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SEROPREVALENCE OF ABORTUS DISEASES THAT COMMIT THE BOVINE REPRODUCTIVE EFFICIENCY IN TWO DAIRY AREAS OF CHIAPAS

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— Abstract—

The objective of the present study was to determine the seroprevalence of antibodies against Brucella abortus, Leptospira spp, and Neospora caninum in cattle in two dairy areas of the state of Chiapas. Blood serum was collected from 76 and 103 female bovines from the municipalities of Tecpatán and Juárez respectively. The detection of antibodies against Brucella abortus was performed by the Card test and its confirmation with Rivanol, microplate agglutination against seven serovars of Leptospira, and enzyme immunoassay against Neospora caninum. The results found show a seroprevalence of antibodies for Brucellosis of 0 and 6.8%; 29 and 63% for leptospirosis for the municipality of Tecpatán and Juárez respectively, being the serovars with the highest frequency of seropositivity Icteroahemorrhagiae and Tarassovi, in both municipalities, while seropositivity against Neospora caninum was 46 and 21% respectively. It is concluded that the presence of antibodies against Brucella, Leptospira, and Neospora caninum may be related to the reproductive efficiency of cattle in the municipalities of Tecpatán and Juárez, in the state of Chiapas.

Keywords

Ruminants, Brucellosis, Leptospirosis, Neosporosis..

Abortion is one of the main causes affecting the economics and competitiveness of dairy production. It is a problem of growing importance that significantly impacts herd productivity by decreasing herd viability and productive performance, reducing the potential number of replacement heifers and milk production, as well as increasing costs associated with feeding, treatments, insemination, and premature culling. It can occur sporadically, endemically, or in the form of an outbreak, among its causes can be mentioned some viral, bacterial, or parasitic infections, although determining exactly what causes it is complex. In Mexico, more than 70% of abortions are classified as of unknown origin (Escamilla *et al*., 2007), which limits the implementation of strategies to reduce their occurrence in herds.

Brucellosis is a zoonotic disease caused by bacteria of the genus *Brucella*, being *Brucella abortus* the main species in cattle. Abortions generally occur between 6 and 9 months of age. However, it is estimated that in unvaccinated cows infected in the first trimester of gestation, up to 80% of abortions can occur. Diagnosis can be made by isolation of the bacteria from uterine fluid, milk, placenta, fetal lung, fetal stomach contents, or by serological or milk agglutination techniques.

Another disease of worldwide distribution is leptospirosis, which is more common in tropical and subtropical countries with high humidity, where conditions for its transmission exist (Artiushin *et al*., 2004), especially during the rainy season, where the number of infections increases (Subharat *et al*., 2012). Other risk factors reported in dairy production systems include high animal density, even for short periods of time, and defects in the integrity of the facilities that facilitate the accumulation of excreta, contributing to the spread and maintenance of infection, making vaccination and antibiotic use inefficient (Martíns *et al*., 2012; Llanco *et al*., 2017).

Leptospira hardjo has been identified as the main responsible, individually or in association with other pathogens such as *Neospora caninum* or *Brucella abortus*, for causing bovine abortion in Mexico (Escamilla *et al*., 2007). On the other hand, in some Latin American countries such as Venezuela, Mexico, Colombia, and Brazil, seroprevalences of 42, 10, 61, and 45%, respectively, have been reported (Godoy *et al*., 1997; Nilson, 2003; León *et al*., 2008; Martins *et al*., 2012).

The most important economic impact on cattle farms is the reproductive problems that are mainly manifested by the presentation of abortions, the birth of weak animals, and a decrease in reproductive efficiency.

Bovine neosporosis is a parasitic disease caused by the protozoan *Neospora caninum*, which is characterized by causing abortion at any stage of gestation and can occur more than once in the reproductive life of cattle (Dubey *et al*., 2007). Neosporosis can be acquired by postnatal exposure,

after ingestion of food contaminated with tachyzoites from abortions, infected placentas, or oocysts present in the feces of dogs (Wouda, 2000). It is recognized as one of the main causes of economic losses in production units because it is associated with embryonic losses and is considered one of the main causes of abortion (Quiroz, *et al*., 2011). In studies of neosporosis seroprevalence in cattle in the municipality of Villaflores, Chiapas, 26% was reported (Santiago and Velasco, 2014) and the Istmo-Costa region (Girón and González, 2017) reported 8.33% of seropositive animals.

With this background, it becomes necessary to perform serological tests that allow the identification of the different agents involved as a cause of reproductive failures in the bovine dairy of the region. The objective of the present study was to determine the seroprevalence of antibodies against *Brucella abortus*, *Leptospira* spp, and *Neospora caninum*, in cattle with a history of reproductive problems in two dairy areas of the state of Chiapas.

