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Cows of reproductive age in the high Andean region of Peru have seroprevalence of antibodies against bovine herpesvirus 1 and *Neospora caninum*

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Objective
To determine the seroprevalence of infectious agents with reproductive implications in cattle from 3 districts of the high Andean region of Peru over 3,500 m above level sea.

Methods
361 blood samples were collected from cows in 3 districts of the Apurimac department using nonprobabilistic stratified sampling for an exploratory study. Antibodies against *Brucella* spp, *Mycobacterium avium* subsp *paratuberculosis*, bovine viral diarrhea virus, bovine leukemia virus, bluetongue virus, bovine herpesvirus 1 (BHV), and *Neospora caninum* (NC) were detected by ELISA. Analysis of independence, multiple correspondence, and logistic regression were performed for risk factors, with district, age, and phenotypic traits as predictors.

Results
The highest seroprevalences were 18.28% BHV and 6.93% NC, with a similar trend at the sector and herd levels. Bovine herpesvirus 1 was predominant in Cotaruse and Oropesa and NC in Oropesa and San Jerónimo. The multiple correspondence analysis explained 42.40% of the structural variability, with greater dispersion observed between seropositives for NC and BHV, without a significant association between seropositivities. The district was a risk factor for NC, with a 95% CI of 0.11 to 0.88 in Cotaruse, although the 95% CI for BHV was 7.33 to 630.75 in Cotaruse and 14.11 to 1,289.94 in Oropesa.

Conclusions
BHV and NC are seroprevalent in cows from the sampled high Andean herds, and district location is the strongest risk factor.

Clinical Relevance
Environmental conditions and livestock management practices of the region could explain the seroprevalence rates; however, strengthening surveillance against BHV and NC is imperative to prevent risks of acute or subclinical infections.

Keywords: high Andean cattle farming, epidemiology, ELISA, bovine herpesvirus 1, *Neospora caninum*

Cattle farming in Peru has a population of around 5,866,168 heads,[1] of which 36.45% are purebred and 63.55% correspond to Creole cattle, mainly distributed along the Andean region under the care of small- and medium-scale breeders in extensive or semi-intensive breeding systems. One of the main challenges in small-scale farming is the presence of infectious diseases, which affect herd productivity and cause reproductive issues.[2] There is limited information on the sanitary status of Creole cattle herds in high Andean regions as well as on the factors limiting productivity and development in these breeding systems under harsh environmental conditions.

Infectious diseases reduce cattle productivity, with reproductive impacts reflected in a lower number of calves born, an increased number of inseminations per conception, estrus repetition, infertility, embryonic death, congenital malformations, abortions, reduced milk production, economic losses, and costs associated with premature culling.[3] The etiology of infectious diseases is multifactorial, involving various

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causative agents, such as bacteria, viruses, fungi, and parasites. Infectious agents in cattle farming include a great diversity and are widely distributed: bovine viral diarrhea virus (BVDV), bovine herpesvirus 1 (BHV), *Neospora caninum* (NC), *Brucella* spp (BR), *Leptospira* sp, *Mycobacterium avium* subsp *paratuberculosis* (MAP), etc.[4] Numerous reports[5–12] worldwide describe varying seroprevalence rates across different latitudes and altitudes.

At the level of Peru, high seroprevalence rates of BVDV and NC have been reported in dairy cattle from the valleys of Lima, Arequipa, and Mantaro, identifying these pathogens as major causes of reproductive disorders and abortions.[13] *Brucella* spp and *Leptospira* sp have also been detected in dairy cattle from Lima, Arequipa, and other milk-producing regions, though sporadically.[14] Infectious diseases with reproductive implications are associated with calf losses, reduced milk production, increased feed costs, expenses for reproductive treatments, and long-term impacts on female reproductive efficiency, ultimately leading to premature culling.[3,15]

In the Andean region, varying seroprevalence rates of antibodies against major infectious agents affecting cattle have been reported to assess the sanitary status across different altitudinal zones. However, climate pattern variations due to global warming, along with dynamic changes in live animal movements, could alter life zones, host migration patterns, and the distribution of infectious agent vectors at higher elevations. Additionally, differences in the prevalence of infectious agents among populations could be influenced by environmental conditions and breeding systems. The majority of cattle in Peru's high Andean region are Creole, and monitoring herd health is crucial due to the socioproductive benefits of this species in subsistence breeding systems. Therefore, this study aimed to determine the seroprevalence of antibodies against infectious agents with reproductive implications in cows from the high Andean region of Peru over 3,500 m above level sea, specifically in the districts of San Jerónimo, Cotaruse, and Oropesa, in the department of Apurímac.

