Risk factors associated with testing positive for brucellosis and occurrence of abortion in high-yielding Holstein heifers

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Abstract

This retrospective study aimed to estimate the seroprevalence of bovine brucellosis (bBR) in high-yielding Holstein heifers and identify risk factors associated with seropositivity to this disease. An additional aim was to assess the heifer-related risk factors for abortion. This study was carried out on a commercial dairy farm in northern Mexico (25° N) using 3848 heifers from 2018 to 2021. The seroprevalence of bBR was 30% (95% CI = 29–32%) for lactating heifers (card test). Multiple logistic regression models indicated that heifers with > 680 kg at calving had half the risk of being positive to bBR than heifers with < 680 kg at calving (22.7 *vs*. 36.1%). Heifers suffering from diarrhea during the growing period were 1.3 times more likely to be seropositive to bBR than heifers not diagnosed with this disease (34.1 *vs*. 29.4%; P < 0.01). The risk of being seropositive to bBR increased in heifers suffering from retained placenta compared with heifers not suffering from this reproductive disorder (OR = 2.46; 47.5 *vs.* 26.3%). Heifers vaccinated with two doses of commercial vaccines against brucellosis were associated (P < 0.01) with 2.2 times higher odds of being seropositive to bBR than heifers vaccinated only once. Heifers seropositive to bBR were 3.1 times more likely to abort than heifers seronegative to this disease (38.5 *vs*. 16.2%; P < 0.01). Heifers with body condition score (BCS) ≥ 3.5 at calving (OR = 0.3) had a protective role for seropositive to bBR. The likelihood of having an abortion was 3.1 times higher in heifers diagnosed with retained placenta than in those not having this reproductive disorder. It was concluded that brucellosis control programs should focus on age at first calving, retained placenta, and the number of brucellosis strain RB51 vaccinations applied to heifers. Increasing BCS was advantageous to achieve lower abortion rates.

Introduction

Bovine brucellosis (bBR) is a significant zoonosis with important repercussions for both public health (Dadar et al. 2019; Mitiku and Desa 2020) and economic cost for milk producers in medium to low-income countries (Deka et al. 2018). The economic losses arise from the direct reduction in milk yield (Herrera et al. 2008) and other costs derived from abortions, increased inter-calving period, the presence of fewer animals in milk at any given time, repeat breeding, and culling of cows seropositive to brucellosis. Additional economic harm arises from the hindrance of free animal movement, which impedes the import and export of livestock, and increases replacement cost, among other causes (Deka et al. 2018; Bardhan et al. 2020; Ibrahim et al. 2021). Additional negative repercussions of brucellosis are the occurrence of stillbirths, abortion, birth of weak calves, infertility, epididymitis and orchitis in bulls (Arif et al. 2017; Choudhary et al. 2019; Meng and Zhuqing 2020), veterinary and medical expenditures, and expenses for the control program of this disease (Tamba et al. 2021). However, most cows are asymptomatic, having this hidden infection without displaying clinical signs; thus, contributing to the spread of the illness in the herd. bBR seroprevalence in intensive dairy herds in Mexico is 23 to 35% (Mellado et al. 2014), which constitutes a risk to public health and an economic hampering for intensive dairy operations. Even though the abundant resources and efforts for some decades of intensive dairy enterprises in Mexico for controlling bBR, this chronic disease is still widespread in this country (Luna-Martínez and Mejía-Terán 2002).

Many factors have been identified associated with bBR seropositivity, which can be generally classified into three groups: management factors (Herrán Ramirez et al. 2020), host factors (Patel et al. 2014; Yanti et al. 2021), and agroecological factors (Chiebao et al. 2015). However, there is presently a paucity of information on unexplored risk factors for the occurrence of bBR and abortion in high-yielding dairy heifers in intensive farming systems in hot environments. A better understanding of bBR in dairy cows would increase the understanding of bBR and abortion risk factors for dairy cattle and stimulate local awareness programs and guideline practices on bBR control interventions (Lindahl et al. 2019).

