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## SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH BOVINE BRUCELLOSIS AND ABORTION IN HOLSTEIN HEIFERS

Jesús Mellado<sup>1</sup>, Narciso Y. Ávila-Serrano<sup>2</sup>, Ángeles de Santiago<sup>3</sup>, Ulises Macías-Cruz<sup>4</sup>,  
Leonel Avendaño-Reyes<sup>4</sup>, Perpetuo Álvarez<sup>5</sup>, and Miguel Mellado<sup>1\*</sup>

<sup>1</sup> *Department of Animal Nutrition, Autonomous Agrarian University Antonio Narro,  
25315 Saltillo, Mexico*

<sup>2</sup> *Department of Animal Science, Sea University,  
71980 Puerto Escondido, Mexico*

<sup>3</sup> *Department of Veterinary Medicine, Autonomous Agrarian University Antonio Narro,  
27054 Torreon, Mexico*

<sup>4</sup> *Institute of Agriculture Science, Autonomous University of Baja California,  
21705 Mexicali, Mexico*

<sup>5</sup> *Department of Renewable Natural Resources, Autonomous Agrarian University Antonio Narro,  
25315 Saltillo, Mexico*

*Email: jmelladobosque@hotmail.com, valear68@yahoo.com.mx,  
angelesdesantiago867@gmail.com, umacias@uabc.edu.mx, lar62@uabc.edu.mx,  
perpetuo.alvarezv@uaaan.edu.mx, melladomiguel07@gmail.com\**

*ORCID: <http://orcid.org/0000-0003-2093-1729>,  
<http://orcid.org/0000-0003-4043-501X>,  
<https://orcid.org/0000-0002-5491-0662>,  
<https://orcid.org/0000-0002-6947-2247>,  
<https://orcid.org/0003-2666-3999>,  
<https://orcid.org/0000-0001-5477-5707>*

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**Aim:** To estimate the seroprevalence of bovine brucellosis (bBR) and identify risk factors associated with seropositivity to this disease. An additional aim was to evaluate heifer-related risk factors for abortion. **Methods:** A total of 3,848 Holstein heifers with a single lactation from a large commercial dairy herd in northern Mexico (25°N) participated in this study. Multiple regression models were employed to analyze risk factors for brucellosis seropositivity rate and to calculate odds ratios, quantifying the strength of the risks. **Results:** The seroprevalence of bBR was 30% (95% CI=29–32%) for lactating heifers (card test). Heifers weighing more than 680 kg at calving had half the risk of testing positive for bBR compared to those weighing less than 680 kg (22.7% vs. 36.1%). The risk of being seropositive for bBR increased in heifers with retained placenta compared to those without this reproductive disorder (OR = 2.46; 47.5% vs. 26.3%). Heifers vaccinated with two doses of a commercial brucellosis vaccine had 2.2 times higher odds of being seropositive for bBR compared with those vaccinated only once ( $P < 0.01$ ). Seropositive heifers for bBR were 3.1 times more likely to abort than seronegative heifers (38.5% vs. 16.2%;  $P < 0.01$ ). Heifers with a body condition score (BCS) of 3.5 or higher at calving were less likely to abort than heifers with BCS < 3.5. **Conclusions:** Brucellosis control programs should prioritize age at first calving, placenta retention, and the number of RB51 vaccinations administered to Holstein heifers.

**Keywords:** body condition score, diarrhea; metritis, pneumonia; RB51 vaccine, retained placenta.

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## INTRODUCTION

Bovine brucellosis (bBR) is a significant zoonosis with important implications for public health (Dadar et al., 2019; Mitiku and Desa, 2020) and substantial economic costs for milk producers in middle- to low-income countries (Deka et al., 2018). Economic losses arise from the direct reduction in milk production (Martínez-Guerrero et al., 2025) and from other costs associated with abortions, longer intercalving periods, repeat breeding, fewer animals in milk, and the culling of cows diagnosed with brucellosis. Additional financial harm results from restricted animal movement, which hinders livestock import and export and increases replacement costs, among other issues (Ibrahim et al., 2016; Deka et al., 2018; Bardhan et al., 2020). Other negative effects of brucellosis include stillbirths, abortions, weak calves at birth, infertility, epididymitis, and orchitis in bulls (Arif et al., 2016; Choudhary et al., 2019; Meng and Zhuqing, 2020), increased veterinary and medical expenses, and costs for disease control programs (Tamba et al., 2021).