Methods

Location

The study was conducted in the districts of San Jerónimo, Cotaruse, and Oropesa, located in the department of Apurímac, Peru **(Figure 1)**. The districts were located within the range of 2,915 to 5,438 m above level sea, and the herds were located above 3,500 meters. Sample collection was carried out from April through December 2023. The region is classified as a subalpine subhumid and alpine tundra climate (Köpper-Geiger), with average daily temperatures ranging from a maximum of 29.1 °C to a

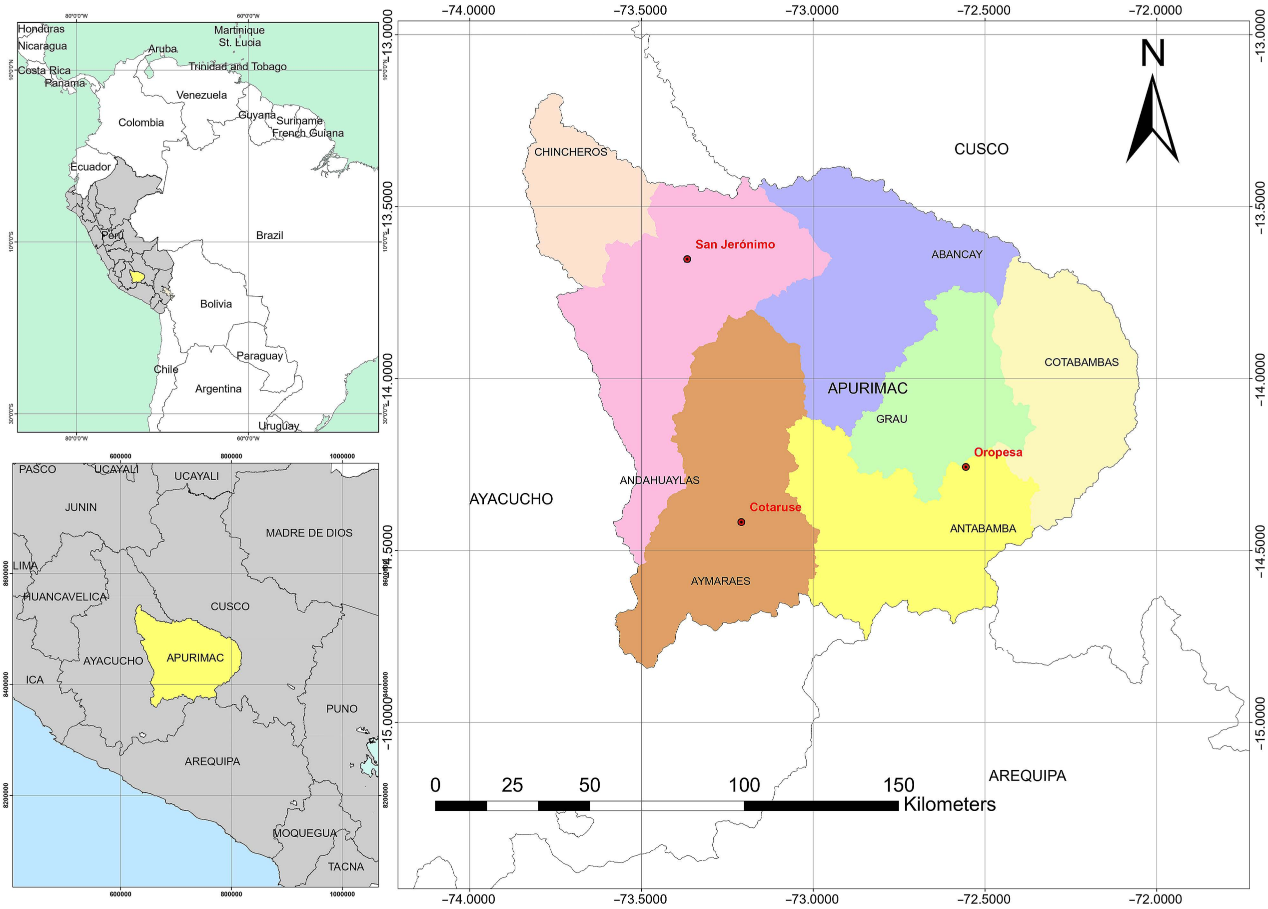

Figure 1—Location map of the districts of San Jerónimo, Cotaruse, and Oropesa in the department of Apurímac, Peru.