Therefore, the hypotheses were: (1) heifers with adequate BCS at mating, older age at first calving, not calving in summer, receiving a single vaccination against brucellosis, presenting higher growth rates, and not suffering from infectious diseases before or at calving would have reduced risk of seropositivity to bBR; (2) cows seronegative to bBR, with adequate BCS at mating, older age at first calving, receiving a single vaccination against brucellosis, presenting higher growth rates and not suffering from infectious diseases before or at calving would have reduced risk of abortion; (3) the seroprevalence to 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 bBR in lactating heifers in a zone where the incidence rate of bovine brucellosis is very high, (2) to identify heifer-level risk factors associated with positivity to brucellosis in high yielding Holstein heifers, (3) to determine the heifer-related risk factors for the occurrence of abortion. These results can help to design age-specific risk-based bBR management strategies.

Material And Methods

Animals and herd management

This study was approved by the Ethics Committee of the Research Department of the Autonomous Agrarian University Antonio Narro (protocol 42520-3001-2138). The study was carried out on a commercial dairy farm (≈ 3000 milking cows) in a hot zone of northern Mexico (25° N, 103° W; mean annual temperature 23.7 ºC). A total of 3848 heifers with a single lactation per animal were included in this retrospective study from 2018 to 2021. The herd annual daily average milk yield production was 33.2 kg.

Cows were kept in open dirt-based pens with metal framework shades in the center of pens and feeding allies. Hot weather's adverse effects in this location were minimized by providing lactating cows with fans and sprayed water. Lactating cows were fed ad libitum total mixed rations (TMR) twice per day, and approximately 2.5% of feed refusals were removed before each feeding. Ration formulation was based on recommendations of the National Research Council (2001) to support milk production of 42 kg/d for cows consuming 25 kg of DM/d. Cows were milked daily at 0600, 1400, and 2100 h. The TMR included corn silage, alfalfa hay, and a concentrate containing corn grain, cotton-seed meal, soybean meal, and minerals.

Calves were immediately separated from their mothers after calving and were placed into individual (1.2 × 1.7 m) partially roofed dirt-floor open-sided wood pens. A shade cloth about 3 m above the pens protected calves from solar radiation. Calves were fed 4 L of high-quality pasteurized colostrum within the first hour postpartum; thereafter, they ingested whole milk using open 5-L plastic buckets until 60 days of age.

Health management and diseases recording

Heifers were vaccinated against infectious bovine rhinotracheitis, bovine respiratory syncytial virus, bovine viral diarrhea types 1 and 2, para-influenza 3, and leptospirosis caused by five Leptospira serovars (CattleMaster Gold FP5®, Zoetis, Mexico D.F., Mexico). Heifers were also vaccinated against leptospirosis (5-serovars; LEPTAVOID-H®; Merck Sharp and Dohme Corp., Mexico, D.F.). In addition, heifers were vaccinated subcutaneously with *Brucella abortus* (strain RB51; MSD Salud Animal Mexico, Mexico City, Mexico) vaccine at an average (± SD) age of 21.2 ± 2.9 months. Most heifers (88%) received a second *B. abortus* vaccine 10.8 ± 3.1 months after the first vaccination. Heifers were also vaccinated to prevent blackleg caused by *Clostridium chauvoei*, malignant edema caused by *C. septicum*, the black disease caused by *C. novyi*, gas-gangrene caused by *C. sordellii*, enterotoxemia and enteritis caused by *C. perfringens* Types B, C, and D, and disease caused by *Histophilus somni* (*Haemophilus somnus*) (Ultrabac 7 Somubac®, Zoetis, Guadalajara, Mexico). Finally, heifers received vaccination against anthrax (Bayovac Thraxol 2®, Bayer, CD Mexico, Mexico).

