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Co-infection with bovine herpesvirus type 1 and bovine leukemia virus in dairy cattle in Cajamarca, Peru: A seroprevalence study

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Abstract

Bovine herpesvirus type 1 (BoHV-1) and bovine leukemia virus (BLV) are among the most important pathogens affecting dairy cattle, causing significant economic losses worldwide. The present study determined the seroprevalence of IBR (Infectious Bovine Rhinotracheitis) and BLV in dairy herds in Cajamarca, Peru. A total of 464 animals were sampled, and the presence of antibodies against BoHV1 and BLV was determined using commercial indirect enzyme-linked immunosorbent assay (I-ELISA) kits. Among the sampled animals, the overall seroprevalence was 8.84% (95% CI: 0.5–35.12) for BoHV1 and 7.54% (95% CI: 5.13–9.95) for BLV. The highest BLV seroprevalence was observed in Cajamarca (23.68%), while no positive cases were detected in four provinces. Regarding BoHV1 seroprevalence, it was highest in Cajamarca (26.32%) and Santa Cruz (28.13%), with no cases detected in five of the ten provinces evaluated. This indicates that seroprevalence rates varied among the different provinces studied.. Furthermore, 3.66% (95% CI: 1.95-5.38) of the animals tested positive for both BoHV1 and BLV, indicating that they were infected with both viruses simultaneously. The study found that BoHV1 and BLV infections are widespread in numerous provinces of the Cajamarca region, making it necessary to undertake control programmes to prevent the further spread of those two viruses in bovine.

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Introduction

Worldwide, among the most significant endemic infectious agents in dairy cattle, causing substantial economic losses and compromising animal health, are bovine herpesvirus 1 (BHV-1) and bovine leukosis virus (BLV) (1). BHV-1, also known as red nose, is a double-stranded DNA virus from the Herpesviridae family that causes infectious bovine rhinotracheitis (IBR) (2). Although the first case of IBR was reported in the United States around the 1950s, today it has a global distribution with significant differences in incidence and prevalence. In African countries, Latin America and some Asian countries its prevalence is high while in European countries such as Norway or Denmark it has been eradicated and in others its prevalence is quite low due to the implementation of control

programs (3). As for BLV, the first report of the disease dates back to 1871 in Germany and it has been spreading due to the trade of cattle destined for meat and dairy production. Currently, it has been successfully eradicated in several European countries, but according to recent studies, infection rates have increased in several countries, including countries in the region, such as Argentina and Brazil, where there is a prevalence of more than 40% (1). It is known to result in various clinical conditions in cattle, including abortions, infertility, conjunctivitis, encephalitis, mastitis, reduced milk production, enteritis, and dermatitis (4). The virus can be manifested in different forms in cattle, including respiratory, conjunctival, infectious pustular vulvovaginitis affecting the caudal reproductive tract, infectious balanoposthitis of the male external genitalia, endemic abortions, and neonatal septicemic forms, which is characterized by encephalitis and focal plaque necrosis on the tongue. It is also considered the leading cause of reproductive and respiratory tract diseases in dairy and beef herds, causing significant economic and health impacts worldwide (5,6). On the other hand, BLV, an oncogenic B-lymphotropic retrovirus from the Retroviridae family (7), is the causative agent of Enzootic Bovine Leukosis (EBL), the most common neoplasm in dairy and beef cattle (1,8). This virus primarily affects lymphoid cells, specifically B lymphocytes (9), and is mainly transmitted through the iatrogenic route (10). It manifests through neoplasms or can remain asymptomatic, affecting cattle of all ages. Its socio-economic impact lies in reduced milk production, reproductive losses, and the seizure of cattle, preventing the export of cattle or cattle by-products (7). The typical mode of transmission is horizontal, through direct or indirect exposure to infected lymphocytes in blood or milk. Following infection, animals typically appear clinically healthy for the first few years, but 30 to 70% may develop persistent lymphocytosis, and 0.1 to 10% may develop lymphosarcoma. This infection is detected through serological tests, usually the enzyme-linked immunosorbent assay (ELISA) (10). But recent epidemiological studies report the use of real-time PCR (qPCR) as it is able to detect the disease in asymptomatic or early-stage animals (1). In the case of HBV-1, diagnostic methods include indirect ELISA (6), blocking ELISA (5) and also PCR (2) due to its high specificity, this technique has become essential in epidemiological surveillance. Both diseases have been associated with economic losses in cattle herds, primarily due to decreased milk production, reproductive inefficiency, increased slaughter rates, and trade restrictions for health reasons (10,11). The often-underestimated economic impact of BLV is mainly due to its asymptomatic nature and chronic course, with minimal mortality (12). This condition increases the likelihood of infected cattle being slaughtered before reaching their maximum productive lifespan (11). In contrast, BHV-1 is important due to its ability to establish lifelong latent infections in the sensory ganglia of the peripheral nervous system after acute infection (13). This latency is characterized by periodic reactivations, facilitating virus transmission and causing transient immunosuppression (14). Even a single exposure to BHV-1 can have a significant economic impact and considerable commercial relevance for artificial insemination (AI) centers and breeding operations (15). Both viruses can coexist in endemic regions, further complicating the clinical and epidemiological picture (16). HBV-1 and BLV co-infections may have synergistic effects that increase the severity of the disease (17). For example, HBV-1-induced immunosuppression is characterized by synergism with other bacterial or viral infections such as bovine viral diarrhea (BVD) (3), while BLV can alter immune function by increasing susceptibility to infections such as mastitis (1). Likewise, these diseases are also of concern because of their public health implications due to the zoonotic risks they could pose. Thus, in 2014, a case was reported where BLV was detected in human breast tissue samples (18) and a review article also found an overall incidence of BLV in human samples of 27% (19). A meta-analysis also showed that BLV infection is associated with an increased risk of breast cancer (20). In Cajamarca, in 2006, the seroprevalence of IBR in cattle was reported for first time at 0.62% (21). More recent studies from 2024 report a higher antibody prevalence of 2.74 ± 1.87 % (22).