minimum of −0.5 °C between June and August and from a maximum of 28.1 °C to a minimum of 3.1 °C between September and May.[16]

Design

The study was exploratory with the main objective of identifying the preliminary seroprevalence of antibodies against the infectious agents BR, MAP, BVDV, bovine leukemia virus (BLV), bluetongue virus (BTV), BHV, and NC in a population of reproductive-age cows, with homogeneous conditions of extensive breeding and direct mating for reproduction.

Population and sample

The total population of Creole cows in the 3 districts was 6,105: 2,146 in San Jerónimo, 2,498 in Cotaruse, and 1,461 in Oropesa.[17] The sample size was calculated with a 95% CI, 50% positive diagnosis probability, 50% negative diagnosis probability, and 5% maximum margin of error. A sample of 361 cows was calculated, and all of them were tested for all infectious agents. The sample was stratified by proportional fractioning according to the population in each district: 126 in San Jerónimo (35%), 148 in Cotaruse (41%), and 87 in Oropesa (24%). Sampling was nonprobabilistic as accessibility and the willingness of breeders to participate in the study were limiting, so cows were sampled from 93 herds (51 in San Jerónimo, 18 in Oropesa, and 24 in Cotaruse) located in 23 sectors (5 in San Jerónimo, 2 in Oropesa, and 16 in Cotaruse).

Sample collection and serological analysis

All handling procedures were carried out in accordance with the Animal Research: Reporting of In Vivo Experiments 2.0 guidelines and the Peruvian government's Animal Protection and Welfare Law (30407). Blood samples were collected from cows aged 2 to 7 years with a history of natural breeding. During the sampling, age was recorded within the age groups of 2 to 3 years, 4 to 5 years, or 6 to 7 years, and phenotypic traits were recorded as Creole phenotype and Brown Swiss phenotype. Each animal was properly identified and carefully restrained, and the sampling site was disinfected before drawing blood from the caudal vein using a Vacutainer needle. The human-animal interaction was limited to the extraction of blood samples, taking a time of 15 to 20 seconds for each animal, so the protocol was not submitted to an ethical committee for experimentation. Samples were labeled and refrigerated for transport to the Laboratorio de Sanidad Animal del Servicio Nacional de Sanidad Agraria in Lima, Peru. Serum was used to detect antibodies against infectious agents. *Brucella* spp was detected using a competitive ELISA (DEX-UCDSA/Bac.19; SVANOVIR Brucella-Ab C-ELISA; SVANOVA). *Mycobacterium avium* subsp *paratuberculosis* was detected using an indirect ELISA (DEX-UCDSA/Bac.90; ID Screen® Paratuberculosis Indirect Screening Test; IDvet Innovative Diagnostics). Bovine viral diarrhea virus was detected via antigen-detection ELISA (DEX-UCDSA/Vir-07; BVD-SERO-II kit; IDEXX Laboratories). Bovine leukemia virus was detected using an antibody-detection ELISA (DEX-UCDSA/Vir-03; Bovine Leukosis Virus Antibody Detection Kit; Synbiotics Corp). Bluetongue virus was detected through a solid-phase competitive ELISA (DEX-UCDSA/Vir-10; cELISA Bluetongue Virus Antibody Test kit; IDEXX Laboratories). Bovine herpesvirus 1 was detected using an antibody-detection ELISA (DEX-UCDSA/Vir-21; Infectious Bovine Rhinotracheitis Virus Antibody Test Kit; IDEXX Laboratories). *Neospora caninum* was detected using a competitive ELISA (DEX-UCDSA/Par-17; ID Screen *Neospora caninum* Competition; IDvet Innovative Diagnostics).

Statistical analysis

Positive serological diagnoses were organized into frequency tables, and seroprevalence by district, age groups, and phenotypic traits seroprevalence was analyzed using the χ^2 test for independence in SPSS, version 15.0 (IBM Corp). Sector-level and herd-level seropositivity (with at least 1 seropositive case) were presented as case count and percentage. Nominal correlations between BHV and NC were evaluated using the Cramér V in SPSS, version 15.0. A multiple correspondence analysis (MCA) was performed with these same variables to assess the joint variability of diseases and identify coinfection trends. Logistic regression and OR analysis were applied to the most prevalent infectious agents (BHV and NC) to determine risk factors using district, age groups, and phenotypic traits as predictors. The Hosmer-Lemeshow test ($P > .05$) was used to assess the goodness of fit, and the McFadden test evaluated the predictive capacity of the model. Multivariate analyses were performed in R (version 4.4.3; R Foundation for Statistical Computing) using the *FactoMineR* package[18] for MCA and the *pscl*[19] and *stats*[20] packages for logistic regression.