MATERIALS AND METHODS

The present work was carried out in Tecpatán and Juárez, Chiapas, two of the most important municipalities in the production of milk and its derivatives in the state. The municipality of Tecpatán is located at 17° 098'' N and 93° 19'' W, at an altitude of 320 masl. The climate is hot and humid with rainfall throughout the year. The municipality of Juarez is located at 17°36'27''N 93°11'35''W at an altitude of 150 masl. It presents a warm humid climate with rainfall all year round. (INAFED 2018).

Sample size determination

To determine the sample size, the formula described by Milian (1998) was used, where N = 3.84 P (1-P) / t^2. where N = Sample size; P = Is the prevalence estimate (.28), 3. 84 = Z value of the standard normal distribution; t^2 = Limit within which the prevalence estimate is desired (.10) with a confidence level of 95%, obtaining a total of 76 animals to be sampled in 16 herds in the municipality of Tecpatán and 103 animals in 22 herds in the municipality of Juárez, Chiapas.

Collection of samples

Blood samples were obtained by puncture of the coccygeal vein from dual-purpose cows (*Bos taurus X Bos indicus*), all of them with a history of abortion, repeated estrus or anestrus, with a minimum age of 4 years, and without a history of vaccination against brucellosis and leptospirosis. The collected samples were centrifuged at 2500 rpm for 5 min to obtain blood serum.

Antibody Detection

The detection of antibodies against *Brucella spp* was performed by the 8% card test and its confirmation by Rivanol, with the microagglutination test (MAT) antibodies against six serovars of *Leptospira spp* of national importance were detected.

For the detection of IgG antibodies specific to *Neospora caninum*, the enzyme-linked immunoassay test (ELISA) was used, using the commercial package neospora 2/strip anti-N. *caninum* (IDDEX® Laboratories, Inc), with a sensitivity of 98.6% and specificity of 98.8%. The test was performed with a single 1:100 dilution, identifying positives and negatives at the absorbance of 450 nm. Sera were tested paired and the cut-off point was 0.50, with those with mean readings of ≥ 0.50 being considered positive.

The study was descriptive cross-sectional, the sampling design was simply random, observing the frequency with which antibodies against the diseases studied were present. The positive results were expressed in terms of the Prevalence Rate for each municipality considered in this study.

RESULTS AND DISCUSSION

Seroprevalence of Brucellosis. In none of the serums (0/76) from the 16 herds considered for the study in the municipality of Tecpatán, antibodies against *Brucella spp*. were detected; however, in the serums from the municipality of Juárez, the positive seroprevalence rate was 6.8% (7/103), after performing the Rivanol test for confirmation, four of them showed the presence of antibodies against this bacteria, which belonged to 3 of the 22 herds considered for the study (Image 1).

Gonzalez *et al*. (2006) mentioned that the 8% Card test is highly sensitive but not very specific, that is why the Rivanol test is used for the confirmation of brucellosis in cattle; however, the specificity of the latter can be diminished when used shortly after vaccination of animals with the S19 strain or due to revaccination. The serological results found during this study in cattle in the municipality of Juárez show that although the samples came from negative herds free of brucellosis, the abortion problems reported in the herds sampled could be caused by the disease since there is no history of vaccination in these herds. It is important to highlight that the clinical signs of brucellosis are frequently observed during the first gestation and in subsequent calvings remain asymptomatic carriers and continue to eliminate the bacteria through milk, calving products, and vaginal exudate; these characteristics put calves that will be considered as new replacements at risk of contagion, so it would be advisable to establish a correct serological

monitoring and segregation program within herds, especially in those where there are few biosecurity measures (Fernandez *et al*., 2018).

Seroprevalence of Leptospirosis. Twenty-nine percent (22/76) of bovine blood serums from the municipality of Tecpatán showed antibodies to at least one serovar of *Leptospira spp*, while the seroprevalence rate of positive cases in the municipality of Juárez was 63% (65/103), (Image 1).

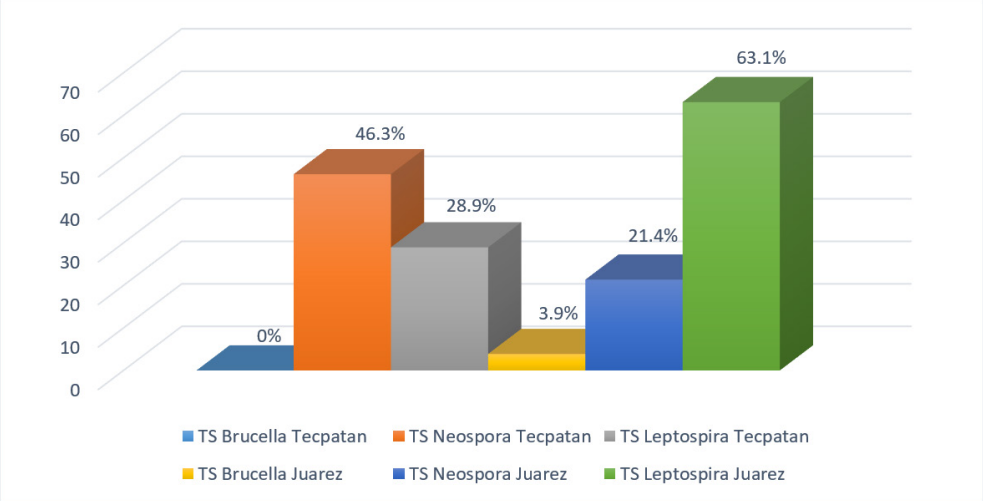

Image 1. Seroprevalence rate of bovine reproductive diseases in the municipalities of Tecpatán and Juárez, Chiapas. Source: Own elaboration