Blood samples (five mL) from coccygeal vessels from each heifer were obtained with vacuum tubes coated with a clot activator (BD Vacutainer, Franklin Lakes, NJ, USA) for serum collection. The sampling period occurred at 32 ± 6 months of age. Blood was allowed to clot and then placed in ice and transported to an accredited laboratory. The serum samples were examined by the brucellosis card test; noticeable agglutination was taken as a positive test result. We acknowledge that the validation of such diagnostic tests is an issue for assessing the prevalence of this disease because no confirmatory test was used; therefore, the true prevalence for brucellosis in these heifers was calculated according to the following formula:

True Prevalence = PO + Specificity – 1/ Sensitivity + Specificity – 1, where PO = prevalence observed by the test. Values for sensitivity (91%) and specificity (99.6%) were taken from previous studies on dairy cows (Rahman et al. 2019).

Subclinical ketosis was determined using the Ketostix® (Bayern, CD. Mexico, Mexico); urine strip test was directly moistened from urine after spontaneous or stimulated micturition approximately 7 days postpartum. The result (traces or high concentrations of acetoacetate) was read after 5 to 10 s of contact and was recorded. In addition, the mammary gland health was monitored regularly using the California mastitis test.

Identification of lame cows was made on those heifers showing noticeable limp. The full-time herd veterinarian recorded lameness data, and the appropriate protocol for this disease was applied. Retained fetal membranes were defined as the presence of placental tissues 24 h after calving, and this event was recorded by the attending veterinarian. The diagnosis of clinical metritis was based on characteristics of the presence of watery, fetid, reddish-brownish vaginal discharge within 21 days in milk and uterine examination (size and tone) by palpation per rectum. All heifers with metritis received ceftiofur until recovery. Heifers were diagnosed with diarrhea (watery discharges that sifts through the ground) and pneumonia (rectal temperature >39.4°C, nasal discharge, ocular discharge, coughing, and increased respiratory rate) by the herd veterinarian.

Statistical analyses

To analyze factors contributing to the seropositivity to bBR (binary outcome; heifers classified as either seronegative or seropositive to bBR during their first lactation), a full preliminary model with heifers as a random effect was screened using a multivariable logistic regression model of SAS (SAS Inst. Inc., Cary, NC), applying a backward stepwise elimination using the LOGISTIC procedure Variables were removed from the model by the Wald statistic criterion if the significance was greater than 0.05.

Multivariate mixed logistic regression models produced odds ratios (OR) and 95% confidence intervals to estimate the strength of association between the potential risk factors and seropositivity to bBR. Continuous variables were used as categorized in the final multivariable models using their mean as a separating point. The preliminary model for seropositivity to bBR contained 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 months being December–February; spring, March-May; summer, June–August; and fall, September–November), body condition score 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.

Two-way biologically plausible interactions between pre-selected explanatory variables were included in the multivariable model. None of the interactions were statistically significant. For abortion, the same variables were included in the model with the addition of seropositivity to bBR. Year of calving was included in the models for brucellosis and abortion as covariate. The association between age at first calving and seropositivity to bBR was assessed using the CurveExpert Professional 2.5.6 software (Hyams Development, Madison, AL).

Results

Of the 3848 sera tested, antibodies to bBR were detected in 1157 lactating heifers from 2018 to 2021 (30.1%; 95% CI = 29–32%). The adjusted prevalence of this disease was 33.2%. The risk factors related to seropositivity to bBR are listed in Table 1. Heifers with > 680 days of age at first calving had half the risk to be seropositive to bBR compared to younger heifers at first calving. The seroprevalence to brucellosis abruptly declined with increasing age at first calving with 30 percentage points of difference in incidence rate between the youngest and the oldest heifers at first parturition (Fig. 1). Heifers suffering from preweaning diarrhea were 1.2 times more ($P < 0.01$) likely to become seropositive to bBR than heifers not having this disease. The proportion of heifers seropositive to bBR was nearly three times as much in heifers suffering from retained placenta compared with that observed in heifers not diagnosed with this reproductive disorder. Heifers receiving two vaccinations against bBR were 2.2 more likely ($P = 0.01$) to be seropositive to bBR than heifers receiving a single vaccine against bBR. In their first lactation, heifers calving in summer had 1.2 times more risk ($P = 0.02$) to be seropositive to bBR as had heifers calving in other seasons of the year.

