

## Presence of IgG antibodies against reproductive infections in breeding bulls of Magdalena Medio, Colombia<sup>□</sup>

*Presencia de anticuerpos tipo IgG contra infecciones reproductivas en toros reproductores de la región del Magdalena Medio, Colombia*

*Presença de anticorpos IgG contra infecções reprodutivas de touros reprodutores do Magdalena Médio, Colômbia*

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### Summary

**Background:** cattle farming is an important economic activity in several regions of Colombia. However, farmers are unaware of their cattle health status, and particularly of bacterial, parasitic, and viral diseases, which affect reproduction and profitability. **Objective:** to assess the presence of IgG antibodies against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), bovine leukosis, leptospirosis, brucellosis, and *Neospora caninum* in breeding bulls in the Magdalena Medio of Colombia, according to age, breed, and origin. **Methods:** a descriptive, cross-sectional study was conducted with 174 male breeding cattle from several subspecies. Animals were above 18 months of age and belonged to dual-purpose farms in the Magdalena Medio region. Presence of IgG anti-IBR, BVD, brucellosis, neosporosis, and leukosis antibodies were determined with an enzyme-linked immunosorbent assay (ELISA). A sample of 156 males was assessed for leptospirosis using a microscopic agglutination test (MAT) in a dark field. **Results:** IgG anti-IBR antibodies were found in 92.5% of the animals, anti-BVD in 62.6%, anti-*Neospora caninum* in 79.3%, anti-*Brucella abortus* in 4.02%, anti-*Leptospira pomona* in 8.33%, and anti-*L. hardjo* in 15.38%, with >1:100 titres. Besides, 64.94% prevalence was observed for bovine leukosis. **Conclusions:** a high prevalence of IgG anti-IBR, IgG anti-BVD, IgG anti-*Neospora caninum*, IgG anti-leukosis, and IgG anti-leptospirosis antibodies were observed in Magdalena Medio bulls. With respect to *B. abortus*, confirmatory and competitive ELISA test should be performed and

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sanitary standards need to be established. Detection of IgG antibodies among bulls reveals poor sanitary conditions, absent of protective-vaccination antibodies and an urgent need for health control measurements.

**Keywords:** *bovine viral diarrhoea, brucellosis, enzyme-linked immunosorbent assay, infectious bovine rhinotracheitis, leptospirosis, Neospora caninum.*

### Resumen

**Antecedentes:** la ganadería bovina es una importante actividad económica desarrollada en varias regiones del país, sin embargo, los ganaderos desconocen el estado sanitario de los bovinos con respecto a enfermedades bacterianas, parasitarias y virales, lo que afecta la reproducción y la rentabilidad. **Objetivo:** evidenciar la presencia de anticuerpos tipo IgG anti-rinotraqueitis infecciosa bovina (IBR), diarrea viral bovina (BVD), leucosis bovina, leptospirosis, brucelosis y *Neospora caninum* en los toros reproductores del Magdalena Medio colombiano, en asociación con variables de edad, raza y procedencia. **Métodos:** se realizó un estudio descriptivo de corte transversal en un grupo poblacional de 174 machos reproductores bovinos de diferentes subespecies. Los animales eran mayores de 18 meses en fincas destinadas a ganadería doble propósito ubicadas en la región del Magdalena Medio. Mediante un ensayo de inmunoabsorción enzimática (ELISA) se determinó la presencia de anticuerpos tipo IgG anti-IBR, BVD, brucelosis, neosporosis y leucosis. Para el caso de la leptospirosis, a 156 de los machos se les realizó la prueba de microaglutinación en campo oscuro (MAT). **Resultados:** se hallaron anticuerpos tipo IgG anti-IBR en un 92,5%, anti-BVD en el 62,6%, anti-*Neospora caninum* 79,3%, anti-*Brucella abortus* 4,02%, anti-*Leptospira pomona* 8,33% y anti-*L. hardjo* 15,38%, con títulos >1:100. Además, para leucosis bovina se obtuvo una prevalencia del 64,94%. **Conclusión:** se observaron altos porcentajes de casos con presencia de anticuerpos IgG anti-IBR, BVD, *N. caninum*, leucosis y leptospirosis en el ganado bovino del Magdalena Medio colombiano. Para *B. abortus* debe realizarse la prueba confirmativa ELISA competitiva y aplicar la norma sanitaria. La determinación de anticuerpos tipo IgG permitió conocer el delicado estado sanitario de los reproductores bovinos, teniendo en cuenta que no han recibido ningún esquema de vacunación y se hace necesario un adecuado control sanitario.

