Incidence of bovine abortion in dairy cattle from Costa Rica and consequent potential productive and reproductive losses

Emilia Vindas Van der Wielen

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Wageningen University & Research

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Emilia Vindas Van der Wielen
1115723

QVE-80436
36 ECTS

Supervisors:
Gustavo Monti (WUR)
Juan José Romero Zúñiga (UNA-CR)

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Quantitative Veterinary Epidemiology Group

Wageningen University & Research

Table of contents

Abstract

Cows' reproductive efficiency and milk yield are among the most crucial aspects of a dairy farm's profitability; nevertheless, this can be negatively affected by abortions. Although abortion frequency has been estimated in many countries, information about the burden on dairy cattle from Costa Rica is limited. Also, it is known that abortions can cause economic loss; however, the reproductive and productive losses caused by abortion have not been estimated in dairy cattle from tropical productive conditions like in Costa Rica. Therefore, the objectives of this study were (1) to estimate the incidence and recurrence of bovine abortion and (2) to estimate the potential productive and reproductive losses caused by abortions in dairy cattle from Costa Rica. The frequency of abortion, estimated by the incidence rate (IR) and recurrence rate (RR) expressed per 100 cow-months at risk, was assessed using data of lactations between 2010 and 2022 recorded in the Veterinary Automated Management and Production control Program (VAMPP). The dataset included 1,032,457 lactations from 330,265 cows belonging to 1,134 specialized Costa Rican dairy herds. The abortions were classified as early foetal mortality (EFM) and late foetal mortality (LFM), and stratifications were made based on cow breed, lactation number, and ecological zone the farm belongs to. The reproductive and productive losses caused by abortion were estimated using a dataset with 953,181 complete lactations from 322,873 cows belonging to 1,133 Costa Rican specialized dairy herds. The reproductive losses were indicated by the number of services per conception (NSC), and the production losses were indicated by the days in milk (DIM) per lactation and per cow, 305-day milk production per lactation, total milk production per lactation, and total life milk production per cow. The reproductive and productive losses of abortion were estimated using generalized linear mixed models adjusting for cow breed, lactation number, and ecological zone and using farm and cow as random effects. The IR of general abortion, EFM, and LFM cases were 0.98, 0.41, and 0.57, respectively. No differences were found in IR among cow breed, lactation number, and ecological zone, and a trend of abortions across the calving years was not observed. The single RR was 0.95 and the second RR was 1.41. A lactation with a case of abortion had an increase of 0.72 services per conception and a decrease of 17.4 DIM per lactation, 428L of 305-day milk production per lactation, and 791L of total milk production per lactation. Also, in general, as the number of lactations with abortions increased from 0 to 3 or more, the DIM per cow increased, but there was no significant difference in the total life milk production per cow when the number of lactations with abortions increased. These results detail the abortion frequency in dairy cattle from Costa Rica, possibly representing the reality of other tropical specialized dairy systems in Latin America and illustrating their impact on the cows' reproductive and productive performance.

1. Introduction

The cows' reproductive efficiency and milk production are among the most critical aspects of a dairy farm's profitability; however, this profitability can be negatively affected by bovine abortions. These events have numerous causes and can limit the farm's milk production since they decrease milk yield and the number of potential replacements of the herd. Additionally, bovine abortions increase treatment costs, feeding, number of services per conception (NSC), and involuntary culling of cows (El-Tarabany, 2015; Gädicke et al., 2010; Lee and Kim, 2007).

Bovine abortion can be defined as the foetus' death between pregnancy days 42 and 260 (Markusfeld-Nir, 1997; Mee, 2020). However, many studies have used varying definitions of bovine abortion (Table S1) with consequent variable frequency of occurrence of the abortion cases confounding their comparison. Furthermore, the morbidity measure used to evaluate the occurrence of abortion cases also differ among studies, mainly the cumulative incidence, prevalence, and incidence rate (IR) were used (El-Tarabany, 2015; Forar et al., 1995; Gädicke and Monti, 2013; Markusfeld-Nir, 1997; Norman et al., 2012; Thobokwe and Heuer, 2004; Zobel et al., 2011). The estimated IR of abortion calculated in dairy farms in Chile (1.74 per 100 cow-months at risk) (Gädicke and Monti, 2013) was higher than in the United States of America (USA) (1.41 per 100 cow-months at risk) (Forar et al., 1996) and Israel (1.17 per 100 cow-months at risk) (Markusfeld-Nir, 1997) (Table S2). These studies used similar definitions of abortion and considered both observed and non-observed abortions. The cause of abortions are diverse and include infectious diseases, metabolic disorders, and genetic predisposition among many others (Hovingh, 2009). Therefore, the difference in IR could arise from the differing farm management practices, climatic conditions, and cows' status regarding infectious diseases, but also from methodological approaches like inferred abortions calculation (Table S1).

The estimated proportion of cows that suffered an abortion in dairy cattle from various countries ranges from 1.3-15.4% (Table S2) (Chebel et al., 2004; Keshavarzi et al., 2020, 2017; Labèrnia et al., 1996; Lee and Kim, 2007; López-Gatius et al., 2002; Markusfeld-Nir, 1997; Norman et al., 2012; Zobel et al., 2011). Besides the potential frequency of the causes of abortion, the differences in sample size and intensification level of the dairy herds could have contributed to this wide proportion range. In addition, the different definitions of abortion used in the studies could have added more variation to the significant discrepancy observed between the estimated proportion of aborted cows in Iran (12.5-15.4%) (Keshavarzi et al., 2020, 2017) and the USA (1.3%) (Table S2) (Norman et al., 2012). For example, the studies performed in Iran considered a case of abortion when the foetal death occurred between days 63-252 (Keshavarzi et al., 2017) and 60-260 of pregnancy (Table S1) (Keshavarzi et al., 2020). Contrastingly, the study performed in the USA considered a case of abortion when the death of the foetus occurred at a minimum of 152 days of pregnancy (Table S1) (Norman et al., 2012), excluding the early foetal mortality (EFM), which occurs in the days 42 to 120 of gestation (Mee, 2020). While bovine abortion frequency has been estimated in dairy cattle from many countries, information about burden and impact in dairy cattle from Costa Rica is scarce.

Due to its different economic implications, it is essential to distinguish and classify the bovine abortions on whether they were observed (Gädicke and Monti, 2013), if the cow had suffered a previous abortion (Keshavarzi et al., 2017), and if the abortion originated a new lactation (Keshavarzi et al., 2020). Observed abortions usually occur when the foetus is between 120 and 260 days of pregnancy; on days 42 to 120, the foetus is generally so small that the farmer does not observe it, or is reabsorbed (Mee, 2020). When the foetus is reabsorbed, the only evident indirect sign of abortion is the cow's return to service; therefore, it can be referred to as inferred abortion (Mee, 2020). The IR of inferred abortions can be greater than observed abortions; for example, in Chilean dairy cattle, the IR of inferred abortion (1.4 per 100 cow-months at risk) was more significant than observed abortion (0.3 per 100 cow-months at risk) (Table S2) (Gädicke and Monti, 2013). Inferred abortions should not be ignored due to their contribution to the overall abortion IR and the negative impact they can have on the reproductive parameters such as the increase of NSC, calving interval

(CI), and days open, and consequently, on the farm's profitability. Knowing if cows had a previous abortion and if the abortion started a new lactation is also essential from an economic perspective. Cows with a previous abortion have an increased risk of abortion (Rafati et al., 2010), and cows that undergo an abortion that starts a new lactation have a greater decrease in milk yield and consequently a greater economic loss than cows that undergo an abortion and do not start a new lactation (Keshavarzi et al., 2020). However, these frequency measures can be affected by the farms' culling policies regarding cows that have undergone pregnancy loss since aborted cows have an increased risk of being culled (Bell et al., 2010; Keshavarzi et al., 2020).

Several determinants are associated with different risks of abortion in cattle. The risk of abortion during the gestation time is not the same since abortion frequency in dairy cattle has been estimated to be the highest in the early gestation days (Forar et al., 1996; Gädicke and Monti, 2013; Garcia-Ispierto and López-Gatius, 2019; López-Gatius et al., 2004; Markusfeld-Nir, 1997; Norman et al., 2012). The IR of abortion in dairy cattle in Chile and Israel on day 90 or less of gestation (1.39-2.49 per 100 cow-months at risk) was greater compared to gestation days 91-180 (0.36-1.14 per 100 cow-months at risk) (Table S3) (Gädicke and Monti, 2013; Markusfeld-Nir, 1997). Additionally, pregnancy loss frequency in cows having twin pregnancies (21.4-28.8%) was higher than in single pregnancies (7.7-8.2%) (Table S3) (López-Gatius et al., 2004, 2002), possibly due to the decreased space the foetuses have to develop, predominantly in unilateral twins (Garcia-Ispierto and López-Gatius, 2019). Furthermore, different abortion risks have been reported for some breeds. A lower abortion frequency has been estimated in Jersey (1.1%) (Norman et al., 2012) and Brown Swiss cows (3.6%) (El-Tarabany, 2015) compared to Holstein cows (1.3% and 5.3%, respectively) (Table S4). The Holstein breed may have a decreased adaptability to heat stress, particularly in (sub)tropical conditions, and consequently a decreased reproductive performance (El-Tarabany and El-Tarabany, 2015). In addition, different risks due to the animal's age have also been reported. For example, the abortion rate in Israel for heifers was 0.90 per 100 cow-months at risk, whereas the rate for primiparous and multiparous cows ranged from 1.41 to 1.59 per 100 cow-months at risk (Table S4) (Markusfeld-Nir, 1997). It is possible that multiparous cows (Labèrnia et al., 1996; Markusfeld-Nir, 1997; Rafati et al., 2010) have a more significant estimated abortion frequency because of their risk of being in a negative energy balance (NEB), given that the high milk yield increases their nutritional demands (Roche et al., 2018). Cows in a NEB can present decreased progesterone levels in plasma and reduced oocyte and embryo quality (Butler, 2003).

