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Bovine Herpesvirus-1 antibodies levels and associated Risk Factors in unvaccinated Dairy Herds from tropical wet weather, Ecuador

Niveles de anticuerpos frente a Herpesvirus Bovino tipo 1 y factores de riesgo asociados en rebaños lecheros no vacunados en un clima húmedo tropical, Ecuador

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ABSTRACT

In order to determine the antibodies levels against Bovine Herpesvirus type 1 (BHV-1) and associated risk factors in unvaccinated dairy herds of tropical wet weather of Ecuador, an investigation was carried out in thirteen production units (PU), in the Chone Canton, Province of Manabí. The sample size was calculate by cluster sampling and the variables to be evaluated were antibodies levels frequency, age, sex, ocular or vulvovaginal lesions, breed and PU. One hundred eighty three blood samples were taken from the jugular or caudal vein and labeled for transfer to the laboratory. The tubes were centrifuged at 3,220 g for 15 minutes and the sera were transferred to Eppendorf tubes that once labeled were stored at –20°C until processing. Antibodies levels against HBV-1 were measured through of competitive ELISA technique, and calculated with simples statistical. The risk factors were estimated through the Odds ratio and relative risk, and the significance level through the Chi-square. The general antibodies levels frequency was 58.47 %. According age, 28.57 % in animals under two years old (yr.old) and 67.38 % for cattle over two yr.old. According to the sex, males showed 18.18 % and females 82.61 %. The determined risk factors (P<0.05) were age and sex, with a significant relative risk (P<0.05) of 3.31 and 2.32, respectively. The unvaccinated dairy herds of Western lowlands of Ecuador, have high antibodies levels frequency against BHV-1, with females over two yr.old at the highest risk of presenting infectious bovine rhinotracheitis (IBR).

Key words: Dairy herds; BHV-1; seroprevalence; risk factors; unvaccinated

RESUMEN

Con el objetivo de determinar la seroprevalencia frente a Herpesvirus Bovino tipo 1 (BHV-1) y los factores de riesgo asociados en rebaños lecheros no vacunados de un clima húmedo tropical de Ecuador, se llevó a cabo una investigación en 13 unidades de producción (UP), en el cantón Chone de la provincia de Manabí. El tamaño de la muestra fue calculado a través del muestreo por conglomerados y las variables a ser evaluadas fueron: seroprevalencia, edad, sexo, lesiones oculares o vulvovaginales, mestizaje y UP. Un total de 183 muestras de sangre periférica fueron tomadas y rotuladas para su envío al laboratorio. Los tubos fueron centrifugados a 3.220 g por 15 minutos y los sueros transferidos a tubos Eppendorf, una vez rotulados se almacenaron a –20°C, hasta su procesamiento. Los niveles de anticuerpos frente a HBV-1 fueron medidos a través de la técnica de ELISA competitiva y calculados con estadísticos simples. Los factores de riesgo fueron estimados, a través del Odds Ratio y riesgo relativo, la significancia con la prueba de Ji-cuadrado. La seroprevalencia general fue 58,47 %. De acuerdo a la edad, el valor en los animales menores de dos años fue 28,57 %, y en los mayores a dos años 67,38 %. Los machos mostraron un 18,18 % de seroprevalencia, mientras que las hembras 82,61 %. Los factores de riesgo determinados (P<0,05), fueron la edad y el sexo, con un riesgo relativo significativo (P<0,05) de 3,31 y 2,32, respectivamente. Los rebaños lecheros no vacunados evaluados, presentan alta seroprevalencia frente a BHV-1, con hembras mayores a dos años con mayor riesgo de presentar rinotraqueitis infecciosa bovina (RIB).

Palabras clave: Rebaños lecheros; BHV-1; seroprevalencia; factores de riesgo; no vacunados

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INTRODUCTION

Bovine herpesvirus type 1 (BHV-1) is a member of the Herpesviridae family, subfamily Alphadenovirinae [1, 18, 29], which in ruminants, domestic and wild, form a large group of pathogens with high morbidity [20, 22]. BHV-1 is responsible for economic losses in milk production worldwide [5, 24], due to the use of medications, milk discarding, mortalities [3, 10, 20, 25] and reproductive failures, such as embryonic death or abortions [6, 15], increasing the importance, due to the lack of regularization and the globalization of animal markets or their products [13, 16].

Only one BHV-1 serotype has been recognize, but subtypes have been describe based on viral desoxyribonucleic acid (DNA) restriction patterns [18]. BHV-1 is the causative agent of bovine infectious rhinotracheitis (IBR) [26, 29], which manifests as rhinotracheitis and conjunctivitis [28], in addition to others syndromes such as reproductive, encephalic and enteric [4, 8, 20]. IBR is part of the bovine respiratory disease complex, which is caused by a combination of viral and bacterial pathogens [3]. IBR is considered a specific species disease of cattle (*Bos taurus*) [26], although it has been reported that sheep (*Ovis aries*) are sensitive [10], and has cross immunity with other herpesviruses of other animals, such as goat (*Capra hircus*), buffalo (*Bubalus Bubalis*) or cervids [5, 9, 22]. BHV-1 is found in conjunctival, nasal and reproductive secretions [29], transmitted directly through aerosols or by contact with infected animals, and indirectly by water and food contaminated with body secretions, and even by semen used in artificial insemination or embryo transfer [1, 5, 24, 26].