Most cows are asymptomatic, carrying this hidden infection without showing clinical signs, thereby

facilitating the spread of the disease within herds (Franca et al., 2025). The seroprevalence of bBR in intensive dairy herds in Mexico ranges from 23% to 35% (Mellado et al., 2014), posing a risk to public health and creating economic challenges for intensive dairy farms. Despite extensive resources and decades of effort to control bBR in Mexican dairy enterprises, this chronic disease remains widespread (Luna-Martínez et al., 2002), with only 13% of the territory free of it (**Fig. 1**). Several factors associated with bBR seropositivity rate have been identified, generally categorized into management factors (Herrán Ramírez et al., 2020) and agroecological factors (Chiebao et al., 2015). Nonetheless, there is a lack of information on unknown risk factors for bBR occurrence and abortion in dairy heifers within intensive farming systems in hot environments. Given the negative health implications for cattle and humans, economic implications for the farmers and its widespread occurrence in cattle and wildlife populations in the study area, gaining a better understanding of bBR in Holstein heifers would improve knowledge about risk factors for both bBR and abortion in dairy



**Fig. 1.** Zoosanitary status for bovine brucellosis in Mexico. Control: serological testing, vaccination, and slaughter of infected animals. Eradication: total elimination of *Brucella abortus* in cattle, through the slaughter of infected animals, vaccination, and quarantine. Free: herds officially certified after demonstrating, through diagnostic tests, that cattle are free of *Brucella*. National Service for Agrifood Health, Safety and Quality (SENANSICA; <https://www.gob.mx/senasica/documentos/situacion-actual-del-control-de-la-brucelosis-en-mexico>)

cattle and support the development of local awareness programs and guideline practices for controlling bBR (Lindahl et al., 2019).

Therefore, the hypotheses were: (1) heifers with adequate body condition score (BCS) at mating, older age at first calving, not calving in summer, receiving a single vaccination against bBR, having higher growth rates, and not suffering from infectious diseases before or during calving would have a lower risk of seropositivity to bBR; (2) heifers that are seronegative to bBR, with adequate BCS at mating, older age at first calving, receiving a single vaccination against bBR, having higher growth rates, and not suffering from infectious diseases before or during calving would have a lower risk of abortion; (3) the seroprevalence of bBR in heifers would be higher than previously reported in the literature due to the high endemicity of this disease in the study area.

The objectives of this study were: (1) to assess the prevalence of bBR in lactating heifers in an area with a very high incidence of bBR, (2) to identify heifer-level risk factors associated with bBR seropositivity in Holstein heifers, (3) to determine the heifer-related risk factors for the occurrence of abortion. These findings can help in developing age-specific, risk-based bBR management strategies.

## MATERIAL AND METHODS

**Animals and herd management.** This study received approval from the Ethics Committee of the Research Department at the Autonomous Agrarian University Antonio Narro (protocol 42520-3001-2138). It was conducted on a commercial dairy farm with approximately 3,000 milking cows in a hot region of northern Mexico (25° N, 103° W; mean annual temperature 23.7°C). A total of 3,848 heifers, each with a single lactation, participated in this retrospective study from 2018 to 2021. The herd's average daily milk production was 33.8 kg.

Heifers were kept in open, dirt-floored pens with metal-frame shades in the center and along the feeding alleys. The negative effects of hot weather in this location were reduced by providing lactating heifers with fans and sprayed water. Lactating heifers were fed ad libitum total mixed rations (TMR) twice daily, and about 2.5% of feed refusals were removed before each feeding. Ration formulation followed the National Research Council (NRC, 2001) recommendations to support milk production of 36 kg/day for heifers consuming 25 kg of dry matter/day. The

heifers were milked daily at 0600, 1400, and 2100 hours. The TMR included corn silage, alfalfa hay, and a concentrate containing corn grain, cottonseed meal, soybean meal, and minerals.

Calves were promptly separated from their mothers after birth and placed in individual open-sided wooden pens (1.2 × 1.7 m) with dirt floors and partial roofs. A shade cloth about 3 meters above the pens protected calves from sunlight. Calves were fed 4 liters of high-quality pasteurized colostrum within the first hour after birth; afterward, they drank whole milk from open 5-liter plastic buckets until 60 days old.