Despite these findings, Cajamarca lacks epidemiological surveillance and control programs for these diseases, regional or national research on their prevalence is very limited. Therefore, the objective of this study was to determine the seroprevalence of BHV-1 and BLV in dairy cattle in Cajamarca, Peru.

Materials and methods

Ethical statement

This study was approved by the Institutional Research Ethics Committee (CIEI) of the Universidad Nacional Toribio Rodríguez de Mendoza, CIEI-N° 00102/2024. Animals were restrained by specialists avoiding sudden movements according to the guidelines of Animal Research: Reporting of in vivo Experiments 2.0.

Study location

Sampling was conducted in 42 districts of the Cajamarca region; 6 in Cajamarca province, 3 in Cajabamba province, 6 in Celendín province, 5 in Chota province, 5 in Cutervo province, 2 in Hualgayoc province, 3 in San Marcos province, 5 in San Miguel province, 3 in San Pablo province and 4 in Santa Cruz province, during the months of April to August in the year 2024 (Figure 1). The region has an average maximum temperature of 20.8 °C, average annual temperature of 14.75°C, average minimum temperature of 8.5 °C, average annual relative humidity of 68.3 %, with annual rainfall of 801.8 mm at an altitude of 2 760 masl.

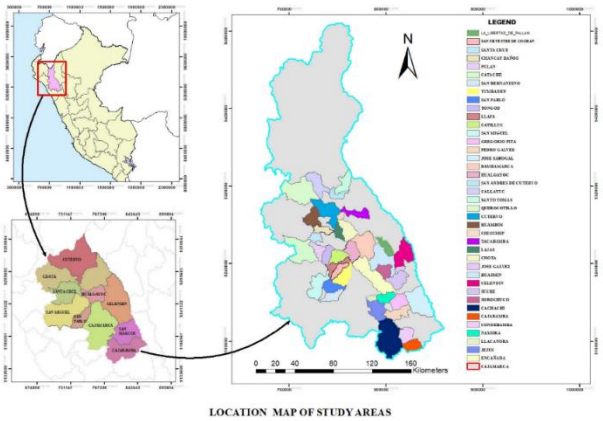

Figure 1: Map of animal sampling location, Cajamarca region.