Several determinants are associated with abortion in cows. One of them is the infectious agents that can cause abortion in cows; they include viruses like bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR), bacteria such as *Brucella abortus*, *Actinomyces pyogenes*, *Campylobacter* spp., *Arcanobacterium pyogenes*, pathogenic *Leptospira* spp., and *Coxiella burnetii*, fungi like *Aspergillus fumigatus* and *Mucor* spp., and parasites including *Tritrichomonas foetus* and *Neospora caninum* (Table S5) (Anderson, 2007; Anderson et al., 1990; BonDurant, 2007; Clothier and Anderson, 2016; Khodakaram-Tafti and Ikede, 2005; Macías-Rioseco et al., 2019; Romero et al., 2005). Several studies showed that bacteria is one of the most common infectious causes of abortion (14.5-24.4%) (Anderson et al., 1990; Campero et al., 2003; Jamaluddin et al., 1996; Khodakaram-Tafti and Ikede, 2005; Kirkbride, 1992) and fungi is one of the least common (0.3-5.3%) (Table S5) (Anderson et al., 1990; Campero et al., 2003; Clothier and Anderson, 2016; Jamaluddin et al., 1996; Kirkbride, 1992). However, the frequency of abortion caused by infectious agents depends on various factors, including the epidemiologic situation of each infectious agent in the country, farm's management practices adopted, and the production intensification level. For instance, in Uruguay and Denmark, the most common infectious cause of abortion was *Neospora caninum* (19.1-29.4%) and the least common included viral causes (0.6-1.0%) (Table S5) (Macías-Rioseco et al., 2020; Wolf-Jäckel et al., 2020). Nevertheless, for many cases (47.0-70.5%), the exact cause of abortion cannot be determined (Table S5) (Anderson et al., 1990; Campero et al., 2003; Clothier and Anderson, 2016; Jamaluddin et al., 1996; Khodakaram-Tafti and Ikede, 2005; Kirkbride, 1992; Macías-Rioseco et al., 2020).

The herd's reproductive efficiency is essential to a cattle farm's profitability (Britt, 1985; Inchaisri et al., 2010; Meadows et al., 2005). However, since milk production and fertility have a negative correlation (Nebel and McGilliard, 1993), dairy farms can suffer economic losses due to sub-fertile animals. Low conception rates, increased CI, premature culling of sub-fertile cows, and pregnancy losses can decrease the productivity and profitability of the farms (Inchaisri et al., 2010; Krpalkova et al., 2016; Lee and Kim, 2007; Meadows et al., 2005; Tadesse et al., 2022). The estimations of the financial loss associated with abortion ranged from US$ 143 to 2,333 per abortion (De Vries, 2006; Gädicke et al., 2010; Lee and Kim, 2007). This loss includes treatment costs, feeding, labour, replacement heifers, loss of the calf, NSC, and premature culling (El-Tarabany, 2015; Gädicke et al., 2010; Lee and Kim, 2007). Although the direct consequence of abortion is producing fewer calves, other indirect effects lead to further loss. A case of abortion can increase the CI by 46-120 days and the NSC by 1.0-1.6 doses (Gädicke et al., 2010; Keshavarzi et al., 2020). Furthermore, a case of abortion can decrease the 305-day milk yield by 893.6-950.0 kg (El-Tarabany, 2015; Keshavarzi et al., 2020), the average milk yield by 0.8L/day, and the productive lifetime of the cow by 0.5 years (Gädicke et al., 2010). Nonetheless, the productive and reproductive losses can vary with the cows' characteristics. For instance, more significant losses were observed in first parity cows (Gädicke et al., 2010) and for Holstein cows compared to Brown Swiss and their cross (El-Tarabany, 2015). Next to that, as the gestation and lactation length increased, the economic loss caused by a case of abortion also increased (De Vries, 2006). Although it is known that abortions are an important source of financial loss in cattle farms, it has not been estimated the potential reproductive and productive losses caused by abortion in dairy cattle from productive tropical conditions like in Costa Rica.

The main objectives of this study are to estimate the incidence and recurrence of bovine abortion in Costa Rican dairy cattle and to estimate the potential productive and reproductive losses caused by bovine abortions in dairy cattle from Costa Rica.

2. Materials and methods

2.1 Herds and management

This study used a retrospective design using data from 01/01/2010 to 31/12/2022, collecting all lactations from cows present in those years. The study population consisted of 1,134 specialized dairy cattle herds from Costa Rica. A total of 1,032,457 lactations were analyzed, corresponding to 330,265 cows. The dairy herds studied were located in 10 ecological zones (ecozones) classified according to Holdridge's classification of life areas (Holdridge et al., 1978) as (1) moist low-mountain forest, (2) very moist low-mountain forest, (3) rainy low-mountain forest, (4) moist pre-mountain forest, (5) very moist pre-mountain forest, (6) very moist mountain forest, (7) rainy forest, (8) dry tropical forest, (9) moist tropical forest and (10) very moist tropical forest (Figure S1). The ecozones are classified according to their altitude, latitude, temperature, and precipitation (Holdridge et al., 1978), management practices, grazing pastures, cow breeds, and endemic diseases. The altitude of Costa Rican dairy farms tends to be between 500 to 2,500 meters above sea level (Vargas-Leitón et al., 2013), and a single ecozone can be present in various regions of the country.

In Costa Rica, the majority (79.4%) of bovine dairy production systems are based on pasture (INEC, 2022) and offer, on average, 4.5 kg of concentrate per cow daily (Vargas-Leitón et al., 2013). The cow breeds used in this study include Holstein (28.8%), Jersey (30.9%), Holstein x Jersey crossbred cows (10.7%), and other breeds (e.g., Gyr, Fleckvieh, Brown Swiss) and their crosses (29.6%). The service of the cows included natural mating (NM) (46.2%), artificial insemination (AI) (53.5%), and embryo transfer (ET) (0.3%). However, no information was available regarding whether hormonal synchronization protocols were used. The vaccines applied to the cows in this study were unknown as most farmers did not record this information in their system. Nonetheless, bovine vaccines available in Costa Rica include those against *Brucella abortus*, *Clostridium* spp., *Leptospira* spp., bovine respiratory syncytial virus, BVD, IBR, and bovine parainfluenza-3 viruses (Castillo-Badilla et al., 2019).

2.2 Data collection

The inclusion criteria for a farm being considered in this study were to show regular veterinary visits, to use the Veterinary Automated Management and Production control Program (VAMPP) (Noordhuizen and Buurman, 1984) herd management information system, and to have complete records of at least four years within the study period. In addition, the pregnancy had to be confirmed by any method (transrectal palpation or ultrasonography) between 35 and 60 days after the service/mating. During the routine visits, the veterinarians collected the reproductive information to be entered into the system and the information regarding the abortions was collected by the farmers/farm workers or calculated by VAMPP software as half of the interval between the last positive pregnancy diagnosis and the registration of a new service or a non-pregnant diagnosis by the veterinarian.

Inclusion criteria for lactations of cows in each farm were that it started between January 1st of the first year of records until three months before the end of the last year of records, and to have complete records of the lactation. In addition, lactations on course at the start of the record collection must have at least three months of records. The database was created by retrieving information from the VAMPP database, which belongs to the Regional Center of Informatics for Sustainable Animal Production (CRIPAS), a project from the School of Veterinary Medicine of the Universidad Nacional de Costa Rica, and includes data recorded since 1986 from almost 1,500 Costa Rican farms (Romero Zúñiga et al., 2019).

2.3 Dataset descriptions

We built two datasets for being analyzed. Dataset A consisted of 1,134 specialized dairy cattle herds from Costa Rica, and it was used to calculate the incidence and recurrence rates of abortion. This dataset included 1,032,457 lactations from 330,265 cows. Dataset B was created using only the complete lactations from dataset A (excluding censored lactations) to estimate the reproductive and productive losses caused by abortion. This dataset included 1,133 specialized dairy cattle herds from Costa Rica and 953,181 lactations from 322,873 cows.

2.4 Case definitions

A case of abortion was defined as the disruption of gestation between 42 to 260 days after conception (Markusfeld-Nir, 1997; Mee, 2020). The abortion could be observed by the farmer or inferred when a cow was inseminated or diagnosed as non-pregnant after a previously confirmed pregnancy (Markusfeld-Nir, 1997). Abortions were classified as early foetal mortality (EFM) when the disruption was between 42 and 120 days of gestation (Mee, 2020) and late foetal mortality (LFM) when the disruption was between 121 and 260 days of gestation. The time-at-risk was calculated from 42 days after conception until:

(1) for those lactations without abortion, 260 days after their last service (end of foetal period),

(2) for those lactations with an observed abortion, half of the interval between conception to observed abortion date,

(3) for those lactations with a non-observed abortion (noticed by a new service or a non-pregnant diagnosis after a previously confirmed pregnancy), half of the interval between the last positive pregnancy diagnosis and the registration of a new service or a non-pregnant diagnosis (since the exact day of the abortion was not known), or

(4) for those lactations that the cow left the herd due to being sold, death, or culling, the last day present in the herd.

The first trimester of gestation was defined as the period between days 42 to 90 of gestation, the second trimester included the period between days 91 to 180 of gestation, and the third trimester included the days 181 to 260 of gestation. The NSC refers to the number of services needed to obtain the conception that gave origin to the lactation, even if the gestation ended in abortion.

The 305-day milk production of each lactation was estimated by VAMPP for lactations with records of at least 200 days, but not for lactations of less than 200 days. The lactations of less than 200 days were not included in the analysis of the effect of abortion on the 305-day milk production per lactation. For lactations with milk production records between 200 and 304 days, the 305-day milk production was estimated using an extrapolation of the last milk record, and for cows with milk production records longer than 305 days, the production at 305 days was cut and the first sample after 305 days and the last milk record were interpolated (Romero et al., 2005). The total milk production per lactation refers to the sum of the liters of milk produced by a cow for the duration of the lactation. The total life milk production per cow was calculated by VAMPP as the sum of the total milk production per lactation of all the recorded lactations per cow. The days in milk (DIM) per lactation refers to the duration of the lactation, considering lactations that started after calving or after abortion. The DIM per cow was calculated as the sum of the DIM per lactation of all the lactations recorded per cow.

2.5 Statistical analysis

All retrieved records were edited and validated for biological plausibility. First, a descriptive analysis was performed including general characteristics of the farms and animals. However, the number of observations per variable/category differs since farm records varied in periodicity and completeness of the data collection and record keeping. Using dataset A, the morbidity of bovine abortion cases was estimated using crude and specific indicators such as incidence rate (IR), cumulative incidence (CIR), and recurrence rate (RR). The overall IR was calculated as new abortion cases over the population at risk (expressed in 100 cow-months at risk); for all the pregnant cows in the population (Henken et al., 2017). In addition, specific IR for EFM and LFM were calculated, and the CIR of abortion was calculated as new abortion cases over the total number of cows at risk. Moreover, for the overall abortions and the foetal mortality rates, specific rates based on cow breed, lactation number, and ecozone were estimated. The different IR by cow breed, lactation number, and ecozone were statistically evaluated using generalized linear mixed models with a Poisson distribution, adjusted for herd size and the year of calving and using the farm that the animal belongs as random effect. The different IR per farm by year of calving were evaluated using a generalized linear mixed model with a Poisson distribution, adjusted for herd size and farm as a random effect. A $P<0.05$ was considered statistically significant.

The RR of abortion in a given lactation was calculated as:

(1) single RR: new abortion cases over the population at risk (expressed in 100 cow-months at risk); for cows that have had a previous abortion during the lactation;
(2) second RR: new abortion cases over the population at risk (expressed in cow-months at risk); for cows that have had two previous abortions during the lactation (Glynn and Buring, 1996).

Finally, a descriptive analysis was performed regarding the cows with recurrent abortions in different lactations according to the farm and ecozone they belong to.