Results

Serum samples from 361 reproductive-age cows across 93 herds, 23 sectors, and 3 districts of the Apurímac department in the Peruvian high Andean region were analyzed. No antibodies against BR or MAP were detected in any district **(Table 1)**. The overall seroprevalence of antibodies against BHV was 18.28%, showing a significant association with district ($P < .01$). The highest seroprevalence was observed in Oropesa (37.93%), followed by Cotaruse (21.62%) and San Jerónimo (0.79%).

According to Table 1, the overall seroprevalences of antibodies against BVDV, BLV, and BTV were 0.83%, 0.83%, and 0.55% (3, 3, and 2 cases, respectively). The overall seroprevalence of antibodies against NC was 6.93%, with a higher frequency in San Jerónimo (10.32%), followed by Oropesa (6.90%) and Cotaruse (4.05%), though not statistically significant ($P > .05$).

The cows' ages ranged from 2 to 7 years, with a higher percentage of BHV antibodies found in the 4-to-5-years (19.47%) and 6-to-7-years (23.33%) age groups, although not significantly. Antibodies against NC were more prevalent in cows in the 2-to-3-years

Table 1—Seroprevalence of antibodies against infectious agents in reproductive-age cows in the high Andean region.

Variable	n	BR	MAP	BHV	BVDV	BLV	BTV	NC
District								
San Jerónimo	126	0 (0)	0 (0)	0.79% (1; −0.8 to 3.4)	0 (0)	0.79% (1; −0.8 to 2.4)	0 (0)	10.32% (13; 4.9 to 15.7)
Cotaruse	148	0 (0)	0 (0)	21.62% (32; 14.9 to 28.3)	0.68% (1; −0.7 to 2.0)	1.35% (2; −0.5 to 3.2)	1.35% (2; −0.5 to 3.2)	4.05% (6; 0.8 to 7.3)
Oropesa	87	0 (0)	0 (0)	37.93% (33; 27.5 to 48.3)	2.30% (2; −0.9 to 5.5)	0 (0)	0 (0)	6.90% (6; 1.5 to 12.3)
P value		—	—	< .01	—	—	—	.13
Age group								
2–3 y	218	0 (0)	0 (0)	16.97% (37; 12.0 to 22.0)	0.92% (2; −0.4 to 2.2)	0.92% (2; −0.4 to 2.2)	0 (0)	7.34% (16; 3.9 to 10.8)
4–5 y	113	0 (0)	0 (0)	19.47% (22; 12.1 to 26.9)	0.88% (1; −0.9 to 2.6)	0.88% (1; −0.9 to 2.6)	0 (0)	7.08% (8; 2.3 to 11.9)
6–7 y	30	0 (0)	0 (0)	23.33% (7; 7.3 to 39.4)	0 (0)	0 (0)	6.67% (2; −2.8 to 16.1)	3.33% (1; −3.5 to 10.2)
P value		—	—	0.65	—	—	—	.72
Phenotypic traits[a]								
Creole	232	0 (0)	0 (0)	17.24% (40; 12.3 to 22.1)	0 (0)	0.43% (1; −0.4 to 1.3)	0.86% (2; −0.3 to 2.1)	8.62% (20; 5.0 to 12.3)
Brown Swiss	115	0 (0)	0 (0)	14.78% (17; 8.2 to 21.4)	2.61% (3; −0.4 to 5.6)	1.74% (2; −0.7 to 4.2)	0 (0)	4.35% (5; 0.6 to 8.1)
P value		—	—	.56	—	—	—	.15
Total	361	0 (0)	0 (0)	18.28% (66)	0.83% (3)	0.83% (3)	0.55% (2)	6.93% (25)

Independence analysis using the χ^2 test ($P < .01$). Percentages are presented, with counts and 95% CIs shown in brackets.

