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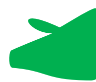

Production of serum antibodies in response to vaccination against infectious bovine rhinotracheitis and bovine viral diarrhea viruses with a commercial vaccine

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Abstract:

The objective was to determine the prevalence of serum antibodies (PSA) in response to vaccination against infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) viruses in dairy cows under subtropical conditions. A commercial polyvalent vaccine with inactivated BVD virus and modified active viruses of IBR, parainfluenza 3 and bovine respiratory syndrome was used. Two groups were formed: vaccinated (VEG) and unvaccinated (UEG) experimental group, which were homogeneous in PSA against IBR and BVD before vaccination. VEG was immunized on d 0 and d 30 after the first

vaccination (booster vaccine). To detect antibodies, serum samples were collected 30 d after the first and second vaccinations. Serum antibodies against IBR and BVD were determined by the ELISA test. The average PSA against IBR and BVD before vaccination was 16 (18 % in VEG *vs* 14 % in UEG; $P>0.05$) and 8 % (10 % in VEG *vs* 6 % in UEG; $P>0.05$), respectively. The first and second vaccinations against IBR induced the formation of antibodies 30 d after their application; with the first vaccination, PSA in vaccinated cows was 36 percentage units higher ($P<0.05$) than in unvaccinated cows (58 *vs* 22 %) and with the booster vaccine, PSA in vaccinated cows was 66 percentage units higher ($P<0.05$) than in unvaccinated cows (94 *vs* 28 %). The commercial vaccine did not induce the production of antibodies against BVD with either immunization.

Keywords: Infectious bovine rhinotracheitis, Bovine viral diarrhea, Polyvalent vaccine, Modified active virus, Inactivated virus, Serum antibodies, Dairy cows.

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Introduction

Infectious bovine rhinotracheitis (IBR), also known as infectious pustular vulvovaginitis, is a disease that affects domestic cattle and wild ruminants and is caused by Bovine Herpesvirus Type 1 (BHV-1), a member of the genus *Varicellovirus* of the family Herpesviridae. This virus causes respiratory (virus subtype 1.1), genital (virus subtype 1.2) and neurological (virus subtype 1.3) infections; however, although several subtypes have been distinguished, there is only one antigenic type important in the reproduction of cattle, BHV-1[1]. The importance of detecting the BHV-1 type in cattle herds is that it causes a decrease in cow productivity, due to reproductive failures, and respiratory conditions in young calves[2]. IBR can go unnoticed when the animals do not show clinical signs, but the virus remains latent, lodged in target organs, so that if the infection is acquired via the genitals, it replicates in the vaginal or preputial mucosa and establishes itself in the sacral nodes. Stress due to calving, transport or handling induces the reactivation of the infection and animals, even without signs of the disease, eliminate the virus into the environment, acting as apparently healthy carriers, which constitute the main risk factor for the disease[1].

In Mexico, IBR has been identified in dairy herds of the highlands[3] and its relationship with reproductive problems has been documented[4-7]. In herds of the humid tropics, the

presence of IBR has been identified and its prevalence and incidence have been studied[8,9], but it has not been reported whether the production of antibodies in response to vaccination is appropriate, so it is convenient to perform this type of study in cattle of the tropics and subtropics, to control the transmission of the disease.

Additionally, bovine viral diarrhea (BVD) has also been identified as a disease that affects reproduction in cows, which show low fertility, estrus repetition, embryo resorption and abortions[6,10]. After birth, pneumonia, conjunctivitis and ulcers in the nose and mouth are frequent in calves[6,11,12], so vaccination has been used to control BVD in cattle herds[13], but one must be certain about the effectiveness of the vaccine by measuring antibodies generated in the herd, because the recommended type of vaccine has been that of inactivated virus, for not causing abortion in pregnant cows or heifers and not generating vaccine infection in animals[14]. In herds of the humid tropics, the presence of BVD has been identified, studying the prevalence and incidence of this disease[9,15]; however, the production of antibodies in response to vaccination, to ensure immune protection and establish a control mechanism, has not been determined.

The objective was to determine the prevalence of serum antibodies in response to subcutaneous vaccination against infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) viruses in dairy cows under subtropical conditions in Mexico.

Material and methods

Study location

The study was carried out in a dairy herd of the Las Margaritas Research Station, belonging to the National Institute for Forestry, Agricultural and Livestock Research (INIFAP, for its acronym in Spanish), located in the eastern region of the state of Puebla at 20° 00' 07.86" N and 97° 18' 19.08" W, at 545 m asl, with an average annual temperature of 21°C and annual rainfall of 2,500 mm.