With dataset B, the reproductive losses expressed by the NSC and productive losses expressed by the DIM per lactation and per cow, 305-day milk production per lactation, total milk production per lactation, and total life milk production caused by a case of abortion were estimated using different models. Given the hierarchical structure of the data (lactation nested on cow nested on farm) combined with repeated type of data at cow level, generalized mixed models were used. For all models except for those analyzing the total life production of a cow and the total DIM per cow, farm and cow were used as random effects. For those two, only farm was considered as a random effect. The final models were adjusted for fixed effects such as cow breed, lactation number, and the ecozone the farm belongs to. Significant two-way interactions between the variables were included in the final model if significant (Table S6). The best models were obtained using a backward model building strategy and goodness-of-fit of different fitted models during the process were compared using the Akaike information criterion (AIC) or Quasi information criterion (QIC). For the losses related to the additional NSC, a generalized linear mixed model with a Poisson distribution was fitted and QIC was used. A generalized linear mixed model with normal distribution was fitted for the effect of abortion on the DIM per lactation, 305-day milk production per lactation, and total milk production per lactation and AIC was used.

The general statistical model was:

$y_{ijklm} = \mu_0 + abortion_i + breed_j + lactation_k + eco_l + a_m + e_{ijklm}$

where:

y_{ijklm} is the NSC, DIM per lactation, 305-day milk production per lactation, and total milk production per lactation;

μ_0 the general mean;

$abortion_i$ a binary fixed effect of *i*th lactation (*i* = 0=no, 1=yes);

$breed_j$ the fixed effect of *j*th breed (*j* = 0=Holstein, 1=Jersey, 2=Holstein x Jersey, 3=Others);

$lactation_k$ the fixed effect of the *k*th lactation number (*k* = 1= first lactation, 2= second lactation, 3= third lactation, 4= fourth lactation or greater);

eco_l the fixed effect of the *l*th ecozone (*l*=1-10);

a_m the random effect of *m*th cow identification (*m*=1-322,873) nested within herd;

e_{ijklm} the random residual effect.

The effects of abortion per lactation number and the number of lactations with abortion in the cow's lifetime on the total life milk production per cow and the total DIM per cow were evaluated using the QIC and a generalized linear mixed model with normal distribution using farm as a random effect and adjusted for fixed effects as cow breed, total calving number, and ecozone the farm belongs to (Table S6).

The general statistical models were:

$y_{ijklm} = \mu_0 + abortion \times lactation_i + breed_j + calving_k + eco_l + a_m + e_{ijklm}$

where:

y_{ijklm} is the total life milk production per cow and the total DIM per cow;

μ_0 the general mean;

$abortion \times lactation_i$ the fixed effect of *i*th number of lactation where the abortion occurred (*i* = 0=no abortion, 1=abortion in first lactation, 2=abortion in second lactation, 3=abortion in third lactation, 4=abortion in fourth lactation or greater);

$breed_j$ the fixed effect of *j*th breed (*j* = 0=Holstein, 1=Jersey, 2=Holstein x Jersey, 3=Others);

$calving_k$ the fixed effect of the *k*th total number of calving (*k* = 1= one calving, 2= two calvings, 3= three calvings, 4= four calvings or greater);

eco_l the fixed effect of the *l*th ecozone (*l*=1-10);

a_m the random effect of *m*th herd (*m*=1-1,133);

e_{ijklm} the random residual effect.

and:

$y_{ijklm} = \mu_0 + nabortion_i + breed_j + calving_k + eco_l + a_m + e_{ijklm}$

where:

y_{ijklm} is the total life milk production per cow and the total DIM per cow;

μ_0 the general mean;

$nabortion_i$ the fixed effect of *i*th number of lactations with abortion (*i* = 0=no abortion, 1= one lactation with abortion, 2= two lactations with abortion, 3= three or more lactations with abortions);

$breed_j$ the fixed effect of *j*th breed (*j* = 0=Holstein, 1=Jersey, 2=Holstein x Jersey, 3=Others);

$calving_k$ the fixed effect of the *k*th total number of calving (*k* = 1= one calving, 2= two calvings, 3= three calvings, 4= four calvings or greater);
eco_l the fixed effect of the *l*th ecozone (l=1-10);
a_m the random effect of *m*th herd (*m*=1-1,133);
e_{ijklm} the random residual effect.

Data processing and statistical analysis were performed using the Statistical Analysis System OnDemand for Academics (SAS ODA).

3. Results

3.1 Morbidity of general abortion, early foetal mortality, and late foetal mortality cases

Out of the 1,032,457 lactations, there was a total of 65,502 (6.3%; 95% CI 6.3-6.4%) lactations with at least one case of abortion. Of the 65,502 lactations with abortions, 27,416 (41.9%) were classified as EFM and 38,086 (58.1%) were classified as LFM (Table 1).

Table 1. Cumulative incidence (%) (CIR) and incidence rate (IR: number of new abortion cases per 100 cow-months at risk) for overall, early foetal mortality (EFM), late foetal mortality (LFM), per trimester of gestation, and recurrence rate (RR) within a lactation, with their respective 95% confidence interval (95%CI).

Variable	Abortions (n)	At risk (n)	CIR	95% CI	IR	95% CI
Overall	65,502	1,032,457	6.3	6.3-6.4	0.98	0.97-0.99
EFM	27,416	1,032,457	2.7	2.5-2.8	0.41	0.40-0.41
LFM	38,086	1,032,457	3.7	3.5-3.8	0.57	0.56-0.57
1st trimester	21,928	1,032,457	2.1	2.1-2.2	0.33	0.32-0.33
2nd trimester	17,808	1,032,457	1.7	1.7-1.7	0.27	0.26-0.27
3rd trimester	25,766	1,032,457	2.5	2.5-2.5	0.38	0.38-0.39
RR1*	3,488	65,502	5.3	5.2-5.5	0.95	0.92-0.98
RR2**	236	3,488	6.8	5.9-7.6	1.41	1.23-1.59

*Second abortion case in a single lactation. **Third abortion case in a single lactation.

In addition, 15,644 cases of pregnancy loss occurred before 42 days of conception and 860 cases of pregnancy loss occurred after 260 days of conception. However, these cases were not included in the further analysis. The abortion cases were not distributed evenly across the 42-to-260-day gestation period; the minimum number of abortion cases (115) occurred at day 121 of gestation and the maximum number of abortion cases (775) occurred at day 259 of gestation (Figure 1). Moreover, the IR of abortion was the greatest in the third trimester of gestation (0.38 cases per 100 cow-months at risk; 95% CI 0.38-0.39) (Table 1).

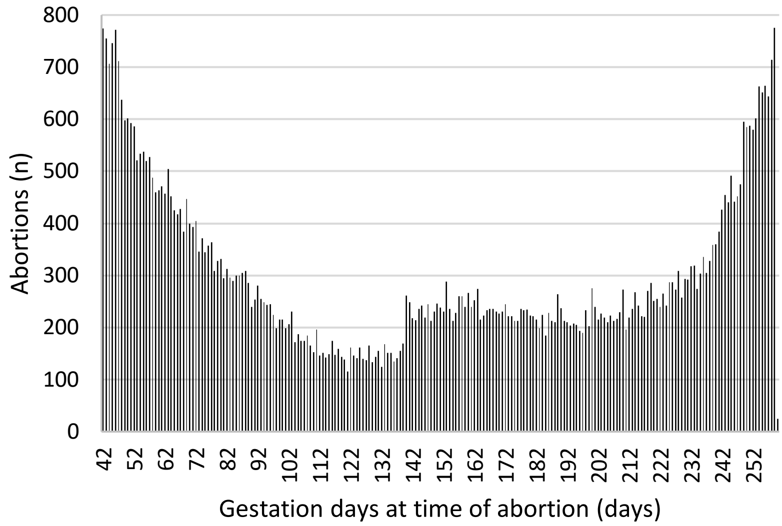

Figure 1. Distribution of abortion cases according to the days of gestation at the time of the abortion diagnosis.

From the total number of lactations, 6.8% were censored. Of all the lactations without abortion, 3.8% ended with the cow's culling whereas 9.6% of the lactations with abortion ended with the cow's culling. From the lactations that ended with the cow's culling, 14.5% underwent an abortion; 89.8% of these cows had a single abortion during the lactation, 9.2% had two abortions during the lactation, and 1.0% had 3 or more abortions during the lactation. Also, of the cows that underwent an abortion and were culled, 94.6% had one lactation with abortion, 5.0% had two lactations with abortions, and 0.4% had 3 or more lactations with abortions. Finally, of the cows that underwent an abortion and were culled, 54.6% had 4 or more calving. The time elapsed between the last abortion in the lactation and the culling of the cow ranged from 23 to 2,933 days, with an interval of 116 days as the most frequent (Figure 2).

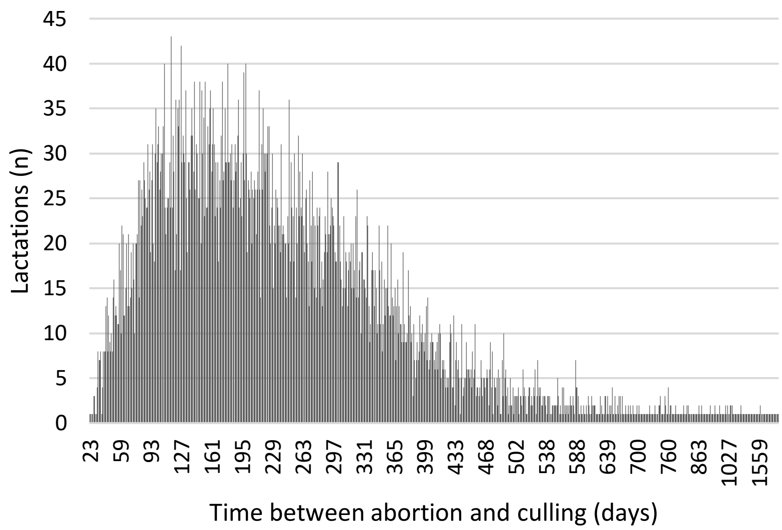

Figure 2. Distribution of the time between the last abortion in a lactation and the culling of the cow.

Holstein cows had the largest lactational CIR (7.5%) and other and crossbred cows had the lowest (4.7%). Furthermore, the CIR ranged from 6.0-7.0% between lactation number, with a median of 6.3%, being the largest for the second lactation. Finally, the CIR ranged from 5.0 to 8.2% between ecozones with a median of 6.1%, being the largest at the very moist mountain forest (Table 2).

Table 2. Cumulative incidence (%) (CIR) and incidence rate (IR: new abortion cases per 100 cow-months at risk) of overall abortion, early foetal mortality, and late foetal mortality by cow breed, lactation number, and ecozone, with their respective 95% confidence interval (95%CI).