BR = *Brucella* spp. BHV = Bovine herpesvirus 1. BLV = Bovine leukemia virus. BTV = Bluetongue virus. BVDV = Bovine viral diarrhea virus. MAP = *Mycobacterium avium* subsp *paratuberculosis*. NC = *Neospora caninum*.

[a] Percentages calculated based on 347 cows with records of phenotypic traits.

(7.34%) and 4-to-5-years (7.08%) age groups, although these associations were not significant ($P > .05$; Table 1). On the other hand, BTV antibody-positive cases were found in cows in the 6-to-7-years age group, BVDV and BLV antibody-positive cases were mostly concentrated in the 2-to-3-years and 4-to-5-years age groups, and BVDV antibody-positive cases were concentrated in cows with Brown Swiss phenotypic traits. However, these findings had no inferential value due to the few cases found. Higher frequencies of BHV and NC antibody-positive cases were observed in cows with Creole phenotypic traits, but these associations were not significant ($P > .05$).

A general sector-level seropositivity of 65.20% for BHV and 34.80% for NC was observed **(Table 2)**. A lower seropositivity rate for BHV was found in sectors of the San Jerónimo district, whereas lower seropositivity for NC was observed in sectors of the Cotaruse district. At the herd level, 28.00% of the herds tested positive for BHV and 19.40% for NC.

The MCA showed that 2 dimensions explained 42.40% of the data variability (22.10% and 20.30%; **Figure 2**). Negative cases for all tests clustered together, and seropositive cases for BHV, BLV, and BVDV tended to be together on the dimension 1 axis. The dispersion between seropositive cases for BTV and NC on the dimension 2 axis represented more heterogeneous cows, which could be associated with different factors influencing their seropositivity. However, the distributions and relationships of seropositivity between BLV, BVDV, and BTV should be interpreted cautiously due to the low frequency found.

The nominal correlation values among positive antibody cases for BHV and NC were weak and nonsignificant ($P > .05$) within each district **(Table 3)**.

The risk for seroprevalence of BHV and NC was assessed due to their higher frequency of positive cases. According to **Table 4**, the district could be a significant risk factor, where cows from Cotaruse

Table 2—Sector and herd seropositivity rates for infectious agents in reproductive-age cows by district in the high Andean region.

Seropositivity level	District	n	BHV	BVDV	BLV	BTV	NC
Sector-level seropositivity	San Jerónimo	5	20.00% (1)	0 (0)	20.00% (1)	0 (0)	80.00% (4)
	Oropesa	2	100.00% (2)	100.00% (2)	0 (0)	0 (0)	50.00% (1)
	Cotaruse	16	75.00% (12)	6.30% (1)	12.50% (2)	12.50% (2)	18.80% (3)
	Total	23	65.20% (15)	13.00% (3)	13.00% (3)	8.70% (2)	34.80% (8)
Herd-level seropositivity	San Jerónimo	51	2.00% (1)	0 (0)	2.00% (1)	0 (0)	21.60% (11)
	Oropesa	18	55.60% (10)	11.10% (2)	0 (0)	0 (0)	16.70% (3)
	Cotaruse	24	62.50% (15)	4.20% (1)	8.30% (2)	8.30% (2)	16.70% (4)
	Total	93	28.00% (26)	3.20% (3)	3.20% (3)	2.20% (2)	19.40% (18)

Independence analysis using the χ^2 test ($P < .01$). Percentages are presented, with counts shown in brackets.