**Health management and disease recording.** Heifers were vaccinated against infectious bovine rhinotracheitis, bovine respiratory syncytial virus, bovine viral diarrhoea types 1 and 2, and para-influenza 3 (CattleMaster Gold FP5<sup>®</sup>, Zoetis, Mexico D.F., Mexico). They were also vaccinated against leptospirosis (5 serovars; LEPTAVOID-H<sup>®</sup>; Merck Sharp and Dohme Corp., Mexico, D.F.). Additionally, heifers received a subcutaneous immunization with *Brucella abortus* (strain RB51; MSD Salud Animal Mexico, Mexico City, Mexico) vaccine at an average (±SD) age of 15.2 ± 2.3 months. Most heifers (88%) received a second *B. abortus* vaccine approximately 10.8 ± 3.1 months after the first dose. In all cases, the bBR test was performed approximately 4 months after the last vaccination, and all heifers were sampled at comparable physiological stages. Heifers were also vaccinated to prevent blackleg caused by *Clostridium chauvoei*, malignant edema caused by *C. septicum*, black disease caused by *C. novyi*, gas gangrene caused by *C. sordellii*, enteritis and enterotoxemia caused by *C. perfringens* Types B, C, and D, and disease caused by *Histophilus somni* (*Haemophilus somnus*) (Ultrabac 7 Somubac<sup>®</sup>, Zoetis, Guadalajara, Mexico). Finally, heifers received an anthrax vaccine (Bayovac Thraxol 2<sup>®</sup>, Bayer, CD Mexico, Mexico).

Blood samples (5 mL) from coccygeal vessels of each heifer were collected using vacuum tubes coated with a clot activator (BD Vacutainer, Franklin Lakes, NJ, USA) for serum separation. The sampling was conducted at 32 ± 6 months of age. Blood was allowed to clot, kept on ice, and transported to a qualified laboratory. The serum was tested using the brucellosis card test; visible agglutination was interpreted as positive. We acknowledge that the brucellosis card test has several limitations, such as lower specificity in endemic areas, inability to distinguish between active and past infection, potential for false results,

and limited effectiveness for chronic, deep-seated infections. Validating such diagnostic tests is important for assessing the disease's prevalence because no confirmatory test was performed; therefore, the true prevalence of brucellosis in these heifers was estimated using the following formula:

$$\text{True Prevalence} =$$

$$= \text{PO} + \text{Specificity} - 1 / \text{Sensitivity} + \text{Specificity} - 1,$$

where PO = prevalence observed by the test.

Values for sensitivity (91%) and specificity (99.6%) were derived from previous studies on dairy cows (Rahman et al., 2019).

Subclinical ketosis was identified using the Ketostix® (Bayern, CD. Mexico, Mexico); the urine strip test was directly moistened with urine obtained after spontaneous or stimulated micturition approximately 7 days postpartum. The result (traces or high concentrations of acetoacetate) was read after 5 to 10 seconds of contact and recorded. Additionally, mammary gland health was monitored regularly using the California Mastitis Test.

Identification of lame heifers was based on heifers displaying a noticeable limp. The full-time herd veterinarian recorded lameness data, and the appropriate protocol for this condition was followed. Retained fetal membranes were defined as the presence of placental tissue 24 hours after calving, and the attending veterinarian documented this event. The diagnosis of clinical metritis relied on the presence of watery, foul-smelling, reddish-brown vaginal discharge within 21 days in milk and on uterine examination (size and tone) by rectal palpation. All heifers diagnosed with metritis were treated with ceftiofur until they recovered. Heifers with diarrhea exhibited watery discharges that sifted through the ground, while those with pneumonia were identified by a rectal temperature exceeding 39.4°C, nasal or ocular discharge, coughing, and increased respiratory rate.

**Statistical analyses.** To analyze factors contributing to seropositivity to bBR (a binary outcome in which heifers are classified as either seronegative or seropositive during their first lactation), a comprehensive preliminary model with heifers as a random effect was evaluated using multivariable logistic regression in SAS (SAS Inst. Inc., Cary, NC, USA). This involved applying a backward stepwise elimination process with the LOGISTIC procedure. Variables were removed from the model based on the Wald statistic criterion if their P-values exceeded 0.05.