		Overall			**Early foetal mortality**		**Late foetal mortality**	
Variable	**Category**	**Abortions (n)**	**CIR (95%CI)**	**IR (95%CI)**	**CIR (95%CI)**	**IR (95%CI)**	**CIR (95%CI)**	**IR (95%CI)**
Cow breed	*Holstein*	22,319/ 297,339	7.5 (7.4-7.6)	1.17 (1.15-1.18)	2.9 (2.9-3.0)	0.46 (0.45-0.46)	4.6 (4.5-4.7)	0.71 (0.70-0.73)
	Holstein x Jersey	8,042/ 110,627	7.3 (7.1-7.4)	1.13 (1.11-1.16)	2.7 (2.6-2.8)	0.42 (0.40-0.43)	4.6 (4.5-4.7)	0.71 (0.69-0.73)
	Jersey	20,725/ 319,375	6.5 (6.4-6.6)	1.01 (0.99-1.02)	3.0 (2.9-3.0)	0.46 (0.45-0.47)	3.5 (3.4-3.6)	0.54 (0.53-0.55)
	Other	14,416/ 305,116	4.7 (4.6-4.8)	0.72 (0.70-0.73)	2.0 (2.0-2.1)	0.31 (0.30-0.32)	2.7 (2.6-2.7)	0.41 (0.40-0.42)
Lactation number	*1*	12,275/ 205,938	6.0 (5.9-6.1)	0.87 (0.85-0.89)	1.9 (1.9-2.0)	0.28 (0.27-0.29)	4.0 (4.0-4.1)	0.59 (0.58-0.60)
	2	15,848/ 225,596	7.0 (6.9-7.1)	1.09 (1.07-1.11)	2.9 (2.8-3.0)	0.45 (0.44-0.46)	4.1 (4.1-4.2)	0.64 (0.63-0.66)
	3	11,762/ 183,607	6.4 (6.3-6.5)	1.00 (0.98-1.01)	2.8 (2.7-2.8)	0.43 (0.42-0.44)	3.6 (3.6-3.7)	0.57 (0.55-0.58)
	≥4	25,617/ 417,315	6.1 (6.1-6.2)	0.97 (0.95-0.98)	2.8 (2.8-2.9)	0.45 (0.44-0.46)	3.3 (3.2-3.3)	0.52 (0.51-0.53)
Ecozone	*Moist low-mountain forest*	4,391/ 71,933	6.1 (5.9-6.3)	0.95 (0.92-0.98)	3.3 (3.2-3.5)	0.52 (0.50-0.54)	2.8 (2.7-2.9)	0.43 (0.41-0.45)
	Very moist low-mountain forest	4,015/ 65,683	6.1 (5.9-6.3)	0.94 (0.91-0.97)	2.8 (2.7-3.0)	0.44 (0.42-0.46)	3.3 (3.1-3.4)	0.51 (0.48-0.53)
	Rainy low-mountain forest	5,342/ 87,088	6.1 (6.0-6.3)	0.94 (0.92-0.97)	2.6 (2.5-2.8)	0.39 (0.38-0.41)	3.6 (3.5-3.7)	0.55 (0.53-0.57)
	Moist pre-mountain forest	6,689/ 116982	5.7 (5.6-5.9)	0.87 (0.85-0.89)	2.2 (2.1-2.3)	0.33 (0.32-0.35)	3.5 (3.4-3.6)	0.53 (0.52-0.55)
	Very moist pre-mountain forest	20,101/ 311,859	6.4 (6.4-6.5)	1.00 (0.98-1.01)	2.7 (2.6-2.7)	0.41 (0.40-0.42)	3.8 (3.7-3.8)	0.58 (0.57-0.59)
	Very moist mountain forest	1,611/ 19621	8.2 (7.8-8.6)	1.32 (1.25-1.38)	4.9 (4.6-5.2)	0.78 (0.73-0.83)	3.3 (3.1-3.6)	0.53 (0.49-0.58)
	Rainy forest	197/ 3,942	5.0 (4.3-5.7)	0.75 (0.64-0.85)	1.0 (0.7-1.3)	0.15 (0.10-0.20)	4.0 (3.4-4.6)	0.60 (0.50-0.69)
	Dry tropical forest	1,774/ 35608	5.0 (4.8-5.2)	0.75 (0.72-0.79)	2.1 (2.0-2.3)	0.32 (0.30-0.34)	2.9 (2.7-3.0)	0.43 (0.40-0.46)
	Moist tropical forest	18,486/ 270,679	6.8 (6.7-6.9)	1.06 (1.04-1.07)	2.6 (2.6-2.7)	0.40 (0.40-0.41)	4.2 (4.1-4.3)	0.65 (0.64-0.67)
	Very moist tropical forest	2,893/ 48,155	6.0 (5.8-6.2)	0.92 (0.89-0.95)	2.5 (2.4-2.7)	0.39 (0.37-0.41)	3.5 (3.3-3.6)	0.53 (0.50-0.56)

The IR, expressed per 100 cow-months at risk, was 0.98 (95% CI 0.97-0.99) for overall abortion cases, 0.41 (95% CI 0.40-0.41) for EFM cases, and 0.57 (95% CI 0.56-0.57) for LFM cases (Table 1). The IR of overall abortion cases per farm ranged from 0 to 4.97 cases with a median of 0.89 (Figure 3). However, the IR of overall, EFM, and LFM abortion cases did not differ ($P>0.05$) among cow breeds, lactation number, or ecozones (Table 2).

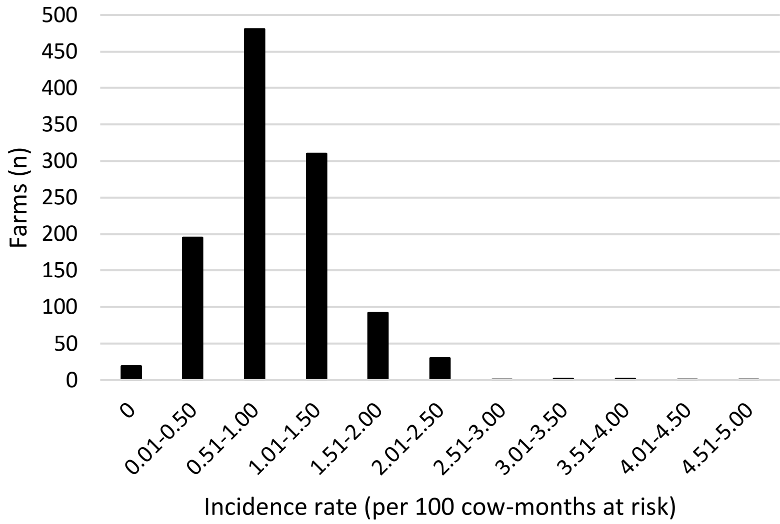

Figure 3. Distribution of the abortion incidence rate (expressed per 100 cow-months at risk) per farm.

Additionally, the proportion of abortions over the total number of gestations that ended (including calving and abortions that started a new lactation) per month ranged from 7.6 to 10.0% (P<0.001); November and December had the minimum and June and July had the maximum (Figure 4). Finally, based on the year the abortion occurred, a trend of abortions was not observed over time (P=1.00) (Figure 5).

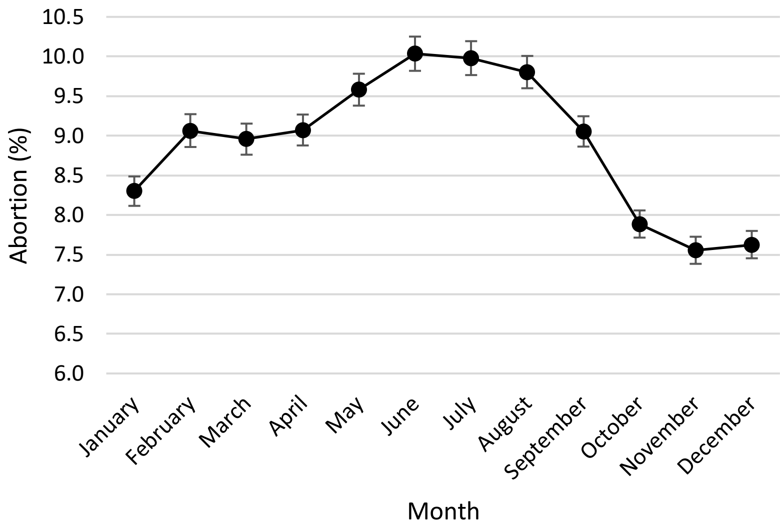

Figure 4. Proportion of abortions over the total number of gestations ended (including calving and abortion that started a new lactation) per month.

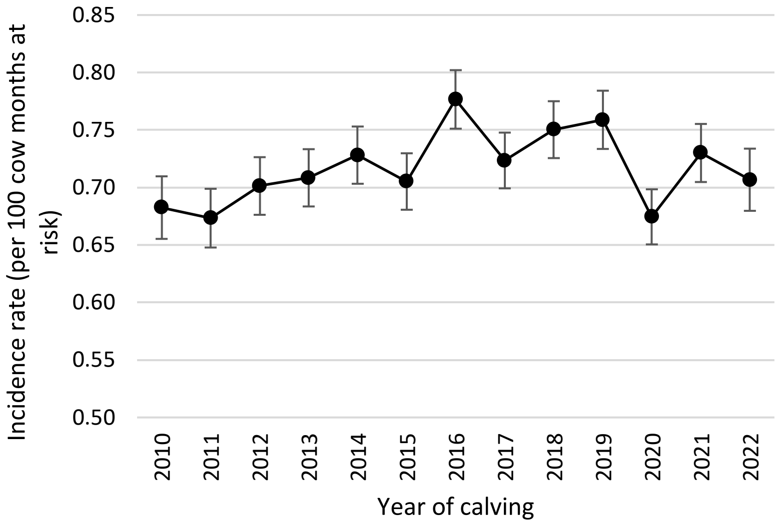

Figure 5. Abortion incidence rate (expressed per 100 cow-months at risk) by the year the abortion occurred.

From the 65,502 lactations with abortion, 62,014 (94.7%) had a single abortion in the lactation, 3,488 (5.3%) had 2 abortions within a lactation, and 236 (0.3%) had 3 or more abortions in a given lactation. Therefore, the single abortion RR was 0.95 (95% CI 0.92-0.98) per 100 cow-months at risk while the second RR was 1.41 (95% CI 1.23-1.59) per 100 cow-months at risk (Table 1).

Furthermore, the number of farms varied among ecozones with a median of 79 farms per ecozone; the very moist mountain forest had the minimum number of farms (14), and the very moist pre-mountain forest ecozone had the maximum number of farms (388). Therefore, the median number of cows with abortion per ecozone was 3,684, ranging from 186 to 17,621 aborted cows per ecozone. In addition, 99.7% (1,112/1,115) of the farms had cows with one abortion and the proportion of farms with 1 to 6 recurrent abortions ranged from 0.1 to 75.6%. However, 87.0% (49,475/56,954) of the cows that had an abortion did not have a recurrent abortion between lactations (Table 3).

Table 3. Descriptive statistics of the number of abortions per cow by farm and ecozone.