**Palabras claves:** *brucelosis, diarrea viral bovina, ensayo de inmunoabsorción enzimática, leptospirosis, Neospora caninum, rinotraqueitis infecciosa bovina.*

### Resumo

**Antecedentes:** a pecuária é uma atividade econômica importante em várias regiões do país, no entanto, os agricultores não têm conhecimento do estado de saúde do gado em relação a doenças bacterianas, parasitárias e virais. É por esta razão que as perdas econômicas aumentam e diminuem a qualidade de reprodução. **Objetivo:** demonstrar a presença de anticorpos IgG para rinotraqueíte-viral bovina (IBR), diarréia viral bovina (BVD), leucose bovina, leptospirose, brucelose e *Neospora caninum*. Nos machos reprodutores do Magdalena Medio tendo em conta as variáveis idade, raça e origens. **Método:** foi realizado estudo descritivo transversal em duas populações de 174 machos reprodutores de diferentes subespécies de gado com mais de 18 meses de idade procedentes de fazendas utilizadas para a pecuária de duplo propósito, localizadas na região do Magdalena Médio. Para detectar a presença de anticorpos IgG anti-IBR, BVD, brucelose, e enzoótica, neosporose foi determinada pela técnica de ensaio imunoenzimático (ELISA). Para o caso de leptospirose foi realizado teste de microaglutinação em campo escuro (MAT). **Resultados:** anticorpos IgG anti-IBR foram encontrados em 92,5%, anti-BVD num 62,6%, anti-*Neospora caninum* num 79,3%, anti-*Brucella abortus* 4,02%, anti-*Leptospira pomona* num 8,33% e anti-*L. hardjo* 15,38% com títulos > 1:100. Para leucose bovina, uma percentagem de 64,94%, foi obtido. **Conclusão:** foram observados altos percentagens de casos na presença de anticorpos IgG anti-IBR, BVD, *N. caninum*, leptospirose e leucose nos bovinos do Magdalena Médio colombiano, o teste confirmatório ELISA competitivo. Para a determinação de *B. abortus* deve ser realizado e dar cumprimento a normatividade sanitária. A determinação de anticorpos IgG tem permitido conhecer o delicado estado sanitario dos reprodutores bovinos, considerando que eles não receberam qualquer vacinação é necessário um adequado controle de saúde.

**Palavras chave:** *brucelose, diarréia viral bovina, ensaio imunoenzimático, leptospirose, Neospora caninum, rinotraqueíte infecciosa bovina.*

## Introduction

There is limited epidemiological data establishing a relationship between infected cattle and variables such as age, subspecies and origin. Colombian's livestock industry plays an important role in the national economy. It represents 27 and 64% of the agricultural and livestock gross domestic product (GDP) of the country, respectively (Gomez *et al.*, 2011).

Viral infectious diseases have a negative impact on livestock due to frequent stillbirths and birth defects that cause large economic losses. Parasitic and bacterial infections, including neosporosis, leptospirosis and brucellosis also affect cattle productivity and are associated with male infertility. The objective of this study was to evaluate the presence of IgG antibodies, IgG anti-infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), bovine leukosis, leptospirosis, brucellosis, and IgG anti-*Neospora caninum* in breeding cattle in the Magdalena Medio.

## Materials and methods

A descriptive, cross-sectional study was conducted in 22 dual-purpose cattle farms located in the Magdalena Medio region of Colombia, spanning from the provinces of Bolívar and Cesar to Norte de Santander and Santander.

### *Animals*

Healthy breeding cattle over 18 months of age were used to evaluate leukosis, brucellosis, IBR, neosporosis, and BVD infections. From this group, 156 males were evaluated for leptospirosis.

### *Sample size calculation*

Calculation of the sample size was performed with EPIDAT 3.0 program. A population of 10,300 breeding bulls was used, with an expected 65% prevalence (Carrizales, 2002), 95% confidence, and 7% precision. Sample size was estimated in 174 animals. After considering that sample size was insufficient, a second population of 156 animals was examined for determination of *Leptospira hardjo* and *L. pomona*.

### *Survey*

Each farm owner was surveyed to obtain basic data of the animals including age, breed, and origin of each individual, and vaccine status. Breed was recorded as *Bos taurus* (BT), *Bos indicus* (BI), or crossbreed cattle (BT x BI).