Table 1
Adjusted odds ratios and 95% confidence interval (CI) for the effects of various factors on the seropositivity to brucellosis of Holstein heifers in a hot environment (n = 3848), 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		

Table 2
Adjusted odds ratios and 95% confidence interval (CI) for the effects of various factors on the seropositivity to brucellosis of Holstein heifers in a hot environment (n = 3848), using multivariable logistic regression analyses.

Variables	Prevalence	Odds ratio (OR)	95% CI OR	*P*-value
Positive to 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		

Heifers seropositive to bBR were 3.1 more likely ($P < 0.01$) to have an abortion than seronegative heifers to bBR (Table 1). Heifers with a BCS ≥ 3.5 at calving had less than half the risk to have an abortion compared with heifers with a BCS < 3.5 at calving. Heifers suffering from retained fetal membranes had 3.1 times more ($P < 0.01$) risk of abortion than cows not suffering from this reproductive disorder. Heifers diagnosed with pneumonia during the growing period were 1.6 more ($P < 0.01$) likely to have an abortion than heifers not presenting this respiratory disease. Heifers diagnosed with clinical metritis had 1.3 times the risk of abortion as had heifers not having this uterine disease (Table 1).

Discussion

The observed seroprevalence of bTB in brucellosis-vaccinated cows in this herd was above the maximum 26% reported data in Holstein herds with high bBR prevalence in the same zone where the current study took place (Mellado et al. 2014). Despite the existence of a program to control this disease in this large dairy basin, the value of RB51 whole-herd vaccination, in combination with test-and-slaughter in controlling bovine brucellosis, has not been demonstrated. However, it has been observed that some farmers are reluctant to get rid of seropositive cows to bBR since they do not perceive severe symptoms in their cows as causing drastic reduction in productivity (Mellado et al. 2021). These seropositive cows are latent carriers of *B. abortus* within the herd regardless of the generalized vaccination of all cows. Thus, this failure to adequately control animal brucellosis might explain the extremely high proportion of brucellosis vaccinated cows seropositive to bBR. It has been observed that in brucellosis endemic areas, under intermediate prevalence conditions, there are high percentages of reactors, as well as abortions.

The interval between vaccination and the assay was very close in some cows, but cattle vaccinated with strain RB51 fail to produce antibodies that can be detected by conventional serologic tests (Stevens et al. 1995; Tittarelli et al. 2008). Thus, it is not expected that RB51 induces antibodies against smooth lipopolysaccharide (LPS) detectable by the serological tests used.

Older heifers at first calving in the current study were less likely to be seropositive to bBR than younger heifers at first calving. This response may be associated with an increased risk of dystocia in lighter heifers (Atashi et al. 2021) and the sequelae of this reproductive disorder, such as digestive and respiratory disorders, and retained placenta, uterine diseases and mastitis (Lombard et al. 2007). This physiological imbalance alters the immune function (Ingvartsen and Moyes 2013), which may predispose younger heifers to result seropositive to bBR. It is also likely that heifers calving at younger ages are more likely to be positive to bBR because they partition greater energy towards growth during their first lactation than heifers older at their first calving (Van Amburgh et al. 1998).

One of the most surprising findings in the present study was that neonatal diarrhea was associated with higher odds for seroprevalence to bBR. The opposite would be suspected, because preweaning diarrhea is a major factor for increasing the age of heifers for attaining puberty (Heinrichs and Heinrichs 2013), and older heifers at first calving were less susceptible to be seropositive to bBR in the current study. However, it is unclear how preweaing diarrhea could be associated with seropositivity to bBR. Its effect could be indirect via the occurrence of other diseases linked to diarrhea, such as bovine respiratory disease (Pardon et al. 2013), and these diseases may impair the immune function that could predispose heifers to acquire bBR.

