions and other infectious diseases have not been evaluated in the present study. Thus, diseases investigated serologically may not reflect all factors associated with reproductive failures of the herds evaluated.

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Statement of animal rights

The manuscript does not contain clinical studies or patient data.

Conflict of interested statement

The authors declare no conflict of interest.

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