Vaccination programs, parity, and calving season as factors affecting the risk of fetal losses and mummified fetuses in Holstein cows

Miguel Mellado1, Omar Nájera1, Jesús Mellado1, José E. García1, Ulises Macías-Cruz2, Álvaro F. Rodríguez1, Cesar A. Meza-Herrera3 and Leonel Avendaño-Reyes2

1Autonomous Agrarian University Antonio Narro, Dept. of Animal Nutrition, Saltillo, Mexico. 2Institute of Agricultural Sciences, Autonomous University of Baja California, Mexicali, Mexico. 3University Regional Unit of Arid Zones, Autonomous University Chapoing, Bermejillo, Mexico.

Abstract

Aim of study: To investigate vaccination programs, parity, and calving season as factors affecting the risk of abortion and mummified fetuses in Holstein cows.

Area of study: Hot zone of Northeast Mexico.

Material and methods: Multiple logistic regression models were used to examine the relationship between peripartum disorders, parity, previous occurrence of abortion, season of calving, vaccination program, incidence of abortion, and mummified fetuses in Holstein cows.

Main results: For 7014 pregnancies (2886 cows), the percentage of cows aborting and having mummified fetuses was 17.7% and 1.1%, respectively. As the number of brucellosis vaccinations increased, the incidence of abortion increased (10.4% for a single vaccination and 38.0% for 6 accumulated vaccinations). Abortion for cows having 1-2 previous abortions (56%) and >2 abortions (77%) was fivefold and sevenfold greater ($p<0.01$), respectively, than that for cows without previous abortion. Other important risk factors for abortion were number of calvings (19.8% for nulliparous and primiparous vs. 13.8% for >3 parturitions; OR=1.7, $p<0.01$), leptospirosis vaccine application <55 days postpartum (dpp; OR=1.3, $p<0.05$), viral vaccine application >37 dpp (OR=1.3, $p<0.01$), brucellosis vaccine application >20 dpp (OR=1.6, $p<0.01$), and no application of clostridial vaccine (OR=3.7, $p<0.01$). Significant risk factors for mummified fetuses were application of ≥3 brucellosis vaccinations (OR=3.3, $p<0.01$), no application of 10-way clostridial vaccine (OR=2.3, $p<0.01$), >2 previous abortions (OR=18.4, $p<0.01$), and calving in autumn (OR=0.4, compared to winter, $p<0.05$).

Research highlights: Risk of abortion and mummified fetuses in Holstein cows has been found to be related to vaccination programs.

Additional key words: bovine abortion; clostridial vaccination; Brucella abortus RB51 vaccine; repeated abortion; Leptospira vaccine

Abbreviations used: dpc (days post-calving); dpp (days postpartum) OR (odds ratio); CI (confidence intervals).

Authors’ contributions: Data acquisition: ON, CAMH. Study design and drafted the manuscript: MM. Analyzed the results: JM, MM. Revised the manuscript and reviewed the pertinent literature: AFR, UMC, JEG, LAR. All authors read and approved the final version of the manuscript.

Citation: Mellado, M; Nájera, O; Mellado, J; García JE; Macías-Cruz, U; Rodríguez, AF; Meza-Herrera, C; Avendaño-Reyes L, 2021. Vaccination programs, parity, and calving season as factors affecting the risk of fetal losses and mummified fetuses in Holstein cows. Spanish Journal of Agricultural Research, Volume 19, Issue 3, e0402, 13 pages (2021)

Received: 11 Mar 2020. Accepted: 22 Jul 2021.

Copyright © 2021 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

Funding: The authors received no specific funding for this work.

Competing interests: All authors declare no actual or potential conflicts between the authors and other people or organizations that could inappropriately bias their work.

Correspondence should be addressed to Miguel Mellado: melladomiguel07@gmail.com

Introduction

Reproductive efficiency plays a crucial part in the profitability of intensive dairy operations, as it affects milk yield and the productive lifetime of dairy cows which affects the net returns in dairy herds (De Vries, 2006). Components of reduced reproductive performance of high-milk yielding cows in intensive dairy operations include delayed resumption of ovulation after parturition (Crowe et al., 2014; Santos et al., 2016), lower expression of estrus signs (Madureira et al., 2015), uterine health (Krause et al., 2014; Moore et al., 2014), lower pregnancy rates to first and subsequent inseminations (Flores et al., 2019), increased incidence of embryonic death (Diskin et al., 2016) and abortion (Mellado et al., 2016, 2019). The latter is very costly because it leads to significant economic losses to dairy producers. After all, rebreeding of aborted cows result in long calving intervals, loss of calf returns, culling of cows, and the replacement cost if the cow is culled (Lee & Kim, 2007).

An adequate vaccination program may avoid the infectious causes of abortion; however, non-infectious
causes of abortion in bovines are more difficult to prevent in intensive dairy herds (Grimard et al., 2006). Non-infectious causes of abortion include high milk production (Grimard et al., 2006; Mellado et al., 2019), heat stress (Garcia-Ispierto et al., 2006; Mellado et al., 2016), parity (Labèrnia et al., 1996; López-Gatius et al., 2009), previous postpartum disorders (López-Gatius, 2012) and twin pregnancies (López-Gatius et al., 2004; Mellado et al., 2019).

Fetal mummification does not account for a substantial loss in earnings in intensive dairy farms; however, this gestational disorder is a cause of failure to yield a calf per year per cow and imposes economic loss by extending the inter-calving period, the occurrence of fetal loss, and treatment to remove the mummified fetus, which frequently includes a cesarean section (Dutt et al., 2018). Therefore, it is essential to know the causes of mummified fetuses to help rule out particular situations, increasing this reproductive disorder's risk. Various risk factors have been described for bovine abortion/fetal loss (Mellado et al., 2016, 2019); however, risk factors associated with vaccination programs in adult dairy cows are unknown. Therefore, the objective of this investigation was to discern whether vaccination programs under field conditions and in a hot environment, parity, previous abortion, time from calving to vaccination, and calving season would influence the risk for abortion or mummified fetuses. The hypothesis was that adult cows repeatedly vaccinated against brucellosis, the time from calving to vaccination against abortifacient organisms, and cows with previous abortions would increase the risk of future abortion. Also, we hypothesized that season and time from calving to application of vaccinations would increase the occurrence of mummified fetuses. These hypotheses are proposed because it is plausible to expect that repeated cases of abortion in cows (Keshavarzi et al., 2017), greater parity (Norman et al., 2012), late gestation during warm months (Norman et al., 2012), and repeated inoculation against Brucella abortus (Sanz et al., 2010) will increase the risk of the premature expulsion of the fetus.