An important finding of this study was that retained fetal membranes were significantly associated with seropositivity to bBR. This finding is consistent with Merga Sima et al. (2021). This association could be due to the immunosuppression experienced by the dairy cows during the transition period, which represents a time of physiological stress that results in defective separation of fetal membranes and, consequently, their retention post-calving. In fact, an important pathogenic factor causing retained fetal membranes is immune alteration during parturition (Mordak and Anthony 2015; Dervishi et al. 2016; Lu et al. 2020), although other reports indicate that cows with or without retained placenta showed similar immune function (Yazlık et al. 2019). Thus, it could be that disruption of the immune response in brucellosis-vaccinated cows with retained placenta may predispose these animals to result seropositive to bBR.

The odds of seropositivity to bBR in the group of heifers receiving two doses of the brucellosis vaccine were markedly greater than that of heifers that were vaccinated once. It has been stated that RB51 is an attenuated rough strain vaccine that, after vaccination, gives no false positives with the conventional serological assays. Thus, this vaccine is not supposed to interfere in brucellosis surveillance (Herrera-Lopez et al. 2010). However, in a field trial, 49% of pregnant cows from a brucellosis-free herd, revaccinated with the RB51vaccine, and introduced into an infected herd were seropositive to bBR using the card test (Leal-Hernandez et al. 2005). These data tear down the notion that RB51 vaccination does not induce antibodies that interfere with Brucellosis diagnosis.

After a first vaccination with strains RB51, a second inoculation induce a strong and complex immune response (Dorneles et al. 2015; Boggiatto et al. 2019). Furthermore, the presence of seropositive cows post-vaccination represents a secondary response that does not necessarily indicate brucellosis infection when confirmed by the Rivanol test (Cantú et al. 2007). Thus, in the current study, revaccination with the RB51 vaccine could have created persistent titres that confounded the identification of infected heifers by the card tests.

Heifers calving in summer were more likely to become seropositive to bBR than heifers calving during all other seasons, suggesting that heat stress in the lactating dairy heifers is positively associated with seropositivity to bBR. This hypothesis is proposed due to cows' immunosuppression due to heat stress (temperature-humidity index in summer > 88 units in the study site; Bagath et al. 2019; Lendez et al. 2021). Additionally, hot environments facilitate infectious microorganisms shedding (Hamel et al. 2021). Indirectly, hot weather can influence seropositivity to bBR mediated through reduced dry matter intake, and the negative energy balance (Wheelock et al. 2010) impairs immune function (Ingvartsen and Moyes 2013).

The risk of abortion was more likely in heifers seropositive to bBR than in seronegative animals. This response agrees with other studies, where positivity to brucellosis constitutes a major factor for abortion in cattle (Kardjadj 2018; Sarangi et al. 2021). However, even in seronegative heifers, a high percentage of abortions occurred, suggesting that vaccination with RB51 did not completely prevent abortion in heifers. These results agree with Poester et al. (2006) data, who observed that vaccination with RB51 partially prevented abortions in crossbreed virgin heifers.

Heifers with a BCS $\geq$ 3. 5 units at parturition had a much lower risk of abortion than heifers with BCS < 3.5 units. These results are in line with observations of Starbuck et al. (2004), who observed that cows in average body condition (2.75–3.25) sustained 92.1% of pregnancies, whereas those with BCS $\leq$ 2.50 maintained 84.2% of pregnancies. Likewise, Mellado et al. (2019) reported 10 percentage points higher fetal losses in cows with BCS < 3.5 than cows with higher BCS. Nutritional status influences the establishment and maintenance of a pregnancy to term in dairy cows (Meikle et al. 2018). BCS around 3.5 minimize BCS-related health and fertility disorders (O'Hara et al. 2015), which would explain the lower likelihood of abortion in heifers with greater body energy reserves at calving. Additionally, heifers with lower BCS tend to eat more, which increases progesterone clearance, resulting in decreased blood concentration of this hormone (Reksen et al. 2002), which is essential for pregnancy maintenance. Also, reduced BCS has long-term carryover detrimental effects on embryo quality (Carvalho et al. 2014). It is worth mentioning that no interactions were observed between various heifers-related variables and abortion, thus, suggesting an independent effect of BCS on the occurrence of this reproductive disorder.

