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ABSTRACT

Worldwide distributed Bovine Viral Diarrhea Virus (BVDV) represents a high risk of infection in most bovine farms, in which it is associated with gastrointestinal, respiratory, and reproductive diseases. The purpose of this research was to establish the seroprevalence and the main risk factors associated with the presentation of BVDV in the municipality of Sotaquirá, Colombia. Samples were taken from 1000 cattle of Holstein, Ayrshire, Jersey, Normande Gyr and Holstein x Gyr. Epidemiological surveys were implemented, reproductive and management variables were taken into consideration. Indirect ELISA was performed to detect specific antibodies against BVDV using the commercial kit SERELISA® BVD p80 Ab Mono Blocking. The overall seroprevalence of antibodies against BVDV was 42.5% (425/1000), where the Gyr breed (59.1% apparent prevalence (AP); 60.3% real prevalence (PR)) and the age group > 4 years (53.0% PA; 54.4% PR) presented the highest seroprevalences. A significant statistical association was found for the breed, age, management practices evaluated and the presentation of PI3 ($p \leq 0.05$). Age group > 4 years, Normande breed, presentation of PI3 and grazing lease were established as risk factors associated with BVDV in the herds. These infections are mainly associated with dairy cattle and herds with many animals, so it is important to consider vaccination plans as a preventive system and follow up on the most common diseases.

1. Introduction

Bovine Viral Diarrhea Virus (BVDV) belongs to *Pestivirus* genus of the Flaviviridae family, has been classified into 2 biotypes (cytopathic and non-cytopathic) according to its behavior in cultured cells, and into 2 genotypes (I and II) based on its genetic sequence. Depending on the infecting strain, a particular medical profile, severity varies from subclinical form, passing through the clinical form, and even producing fatal mucosal disease or causing deleterious effects on the fetus (de Oliveira, Mechler-Dreibi, Almeida & Gatto, 2020; Villamil, Ramírez, Vera & Jaime, 2018).

The virus is worldwide distributed, causes a high risk of infection in cattle herds. It is associated with gastrointestinal, respiratory and reproductive diseases, causing continuous economic losses, mainly due to the decrease in reproductive efficiency, thus generating great pressure in the livestock sector (Duan et al., 2020). In addition, its ability to cross the transplacental barrier frequently resulting in the birth of cattle persistently infected (PI), process that occurs in the first third of gestation because at this stage, the fetus has not developed the adaptive immune system and the virus is recognizing as self, without generating a response against the agent (Khodakaram-Tafti & Farjanikish, 2017). Consequently, the birth of an asymptomatic animal tends to proliferate the disease. However, the virus can also cause embryonic death, mummification and abortion (Spetter et al., 2020; Valdez et al., 2018a).

The prevalence of this disease variates across countries due to climatic and geographic factors and the different breeds and crossbreeds

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used in cattle production. It can also vary between geographical regions within a country, where the prevalence of antiviral antibodies can be higher than 90% if vaccination is a common practice (Selim, Elhaig, Moawed & El-Nahas, 2018).

Over the past years different infection agents have been diagnosed in some municipalities of Boyacá, especially in farms with high milk production and also with a background of reproductive disorders such as abortions, where BVD presents a important seropositivity of 76.4% (Moreno Figueredo, Benavides Ortiz, Guerrero & Cruz Carrillo, 2017). It is unclear the behavior of the disease and its participation in the reduction of reproductive efficiency, therefor, an improved understanding of the dynamics of serological patterns in BVD positive herds is important as a basis to implementation of integral sanitary protocols for detection and control of BDV (Abad-Zavaleta, Ríos-Utrera, Rosete-Fernández, García-Camacho & Zárate-Martínez, 2016). This research aimed to establish the seroprevalence and the main risk factors for the presence of BVDV in the municipality of Sotaquirá, Colombia.