Experimental groups

One hundred female bovines of the Brown Swiss and Holstein breeds under rotational grazing of African Star grass (*Cynodon plectostachyus*), which had never been vaccinated

against IBR and BVD, were used. The females were divided into two experimental groups with 50 individuals each. One group was applied a commercial vaccine; the other group consisted of unvaccinated control females. The conformation of the two experimental groups was similar, so that the vaccinated group had 33 lactating cows (7 pregnant and 26 open), 10 pregnant dry cows and 7 heifers, while the unvaccinated group consisted of 34 lactating cows (7 pregnant and 27 open), 9 pregnant dry cows and 7 heifers. In addition, to homogenize the two groups in terms of prevalence of serum antibodies, they underwent a first serological diagnosis of antibodies against IBR and BVD viruses before vaccination. Care was taken that the body condition (1=emaciated; 5=obese) did not fall below 2.5 units.

Vaccination protocol

The vaccinated experimental group was immunized for the first time on day 0; subsequently, it was immunized on d 30 with a booster vaccine, subcutaneously applying 2 ml of a commercial polyvalent vaccine at each immunization. However, the vaccination protocol recommended by the laboratory consists of two subcutaneous applications of 2 ml each with an interval of 21 d. The vaccine used in the two immunizations was from the same batch. This consisted of two independent fractions; a lyophilized preparation of chemically modified active strains of the viruses of IBR, parainfluenza 3 and bovine respiratory syncytial syndrome, and a liquid preparation (diluent) of inactivated BVD virus types 1 and 2 (cytopathic and noncytopathic strains). The viral antigens were propagated in a cell line established by the laboratory. In addition, the vaccine contained a combination of adjuvants, not described by the manufacturer, including Amphigen as an immune response enhancer. Since its acquisition, the vaccine was kept at 4 °C until the time of its application. During vaccination, it was kept in the shade in a cooler with abundant refrigerants (5 to 7 °C), according to the manufacturer's specifications. During the development of the experiment, no animal showed clinical signs attributable to diseases related to the vaccine or any other disease.

Post-vaccination blood sampling and serum collection

Thirty (30) days after each immunization, blood samples were taken from the two experimental groups to determine antibody production in response to the first vaccination and booster vaccine. Blood samples were collected in 6 ml vacuum tubes that contained clot-separating gel. The samples were centrifuged at 4,000 rpm for 10 min to obtain blood

serum. The collected sera were deposited in 6 ml polypropylene vials and then frozen at -20 °C until laboratory analysis.

Laboratory analysis

The serological diagnosis for the detection of antibodies against IBR and BVD viruses was carried out with the CIVTEST BOVIS IBR and CIVTEST BOVIS BVD/BD P80 kits (Laboratorios Hipra, S.A., Mexico), based on the ELISA test, whose sensitivity and specificity is 96.3 and 99.5 %, respectively. In both serological diagnoses, the reading was performed at an optical density of 450 nanometers, in a BioTek ELx800 spectrophotometer (BioTek Instruments, Inc., USA).

Response variables and statistical analyses

For each disease, three response variables were analyzed: 1) Prevalence of serum antibodies before vaccination (day 0); 2) Prevalence of serum antibodies 30 d after the first vaccination (d 30), which was considered as the production of antibodies in response to the first vaccination; and 3) Prevalence of serum antibodies 30 d after the second vaccination (d 60), which was considered as the production of antibodies in response to the booster vaccine. Response variables were treated as binary variables, so antibody prevalence was recorded as 1 when a female had serum antibodies on day 0 before vaccination, 30 d after the first vaccination, or 30 d after the booster vaccine; otherwise, the prevalence of serum antibodies was recorded as 0. The information was analyzed with the GENMOD procedure (PROC GENMOD) of the SAS program, fitting a logistic regression model that included the fixed effect of vaccination or treatment (vaccinated experimental group, unvaccinated experimental group), in a binomial distribution and applying a logit link function. The convergence criterion was 10^{-8} in the six statistical analyses. In preliminary analyses, it was determined that the status of the female (in lactation, dry, heifer) did not affect any of the response variables analyzed ($P>0.05$), so it was not included in the definitive model.

