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

Jorge Víctor Rosete Fernández a
Guadalupe Asunción Socci Escatell b
Abraham Fragoso Islas a
Sara Olazarán Jenkins a
Ángel Ríos Utrera c*

a Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Sitio Experimental Las Margaritas. Km. 9.5 carretera Hueytamalco-Tenampulco, Hueytamalco, Puebla, México.

b INIFAP. CENID Salud Animal e Inocuidad. Ciudad de México, México

c Universidad Veracruzana. Facultad de Medicina Veterinaria y Zootecnia. Veracruz, Veracruz, México.

*Corresponding author: ariosu@hotmail.com

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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Before vaccination</th>
<th>At the first vaccination</th>
<th>At the booster vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBR</td>
<td>0.5850</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BVD</td>
<td>0.4588</td>
<td>0.1769</td>
<td>0.1042</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of animals</th>
<th>Positive animals</th>
<th>Prevalence of antibodies</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>50</td>
<td>9</td>
<td>18.0 ± 5.4 (^a)</td>
<td>9.1 - 31.9</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>50</td>
<td>7</td>
<td>14.0 ± 4.9 (^a)</td>
<td>6.3 - 27.4</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>16</td>
<td>16.0 ± 5.5</td>
<td>9.7 - 25.0</td>
</tr>
</tbody>
</table>

\(^a\) 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

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of animals</th>
<th>Positive animals</th>
<th>Prevalence of antibodies</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>50</td>
<td>5</td>
<td>10.0 ± 4.2 a</td>
<td>4.2 - 21.9</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>50</td>
<td>3</td>
<td>6.0 ± 3.4 a</td>
<td>1.9 - 17.0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>8</td>
<td>8.0 ± 3.8</td>
<td>3.0 - 19.4</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of animals</th>
<th>First immunization</th>
<th>Booster immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Prevalence</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>50</td>
<td>29</td>
<td>58.0 ± 7.0 a</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>50</td>
<td>11</td>
<td>22.0 ± 5.9 b</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>No. of animals</th>
<th>First immunization</th>
<th>Booster immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Prevalence CI</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>50</td>
<td>7</td>
<td>14.0 ± 4.9 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.8 -</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>50</td>
<td>3</td>
<td>6.0 ± 3.4 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.9 -</td>
</tr>
</tbody>
</table>

* 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:


