

Seroprevalence of reproductive and infectious diseases in cattle: the case of Madre de Dios in the Peruvian southeastern tropics

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OBJECTIVE

The objective of this study was to determine the seroprevalence of reproductive and infectious diseases in tropical cattle in the Tambopata and Tahuamanu Provinces in the department of Madre de Dios, Peru.

SAMPLE

156 bovines from 7 cattle farms were sampled. These farms used exclusive grazing for food and natural mating for reproduction and did not have sanitary or vaccination programs.

METHODS

The serum of blood samples was subjected to ELISA with commercial kits for the detection of antibodies against *Neospora caninum*, *Mycobacterium avium* subsp *paratuberculosis* (MAP), *Leptospira interrogans*, pestivirus bovine viral diarrhea virus-1, retrovirus bovine leukemia virus (BLV), orbivirus bluetongue virus (BTV), and herpesvirus bovine herpes virus-1 (BHV). The data were analyzed by means of association tests with χ^2 ($P < .05$) and Spearman rank correlation ($P < .05$) in the SPSS v.15.0 software (IBM Corp).

RESULTS

A low prevalence of antibodies to *L interrogans*, *N caninum*, *M avium* subsp *paratuberculosis*, bovine viral diarrhea virus-1 was found, but it was high to BTV, BLV, and BHV (100%, 53.85%, and 72.44%, respectively). The presence of BLV and BHV was higher in the Las Piedras District, bovines less than 5 years old, and cattle with breed characteristics of zebu and crossbred ($P < .01$). In addition, there was a significant correlation between both infections, showing 83.3% of BLV positivity that were also BHV positive ($P < .01$).

CLINICAL RELEVANCE

The high prevalence of antibodies to BTV, BHV, and BLV could be due to livestock management practices, direct contact with infected animals, and variation of the presence of vectors and natural reservoirs in the context of climate change in the tropics.

Keywords: bluetongue, natural mating, enzootic bovine leukosis, IBR, tropical livestock

The presence of reproductive and infectious diseases (RID) in livestock determines the productive efficiency of bovine herds. The diversity of RID is quite wide and includes causative agents such as viruses, bacteria, protozoa, and fungi.¹ The way of

RID's transmission is multiple, due to the difficulties in implementing good management practices (dehorning, castration, gynecological examination, etc), contaminated pastures, direct contact with disease-carrying animals introduced into the herds, greater interaction with transmission vectors and wildlife, use of infected breeders in natural mating, or straws of semen and embryos without any guarantee of sanitary control or even the transplacental route from mother to offspring.²⁻⁴

Received August 3, 2023

Accepted January 24, 2024

doi.org/10.2460/ajvr.23.08.0177

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The greatest alterations due to RID can occur in the reproductive process, such as failure during service and conception, embryo mortality, abortions, and neonatal mortality. Typical clinical signs of infectious diseases manifest as fever, emaciation and anorexia, diarrhea, pneumonia, embryonic death or resorption, fetal malformations, early abortion and, at advanced ages, weak neonates, placental retention, genital lesions, and even temporary infertility.⁵⁻⁷ Some RIDs cause death in severe cases because they do not have effective treatments, due to anorexia and neurological damage; however, the greatest economic impact occurs in subclinical and latent infections, since they affect the immune system and decrease milk production and weight gain and infected animals become reservoirs of infectious agents.^{8,9} The main control strategy is strict monitoring of incoming animals, avoiding the entry of animals with unknown health status, a quarantine period, annual serological analysis, and early culling of infected animals.¹⁰

In the department of Madre de Dios, Peru, a population of 28,197 bovines was reported in 1994,¹¹ and rose to 50,145 in 2012,¹² denoting a substantial increase in livestock activity in recent years in the region. The cattle farms in the research area have as their main purpose meat production by raising *Bos indicus* cattle, some crosses with *Bos taurus*, and Creole cattle.¹³ However, the environmental and ecological characteristics of the tropical zone are favorable for the development of natural reservoirs of infectious agents (viruses, bacteria, and parasites) and transmission vectors of RID.¹⁴ The objective of this research was to determine seroprevalence of

bovine neosporosis (BN), bovine leptospirosis (BL), bovine paratuberculosis (PTBC), bovine viral diarrhea (BVD), bluetongue (BT), enzootic bovine leukosis (EBL), and infectious pustular vulvovaginitis (IPV) by detecting antibodies for the causal agents through ELISA, in cattle from the provinces of Tambopata and Tahuamanu in Madre de Dios.