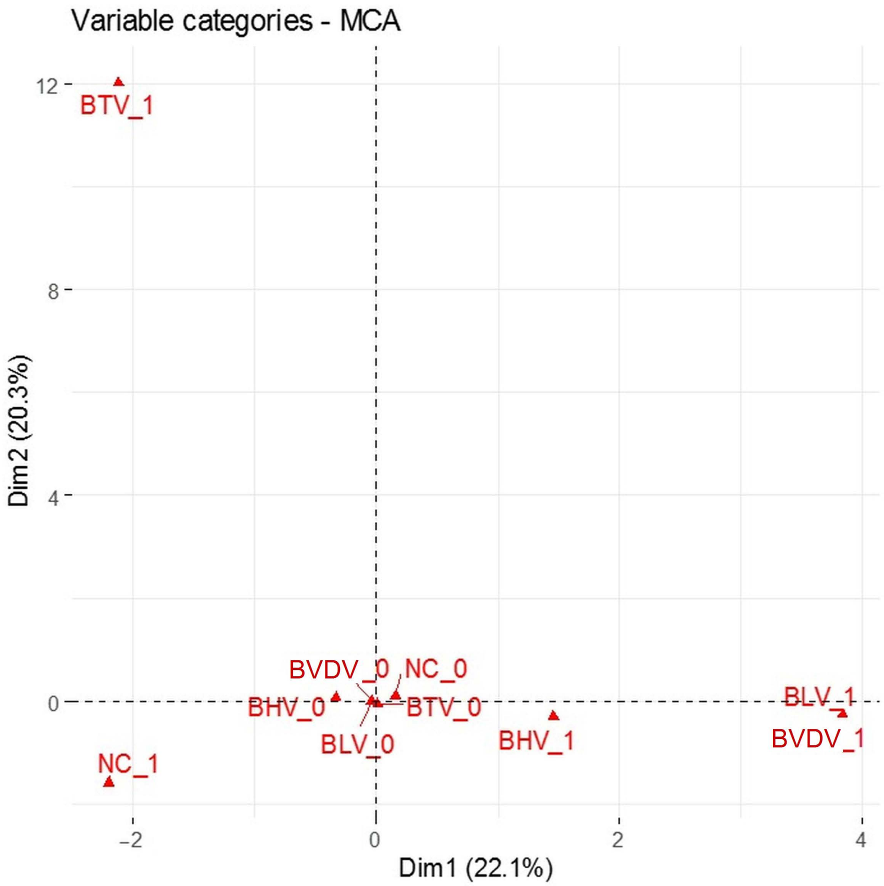

Figure 2—Distribution of seropositive (1) and negative (0) cases of infectious agents in the multiple correspondence analysis (MCA) in the high Andean region. BHV = Bovine herpesvirus 1. BLV = Bovine leukemia virus. BTV = Bluetongue virus. BVDV = Bovine viral diarrhea virus. Dim = Dimension. NC = *Neospora caninum.*

Table 3—Cramér V correlation values and significance for BHV and NC seropositivity by district in reproductive-age cows in the high Andean region.

		District		
		Oropesa	**Cotaruse**	**San Jerónimo**
Seropositivity	**Measures**	**NC**	**NC**	**NC**
BHV	Cramér V	0.12	0.03	0.03
	Significance	0.27	0.77	0.73

Table 4—Risk factors for BHV and NC seroprevalence in reproductive-age cows in the high Andean region.

		BHV			**NC**		
Factors		**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
	(Intercept)	0.01	< 0.01–0.04	< .01**	0.17	0.08–0.33	< .01**
San Jerónimo	Cotaruse	35.11	7.33–630.75	< .01**	0.33	0.11–0.88	.03*
	Oropesa	70.76	14.11–1,289.94	< .01**	0.94	0.31–2.59	.91
2–3 y	4–5 y	1.58	0.80–3.09	.18	0.81	0.31–1.94	.64
	6–7 y	0.90	0.24–2.81	.87	0.37	0.02–2.02	.36
Creole	Brown Swiss	0.70	0.33–1.39	.33	0.38	0.12–1.02	.07

The risk of infection is significant at the $P < .05$ (*) and $P < .01$ (**) levels.

were 35.10 times more likely to be infected with BHV compared to San Jerónimo ($P < .01$), and cows from Oropesa were 70.80 times more likely than those from the same district ($P < .01$). However, the CIs were wide, likely due to the low number of positive cases found in San Jerónimo. Regarding NC, cows from Cotaruse were 0.33 times less likely to be infected compared to those in San Jerónimo. The predictors "age group" and "phenotypic traits" were not significant ($P < .05$). The McFadden values indicate that the models have modest predictive power for BHV ($P < .177$) and NC ($P < .049$) seroprevalence.

Discussion

This study simultaneously analyzed the presence of antibodies against 7 etiological agents with reproductive implications in cattle from the high Andean region of Peru in herds located above 3,500 m above sea level. The absence of seropositive cases for BR could be attributed to the effectiveness of animal transit regulations and national surveillance programs[21] as well as environmental factors, such as accessibility, climate, and extensive grazing systems, that hinder the transmission of MAP. In other regions, various reports[5,22] of BR seroprevalence have been documented, including 0% in Oxapampa, Pasco, and 0.88% in Formosa, Argentina. Regarding MAP, seroprevalence rates of 1.28% have been reported[9,23] in crossbred Zebu cattle in Madre de Dios and 0.55% nationwide in Peru, among others. Bovine paratuberculosis significantly affects the productive and reproductive performance of intensively reared cattle, impacting dairy farm profitability.[24,25] Along with bovine brucellosis, it is relevant to public health and local economies. Therefore, it is crucial to maintain periodic monitoring and prevention strategies for these diseases.