### *Sampling*

Five ml of blood were collected by venipuncture of the coccygeal vein in tubes without anticoagulant by using Vacutainer® vacuum system. Each sample was properly labeled with designated label codes and transported to the laboratory in ice containers. Blood samples were centrifuged at 1500 x g and aliquoted in 1.5 ml vials. Samples were stored at -20 °C for further processing.

### *Enzyme-linked immunosorbent assay (ELISA)*

ELISA tests were carried out with SVANOVIR® (Svanova Biotech, Uppsala, Sweden) commercial kits. For each assay, 96-well microplaques were sensitized with BVD, IBR, *Brucella* spp, *N. caninum*, and leukosis antigen, following the manufacturer's recommendations. Positive and negative controls were included with each assay.

### *Microscopic agglutination test (MAT) in dark field*

For each sample, 40 µl of serum was added to 1960 µl Phosphate buffered Saline (SAF) and incubated 20 minutes at room temperature. Serum dilution was mixed 1:1 in microplates with each antigen serovar in a total volume of 100 µl and incubated for 2 hours at 28 - 30 °C or 18 hours at 4 - 8 °C. Then, 50 µl of mixture was placed in a filter paper and read with a dark-field microscopy using 10X magnification. Titers  $\geq 1100$  and 50% *Leptospira* agglutination was considered as a positive result (OIE, 2008).

### *Data analysis*

For the data analysis, variable factors such as age, breed and origin were included. Also, measurements such as frequency, percentage and confidence interval (95%) were established for each of the evaluated infectious event. To establish possible association

between positivity of serological tests and the animal characteristics, the chi-square test or the Fisher's exact test was applied as appropriate. No vaccination against IBR, BVD, *L. pomona*, and *L. hardjo* were applied to the bovines in the present study.

## Results

A total of 174 dual-purpose male cattle made part of the present study. Of the 174 assessed, 92.5% was positive for IBR, 62.6% for BVD, and 79.3% for *N. caninum*. Furthermore, 4.02 and 64.94% of the cattle were found positive for *Brucella abortus* and bovine leucosis, respectively. Among the 156 males assessed for *L. pomona* and *L. hardjo*, the percentages of positive results were 8.33 and 15.38%, respectively.

Table 1 shows the province location of the livestock farms (n = 22) in the study: Bolívar, Cesar, Santander, and Norte de Santander. With the Fisher's exact test, it was evidenced that there is no statistically significant differences between infection and geographic origin (p = 0.17).

Subspecies and infection by IBR showed statistically significant differences (p < 0.05), specifically among *Bos indicus* subspecies. IgG positive antibodies against BDV, *N. caninum*, leucosis, and *B. abortus* were present in three breed

categories. However, there were no differences among groups (p = 0.166), as seen in Table 2.

## Discussion

The present study determined IgG antibodies anti-IBR, BVD, *N. caninum*, *B. abortus*, and IgG anti-leukosis in breeding bulls from dual-purpose livestock farms from the Magdalena Medio region. In this region, viral diseases such as BVD, IBR, and leukosis represent major infection, threatening the livestock sector with significant economic losses. Moreover, lack of prevention measures, epidemiological data and unavailability of vaccines allow successful proliferation of these diseases (OIE, 2005). Several studies have focused on the diagnosis of female cattle without giving attention to males. The results obtained were significantly higher than those conducted in Montería (Colombia) with 21% seroprevalence for BLV (Sánchez *et al.*, 2003; Betancur and Rodas, 2008; Peralta, 2009).

We determined IgG type antibodies of viral diseases (BVD and IBR) by indirect ELISA technique, where 62.6% positivity for BVD and 92.5% for IBR were obtained. These results are similar to a study in Peru, where 48.7% IgG anti BVD and 92.5% anti IBR of a total of 347 samples of males and females were reported as positive, respectively (Quispe *et al.*, 2002). Cárdenas *et al.* (2011) in Peru showed that 56.2% had

