

# Seroprevalence and risk factors associated to BHV-1 and DVVB in dairy herds in Pasto, Colombia, in 2011\*

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## Abstract

**Introduction.** The bovine herpes virus type 1 (BHV-1) causes the disease called Infectious Bovine Rhinotracheitis (IBR), infects cattle and presents clinical manifestations such as pustular vulvo-vaginitis, abortion, rhinotracheitis and meningoencephalitis. **Objective.** This research work aims to determine the prevalence of serum antibodies to bovine herpesvirus-1 (BHV-1) and bovine diarrhoea (BVD) virus and risk factors associated to these infections in dairy herds in the municipality of Pasto, Colombia. **Materials and methods.** Farms above 2527 meters over the sea level were selected. A total of 238 serum samples were collected and analyzed using the ELISA test to determine the seropositivity against BHV-1 and BVD virus. A questionnaire, which included variables related to cattle, plus a health and a management measure were filled out in each herd. A multivariate analysis binary logistic regression was used with a confidence interval of 95% ( $p < 0.05$ ) using the program SPSS19®. **Results.** The estimated prevalence of exposure to BHV-1 in the municipality of Pasto was 17.65% and 32.77% for bovine viral diarrhoea at the herd level. The binary logistic regression showed that the use of a bull instead of artificial insemination (OR = 30.56, CI 6.87, 135.98,  $P < 0.0001$ ) is a risk factor with BHV-1, and abortion (OR = 22.70, CI 4.21, 122.42,  $P < 0.0001$ ) and acquisition of new animals (OR = 34.90, CI 6.30, 193.43,  $P < 0.0001$ ) are risk factors with BVD. **Conclusions.** The prevalence of infectious rhinotracheitis and bovine viral diarrhoea in dairy herds in Pasto does not indicate the existence of the disease in animals serologically positive, but suggests that at some point in their lives they were exposed to the virus. From the risk factors identified in this work, recommendations can be established for an effective control of reproductive diseases in the region.

**Keywords:** abortion, bovine viral diarrhoea virus, dairy cattle, rhinotracheitis

## Factores de riesgo asociados a la seroprevalencia de BHV-1 y DVVB en hatos lecheros en Pasto, Colombia, en el 2011

### Resumen

**Introducción.** El herpes virus bovino tipo I (BHV-1) causa la enfermedad llamada rinotraqueitis infecciosa bovina (IBR), infecta al ganado vacuno y presenta manifestaciones clínicas tales como la vulvovaginitis purulenta, el aborto, la rinotraqueitis y la meningoencefalitis. **Objetivo.** Determinar la seroprevalencia de anticuerpos contra herpesvirus-1 bovino (BHV-1) y diarrea viral bovina y los factores de riesgo asociados a estas infecciones en los hatos lecheros del municipio de Pasto, Nariño. **Materiales y métodos.** Se seleccionaron fincas sobre 2527 msnm. Se tomaron 238 muestras de suero y se analizaron mediante la prueba de ELISA para determinar la seropositividad de HVB-1 and DVVB. En un cuestionario se recolectó la información en cada finca, donde se incluyeron variables relacionadas al ganado, salud y prácticas de manejo. Con un análisis multivariado mediante regresión logística binaria con un Intervalo de Confianza del 95% ( $p < 0.05$ ) utilizando el programa SPSS19® se determinó el Odds Ratio de cada variable. **Resultados.** La prevalencia encontrada en el municipio de Pasto para el virus de rinotraqueitis infecciosa bovina fue de 17,65% y 32,77% para diarrea viral bovina. Se determinó para BHV-1 como factor de riesgo asociado a la presencia de IBR el uso de toros (OR = 30.56, IC 6.87, 135.98,  $P < 0,0001$ ) y para DVVB (OR = 22.70, IC 4.21, 122.42,  $P < 0,0001$ ) al igual que la adquisición de nuevos animales

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(OR=34.90, IC 6.30, 193.43. P<0,0001). **Conclusiones.** La prevalencia de rinotraqueítis infecciosa y diarrea viral bovina en hatos lecheros del municipio de Pasto no indica la enfermedad existente en los animales positivos, pero sugiere que en algún momento de su vida fueron expuestos al agente causal. A partir de los factores de riesgo identificados en el presente trabajo se pueden hacer recomendaciones para establecer un control efectivo de enfermedades reproductivas presentes en la región.