2. Materials and methods

2.1. Study area

The study was carried out between May and July 2019. Sotaquirá town is located in the department of Boyacá (Colombia). Its altitude is 2860 m above sea level, the average temperature is 14 °C, the annual pluvial precipitation is 1100 mm and the relative humidity is 88%. The town area is 268.65 km^2, where 0.1 km^2 correspond to the urban area, and 268.55 km^2 correspond to the rural area (Alcaldía Sotaquirá, 2019).

2.2. Sample size

According to the town's cattle population census (ICA, 2019), a total of 19,333 cattle were reported. A sample of 1000 individuals was determined with a confidence interval of 95% and an accepted error of 3.1%, considering the following formula:

$$n = \left(\frac{Z_{\alpha/2}\sqrt{p(1-p)}}{E}\right) = \frac{Z^2\alpha/2{\cdot}p(1-p)}{E^2}$$

Where: n = sample size; E = accepted error; p = expected value of the proportion; α = tail probability. Cattle Holstein (601), Ayrshire (11), Jersey (21), Normande (257), Gyr (22) and Holstein x Gyr (88) distributed in 65 herds with no vaccination history were sampled. Before sampling, epidemiological survey were implemented, the variables related to the animal were included, considering the age group, breed and gender of the cattle evaluated and the variables related to management practices such as livestock owned by other owners, artificial insemination, pasture leasing, livestock exhibitions, purchase of animals and herd size.

2.3. Sample collection and processing

Blood samples were extracted by puncture of the coccygeal vein using 21-gage vacutainer needle. Seven ml of blood were collected and deposited in vacutainer® tubes without anticoagulant (red cap). These were identified and kept refrigerated (4 °C) to be transported to the Veterinary Parasitology laboratory of the Universidad Pedagógica y Tecnológica de Colombia. Subsequently, the tubes were centrifuged at 2500 rpm for 10 min to separate the serum and transferred to an Eppendorf storage tube and frozen at -20 °C until the tests were performed.

An indirect enzyme-linked immunosorbent assay (ELISA) was performed for detection of specific antibodies against BVDV, using the commercial kit SERELISA® BVD p80 Ab Mono Blocking, the sensitivity of 98% and specificity of 100%, following the manufacturer's protocol. Positive cases were determined as those individuals with a competence percentage ≥ of 50% in the test and as negative those bovines in which this value was < 30%.

2.4. Statistical analysis

It is an observational, descriptive, cross-sectional study with simple random sampling of animals, where the sampling unit was bovines. The apparent prevalence and real prevalence were determinate with WinEpi statistical program; after consolidating and filter the database, the data were analyzed in the Epi Info® 7.2.4.0 version statistical program. The ratio of animals and herds affected by BVDV that was exposed to a factor was compared with the same proportion of a non-exposed population to that factor to estimate prevalence ratios (PR). This PR was used to measure the association between BVDV and the hypothetical causal factors, as well as the significance of these associations using a Chi-square test (Thrusfield, 2005). PR values higher than 1 (lower confidence interval LCI 95% > 1) and with $p < 0.05$ were considered as risk factors, while PR values lower than 1 (upper confidence interval UCI 95% < 1) and with $p < 0.05$ were considered protective factors. The dependent variable included the ELISA BVD serological results obtained; the independent variables were all the determining factors established in the epidemiological survey applied during sample collection such as age group, breed, gender, livestock owned by other owners, artificial insemination, pasture leasing, livestock exhibitions, purchase of animals, herd size and infectious diseases. Once these factors were established, a stratified logistic regression was performed to test for confounding and to identify the simultaneous interaction between the variables significantly associated with BVDV (Martin, Meek & Willeberg, 1997).

2.5. Ethical considerations

The study was conducted under the regulations of laws 576 of 2000 and 84 of 1989 of the Republic of Colombia. Informed consent was obtained from the owners of the cattle prior to sample collection.