Multivariate mixed logistic regression models produced odds ratios (OR) and 95% confidence intervals to estimate the strength of the association between potential risk factors and seropositivity to bBR. Continuous variables were categorized in the final multivariable models using their means as cutoff points. The initial model for seropositivity to bBR included the following potentially explanatory variables: birth weight (<37 or >37 kg), weaning weight (<71 or >71 kg), pre-weaning average daily weight gain (<560 or >560 g), season of calving (winter, December to February; spring, March to May; summer, June to August; and fall, September to November), BCS at calving (>3.5 vs. ≥3.5 units), age at first calving (<680 vs. ≥680 days), number of brucellosis vaccines (1 or 2), and the occurrence (yes vs. no) of pneumonia, subclinical ketosis, mastitis, diarrhea, retained placenta, puerperal clinical metritis, and laminitis. Retained placenta could be confounded by dystocia, and it is a primary confounder of developing metritis. Since records on dystocic parturition were unavailable, we acknowledge that the impact of metritis and retained placenta on the brucellosis seropositivity rate cannot be accurately measured without separating it from the effect of dystocia itself.

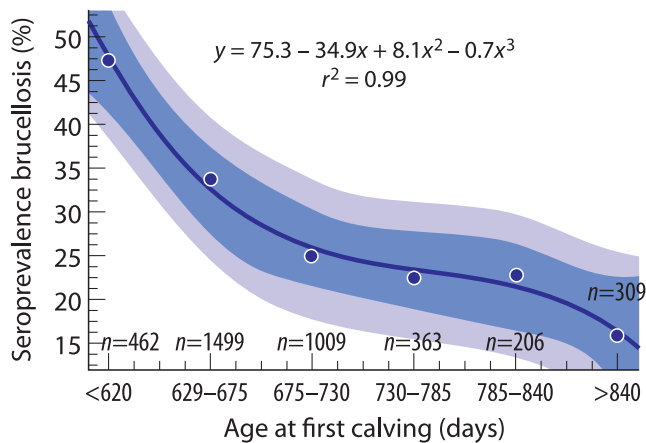
Two-way biologically plausible interactions between pre-selected explanatory variables were included in the multivariable model. Not all interactions were statistically significant. For abortion, the same variables were used in the model with the addition of seropositivity to bBR. The year of calving was included as a covariate in the models for bBR and abortion. The relationship between age at first calving and seropositivity to bBR was evaluated using CurveExpert Professional 2.5.6 software (Hyams Development, Madison, AL).

## RESULTS

Of the 3,848 sera tested, antibodies to bBR were detected in 1,157 lactating heifers from 2018 to 2021 (30.1%; 95% CI=29...32%). The adjusted occurrence of this illness was 33.2%. The risk factors associated with bBR seropositivity are listed in **Table 1**. Heifers older than 680 days at first calving had half the risk of being seropositive to bBR compared with younger heifers. The seroprevalence of bBR decreased sharply with increasing age at first calving, with a 30-percentage-point difference in incidence rate between the youngest and oldest heifers at first parturition (**Fig. 2**). Heifers with preweaning diarrhea were

**Table 1.** Adjusted odds ratios and 95% confidence interval (CI) for seropositivity to brucellosis of lactating Holstein heifers in a hot environment ( $n=3848$  calvings), using multivariable logistic regression analyses

| Variables                   | Seroprevalence   | Odds ratio (OR) | 95% CI OR | P-value |
|-----------------------------|------------------|-----------------|-----------|---------|
| Age at first calving (days) |                  |                 |           | <.0001  |
| >680                        | 22.7 (392/1731)  | 0.57            | 0.49–0.66 |         |
| <680                        | 36.1 (765/2117)  | Reference       |           |         |
| Diarrhea                    |                  |                 |           | 0.0194  |
| Yes                         | 34.1 (191/561)   | 1.27            | 1.04–1.54 |         |
| No                          | 29.4 (966/3287)  | Reference       |           |         |
| Retained placenta           |                  |                 |           | <.0001  |
| Yes                         | 47.5 (326/686)   | 2.48            | 2.1–2.8   |         |
| No                          | 26.3 (831/3162)  | Reference       |           |         |
| Number of RB51 vaccines     |                  |                 |           | <.0001  |
| 2                           | 31.5 (1071/3404) | 2.2             | 1.7–2.8   |         |
| 1                           | 19.4 (86/444)    | Reference       |           |         |
| Season                      |                  |                 |           | 0.0198  |
| Summer                      | 35.3 (319/904)   | 1.22            | 1.03–1.44 |         |
| Autumn, Winter, Spring      | 28.5 (838/2943)  | Reference       |           |         |



**Fig. 2.** Association between age at first calving and seroprevalence of brucellosis in high-yielding Holstein heifers in a hot environment. Dark bands are 95% confidence intervals for estimated values. Light bands are 95% confidence intervals for actual values

1.2 times more likely ( $P<0.01$ ) to be seropositive for bBR than heifers without preweaning diarrhea. The proportion of heifers seropositive to bBR was nearly three times higher in heifers suffering from retained placenta compared to those without this reproductive disorder. Heifers receiving two bBR vaccinations were 2.2 times more likely ( $P=0.01$ ) to be seropositive than those receiving only one vaccination. In

their first lactation, heifers calving in summer had a 1.2 times higher risk ( $P=0.02$ ) of being seropositive for bBR than those calving in other seasons.