Results

The statistical significance of the treatment effect, by response variable and disease, is shown in Table 1. Prior to vaccination, the prevalence of serum antibodies against the IBR

virus, as well as against the BVD virus, was similar (P>0.05) in the vaccinated and unvaccinated experimental groups, so the two groups were homogeneous in antibodies against IBR and BVD viruses prior to vaccination. Treatment affected (P<0.001) the production of antibodies against the IBR virus at the first and second (booster vaccine) vaccinations; however, contrary to what was expected, it did not affect (P>0.05) the formation of antibodies against the BVD virus at either immunization.

Table 1: Statistical significance of treatment effect, by response variable and disease

	Response variable (prevalence of antibodies)		
Disease	**Before vaccination**	**At the first vaccination**	**At the booster vaccine**
IBR	0.5850	0.0002	<0.0001
BVD	0.4588	0.1769	0.1042

IBR= infectious bovine rhinotracheitis; BVD= bovine viral diarrhea.

Table 2 presents the prevalences of serum antibodies against the IBR virus and their standard errors and 95 % confidence intervals, before vaccination, by experimental group. The prevalences were 18 and 14 % for the vaccinated and unvaccinated experimental groups, respectively; the average prevalence of the two groups was 16 %.

Table 2: Prevalences (%) of serum antibodies against infectious bovine rhinotracheitis virus and their standard errors and 95% confidence intervals, before vaccination, by experimental group

Experimental group	**Number of animals**	**Positive animals**	**Prevalence of antibodies**	**Confidence interval**
Vaccinated	50	9	18.0 ± 5.4 [a]	9.1 - 31.9
Unvaccinated	50	7	14.0 ± 4.9 [a]	6.3 - 27.4
Total	100	16	16.0 ± 5.5	9.7 - 25.0

[a] Prevalences with the same literal are not different (P>0.05).

The prevalences of serum antibodies against the BVD virus and their standard errors and 95% confidence intervals, before vaccination, by experimental group, are shown in Table 3. The prevalences for the vaccinated and unvaccinated experimental groups were 10 and 6 %, respectively; the average prevalence of the two groups was 8 %.

Table 3: Prevalences (%) of serum antibodies against bovine viral diarrhea virus and their standard errors and 95% confidence intervals, before vaccination, by experimental group

Experimental group	Number of animals	Positive animals	Prevalence of antibodies	Confidence interval
Vaccinated	50	5	10.0 ± 4.2 [a]	4.2 - 21.9
Unvaccinated	50	3	6.0 ± 3.4 [a]	1.9 - 17.0
Total	100	8	8.0 ± 3.8	3.0 - 19.4

[a] Prevalences with the same literal are not different (P>0.05).

Table 4 shows the prevalences of serum antibodies against the IBR virus at the first and second vaccinations. The first and second vaccinations against the IBR virus induced the production of antibodies 30 d after their application; with the first vaccination, the prevalence of serum antibodies in vaccinated cows was 36 percentage units higher (P<0.05) than in unvaccinated cows (58 *vs* 22 %); with the booster vaccine, the prevalence of serum antibodies in vaccinated cows was 66 percentage units higher (P<0.05) than in unvaccinated cows (94 *vs* 28 %).

Table 4: Prevalences (%) of serum antibodies against infectious bovine rhinotracheitis virus and their standard errors and 95% confidence intervals, by experimental group

Experimental group	No. of animals	First immunization			Booster immunization		
		Positive	Prevalence	CI	Positive	Prevalence	CI
Vaccinated	50	29	58.0 ± 7.0 [a]	44.1 - 70.8	47	94.0 ± 3.4 [a]	83.0 - 98.1
Unvaccinated	50	11	22.0 ± 5.9 [b]	12.6 - 35.5	14	28.0 ± 6.4 [b]	17.3 - 41.9

CI= confidence interval.

[ab] Prevalences with different literals are different (P<0.05).

The prevalences of serum antibodies against the BVD virus at the first and second vaccinations are shown in Table 5. Antibody production in vaccinated females was not satisfactory with either immunization; with the initial vaccine, the prevalences of serum antibodies for the vaccinated and unvaccinated experimental groups were 14 and 6 %, respectively; with the booster vaccine, they were 16 and 6 %, respectively.

Table 5: Prevalences (%) of serum antibodies against bovine viral diarrhea virus and their standard errors and 95% confidence intervals, by experimental group

Experimental group	No. of animals	First immunization			Booster immunization		
		Positive	Prevalence	CI	Positive	Prevalence	CI
Vaccinated	50	7	14.0 ± 4.9 [a]	6.8 - 26.6	8	16.0 ± 5.2 [a]	8.2 - 28.9
Unvaccinated	50	3	6.0 ± 3.4 [a]	1.9 - 17.0	3	6.0 ± 3.4 [a]	1.9 - 17.0

[a] Prevalences with the same literal are not different ($P>0.05$).