During the pathogenesis of BHV-1 infection, the infected animal goes through the acute or viremia phases, of latency that can generate long-term infections, and periodic viral reactivation in the host [26, 29], where it can present sporadic periods of viral excretion and serve as a potential source of transmission [1]. Antibodies obtained by passive immunity through colostrum do not prevent initial viral replication and follow the establishment of latency, becoming latent seronegative carriers, until seroconversion or viral reactivation occurs [14]. The main consequence of BHV-1 infection, after accessing a wide range of organs and tissues, is to cause a variety of symptoms such as keratoconjunctivitis, tracheitis, enteritis, infertility and abortions [12, 15, 26].

Antibodies levels against BHV-1, has a wide range in the reported studies of different herds, which ranges from moderate, between 20 and 30 % [5, 15], to high frequency greater than 50 % [1-3, 6, 12, 21, 24]. These differences between studies could be due to types of productive systems [20, 24], preventive measures used [1, 24], or the viral isolate involved [20]. Among the factors that have been reported as risk, there is mainly age, where older animals are the ones with the highest seroprevalence [6, 7, 12, 24, 29], sex [1, 24], the history of reproductive problems in herds [12, 20], and the non-implementation of preventive measures [14, 15, 18, 20, 28, 29]. BHV-1 antibodies levels frequency in Ecuador was established at 43 % [7], but additional studies have not been reported and the current status of the disease is unknown. In the Ecuador Coast Region were used largely for cattle meat and milk production, where one of the main production areas is the Chone Canton of the Province of Manabí, being its economic axis. Cattle production is mostly traditional, where preventive measures such as vaccination of different diseases, including IBR, are not carried out. For this reason, an investigation was carried out to determine seroprevalence against BHV-1 and associated risk factors, in unvaccinated herds of the tropical wet weather of Ecuador.

MATERIALS AND METHODS

Ethical considerations

To carry out this research, the Norwegian National Research Ethics Committees guidelines for the use of animal in research were considered [17].

Study location

The research work was carried out in the Convent Parish, in the Chone Canton in the Province of Manabí, Ecuador. Geographically it is between 0°11'35.31" South and 79°54'01.60" West, at 296 meters above sea level and an average annual temperature ranging from 23 to 30°C. The climate is humid tropical with an annual average of 1,240 cubic millimeters (mm) [11]. It is an area of cattle production, with extensive grazing in grasslands of introduced pastures (*Panicum maximun*, *Brachiaria* spp.), with sources of water from springs, rivers, dams and underground wells, with low use of technologies and no history of prevention against IBR.

Study design

Thirteen dairy PU were used, which had 1,010 animals over six month's (mos) olds, at study time [20, 24]. The size of the sample was determined by cluster sampling, where it is calculated by combining all the animals of herds under study, using the probability proportional to the size of each herd [30], and the cattle to be sampled were randomly selected according to age group in proportion to percentage of different age groups within each herd [27]. The dependent variable studied was antibodies levels against BHV-1 frequency and the independents were age (greater than two yr., less than two yr.), sex (male, female), presence of vulvovaginal or ocular lesions, breed according to their phenotypic characteristics (*Bos taurus taurus* and *B. t. indicus*) and PU.

Population sample size

The sample size of the bovine population of the 13 PU was 183 animals, of which 141 were older than two yr old. and 42 were younger, 22 males and 141 females, 107 had vulvovaginal or ocular lesions, and the animals' number according to their racial predominance was: 98 *B. t. indicus* and 85 *B. t. taurus*, respectively.

Blood serum sample collection and storage

Blood sampling was collected via jugular vein in the case of young animals, and the caudal vein in adult, using Vacutainer® system. The samples were labeled and transferred in refrigeration in cellar at 4°C (Ecoshel, C800D, Mexico), to animal health laboratory of the Universidad Eloy Alfaro de Manabí Extension Chone. The samples were centrifuged at 3,220 g for 15 minutes (min), for blood serum extraction, which was deposited in Eppendorf® tubes, identified and stored at -20°C (Thermo Scientific, 25LCETSSA, USA) until its processing [29].

Competitive Elisa technique

For serological diagnosis, the sera were thawed at room temperature and analyzed using the competitive ELISA technique. The entire technique procedure was performed following the instructions of the manufacturer's protocol. The ID Screen® IBR gB competition Kit from the ID laboratory was used (Vet Innovative Diagnostics, Grabels-FRANCE), for the detection of anti-gB BHV-1 antibodies in serum samples [19].