Methods

Location

The study was carried out in 7 cattle farms, 4 in the province of Tambopata and 3 in the province of Tahuamanu in the department of Madre de Dios, in 2021. The provinces are located on the Interoceanic Highway and border to the north with the Republic of Brazil, to the east with the Republic of Bolivia, to the south with the province of Manu, and to the west with the department of Ucayali (**Figure 1**). The region has an Amazonian geography, with a tropical, warm, and humid climate, with annual rainfall that exceeds 1,000 mm, an average annual temperature of 26 °C, with maximums that reach 38 °C between August and September and minimums of 5 °C between May and August.¹⁵

Sample

The study was cross-sectional and descriptive of a single cohort. According to the IV National Agricultural Census,¹² in the districts where the study was carried out, the cattle populations was a total of 35,520 heads in 3,303 herds (or agricultural units): 5,439 in Iberia, 6,933 in Iñapari, 9,127 in Las Piedras,

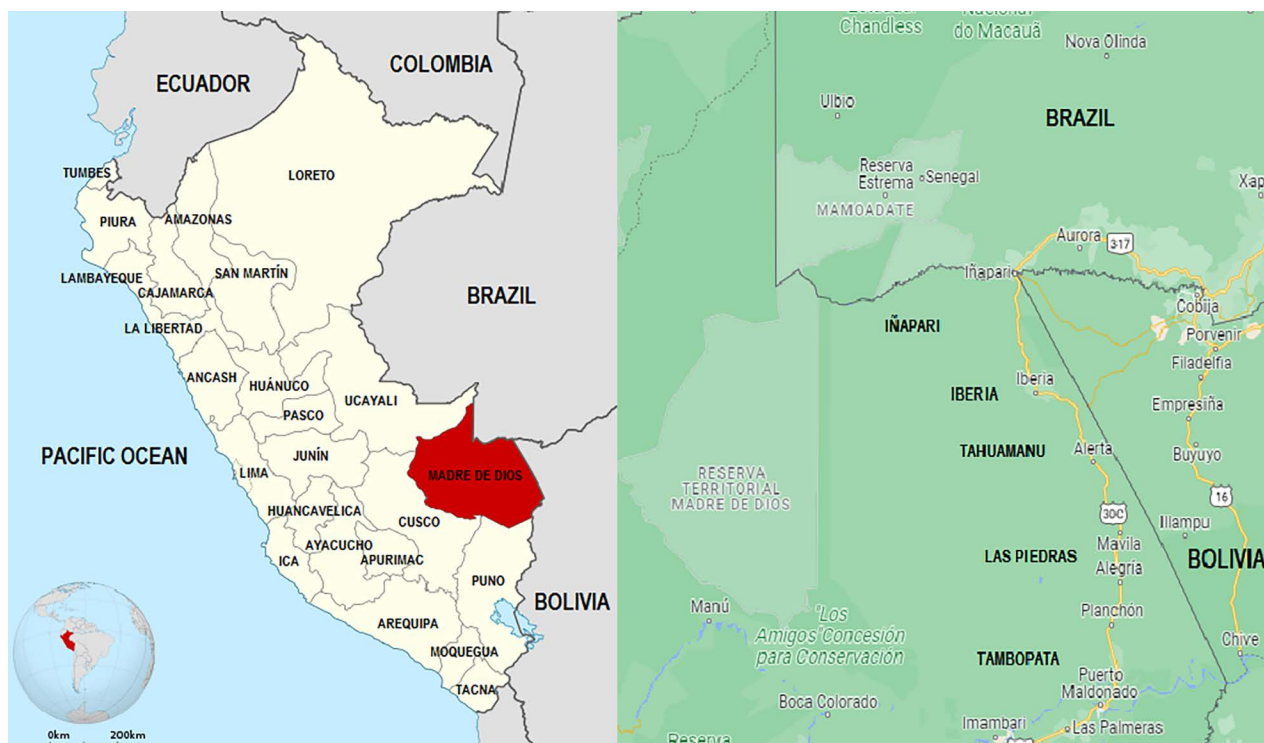


Figure 1—Location map of Iberia and Iñapari Districts (Tahuamanu Province) and the Las Piedras and Tambopata Districts (Tambopata Province) in the department of Madre de Dios, where the sampling was carried out.