Discussion

Infectious bovine rhinotracheitis

In the present study, the average prevalence of serum antibodies against the IBR virus was 16.0 %, which is considerable for a herd with no history of vaccination; therefore, the animals should be included in a vaccination program to protect them from the disease and avoid reproductive problems. The prevalence of serum antibodies against the IBR virus observed in this study was lower than those observed in grazing cattle in the state of Veracruz, with values of 58.6[16] and 76.3 %[17]; however, it is higher than that observed (5.3 %) in Zebu, Brown Swiss and Holstein cattle in Tizimín, Yucatán[18]. These prevalences identified in cattle kept in a tropical climate, as well as those observed in the Mexican highlands, particularly that of the state of Hidalgo, which was 35.2 %[19], highlight the circulation of the virus in the herd with the risk of the animals getting sick and the need for its control in Mexico. In a literature review that summarized information from Mexican studies published from 1975 to 2016, a prevalence of antibodies against the IBR virus of 56.4 % was estimated[20].

Outside Mexico, in dairy herds of Toca-Boyacá and Caquetá, Colombia, prevalences of 35.7[21] and 90.0 %[22], respectively, were found; in Valle de Cauca, in beef cattle, a prevalence of 69.8 %[23] was found. In Peru and Chile, prevalences of 29.0[24], 67.6[25], 36.0[26] and 76.0 %[27] have been found. Due to its detection in these and many other countries, the IBR virus (BHV-1) is considered one of the most widely distributed pathogens in the world[28]. Nevertheless, despite the fact that there are multiple biological products for the immunization of animals[29], it is thought to be one of the largest generators of economic losses in livestock production, both beef and milk. Asymptomatic cattle are

the most important reservoir because they can excrete the virus intermittently and transmit it to healthy cattle[30].

With the first vaccination, the prevalence of antibodies in vaccinated cows (58 %) was higher ($P<0.05$) than the prevalence of antibodies observed in unvaccinated cows (22 %). This allows to interpret that there was production of antibodies in response to the first vaccination against the IBR virus, but relatively mild. However, with the booster vaccine, the prevalence of antibodies in vaccinated cows increased considerably up to 94 %, a prevalence that was much higher ($P<0.05$) than that found in unvaccinated cows (28 %), so the substantial increase in antibodies reinforces the interpretation of a favorable antibody production with the second immunization; therefore, it can be inferred that the modified active virus of the commercial vaccine did produce immune protection against the IBR virus. With this vaccination protocol (initial vaccine + booster vaccine), it is considered feasible to protect cattle against the IBR virus, particularly females, which are the ones that, due to the disease, suffer from genital tract infections, such as infectious pustular vulvovaginitis, metritis[8], mastitis, abortions, repetition of services, fetal infection and anestrus[31,32].

The magnitude of antibody production observed in the present study was not achieved in a study where an intranasal vaccine of attenuated viruses of IBR and PI3 (TSV-2) was used in a single dose, which was poorly immunogenic, since only 33.3 % of the animals produced antibodies 28 d after vaccination[33]; it is likely that, with the intranasal route, a second or more immunizations will be required to achieve a better immune response. In another study where a vaccine with modified active virus was used, the percentage of abortions in vaccinated females was 5 % and in unvaccinated 73 %[34], so it can be inferred that the vaccine substantially prevented abortion. Something similar happened in the present study, since no abortion was observed; nevertheless, in the cited study[34], it was not determined if the low percentage of abortions (5 %) was due to the effect of vaccination, so it could be due to other factors. Consequently, it seems better to use a vaccine with modified active virus to protect against the IBR virus, especially in females of reproductive age, since when the virus replicates within the host cells, protective immunity increases[35].

In Argentina, a study was carried out where two types of intradermal vaccines made with inactivated BHV-1 that contained the sequence of the secreted version of glycoprotein D were used, one made with adjuvant and the other not, which were applied with a booster at 20 and 33 d, assuming that both increased the humoral immune response; however, only the one that had adjuvant improved the cellular immune response[36].

Bovine viral diarrhea

The average prevalence of serum antibodies against the BVD virus obtained in the present study (8 %) was lower than those reported (69.0 and 60.3 %) by other authors[37,38] for grazing cattle in the state of Veracruz. In studies conducted in Mexico with dairy cows in the states of Hidalgo and Aguascalientes, prevalences of 32.8[7] and 48.6 %[19] were found. In a literature review that summarized information from Mexican studies published from 1975 to 2016, a prevalence of antibodies against the BVD virus of 59.3 % was estimated[39].