Material and methods

Animals and herd management

The experimental procedures and animal care conditions were approved by the Ethics Committee of the Research Department of the Autonomous Agrarian University Antonio Narro (protocol 42520-3001-2258). Holstein cows from a single large commercial dairy farm (3000 milking animals) located in a hot-arid environment of northern Mexico (25° N, 103° W, elevation 1140 m, mean annual rainfall 230 mm, mean annual temperature 23.7 °C) were included in this retrospective study. The herd’s annual daily milk yield was 34 kg/day, and the mean annual culling rate was 34%. Due to the low fertility of this herd because of the high ambient temperature, abortion was not a cause for culling, and therefore, cows often presented extended lactations (>500 days). Thus, two abortions never occurred in the same lactation.

A total of 7014 pregnancies from 2886 cows were included in this retrospective investigation from 2016 to 2019. Cows were housed in open-dirt pens equipped with a fixed metal framework shades in the center of the pens and additional shades covering the feed alleys. Lactating cows were fed total mixed rations twice per day, and ≈5% of feed refusals were removed immediately before each morning feeding.

Calves were open bucket fed and had access to free-choice water and pelleted calf starter. Bodyweight was registered with a mechanic scale at birth before colostrum ingestion and at weaning. Female calves were vaccinated subcutaneously with a standard label dose (10 × 10⁶ CFU) of brucellosis vaccine strain RB51 (MSD Salud Animal Mexico, Mexico City, Mexico) at ≈4 months of age (this vaccination was considered for getting the sum of the total number of vaccinations against B. abortus applied to cows). All animals included in this investigation were revaccinated against brucellosis every year before the first breeding post-calving. It has been suggested that booster RB51 vaccination is required between 4 and 5 years of age to maintain high levels of protection after calfhood vaccination (Olsen & Stoffregen, 2005), although no improvement in the immunological response resulting from RB51 revaccination has been demonstrated in adult cattle (Dorneles et al., 2014). The practice of annual revaccination against B. abortus in northern Mexico arises from a widespread dogma among veterinarians involved in dairy practice who have come to believe that repeated vaccinations against B. abortus strengthen the immunity of cows against this bacteria.

Some cows were vaccinated with a 10-way clostridial vaccine (toxoids of Clostridium perfringens types A, B, C and D, C. septicum, C. sordelli, C. novyi, C. haemolyticum, C. tetani, and inactivated cells of C. chauvoei; MSD Salud Animal México, Mexico City) previously to the first breeding and previously to the first service after calving. No particular criteria existed for applying this vaccine; inventory availability dictated the use of this vaccine.

All animals were vaccinated annually 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 DF, Mexico). Cows were also annually vaccinated against leptospirosis
(5-serovars; LEPTAVOID-H®; Merck Sharp & Dohme Corp., Mexico DF) 30 days postpartum (dpp). All cows were periodically tested for brucellosis, and seropositive reactors to the card tests were culled. The herd prevalence of brucellosis was 1.5%, and none of the seropositive animals were included in the investigation.

Estrus was detected by direct observation and with the aid of tail chalk that was applied daily. After a voluntary waiting period of 50 dpp, cows in estrus were artificially inseminated following the a.m./p.m. guideline. Controlled breeding programs (Ovsynch) were used in all repeat-breeding animals. Commercial frozen-thawed semen from 69 high genetic merit bulls from the USA was used. Pregnancy diagnoses were performed at 45±3 days from their last recorded AI by the herd veterinary. Second palpation was carried out between 105 and 145 d following insemination.

Loss of pregnancy between 42-48 (pregnancy diagnosis by palpation per rectum) and 260 days of gestation was considered an abortion (Mee, 2020). Most of these cows displayed expelled recognizable lifeless fetuses, had the presence of extraembryonic membranes and vaginal discharges, or resulted negative to the second palpation displayed expulsed recognizable lifeless fetuses, had considered an abortion (Mee, 2020). Most of these cows sis by palpation per rectum) and 260 days of gestation was following insemination.

Abortion in Holstein cows as a function of vaccination programs

Statistical analyses

The response variable for the cow-level risk factors was binomial, with cows classified as presenting abortion or mummified fetuses or having normal parturition. A screening process to detect explanatory candidate variables (p≤0.15) was performed using univariate logistic regression analyses using SAS (SAS Inst. Inc., Cary, NC, USA) to subsequently build a multivariate model. The preliminary models contained the following potentially explanatory variables: number of brucellosis vaccinations (1, 2-3, >3) per cow, parity (1, 2-4, >4), days from delivery to leptospirosis vaccination (≤55 and >55 days), the interval from calving to various virus vaccination (≤37 and >37 days), days from delivery to brucellosis vaccination (≤20 and >20 days), application of 10-way clostridial vaccine (yes vs. no), occurrence of puerperal metritis (yes vs. no), number of previous abortions (0, 1-2, >2), season of delivery, age at first calving (≤700 and >700 days), birth weight (≤37 and >37 kg), weaning weight (≤77 and >77 kg), occurrence of ketosis (yes vs. no), dystocic parturition (yes vs. no) and season of calving (winter months being January–March; spring, April–June; summer, July–September; and fall, October–December). Also, two-way interactions were included in the model. The cut-off values for the time of postpartum application of various vaccinations were based on the mean interval between calving and vaccination (values above and below the mean).

Because gestations of the same cow cannot be considered independent events, for cows with various pregnancies, the sampling unit (pregnancy) was nested within cows to account for the cluster effect. Analysis of putative risk factors was performed using PROC GLIMMIX of SAS. The multivariate models were assessed using a binary distribution for the occurrence of abortion and mummified fetuses. The main confounders identified were the number of calvings per cow and the previous number of abortions; therefore, this set of baseline variables was included in the multivariate model for controlling these confounders. Additional covariates that caused effect modification of explanatory variables were added to the model based on significant interactions. All covariates included in the model were significant at p<0.05. Cow within gestation number was a random effect, and other main effects and their interactions were considered fixed.

Multivariate mixed logistic regression models using the GLIMMIX procedure produced odds ratios (OR) as estimates of the strength of association between the potential risk factors and the reproductive disorders studied. The Cochran-Armitage trend test (PROC FREQ of SAS) was used to test the null hypothesis that abortion is independent of days of gestation. The cumulative probability of pregnant cows aborting to defined times of pregnancy was determined using Kaplan-Meier survival curves produced with the Statgraphics Centurion XV statistical software (Statpoint Technologies Inc., Warrenton, VA, USA). Finally, the association between the number of B. abortus vaccinations received per cow and the abortion rate was assessed using the CurveExpert Professional 2.5.6
software (Hyams Development, Madison, AL, USA). For all statistical analyses, values with \( p<0.05 \) were regarded as statistically significant.