Heifers seropositive to bBR were 3.1 times more likely ( $P<0.01$ ) to have an abortion than seronegative heifers for bBR (**Table 2**). Heifers with a BCS of 3.5 or higher at calving had less than half the risk of abortion compared to those with a BCS below 3.5 at calving. Heifers suffering from retained fetal membranes had a 3.1 times higher ( $P<0.01$ ) risk of abortion than heifers without this reproductive disorder. Heifers diagnosed with pneumonia during the growing period were 1.6 times more ( $P<0.01$ ) likely to experience abortion than those without this respiratory disease. Heifers diagnosed with clinical metritis had a 1.3 times greater risk of abortion than heifers not affected by this uterine disease (**Table 2**).

## DISCUSSION

The observed seroprevalence of bBR in brucellosis-vaccinated heifers in this herd was higher than the maximum 26% reported in Holstein herds with high bBR prevalence in the same zone as the current study (Mellado et al., 2014). Despite a program to control this disease in this large dairy basin, the value of RB51 whole-herd vaccination combined with test-and-slaughter in controlling bBR has not been proven. However, it has been observed that some farmers

**Table 2.** Adjusted odds ratios and 95% confidence interval (CI) for abortion in lactating Holstein heifers in a hot environment ( $n=3848$  calvings), using multivariable logistic regression analyses

| Variables                | Prevalence      | Odds ratio (OR) | 95% CI OR | P-value |
|--------------------------|-----------------|-----------------|-----------|---------|
| Positive for brucellosis |                 |                 |           | <.0001  |
| Yes                      | 38.5 (445/1157) | 3.1             | 2.6–3.7   |         |
| No                       | 16.0 (431/2691) | Reference       |           |         |
| BCS at parturition       |                 |                 |           | <.0001  |
| >3.5                     | 20.8 (742/3563) | 0.3             | 0.23–0.38 |         |
| <3.5                     | 47.0 (134/285)  | Reference       |           |         |
| Placental retention      |                 |                 |           | <.0001  |
| Yes                      | 39.7 (272/686)  | 3.1             | 2.6–3.7   |         |
| No                       | 19.1 (604/3162) | Reference       |           |         |
| Pneumonia                |                 |                 |           | 0.0003  |
| Yes                      | 31.8 (129/406)  | 1.6             | 1.2–2.0   |         |
| No                       | 21.7 (747/3442) | Reference       |           |         |
| Metritis                 |                 |                 |           | 0.0040  |
| Yes                      | 24.0 (394/1641) | 1.3             | 1.1–1.6   |         |
| No                       | 21.8 (394/1641) | Reference       |           |         |

BCS = Body condition score.

are reluctant to cull seropositive cows for bBR, as they do not observe severe symptoms in their cows that would cause a significant drop in productivity (Mellado et al., 2021). These seropositive cows are latent carriers of *B. abortus* within the herd, despite widespread vaccination of all cows. Therefore, this failure to effectively control bBR might explain the extremely high proportion of brucellosis-vaccinated cows that are seropositive for bBR. It has also been observed that in brucellosis-endemic areas with moderate prevalence, there are high percentages of reactors, as well as abortions.

The interval between vaccination and the assay was very short in some heifers. Still, cattle vaccinated with strain RB51 are not expected to produce antibodies detectable by conventional serologic tests (Stevens et al., 1995; Tittarelli et al., 2008). Therefore, it is unlikely that RB51 induces antibodies against smooth lipopolysaccharide (LPS) that are detectable by the serological tests used.

In the current study, older heifers at first calving were less likely to be seropositive for bBR than younger heifers. This response may be linked to a higher risk of dystocia in lighter heifers (Atashi et al., 2021) and the subsequent consequences of this reproductive disorder, such as digestive and respira-

tory issues, retained placenta, uterine diseases, and mastitis (Ingvarlsen and Moyes, 2013). This physiological imbalance affects immune function (Lombard et al., 2007), potentially making younger heifers more prone to seropositivity for bBR. It is also probable that heifers calving at a younger age are more likely to test positive for bBR because they allocate more energy toward growth during their first lactation than older heifers at their first calving (Heinrichs and Heinrichs, 2011).