Sample

In the study area according to CENAGRO, there is a cattle population (Elementary Sampling Unit - ESU) of 628,066 head of cattle and 161,092 (Primary Sampling Unit - PSU) or farms (23). The minimum sample size was calculated using the formula for estimating the proportions of a known population (15) with a confidence level of 95 % and an expected error of 5 %. For the present study, a prevalence of 0.5, a cattle population of 628,066 head of cattle, distributed in 161,092 PSUs, was considered as a reference. An expected prevalence of 50% was considered since there is no previous reliable information about the real prevalence in the study area. A sample size of 384 cattle (UEM) was determined to be sampled in 98 cattle farms (UPM) randomly selected from the UPM register, in the districts of each province involved in the study, for which a simple stratified random sampling was defined in the geographical area, having considered expanding the sample size to 464 in 116 farms, based on the average of 4 cattle per farm. The animals sampled were cattle older than 8 months, carrying out a basic, descriptive and cross-sectional study. All animals were sampled from cattle with no vaccination history identified by the Servicio Nacional de Sanidad Agraria.

Serum samples

Blood samples were taken from the middle coccygeal vein, with a 20G x 1" needle from each bovine, using the vacutainer system in sterile 6 mL tubes without anticoagulant (VACUETTE®), the samples were homogenised using the inversion method and transferred under refrigerated conditions (4°C), The samples were centrifuged for 5 minutes at 1500 rpm to obtain the blood serum and collected in 1.5 mL microtubes.5 mL microtubes, the serum was stored at -20°C until analysis, the same protocol was followed for both diseases. A competitive ELISA using the commercial ID Screen® BLV Competition kit was used to detect antibodies against gp51 of Enzootic Bovine Leukosis virus in bovine serum. An indirect ELISA using the commercial ID Screen IBR Indirect kit was also used to detect anti-IBR antibodies.

Sample processing

The procedure was performed according to the instructions provided in each commercial kit. First, a competitive ELISA was performed to detect antibodies against BLV gp51. To do this, 80 µl of diluent was placed in each well of the microplate, 20 µl of positive control was added to duplicate wells (A1 and B1), 20 µl of negative control was added to wells (C1 and D1), and 20 µl of sample was added to the remaining wells. They were then incubated at 21°C (±5°C) for 45 minutes (±4 min), each well was emptied, and three washes were performed using 300 µl of wash solution. Add 100 µl of 1X conjugate to each well, incubate for 30 minutes (±3 min) at 21°C (±5°C), and remove the contents of each well. The wells were washed three times using 300 µl of wash solution, 100 µl of the developing solution was added to each well, and they were incubated for 15 minutes (±2 min) at room temperature in the dark. Subsequently, 100 µl of stop solution was added. Finally, the reading was taken at 450 nm in an ELISA microplate reader, and the optical density of each sample was recorded. An indirect ELISA was also performed to detect antibodies against BHV-1. Ninety µl of diluent was placed in each well of the microplate, 10 µl of negative control in duplicate wells (A1 and B1), 10 µl of positive control in wells (C1 and D1), and 10 µl of sample in the remaining wells. The plate was incubated at 37°C (±3°C) for 45 minutes, each well was emptied, and three washes were performed using 300 µl of wash solution. Add 100 µl of the conjugate to each well and incubate for 30 minutes (±3 min) at 37°C (±3°C), then remove the contents of each well. The wells were washed three times using 300 µl of wash solution, 100 µl of the developing solution was added to each well and incubated for 15 minutes at 21°C (±5°C) in the dark, and 100 µl of stop solution was added. Finally, the reading was taken at 450 nm in an ELISA microplate reader, and the optical density of each sample was recorded. The percentage of competition (S/N%) for BLV and (S/P%) for IBR for each sample was calculated and interpreted according to the manufacturer's guidelines.

Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to determine the presence of specific antibodies against IBR (ID.vet, France) and BLV (ID.vet, France). The reading was performed on a microplate reader (BENCHMARK SCIENTIFIC, MR9600-E, USA) with a wavelength of 450 nm. To determine positive and negative cases against BLV, samples with percentages less than or equal to 50% were considered negative, those with values between 50% and 60% were considered suspicious, and those with values equal to or greater than 60% were considered positive for BLV antibodies. As for IBR, samples

whose absorbance value was higher than the cut-off point established by the manufacturer were considered positive, and those whose absorbance value was below the cut-off point were considered negative. Finally. data processing and analysis was performed with the ID SOFT Ver. 5.20.8 program.