and 14,021 in the Tambopata District. Due to the high heterogeneity of the number of cattle in each herd, the sample was calculated based on the number of cattle per district. The sample size formula for finite populations was used,¹⁶ with 95% confidence level, 7% error, and 50% probability of success; finally, a sample of 150 animals was obtained. The sampling was carried out with the informed consent of the farmers, who were made aware of the pertinent information about the objectives of the study, the destination of the collected samples, and confidentiality in all cases. The type of sampling was intentional, and the inclusion criteria were the acceptance of the farmers to take the sample and the availability of handling corals on their farms. The herds were selected at random from the farms that had good vehicular access, and then we determined if they met the proposed inclusion criteria. One hundred fifty-six bovines between males (8) and females (148) over 1 year old were sampled. The sample corresponded to subsets of the selected herds that met the age range and breed characteristics, as follows: 108 were from the Tambopata District (from 3 herds), 22 from the Las Piedras District (from 1 herd), 19 were from the Iberia District (from 2 herds), and 7 from the Iñapari District (from 1 herd). The breed group included bovines with Nelore- and Brahman-breed characteristics (zebu cattle), Brown Swiss-breed characteristics, and undetermined characteristics (crossbred cattle).

Animal care and production system

The study protocol was not submitted to an ethical committee for animal experimentation, because the human-animal interaction was limited to the extraction of blood samples, taking a maximum time of 15 to 30 seconds for each animal. The study was nonexperimental and descriptive of a single cohort in which the animals were carefully restrained in a restraint box inside the cattle stable to avoid sudden movements according to Animal Research: Reporting of In Vivo Experiments 2.0 guidelines. The farms had an extensive production system, with exclusive grazing and no provision of any nutritional supplements, although mineral salt blocks are occasionally provided. Cows arrive at their first calving at 3.5 years old and have a productive life of approximately 8 years. The reproduction system is by natural mating, using permanent bulls in the female herds. The productive purpose is mixed since economic income is received from the sale of milk and cattle for meat. Regarding the sanitary management system, farmers carry out deworming programs and vitamin supplementation once a year; however, vaccination programs are not carried out.

Blood extraction and serological analysis

Blood samples were drawn by direct puncture of the middle coccygeal artery, using sterile 10-mL Vacutainer tubes without anticoagulant. After a period of centrifugation of the samples, the serum was stored in 5-mL vials and frozen at -20°C until analysis. The serum vials were sent to the Virology,

Parasitology and Bacteriology Laboratory of SENASA (National Agrarian Health Service of Peru). For detection and quantification of specific antibodies for *Neospora caninum* (agent causal of BN) trachozoites, a competition ELISA was used with the cELISA VMRD Kit (DEX-UCDSA/par3). For the detection of *Mycobacterium avium* subsp *paratuberculosis* (MAP) antibodies (agent causal of PTBC), indirect ELISA was used with the DEX-UCDSA/Bac.77.PARACHECK 2-Prionics AG Kit. For diagnosis of antibodies to *Leptospira interrogans* (agent causal of BL), a microagglutination technique using the DEX-UCDSA/Bac.11 Kit. For determination of antibodies to bovine leukemia virus (BLV; agent causal of EBL), bluetongue virus (BTV; agent causal of BT), bovine herpes virus (BHV; agent causal of IPV), and bovine viral diarrhea virus (vBVD; agent causal of BVD), the DEX-UCDSA/Vir-03, DEX-UCDSA/Vir-10, DEX-UCDSA/Vir-21, and DEX-UCDSA/Vir-07 BVD SERO-II kits were used, respectively.