Lactation with abortion (n)	Farms (n)	Cows (n)	Cows per farm			Cows per ecozone		
			Median	Minimum	Maximum	Median	Minimum	Maximum
1	1,112	49,475	25	1	629	3,218	175	15,428
2	843	6,566	4	1	162	422	11	2,005
3	336	770	1	1	22	39	25	236
4	67	134	1	1	15	4	2	59
5	6	6	1	1	1	1	1	2
6	2	2	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1
Total	**1,115**	**56,954**						

3.2 Reproductive and productive losses caused by an abortion case

Number of services per conception

For the losses related to the additional NSC, the final model included 4 variables and 6 interaction terms (Table S6). In general, a lactation with a case of abortion (2.43±0.02) needed 0.72 more services per conception than a lactation without it (1.71±0.004). The difference of NSC between lactations with and without abortions among cow breeds ranged from 0.63 to 0.83 with the lactations from Holstein x Jersey cows having the least difference. In addition, the difference in NSC between lactations with and without abortions among lactation number ranged from 0.68 to 0.73, with first-lactation cows having the least difference. Finally, the difference in NSC between lactations with and without abortions among ecozones ranged from 0.29 to 1.03, having the largest difference in the very moist mountain forest ecozone (Table 4). In the first lactation, the Holstein x Jersey breed had the lowest NSC (1.51±0.01) compared to the other breeds studied; although the NSC of Holstein, Jersey, and other and crossbred breeds were not significantly different. In the second, third, and fourth lactation or greater, Holstein cows had the largest NSC (2.26-2.34) while other and crossbred cows had the least NSC (2.04-2.11). Furthermore, Holstein (1.51±0.05), Holstein x Jersey (1.50±0.05), and Jersey (1.53±0.04) cows had the least NSC in the rainy forest ecozone while other and crossbred breeds (1.62±0.01) had the least NSC in the moist pre-mountain forest. The greatest NSC for Holstein x Jersey (2.24±0.03) and for Jersey (2.25±0.02) cows were in the rainy low-mountain forest while the greatest NSC for Holstein (2.67±0.04) and other and crossbred cows (2.53±0.05) were in the very moist mountain forest. Finally, the rainy forest ecozone had the lowest NSC for all the lactation numbers (1.29-1.70) and the very moist mountain forest had the greatest (2.17-2.60).

Table 4. Estimated least square means (LSM) (± standard error (SE)) for the effects of abortion on the number of services per conception per cow breed, lactation number, and ecozone in dairy herds from Costa Rica adjusted for cow breed, lactation number, ecozone, and significant interactions with farm and cow identification as random effects.

Variable	Category	LSM (±SE) Lactation without abortion	LSM (±SE) Lactation with abortion	Difference [a]
Overall		1.71 (±0.004)	2.43 (±0.02)	0.72*
Cow breed	*Holstein*	1.84 (±0.01)	2.49 (±0.02)	0.65*
	Holstein x Jersey	1.67 (±0.01)	2.30 (±0.03)	0.63*
	Jersey	1.73 (±0.01)	2.47 (±0.02)	0.74*
	Other	1.62 (±0.01)	2.45 (±0.02)	0.83*
Lactation number	*1*	1.36 (±0.004)	2.04 (±0.02)	0.68*
	2	1.80 (±0.01)	2.50 (±0.02)	0.70*
	3	1.87 (±0.01)	2.58 (±0.02)	0.71*
	≥4	1.89 (±0.01)	2.62 (±0.02)	0.73*
Ecozone	*Moist low-mountain forest*	1.80 (±0.01)	2.59 (±0.03)	0.79*
	Very moist low-mountain forest	1.68 (±0.01)	2.45 (±0.03)	0.77*
	Rainy low-mountain forest	1.81 (±0.01)	2.65 (±0.03)	0.84*
	Moist pre-mountain forest	1.59 (±0.005)	2.29 (±0.02)	0.70*
	Very moist pre-mountain forest	1.76 (±0.003)	2.44 (±0.01)	0.68*
	Very moist mountain forest	1.95 (±0.02)	2.98 (±0.06)	1.03*
	Rainy forest	1.43 (±0.02)	1.72 (±0.08)	0.29 **
	Dry tropical forest	1.71 (±0.02)	2.59 (±0.05)	0.88*
	Moist tropical forest	1.77 (±0.004)	2.42 (±0.01)	0.65*
	Very moist tropical forest	1.68 (±0.01)	2.34 (±0.03)	0.66*

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. ** P<0.05. Quasi information criterion = 1,401,043.5.

Days in milk

For the effect of abortion during a lactation on the DIM per lactation, the final model included 4 variables and 2 interactions (Table S6). With a case of abortion during a lactation, the DIM per lactation additionally decreased by 17.4 days (±0.9). Moreover, cow breed, lactation number, and ecozone had a significant effect on the DIM per lactation as well. Holstein cows (215.9 days ±0.5) spent the longest DIM while other and crossbred breeds (165.8 days ±0.5) spent the shortest. Cows in the second and third lactation spent the longest DIM (200.5-202.7 days) while cows in the first lactation spent the shortest (189.6 days ±0.7). In addition, cows in the very moist mountain forest spent the longest DIM (250.9 days ±1.1) while cows in the rainy forest spent the shortest (116.8 days ±2.4) (Table 5). Furthermore, for all lactations, Holstein cows spent the longest DIM (212.3-221.5 days), and other and crossbred cows spent the shortest (153.6-173.6 days). Finally, for all the lactation numbers, cows in the rainy forest spent the shortest DIM (84.0-133.9

days) while for all the lactation numbers except the first lactation, cows in the very moist mountain forest spent the longest (240.9-262.2 days). For the first lactation, cows in the moist low-mountain forest (244.4 days ±1.2) spent the longest DIM per lactation.

Table 5. Estimated least square means (LSM) (± standard error (SE)) for the effects of abortion on the days in milk (days) in dairy herds from Costa Rica adjusted for cow breed, lactation number, ecozone, and significant interactions with farm and cow identification as random effects.

Variable	Category	LSM (±SE)	Difference [a]
Abortion during the lactation	*No*	205.4 (±0.3)	Reference
	Yes	188.0 (±0.6)	-17.4 (±0.6) *
Cow breed	*Holstein*	215.9 (±0.5)	Reference
	Holstein x Jersey	194.4 (±0.6)	-21.5 (±0.5) *
	Jersey	210.6 (±0.5)	-5.3 (±0.4) *
	Other	165.8 (±0.5)	-50.1 (±0.4) *
Lactation number	*1*	189.6 (±0.7)	Reference
	2	200.5 (±0.7)	10.9 (±0.9) *
	3	202.7 (±0.7)	13.1 (±1.0) *
	≥4	193.9 (±0.5)	4.3 (±0.8) *
Ecozone	*Moist low-mountain forest*	242.8 (±0.6)	Reference
	Very moist low-mountain forest	209.7 (±0.6)	-33.1 (±0.8) *
	Rainy low-mountain forest	198.6 (±0.6)	-44.2 (±0.8) *
	Moist pre-mountain forest	180.6 (±0.5)	-62.2 (±0.7) *
	Very moist pre-mountain forest	210.3 (±0.4)	-32.5 (±0.6) *
	Very moist mountain forest	250.9 (±1.1)	8.1 (±1.2) *
	Rainy forest	116.8 (±2.4)	-126.0 (±2.5) *
	Dry tropical forest	145.8 (±0.8)	-97.0 (±1.0) *
	Moist tropical forest	197.2 (±0.4)	-45.6 (±0.6) *
	Very moist tropical forest	214.1 (±0.7)	-28.7 (±0.9) *

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. Akaike information criterion =12,078,039. Covariance parameter estimate = 18,650 (±27.0).

For the effect of abortion per lactation number on the total DIM per cow, the final model included 4 variables and 5 interaction terms (Table S6). Overall, cows without abortions spent less total DIM in their productive lifetime. When the abortion occurred on the fourth lactation or greater the DIM was greater than when the abortion occurred in the first lactation (681.7 days ±57.5; P<0.0001). This pattern was evident among the cow breeds studied. However, the effect of abortion per lactation number on the total DIM per cow is also influenced by the number of calving. Cows had greater DIM when they had undergone an abortion than when they did not have an abortion during their productive lifetime (Table 6). Nonetheless, for cows with four or more calving, there was no significant difference (P>0.05) in the DIM when the abortion occurred in the first, second, or fourth lactation. For all number of calving, there were no significant differences (P>0.05) in the total DIM per cow among cow breeds and for all cow breeds, there were no significant differences (P>0.05) in the total DIM per cow among ecozones.

Table 6. Estimated least square means (LSM) (± standard error (SE)) for the effect of abortion per lactation number on the total days in milk in the productive lifetime of a cow in dairy herds from Costa Rica adjusted for cow breed, number of calving, ecozone, and significant interactions with farm as random effect.

Variable	Category	LSM (±SE) No abortion	LSM (±SE) Abortion 1st lactation	LSM (±SE) Abortion 2nd lactation	LSM (±SE) Abortion 3rd lactation	LSM (±SE) Abortion ≥4th lactation
Overall		490.8 (±10.0)	631.5 (±16.1)	726.2 (±32.4)	971.4 (±47.9)	1,313.2 (±65.6)
	Difference [a]	*Reference*	*140.7 (±10.1)**	*235.5 (±27.0)**	*480.6 (±44.8)**	*822.5 (±61.2)**
Cow breed	*Holstein*	521.3 (±14.4)	650.0 (±21.0)	741.3 (±40.9)	989.3 (±51.1)	1,297.5 (±69.0)
	Difference [a]	*Reference*	*128.7 (±11.9)**	*220.0 (±32.3)**	*468.0 (±45.8)*	*776.22 (±62.4)**
	Holstein x Jersey	456.7 (±14.9)	627.2 (±29.0)	703.5 (±36.7)	938.3 (±49.8)	1,301.3 (±73.4)
	Difference [a]	*Reference*	*170.4 (±22.9)**	*246.8 (±29.6)**	*481.6 (±45.1)**	*844.5 (±66.8)**
	Jersey	524.0 (±14.1)	625.4 (±18.2)	715.1 (±32.9)	965.9 (±50.2)	1,311.0 (±64.7)
	Difference [a]	*Reference*	*101.4 (±9.8)**	*191.1 (±26.2)**	*441.9 (±46.3)**	*787.0 (±59.9)**
	Other	461.1 (±18.1)	623.5 (±23.4)	745.0 (±34.4)	991.9 (±51.8)	1,343.2 (±65.5)
	Difference [a]	*Reference*	*162.3 (±25.8)**	*283.9 (±29.2)**	*530.8 (±48.9)**	*882.1 (±62.5)**
Number of calving	*1*	158.0 (±11.5)	150.2 (±11.9)			
	Difference [a]	*Reference*	*-7.7 (±5.1)*			
	2	341.8 (±9.9)	442.7 (±13.8)	355.8 (±11.9)		
	Difference [a]	*Reference*	*100.9 (±9.1)**	*14.0 (±6.0) **		
	3	545.1 (±12.3)	700.9 (±20.7)	627.5 (±18.4)	573.4 (±15.0)	
	Difference [a]	*Reference*	*155.9 (±13.9)**	*82.4 (±11.0)**	*28.3 (±9.1)*	
	≥4	918.3 (±21.5)	1,232.1(±33.5)	1,179.0(±27.9)	1,150.7 (±27.3)	1,173.0 (±25.9)
	Difference [a]	*Reference*	*313.9 (±22.0)**	*260.8 (±14.6)**	*232.4 (±13.9)**	*254.7 (±11.9)**

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. Quasi information criterion = 338,023.7.