Data analysis

Data were processed using descriptive statistics. Positive cases are presented as percentages with 95% confidence intervals. The association of the concurrent presence of antibodies was determined by the Kruskall-Wallis test. The degree of linear relationship between IBR seropositivity and BLV was identified with the Phi correlation. Data were analysed using InfoStat statistical software version 2020 and a *P* value of $P < 0.01$ was considered significant.

Results

The prevalence determined in the department of Cajamarca for IBR and BLV was 8.84% (41/464) and 7.54% (35/464) respectively, with different prevalence according to the provinces, the Holstein breed presented 15% for both diseases, the animals older than 5 years presented 13% (BLV) and 14% (IBR), the females presented 8.62% (BLV) and 9.85% (IBR). In addition, 3.66% (17/464) of coinfection was found, as shown in Table 1.

Table 1: Seropositivity (%) for BLV and IBR in dairy cattle

Variable	Category	No	Bovine Viral Leukosis		Infectious Bovine Rhinotracheitis		Association		Phi Coefficient
			Positives	% (95% CI)	Positives	% (95% CI)	Positives	% (95% CI)	
Province	Cajabamba	28	1	3.57 (0 - 10.90) a	0	0 a	0	0 a	sd
	Cajamarca	76	18	23.68 (13.90 - 33.46) b	20	26.32 (16.19 - 36.45) b	11	14.47 (6.38 - 22.57) b	0.44
	Celendín	40	2	5 (0 - 12.06) a	6	15.00 (3.43 - 26.57) ab	1	2.5 (0 - 7.56) a	0.22
	Chota	92	6	6.52 (1.38 - 11.66) a	0	0 a	0	0 a	sd
	Cutervo	56	0	0 a	0	0 a	0	0 a	sd
	Hualgayoc	32	0	0 a	0	0 a	0	0 a	sd
	San Marcos	32	4	12.50 (0.39 - 24.61) ab	4	12.50 (0.39 - 24.61) ab	3	9.38 (0 - 20.05) ab	0.71
	San Miguel	44	0	0 a	0	0 a	0	0 a	sd
	San Pablo	32	4	12.50 (0.39 - 24.61) ab	2	6.25 (0 - 15.12) a	2	6.25 (0 - 15.12) ab	0.68
	Santa Cruz	32	0	0 a	9	28.13 (11.66 - 44.59) b		0 a	sd
	p value			*<0.0001*		*<0.0001*		*<0.0001*	
Race	Holstein	120	18	15.00 (8.52 - 21.48) b	18	15.00 (8.52 - 21.48) b	11	9.17 (3.93 - 14.40) b	0.54
	Brown Swiss	71	2	2.82 (0 - 6.76) a	10	14.08 (5.79 - 22.38) ab	1	1.41 (0 - 4.22) a	0.18
	Crusader	233	15	6.44 (3.26 - 9.61) a	11	4.72 (1.98 - 7.46) a	5	2.15 (0.27 - 4.02) a	0.35
	Fleckvieh	40		0 a	2	5.00 (0 - 12.06) ab	0	0 a	sd
	p value			*0.0013*		*0.0032*		*0.00*	
Age group	< 2 years	154	4	2.60 (0.06 - 5.14) a	3	1.95 (0 - 4.16) a	0	0 a	sd
	2-5 years	250	18	7.20 (3.97 - 10.43) a	24	9.60 (5.92 - 13.28) b	7	2.80 (0.74 - 4.86) a	0.28
	> 5 years	60	13	21.67 (10.93 - 32.40) b	14	23.33 (12.32 - 34.35) c	10	16.67 (6.96 - 26.38) b	0.67
	p value			<0,0001		<0.0001		<0.0001	
Sex	Female	406	35	8.62 (5.88 - 11.36) b	40	9.85 (6.94 - 12.76) b	17	4.19 (2.23 - 6.14) a	0.4
	Male	58	0	0 a	1	1.72 (0 - 5.18) a	0	0 a	sd
	p value			*0.02*		*0.0414*		*0.1128*	
Total		464	35	7.54 (5.13 - 9.95)	41	8.84 (6.24 - 11.43)	17	3.66 (1.95 - 5.38)	0.4

[ab]For each variable different letters between their levels are significant differences, in each factor (Kruskall-Wallis, P<0.05).