**Palabras clave:** aborto, diarrea viral, ganado de leche, rinotraqueítis

### Fatores de risco associados à seroprevalencia de bhv-1 e dnb em hatos leiteiros em Pasto, Colômbia, em 2011

#### Resumo

**Introdução.** O vírus herpes bovino tipo I (BHV-1) faz com que a doença chamada Rinotraqueíte Infecciosa Bovina (IBR), infecta bovinos e apresenta manifestações clínicas como vulvo-vaginite pustular, aborto rinotraqueíte, e meningoencefalite. **Objetivo.** Determinar a seroprevalencia de anticorpos contra herpesvirus-1 bovino (BHV-1) e diarreia viral bovina e os fatores de risco associados a estas infecções nos currais leiteiros do município de Pasto, Nariño.

**Materiais e métodos.** Seleccionaram-se herdades sobre 2527 msnm. Tomaram-se 238 mostras de soro e se analisaram mediante a prova de ELISA para determinar a seropositividade de HVB-1 e DVB. Num questionário se coletou a informação em cada herdade, onde se incluíram variáveis relacionadas ao gado, saúde e práticas de manejo. Com uma análise multivariada, mediante regressão logística binária com um Intervalo de Confiança de 95% (p<0.05) e utilizando o programa SPSS19®, determinou-se o Odds Ratio de cada variável. **Resultados.** A prevalência encontrada no município de Pasto para o vírus de rinotraqueítis infecciosa bovina foi de 17,65%, e 32,77% para diarreia viral bovina. Determinou-se para BHV-1 que o uso de touros é um fator de risco associado à presença de IBR (OR =30.56, IC 6.87, 135.98, P<0,0001) e para VDVB, (OR= 22.70, IC 4.21, 122.42, P<0,0001) ao igual que a aquisição de novos animais (OR=34.90, IC 6.30, 193.43. P<0,0001). **Conclusões.** A prevalência de rinotraqueítis infecciosa e diarreia viral bovina em currais leiteiros do município de Pasto não indicam a doença existente nos animais positivos, mas sugere que em algum momento de suas vidas foram expostos ao agente causal. A partir dos fatores de risco identificados no presente trabalho se pode estabelecer recomendações para estabelecer um controle efetivo de doenças reprodutivas presentes na região.

**Palavras importantes:** aborto, diarreia viral, gado de leite, rinotraqueítis

## Introduction

The bovine herpes virus type I (BHV-1) causes the disease called Infectious Bovine Rhinotracheitis (IBR), which infects cattle and presents clinical manifestations such as pustular vulvovaginitis, abortion, rhinotracheitis and meningoencephalitis<sup>1</sup>. IBR is a disease that can decrease the reproductive efficiency, mainly by abortions, embryonic death and impaired reproductive indicators: increased in number of services per conception, increase of the number of open days and decreases in fertility and birth rates. BHV-1 has a tropism to the placenta and fetal tissues. IBR abortion is common during the second half of pregnancy but the fetus is susceptible at any age and abortion or embryonic mortality in the first 60 days<sup>2</sup> can take place. An important feature is that it establishes latent infection in sensory neurons in the trigeminal or sacral ganglia and tonsils<sup>3</sup>. BHV-1 can be reactivated and may be shed and induce primary infections in new susceptible animals. BHV-1 can spread easily within farms, among