**Results**

**Occurrence of abortion**

The proportion of pregnant cows presenting abortion from 2016 to 2019 was 17.7 (1242/7014). The median time from calving to abortion was 129 days with two well-defined (\( p<0.01 \)) peaks at around 86 and 168 days of gestation (Fig. 1). The risk factors adjusted for parity and previous abortion for current abortion are listed in Table 1. Throughout their productive life, cows with \( \geq 2 \) brucellosis vaccinations had 7.4 times more risk of abortion as had cows with one vaccination against *B. abortus* (adjusted for the effects of parity, previous abortion, annual clostridial vaccination, and days to postpartum leptospirosis vaccination; \( p<0.01 \)). The impact of repeated vaccination against brucellosis on the occurrence of abortions was more pronounced for cows receiving five or six vaccinations (Fig. 2). Furthermore, the interval from breeding to abortion depended on the number of *B. abortus* vaccinations applied. Cumulative *B. abortus* vaccinations were associated with a decreased interval from breeding to abortion (Fig. 3).

There was an interaction between the number of brucellosis vaccinations applied to cows and the pre-breeding vaccination of cows for leptospirosis (\( p<0.01 \)) for the incidence of abortions. The proportion of abortions of unknown cause was twice as much in cows receiving the leptospirosis vaccine >55 d post-calving in cows with two or more *B. abortus* vaccine applications compared with that observed in cows receiving the annual leptospirosis booster vaccine >55 days post-calving (dpc) but with single brucellosis vaccination.

Also, there was a significant interaction detected between the number of brucellosis vaccinations per cow and the application of the clostridial vaccine. A remarkable low (0.7%; \( p<0.01 \)) incidence of abortion was observed in cows with >3 application of brucellosis vaccine and given the multicomponent clostridial vaccine compared with cows getting a single *B. abortus* RB51 vaccine and receiving the clostridial vaccine (7.7%). The interaction between the number of brucellosis vaccinations per cow and parity was significant (\( p<0.05 \)). Despite the interdependence between these variables (the number of vaccinations against *B. abortus* increased linearly with parity), the interaction occurred because abortion rate followed a cubic trend relative to parity.

Comparisons among the different parity groups showed that nulliparous and primiparous cows were 1.6 more likely (\( p<0.01 \)) to have an abortion than multiparous cows (Table 1). Cows vaccinated against leptospirosis <55 dpp were 3.4 more likely to have an abortion when compared with cows immunized against this disease >55 dpp (Table 1). There was a significant interaction between dpc vaccination against leptospirosis and the occurrence of previous abortion. Abortions were 56.5% for cows with >2 abortions receiving the leptospirosis vaccination <55 dpc compared with 82.8% for cows with >2 abortions receiving the leptospirosis booster vaccine >55 dpc.

Cows vaccinated <37 dpp against various viral diseases-causing abortion had a lower (\( p<0.01 \)) risk of abortion relative to cows vaccinated >37 dpc (Table 1). There was a significant interaction of viral vaccine applied at different times post-calving with previous abortion: cows with two previous abortions that received the viral vaccination <37 dpp had a reduced risk of abortion (OR=0.35; \( p<0.01 \)) compared with cows with two previous abortions and receiving the viral vaccination >37 dpp.

Cows vaccinated >20 dpc against brucellosis had 1.8 times more risk of abortion than cows vaccinated <20 dpp (\( p<0.01 \)). Cows no receiving clostridial vaccines (adjusted for parity and number of previous abortions) had nearly 4 times the risk of abortion as had cows receiving this vaccine (Table 1). There was a significant interaction of the application of the clostridial vaccine with the occurrence of previous abortion: cows that received the clostridial vaccine with two previous vaccinations against brucellosis had a reduced (\( p<0.01 \)) risk of abortion (OR=0.37) than cows receiving the clostridial vaccine with one or >3 previous vaccinations against brucellosis.

Cows not experiencing puerperal metritis had a reduced risk of abortion as had cows free of this postpartum disease (Table 1). Previous abortions had the strongest association with subsequent abortions (adjusted for days to...
Abortion in Holstein cows as a function of vaccination programs

Variables | Abortions, n (%) | Odds ratio (OR) | 95% CI OR | p
--- | --- | --- | --- | ---
Brucellosis vaccinations per cow\(^1,2,3,4\) | | | | <0.0001
>3 | 559/2556 (21.9) | 7.4 | 5.7 – 9.8 | |
2-3 | 430/2028 (21.2) | 4.3 | 3.3 – 5.6 | |
≤2 | 253/2430 (10.4) | Reference | | |
Parity | | | | <0.0001
Nulliparous and primiparous | 905/4571 (19.8) | 1.6 | 1.2 – 2.1 | |
Two to three parturitions | 169/1214 (13.9) | 1.0 | 0.8 – 1.3 | |
>3 parturitions | 168/1229 (13.7) | Reference | | |
Days postpartum leptospirosis vaccination\(^5,6\) | | | | 0.0272
<55 | 1163/6450 (18.0) | 3.4 | 2.4 – 4.7 | |
>55 | 84/585 (14.4) | Reference | | |
Days postpartum viral vaccine\(^7,8\) | | | | <0.0001
<37 | 754/4644 (16.2) | 0.6 | 0.5 – 0.7 | |
>37 | 488/2370 (20.6) | Reference | | |
Days postpartum to B. abortus vaccine | | | | <0.0001
>20 | 815/3985 (20.4) | 1.8 | 1.5 – 2.1 | |
<20 | 427/3029 (14.1) | Reference | | |
Application of 10-way clostridial vaccine\(^9,10\) | | | | <0.0001
No | 1103/5041 (21.9) | 3.7 | 3.1 – 4.5 | |
Yes | 139/1973 (7.1) | Reference | | |
Puerperal metritis | | | | 0.0035
No | 1130/6517 (17.3) | 0.72 | 0.6 – 0.9 | |
Yes | 112/497 (22.5) | Reference | | |
Number of previous abortions | | | | <0.0001
>2 | 218/283 (77.0) | 26.1 | 19.6 – 34.8 | |
1-2 | 323/577 (56.0) | 9.9 | 8.2 – 11.9 | |
0 | 701/6154 (11.4) | Reference | | |

\(^1\) *Brucella abortus* strain RB51 vaccine, including the vaccine applied at four months of age.

Significant (*p*<0.05) interactions: \(^2\)number of brucellosis vaccinations per cow × postpartum leptospirosis vaccination; \(^3\)number of brucellosis vaccinations per cow × application of the clostridial vaccine; \(^4\)number of brucellosis vaccinations per cow × parity.

Significant (*p*<0.05) interactions: \(^5\)days post-calving vaccination against leptospirosis × occurrence of previous abortion; \(^6\)days postpartum for leptospirosis vaccination × occurrence of previous abortion.

\(^7\) Significant (*p*<0.05) interactions of time of viral vaccination post-calving × occurrence of previous abortion.

\(^8\) Significant (*p*<0.05) interactions of application of clostridial vaccine × occurrence of previous abortion.

\(^9\) Significant (*p*<0.05) interactions of application of clostridial vaccine × occurrence of previous abortion.