For the effect of the number of lactations with abortions on the total DIM in the productive lifetime of a cow, the final model included 4 variables and 5 interaction terms (Table S6). Overall, as the number of lactations with abortions increased from 0 to 3 or more, the DIM per cow also increased. This increase in DIM as the number of lactations with abortions increased was evident in all the cow breeds studied and all the number of calving (Table 7). With no abortions during the cows' lifetime, Jersey and Holstein cows had the greatest DIM (Table 6; Table 7). However, for all number of calving, there were no significant difference (P>0.05) in the DIM among cow breeds or ecozones.

Table 7. Estimated least square means (LSM) (± standard error (SE)) for the effect of the number of lactations with abortions on the total days in milk in the productive lifetime of a cow in dairy herds from Costa Rica adjusted for cow breed, number of calving, ecozone, and significant interactions with farm as random effect.

Variable	Category	LSM (±SE) No abortion	LSM (±SE) 1 abortion	LSM (±SE) 2 abortions	LSM (±SE) ≥3 abortions
Overall		491.1 (±10.0)	581.5 (±14.9)	786.6 (±39.1)	1,091.3 (±115.3)
	Difference [a]	*Reference*	*90.5 (±8.2) **	*295.6 (±36.9) **	*600.3 (±119.4) **
Cow breed	*Holstein*	522.0 (±14.6)	588.9 (±22.1)	773.9 (±39.1)	1,036.7 (±120.8)
	Difference [a]	*Reference*	*66.9 (±12.7) **	*251.9 (±35.2) **	*514.7 (±127.4) ***
	Holstein x Jersey	458.1 (±14.7)	559.9 (±22.9)	803.1 (±56.7)	1,061.7 (±132.3)
	Difference [a]	*Reference*	*101.9* (±15.6) *	*345.1 (±51.9) **	*603.6 (±135.8) ***
	Jersey	524.4 (±14.3)	589.2 (±17.0)	727.8 (±42.1)	1,005.4 (±124.0)
	Difference [a]	*Reference*	*64.9 (±7.9) **	*203.5 (±38.5) **	*481.1 (±128.8) ***
	Other	459.7 (±18.1)	588.0 (±22.1)	841.6 (±44.5)	1,261.5 (±124.8)
	Difference [a]	*Reference*	*128.2 (±13.2) **	*381.9 (±42.4) **	*801.8 (±125.9) **

[a] Tukey-Kramer adjustment for multiple comparison. * P<0.0001. ** P≤0.01. Quasi information criterion = 338,018.9.

Table continued on the next page.

Variable	Category	LSM (±SE) No abortion	LSM (±SE) 1 abortion	LSM (±SE) 2 abortions	LSM (±SE) ≥3 abortions
Number of calving	*1*	160.2 (±12.1)	179.0 (±16.5)		
	Difference [a]	*Reference*	*18.9 (±9.2)*		
	2	341.2 (±9.9)	393.8 (±13.8)	482.0 (±21.6)	
	Difference [a]	*Reference*	*52.6 (±7.7) **	*140.8 (±19.4) **	
	3	544.7 (±12.3)	617.0 (±17.7)	678.1 (±21.0)	819.0 (±71.5)
	Difference [a]	*Reference*	*72.3 (±9.9) **	*133.4 (±16.6) **	*274.3 (±69.6) ***
	≥4	918.1 (±21.6)	1,136.2 (±25.7)	1,286.4 (±30.4)	1,460.8 (±39.6)
	Difference [a]	*Reference*	*218.0 (±10.7) **	*368.3 (±18.6) **	*542.7 (±33.7) **

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. ** P≤0.01. Quasi information criterion = 338,018.9.

Milk production

For the effect of abortion during a lactation on the 305-day milk production per lactation, the final model included 4 variables and 2 interactions (Table S6). For those lactations with a case of abortion, the 305-day milk production per lactation decreased on average by 428.0L (±11.6). Additionally, cow breed, lactation number, and ecozone had a significant effect on the 305-day milk production per lactation. Holstein cows had the greatest 305-day milk production (6,466.9L ±11.0) and other and crossbred breeds had the least (4,782.4L ±11.6). Cows in the third and fourth lactation or greater had the greatest 305-day milk production (5,733.8-5,780.4L) and cows in the first lactation had the least (4,616.8L ±21.4). Also, cows in the very moist tropical forest had the greatest 305-day milk production (6,586.6L ±12.8) while cows in the rainy forest and rainy low-mountain forest had the least (4,576.4-4,590.5L) (Table 8). In addition, for all lactation numbers, Holstein cows had the greatest 305-day milk production per lactation (5,524.4-6,873.7L) while other and crossbred cows had the lowest (4,143.2-5,146.7L). Finally, for all lactation numbers, the cows in the very moist tropical forest had the greatest 305-day milk production (5,501.8-7,135.2L) and for all lactation numbers except the third lactation, the rainy forest had the lowest 305-day milk production (3,851.6-4,842.8L). For the third lactation, cows in the rainy low-mountain forest had the lowest 305-day milk production per lactation (4,809.5L ±22.7).

Table 8. Estimated least square means (LSM) (± standard error (SE)) for the effects of abortion on the 305-day milk production (L) per lactation in dairy herds from Costa Rica adjusted for cow breed, lactation number, ecozone, and significant interactions with farm and cow identification as random effects.

Variable	Category	LSM (±SE)	Difference [a]
Abortion during the lactation	*No*	5,588.2 (±9.1)	Reference
	Yes	5,160.2 (±14.2)	-428.0 (±11.6) *
Cow breed	*Holstein*	6,466.9 (±11.0)	Reference
	Holstein x Jersey	5,227.6 (±13.6)	-1,239.3 (±10.5) *
	Jersey	5,019.9 (±10.9)	-1,447.0 (±6.6) *
	Other	4,782.4 (±11.6)	-1,684.5 (±7.6) *
Lactation number	*1*	4,616.8 (±21.4)	Reference
	2	5,365.9 (±18.0)	749.1 (±27.0) *
	3	5,780.4 (±20.1)	1,163.7 (±28.4) *
	≥4	5,733.8 (±15.2)	1,117.0 (±25.2) *
Ecozone	*Moist low-mountain forest*	6,261.9 (±10.1)	Reference
	Very moist low-mountain forest	5,792.9 (±11.7)	-469.0 (±13.0) *
	Rainy low-mountain forest	4,590.5 (±11.1)	-1,671.4 (±13.0) *
	Moist pre-mountain forest	5,545.3 (±10.0)	-716.6 (±11.7) *
	Very moist pre-mountain forest	5,155.4 (±7.2)	-1,106.5 (±9.7) *
	Very moist mountain forest	5,799.4 (±16.8)	-462.6 (±17.7) *
	Rainy forest	4,576.4 (±81.8)	-1,685.5 (±82.0) *
	Dry tropical forest	4,774.3 (±22.6)	-1,487.6 (±23.6) *
	Moist tropical forest	4,659.4 (±7.8)	-1,602.5 (±10.1) *
	Very moist tropical forest	6,586.6 (±12.8)	324.7 (±14.1) *

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. Akaike information criterion =7,253,369. Covariance parameter estimate = 2,777,203 (±6,131.2).

For the effect of abortion during a lactation on the total milk production per lactation, the final model included 4 variables and 1 interaction term (Table S6). For those lactations with a case of abortion during the lactation, the total milk production decreased on average by 791.3L (±15.0). Additionally, cow breed, lactation number, and ecozone had a significant effect on the total milk production per lactation. Holstein cows had the greatest total milk production per lactation (5,788.4L ±13.9) while other and crossbred cows had the least (3,935.7L ±14.5). Cows in the third lactation had the greatest total milk production (5,055.5L ±24.6) and cows in the first lactation had the least (4,056.3L ±26.0). Furthermore, cows in very moist tropical forest had the greatest total milk production per lactation (5,897.7L ±17.7) and in the rainy forest ecozone the least (3,401.83L ±99.3) (Table 9). Finally, for all lactation numbers, cows in the very moist tropical forest had the greatest total milk production per lactation (5,030.1-6,386.2L) and in the rainy forest the least (3,043.3-3,674.0L).

Table 9. Estimated least square means (LSM) (± standard error (SE)) for the effects of abortion on the total milk production (L) per lactation in dairy herds from Costa Rica adjusted for cow breed, lactation number, ecozone, and significant interactions with farm and cow identification as random effects.

Variable	Category	LSM (±SE)	Difference [a]
Abortion during the lactation	*No*	5,054.6 (±11.2)	Reference
	Yes	4,263.3 (±18.0)	-791.3 (±15.0) *
Cow breed	*Holstein*	5,788.4 (±13.9)	Reference
	Holstein x Jersey	4,487.4 (±17.0)	-1,301.0 (±13.4) *
	Jersey	4,424.4 (±13.7)	-1,364.0 (±8.8) *
	Other	3,935.7 (±14.5)	-1,852.7 (±9.8) *
Lactation number	*1*	4,056.3 (±26.0)	Reference
	2	4,761.8 (±23.0)	705.5 (±33.3) *
	3	5,055.5 (±24.6)	999.3 (±34.4) *
	≥4	4,762.1 (±17.8)	705.8 (±30.0) *
Ecozone	*Moist low-mountain forest*	5,794.3 (±14.0)	Reference
	Very moist low-mountain forest	5,135.6 (±16.0)	-658.8 (±18.4) *
	Rainy low-mountain forest	3,967.8 (±14.9)	-1,826.5 (±18.1) *
	Moist pre-mountain forest	4,831.8 (±13.6)	-962.5 (±16.6) *
	Very moist pre-mountain forest	4,463.5 (±9.5)	-1,330.9 (±13.7) *
	Very moist mountain forest	5,371.5 (±23.4)	-422.8 (±25.1) *
	Rainy forest	3,401.8 (±99.3)	-2,392.5 (±99.8) *
	Dry tropical forest	3,867.4 (±29.3)	-1,927.0 (±31.1) *
	Moist tropical forest	3,857.9 (±10.2)	-1,936.4 (±14.2) *
	Very moist tropical forest	5,897.7 (±17.7)	103.4 (±20.0) *

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. Akaike information criterion =10,260,869. Covariance parameter estimate = 6,915,607 (±13,163).