cows<sup>4</sup>. The most common way of transmission is the introduction of latently infected animals in a farm. IBR seroprevalence varies among countries and herds. In South America it is ranged from 51 to 99% in Peru and Uruguay, respectively. In Yucatán, Mexico, in 1997, they found a prevalence of 5.33%<sup>5</sup>. Another study in Canada in 2004, about the status of *Neospora caninum*, bovine viral diarrhea and infectious bovine rhinotracheitis showed that samples tested for antibodies against IBR, 512 (20.4%) had relatively high degrees, 1621 (64.6%) were low and 378 (15.1%) were negative. The average prevalence in herds against IBR was 19.8%. Cows that were purchased had higher antibody titers to IBR than those born on the farm. The age of the cow was the only risk factor significantly associated with the status of cows to IBR<sup>6</sup>. In Colombia in 2006 in Montería, a seroprevalence of 74, 7% to IBR in cattle with a clinical history of infertility was reported. This research found that sex has an association with IBR. 72% of females and 95% of bulls were positive for IBR, therefore infected bulls

may be an important source of transmission of IBR<sup>7</sup>. Likewise, a study in Antioquia and Valle in 2009 found a 100% prevalence of serological herds for BHV-1 and an individual prevalence of 75.63%. The prevalence for cattle farms in the departments of Antioquia and Valle was 85.51% and 69.84%, respectively<sup>8</sup>. In Nariño, in 1999, the serological prevalence in herds was 49%, the individual prevalence was 8.4% and it was 19.4% for Pasto<sup>9</sup>.

Bovine Viral Diarrhea (BVDV) is a pestivirus infection affecting cattle and presents a wide range of clinical manifestations. In pregnant cows the range of signs goes from embryonic death, abortion or stillbirths to the birth of persistently infected (PI) calves<sup>10</sup>. A study performed in 2002 on the distribution of BVDV in the genital system indicated that the specific antigen is found in cells such as macrophages, around the outside of the ovaries<sup>11, 12</sup>. Seroprevalence in no vaccinated herds differs among areas or countries, ranging between 20 and 90%<sup>13, 14</sup>.

The BVD virus is usually transmitted between animals by inhalation or ingestion of nasal secretions, saliva, urine or feces. The virus can also be transmitted generally in semen from an infected bull or by transfer of contaminated embryos. Exposure of the fetus can result in embryonic absorption, mummifications, abortions, congenital malformations or the birth of apparently normal calves that are persistently infected and shed large amounts of BVD<sup>15-18</sup>.

In Cuba, in 2010, the presence of BVDV had a morbidity of 17.9% bovine reproductive performance problems. With a risk factor for 63% of the exposed population, there is a chance to manifest the disease and the clinical symptoms. The mortality rate is 5.5% and there is a 68% relative risk to the herd in which the disease is serious or fatal<sup>19</sup>. In Chile, in 2009, the prevalence and distribution of brucellosis, bovine leucosis, bovine viral diarrhea and infectious bovine rhinotracheitis was established, by using a methodology that determines milk with 3 samples. 187, 216 herds and 246 for the third sampling. BVDV antibodies were found as follows: 98%, 96%, 93%, respectively<sup>20</sup>.

In 2007 in Montería, Colombia, serum samples were collected from 150 female and 20 male animals from 32 farms. It was found that 29.4%

of the animals were seropositive for BVDV, just as the study identified the dependence between the presences of bovine viral diarrhea and sex<sup>21</sup>. Therefore, the objective of this study is to determine the prevalence of infectious rhinotracheitis virus and bovine viral diarrhea, and the associated risk factors in dairy herds in Pasto, Nariño.

## Materials and methods

**Study Site:** The study was conducted in 10 dairy farms in the rural municipality of Pasto (Nariño-Colombia), a city located nearby the Galeras volcano, belonging to an ecosystem of lower mountain dry forest according to the Holdridge classification. The city has a 700 mm annual precipitation, average temperature of 13.3°C and humidity of 60% to 88%. These farms were extensively managed, cows appending most of the time on fenced pastures, with no physical separation between heifers and adult cows.

