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Short Communication

Initial Seroprevalence Records of Infectious Agents Implicated in Reproductive Issues in High-altitude Cattle from Two Districts in Cajamarca, Peru

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Abstract

In high Andean regions with subsistence livestock farming, diagnostic studies are rarely conducted for reproductive issues in extensively bred cows. This research aimed to determine the seroprevalence of *Neospora caninum*, bovine viral diarrhea virus (BVDV), and bovine herpesvirus (BHV-1) in cattle above 3300 meters in Peru. Blood from 292 cattle across categories and breeds was collected. Antibody prevalence was 13.70±3.94% for *N. caninum*, 30.14±5.26% for BVDV, and 2.74±1.87% for BHV-1. District-wise differences were not significant (P>0.05). Cow and Creole breed were the most affected, but without statistical variance (P>0.05). These results confirm *N. caninum*, BVDV, and BHV-1 presence in reproductive issues in high altitudes cattle in Cajamarca and Celendín provinces.

Keywords: Cattle, High altitude, BHV-1, BVDB, *Neospora caninum*, Reproductive problems, Serology

Introduction

The management of bovine reproduction is the cornerstone of herd health provision and the success in modern veterinary practice for large animals. However, over the last decades, fertility has steadily declined, despite increasing veterinary intervention [1]. The primary factors negatively affecting cattle reproduction are attributed to pathogenic microorganisms.

Protozoan parasites such as *Neospora caninum* directly impinge upon bovine reproductive health [2]. In cattle, they manifests considerable global economic losses attributable to reproductive disorders including abortion, retention of fetal membranes, metritis, estrus repetitions, and temporary anestrus [3]. Furthermore, viruses such as bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BHV-1) exert significant adverse reproductive impacts on cattle health. Within the reproductive tract, BVDV induces suboptimal fertility, abortions, and fetal deformities during gestation [4]. Conversely, BHV-1 precipitates systemic infections, fever, anorexia, nasal mucosa reddening, cough, and conjunctivitis, often culminating in abortions occurring. Early embryonic demise can also ensue as a consequence of the infection [5].

Moreover, climatic conditions exert a discernible influence on animal reproduction. Productive efficiency of dairy cows is diminished at higher altitudes compared to lower altitude areas [6]. Extreme weather conditions disrupt the energy exchange between the animal and its environment,

negatively impacting reproduction. Conception rates decline under heat and cold stress, with endocrine functions being perturbed by climatic extremes [7].

The raising of dairy cattle in the highlands of the Peruvian mountains is centered on an extensive system, with an average of six to seven animals per farm and distinct health and reproductive management practices compared to valleys or intensive systems, including the use of dogs for herding and livestock care. A study conducted at 3200 meters above sea level in hamlets of the provinces of Cajamarca, Celendín, and Hualgayoc evaluated the effectiveness of estrus synchronization and artificial insemination, achieving a pregnancy rate of 42.82% (301/703) [8]. However, after the study, producers frequently reported cases of infertility, abortions, and retained placentas.

Given the background of reproductive problems in cows from the mentioned provinces and with the aim of pinpointing whether the causes could be of infectious origin, this study determines the seroprevalence of three etiological agents involved in reproductive issues: *N. caninum*, BVDV, BHV-1, in extensively raised dairy cattle located above 3300 meters above sea level in hamlets of two districts of the provinces of Cajamarca and Celendín.

Material and Methods

Ethical Considerations

The owners of the cattle taken for consultation were informed and agreed to the participation of their animal in the study. Sampling was performed taking all biosafety and animal welfare measures, according to the guidelines of the Ley de Protección y Bienestar Animal - N° 30407, of the Peruvian State.

Study and Sampling Area

This is an exploratory study in which a total of 292 cattle were evaluated. Cattle of varying age categories and breeds were randomly sampled, situated at altitudes ranging from 3311 to 3979 meters above sea level, and distributed across hamlets within the La Encañada and Sorochuco districts of the Cajamarca and Celendín provinces, respectively. Among the total sampled animals, six of them had a history of abortion (n=5) and infertility (n=1), as per information provided by the livestock producers. The study area exhibits an average annual temperature of 7.85°C and a relative humidity of 83.93%. GPS technology (GPSMAP® 66i, USA) was employed to ascertain the altitude of each hamlet.

The hamlets under investigation in this study are situated within mining-influenced zones that promote livestock husbandry improvement through cattle acquisition and the implementation of reproductive biotechnology programs. These areas boast a diverse array of wildlife, including Andean foxes, Andean deer, and others. The cattle were reared in an extensive husbandry system, with interactions occurring among ovine, caprine, equine, porcine, and, in some instances, alpacas. All livestock keepers had dogs without health controls, which they employed for both companionship and assistance in livestock management. On the other hand, it was observed that the pasture intended for cattle consumption exhibited poor quality and limited quantity *(Fig. 1)*.