\(^10\) Significant (*p*<0.05) interactions of application of clostridial vaccine × occurrence of previous abortion.

leptospirosis vaccination post-calving, number of brucellosis vaccinations applied during the productive life, parity, and dpp to brucellosis vaccination), which indicates that some cows are predisposed to recurrent abortion. The interaction between previous abortion and dpp of leptospirosis vaccination was significant (*p*<0.01). The occurrence of previous abortion by-number of anti-brucellosis vaccinations was significant (*p*<0.05) for abortion, indicating that cows with more than one previous abortion had a higher abortion rate than cows with just one abortion.
The incidence of mummified fetuses was 1.1% (77/7014). The risk factors for mummified fetuses (adjusted for parity and number of previous abortions) are listed in Table 2. The odds of mummified fetuses in cows with >2 Brucella abortus S19 vaccine were three times higher (p<0.01) than cows receiving a single vaccination. In addition, there was an interaction between number of brucellosis vaccinations per cow and the annual boosting leptospirosis vaccination (p<0.05).

Cows no receiving clostridial vaccines were four times more likely (p<0.01) to present mummified fetuses than cows immunized against these bacteria (Table 2). Cows no receiving bovine leptospirosis vaccines postpartum had about half the risk (p<0.01) of presenting mummified fetuses than cows receiving this vaccine. As in the case of abortion, the higher the number of previous abortions, the higher (p<0.01) the risk of mummified fetuses. Calving in autumn decreased (p<0.05) the risk of mummified fetuses (Table 2). Cows with a single vaccination against brucellosis had half the chance of presenting mummified fetuses than cows repeatedly vaccinated against this disease.

**Discussion**

**Occurrence of abortion**

Abortion in the present study was defined as the loss of a fetus that occurs from the moment of pregnancy diagnosis by palpation per rectum until 260 days of gestation (the point at which the fetus can survive outside the uterus). We caution about this definition because there are numerous abortion definitions used internationally, including early ultrasonographic pregnancy diagnosis (<30 days).

This study showed that despite the sound vaccination program for reproductive diseases and protection of the developing fetus, abortions of unknown cause in this farm were two to three-fold higher than the abortion/fetal loss rates of 1.3-5.0% reported in North-American (Forar et al., 1995; Joussan et al., 2005; Norman et al., 2012), 1.7-10.3% found in European (Mee, 1992; Andreu-Vázquez et al., 2012; Barański et al., 2012), 6-7% in New Zealand and Australian (Norton et al., 1989; McDougall et al., 2005) and 6.9% observed in Asian (Lee & Kim, 2007) intensive dairy herds. Nonetheless, abortions observed in the present investigation are close to those observed in Holstein herds, where the etiology of abortions was not investigated in the temperate zone of Mexico (24-29%; Albuja et al., 2019; Mellado et al., 2019).

Abortions had bimodal days of gestation distribution. The first abortion peak around 86 days of pregnancy agrees with previous studies, where the occurrence of abortions of unknown etiology peaks during the first three months of pregnancy (Albuja et al., 2019). However, twinning has been a significant contributor to pregnancy loss during this period (López-Gatius et al., 2004). The second peak of abortions occurred in the second trimester of gestation, which agrees with García-Ispierto & López-Gatius (2019), who observed the highest occurrence of abortion between 135 and 154 days of pregnancy, being unicorunal twins a significant cause of fetal losses during this period. In the present study, abortions were likely due to many bacterial, viral, protozoan, and fungal infections because bovine abortion during mid-to-late-gestation is primarily due to infectious causes (Anderson, 2007; Reichel et al., 2018).

For a given pregnancy, abortions were higher among cows who previously had this reproductive disorder than those who had not. These results align with Markusfeld-Nir (1997) observations, where the proportion of aborted cows...
Abortion in Holstein cows as a function of vaccination programs

Abortion happens due to many causes, although infections are the most common origin of this reproductive disorder (Wolf-Jäckel et al., 2020). Some infectious abortions by one single pathogen only occur once in the cow’s life due to acquired immunity after their first abortion (Megid et al., 2010). Yet, Neospora caninum is now recognized as the most common cause of repeated abortions in cattle. In the present study, few cows were culled because of abortion; thus, the permanence of aborting cows in the herd, possibly due to N. caninum, increased the abortion risk in subsequent pregnancies. The explanation for this response could be the endemic infections of N. caninum in cattle populations of Mexico (García-Vázquez et al., 2005; Medina-Esparza et al., 2018), as chronic seropositive cows to this protozoa are reported to have recurrent abortions (Corbellini et al., 2006; Pabón et al., 2007). Given that N. caninum does not induce abortion below day 90 of pregnancy (Wilson et al., 2016; Melendez et al., 2020), abortions before 90 days of pregnancy could have resulted from advanced age, chronic endometritis, luteal phase deficiency, uterine anomalies, unicorneal twining or abnormal immunologic response.

Annual revaccination of adult non-pregnant cattle with Brucella abortus strain RB51 markedly increased abortions in cows, suggesting that this vaccine should not be applied annually. Even though retrospective analysis has methodological limitations, these data were abundant, complete, balanced, and accurate on both the risk and outcome factor to answer the study question; therefore, it is considered that these data provide valid information about causal effects of factors used in this study using observational evidence.

Fluegel Dougherty et al. (2013) found that vaccination of adult pregnant beef cattle with B. abortus RB51 resulted in 5.3% pregnancy losses. Likewise, field reports have shown the brucellosis vaccine strain RB51 in

Table 2. Final multivariate logistic regression model for factors associated with the occurrences of mummified fetuses in high-yielding Holstein cows in a hot arid environment.

| Variables                                      | Mummified fetuses, n (%) | Odds ratio (OR) | 95% CI OR     | p          |
|------------------------------------------------|--------------------------|-----------------|---------------|------------|
| Number of brucellosis vaccinations per cow¹   |                          |                 |               | <0.001     |
| >3                                             | 36/2556 (1.4)            | 3.0             | 1.2–7.0       |            |
| 2-3                                            | 30/2028 (1.5)            | 3.3             | 1.7–6.6       |            |
| 1                                              | 11/2430 (0.5)            | Reference       |               |            |
| Application of 10-way clostridial vaccine²     |                          | 0.0005          |               |            |
| No                                             | 70/5041 (1.4)            | 4.0             | 1.8–8.6       |            |
| Yes                                            | 7/1973 (0.4)             | Reference       |               |            |
| Leptospirosis vaccination³                      |                          | 0.0284          |               |            |
| No                                             | 8/1456 (0.6)             | 0.4             | 0.2–0.9       |            |
| Yes                                            | 69/497 (1.2)             | Reference       |               |            |
| Number of previous abortions                   |                          | <0.0001         |               |            |
| >2                                             | 27/283 (9.5)             | 20.2            | 12.0–34.2     |            |
| 1-2                                            | 15/577 (2.6)             | 3.7             | 1.9–7.1       |            |
| 0                                              | 35/6154 (0.6)            | Reference       |               |            |
| Season of delivery                             |                          | 0.0425          |               |            |
| Spring vs. winter                              | 1.51 vs. 1.44            | 1.1             | 0.5–2.1       |            |
| Summer vs. winter                              | 1.44 vs. 1.44            | 1.0             | 0.5–1.9       |            |
| Autumn vs. winter                              | 0.62 vs. 1.44            | 0.4             | 0.2–0.8       |            |