Contrary to what was expected, in the present study it was evident that vaccinated females did not satisfactorily produce antibodies against the inactivated BVD virus, since a prevalence of serum antibodies of not less than 94 % was expected as a humoral response, as in immunization against the IBR virus, so with the commercial vaccine evaluated, it is not possible to ensure immunological protection in cattle, particularly in cows, which are the ones that, due to the disease, suffer from infections that affect reproduction[6,10], and replacement heifers, which can become infected from birth, suffering from pneumonia, conjunctivitis and ulcers in the nose and mouth[6,11,12], they can even die due to the infection, which very often goes undetected and undiagnosed. Therefore, although in recent years vaccines with inactivated virus have been improved by adding potent adjuvants, the low production of antibodies in this study in response to vaccination with inactivated virus makes it necessary to try other control options, as it has been suggested that a good strategy to overcome weak production of antibodies in response to vaccination with inactivated virus is the alternation of repeated immunizations with inactivated virus vaccines and modified active virus vaccines, or vice versa[40], as demonstrated in an experiment where a vaccination protocol for heifers was used, which consisted of initially immunizing with inactivated virus, four weeks later with modified active virus, and subsequently revaccinated annually with inactivated virus, improving the immune response considerably[41].

On the other hand, it has been reported that vaccination against BVD as the only control measure is not sufficient to prevent the circulation of field virus in cattle[42,43,44], since the elimination of persistently infected (PI) animals should be included as an important action and effective vaccination strategies should be used to reduce this type of animals, and thus control BVD more efficiently[45] after a program to detect PI animals from birth[46], as these animals are immunotolerant to homologous non-cytopathogenic viruses[47]. Therefore, the failure to observe adequate antibody production in vaccinated animals could be due to the fact that the experimental herd used had a significant proportion of PI animals, which does not develop antibodies, since the immune system does not consider the virus as a foreign agent to the organism[47]. Another reason could be that the inactivated virus vaccine

induced short-lived antibody production, as it does not promote immunological memory compared to active virus vaccines[48]; consequently, it is very likely that antibodies induced by the inactivated BVD virus vaccine were at undetectable levels at the time the ELISA test was performed, considering that, in a study in which two groups of animals were immunized with two inactivated virus vaccines, a prevalence of serum antibodies of 0 and 12.5 % was obtained after using an ELISA kit to detect antibodies against the p80 protein of the virus (as in the present study); conversely, when an ELISA kit for antibodies against the whole virus was used, antibodies were detected in 80 and 100 % of the animals[49]. However, the authors of the study[49] commented that there is a discrepancy in the results obtained, since there are previous studies in which the detection of antibodies was achieved with this ELISA kit, arguing that the p80 protein is mostly expressed during viral replication, but replication does not occur if inactivated virus vaccine is applied. Therefore, if the ELISA test adequately detects specific antibodies against the p80 protein of the BVD virus, and knowing that the highest proportion of antibodies present in the serum are of the IgG class, which are the ones that increase significantly after a natural infection or vaccination, regardless of whether it is a vaccine of active or inactivated virus, the ELISA test used in the present study is considered to be effective in detecting antibodies against the BVD virus induced by the inactivated virus vaccine.

Additionally, it has been mentioned that the safety and efficacy of inactivated vaccines may be attributable to several factors, among which are the type of strain, the inactivation technique, the viral titer and the adjuvant used[50], so perhaps some of these factors may also have influenced the observed antibody production. Finally, in this work it was expected that immunization would induce the formation of antibodies in animals to establish a “herd immunity”, which would prevent the virus from circulating in animals and, thus, prevent the manifestation of clinical signs of the diseases, since the proportion of infected animals was known before applying the treatments.

Conclusions and implications

The commercial polyvalent vaccine induced the production of satisfactory levels of antibodies against the IBR virus; nevertheless, it was necessary to apply a second booster immunization to increase the percentage of animals with serum antibodies (more than 90 %). On the contrary, this vaccine did not induce an adequate production of serum antibodies against the BVD virus, so it is of utmost importance to find out if there are PI animals within the herd studied. It is possible that by applying the vaccine with inactivated virus, with modified active virus boosters, or vice versa, the antibody production in response to the vaccination against the BVD virus is improved.