Statistical analysis

The relative frequencies of positive cases of BN, PTBC, BL, EBL, BT, IPV, and BVD by the presence of antibodies to causative agents were determined according to province, district, sex, age, and breed group to evaluate the association degree with χ^2 test ($P < .05$). In addition, the correlations between disease seropositivity in cattle were calculated using Spearman ordinal rank coefficients ($P < .05$) to determine the cases of coinfection and the relative frequencies between paired variables. Analyses were carried out in the SPSS v.15.0 software (IBM Corp).

Results

Sampling of 156 bovines was carried out, where 16.67% (26) were in the Tahuamanu Province and 83.33% (130) were in the Tambopata Province. There were 63.23% (108) of cattle in the Tambopata District, 14.10% (22) in the Las Piedras District, 12.18% (19) in the Iberia District, and 4.48% (7) in the Iñapari District; 94.87% (148) were female, and 5.13% (8) were male. Regarding the classification by age group, 51.28% (80) were between 2 and 5 years old, and 48.72% (76) were over 5 years old. Regarding breed characteristics, 73.72% (115) were crossbred cattle, 6.41% (10) were Zebuino, and 19.87% (31) had Brown Swiss characteristics.

From the analysis of blood samples, a high prevalence of BTV antibodies was found in the study area, reaching 100% positive diagnosis. No positive case of *L. interrogans* antibodies was found (0% prevalence). There is a high presence of antibodies to BHV (72.44%), which is associated with the district ($P < .01$), reaching 100% prevalence in Las Piedras and Iñapari. The presence of antibodies to BLV is also high (53.85%), and it is higher in the Iberia and Las Piedras Districts than the others ($P < .01$). The prevalence of antibodies to *N. caninum* and MAP was lower (6 cases and 2 cases, respectively), but a significant association was found by district ($P < .01$), which was higher in Las Piedras and Iñapari. Only 1 positive

case of antibodies to vBVD (0.64%) was found in the Las Piedras District (**Table 1**).

An association analysis of the positive diagnosis of antibodies to causal agents of diseases with the sex, age, and breed group of the sampled animals was performed (**Table 2**). No significant association of any disease with sex was found, but it was found according to age group. There was a higher presence of antibodies to *N caninum* ($P < .05$) and antibodies to BHV ($P < .01$) in cattle from 2 to 5 years old (7.50% and 87.50%, respectively). According to the breed group, crossbred cattle had a higher presence of antibodies to BLV (64.35%) and BHV (87.83%), and in Zebu cattle (Nelore and Brahman), 100% were positive for BHV ($P < .01$).

The correlation between positive cases of antibodies to *N caninum*, MAP, vBVD, BLV, and BHV was evaluated (**Table 3**), and no relationship was found between *N caninum*, MAP, or vBVD; however, a significant correlation was found between the presence of antibodies to BLV and BHV ($P < .01$).

Table 3—Correlation coefficient and significance of antibodies for reproductive and infectious diseases in cattle.

| | BN | PTBC | BVD | EBL | IPV |
|------|-------|--------|--------|--------|--------------------|
| BN | 1.000 | -0.023 | -0.016 | -0.015 | 0.049 |
| | | 0.778 | 0.842 | 0.848 | 0.545 |
| PTBC | | 1.000 | -0.009 | 0.106 | 0.070 |
| | | | 0.910 | 0.190 | 0.383 |
| BVD | | | 1.000 | 0.074 | 0.050 |
| | | | | 0.356 | 0.539 |
| EBL | | | | 1.000 | 0.263 ^a |
| | | | | | 0.001 |
| IPV | | | | | 1.000 |

BN = Bovine neosporosis. BVD = Bovine viral diarrhea. EBL = Enzootic bovine leukosis. IPV = Infectious pustular vulvovaginitis. PTBC = Bovine paratuberculosis.

^aSignificant correlation at the $P < .01$ level with Spearman's rank coefficients.