**Cattle population:** Farm prevalence levels were obtained by a cross-sectional study using a strategy of simple random sampling of animals from dairy farms in the municipality of Pasto, as follows:

$$n = N * Z_2 * P * (1 - P)$$

$$N * e_2 + Z_2 * P * (1 - P)$$

Where:

N is the number of dairy cows in the city.

Q: Expected prevalence.

e: Acceptable Error (in this study, 10%).

Z: confidence level (95% = 0.05).

For the number of animals needed to sample for estimating the individual level prevalence, we assumed an expected prevalence of 30% combined with an acceptable error of 10% and a confidence level of 95%. 238 Holstein cows, which had never been vaccinated against BVDV or IBR infection, were selected. The cows had a moderate level of milk production (15 kg/cow/day), registration of reproductive events and identification of the animal. Among the inclusion criteria were: breeding cows that have calved at least once and have been in the farms for more than 6 months.

**Variables:** Epidemiological data were collected through a structure fill in questionnaire, obtained by a direct interview with the cattle farmer. The variables included were group by topics for BVDV and IBR: Management: Type of reproduction (natural or artificial insemination), synchronization and culling. Health: Annual abortion, deworming and vaccination. Pasture management: Organic fertilizer, manure as fertilizer. Origin of replacements cows: External, same farm or mixed. Biosecurity: Disposal of fetuses and placentas, and water source. Presence of animals: Sheep, horses, pigs, cats, dogs.

**Sample collection and serological examination:** Blood samples (10 ml) were collected by puncture of the tail vessel using sterile tubes

without anticoagulant (Vacutainer), which were subsequently transported and processed to separate the serum by centrifugation (1500 rpm / 5 minutes) and stored at -20 ° C until their analysis in the veterinarian clinical diagnostic laboratory of the University of Nariño.

The presence of antibodies against IBR virus and DVB were tested using a commercial indirect enzyme-linked immunosorbent assay (ELISA) kits (SVANOVA Biotech ®), following the manufacturer's specifications. The sensitivity and specificity of the test were 99% and 96%, respectively. The plates were read at 450nm and the results were given in optical density values expressed in percentage of positivity for antibodies to IBR and BVD. The formula used was:

$$(pp)\% \text{ positivity values} = \frac{\text{sample or negative control (OD corrected)}}{\text{positive control (OD corrected)}} \times 100$$

The optical density (OD) readings for IBR gave percentages of positivity cut-off points at 10. When the result was above 10 it was considered positive and if it was below 10, it was considered negative. For the percentage of positivity of DVB  $\leq 10$  they were considered negative,  $\geq 25$  positive and those between  $> 10$  and  $< 25$  were considered doubtful.

**Data analysis:** The apparent prevalence of antibodies to BVDV and IBR was estimated from the ratio of positive results to the total number of cattle examined. The association between seroprevalence and risk factors was quantified by the use of a multivariate binary logistic regression, with a confidence interval of 95%. The significance of the association was estimated by determining the Odds Ratio (OR) of each factor with a *P* value  $< 00.5$ . The goodness of fit was assessed with statistics Hosmer - Lemeshow. Calculations, which were performed by the use of SPSS ® version 19.

## Results

Sampled herds were characterized by small size and a moderate level of milk production (15 kg/cow/day) that would correspond to tradi-

tionally managed herds with low specialization levels. The percentage of abortions in this study was 7%. The medical records of the farms reported placental retention, to estrus after service increased services per conception interacting directly with the open days, ranging from  $140 \pm 20$  days. The farms only had records of vaccination against mouth and foot disease and brucellosis. Forty two cows were ELISA seropositive; the animal level prevalence for antibodies to BHV-1 was estimated at 17.65% and seventy eight cows were seropositive; the prevalence for antibodies to BVDV was 32.77% (table 1).