Literature cited:

1. OIE. Office International des Epizooties. Rinotraqueitis bovina infecciosa/vulvovaginitis pustular infecciosa (2.3.5.). Manual de la OIE sobre animales terrestres. 2004:514-525.

2. Gu X, Kirkland PD. Infectious bovine rhinotracheitis. Australian and New Zealand Standard Diagnostic Procedure. 2008: 1-18. https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/animal/ahl/ANZSDP-Infectious-bovine-rhinotracheitis-IBR.pdf. Consultado 3 Abr, 2020.

3. Reyes JM, Vázquez R, García JA. Seroprevalencia de IBR y DVB en hatos muestreados en México. 2002-2003. En: Posadas ME editores. XXVIII Congreso Nacional de Buiatría. Morelia, Michoacán, México. 2004:112.

4. Armas CA, Muñoz MCCR, Bolaños LDJ, Iñiguez MG. Determinación de agentes infecciosos causantes de abortos fetales en un hato lechero en Tizayuca, Hidalgo, México. En: Posadas ME editores. XXVIII Congreso Nacional de Buiatría. Morelia, Michoacán, México. 2004:135.

5. Escamilla HP, Morales SE, Martínez JJM, Medina CM. Frecuencia y causas de aborto de origen infeccioso en hatos de bovinos en el estado de Querétaro. En: Posadas ME editores. XXVIII Congreso Nacional de Buiatría. Morelia, Michoacán, México. 2004:111.

6. Waldner CL. Serological status for *N. caninum*, bovine viral diarrhea virus and infectious bovine rhinotracheitis virus at pregnancy testing and reproductive performance in beef herds. Anim Reprod Sci 2005;90(3-4):219-242.

7. Meléndez SRM, Valdivia FAG, Rangel MEJ, Díaz AE, Segura-Correa JC, Guerrero BAL. Factores de riesgo asociados a la presencia de aborto y desempeño reproductivo en ganado lechero de Aguascalientes, México. Rev Mex Cienc Pecu 2010;1(4):391-401.

8. Betancur HC, Gonzales TM, Reza GL. Seroepidemiología de la rinotraqueitis infecciosa bovina en el municipio de Montería, Colombia. Rev MVZ Córdoba 2006;11(2):830-836.

9. Zárate MJP, Rosete FJV, Ríos UA, Barradas PFT, López ER, Olazarán JS, *et al*. Estado reproductivo y prevalencia de IBR y DVB en hembras bovinas en tres épocas del año en la zona centro de Veracruz. En: Posadas ME editores. XXXVIII Congreso Nacional de Buiatría. Acapulco, Guerrero, México. 2013;759-766.

10. Lértora WJ. Diarrea viral bovina: Actualización. Rev Vet FCV UNNE 2003;14(1):1-11.

11. Liebler-Tenorio EM. Pathogenesis. In: Goyal SM, Ridpath JF. editors. Bovine viral diarrhea virus: diagnosis, management and control. Ames, Iowa, USA: Blackwell Publishing Professional; 2005:121-143.

12. Rubio GJL, Betancourt MA, Karg G. Síndrome respiratorio y digestivo en terneras trasladadas a la recría. REDVET 2009;10(8):1-7.

13. Vargas DS, Jaime J, Vera VJ. Perspectivas para el control del virus de la diarrea viral bovina (BVDV). Rev Colomb Cienc Pecu 2009;22:677-688.

14. Gasque GR. Diarrea viral bovina. En: Gasque GR editor. Enciclopedia Bovina. 1ª edición. Distrito Federal, México; 2008:126-127.

15. Rosete FJV, Granados ZL, Zárate MJP, Ríos UA, Banda RVM, Socci EGA, *et al*. Prevalencia e incidencia a diarrea viral bovina en vacas de doble propósito en pastoreo en trópico húmedo. VII Reunión Científica-Tecnológica Forestal y Agropecuaria Tabasco 2015 y IV Simposio Internacional en Producción Agroalimentaria Tropical. Tabasco, México. 2015:246-251.

16. De La Trinidad SH. Seroprevalencia y factores de riesgo de rinotraqueitis infecciosa bovina en ranchos ganaderos de Las Choapas, Minatitlán y Moloacán ubicados en la zona sur del estado de Veracruz, México [tesis de licenciatura]. Veracruz, Veracruz: Universidad Veracruzana; 2010.