Fig 1. Presentation of cattle farming at high altitudes. Buena Vecindad village: Extensive cattle farming is observed alongside alpacas and donkeys (A, D). El Porvenir village: Cattle are seen in fields with low vegetation coverage and small-sized pastures (B, C)

Sampling and Diagnostics

The animals were identified using information provided by the livestock owner (names and ear tag numbers). These animals had not been vaccinated at any point against BVDB or BHV-1. From each animal, 5 mL of blood was collected by coccygeal venipuncture using the vacuum tube system (Vacutainer) into additive-free tubes. The tubes were placed in a rack and transported in a thermal box to the Laboratory of the Fondo de Crédito para el Desarrollo Agroforestal (FONCREAGRO), Cajamarca.

The tubes were centrifuged at 2500 rpm for 10 min. The serum was transferred to 2 mL Eppendorf tubes, frozen at -8°C, and subsequently transported by air to the Microbiology and Parasitology Laboratory of the Faculty of Veterinary Medicine (FMV) at the National University of San Marcos (UNMSM) in Lima, Peru.

Antibodies against *N. caninum* were detected using a commercial competitive ELISA kit *(N. caninum* Antibody Test Kit, cELISA, VMRD, USA). The procedure was conducted according to the manufacturer's instructions. Serum samples with an inhibition percentage of 30% or higher were classified as positive. Antibodies against Bovine Viral Diarrhea virus (BVD) and Bovine Herpesvirus

(BHV-1) were detected using viral neutralization. Fetal bovine nasal turbinate cell cultures, free of BVD virus, were employed as indicator systems in both cases. The cells were cultured using Minimum Essential Medium (MEM) and Leibovitz (L-15) Medium (SIGMA, USA), in a 50:50 ratio supplemented with 10% BVD-free fetal bovine serum and antibiotics (SIGMA, USA).

The technique described by the OIE [9] and the protocol of the Virology Laboratory of the FMV, UNMSM, were followed. For the detection of antibodies against BVD, the NADL strain, CP biotype genotype I, with a titer of 10^{-5} DI50 CC/50 µL, was employed as the antigen. The Cooper strain (Ames, USA), prototype strain of BHV-1 with a titer of 10^{-5} DI_{50} CC/50 mL, was used as the antigen in the diagnosis of antibodies against BHV-1.

Samples were considered positive for BVD and BHV-1 antibodies when the serum titers were equal to or greater than 1:2, as evidenced by the absence of cytopathic effects on the indicator cells.

Statistical Analysis

The results were organized in MS Excel 2019, and seroprevalence along with a 95% confidence interval was calculated. Using the SPSS Statistics 27.0.1 software, the independence of results between districts was assessed using the Mann-Whitney U test, and P-values were adjusted using the Bonferroni correction. Age category and breed were analyzed using the non-parametric Kruskal-Wallis test, and in cases where statistical differences were identified, the Mann-Whitney U test was employed to pinpoint the differing group.

Results

Calf (0 - 12 months), heifer (>12 months - up to before pregnancy), and cow (≥parturitions) of Brown Swiss, Holstein, and Creole breeds were sampled, distributed across fifteen hamlets located between altitudes of 3311 to 3979 meters above sea level, all of which had not been previously vaccinated. Antibodies against *N. caninum* (13.70 [95% CI, 9.75-17.64]), Bovine Viral Diarrhea Virus - BVD (30.14 [95% CI, 24.87 - 35.40]), and Bovine Herpesvirus - BHV-1 (2.74 [95% CI, 0.87-4.61]) were detected in the districts *(Table 1, Fig. 2)*.

With the exception of the calf age group, which did not show cases of BHV-1, all other categories exhibited antibodies against *N. caninum*, BVD, and BHV-1 *(Table*

Table 1. *Prevalence (%) of seropositive animals for Neospora caninum, BVD, and BHV-1, by provinces in extensively raised dairy cattle located above 3300 meters above sea level*