Discussion

The results obtained constitute the first report on the study of IBR and BLV co-infection in dairy cattle in the Cajamarca region. The seroprevalence of IBR in animals without vaccination history reported in this study is 8.84% as well as the prevalence of BLV with 7.54%. With respect to IBR seroprevalence, it is significantly low in relation to studies from other regions of Peru, such as Ayacucho 59.56-67.6% (24,25), Ucayali 46.3% (26) and Lima 36.2% (27). These significantly lower results could be related to the long history of animal introduction in Cajamarca and the semi-stabled farming system, factors that favor the spread of the disease to other dairy areas. Internationally, our values are considerably lower than those of Colombia 57.5-65.5% (4,28), Brazil 48.6% (29), Egypt 22.5% (30) and Ethiopia 79.1% (31). This could reflect differences in management practices, herd size and health control (32). The low

prevalence in Cajamarca does not rule out an increase since neighboring countries such as Ecuador report a high seroprevalence 23.4% (33), so it is advisable to maintain control measures and control the movement of animals (34). Likewise, evolutionary epidemiology studies are necessary to help understand the origin, spread, and persistence of viral diseases (35).

An analysis of IBR prevalence by province showed that there was great variation, with Santa Cruz and Cajamarca provinces having the highest prevalence 28.13 and 23.7%, respectively, followed by San Marcos and San Pablo with 12.5%. On the other hand, the provinces with the lowest prevalence were Chota with 6.5%, Celendín with 5.0% and Cajabamba with 3.6%. Meanwhile, Cutervo, Hualgayoc, San Miguel and Santa Cruz reported 0%. These data indicate that the disease is concentrated in certain geographic areas with different levels of seropositivity. However, this is not indicative of a degree of resistance, but may be related to management conditions and animal movement, as shown in a study carried out in Ecuador, where the high prevalence (23.40%) is related to poor management and high transhumance of animals without health certificates (33). This is very similar to the situation in Cajamarca, where there is no adequate animal health management. Therefore, although seroprevalence is low or non-existent in many provinces, these areas remain prone to infection and dissemination of the disease if vaccination and monitoring plans are not implemented. Likewise, it can be affirmed that since the antecedents on the prevalence of IBR in the provinces of Cajamarca are very scarce and outdated, it is not possible to determine the degree of variation with respect to other studies carried out that are statistically different from the other provinces.

In the analysis by breed, it was found that the highest IBR seroprevalence was observed in Holstein (15%) and Brown Swiss (14%) cattle, which is in agreement with studies that indicate the existence of a higher susceptibility in specialized breeds given their genetic predisposition and semi-stabled breeding systems that favor contagion (4). In contrast, it was found that crossbred cattle had a lower prevalence of 4.72%, which is a low percentage in relation to reports from the Ucayali Region that show 46.3% in zebu and crossbred animals (26), and in India in Holstein crossbreeds with 29.50% (36). On the other hand, for the Fleckvieh breed, the prevalence was 5% for IBR and 0% for BLV. These low rates could be due to the presence of resistance genes since previous research corroborates the existence of differences in susceptibility to IBR among breeds and that there is a wide genetic variation for antibody response to BoHV-1 (37). However, variations in our results could also be due to breeding systems, population differences, malnutrition or some parasitic infestations (31) and in some cases the underdiagnosis of subclinical infections (38).

For BLV, by competitive ELISA, the highest prevalences were found in the provinces of Cajamarca with 23.68%, San Marcos and San Pablo with 12.50%, being lower in Chota with 6.52%, Celendín with 5% and Cajabamba with 3.57%. In the provinces of Cutervo, San Miguel, Hualgayoc and Santa Cruz, the prevalence was 0%. This could be related to the different preventive practices of farmers and authorities. The BLV seroprevalence found in the Cajamarca region (7.54%) was lower than that reported in two districts of the Amazonas region, where an overall prevalence of 14.1% was found (39). It is also lower than the data reported in Madre de Dios (53.85%) (39,40) and international studies such as that of Canada (26.9%) (41) and the Colombian tropical region (21.01%-35.1%, 14.64%) (42,43). This suggesting the existence of differences in management and sanitary control practices, taking into account that BLV transmission has been strongly linked to biosecurity practices at farm level (such as the reuse of materials contaminated with blood) rather than to environmental or geographical factors (44-46).