¹ Brucella abortus strain RB51 vaccine, including the vaccine applied at four months of age. ² Toxoids of Clostridium perfringens types A, B, C and D, C. septicum, C. sordelli, C. novyi, C. haemolyticum, C. tetani, and inactivated cells of C. chauvoei. ³ Number of brucellosis vaccinations per cow × annual boosting leptospirosis vaccination interaction detected (p<0.05).
the abortion of some cows (Yazdi et al., 2009; Sanz et al., 2010). Thus, it could be that the attenuated strain *B. abortus* RB51 could be harbored in non-pregnant cows, and once pregnancy is established, *B. abortus* can cause fetal infection and abortion, as it has been reported by Van Metre et al. (1999) and Yazdi et al. (2009). Thus, another explanation for the harmful effects of repeated RB51 immunization is that the recurrent use of the RB51 vaccine could increase the risk of reversion of this attenuated strain RB51 vaccine to wild-type virulence.

Hitherto no extensive study has proven the vaccine's responsibility against *B. abortus* strain RB51. This investigation shows strong evidence that annual revaccination against this disease is not safe for non-pregnant Holstein cows in early lactation. This study also indicates that the accumulation of *B. abortus* vaccinations markedly increases the risk of abortion and triggered abortion earlier in pregnancy. These results suggest that this live *B. abortus* strain RB51 vaccine applied repeatedly can cause abortion. These results would be similar to the live vaccine strain 1B of *Chlamydia abortus* used to prevent abortion in small ruminants, which may enter circulation and cause abortion in some vaccinated animals (Longbottom et al., 2018). It could be that the reaction of cows to repeated vaccinations against *B. abortus* is not due to the agent but to an immune-mediated response by susceptible cows to adverse reactions to vaccines.

Additionally, revaccination with *B. abortus* RB51 after 20 dpp increased the risk of fetal loss in cows, compared to cows vaccinated <20 dpc. This greater incidence of *B. abortus* RB51-induced abortion in cows revaccinated closer or during breeding indicates that vaccination of adult non-pregnant cattle with RB51 does not seem advisable if more than 20 days have elapsed between calving and vaccination. This response is unknown, but perhaps the depression of the immune system in high-yielding cows during the transition period (Esposito et al., 2014) could have contributed to the higher *B. abortus* RB51-induced abortion (Uzal et al., 2000; Fluegel Dougherty et al., 2013). Further analyses of the association of abortions and cows vaccinated after 20 dpp are required to clarify this relationship.

In the present study, the time of postpartum immunization against viruses linked to abortion was an important factor affecting this reproductive disorder. Possibly, impairment of the immune response in the immediate postpartum period of dairy cows (Mann et al., 2018), which coincided with revaccination (>37 dpp), is cause-and-effect related. Unfortunately, few data are available from controlled clinical trials that have assessed the efficacy of this vaccine for protection against abortion in adult cows. Fetal immunity against viral infection is not absolute in vaccinated animals (Van Campen et al., 2000). Other reports have demonstrated insufficient fetal protection of inactivated viral vaccines (Grooms, 2004; Rodning et al., 2010). Thus, these results suggest that the appropriate window to vaccinate cows postpartum and pre-breeding against viral diseases linked to abortion is <37 dpc. This is so because the onset of immunity from inactivated virus vaccines may be delayed four to six weeks from the time of initial vaccination, which may result in vaccination failure (Newcomer et al., 2017).

An interesting finding of this investigation was that, for a given gravidity, abortions were lower among cows that were revaccinated against different *Clostridium* species compared with cows that did not receive this vaccine. This finding has to be seen in light of some limitations of the present study, such as whether vaccination or not vaccination against clostridial infections was not controlled at all. Most commercial clostridial vaccines recommend annual revaccination in cattle, but this recommendation is largely ignored (Uzal, 2012). Results of the present investigation suggest that the antibody responses elicited by the 10-way clostridial vaccine somehow reduced the incidence of abortion. Antibody titers against clostridial antigens have been considered the primary connection of vaccine-induced protection against these infectious diseases. Given that antibody levels after applying clostridial vaccines remain above the minimal level required for protection only for one year, annual booster vaccinations are required to protect against clostridial diseases. In the present study, the protection of this vaccine apparently extended to the safety of the growing fetus while in the uterus. However, causality between clostridial diseases and abortion in ruminants has not been established (Anderson, 2007; Borel et al., 2014; Vidal et al., 2017). Thus, probably cross-reactivity (immune response against abortifacient organisms not specifically targeted by the vaccine antigen) and cross-protection (defense against non-vaccine microorganism varieties) could have happened as it is the case of protection against *M. leprae* by *M. tuberculosis*, or protection against *N. gonorrhoeae* by *M. meningitis*, just to mention a few examples (Vojtek et al., 2019).

Cow parity was associated with the occurrence of abortions in this investigation. The incidence of abortion was greatest in primiparous cows, contrary to observations of Thurmond et al. (1990) and Lee & Kim (2007), who found that fetal losses were higher in cows with three or more parturitions. Other studies have reported no effect of parity on pregnancy losses (Labernia et al., 1996; Moore et al., 2005), whereas observations of Jousan et al. (2005) and Gehrke & Zbylut (2011) show that the highest risk of pregnancy/fetal loss occurs in first or second-lactation cows. The contrasting results among studies on this matter seem to be due to the different definitions of pregnancy loss, the methods of pregnancy diagnosis (manual, laboratory, ultrasound), the criteria used for time of fetus mortality, and the signs used to diagnose abortion (late-term abortions are the easiest to notice, but abortions
Abortion in Holstein cows as a function of vaccination programs

Spanish Journal of Agricultural Research September 2021 • Volume 19 • Issue 3 • e0402

can happen at any stage of pregnancy). It is worth noting that despite the higher abortion rate in nulliparous/primiparous heifers, the abortion rate increased with the number of B. abortus vaccinations, which implies that the older the cows, the higher the abortion rate. This apparent contradiction may be due to uncontrolled interferences exerted by other factors included in the model, or that heifers are more susceptible to infections leading to abortion compared with primiparous and multiparous cows (Alfieri & Alfieri, 2017).

Cows that received the pentavalent leptospira vaccine >37 dpp had an increased risk of fetal loss than cows vaccinated <37 dpc. However, given the observational nature of this study, the occurrence of this association does not necessarily imply causation. Therefore, limitations exist in the present observational research in assessing causal associations. For dairy cows, energy balance during the postpartum period directly influences fertility and susceptibility to infectious disease (Mulligan & Doherty, 2008). Therefore, it is believed that the loss of body condition score peripartum (>37 dpp; Chebel et al., 2018) was a predisposing factor associated with a lesser response of cows to the leptospira vaccine, which apparently resulted in a greater occurrence of abortion.