Province: District	Village	Altitude (masl)	Nº	*N. caninum*		BVD		BHV-1	
				Positive	Prevalence (95% CI)	Positive	Prevalence (95% CI)	Positive	Prevalence (95% CI)
Cajamarca: La Encañada	El Porvenir	3791	26	4	15.38 (1.52-29.25)	8	30.77 (13.03-48.51)	0	0.00 (0.00-0.00)
	Buena Vecindad	3476	11	1	9.09 (0.00-26.08)	0	0.00 (0.00-0.00)	0	0.00 (0.00-0.00)
	El Valle	3311	5	2	40.00 (0.00-82.94)	0	0.00 (0.00-0.00)	0	0.00 (0.00-0.00)
	Yerba Buena Chica	3719	44	4	9.09 (0.60-17.59)	16	36.36 (22.15-50.58)	4	9.09 (0.60-17.59)
	Lagunas de Combayo	3979	23	2	8.79 (0.00-20.21)	9	39.13 (19.18-59.08)	2	8.70 (0.00-20.21)
	Quinuapampa	3828	24	1	4.17 (0.00-12.16)	15	62.50 (43.13-81.87)	1	4.17 (0.00-12.16)
	San Juan de Yerba Buena	3561	28	5	17.86 (3.67-32.04)	15	53.57 (35.10-72.04)	0	0.00 (0.00-0.00)
Subtotal			161	19	11.80 (6.82-16.78)[a]	63	39.13 (31.59-46.67)[a]	7	4.35 (1.20-7.50)[a]
Celendín: Sorochuco	San Nicolás de Challuagón	3705	10	1	10.00 (0.00-28.59)	2	20.00 (0.00-44.79)	0	0.00 (0.00-0.00)
	Chugurmayo	3713	18	3	16.67 (0.00-33.88)	5	27.78 (7.09-48.47)	0	0.00 (0.00-0.00)
	Alto Cruz Pampa	3547	10	0	0.00 (0.00-0.00)	0	0.00 (0.00-0.00)	0	0.00 (0.00-0.00)
	Agua Blanca	3667	62	11	17.74 (8.23-27.25)	13	20.97 (10.83-31.10)	1	1.61 (0.00-4.75)
	El Tingo	3414	3	1	33.33 (0.00-86.68)	1	33.33 (0.00-86.68)	0	0.00 (0.00-0.00)
	La Chorrera	3688	5	1	20.00 (0.00-55.06)	1	20.00 (0.00-55.06)	0	0.00 (0.00-0.00)
	Uñigan Pululo	3744	23	4	17.39 (1.90-32.88)	3	13.04 (0.00-26.81)	0	0.00 (0.00-0.00)
Subtotal			131	21	16.03 (9.75-22.31)[b]	25	19.08 (12.35-25.81)[b]	1	0.76 (0.00-2.25)[b]
Total			**292**	**40**	**13.70 (9.75-17.64)**	**88**	**30.14 (24.87-35.40)**	**8**	**2.74 (0.87-4.61)**

[a,b]*Different letters indicate statistical differences between districts for each disease agent (Mann-Whitney U test + post hoc test [Bonferroni correction], P<0.05)*

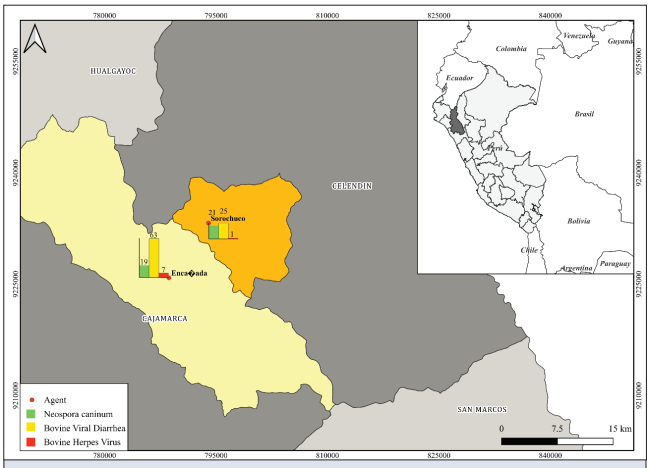

Fig 2. Distribution of seropositive cases in the villages of the two districts, La Encañada and Sorochuco

Table 2. *Prevalence (%) of seropositive animals for Neospora caninum, BVD, and BHV-1, by age group and breed in extensively raised dairy cattle located above 3300 meters above sea level*

Classification	Category	Nº	*N. caninum*		BVD		BHV-1	
			Positive	Prevalence (95% CI)	Positive	Prevalence (95% CI)	Positive	Prevalence (95% CI)
Age group	Calf	15	1	6.67 (0.00-19.29)[a]	3	20.00 (0.00-40.24)[a]	0	0.00 (0.00-0.00)[a]
	Heifer	22	5	22.73 (5.22-40.23)[a]	10	45.45 (24.65-66.26)[a]	1	4.55 (0.00-13.25)[a]
	Cow	255	34	13.33 (9.16-17.51)[a]	75	29.41 (23.82-35.00)[a]	7	2.75 (0.74-4.75)[a]
Breed	Brown Swiss	148	11	7.43 (3.21-11.66)[b]	46	31.08 (23.62-38.54)[a]	6	4.05 (0.88-7.23)[a]
	Creole	128	25	19.53 (12.66-26.40)[a]	39	30.47 (22.49-38.44)[a]	2	1.56 (0.00-3.71)[a]
	Holstein	16	4	25.00 (3.78-46.22)[a]	3	18.75 (0.00-37.88)[a]	0	0.00 (0.00-0.00)[a]
Total		**292**	**40**	**13.70 (9.75-17.64)**	**88**	**30.14 (24.87 - 35.40)**	**8**	**2.74 (0.87-4.61)**