On the other hand, the analysis of coinfection revealed that there was a higher prevalence of both viruses in animals older than 5 years (21.67% for BLV and 23.33% for IBR), coinciding with studies that point to age as a risk factor due to increased viral exposure throughout life (32,46). In addition, females presented more positive cases than males ($P<0.05$), a finding consistent with research in Iran (47), Egypt (48), India (36) and Colombia (4). Although other studies report contradictory results with a higher number of cases in males (49), it is important to note that there is evidence that semen has been identified as a vehicle for viral dissemination (47). However, it is important to take these findings with caution as it could also be attributed to various conditions, such as the effect of the size of the animals sampled of each sex and the breeding affinity of a certain sex in certain areas. In relation also to simultaneous IBR and BLV infection, Also, BLV-infected animals have been reported to be more susceptible to co-infections with BVDV and BoHV-1 (50). A study in Mexico, observed that 12.20% of the animals were co-infected with BLV and IBR (51). However, no statistical association was found between both viruses ($p>0.05$). In contrast, the present investigation reports a significant association ($P<0.05$) by province, breed and age group, which highlights the need for further studies to identify the associated risk factors and other epidemiological characteristics of both viruses, in order to take preventive measures to mitigate their spread in the cattle population of Cajamarca. This is especially true given that disease negatively affect animal welfare and are often cited as the leading cause of cattle death, causing direct or indirect economic losses to breeders (52,53).

Conclusion

The seroprevalence in the Cajamarca region was 8.84% for IBR and 7.54% for BLV in extensively reared dairy cattle. However, there are differences between provinces so there is a need to implement more studies in order to propose

comprehensive control programmes and mainly prevention through herd biosecurity measures, as well as immunization through vaccination.

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Conflict of interest

There is no conflict of interest.

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العدوى المشتركة مع فيروس الهربس البقري من النوع ١ وفيروس اللوكيميا البقري في الأبقار الحلوب في كاخاماركا، بيرو: دراسة الانتشار المصلي

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الخلاصة

يعد فيروس الهربس البقري من النوع ١ (BoHV-1) وفيروس اللوكيميا البقري من بين أهم مسببات الأمراض التي تؤثر على الأبقار الحلوب، مما يتسبب في خسائر اقتصادية كبيرة في جميع أنحاء العالم.

حددت الدراسة الحالية الانتشار المصلي لالتهاب الأنف والقصبة البقري المعدي في قطعان الحليب في كاخاماركا، بيرو. تم أخذ عينات من ما مجموعه ٤٦٤ حيوانا، وتم تحديد وجود الأجسام المضادة ضد BoHV-1 و BLV باستخدام مجموعات المقايسة المناعية غير المباشرة المرتبطة بالإنزيم (I-ELISA). من بين الحيوانات التي تم أخذ عينات منها، كان الانتشار المصلي الإجمالي ٨,٨٤ ٪ (٩٥ ٪ سي: ٠,٥–٣٥,١٢) لـ BoHV-1 و ٧,٥٤ ٪ (٩٥ ٪ سي: ٥,١٣–٩,٩٥) لـ BLV. وقد لوحظت أعلى نسبة انتشار مصلي للفيروس في كاخاماركا (٢٣,٦٨٪)، في حين لم يتم الكشف عن حالات إيجابية في أربع مقاطعات. فيما يتعلق بالانتشار المصلي لفيروس BoHV-1، كان أعلى مستوى في كاخاماركا (٢٦,٣٢٪) وسانتا كروز (٢٨,١٣٪) ، مع عدم اكتشاف حالات في خمس من المقاطعات العشر التي تم تقييمها. يشير هذا إلى أن معدلات الانتشار المصلي تباينت بين المقاطعات المختلفة التي تمت دراستها. علاوة على ذلك، ثبتت إصابة ٣,٦٦ ٪ (٩٥ ٪ سي: ١,٩٥-٥,٣٨) من الحيوانات لكل من BoHV-1 و BLV، مما يشير إلى أنهم أصيبوا بكلا الفيروسين في وقت واحد. ووجدت الدراسة أن عدوى فيروس التهاب الكبد الوبائي ١ وفيروس التهاب الكبد الوبائي منتشرة على نطاق واسع في العديد من مقاطعات منطقة كاخاماركا، مما يجعل من الضروري تنفيذ برامج مكافحة لمنع انتشار هذين الفيروسين في الأبقار.