The occurrence of puerperal metritis was a risk factor associated with abortion. However, this association is not clear, as cows with metritis treated with antimicrobial therapy present a high recovery rate from this disease (Ornell et al., 2016). Additionally, uterine infections in postpartum dairy cows may interrupt successful reproduction at several crucial stages, although these detrimental effects do not proceed beyond the early embryo development (Gilbert, 2012). Puerperal metritis could be mediated by a diminished ovarian function altered by bacterial products or inflammatory agents acting directly on the ovary or indirectly on the hypothalamus or pituitary gland (Sheldon et al., 2006; Cheong et al., 2017). This hampered ovarian function can cause smaller dominant follicles and corpus luteum, with the consequent lower blood estradiol and progesterone concentrations (Williams et al., 2007) which could ultimately affect early fetus survival.

Mummified fetuses

So far, no specific causes for the mummification of bovine fetuses have been recognized (Drost, 2007), basically because of tissue degeneration and autolysis. However, in the present study, it was found that the risk of the occurrence of mummified fetuses increased with increasing the number of B. abortus vaccinations applied to adult animals. This response is intriguing. Given that fetal mummification occurs after the development of the placenta and fetal ossification (after 70 days of pregnancy), it could be that attenuated B. abortus bacteria reacti
tivated after the third month of pregnancy and infected maternal and fetal tissues, as has been the case of previous studies in cattle (Palmer et al., 1996; Fluegel Dougherty et al., 2013).

It was found a markedly increased risk of mummified fetuses in those cows receiving the bovine clostridial vaccine. However, this association may not be as straightforward as it may appear, considering that this was not a randomized controlled trial. However, several studies assessing agreement between similar hypotheses tested using randomized controlled trials compared to observational designs have found that agreement between the two is high (Boyko, 2013). The exact manner of the harmful effect of this multi-way clostridial vaccine is unknown. It could be that the inactivated toxins (toxoid) used to immunize cows against a wide variety of Clostridium species could reach the uterus causing fetal demise and subsequent fetal mummification without causing further contamination and irritation of the uterus. Although Clostridium species are not considered abortifacients, reports in foals (Ortega et al., 2007), mink (Hammer et al., 2017), and sheep (Brozos et al., 2012) have implicated these microorganisms in omphalitis and abortion. Of interest, the time of vaccination post-calving against leptospirosis, various viral diseases, and brucellosis also had higher risks of developing subsequent mummified fetuses.

Conclusions

Repeated postpartum B. abortus RB51 vaccination in adult Holstein cows was not associated with a reduced abortion and mummification rate. Yet, it was associated with a raised risk of these reproductive disorders, emphasizing the importance of modifying vaccination protocols of cows. These findings are of great practical significance because they contradict the view that revaccination of cows increases immunity against this disease. The reasons for the repeated B. abortus RB51 vaccination associated with increased risk for abortion warrant further research. The number of previous abortions was the most crucial risk factor for abortion. Further research to understand the cause of these repeated abortions is highly needed.

References

Albuja C, Ortiz O, López C, Hernández-Cerón J, 2019. Economic impact of pregnancy loss in an intensive dairy farming system. Vet Méx 6(1): 1-8. https://doi.org/10.22201/fmvz.24486760e.2019.1.572

Alfieri AA, Alfieri AF, 2017. Infectious diseases that impact the bovine reproduction. Rev Bras Reprod Anim 41: 133-139.
Anderson ML, 2007. Infectious causes of bovine abortion during mid-to late-gestation. Theriogenology 68: 474-486. https://doi.org/10.1016/j.tleriogenology.2007.04.001

Andreu-Vázquez C, Garcia-Ispierto I, Ganau S, Fricke PM, López-Gatius F, 2012. Effects of twinning on the subsequent reproductive performance and productive lifespan of high-producing dairy cows. Theriogenology 789: 2061-2070. https://doi.org/10.1016/j.tleriogenology.2012.07.027

Barański W, Zduńczyk S, Janowski T, 2012. Embryonic and foetal losses in eight dairy herds in north-east Poland. Polish J Vet Sci 15: 735-739. https://doi.org/10.2478/v10181-012-0112-5

Borel N, Frey CF, Gottstein B, Hilme B, Pospischil A, Franzoso FD, Waldvogel A, 2014. Laboratory diagnosis of ruminant abortion in Europe. Vet J 200: 218-229. https://doi.org/10.1016/j.vetj.2014.03.015

Boyko EJ, 2013. Observational research - Opportunities and limitations. J Diabetes Comp 27(6): 642-648. https://doi.org/10.1016/j.jdiacomp.2013.07.007

Brozos CN, Lazaridis L, Karagiannis I, Kiousis E, Tsousis G, Psychas V, Giadinis ND, 2012. Prolonged dystocia, uterine necrosis, and ovariohysterectomy in a Chios ewe. Tur J Vet Anim Sci 36: 211-213.

Chebel RC, Mendoça LGD, Baruselli PS, 2018. Association between body condition score change during the dry period and postpartum health and performance. J Dairy Sci 101: 4595-4614. https://doi.org/10.3168/jds.2017-13732

Cheong SH, Filho OGS, Absalon-Medina VA, Schneider A, Butler WR, Gilbert RO, 2017. Uterine and systemic inflammation influences ovarian follicular function in postpartum dairy cows. PLoS ONE 12(5): e0177356. https://doi.org/10.1371/journal.pone.0177356

Corbellini LG, Pescador CA, Frantz F, Wunder E, Smith DR, Driemeier D, 2006. Diagnostic survey of bovine abortion with special reference to Neospora caninum infection: Importance, repeated abortion and concurrent infection in aborted fetuses in Southern Brazil. Vet J 172: 114-120. https://doi.org/10.1016/j.tvjl.2005.03.006

Crowe MA, Diskin MG, Williams EJ, 2014. Parturition to resumption of ovarian cyclicity: comparative aspects of beef and dairy cows. Animal 8: 1-14. https://doi.org/10.1017/S1751731114000251

De Vries A, 2006. Economic value of pregnancy in dairy cattle. J Dairy Sci 89: 3876-3885. https://doi.org/10.3168/jds.S0022-0302(06)72430-4

Diskin M, Waters S, Parr M, Kenny D, 2016. Pregnancy losses in cattle: potential for improvement. Reprod Fertil Dev 28: 83-93. https://doi.org/10.1071/RD15366

Dorneles EM, Teixeira-Carvalho A, Araujo MS, Lima GK, Martins-Filho OA, Sriranganathan N, Lage AP, 2014. T lymphocytes subsets and cytokine pattern induced by vaccination against bovine brucellosis employing S19 calfhood vaccination and adult RB51 revaccination. Vaccine 32: 6034-6038. https://doi.org/10.1016/j.vaccine.2014.08.060