**Table 1.** IBR, BVD, *Neospora caninum*, leukosis, and brucellosis infections by geographic origin.

Infection/ place of origin	IBR			BVD			<i>Neospora caninum</i>			Leucosis			<i>Brucella abortus</i>		
	n	%	IC (95%)	n	%	IC (95%)	n	%	IC (95%)	n	%	IC (95%)	n	%	IC (95%)
Cesar	126	94.7	89.46-97.85	82	61.7	52.8-69.9	106	79.6	71.9-86.2	83	76.90	53.59-70.65	4	3.0	0.83-7.52
Santander	18	81.8	59.72-94.81	16	72.7	49.7-89.2	17	94.4	40.7-82.8	19	59.93	65.09-97.09	2	9.0	1.12-29.1
Norte de Santander	16	88.9	65.20-98.62	10	55.6	30.7-78.4	14	63.6	72.7-99.9	10	55.6	30.76-78.47	1	5.5	0.14-27.2
Bolívar	1	100	-	1	100	-	1	100	-	1	100	0.03-1	0	0	-
Total positive	161	91.3	87.56-95.96	109	72.5	55.0-69.8	138	84.4	72.5-85.1	113	73.10	57.4-72.0	7	4.02	1.63-1.11

**Table 2.** IgG antibodies anti-IBR, BVD, *Neospora caninum*, leukosis, and *Brucella abortus* by subspecies.

Infection/ subspecie	IBR				BVD				<i>Neospora caninum</i>			
	n	%	IC	p	n	%	IC	p	n	%	IC	p
BI x BT	17	70.8	48.91 - 87.38		11	45.8	25.55 - 67.18		19	70.4	49.8 - 86.2	
<i>Bos indicus</i>	132	97.1	92.64 - 99.19	>0.05	88	64.7	56.05 - 72.70	>0.05	111	81.6	74.1 - 87.7	>0.05
<i>Bos taurus</i>	12	85.7	57.19 - 98.22		10	71.4	41.89 - 91.61		8	72.7	39.0 - 94.0	
Total positive	161	84.5	87.56 - 95.96		109	60.6	55.00 - 69.84		138	74.9	72.5 - 85.1	

Table 2 continued

Infection/ subspecie	Leucosis				<i>Brucella abortus</i>			
	n	%	IC	p	n	%	IC	p
BI x BT	20	17.7	53.7 - 88.9		0	0		
<i>Bos indicus</i>	82	72.5	55.6 - 68.6	>0.05	7	4.02	1.63-8.11	>0.05
<i>Bos taurus</i>	9	7.9	48.2 - 97.7		0	0		
Total positive	113	32.7	56.2 - 70.9		7	4.02		

antibodies against BVD in 406 samples including bovines older than 6 months. In Chile, Peralta (2009) screened 156 animals and found that 54.0% had antibodies for BVD, whereas in Colombia, only 29.4% was observed as positive for BVD (Betancur *et al.*, 2006). The presence of anti-BVD antibodies in animals without vaccination history indicates a clear exposure to the virus (Peralta, 2009). Accordingly, it is possible to infer that seropositivity of bovine animals for BVD, IBR, and bovine leukosis is due to their contact with these pathogens. It may result in infected carriers that spread the pathogens to other animals (Betancur *et al.*, 2006).

Furthermore, in order to determine neosporosis prevalence, detection of IgG type anti-*N. caninum* antibodies was conducted and a prevalence of 79.3% was observed. This result provides insight to understand the current status of the infection and the impact that generates the disease, since it can cause economic losses. These findings did not demonstrate the existence of the disease in animals that were positive, however, it suggests that the animals were exposed to *N. caninum*, causing the detection of antibodies (Cedeño, 2010). Our results correlate with a recent report by FEDEGAN (Zambrano, 2012),

where seroprevalence of *N. caninum* for both sexes was documented to be 54% in Colombia. In general, the presence of the parasite was observed in more than half of the population of cattle. Our results are also in agreement with previous reports in Pasto, Colombia, where prevalence of 76.9% was obtained in dairy cattle (Cedeño *et al.*, 2010). This allowed establishing the relationship between seropositivity and prior contact of males with the parasite due to a spreading factor, like dogs living in the livestock farms, which would have important consequences in terms of economic losses (Dubey and Lindsay, 1996).

The BLV is a retrovirus associated with enzootic bovine leukosis, which is presented as a globally distributed disease. It is commonly found in adult cattle and characterized by a long incubation period. This study analyzed 174 animals and found 64.94% positive; a higher value when compared to 21% prevalence observed in Córdoba (Colombia) among 137 cattle from 28 dual-purpose livestock farms. Furthermore, in the same province, 15% seroprevalence with an ELISA technique was reported (Betancur *et al.*, 2008). The higher prevalence of leukosis in our study is an alert for establishing control interventions and biosafety measurements

in livestock farms. It also suggests verification of sanitary condition of animals entering the venue. It has been found that maneuvers such as removal of blood, vaccinations and a digital rectal examination, in which work is done with a large number of animals using the same instrumentation, may spread the virus. In the same way, virus transmission has been associated to places where arthropods burden is high, since they can contribute to the spread of illness (Johnson and Kaneene, 1992; Delgado *et al.*, 2009).