17. Abad-Zavaleta J, Ríos-Utrera A, Rosete-Fernández JV, García-Camacho A, Zárate-Martínez JP. Prevalencia de rinotraqueitis infecciosa bovina y diarrea viral bovina en hembras en tres épocas de año en la zona centro de Veracruz. Nova Scientia 2016;8(1):213-227.

18. Calderón VG, Alvarado IA, Vilchis MC, Aguilar SA, Batalla CD. Detección de seropositividad al virus de rinotraqueitis infecciosa bovina (IBR), en ganado del municipio de Tizimín, Yucatán, México. Téc Pecu Méx 1997;35(3):161-164.

19. Sánchez-Castilleja YM, Rodríguez DJG, Pedroso M, Cuello S. Simultaneidad serológica de *Neospora caninum* con *Brucella abortus* y los virus de la rinotraqueitis infecciosa bovina y diarrea viral bovina en bovinos pertenecientes al estado de Hidalgo, México. Rev Salud Anim 2012;34(2):95-100.

20. Ríos-Utrera Á, Rosete-Fernández JV, Zárate-Martínez JP, Fragoso-Islas A, Olazarán-Jenkins S, Granados-Zurita L, *et al*. Rinotraqueítis infecciosa bovina: determinación de la prevalencia de anticuerpos en vacas mexicanas no vacunadas de los estados de Tabasco, Puebla y Veracruz. Rev Científ, FCV-LUZ 2018;28(5):349-359.

21. Ochoa X, Orbegozo M, Manrique AF, Pulido PM, Ospina J. Seroprevalencia de rinotraqueitis infecciosa bovina en hatos lecheros de Toca-Boyacá. Rev MVZ Córdoba 2012;17(2):2974-2982.

22. Motta GJL, Waltero GI, Abeledo MA. Prevalencia de anticuerpos al virus de la diarrea viral bovina, herpesvirus bovino 1 y herpes virus bovino 4 en bovinos y búfalos en el departamento de Caquetá, Colombia. Rev Salud Anim 2013;35(3):174-181.

23. Ruiz-Saenz J, Jaime J, Vera VJ. Prevalencia serológica y aislamiento del herpesvirus bovino 1 (BHV-1) en hatos ganaderos de Antioquia y del Valle de Cauca. Rev Colomb Cienc Pecu 2010;23:299-307.

24. Pariente AE, Ccama SA, Rivera GH. Anticuerpos contra el virus causante de la rinotraqueitis infecciosa en vacunos de la provincia del Melgar, Puno. Rev Inv Vet Perú 2006;17(2):137-143.

25. Zacarías RE, Benito ZA, Rivera GH. Seroprevalencia del virus de la rinotraqueitis infecciosa en bovinos criollos de Parinacochas, Ayacucho. Rev Inv Vet Perú 2002;13(2):61-65.

26. Sánchez TG, Benito ZA, Rivera GH. Seroprevalencia del virus de la rinotraqueitis infecciosa bovina en ganado lechero del Valle de Lima. Rev Inv Vet Perú 2003;14(1):54-60.

27. Felmer A, Zúñiga AJ, López HM. Prevalencia y distribución espacial de brucelosis, leucosis bovina, diarrea viral bovina y rinotraqueitis infecciosa bovina a partir del análisis ELISA de estanques prediales en lecherías de la IX Región, Chile. Arch Med Vet 2009;41:17-26.

28. OIE. Office International des Epizooties. Rinotraqueitis bovina infecciosa/vulvovaginitis pustular infecciosa. (2.4.13). Manual de la OIE sobre animales terrestres. 2008:1-17.

29. Ruiz-Saenz J, Jaime J, Vera VJ. Vacunas contra el herpesvirus bovino-1: una mirada desde el pasado hacia el futuro de la inmunización. Acta Biol Col 2009;14(2):3-20.

30. Pérez S, Inman M, Doster A, Jones C. Latency-related gene encoded by bovine herpesvirus 1 promotes virus growth and reactivation from latency in tonsils of infected calves. J Clinical Microbiol 2005;43:393-401.

31. Elhassan AM, Fadol MA, El-Hussein AM. Seroprevalence of bovine herpes virus-1, bovine herpes virus-4 and bovine viral diarrhea virus in dairy cattle in Sudan. Pak Vet J 2011;31(4):317-320.

32. Gür S, Doğan N. The possible role of bovine herpes virus type-4 infection in cow infertility. Anim Sci J 2010;81(3):304-308.

33. Vilchis MC, Sosa RM, Alvarado IA, Aguilar SA, Hernández VR, Batalla CD. Evaluación de la vacuna tsv-2 de ibr-p13 en bovinos nacionales productores de leche. Téc Pecu Méx 1991;29:19-23.