[a,b]*Different letters indicate statistical differences between categories within each column (Kruskal-Wallis + post hoc test [Mann-Whitney U test], P<0.05)*

2). Similarly, the only group that did not show antibodies against BHV-1 was the one composed of Holstein breed animals *(Table 2)*. However, it is necessary to note that the number of individuals in both of these categories (calves and Holstein breed) was smaller compared to the heifer and cow categories, and to the Brown Swiss and Creole breeds.

Out of the five animals with a history of abortion, four were seropositive for BVD, and one was seropositive for both *N. caninum* and BVD. The remaining animal with a history of infertility was seropositive for BHV-1. Among the positive animals, 5.14% exhibited antibodies against both *N. caninum* and BVD simultaneously. Similarly, 1.71% showed antibodies against both BVD and BHV-1.

Discussion

A global seroprevalence of 13.70±3.94% for *Neospora caninum*, 30.14±5.26% for BVD, and 2.74±1.87% for BHV-1 was found across the two districts. In Sorochuco, a higher number of seropositive cases were observed for *N. caninum*, unlike BVD and BHV-1, which were more prevalent in La Encañada. Numerically, the group of cows exhibited the highest presence of antibodies against the evaluated infectious agents. Creole animals showed the highest reactivity against *N. caninum*, while Brown Swiss exhibited higher reactivity against BVD and BHV-1.

Since all the cattle owners had dogs for companionship and cattle care, these dogs could be one of the causes contributing to the spread and latency of neosporosis in the area. Studies have determined that the presence of dogs on the farm is a determining factor for *N. caninum* infection in cattle [10,11].

A common practice among livestock farmers in the assessed areas is to introduce animals by purchasing them from local, regional, and national herds to improve genetics. This practice may introduce pathogens due to the lack of sanitary control before acquiring the

animals. Furthermore, various reproductive techniques, such as artificial insemination, have been employed to optimize cattle reproduction, achieving a pregnancy rate effectiveness of 42.82% (301/703) in a previous study using estrus synchronization and artificial insemination. However, before and after the study, producers frequently reported cases of infertility, abortions, and retained placentas [8]. It is possible that these actions have introduced and disseminated BVDV, as this virus can be transmitted directly to the reproductive tract through semen or through insemination or embryo transfer materials. Additionally, most producers, especially those who disagree with artificial insemination or embryo transfer programs, rely on natural mating in their herds. In such cases, infected bulls transmit and spread BVDV and BHV-1 to the cows [12]. Consequently, the virus can remain in constant transmission from mother to fetus [4].

It is plausible that altitude may not serve as a predisposing factor for the presence of BHV-1 and BVD, given that both diseases have been reported across various altitudinal ranges [13,14]. Generally, in more remote areas far from major cities, there is limited animal movement due to poor road development and access routes, which might prevent disease dissemination.

An important factor to consider is the effect of pasture quality on the normal physiology of animals. *Fig. 1* illustrates agricultural conditions with underdeveloped and poor-quality pastures. It has been demonstrated that poor food quality adversely affects the fertility of cows [15]. Therefore, in addition to *N. caninum*, BVD, and BHV-1, the impact of low-quality pastures on the reproductive performance of cows in the evaluated districts should also be taken into account. On the other hand, extensive breeding of different animal species could potentially contribute to the conservation and dissemination of neosporosis, BVD, and BHV-1 in the livestock of the La Encañada and Sorochuco districts.

In conclusion, the presence of antibodies against *N. caninum*, Bovine Viral Diarrhea Virus, and Bovine Infectious Rhinotracheitis Virus type 1 was evidenced for the first time in extensively reared cattle in the high-altitude areas (>3300 meters above sea level) of two districts in the provinces of Cajamarca and Celendín. These agents could be contributing to the occurrence of reproductive issues in the cattle population of the region.

Declerations

Availability of Data and Materials: All relevant data are contained in this manuscript. However, for detailed information, please contact the corresponding author (L. Vargas-Rocha).

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Ethical Considerations: The owners of the cattle taken for consultation were informed and agreed to the participation of their animal in the study. Sampling was performed taking all biosafety and animal welfare measures, according to the guidelines of the Ley de Protección y Bienestar Animal - N° 30407, of the Peruvian State.

Competing of Interests: The authors declare the absence of any known competing financial interests or personal relationships that might have seemed to influence the work reported in this paper.

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