Table 1—Seroprevalence rate of reproductive and infectious diseases according to province and district in the department of Madre de Dios.

| Variable/level | BN | BL | PTBC | BVD | BT | EBL | IPV |
|----------------|--------------------|-------|--------------------|----------|-----------|--------------------|--------------------|
| Province | | | | | | | |
| Tambopata | 3.08 (4) | 0 (0) | 3.85 (1) | 0.77 (1) | 100 (130) | 51.54 (67) | 73.85 (96) |
| Tahuamanu | 7.69 (2) | 0 (0) | 0.77 (1) | 0 (0) | 100 (26) | 65.38 (17) | 65.38 (17) |
| <i>P</i> value | .26 | - | .20 | .65 | - | .20 | .38 |
| District | | | | | | | |
| Tambopata | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 100 (108) | 44.44 (48) | 68.52 (74) |
| Las Piedras | 18.18 (4) | 0 (0) | 4.55 (1) | 4.55 (1) | 100 (22) | 86.36 (19) | 100 (22) |
| Iberia | 5.26 (1) | 0 (0) | 0 (0) | 0 (0) | 100 (19) | 73.68 (14) | 52.63 (10) |
| Iñapari | 14.29 (1) | 0 (0) | 14.29 (1) | 0 (0) | 100 (7) | 42.86 (3) | 100 (7) |
| <i>P</i> value | < .01 ^a | - | < .01 ^a | .11 | - | < .01 ^a | < .01 ^a |
| Total | 3.85 (6) | 0 (0) | 1.28 (2) | 0.64 (1) | 100 (156) | 53.85 (84) | 72.44 (113) |

The numbers in parentheses represent the count of positive cases.

BL = Bovine leptospirosis. BN = Bovine neosporosis. BVD = Bovine viral diarrhea. BT = Bluetongue. EBL = Enzootic bovine leukosis. IPV = Infectious pustular vulvovaginitis. PTBC = Bovine paratuberculosis.

- = Not applicable.

^aSignificant association at the $P < .01$ level.

Table 2—Seroprevalence rate of reproductive and infectious diseases according to sex, age, and breed group.

| Variable/level | BN | PTBC | BVD | EBL | IPV |
|----------------|------------------|------------------|----------|--------------------|--------------------|
| Sex | | | | | |
| Female | 4.05 (6) | 1.35 (2) | 0.68 (1) | 54.73 (81) | 70.95 (105) |
| Male | 0 (0) | 0 (0) | 0 (0) | 37.50 (3) | 100 (8) |
| <i>P</i> value | .56 | .74 | .82 | .34 | .07 |
| Age | | | | | |
| 2-5 y | 7.50 (6) | 1.25 (1) | 1.25 (1) | 52.50 (42) | 87.50 (70) |
| > 5 y | 0 (0) | 1.32 (1) | 0 (0) | 55.26 (42) | 56.58 (43) |
| <i>P</i> value | .01 ^a | .97 | .33 | .73 | < .01 ^b |
| Breed group | | | | | |
| Crossbred | 4.35 (5) | 0.87 (1) | 0.87 (1) | 64.35 (74) | 87.83 (101) |
| Zebuino | 10.00 (1) | 10.00 (1) | 0 (0) | 30.00 (3) | 100 (10) |
| Brown Swiss | 0 (0) | 0 (0) | 0 (0) | 22.58 (7) | 6.45 (2) |
| <i>P</i> value | .31 | .04 ^a | .84 | < .01 ^b | < .01 ^b |
| Total | 3.85 (6) | 1.28 (2) | 0.64 (1) | 53.85 (84) | 72.44 (113) |

The numbers in parentheses represent the count of positive cases.

BN = Bovine neosporosis. BVD = Bovine viral diarrhea. EBL = Enzootic bovine leukosis. IPV = Infectious pustular vulvovaginitis. PTBC = Bovine paratuberculosis.

^aSignificant association at the $P < .05$ level. ^bSignificant association at the $P < .01$ level.

No cases of possible coinfection between BN and PTBC, BN and BVD, or PTBC and BVD were found (**Table 4**). All positive cases of antibodies to MAP and vBVD were positive for BLV and BHV. In addition, 83.3% of positive cases of antibodies to BHV and 50.0% of BLV were also positive for *N caninum*, although not significantly ($P > .05$). A possible coinfection between EBL and IPV was found, where 83.3% of cases were positive for antibodies to BLV were also positive for BHV ($P < .05$).