Antibodies level was calculated according to the percentage of competition (S/N %), which was calculated using the following formula with optical density (OD) data:

$$S/N\ \% = \frac{OD\ simple}{Od\ Negative\ control} \times 100$$

Where, if the result was less than or equal to 45 %, they are considered positive, greater than 45 % and less than 55 %, they are considered doubtful, and greater than or equal to 55 %, they are considered negative [19]. Once the positive and negative animals were obtained, the seroprevalence was calculated with epidemiological formulas.

Analysis of data

BHV-1 antibodies levels were analyzed through simples statistical and the risk factors determination for the independent variables was obtained through of Odds ratio and relative risk calculation [31], and the degree of significance was estimated by the Chi-square test, through the Statistical Analysis System (SAS) [23].

RESULTS AND DISCUSSION

The results of seroprevalence against BHV-1, in dairy herds of the western lowlands of Ecuador, show a high overall seroprevalence in the herds studied, with 107 positive animals, 74 negative and two doubtful, for 58.47 %, as shown in TABLE I.

TABLE I
General BHV-1 antibodies frequency in the herds studied

Results	n	(%)
Positive	107	58.47
Negative	74	40.44
Suspects	2	1.09
Total	183	100.00

In TABLE II, seroprevalence can be observed according to the sampled animals' age, in addition to the value of Odds ratio and relative risk. Seroprevalence was lower for animals younger than two yr-old with 28.57 % and higher in those older than two yr-old with 67.38 %, determining age as a risk factor, showing a relative risk value of 3.31 times higher in adult animals to presence of seropositivity, compared to young animals. Likewise, according to sex, the results show a seroprevalence for males of 18.18 and 82.61 % for females, this variable being a risk factor (P<0.05), since females showed 2.32 times more risk of being seropositive to BHV-1, compared to males (TABLE III).

The seroprevalence according to the presence of vulvovaginal or ocular lesions was 40.19 %, with a relative risk of 1.05 (P>0.05). Likewise, according to the breed predominance (p), the *B. p. indicus* 52.55 % and *B. p. taurus* 51.23 %, respectively, with a relative risk of 1.29 (P<0.05). In the case of PU, 100 % showed animals seropositive to HBV-1.

TABLE II
BHV-1 antibodies frequency according to Age, Odds ratio, Relative Risk

Age	n	Positives	(%)	Odds ratio	Relative Risk
< 2 years	42	12	28.57		
> 2 years	141	95	67.38	5.02*	3.31*
Total	183	107	58.47		

*P Value < 0.05.

TABLE III
BHV-1 antibodies levels frequency according to Sex, Odds ratio, Relative Risk

Sex	n	Positives	(%)	Odds ratio	Relative Risk
Males	22	02	18.18		
Females	161	133	82.61	4.64*	2.32*
Total	183	107	58.47		

*P Value < 0.05.

The seroprevalence against BHV-1 in unvaccinated cattle herds, in western lowlands of Ecuador was determined and had a high value of positive animals with 58.47 %. This value was like that reported in other studies, including unvaccinated herds [1], which showed high seroprevalences, above 50 % [2, 3, 6, 12, 21, 24]. The high number of positive animals may be related to different factors, including the isolate involved [1], the ability of the virus to remain latent, the introduction of infected animals that spread disseminate and perpetuate the virus within the herds [9, 29] and the control measures implemented [15]. Also suggests a viral free circulation in the environment, with continuous or periodic excretions of the infectious agent by latent infected animals or with viral reconversion of the herds [1], which suggests the endemic presence of IBR [15].

The entry of BHV-1 in the herds, can be given in the incorporation of animals, both heifers and bulls [9, 12, 29], that latter copulate with many females, causing rapid viral dissemination, when natural mountaineering is used, although through artificial insemination, transmission could also occur, because semen has been shown to be an important route of excretion of BHV-1 [9].

Among the risk factors studied, age and sex were variables that increased the relative risk of finding animals infected with BHV-1. In the case of age, it has been reported by different authors that the adults' animals have highest risk of being infected [24, 29]. Antibodies against the virus in calves under six mos old of age, probably correspond to passive maternal immunity from colostrum, which is lost around the yr of life, starting the increase in serological profile against BHV-1 in the second yr., due to active infection [29]. Likewise, lasting viral exposure by adult animals increases seroprevalence [1], without ruling out the additive effect of reinfections and the fact that adults are in reproductive age, increasing the risk of acquire the infection through natural mounts or artificial insemination [9].

Sex increased the relative risk of acquiring the infection, with females more likely than males, as was shown by Saravanajayam *et al*. [24]. These results suggest that females are mostly exposed to the virus, since under traditional conditions of bovine exploitation, there is a male: female ratio of 1:25, which suggests greater contact between an infected bull with several cows, relative to a cow infected with the rest of its herd. The presence of ocular or vulvovaginal lesions was not related to seroprevalence, which may be due to the fact that the circulating virus is of low virulence [3].

CONCLUSION

The unvaccinated bovine herds of tropical wet weather of Ecuador, have high seroprevalence against BHV-1, with females over two years old of age with highest risk of presenting serum antibodies. Vulvovaginal or ocular lesions, breed and PU, were not determinants when assessing the risk factors on the seroprevalence of BHV-1 infection.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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