In agreement with the conclusions of Mellado et al. (2019), in high-yielding Holstein cows, retained fetal membranes increased the risk of abortion in the current study. This response is not clear because current therapies to treat retained placenta effectively prevent a reduction of the reproductive performance in cows suffering from this reproductive disorder (Mellado et al. 2018). Therefore, it could be a carryover effect of uterine diseases (retained placenta and metritis) on the occurrence of abortion. These carryover effects might last longer than 4 mo and involve reduced oocyte competence and impaired uterine environment due to trauma to the endometrium (Ribeiro et al. 2016; Sheldon and Owens 2017).

The current study presents compelling evidence that heifers suffering from pneumonia during the growing period had an increased risk of abortion. This association is challenging to interpret and explain. It could be that pneumonia could have a greater risk for long-term sequelae as it happens in humans (Grimwood and Chang 2015), or occasionally may have a long-term persistence (Hermeyer et al. 2012), which could cause problems for unborn calves. Furthermore, with the occurrence of clinical diseases (calving problem, metritis, clinical endometritis, mastitis, pneumonia, digestive problems, and lameness), Ribeiro et al. (2011) documented the increased pregnancy loss, which suggests that these diseases have a profound impact on the maintenance of gestation.

Clinical metritis had a marked impact on the occurrence of abortion in heifers, which suggests that uterine infection is associated with fetal losses in dairy cows, even after the resolution of infection. These results align with Figueiredo et al. (2021), who reported that pregnancy loss tended to be greater for cows diagnosed with metritis but with failure of clinical cure following antimicrobial therapy. Likewise, Giuliodori et al. (2019) documented a greater likelihood of late embryonic loss in dairy cows diagnosed with clinical metritis. However, the mechanisms responsible for this persistent infertility are unclear and complicated to elucidate because metritis is associated with other peripartum disorders and metabolic challenges associated with the onset of lactation. This response could be explained by the potentially greater endometrial injury or incomplete uterine recovery in heifers undergoing clinical metritis. Also, uterine infections may reduce oocyte quality (Piersanti et al. 2020) and its capacity to develop to morulae (Dickson et al. 2020), which could have a carryover effect that could interfere with the completion of fetal development. An additional possible scenario is that the impact of puerperal metritis on the immune system persists weeks after the uterine inflammation has been resolved (Magata et al. 2016); therefore, there are long-term alterations of systemic immune responses which possibly interfere with the maintenance of pregnancy.

Conclusions

Bovine brucellosis persists at a very high prevalence in Holstein heifers in the study zone. Calving heifers in summer decreased the odds of seropositivity to bBR. There was no optimum age at first calving regarding the risk of seropositivity to bBR, but as age of first calving increased, the risk of seropositivity to bBR markedly decreased. Diarrhea and retained fetal membranes were associated with higher odds of seroprevalence to bBR. Avoiding a second vaccination against brucellosis (strain RB51) is an important management factor in achieving a lower risk for seropositivity to bBR.

Increasing BCS was advantageous to achieve lower abortion rates, but metritis retained placenta and pneumonia greatly increased the odds of this reproductive disorder. Seropositivity to bBR was the most important risk factor for abortion. Thus, the current study indicates that differentiation can be made among heifers in the risk of seropositivity of bBR and abortion based on a combination of heifers-related factors.

Declarations

Acknowledgments

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Availability of data

The data that support the findings of this study are available from the corresponding author, M.M, upon reasonable request.