Table 4—Relative frequency (%) of simultaneous negative and positive cases of reproductive and infectious diseases in cattle.

| | BN | | PTBC | | BVD | | EBL | |
|------|------|------|------|-----|------|-----|------|------|
| | (-) | (+) | (-) | (+) | (-) | (+) | (-) | (+) |
| PTBC | | | | | | | | |
| (-) | 98.7 | 100 | | | | | | |
| (+) | 1.3 | 0 | | | | | | |
| BVD | | | | | | | | |
| (-) | 99.3 | 100 | 99.4 | 100 | | | | |
| (+) | 0.7 | 0 | 0.6 | 0 | | | | |
| EBL | | | | | | | | |
| (-) | 46.0 | 50.0 | 46.8 | 0 | 46.5 | 0 | | |
| (+) | 54.0 | 50.0 | 53.2 | 100 | 53.5 | 100 | | |
| IPV | | | | | | | | |
| (-) | 28.0 | 16.7 | 27.9 | 0 | 27.7 | 0 | 40.3 | 16.7 |
| (+) | 72.0 | 83.3 | 72.1 | 100 | 72.3 | 100 | 59.7 | 83.3 |

BN = Bovine neosporosis. BVD = Bovine viral diarrhea. EBL = Enzootic bovine leukosis. IPV = Infectious pustular vulvovaginitis. PTBC = Bovine paratuberculosis.

Discussion

This is the first report about the simultaneous analysis of various RIDs that affect cattle in the department of Madre de Dios, such as BN, BL, PTBC, BVD, BT, EBL, and IPV. In the tropical region, diagnostic studies of other infectious and reproductive diseases in cattle have been reported, such as Babesiosis,¹⁷ and Brucellosis in the departments of Ucayali and¹⁸ Huánuco¹⁹ and in Colombia.⁷ The seroprevalence of antibodies indicates that the subjects have been previously exposed to infectious agents. In this study, no presence of BL was found (0% of antibodies to *L interrogans*), despite that *L interrogans* is zoonotic and wild animals (rats, weasels, reptiles, etc) are its natural reservoirs. Rivera et al¹⁸ found 52.2% of samples with antibodies to *L interrogans* in the Amazonian department of Ucayali, but in Colombia, lower levels of these antibodies were reported: 28.0% and 23.1%.^{6,7} The department of Madre de Dios is abundant in wildlife biodiversity, which could suggest a high presence of transmitting vectors and natural reservoirs of pathogens for extensive livestock farming; however, in this study, no cases of BL were found. Therefore, it is suggested more investigation is necessary.

A low seroprevalence of BN (3.85%), PTBC (1.28%), and BVD (0.64%) was found, where the frequency of antibodies to *N caninum* showed a

significant association with district (higher prevalence in Las Piedras) and age group (higher prevalence in cattle from 2 to 5 years old). Other studies¹⁸ have already analyzed the presence of these diseases in other areas of the Peruvian Amazon, such as in the department of Ucayali, with 1.5% antibodies against *N caninum* but no antibodies against the vBVD; in Colombia, the seroprevalence of 12.5% for *N caninum*,⁷ 76.4% for vBVD, and 10.7% for *N caninum* was reported.⁶ On the other hand, in the state of Rio Grande do Sul, Brazil, a seroprevalence of 30.0% to 42.5% for BVD and 21.8% to 35.0% for BN were found.²⁰ It is likely that the higher frequency in the Las Piedras District is due to the introduction of an infected individual, where the congenital transmission route maintains the prevalence of *N caninum* at a local level and horizontal transmission would be less frequent. Bovine neosporosis causes abortions, newborn death, or the birth of weak calves with nervous signs and no apparent infection, which are spreaders of the disease in the herd. The fetus can die in utero to be reabsorbed, mummified, or autolyzed; to be born alive and die immediately; or to be born clinically normal but congenitally infected.^{21,22} This disease is characterized to be generally asymptomatic and congenitally transmitted; there is no effective treatment, so the control is based on the prevention of transmission and culling of infected animals.^{23,24} Regarding the lower frequency of BN in cattle over 5 years old, it may be due to the fact that the reproductive problems caused by *N caninum* could motivate the early culling of these animals from the herd. The PTBC results of this study are lower than the reports from Nariño, Colombia, with an 8% prevalence in milking cows, which was associated with the body condition of the animals.²⁵ Bovine paratuberculosis is a disease caused by MAP, which affects sheep and cattle (mainly dairy cattle), and feces, colostrum, and milk are an important source of transmission. It is characterized by chronic enteritis, progressive emaciation, and diarrhea and leads to a state of malnutrition, weakness, and death.²⁶⁻²⁸ In the study areas, the breeding of crossed *Bos taurus* X *Bos indicus* cattle oriented toward meat production predominates, and there are very few herds dedicated to milk production, which would explain the low prevalence of PTBC.