Drost M, 2007. Complications during gestation in the cow. Theriogenology 68: 487-491. https://doi.org/10.1016/j.tleriogenology.2007.04.023

Dutt R, Dalal J, Singh G, Gahalot SC, 2018 Management of fetal mummification/maceration through left flank caesarean section in cows - study of four cases. Adv Anim Vet Sci 6: 12-16. https://doi.org/10.17582/journal.aaevs/2018/6.1.12.16

Esposito G, Irons PC, Webb EC, Chapwanya A, 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim Reprod Sci 144: 60-71. https://doi.org/10.1016/j.anireprosci.2013.11.007

Flores J, García JE, Mellado J, Gaytán L, De Santiago A, Mellado M, 2019. Effect of growth hormone on milk yield and reproductive performance of subfertile Holstein cows during extended lactations. Span J Agric Res 17 (1): e0403. https://doi.org/10.5424/sajar/201917113842

Fluegel Dougherty AM, Cornish TE, O’Toole D, Boerger-Fields AM, Henderson OL, Mills KW, 2013. Abortion and premature birth in cattle following vaccination with Brucella abortus strain RB51. J Vet Diag Invest 25: 630-635. https://doi.org/10.1177/1040638713499570

Forar A, Gay J, Hancok D, 1995. The frequency of endemic fetal loss in dairy cattle: a review. Theriogenology 43: 989-1000. https://doi.org/10.1016/0093-691X(95)00063-E

García-Ispierto I, López-Gatius F, Santolaria P, Yáníz JL, Nogareda C, López-Béjar M, De Rensis F, 2006. Relationship between heat stress during the peri-implantation period and early fetal loss in dairy cattle. Theriogenology 65: 799-807. https://doi.org/10.1016/j.theriogenology.2005.06.011

García-Ispierto I, López-Gatius F, 2019. Abortion in dairy cattle with advanced twin pregnancies: Incidence and timing. Reprod Dom Anim 54: 50-53. https://doi.org/10.1111/rrda.13510

García-Vázquez Z, Rosario-Cruz R, Ramos-Aragón A, Cruz-Vázquez C, Mapes G, 2005. Neospora caninum seropositivity and association with abortions in dairy cows in Mexico. Vet Parasitol 134: 61-65. https://doi.org/10.1016/j.vetpar.2005.07.007

Gehrke M, Zbylut J, 2011. Factors connected with pregnancy loss in dairy cows. Bull Vet Inst Pulawy 553: 13842

Gilbert RO, 2012. The effects of endometritis on the establishment of pregnancy in cattle. Reprod Fert Dev 24: 252-257. https://doi.org/10.1071/RD11915
Abortion in Holstein cows as a function of vaccination programs

Grimard B, Freret S, Chevallier A, Pinto A, Ponsart C, Humblot P, 2006. Genetic and environmental factors influencing first service conception rate and late embryonic/foetal mortality in low fertility dairy herds. Anim Reprod Sci 91: 31-44. https://doi.org/10.1016/j.anireprosci.2005.03.003

Grooms DL, 2004. Reproductive consequences of infection with bovine viral diarrhea virus. Vet Clin N Am-Food A Prac 20: 5-19. https://doi.org/10.1016/j.cvfa.2003.11.006

Hammer AS, Andresen L, Aalbæk B, Damborg P, Weiss V, Christiansen ML, Selsing S, Bahl MI, 2017. Abortion and mortality in farm mink Neovison vison associated with feed-born Clostridium limosum. Vet Microbiol 203: 229-233. https://doi.org/10.1016/j.vetmic.2017.03.017

Jousan FD, Drost M, Hansen PJ, 2005. Factors associated with early and mid-to-late fetal loss in lactating and nonlactating Holstein cattle in a hot climate. J Anim Sci 83: 1017-1022. https://doi.org/10.2527/2005.8351017x

Keshavarzi H, Sadeghi-Sefidmazgi A, Kristensen AR, Stygar AH, 2017. Abortion studies in Iranian dairy herds: I. Risk factors for abortion. Livest Sci 195: 45-52. https://doi.org/10.1016/j.livsci.2016.11.004

Krause ART, Pfeifer LFM, Montagner P, Weschenfelder MM, Schwegler E, Lima ME, et al., 2014. Associations between resumption of parturition ovarian activity, utherine health and concentrations of metabolites and acute phase proteins during the transition period in Holstein cows. Anim Reprod Sci 145: 8-14. https://doi.org/10.1016/j.anireprosci.2013.12.016

Kumar A, Saxena A, 2018. Clinical management of fetal mummification in a cow: a case report. Ind Vet J 95: 81-82.

Labernia J, López-Gatius F, Santolaria P, López-Béjar M, Rutllant J, 1996. Influence of management factors on pregnancy attrition in dairy cattle. Theriogenology 45: 1247-1253. https://doi.org/10.1016/0093-691x(96)00079-9

Lee JI, Kim IH, 2007. Pregnancy loss in dairy cows: The contributing factors, the effects on reproductive performance and the economic impact. J Vet Sci 83: 283-288. https://doi.org/10.4142/jvs.2007.8.3.283

Lefebvre RC, Saint-Hilaire É, Morin I, Couto GB, Francoz D, Babkine M, 2009. Retrospective case study of fetal mummification in cows that did not respond to prostaglandin F2α treatment. Can Vet J 50: 71-76.

Longbottom D, Sait M, Livingstone M, Laroucau K, Sachse K, Harris SR, Thomson N, Seth-Smith, HMB, 2018. Genomic evidence that the live Chlamydia abortus vaccine strain 1B is not attenuated and has the potential to cause disease. Vaccine 25: 3593-3598. https://doi.org/10.1016/j.vaccine.2018.05.042

López-Gatius F, 2012. Factors of a non-infectious nature affecting fertility after artificial insemination in lactating dairy cows. A review. Theriogenology 77: 1029-1041. https://doi.org/10.1016/j.theriogenology.2011.10.014

López-Gatius F, Santolaria P, Yániz JL, Garbayo JM, Hunter RHF, 2004. Timing of early foetal loss for single and twin pregnancies in dairy cattle. Reprod Dom Anim 39: 429-433. https://doi.org/10.1111/j.1439-0531.2004.00533.x

López-Gatius F, Szenci O, Bech-Sábat G, Garcia-Ispierlo I, Serrano B, Santolaria P, Yániz J, 2009. Factors of non-infectious nature affecting late embryonic and early foetal loss in high producing dairy herds in north-eastern Spain. Magy Allator Lapja, 131: 515-531.