Code availability

Not applicable

Authors' contributions

Data acquisition: ML, JEM. Study design and drafted the manuscript: MM. Analyzed the results: UMC, LAR, MM. Revised the manuscript and reviewed the pertinent literature: JEG, UMC, LAR, NT. All authors read and approved the final version of the manuscript.

Ethics approval

Autonomous Agrarian University Antonio Narro Animal Care Advisory Committee (approval number 5-5-30-38111-4250-3001-2419).

Declaration of conflict of interests

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The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Ethics statement

The Autonomous Agrarian University Antonio Narro Institutional Animal Care and Use Committee approved all actions connected with cows used for this study (protocol number 3001-2114).

Credit authorship contribution statement

Data acquisition: M.L., P.A. Study design and drafted the manuscript: M.M., Analyzed the results: J.E.G., J.E. Revised the manuscript and reviewed the pertinent literature: U.M.C., L.A.R. All authors read and approved the final version of the manuscript.

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Figures

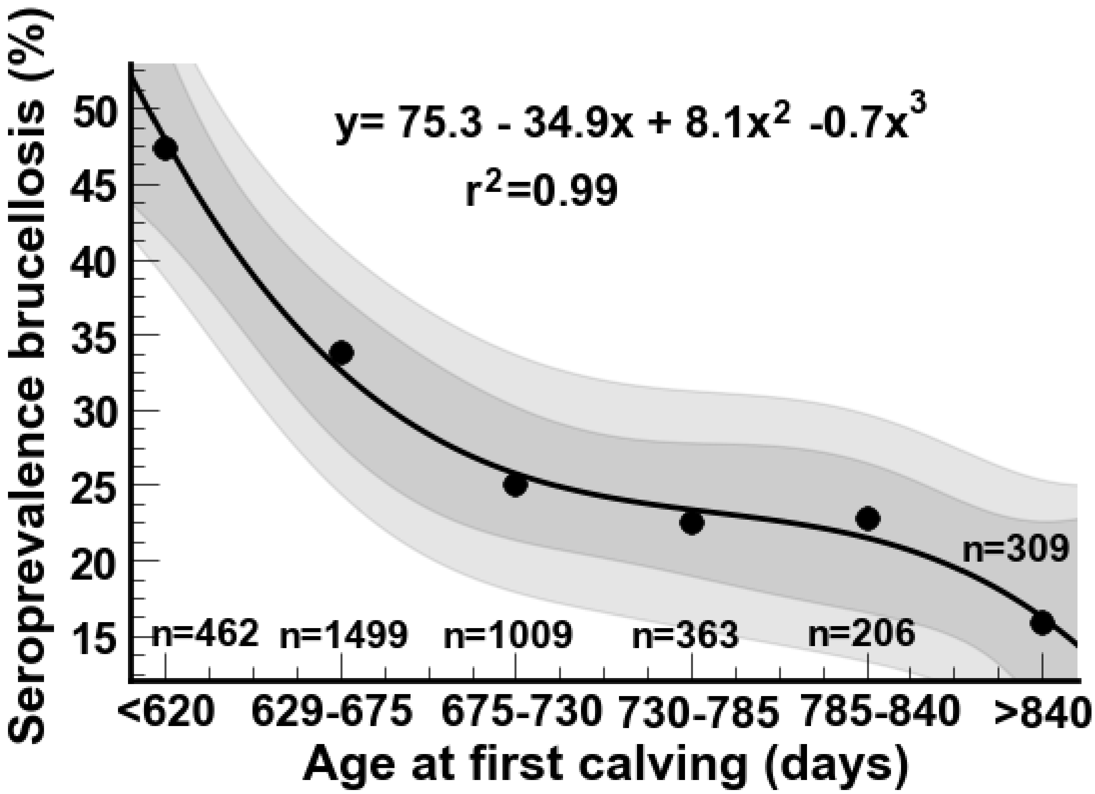

Figure 1

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.