A 100% presence of antibodies to BTV was found in the samples analyzed. BT is an infection caused by a virus of the *Orbivirus* genus of the Reoviridae family, which causes cyanosis of the tongue, fever, inflammation, and hemorrhage in the oral and nasal mucosa and throughout the digestive tract, loss of body condition, inflammation in the coronary bands and sensitive hoof plates, and fetal malformations in infections during the first third of gestation; in domestic and wild ruminants.^{29,30} In the department of Madre de Dios, the presence of antibodies to BTV in Huanganas (*Tayassu pecari*) was already reported in 7.5% and to other antibodies of the same serogroup in 29.2% of sample.³¹ The virus is transmitted by arthropods of the genus *Culicoides* (*Culicoides insignis*) infected by biting animals with

viremia, which makes its prevalence higher in areas with a favorable climate for the development of the vector.²⁹ Vertical transmission of BT from pregnant cows to their offspring and from infected males to females through semen has also been verified. The BTV has a predilection for the reproductive tract, so its disorders generated include embryonic death, abortions, fetal malformations, temporary infertility in males, and virus elimination in semen.^{32,33} BT has been studied mainly in sheep, with variable levels of prevalence in the tropical zone of Peru. A 56.1% BT prevalence in Blackbelly sheep from the department of Junín was found,³⁴ 96.0% in sheep from the department of Ucayali,³⁵ and 99.0% in cattle from Ecuador.³⁶ In a recent study,³⁷ BT prevalence in sheep is not uniformly distributed in Brazil, and there is a higher concentration of cases in the state of Rio Grande do Sul³⁰ and the state of Amazonas. In 2004, no antibodies against the BTV and BHV were detected in deer (*Mazama gouazoubira*) in Bolivia³⁸; however, the recent increase in BT cases in cattle could be due to variations in environmental conditions, temperature patterns, precipitation, and wind, as well as management practices, which would affect the distribution of vectors and hosts in tropical zones.^{30,39} In this regard, Clavijo et al⁴⁰ argue that BTV serotypes may be continuously changing as a function of climate change, and changes in land use in recent years could affect the geographic distribution of domestic and wild ruminants and influence areas where new BTV serotypes may emerge and persist, which could explain the high prevalence of BT in the present study. In addition, the increase in the dynamics of circulation of live cattle in border areas of Peru, Brazil, and Bolivia could contribute to the spread of infected animals as BTV reservoirs.²⁹

A high presence of antibodies to BHV (72.44%) and BLV (53.85%) was found. In addition, both frequencies were significantly correlated ($P < .01$), finding a possible coinfection in 83.3%, where IPV-positive diagnoses were also EBL positive. Infectious bovine rhinotracheitis is produced by BHV-1 that affects the respiratory, reproductive, and nervous systems, which can be transmitted by direct contact with bodily secretions and semen during natural mating, artificial insemination, or embryo transfer.^{41,42} In the respiratory form, infected animals present fever, serous nasal discharge, conjunctivitis, salivation, cough, loss of appetite, depression, necrotic lesions in the nose that can progress to pustules, and ulcers covered by a pseudomembrane that obstructs the upper airways.⁴³ A frequent complication is abortion, between the third and sixth week after infection, mainly in cows from 5 to 8 months of gestation. In the genital form, females develop IPV and males show infectious pustular balanoposthitis.^{20,42} The infection acute phase lasts from 2 to 4 days and recovery from 10 to 14 days after the onset of signs, and the animals remain virus carriers. In this study, a lower prevalence of IPV was found in cattle > 5 years, which could be due to the death or culling of adult cattle. In the nervous form, young animals present meningoencephalitis, lack of coordination, muscle