The seroprevalence of antibodies against BLV, BTV, and BVDV was found to be below 1.00% at the individual level (0.55% to 0.83%). The low percentages found for these infectious agents are not sufficient to allow reliable seroprevalence. A larger sample size in future studies could improve the estimation of their prevalence. Previous reports[6,26] on BLV seroprevalence show high variability in other regions: 14.84% in dual-purpose cattle in the low tropics of Boyacá, Colombia, and 12.80% in dairy farms in the Arequipa Valley. A high seroprevalence of 92.70% was reported in a dairy farm in Lima,[27] and 53.85% in Zebu cattle in the southeastern tropics of Peru in which 83.30% of animals were seropositive for both BLV and BHV.[9] This high coinfection rate is likely due to BLV's immunosuppressive effect, which facilitates BHV infection by reducing immunoglobulin M and immunoglobulin G2 levels.[9,28] Enzootic bovine leukosis is one of the most common tumor diseases in cattle, mainly affecting dairy cattle, and causes significant productivity losses in subclinical infections.[29] Bovine leukemia virus seroprevalence is increasing worldwide except in the European Union, likely due to the absence of measures to minimize transmission and inadequate management practices, which act as risk factors.[24,30]

Regarding BTV, seroprevalence reports[9,31] include 100% in Madre de Dios and 86.00% in Brazil. In China, a 12.20% seroprevalence was reported[7] from 1988 to 2019, with a higher rate in free-ranging cattle (22.50%) compared to confined cattle (1.80%). Clinical signs of BTV infection in sheep include decreased production and abortions,[32] whereas cattle often remain asymptomatic but serve as reservoirs for the virus.[33] Bluetongue virus is endemic to tropical regions where environmental conditions favor the development of the vector *Culicoides insignis.*[31] However, BTV distribution may shift due to environmental changes associated with climate change and inadequate management practices, which create favorable ecosystems for vectors and hosts.[7,34]

Previous reports[2,9,10] on BVDV seroprevalence show high variability: 16.30% in Junín, 0.64% in Madre de Dios, and 2.51% across Peru. Several studies have reported high seroprevalence rates, including; 56.20% in Espinar[14]; 48.70% in Puno[35]; 27.00% in Ecuador[36]; and 32.60% in Ethiopia.[37] The high BVDV seroprevalence in some regions could be attributed to the presence of persistently infected animals within herds, acting as a permanent source of infection.[38] Bovine viral diarrhea is a disease of global importance, associated with gastrointestinal, respiratory, and reproductive disorders in cattle. Its immunosuppressive effect predisposes animals to secondary infections, and subclinically infected animals shed the virus, acting as a continuous source of infection.[39] Pasture leasing and livestock fairs increase the risk of transmission to free populations and even to other species.[40] In this study, BVDV-seropositive cases were found in cows with Brown Swiss phenotypic traits, with no cases detected in Creole cows. The harsh environmental conditions of the high Andean region may challenge the adaptability of certain breeds that are more susceptible to infectious agents.[41] In this regard, seroprevalences of 58.00% in Brown Swiss and 45.30% in Creole cows were reported[8] in animals without clinical signs of bovine viral diarrhea but with some reproductive problems. Similarly, seroprevalences of 27.90% in crossbred Brown Swiss and 22.70% in Creole cattle were reported,[36] although breed was not a significant risk factor. Higher seroprevalence has been reported[42] in Normande cattle. Risk factors for BVDV infection identified in other studies[37,43] include animals aged ≥ 18 months, Holstein Friesian crosses, and herds with ≥ 11 animals in Ethiopia as well as large herds and high animal density. In another study,[39] significant protective factors included Jersey-breed animals, ages between 1 and 2 years, and the use of artificial insemination with infection-free bulls.