Three herds did not have seropositive animals for IBR (70% prevalence antibodies against BHV-1), instead for BVDV all the herds had antibodies against the virus (podemos tener esta frase en español?). Two variables were associated with seropositivity to BVDV: Abortion and the external acquisition of replacements, either at markets or other farms or regions (table 2).

Only one variable was determined as a risk factor associated with the seroprevalence of antibodies for the bovine virus type I (BHV-1): The bull that is used instead of the artificial insemination. The Odds Ratio is described in table 3.

**Table 1. Prevalence of antibodies to BVDV and IBR in Holstein cows in milk in Pasto, Nariño (Colombia)**

| Farm         | Number of cows | Number of positive cows to IBR | Prevalence (%) IBR | Number of positive cows to DVB | Prevalence (%) DVB |
|--------------|----------------|--------------------------------|--------------------|--------------------------------|--------------------|
| A            | 27             | 17                             | 62,96              | 22                             | 81,48              |
| B            | 25             | 9                              | 36,00              | 4                              | 16,00              |
| C            | 19             | 1                              | 5,26               | 10                             | 52,63              |
| D            | 8              | 0                              | 0,00               | 3                              | 37,50              |
| E            | 22             | 8                              | 36,36              | 9                              | 40,91              |
| F            | 15             | 0                              | 0,00               | 6                              | 40,00              |
| G            | 38             | 3                              | 7,89               | 12                             | 31,58              |
| H            | 23             | 2                              | 8,70               | 7                              | 30,43              |
| I            | 47             | 0                              | 0,00               | 4                              | 8,51               |
| J            | 14             | 2                              | 14,29              | 1                              | 7,14               |
| <b>TOTAL</b> | <b>238</b>     | <b>42</b>                      | <b>17,65</b>       | <b>78</b>                      | <b>32,77</b>       |

**Table 2. Risk factors associated with seropositivity of DVB in lactating cows in farms in Pasto, Nariño**

| Variable                        | <sup>2</sup> OR | <sup>1</sup> IC 95% |        | <sup>3</sup> P-Value |
|---------------------------------|-----------------|---------------------|--------|----------------------|
|                                 |                 | Lower               | Upper  |                      |
| Abortion                        | 22.7            | 4,21                | 122,42 | < 0,0001             |
| External origin of replacements | 34.9            | 6,3                 | 193,43 | < 0,0001             |

<sup>1</sup> Confidence interval for OR 95%

<sup>2</sup> Odds ratio

<sup>3</sup> P < 0.05

**Table 3. Risk factors associated with seropositivity of IBR in lactating cows in farms in Pasto, Nariño**

| Variable      | <sup>2</sup> OR | <sup>1</sup> IC 95% |        | <sup>3</sup> P-value |
|---------------|-----------------|---------------------|--------|----------------------|
|               |                 | Lower               | Upper  |                      |
| Used of bulls | 30,56           | 6,87                | 135,98 | < 0,0001             |

<sup>1</sup> Confidence interval for OR 95%

<sup>2</sup>Odds ratio

<sup>3</sup>P < 0.05

## Discussion

Seroprevalence in this cattle population (17.8%) for IBR was much lower than expected. A previous report on seroprevalence to IBR in non

vaccinated dairy herds from the same region showed an animal seroprevalence, in 1999, of 19.4%<sup>22</sup>. High prevalence to IBR in other Colombian regions was observed. In 2006 in Montería, Córdoba reported a seroprevalence

of 74.7%<sup>23</sup> and in 2009, in Antioquia and Valle, it was reported a prevalence of 75.63%<sup>24</sup>. The cattle population chosen in Montería, Córdoba, was of cows with reproductive problems, abortions and reproduction failure. The high seroprevalence (39.7%) to BVDV found in Pasto is higher than that reported by Betancur et al, 2007 in Montería, Córdoba, where seroprevalence was 29.4%<sup>25</sup> in a population of cows with a history of reproductive failures. This probably is an indicator of the presence of PI animals under this type of management.