For the effect of a case of abortion per lactation number during a cow's productive lifetime on the total life milk production per cow, the final model included 4 variables and 6 interaction terms (Table S6). There were no significant differences (P>0.05) in the total life milk production when comparing cows with no abortion and cows with abortion occurring on varying lactation numbers. For cows with 4 or more calvings, the total life milk production was greater by 1,812L (±306) for cows with an abortion occurrence in the fourth or greater lactation in comparison with cows with no abortions (Table 10). For the number of calving 3 and 4 or more, Holstein cows had the greatest total life milk production (13,510-24,554L) and there were no significant differences (P>0.05) among the other breeds studied. For all number of calving, there were no significant differences (P>0.05) in the total life milk production among ecozones.

Table 10. Estimated least square means (LSM) (± standard error (SE)) for the effects of abortion per lactation number on the total life milk production (L) in dairy herds from Costa Rica adjusted for cow breed, number of calving, ecozone, and significant interactions with farm as random effect.

Variable	Category	LSM (±SE) No abortion	LSM (±SE) Abortion 1st lactation	LSM (±SE) Abortion 2nd lactation	LSM (±SE) Abortion 3rd lactation	LSM (±SE) Abortion ≥4th lactation
Overall		10,546 (±272)	10,949 (±413)	10,489 (±351)	10,881 (±560)	11,321 (±609)
	Difference [a]	*Reference*	*403 (±259)*	*-57 (±222)*	*335 (±445)*	*775 (±502)*
Cow breed	*Holstein*	12,611 (±541)	12,520 (±695)	11,881(±575)	12,341(±790)	13,196 (±855)
	Difference [a]	*Reference*	*-92 (±360)*	*-730 (±304)*	*-270 (±481)*	*585 (±548)*
	Holstein x Jersey	9,842 (±404)	10,518 (±684)	9,871 (±585)	9,649 (±714)	10,284 (±760)
	Difference [a]	*Reference*	*676 (±488)*	*28 (±421)*	*-193 (±571)*	*441 (±629)*
	Jersey	10,179 (±311)	10,171 (±393)	9,649 (±388)	9,956 (±549)	10,171 (±596)
	Difference [a]	*Reference*	*-8 (±262)*	*-531 (±268)*	*-223 (±478)*	*-8 (±544)*
	Other	9,550 (±343)	10,587 (±438)	10,555 (±478)	11,577 (±652)	11,634 (±709)
	Difference [a]	*Reference*	*1,037 (±322)*	*1,005 (±344)*	*2,027 (±585)*	*2,084 (±627)*
Number of calving	*1*	3,094 (±155)	3,138 (±287)			
	Difference [a]	*Reference*	*44 (±234)*			
	2	7,138 (±225)	7,550 (±356)	7,068 (±316)		
	Difference [a]	*Reference*	*412 (±252)*	*-70 (±190)*		
	3	11,224 (±316)	11,823 (±471)	11,102 (±408)	11,274 (±455)	
	Difference [a]	*Reference*	*599 (±289)*	*-122 (±225)*	*50 (±307)*	
	≥4	20,727 (±531)	21,287 (±757)	20,467 (±638)	20,335 (±645)	22,539 (±637)
	Difference [a]	*Reference*	*560 (±478)*	*-260 (±329)*	*-392 (±388)*	*1,812 (±306) **
Ecozone	*Moist low-mountain forest*	15,033 (±599)	14,405 (±787)	14,654 (±813)	14,249 (±1,026)	15,360 (±1,074)
	Difference [a]	*Reference*	*-627 (±510)*	*-378 (±620)*	*-784 (±873)*	*327 (±856)*
	Very moist low-mountain forest	11,630 (±686)	12,951 (±1,118)	11,370 (±818)	11,530 (±714)	13,025 (±1,045)
	Difference [a]	*Reference*	*1,321 (±1,061)*	*-259 (±589)*	*-98 (±699)*	*1,395 (±902)*
	Rainy low-mountain forest	8,782 (±586)	9,977 (±596)	10,115 (±729)	10,278 (±714)	11,834 (±935)
	Difference [a]	*Reference*	*1,195 (±499)*	*1,333 (±640)*	*1,496 (±499)*	*3,052 (±833)*
	Moist pre-mountain forest	10,963 (±503)	11,013 (±705)	11,459 (±811)	11,168 (±1,017)	12,593 (±1,189)
	Difference [a]	*Reference*	*50 (±525)*	*497 (±570)*	*206 (±856)*	*1,630 (±1,030)*
	Very moist pre-mountain forest	9,618 (±367)	9,889 (±420)	9,164 (±431)	9.375 (±562)	9,178 (±721)
	Difference [a]	*Reference*	*271 (±282)*	*-454 (±292)*	*-243 (±453)*	*-440 (±591)*
	Very moist mountain forest	13,730 (±826)	12,104 (±1,057)	12,998 (±1,049)	13,559 (±1,263)	16,082 (±1,372)
	Difference [a]	*Reference*	*-1626 (±553)*	*-732 (±645)*	*-172 (±919)*	*2,352 (±1,009)*
	Rainy forest	5,522 (±853)	6,526 (±580)	5,045 (±921)	5,828 (±1,719)	3,365 (±1,052)
	Difference [a]	*Reference*	*1,004 (±973)*	*-478 (±739)*	*306 (±1,837)*	*-2,157(±1,320)*
	Dry tropical forest	8,619 (±1,887)	10,755 (±3,653)	9,032 (±2,653)	10,287 (±3,246)	7,479 (±2,943)
	Difference [a]	*Reference*	*2,136 (±1,591)*	*413 (±929)*	*1,669 (±1,674)*	*-1,139(±1,380)*
	Moist tropical forest	8.337 (±385)	8,811 (±471)	7,987 (±470)	8,353 (±567)	8,584 (±665)
	Difference [a]	*Reference*	*474 (±339)*	*-351 (±326)*	*16 (±376)*	*247 (±467)*
	Very moist tropical forest	13,222 (±932)	13,059 (±993)	13,062 (±1,049)	14,181 (±1,090)	15,712 (±1,667)
	Difference [a]	*Reference*	*-163 (±777)*	*-160 (±813)*	*959 (±1,029)*	*2,489 (±1,431)*

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. Quasi information criterion = 239,864.3

For the effect of the number of lactations with abortions on the total life milk production of a cow, the final model included 4 variables and 5 interaction terms (Table S6). Overall, there were no significant differences (P>0.05) in the total life milk production as the number of lactations with abortions increased from 0 to 3 or more. This is also observed for Holstein, Holstein x Jersey, and Jersey cows. However, for other crossbred cows, as the number of lactations with abortion increased from 0 to 3 or more, the total life milk production also increased. With no abortions during the cows' lifetime, Holstein cows had the greatest total life milk production (12,624L ±552) compared to the other breeds studied (Table 11). For the calving numbers three and four or more, Holstein x Jersey had the lowest total life milk production (10,405-19,640L) and for all number of calving except the first calving, Holstein and other crossbred cows had the greatest total life milk production (8,559-24,654L). For all cow breeds, there were no significant differences (P>0.05) in the total life milk production per cow among ecozones.

Table 11. Estimated least square means (LSM) (± standard error (SE)) for the effects of the number of lactations with abortions on the total life milk production (L) in dairy herds from Costa Rica adjusted for cow breed, number of calving, ecozone, and significant interactions with farm as random effect.

Variable	Category	LSM (±SE) No abortion	LSM (±SE) 1 abortion	LSM (±SE) 2 abortions	LSM (±SE) ≥3 abortions
Overall		10,565 (±281)	10,722 (±300)	11,071 (±464)	12,191 (±820)
	Difference [a]	*Reference*	*157 (±92)*	*506 (±332)*	*1,626 (±820)*
Cow breed	*Holstein*	12,624 (±552)	12,340 (±564)	12,898 (±851)	11,197 (±1,131)
	Difference [a]	*Reference*	*-284 (±229)*	*274 (±559)*	*-1,427 (±1,213)*
	Holstein x Jersey	9,870 (±417)	9,955 (±489)	9,814 (±737)	10,609 (±1,994)
	Difference [a]	*Reference*	*85 (±260)*	*56 (±633)*	*739 (±1,934)*
	Jersey	10,219 (±312)	9,913 (±331)	9,180 (±550)	10,623 (±1,125)
	Difference [a]	*Reference*	*-306 (±142)*	*-1,039 (±458)*	*404 (±1,137)*
	Other	9,545 (±347)	10,680 (±404)	12,390 (±682)	16,336 (±1,372)
	Difference [a]	*Reference*	*1,135 (±216) **	*2,845 (±641) ***	*6,791 (±1,375) **
Number of calving	*1*	3,105 (±158)	2,981 (±181)		
	Difference [a]	*Reference*	*-124 (±92)*		
	2	7,148 (±233)	7,086 (±247)	7,385 (±465)	
	Difference [a]	*Reference*	*-62 (±96)*	*237 (±385)*	
	3	11,240 (±328)	11,275 (±359)	10,671 (±446)	13,765 (±1,634)
	Difference [a]	*Reference*	*35 (±137)*	*-569 (±310)*	*2,525 (±1,557)*
	≥4	20,765 (±541)	21,546 (±585)	21,553 (±692)	22,849 (±1,136)
	Difference [a]	*Reference*	*781 (±203) ***	*788 (±414)*	*2,084 (±934)*

[a] Tukey-Kramer adjustment for multiple comparisons. * P<0.0001. ** P≤0.01. Quasi information criterion = 239,751.1.

4. Discussion and Conclusion

The IR of overall abortion cases estimated in this study was 0.98 cases per 100 cow-months at risk which is lower than that estimated in Israel (IR = 1.17) (Markusfeld-Nir, 1997), Washington, USA (IR = 1.41) (Forar et al., 1996), and Chile (IR = 1.74) (Gädicke and Monti, 2013). For the overall abortion cases, the definitions used in these studies were similar considering the gestational period used to calculate the time-at-risk were the gestation days between 45 to 260 (Markusfeld-Nir, 1997), 31 to 260 (Forar et al., 1996) and 42 to 260 (Gädicke and Monti, 2013) (Table S1). Therefore, the difference in IR could result from the definition of inferred and non-observed abortions, farm management practices, cow breeds used, endemic diseases, and climatic conditions (Forar et al., 1995). Furthermore, the CIR of overall abortion cases in this study was 6.3% which is lower than that estimated in Croatia (7.8%) (Zobel et al., 2011), Spain (7.9-10.6%) (Labèrnia et al., 1996; López-Gatius et al., 2004, 2002), USA (10.8-12.5%) (Chebel et al., 2004; Forar et al., 1996), and Iran (15.4%) (Keshavarzi et al., 2017). However, it is similar to that estimated in Korea (6.9%) (Lee and Kim, 2007), and the median calculated based on 12 studies from 3 different countries (6.5%) (Forar et al., 1995). So, it is likely that morbidity of abortion in Costa Rica's dairy farms is lower than in many other countries.