One of the most surprising findings in this study was that neonatal diarrhea was associated with a higher odds of bBR seroprevalence. Typically, one would expect the opposite because preweaning diarrhea is a major factor in delaying puberty in heifers (Van Amburgh et al., 1998), and older heifers at first calving were less likely to be seropositive to bBR in this study. However, it remains unclear how preweaning diarrhea could be associated with bBR seropositivity. Its effect might be indirect, possibly through the occurrence of other diarrhea-related diseases, such as bovine respiratory disease (Pardon and Buczinski, 2020), which can impair immune function and increase the likelihood that heifers will acquire bBR.

An important finding of this study was that retained fetal membranes were significantly linked to bBR

seropositivity. This finding aligns with (Merga Sima et al., 2021). This connection might be due to immunosuppression in dairy cows during the transition period, a physiological stress that causes defective separation of the fetal membranes and their subsequent retention after calving. A key pathogenic factor leading to retained fetal membranes is immune alteration during parturition (Mordak and Anthony, 2015; Dervishi et al., 2016; Lu et al., 2020), although other reports suggest that cows with or without retained placenta exhibit similar immune function (Yazlık et al., 2019). Therefore, it is possible that immune response disruption in brucellosis-vaccinated heifers with retained placenta may increase their likelihood of being seropositive for bBR.

The likelihood of seropositivity to bBR in heifers receiving two doses of the brucellosis vaccine was significantly higher than in those vaccinated only once. These results are not supported by Fernandes et al., (2024), who did not find differences in the percentage of confirmed seropositive cows to brucellosis receiving one or two doses (6 months apart) of the RB51 strain vaccine.

It has been stated that RB51 is an attenuated rough-strain vaccine that, after vaccination, produces no false positives on standard serological tests. Therefore, this vaccine should not interfere with brucellosis surveillance (Herrera-Lopez et al., 2010). However, in a field trial, 49% of pregnant cows from a brucellosis-free herd, revaccinated with RB51 and introduced into an infected herd, tested seropositive for bBR using the card test (Leal-Hernandez et al., 2005). These findings challenge the idea that RB51 vaccination does not induce antibodies that interfere with brucellosis diagnosis.

After the first vaccination with strain RB51, a second immunization triggers a strong and complex immune response (Dorneles et al., 2015; Boggiatto et al., 2019). The presence of seropositive heifers after vaccination indicates a secondary response and does not necessarily imply brucellosis infection, as confirmed by the Rivanol test (Cantú et al., 2007). Therefore, in this study, revaccination with the RB51 vaccine could have led to persistent antibody levels, making it harder to identify infected heifers using card tests. Also, serological interference (despite expectations for RB51), reverse causality, confounding by management decisions, or imbalance in group sizes among revaccinated heifers could have occurred.

Heifers calving in summer were more likely to become seropositive to bBR than heifers calving during all other seasons, suggesting that heat stress in lactating dairy heifers is positively linked to seropositivity to bBR. This hypothesis is based on the fact that cows are immunosuppressed by heat stress (temperature-humidity index in summer >85 units at the study site (Bagath et al., 2019; Lendez et al., 2021). Additionally, hot environments promote the shedding of infectious microorganisms (Hamel et al., 2021). Indirectly, hot weather can influence seropositivity to bBR through reduced dry matter intake and negative energy balance (Wheelock et al., 2010), which impairs immune function (Ingvarsen and Moyes, 2013).

The risk of abortion was higher in heifers seropositive to bBR than in seronegative animals. This finding aligns with other studies, where positivity to brucellosis is a major factor for abortion in cattle (Kardjadj, 2018; Sarangi et al., 2021). However, even in seronegative heifers, a high percentage of abortions occurred, indicating that vaccination with RB51 did not completely prevent abortion in heifers. These results agree with (Poester et al., 2006), who observed that vaccination with RB51 partially prevented abortions in crossbreed virgin heifers. It is worth noting that bovine viral diarrhea (Gomez-Romero et al., 2021), leptospirosis (Escamilla et al., 2007), neosporosis, and bovine respiratory rhinotracheitis (Millián-Suazo et al., 2016) are other important diseases associated with reproductive failure in dairy herds in Mexico.