3. Results

The apparent prevalence (AP) of BVDV in the study population was 42.5% (425/1000). Forty-one dot five percent of females (361/869) and 48.9% of males (64/131) were seropositive for the virus. A real prevalence (RP) of 43.4% was established with a positive predictive value of 100% and a negative predictive value of 98.5%. In relation to the breeds evaluated, the highest seroprevalences were found in Gyr (59.1% AP; 60.3% RP) and Normande (48.6% AP; 49.6% RP). Likewise, it was established that individuals older than 4 years showed the highest seroprevalence (53.0% AP; 54.4% RP) (Table 1).

Table 1
Apparent prevalence (AP) and real prevalence (PR) of bovine viral diarrhea virus by bovine breed and age group in Sotaquirá, Boyacá.

Category	n	Positives	AP (%)	RP (%)	Positive predictive value	Negative predictive value
Breed						
Holstein	601	237	39.4	40.2	100	98.7
Ayrshire	11	3	27.3	27.9	100	99.2
Jersey	21	8	38.1	38.9	100	98.7
Normande	257	125	48.6	49.6	100	98.1
Gyr	22	13	59.1	60.3	100	97.1
Holstein x Gyr	88	39	44.3	44	100	98.5
Age group						
< 1 year	179	49	27.4	28	100	99.2
1–2 years	209	66	31.6	32.2	100	99.1
2–4 years	112	45	40.2	41	100	98.6
>4 years	500	265	53.0	54.4	100	97.7

Significant statistical association was found between Holstein and Normande cattle; age groups <1 year, 1 to 2 years and >4 years with seropositivity to the disease. Within the management practices evaluated in the farms, it was determined that large herd size (≥11), artificial insemination (AI), pasture leasing, participation in livestock shows, and purchase of animals have a significant association with the seropositivity to the disease, indicating that the occurrence of the virus is related to these practices. After analyzing the presence of other diseases of importance in cattle farms, it was possible to establish that Bovine Parainfluenza Type 3 (PI3) had a significant statistical association with the seroprevalence of BVDV (Table 2).

The PR and CI value identified the Normande breed (1.1608), cattle < 1-year-old (2.3963), >4 years (1.4468), pasture leasing (1.2928), participate in livestock exhibitions (1.5711), purchase of animals (1.2604), and PI3 (1.1208) as possible risk factors for BVDV presentation, while AI (0.7382), Jersey (0.9273) and 1–2 years old (0.7982) was reported as a protective factor for the infectious agent. After the initial identification of risk and protective factors by performing individual Chi-square tests, a stratified logistic regression was performed to look for significant interactions between these factors and their association with the presence of BVDV. The results showed that the interaction between the presence of cattle ⟨ 1-year-old, ⟩ 4 years, Normande breed,pasture leasing and PI3 was significantly associated with the presence of BVDV ($p = 0.0004$, $p = 0.0003$, $p = 0.0212$, $p = 0.0017$ and $p = 0.0484$ respectively) (Table 3).

4. Discussion

In Colombia, apparent prevalence have been reported from 27.1% in the Bogotá savanna (Buitrago Horta, Jiménez Escobar & Zambrano Varón, 2018), 39.59% in Nariño (Puertas Revelo, 2016), 55% in Cesar (Gálvis García, Bautista Amorocho & Vázquez, 2016), up to 76.4% for the department of Boyacá (Moreno Figueredo et al., 2017), Found in the current study is into the range. These differences could result from the variation in the age of the animals sampled, previous exposure of the bovines to the virus, presence of PI animals, number of animals in and management of the herd. Also, it is important to highlight that the cattle sampled, except for the Holstein x Gyr, mostly present a dairy genotype, where the seroprevalence percentages can be associated with those reported by Amelung, Hartmann, Haas and Kreienbrock (2018), who indicate that both dairy production and herds with a large number of animals increase risk of BVDV infection, compared with herds destined for meat production and with lower animal density.

Table 2
Possible risk factors associated with bovine viral diarrhea virus infections, results are shown as prevalence ratio (PR) and 95% confidence interval (95% CI). Significance is denoted by a *p*-value <0.05.