34. Walz PH, Montgomery T, Passler T, Riddell KP, Braden TD, Zhang Y, *et al*. Comparison of reproductive performance of primiparous dairy cattle following revaccination with either modified live or killed multivalent viral vaccines in early lactation. J Dairy Sci 2015;98:8753-8763.

35. OIE. Office International des Epizooties. Rinotraqueitis infecciosa bovina y diarrea viral bovina: repercusiones en la salud animal y el comercio internacional. Animal Diseases Research Institute. 1998:147-156.

36. Quattrocchi V, Soria I, Langellotti CA, Gnazzo V, Gammella M, Moore PD, *et al*. A DNA vaccine formulated with chemical adjuvant provides partial protection against bovine herpes virus infection in cattle. Front Immunol 2017;8(37):1-11.

37. Romero SD, Montiel PT, Aguilar DM, Martínez HDI, García VZS. Prevalencia de diarrea viral bovina en el estado de Veracruz, México. En: Barradas LH, Ceja RI, Vázquez CLM editores. XXII Reunión Científica-Tecnológica Forestal y Agropecuaria. Veracruz, México. 2009:660-667.

38. Milián-Suazo F, Hernández-Ortíz R, Hernández-Andrade L, Alvarado-Islas A, Díaz-Aparicio E, Mejía-Estrada F, *et al*. Seroprevalence and risk factors for reproductive diseases in dairy cattle in Mexico. J Vet Med Anim Health 2016;8(8):89-98.

39. Rosete FJV, Ríos UÁ, Zárate MJP, Olazarán JS, Granados ZL, Fragoso IA, *et al*. Prevalencia de anticuerpos contra diarrea viral bovina en vacas no vacunadas en los estados de Puebla, Tabasco y Veracruz, México. Rev Mex Cienc Pecu 2018;9(3):555-566.

40. Moennig V, Eicken K, Flebbe U, Frey HR, Grummer B, Haas L, *et al*. Implementation of two-step vaccination in the control of bovine viral diarrhoea (BVD). Prev Vet Med 2005;72:109-114.

41. Frey HR, Eicken K, Grummer B, Kenklies S, Oguzoglu TC, Moennig V. Foetal protection against bovine virus diarrhoea virus after two-step vaccination. J Vet Med 2002;49:489-493.

42. Lindberg A, Houe H. Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. Prev Vet Med 2005;72:55-73.

43. Houe H, Lindberg A, Moennig V. Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. J Vet Diag Inv 2006;18:427-436.

44. Wernike K, Gethmann J, Schirrmeier H, Schroder R, Conraths FJ, Beer M. Six years (2011-2016) of mandatory nationwide bovine viral diarrhea control in Germany - A success story. Pathogens 2017;6(50):1-8.

45. Abe Y, Tamura T, Torii S, Wakamori S, Nagai M, Mitsuhashi K, *et al*. Genetic and antigenic characterization of bovine viral diarrhea viruses isolated from cattle in Hokkaido, Japan. J Vet Med Sci 2016;78(1):61-70.

46. Barrett DJ, More SJ, Graham DA, O'Flaherty J, Doherty ML, Gunn HM. Considerations on BVD eradication for the Irish livestock industry. Irish Vet J 2011;64:12.

47. Khodakaram-Tafti A, Farjanikish GH. Persistent bovine viral diarrhea virus (BVDV) infection in cattle herds. Iran J Vet Res 2017;18(3):154-163.

48. Fulton RW, Confer AW, Burge LJ, Perino LJ, d'Offay JM, Payton ME, *et al*. Antibody responses by cattle after vaccination with commercial viral vaccines containing bovine herpesvirus-1, bovine viral diarrhea virus, parainfluenza-3 virus, and bovine respiratory syncytial virus immunogens and subsequent revaccination at day 140. Vaccine 1995;13(8):725-733.

49. González AM, Arnaiz I, Yus E, Eiras C, Sanjuán M, Diéguez FJ. Evaluation of long-term antibody responses to two inactivated bovine viral diarrhoea virus (BVDV) vaccines. Vet J 2014;199:424-428.

50. Walz PH, Riddell KP, Newcomer BW, Neill JD, Falkenberg SM, Cortese VS, *et al*. Comparison of reproductive protection against bovine viral diarrhea virus provided by multivalent viral vaccines containing inactivated fractions of bovine viral diarrhea virus 1 and 2. Vaccine 2018;36:3853-3860.