Heifers with a BCS of 3.5 units or higher at parturition had a much lower risk of abortion than heifers with a BCS below 3.5 units. These results align with observations from Starbuck et al. (2004), who noted that cows in an average body condition (2.75–3.25) maintained 92.1% of pregnancies, while those with BCS of 2.50 or less had a pregnancy rate of 84.2%. Likewise, Mellado et al. (2019) reported 10 percentage points higher fetal loss in Holstein cows with a BCS below 3.5 than in cows with a higher BCS. Nutritional status affects the establishment and maintenance of pregnancy to term in dairy cows (Meikle et al., 2018). A BCS around 3.5 minimizes BCS-related health and fertility issues (O'Hara et al., 2015), which could explain the lower likelihood of abortion in heifers with greater energy reserves at calving. Furthermore, heifers with lower BCS tend to eat more, which increases progesterone clearance and reduces its blood concentration (Reksen et al., 2002),

a hormone essential for sustaining pregnancy. Additionally, reduced BCS is associated with increased fetal losses (Pinedo et al., 2022). It is important to note that no interactions were found between various heifer-related variables and abortion, suggesting that BCS independently influences the occurrence of this reproductive disorder.

In agreement with the conclusions of Mellado et al. (2019), high-yielding Holstein cows with retained fetal membranes showed an increased risk of abortion in this study. This response is poorly understood because current therapies for retained placenta effectively prevent declines in reproductive performance among affected cows (Mellado et al., 2018). It may be a residual effect of uterine diseases, such as retained placenta and metritis, on the incidence of abortion. These residual effects could last longer than 4 months and may involve reduced oocyte quality and a compromised uterine environment caused by trauma to the endometrium (Ribeiro et al., 2016; Sheldon and Owens, 2017).

The current study provides strong evidence that heifers with pneumonia during the growing period face an increased risk of abortion. This link is difficult to interpret and explain. Pneumonia may cause long-term effects in humans (Grimwood and Chang, 2015) or sometimes persist for extended periods (Hermeyer et al., 2012), which could pose problems for unborn calves. Additionally, the occurrence of clinical diseases such as calving problems, metritis, clinical endometritis, mastitis, pneumonia, digestive issues, and lameness (Ribeiro et al., 2016) has been linked to higher rates of pregnancy loss, indicating that these diseases can significantly impact the maintenance of pregnancy.

Clinical metritis significantly influences the occurrence of abortion in heifers, implying that uterine infection is linked to fetal losses in dairy cows even after the infection appears to resolve. These findings agree with Figueiredo et al. (2021), who observed that pregnancy loss was more common in cows diagnosed with metritis but that did not recover clinically after antimicrobial treatment. Likewise, Giuliadori et al. (2013) reported an increased risk of late embryonic loss in dairy cows with clinical metritis. However, the mechanisms behind this ongoing infertility are unclear and difficult to explain because metritis is often associated with other peripartum disorders and metabolic issues related to the start of lactation. This situation could be due to greater endometrial damage

or incomplete uterine healing in heifers with clinical metritis. Additionally, uterine infections may decrease oocyte quality (Piersanti et al., 2020) and the ability of oocytes to develop into morulae (Dickson et al., 2020), potentially affecting fetal development later. Another possible explanation is that the immune system's response to puerperal metritis may persist for weeks after uterine inflammation subsides (Magata et al., 2016); thus, long-term changes in systemic immune responses could disrupt maintenance of pregnancy.

## CONCLUSIONS

Bovine brucellosis remains highly prevalent in Holstein heifers within the study zone. Calving during summer reduced the odds of seropositivity to bBR. There is no specific optimal age at first calving for risk of bBR seropositivity; however, as age at first calving increases, the risk decreases significantly. Diarrhea and retained fetal membranes were linked to higher odds of seroprevalence to bBR. Avoiding a second vaccination against brucellosis (strain RB51) is an important management strategy to reduce the risk of seropositivity to bBR.

Increasing BCS was beneficial for reducing abortion rates, but conditions such as metritis, retained placenta, and pneumonia significantly raised the risk of this reproductive issue. Seropositivity to bBR was the most significant risk factor for abortion. Therefore, the current study suggests that it is possible to differentiate among heifers in terms of bBR seropositivity and abortion risk based on a combination of heifer-related factors.

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## DATA AVAILABILITY

The datasets used in the current study are available from Dr. M. Mellado (email: melladomiguel07@gmail.com) upon reasonable request.

## COMPETING INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this article.

**Authors' contributions:** M. Mellado, conceptualization, formal analysis, supervision, and writing of the original draft. J. Mellado, and L. Avendaño-Reyes, statistical analyses. U. Macías-Cruz, data collection.