Madureira AML, Silper BF, Burnett TA, Polsky L, Cruppe LH, Veira DM, et al., 2015. Factors affecting expression of estrus measured by activity monitors and conception risk of lactating dairy cows. J Dairy Sci 98: 7003-7014. https://doi.org/10.3168/jds.2015-9672

Mann S, Sipka A, Leal Yepes FA, Nydam DV, Overton TR, Wakshlag JJ, 2018. Nutrient-sensing kinase signaling in bovine immune cells is altered during the postpartum nutrient deficit: A possible role in transition cow inflammatory response. J Dairy Sci 101: 9360-9370. https://doi.org/10.3168/jds.2018-14549

Markusfeld-Nir O, 1997. Epidemiology of bovine abortions in Israeli dairy herds. Prev Vet Med 31: 245-255. https://doi.org/10.1016/S0167-5877(96)01142-7

McDougall S, Rhodes FM, Verkerk G, 2005. Pregnancy loss in dairy cattle in the Waikato region of New Zealand. N Z Vet J 53: 279-287. https://doi.org/10.1080/00480169.2005.36561

Medina-Esparza LE, De Luna-Oseguera R, Vitela-Mendoza, IV, Cruz-Vázquez C, 2018. Detection of Neospora caninum from slaughtered dairy cattle in Aguascalientes, Mexico. Rev Mex Cienc Pecu 9: 408-419. https://doi.org/10.22319/rmcpc.v9i3.4538

Mee JF, 1992. Epidemiology of abortion in Irish dairy cattle on six research farms. Irish J Agric Food Res 31: 13-21.

Mee JF, 2020. Investigation of bovine abortion and stillbirth/perinatal mortality - similar diagnostic challenges, different approaches. Irish Vet J 73:20. https://doi.org/10.1186/s13620-020-00172-0

Megid J, Mathias LA, Robles C, 2010. Clinical manifestations of brucellosis in domestic animals and humans. Open Vet Sci J 4: 119-126. https://doi.org/10.2174/1874318801004010119

Melendez P, Ilha M, Coldemeskel M, Graham J, Coarsey M, Baughman D, et al., 2020. An outbreak of Neospora caninum abortion in a dairy herd from the State of Georgia, United States. Vet Med Sci 7 (1): 141-147. https://doi.org/10.1002/vms3.346
Mellado M, López R, de Santiago A, Veliz FG, Macías-Cruz U, Avendaño-Reyes L, García JE, 2016. Climatic conditions, twining and frequency of milking as factors affecting the risk of fetal losses in high-yielding Holstein cows in a hot environment. Trop Anim Health Prod 48: 1247-1252. https://doi.org/10.1007/s11250-016-1084-8

Mellado M, Macías-Cruz U, Avendaño-Reyes L, Veliz FG, Gaytán L, García, JE, Rodríguez AF, 2019. Milk yield, periparturient diseases and body condition score as factors affecting the risk of fetal losses in high-yielding Holstein cows. Span J Agric Res 17 (2) e0404. https://doi.org/10.5424/sjar/2019172-13206

Moore SG, Fair T, Lonergan P, Butler ST, 2014. Genetic merit for fertility traits in Holstein cows: IV Transition period, uterine health, and resumption of cyclici- ty. J Dairy Sci 97: 2740-2752. https://doi.org/10.3168/jds.2013-7278

Newcomer BW, Chamorro MF, Walz PH, 2017. Vaccination of cattle against bovine viral diarrhea virus. Vet Microbiol 206: 78-83. https://doi.org/10.1016/j.vetmic.2017.04.003

Norman HD, Miller RH, Wright JR, Hutchison JL, Olson KM, 2012. Factors associated with frequency of abortions recorded through Dairy Herd Improvement test plans. J Dairy Sci 957: 4074-4084. https://doi.org/10.3168/jds.2011-4998

Norton JH, Lisle AT, Tranter WP, Campbell RSF, 1989. A farming systems study of abortion in dairy cattle on the Atherton Tableland I reproductive performance. Aust Vet J 66: 161-163. https://doi.org/10.1111/j.1751-0813.1989.tb09791.x

Olsen SC, Stoffregen WS, 2005. Essential role of vacci-nation of cattle against bovine viral diarrhea virus. Theriogenology 65: 1516-1530. https://doi.org/10.1016/j.theriogenology.2005.08.021

Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO, 2006. Defining postpartum uterine disease in cattle. Theriogenology 65: 1516-1530. https://doi.org/10.1016/j.theriogenology.2005.08.021

Thurmond MC, Picanos JP, Jameson CM, 1990. Considerations for use of descriptive epidemiology to investigate fetal loss in dairy cows. J Am Vet Med Assoc 197: 1305-1312.

Uzal FA, 2012. Evidence-based medicine concerning efficacy of vaccination against Clostridium chauvbei infection in cattle. Vet Clin N Am-Food A Prac 28: 71-77. https://doi.org/10.1016/j.cvfa.2011.12.006

Van Campen H, Vorpalh P, Huzurbazar S, Edwards J, Cavender J, 2000. A case report: evidence for type 2 bovine viral diarrhea virus BVDV-associated disease in beef herds vaccinated with a modified-live type 1 BVDV vaccine. J Vet Diagn Invest 12: 263-265. https://doi.org/10.1177/104063780001200312

Van Metre DC, Kennedy GA, Olsen SC, Hansen GR, Ewalt DR, 1999. Brucellosis induced by RB51 vaccine in a pregnant heifer. J Am Vet Med Assoc 215: 1491-1493.
Vidal S, Kegler K, Posthaus H, Perreten V, Rodriguez-Campos S, 2017. Amplicon sequencing of bacterial microbiota in abortion material from cattle. Vet Res 48: 64. https://doi.org/10.1186/s13567-017-0470-1

Vojtek I, Buchy P, Doherty TM, Hoet B, 2019. Would immunization be the same without cross-reactivity? Vaccine 37: 539-549. https://doi.org/10.1016/j.vaccine.2018.12.005

Williams EJ, Fischer DP, Noakes DE, England GC, Rycroft A, Dobson H, Sheldon IM, 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68: 549-559. https://doi.org/10.1016/j.theriogenology.2007.04.056

Wilson DJ, Orsel K, Waddington J, Rajeev M, Sweeny AR, Joseph T, Grigg ME, Raverty SA, 2016. Neospora caninum is the leading cause of bovine fetal loss in British Columbia, Canada. Vet Parasitol 218: 46-51. https://doi.org/10.1016/j.vetpar.2016.01.006

Wolf-Jäckel GA, Hansen MS, Larsen G, Holm E, Agerholm JS, Jensen TK, 2020. Diagnostic studies of abortion in Danish cattle 2015-2017. Acta Vet Scand 62: 1. https://doi.org/10.1186/s13028-019-0499-4

Yazdi HS, Kafi M, Haghkhah M, Tamadon A, Behroozikhah AM, Ghane M, 2009. Abortions in pregnant dairy cows after vaccination with Brucella abortus strain RB51. Vet Rec 165: 570-571. https://doi.org/10.1136/vr.165.19.570