In South America, Guarino et al reported in Uruguay, in 2008, a BHV-1 prevalence of 37% and 69% to BVDV in a study conducted with the same diagnostic technique, ELISA<sup>26</sup>, where the population sampled was made of replacement heifers, 3 years old cows and bulls over 2 years. Carbonero; et al in 2008, in Ecuador, carried out a study with 2367 animals belonging to 346 herds, and he reported a BHV-1 seroprevalence of 43.2%<sup>27</sup>. In this work, like in our study, the population included cows older than 6 months to avoid maternal antibodies and false positive. These values are higher compared to the results found in Pasto, but not to those from other regions of Colombia.

In Asturias, Spain, Main; et al<sup>28</sup> in 2005 reported a seroprevalence of 21% for bovine viral diarrhea, lower than those reported in this paper, even though the population studied had a history of reproductive failures.

Among the variables studied in this document, the origin of the replacements reported an OR= 2.80, but the P-value was P = 0.0578, which indicates no significant association. However, Gerdien van Schaik et al in 1998 assume that the most important risk factors for the introduction of BHV-1 are direct and lengthy contacts between unfamiliar cattle, such as cattle purchased and cattle shows. In other counties, it is considered a risk factor for IBR the direct contact between animals or workers and visitors to farms<sup>29</sup>. Other variables that have been reported as risk factors, such as those reported by Carbonero; et al<sup>30</sup>, who found in Ecuador, as a risk factor associated with BHV-1, the farms located under 1800 m over the sea level, and not the herds in colder areas -at a higher altitude- like the farms in this study (that were

over 2500 m over sea level). The same authors found, as a risk factor, the average slopes of the properties (topographic features similar to Pasto), which may be explained by the continuing effort to which animals are subjected in a feeding system based on natural pastures on slopes.

In Yucatán, Mexico, Calderón Solís and Segura Correa<sup>31</sup> determined the interaction of risk factors and the seropositive prevalence to BVDV, such as farm size and origin of the animals. The origin of the animals can be analyzed from the standpoint of introducing the virus to the farm, like the results in our study, in which the external origin of the replacements was a risk factor  $p < 0.001$  for the presentation of DVB. Likewise, Mainar Jaime; et al<sup>32</sup> in 2005 reported as risk factors the age and the origin of animals, in which a seroprevalence was greater for cows that were purchased was 41%, and 18% for the animals from the same farm. In this regard Luzzago; et al<sup>33</sup> in 2008, reported in northern Italy that among the risks of introducing the virus, the cattle trade is 61.3%; 35% is the attendance and 25.8% shows the grazing community. This cattle trade is similar to the way of acquisition of animals reported in this study. Mainar Jaime et al<sup>34</sup> found no significant association between BVDV and abortion but related to other reproductive parameters such as infertility or embryonic death. Melo; et al<sup>35</sup> in Minas Gerais State, Brazil, conducted an investigation which says that the cause of abortion is associated to Neospora, but not to HBV virus-1 or BVDV.

## Conclusions

This study shows a low general and individual seroprevalence to BHV-1 in dairy Holstein herds in Pasto, Colombia. Due to the type of herd management carried out in this population, these results could indicate the absence of PI animal and does not indicate existing disease in animals serologically positive, but suggests that at some point in their lives they were exposed to the disease. The seroprevalence of IBR in Pasto, from 1999 to the present, have not had greater variations in contrast to other regions of the country. BVDV seropositivity is higher in Pasto than in other areas of Colombia, but lower to other studies in South America. Under

these circumstances, and according to this study, dairy health programmes should be directed to avoid the reintroduction of the virus into the herds through persistently or transiently infected animals, because purchasing cattle is the main factor related to seropositivity in the area.

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