A. de Santiago, data collection. N.Y. Ávila-Serrano, review & editing.

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**СЕРОПРЕВАЛЕНТНІСТЬ І ФАКТОРИ  
РИЗИКУ, ПОВ'ЯЗАНІ З БРУЦЕЛЬОЗОМ  
ВЕЛИКОЇ РОГАТОЇ ХУДОБИ ТА ВИКИДНЯМИ  
У НЕТЕЛІВ ГОЛШТИНСЬКОЇ ПОРОДИ**

Jesús Mellado<sup>1</sup>, Narciso Y. Ávila-Serrano<sup>2</sup>,  
Ángeles de Santiago<sup>3</sup>, Ulises Macías-Cruz<sup>4</sup>,  
Leonel Avendaño-Reyes<sup>4</sup>, Perpetuo Álvarez<sup>5</sup>,  
and Miguel Mellado<sup>1\*</sup>

<sup>1</sup> Кафедра годівлі тварин, Автономний аграрний  
університет імені Антоніо Нарро,  
25315 Сальтільо, Мексика

<sup>2</sup> Кафедра тваринництва, Морський університет,  
71980 Пуерто-Ескондідо, Мексика

<sup>3</sup> Кафедра ветеринарної медицини, Автономний  
аграрний університет імені Антоніо Нарро,  
27054 Торреон, Мексика

<sup>4</sup> Інститут сільськогосподарських наук,  
Автономний університет Баха-Каліфорнії,  
21705 Мехікалі, Мексика

<sup>5</sup> Кафедра відновлюваних природних ресурсів,  
Автономний аграрний університет  
імені Антоніо Нарро,  
25315 Сальтільо, Мексика

Email: jmelladobosque@hotmail.com,  
valear68@yahoo.com.mx,  
angelesdesantiago867@gmail.com,  
umacias@uabc.edu.mx, lar62@uabc.edu.mx,  
perpetuo.alvarezv@uaaan.edu.mx,  
melladomiguel07@gmail.com\*

ORCID: <http://orcid.org/0000-0003-2093-1729>,  
<http://orcid.org/0000-0003-4043-501X>,  
<https://orcid.org/0000-0002-5491-0662>,  
<https://orcid.org/0000-0002-6947-2247>,  
<https://orcid.org/0003-2666-3999>,  
<https://orcid.org/0000-0001-5477-5707>

**Мета.** Оцінити серопревалентність бруцельозу великої рогатої худоби (bBR) та ідентифікувати фактори ризику, пов'язані з серопозитивністю до цього захворювання. Додатковою метою було оцінити фактори ризику, пов'язані з викиднями у нетелів. **Методи.** У цьому дослідженні було використано 3848 корів голштинської породи з першою закінченою лактацією з великого комерційного молочного стада на півночі Мексики (25° пн.ш.). Для аналізу факторів ризику щодо рівня серопозитивності на бруцельоз і розрахунку співвідношення шансів, що дає змогу кількісно оцінити ступінь ризику, було застосовано моделі множинної регресії. **Результати.** Серопревалентність bBR становила 30% (95% CI=29–32%) серед лактуючих телиць (кард-тест). Телиці, жива маса яких під час отелення перевищувала 680 кг, мали вдвічі менший ризик отримати позитивний результат тесту на bBR порівняно з тими, жива маса яких була меншою за 680 кг (22,7% проти 36,1%). Ризик отримання серопозитивного результату на bBR був вищим у телиць із затримкою плаценти порівняно з тими, у яких цього репродуктивного розладу не було (OR=2,46; 47,5% проти 26,3%). Телиці, вакциновані двома дозами комерційної вакцини проти бруцельозу, мали у 2,2 рази вищі шанси отримати серопозитивний результат на bBR порівняно з тими, що були вакциновані лише один раз ( $P<0,01$ ). У телиць із серопозитивним результатом на bBR ймовірність викидня була в 3,1 раза вищою, ніж у телиць із негативним результатом (38,5% проти 16,2%;  $P<0,01$ ). Телиці з індексом вгодованості тіла (BCS) 3,5 або вище на момент отелення мали меншу ймовірність викидня, ніж телиці з BCS < 3,5. **Висновки.** Програми контролю бруцельозу повинні надавати пріоритет віку при першому отеленні, затримці плаценти та кількості вакцин RB51, введених телицям голштинської породи.

**Ключові слова:** індекс вгодованості, діарея, метрит, пневмонія, вакцина RB51, затримка плаценти.