The most frequently identified serovars in Tecpatán were *Tarassovi* (47%), *Icteroahemorrhagiae* (23%), and *Bratislava* (20%); in Juárez, the most frequently reported serovars were *Icteroahemorrhagiae* (33%), *Tarassovi* (30%), and *Bratislava* (21%) (Image 2). All herds considered for this study were shown to have at least one animal seropositive for *Leptospira spp*. demonstrating that these serovars should be considered in vaccines against *Leptospira prevention*. Favero *et al*. (2017) reported that the presence of rodents and canines, as well as poor hygiene and the absence of vaccination programs within the herd, increase the probability of infection by *Leptospira spp* in cattle. The results obtained in this study show that in both municipalities the serovars with the highest frequency of seropositivity were *Icteroahemorrhagiae* and *Tarassovi*, both of which have been frequently reported in ruminants in Mexico and are generally associated with interspecies contact: rodent-bovine. On the other hand, *L. hardjo* has been identified as the main responsible, individually or in association with other pathogens such as *Neospora caninum* or B*rucella abortus*, for causing bovine abortion in Mexico (Escamilla *et al*., 2007).

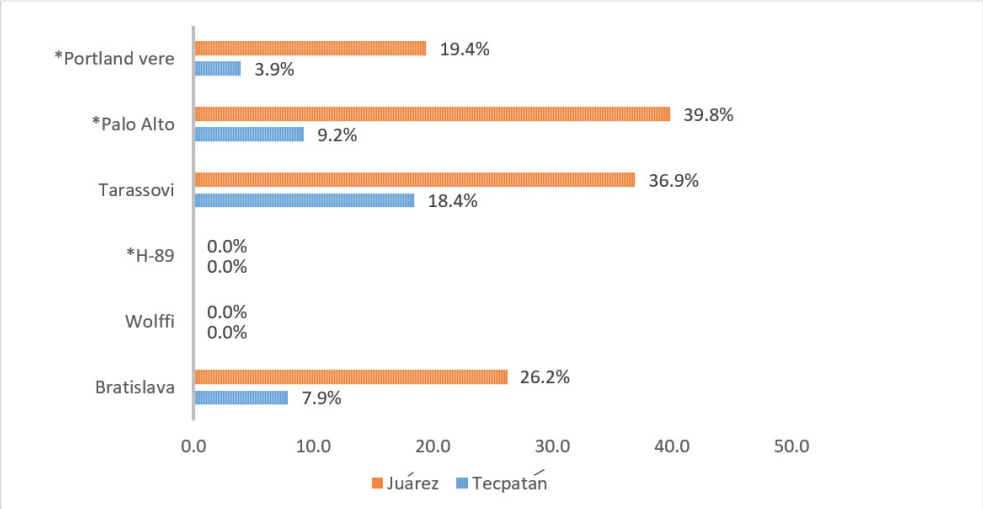

Image 2. Seroprevalence of *leptospira* serovars found in cattle in the municipalities of Tecpatán and Juárez, Chiapas. Source: Own elaboration

Seroprevalence of Neosporosis. In the blood serum samples of cattle from the municipality of Tecpatán, a seroprevalence rate of 46% was observed, that is, 35 of 76 samples showed antibodies against the disease. All herds considered for the study had at least one seropositive animal. In the municipality of Juarez, the seroprevalence of positive cases was 20% (21/103), 13 of the 22 herds had at least one animal with antibodies against *Neospora caninum*. The presence of this disease has already been reported in Mexico, as in other countries, and is related to the coexistence of cattle with dogs. In a study conducted by Sierra *et al*. (2011) and Pulido *et al*. (2017), showed that the presence of dogs seropositive to *Neospora caninum* is a risk factor for cattle in production units located in rural areas, these characteristics were frequently observed in cattle herds of this study, although canines are considered in addition to companion animals, a species used for the control of noxious fauna or as alert animals in most of these herds, it is necessary to raise awareness among producers that their presence can put the health of cattle at risk since they serve as vectors or carriers of some diseases.

CONCLUSION

The presence of antibodies against *Brucella abortus, Leptospira spp*, and *Neospora caninum* suggests that these agents may be related to the reproductive efficiency of cattle in the municipalities of Tecpatán and Juárez, in the state of Chiapas; therefore, it is necessary to implement measures to prevent the spread of these diseases among cattle, as well as to adopt strategies for their control. However, it is also necessary to consider the presence of other non-infectious factors that compromise it.

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