In Colombia, sanitary policies for the prevention, control and eradication lack true rigor, and in some cases, the required reports do not reach the responsible entities. Besides, the economic impact of leukosis infection includes negative effects on milk production, fall in semen value, and low productivity. However, the greatest impact is observed in the international cattle trade restrictions (Delgado *et al.*, 2009; Benavides and Laverde, 2012).

A cross-sectional study with 331 sera of females and 29 males, 201 (60.73%) and 18 (62.07%), respectively, had antibodies against BLV indicating no statistically significant differences between sex and the occurrence of infection with the BLV. This result coincides with the reported by Marin *et al.* (1978), Stöber (1981), and Clerc (2008), where association between sex and BLV infection was not found. On the other hand, Betancur and Rodas (2008) reported differences between seropositivity to the BLV in females and males, 68.6% and 31.4%, respectively ( $p < 0.05$ ), due to a greater male contact with infected females, through the natural mating process.

Regarding age, it was included an age group of  $\leq 3.5$  years, where there were found 21.83% positive, whereas the group of 4-6.5 years was 36.20%, and for the group of 7 - 10 years, 6.89% of prevalence ( $p > 0.05$ ). According to another study where the age group was 1 - 4 years and 21.56% were seropositive, while group  $\geq 5$  years, 65.65% were positive for the serological test, showing significant differences ( $p = 0.0030$ ). This result indicates an association between the age of cattle and positivity to the infection, thus the greater the age the higher is the probability that the animals are seropositive. This could be due to the fact that animals have had more exposure to the virus, as it has been suggested in the literature (Hungerford,

1990; Johnson and Kaneene, 1992; Chamizo, 2005; Betancur and Rodas, 2008; Clerc, 2008).

In cattle, leptospirosis causes economic losses by its effects on reproduction, such as abortions, stillbirths, birth of weak animals and infertility (Coromoto *et al.*, 2004). In studies conducted in Venezuela, 38.2% bovine animals were positive to one or more leptospira serovars and 61.8% were found to be suspicious or negative. In the group of animals positive to one or more serovars, the most frequently observed was *icterohaemorrhagiae* observed 49.2%, followed by *hardjo* (45.0%). In the present study, a higher percentage of seropositivity was observed for *hardjo* (14.74%), while *pomona* was 8.33%. The crossbreed cattle with positive antibodies were 2.56%. Moreover, those who were positive for *pomona* and negative for *hardjo* showed 5.76% prevalence. It is possible that an animal reacts and/or be positive for more than one serovar because there is a probability of cross-reactivity with others. Therefore, the serovar that presents a greater positivity than the other will be identified as the cause of the infection (Van Balen *et al.*, 2009).

For *B. abortus*, the positivity proportion was 4.02%, a value that is lower compared with a study conducted in Mexico (39.6%). However, competitive ELISA was not performed as a confirmatory test (Sánchez-Castilleja *et al.*, 2012). It is recommended to initiate a control program against this disease. *B. abortus*, was included in the 2014 list in force by the OIE, which proposes a greater awareness by livestock farmers and implementation of the required procedures to control the infection (Seleem *et al.*, 2010; OIE, 2014).

In conclusion, the high percentage of positivity of IgG antibodies indicates that cattle have been exposed to the pathogen responsible for the disease. However, the prevalence does not confirm recent or chronic infection. For this reason, it is proposed that the next step should be the validation of seropositivity with confirmatory tests. Likewise, it is suggested to design control policies and eradication plans for IBR, BVD, *N. caninum*, *L. hardjo*, *L. pomona*, *B. abortus*, and leucosis in the Magdalena Medio region. It is also important to mention that this study had limitations by not considering paired samples that could allow an approach to the diagnosis.

Infections by BVD, *B. abortus*, *N. caninum*, leptospirosis, and bovine leukosis did not show any pattern or affinity with respect to the variables like age, breed and origin, and the detection of antibodies. However, IgG anti-IBR was associated with variable subspecies (*Bos indicus*), which may indicate the existence of a susceptibility of cattle to infection by this virus. Still, a paired sample study is necessary to determine the exact values of IgG antibodies and compare them with the studied subspecies.

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### Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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