Variable	Category	PR	95% CI	*p*-value
Breed	Holstein	0.8731	0.7799–0.9775	**0.009649338**
	Normande	1.1608	1.0164–1.3258	**0.01284895**
	Ayrshire	0.7883	0.5468–1.1365	0.239028587
	Gyr	1.4147	0.8537–2.3442	0.085513186
	Holstein x Gyr	1.0358	0.8527–1.2582	0.400295499
	Jersey	0.9273	0.6601–1.3026	0.429243002
Age	< 1 year	2.3963	1.8528–3.0991	**0.0345612**
	1–2 years	0.7982	0.7137–0.8927	**0.000192403**
	2–4 years	0.9563	0.8132–1.1246	0.336326022
	>4 years	1.4468	1.295–1.6164	**0.0078978**
Gender	Male	1.143	0.958–1.3636	0.069397203
	Female	0.8766	0.7134–0.9455	0.063424321
Herd size	Small (≤10)	0,9821	0,9821–1,2648	0.0647231746
	Large (≥11)	0,9010	0,7943–1,0220	**0.0481448517**
Livestock owned by other owners	–	1.0935	0.9276-1.2328	0.0769776638
Artificial insemination	–	0.7382	0.6503–0.8379	**0.00000034**
Pasture leasing	–	1.2928	1.1386–1.4677	**0.000017**
Livestock exhibitions	–	1.5711	1.0615-2.3251	**0.004889953**
Purchase of animals	–	1.2604	1.1386-1.4677	**0.000021**
Neosporosis	–	0.9571	0.8603–1.0648	0.229900501
Bovine Parainfluenza - 3 (PI3)	–	1.1208	1.0032–1.2522	**0.023808998**
Paratuberculosis	–	1.1905	0.843–1.6812	0.180159576

Table 3
Analysis of variables as possible risk factors associated with bovine viral diarrhea virus infections. .

Variable	Odds ratio	Lower confidence interval (LCI 95%)	Upper confidence interval (UCI 95%)	*p*-value
< 1 year	0.5554	0.4019	0.7676	**0.0004**
>4 years	2.3961	1.8527	3.0988	**0.0003**
Normande	1.3984	1.0515	1.8598	**0.0212**
Pasture leasing	2.1097	1.3225	3.3654	**0.0017**
Livestock exhibitions	1.6053	0.571	4.5132	0.3695
Purchase of animals	1.0829	0.7454	1.5732	0.6761
PI3	1.3309	1.002	1.7677	**0.0484**

Global reports are variable but usually show a high prevalence associated with risk factors similar to those observed in Sotaquirá. Management of cattle for dairy production, high densities, age group and poor farm hygiene, seem to be the most common causes of the presentation of BVDV (Demil et al., 2021). In Germany, prevalence is lower than 14% have been reported in some farms. Highlighting that in recent years the prevalence of infection has decreased from 0.68% to 0.04%, as a result of acquired knowledge about the factors associated with a positive status for BVDV and the proper implementation of good bio-sanitary and animal management practices, preventing possible infections and controls the incidence of the virus (Amelung et al., 2018).

Other countries have reported a lower prevalence than Sotaquirá. For instance, Ethiopia has an apparent prevalence of 32.9% (Asmare et al., 2018), Ireland between 11.31% - 25% (Charoenlarp et al., 2018; Sayers, Byrne, O'Doherty & Arkins, 2015) and Scotland 16% (Brülisauer, Lewis, Ganser, McKendrick & Gunn, 2009). The higher prevalence in Sotaquirá may be affected by variables such as management of large herds, nearby productions with the disease, previous exposures to the pathogen, management of productions with dairy cattle or seasonal existence that favor the presence of the virus in herds.