The BHV agent causes infectious bovine rhinotracheitis in cattle, a disease with a significant impact on productive and reproductive efficiency and, consequently, on the economic performance of livestock farms.[44] Bovine herpesvirus 1 is a pneumotropic virus that predisposes animals to secondary respiratory tract infections, primarily in young calves, and remains latent until reactivated under stress conditions, causing new cycles of acute and subclinical infection.[45] The overall BHV seroprevalence in this study was the highest among all infectious agents evaluated (18.28%). This percentage is lower than the 27.40% national seroprevalence,[10] the 59.56% in extensive farming systems in Ayacucho,[11] and the 72.44% observed in crossbred Zebu cattle from Madre de Dios.[9] Similarly, high seroprevalence rates have been reported, such as 67.60% in Parinacochas, Ayacucho,[46] and 73.13% in dual-purpose cattle in Caquetá, Colombia.[44] In this study, BHV seroprevalence varied between districts, with fewer cases reported in San Jerónimo (0.79%), located in northern Apurímac. The high BHV seroprevalence in some populations could be attributed to animal movement between herds, the absence of regulations on livestock mobilization, the lack of vaccination programs, poor disease prevention and control strategies within herds, and the absence of testing for animals entering new herds.[44] Additionally, the lack of physical boundaries between herds under extensive grazing systems and the limited reproductive control (natural mating or artificial insemination) could facilitate virus transmission.[11,47] In high Andean conditions, cases may have originated from the introduction of animals from areas with high BHV seroprevalence. However, further transmission might be limited due to the region's poor accessibility, resulting in a localized seroprevalence in 28.00% of herds (2.00% in San Jerónimo, 55.60% in Oropesa, and 65.50% in Cotaruse).

Bovine neosporosis is the leading cause of abortion in cattle, holding significant economic importance.[12] The seroprevalence of NC reported in this study (6.93%) is higher than the 3.30% reported[2] in Junín and the 3.85% reported[9] in crossbred Zebu cattle in Madre de Dios, Peru. This finding falls is lower than the 20.33% reported[10] nationwide by the Servicio Nacional de Sanidad Agraria; the 12.20% reported[48] in Parinacochas, Ayacucho; the 45.00% reported[49] in dairy cattle from Boyacá, Colombia; and the 58.00% reported[50] in 2020 and 42.00% in 2021 in cows with reproductive issues in Ecuador. *Neospora caninum* transmission is most efficient through the congenital route, though transmission via contaminated feed and water is also significant. Poor livestock management practices, combined with inadequate disposal of carcasses and farm waste, may promote the dissemination of NC. When consumed by domestic dogs, these materials turn them into definitive hosts, perpetuating the parasite's life cycle.

The MCA showed proximity among cows seropositive for BHV, BVDV, and BLV (with lower case frequencies), whereas the distance between BTV, NC, and BHV suggests heterogeneity in the risk factors associated with seropositivity. Due to the low seroprevalence of BVDV, BLV, and BTV, the MCA should be interpreted cautiously; however, its importance in providing information for understanding the status of these infectious agents in the high Andean region is highlighted, particularly with regard to the distribution of BHV and NC cases. The risk factor analysis indicated a significant effect of district on seropositivity for BHV and NC. However, the wide CI for BHV suggests high variability in the estimation, likely due to the low case frequency in San Jerónimo, which served as the reference district. Neither age nor phenotypic traits were significant predictors. However, previous research on a larger population of Belgian cattle identified older age, male sex, cattle origin, and smaller herd size as risk factors for BHV.[47] For NC, Cotaruse appeared to be a protective factor compared to San Jerónimo, potentially due to differences in livestock management and animal movement. In contrast to BHV seropositivity, most NC cases were found in San Jerónimo herds and sectors, although the correlation was not significant. Besides, the nominal correlation analysis revealed no significant association between infectious agents, confirming the distribution mode of MCA and suggesting heterogeneous animals between both seropositivities. The distance between districts may hinder NC transmission, and since congenital transmission is predominant, cases likely originated from animals introduced from high-prevalence areas, becoming localized within certain herds. Expanding the sample size and exploring additional predictors in future studies is recommended to better understand the relationship between BHV and NC seroprevalence.

In conclusion, no seropositive cases were found for BR or MAP; few seropositive cases were found for BVDV, BLV, and BTV (0.55% to 0.83%); and higher seroprevalence rates were observed for BHV (18.28%) and NC (6.93%). District was a significant risk factor for BHV and NC, although the 95% CI indicated greater uncertainty in BHV-seropositive cases. Environmental conditions, livestock management practices, and limited accessibility in the Andean highlands could explain the relatively low overall seroprevalence. However, reinforcing monitoring programs for infectious bovine rhinotracheitis and bovine neosporosis remains imperative to prevent the risks of acute or subclinical infections in the region.

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