tremors, ataxia, and blindness, leading to death.⁴⁴ For its control, it is recommended to monitor the movement of cattle, quarantine period, frequent serological analysis, and culling of positive animals upon diagnosis. EBL is caused by the BLV, which infects B lymphocytes. The majority of individuals infected with BLV never develop clinical disease, although subclinical infection can cause immunosuppression, where T cells infected with BLV increase the expression of immunoinhibitory receptors, which increase their ability to evade the immune response to other infections. Only a small percentage of infected animals develop a tumor (lymphosarcomas), which causes early slaughter and consequent direct economic loss.⁴⁵ The main mechanism of transmission of BLV is through livestock equipment contaminated with blood from infected animals (dehorner, hypodermic needles, surgical instruments, or infested equipment), blood-sucking insects, or vertical transmission from mother to fetus or by ingestion of colostrum from infected cows to suckling calves.^{45,46} The immunosuppressive effect of EBL could explain the high seropositivity of BHV antibodies for IPV, which coincides with Mionetto and Rodríguez,⁸ who also found a significant association between seropositivity between BLV and BHV-1, since BLV would facilitate the entry of other pathogens or would diminish the immunological protection mechanisms such as lower production of IgM and IgG2 against BHV-1. Furthermore, the high seroprevalence of antibodies to BHV may be due to direct contact with respiratory, ocular, or genital secretions of infected cattle, or indirectly through equipment contaminated with the virus, and the transmission through the semen of infected bulls is not ruled out. In other reports, Rivera et al¹⁸ registered the presence of antibodies to BHV-1 in 46.3% of cattle in the department of Ucayali, and Souza et al⁴¹ found a seroprevalence of 54.5% to 60.3% in Rio Grande do Sul²⁰ and 43.3% to 86.2% in Acre, Brazil. The scarce control of the transit of animals in border areas could have contributed to the high prevalence of IPV and EBL, especially in the districts of Iñapari, Iberia, and Las Piedras. There are reports about the significant association of age, breed, and number of births with the EBL and IPV seroprevalence.⁴⁷⁻⁴⁹ In this study, the degree of breed purity of the animals was not certain, and 73.72% corresponded to crossbred cattle, where a higher seroprevalence of antibodies to BHV and BLV was found in zebu breeds (100% and 30.0%, respectively) and in crossbred cattle (87.8% and 64.4%, respectively); cattle with Brown Swiss characteristics had the lowest prevalence of both infections (6.45% and 22.58%, respectively). However, cattle with Brown Swiss characteristics were present in only 1 herd, so the prevalence could be increased if all herds had these types of cattle. Further studies are required to determine genetic resistance to BLV as a control strategy.⁵⁰

In cattle from the Tambopata and Tahuamanu Provinces in the department of Madre de Dios, Peru, a seroprevalence of antibodies to BTV and *L. interogens* was found in 100% and 0% of the sample,

respectively. The seroprevalence of antibodies to *V. caninum*, MAP, and vBVD (3.85%, 1.28%, and 0.64%, respectively) in the study area was lower than BLV and BHV (53.85% and 72.44%, respectively). Antibodies to BLV and BHV were more frequent in the Las Piedras District, BHV in cattle from 2 to 5 years old, BHV in cattle with breed characteristics of zebu, and BLV in crossbred cattle. In addition, a positive correlation was found in cases positive for both infections, where 83.3% of individuals were positive for EBL and positive for IPV.

Acknowledgments

We express our gratitude to Dr. Manuel Antonio Canto Saenz for his kind support in copyediting and language of the manuscript.

Disclosures

The authors have nothing to disclose.

The Google translator tool was used on some occasions during the writing of the text to review some terms in the English language.

Funding

This work was supported by the Dirección General de Ganadería (DGGGA) of MIDAGRI and by the project “Mejoramiento de la disponibilidad y acceso del material genético mediante el uso de técnicas de biotecnología reproductiva en ganado bovino tropical en las regiones de San Martín, Loreto y Ucayali” with CUI 2338934–PROMEG Tropical.

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