Regarding the age, it was established that cattle < 1-year-old, 1 −2 years old and older than 4 years old presented significant statistical association, which indicates that the presence of the virus is related to the age of the cattle. In addition, age was considered a risk factor for the presentation of BVDV. According to Corro, Escalona, Mosquera and Vargas (2017), older animals are 1.79% more likely to be seropositive to the disease due to possible higher exposure to the virus, which is reflected in the increase of antibodies. For animals < 1-year-old, an increase in predisposition to acquire the disease is also reported since these animals are susceptible to be infected transplacentally via or get the disease by direct contact with possible carrier animals, which is reflected in the increase of antibodies against the virus (Evans et al., 2019; Sanchez Ortigoza, 2018).

Holstein and Normande breeds presented a significant statistical association with the presentation of antibodies against the virus. The Normande breed was determined as a risk factor for the pathology; this result agrees with described by Rivera, Rincón and Echeverry (2018), who indicate that the prevalence of BVDV was higher in cattle of the Normande breed compared to other breeds evaluated in the herds, despite being the only one that was not 100% dairy.

When evaluating the association between the presence of other diseases in the area and BVD seropositivity, PI3 seropositivity was established as a risk factor for BVD. This could be explained by a high prevalence of this disease in Colombia, with reports ranging from 11% to 68.9% in dairy and dual-purpose breeds, which is why Betancur-Hurtado, Castañeda-Ternera and González-Tous (2017) suggests that the distribution of the virus has increased, and these coinfections can occur more often.

It is important to highlight that the implementation of biotechnologies such as artificial insemination (AI) directly intervene in animal health, decreasing or preventing diseases (Marizancén & Artunduaga, 2017). Hence, AI is considered a protective factor against BVDV for bovine productions in the municipality of Sotaquirá. It should be emphasized that the implementation of this biotechnology should be done with the adequate analysis of the samples because BVDV still represents a potential problem in AI since semen could be collected from PI bulls or with acute BVDV infections (Arauco Villar & Lozano Salazar, 2018; Barbagelata, 2019).

Likewise, the grazing lease was established as a risk factor for the presentation of BVD in Sotaquirá town; possibly due to farm laborer's that implement management practices or transport animals from one grassland to another are exposed to other herds, which increases the risk of contagion by direct contact with infected animals or with contaminated surfaces, becoming a relevant factor on the transmission of the virus. However, it is important to clarify that transportation does not necessarily result in all cattle being infected by BVDV seropositive animals (Benavides et al., 2020).

Livestock exhibitions and the acquisition of animals were also established as risk factors; this can occur because, during these events, there is high contact between animals from a different origin, types, ages and health conditions. Hence, establishing a potential source of infection, given that animals are bought and sold, not only for consumption but also for breeding purposes (Quispe, Ccama, Rivera & Araínga, 2008). Some being asymptomatic PI, but important factors in spreading the virus (Valdez et al., 2018b).

Finally, it should be taken into account that reproductive diseases that occur in these bovine productions are one of the main problems that affect the economy of the owners. It may not be due only to the presence of a specific disease, but often additional pathologies are not perceived by some diagnostic methods, which makes them go unnoticed (Moreno Figueredo et al., 2017). In addition, the latent character of the virus makes us presume that it will keep circulating indefinitely in farms with seropositive animals with subsequent new infections and reinfections (Vargas-Niño et al., 2018).

5. Conclusion

The serological detection carried out in the town of Sotaquirá, showed a seroprevalence of 42.5% for BVDV, confirming that the problem and alterations that this disease implies persists in dairy herds in this area, affecting both economic and animal health fields. Because of this, it is necessary to intensify the bio-sanitary processes that allow reduce or eliminate the presence of the virus in the region, and it is vital to follow up the groups that were established as risk factors since they are the ones that can mainly perpetuate the disease with its consequent affections or impacts to the competitiveness of the livestock sector in the region.

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Ethical statement

El Comité de ética para la investigación evalúo artículo titulado SEROPREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS (BVDV) IN CATTLE FROM SOTAQUIRÁ, COLOMBIA, y determinó dar el AVAL, teniendo en cuenta que cumple con las condiciones éticas.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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