In this study, the estimated IR, expressed per 100 cow-months at risk, were 0.41 and 0.57 for EFM and LFM cases, respectively. A higher IR was found for LFM cases compared to EFM cases which contrasts with results from previous studies. Previous studies have found a higher abortion IR in the earlier gestation days; the IR, expressed per 100 cow-months at risk, of abortion cases in the first trimester varies from 1.39 (Gädicke and Monti, 2013) to 2.49 (Markusfeld-Nir, 1997) and in the third trimester from 0.81 (Gädicke and Monti, 2013) to 1.56 (Markusfeld-Nir, 1997). This difference in IR could be explained by the burden of the abortifacient infectious agents which are trimester specific. For example, *Brucella abortus*, IBR, and *Neospora caninum* are among the infectious agents generally associated with late-term abortions (Anderson, 2007; Carpenter et al., 2006; Hall et al., 2005; Muylkens et al., 2007; Olsen and Tatum, 2010). In a local study, 94.7% of Costa Rican dairy farms were found to be seropositive to *N. caninum*. However, even though 43.3% of the cows were seropositive, it does not mean they suffered an abortion nor that *N. caninum* caused that abortion (Romero et al., 2005). Furthermore, a local study determined a prevalence of 4.2-4.4% of *B. abortus*-infected Costa Rican dairy herds, estimated by performing an indirect ELISA on the samples positive to Rosa Bengal Test. However, the dairy industry is one of the main contributors in the control of bovine brucellosis in Costa Rica via the application of the RB51 vaccine (Hernández-Mora et al., 2017). Therefore, more research is needed to determine if *N. caninum* and *B. abortus* are the cause of the high LFM IR in dairy cattle from Costa Rica. Additionally, the estimated IR for EFM and LFM cases in this study are much lower than those reported in previous studies; where a higher IR was found especially for earlier gestation days (Forar et al., 1996; Gädicke and Monti, 2013; Markusfeld-Nir, 1997). This could be explained by the countries' different distribution and frequency of abortifacient infectious agents, production intensification levels, farm management practices, incidence of metabolic and reproductive disorders, and climatic conditions (Forar et al., 1995; Hovingh, 2009). Intensive production systems usually have better equipment, infrastructure, housing, and organization which can aid with animal handling and improve animal welfare. In addition, these farms tend to have superior management practices such as greater oestrous detection efficiency, more complete and updated records, better sanitary measures including quarantine of new animals and vaccination protocols, and periodic diagnosis of both pregnancy and of infectious diseases which can help identify or prevent abortions. Moreover, varying climatic conditions, particularly in temperate countries, can have a negative effect on the nutrition, endocrine function, and exposure to specific vectors and pathogens with a consequent increase in the abortion IR. Also, heat stress has been suggested as a possible cause of abortion (Hovingh, 2009; Mellado et al., 2016) and a study from Mexico concluded that cows that calved in the winter and spring had an increased chance of pregnancy loss (Mellado et al., 2016). Therefore, the difference in IR for EFM and LFM cases among studies and countries can arise from various sources.

Even though the different ecozones in Costa Rica can represent different climatic conditions, farm management practices, grazing pastures, and endemic diseases, in this study the IR did not vary among them. In addition, the IR was also similar among cow breeds. It contrasts with other studies where Holstein cows have been found to have an increased frequency of abortion like in countries such as Chile and Egypt (El-Tarabany, 2015; Gädicke and Monti, 2013). Possibly, Holstein cows have a greater milk yield causing them to be at a greater risk of being in a NEB due to unbalanced and/or insufficient diets. It has been reported that cows in a NEB will show decreased oocyte and embryo quality which will probably conclude in an embryo or fetal mortality (Leroy et al., 2008) which could also explain the higher EFM case IR observed in other countries (Gädicke and Monti, 2013; Markusfeld-Nir, 1997). Furthermore, in this study, the abortion IR among lactation number did not differ which is consistent with a study performed in California, USA (Chebel et al., 2004). Nonetheless, other studies have found that cows in the first (Gädicke and Monti, 2013) and second lactation (Markusfeld-Nir, 1997; Rafati et al., 2010) have a higher IR compared to the other lactation numbers which could be explained by the varying immunity status to abortifacient infectious agents. Also, first and second lactation cows can be affected by the increase in nutritional and metabolic demands caused by milk production and their own growth. Finally, a clear trend of abortion IR was not observed over the study period. This could indicate an absence of epidemic disease-induced abortions in the farms studied and no major changes in management practices, climatic factors, or any other possible cause of abortion. However, the minimum IR (0.67), obtained in 2020, could have been due to the Covid-19 pandemic that changed dramatically the way of working and availability of certain products. Whereas the maximum IR (0.78), obtained in 2016, could have been due to specific conditions present in that year, for example, hurricane Otto which had an important impact in the climatic conditions as well as a temporary change in the farm management conditions, possibly favouring abortions. Nonetheless, a specific farm management practices and history analysis must be performed to obtain a more definitive conclusion regarding the cause(s) for the variation in abortion IR across the years.

The CIR of a single recurrent abortion within a lactation (5.3%) estimated in this study was lower to that in Californian (14.5%) (Thurmond et al., 1990) and Israeli dairy farms (17.5%) (Markusfeld-Nir, 1997). Furthermore, 13% of the cows in our study had at least one recurrent abortion between lactations and there was evidence of clustering of cows with recurrent abortions which could indicate deficient farm management, infectious diseases in specific farms, or even a genetic component (Hovingh, 2009; Wijma et al., 2022). Furthermore, when the causes of abortion include infectious agents such as *N. caninum*, it is possible that after the abortion, the cow generated immunity and therefore there is a decrease in the RR of abortion (Dubey et al., 2007). However, the abortion RR can be affected also by the farmer's culling policies for cows with repeated abortions within a lactation and between lactations, and this can vary among farms and countries. In our study, of the lactations with abortions, 9.6% ended with the cow's culling which was more than the double of the proportion of cows culled after a lactation without abortion (3.8%). Although further research is needed, this could suggest that cows with abortion in Costa Rica can have an increased risk of culling and, consequently, it might decrease the risk of recurrence. Similarly, cows that experienced a pregnancy loss in Scotland and Iran had an increased risk of culling (Bell et al., 2010; Keshavarzi et al., 2020). However, there are other reasons for culling cows besides abortion such as increased age/parity, increased calving to conception interval, decreased milk yield and DIM, and health problems like mastitis, ketosis, lameness, retained placenta, ovarian cysts, among many others (Bell et al., 2010; Keshavarzi et al., 2020; Mõtus and Niine, 2022; Rilanto et al., 2020). Nonetheless, 45.4% of the culled cows in our study had less than four calvings which can represent a substantial economic loss for the farmers since the potential of maximum milk yield usually is not achieved until the third lactation (Rilanto et al., 2020; Vijayakumar et al., 2017).

In our study, we estimated that a lactation with a case of abortion needs 0.72 additional services to obtain a conception than a lactation without an abortion. This result is lower to the difference in the number of inseminations per lactation between cows with and without a case of abortion in Chilean (0.97) (Gädicke et al., 2010) and Iranian dairy herds (1.57) (Keshavarzi et al., 2020). However, the difference in NSC was also due to the breed of the cow, the lactation number, and the ecozone. Holstein x Jersey cows had the least difference in NSC which agrees with other studies (Auldist et al., 2007; Coffey et al., 2016) that have concluded that Holstein x Jersey crossbred cows have increased fertility compared to Holstein and Jersey purebred cows. Also, cows in the first lactation had the least difference in NSC which could be caused by the decreased reproductive performance of lactating cows (Lucy, 2001; Nebel and McGilliard, 1993). Any additional NSC increases the costs related to the inseminations and labour and causes an increase in the days open and productive time lost. However, the NSC is a measure that depends on other factors like the oestrus detection efficiency as well as the quality of the semen used (Tadesse et al., 2022).

We estimated that the 305-day milk production per lactation of a lactation with a case of abortion decreased on average 428L. This amount is lower than the decrease reported in other studies (894 to 950 kg) (El-Tarabany, 2015; Keshavarzi et al., 2020). However, milk production is affected, both directly and indirectly, by many factors including cow breed, parity number, genetic factors, nutrition, and climatic conditions (Ramírez-Rivera et al., 2019). Milk production in a lactation with abortion could be decreased due to the mammary glands' reduced development and because the aged or damaged mammary epithelial cells were not replaced in time for the start of the next lactation (Capuco et al., 1997). Another explanation could be the increased risk of cows with abortion of suffering reproductive disorders like retained placenta and metritis (Keshavarzi et al., 2020) which can cause a decrease in the overall health of the cow and therefore the milk production, or it can cause the involuntary culling of the cow. This could also explain the decrease in total milk production per lactation of a lactation with a case of abortion (-791L) observed in our study. These results coincide with previous studies that have determined that the milk production of a cow with abortion decreases (El-Tarabany, 2015; Gädicke et al., 2010; Keshavarzi et al., 2020) as well as the productive lifetime (Gädicke et al., 2010). However, in general, no significant differences were found in the total life milk production when comparing cows with no abortion and cows with abortion on varying lactation numbers in our study. Also, no significant differences were observed in the total life milk production as the number of lactations with abortions increased from no abortions to 3 or more lactations with abortions. Although the 305-day milk production and total milk production per lactation decreased with a case of abortion, it is possible the total life milk production of a cow with and without an abortion did not differ because the milk production in Costa Rican cows is, on average, lower (305-day milk yield=5,562kg) (Romero et al., 2005) than other countries such as Ireland (second lactation milk yield at 304 days=6,550-6,789) (Ferris et al., 2014) and Iran (305-day milk yield=11,556-11,608kg) (Keshavarzi et al., 2020), therefore the milk yield reduction would not be as noticeable as in high producing cows. Another explanation could be that there is no difference between a cow with and without an abortion in her lifetime since the lactation after an abortion can have a higher milk yield (Dijkhuizen et al., 1985) as the dry period of the cow is longer, and the mammary gland has more time to recuperate for the next lactation (Capuco et al., 1997). This increase in milk yield, and possibly DIM, on the lactation after the abortion could also explain the effect of abortion on the DIM found in this study; a case of abortion caused a decreased DIM per lactation of 17 days but an increased total DIM per cow. Nevertheless, a study using a matched pairs design could give more specific information regarding the effect of abortion on the cows' total life milk production and DIM to consider more accurately the number of abortions per lactation, the number of lactations with abortions per cow, and the lactation number when the abortion (s) occurred.

Bovine abortion frequency in dairy cattle from Costa Rica and the consequent reproductive and productive losses have been estimated in this study using an extensive dataset. This study is of great importance not only to Costa Rican dairy producers but also to dairy farmers from other Central American countries that produce under similar conditions regarding climate, management and productive practices, since the majority of the studies available are based on temperate countries and usually focus on specific infectious agents (Björkman et al., 2000; Moore et al., 2008; Okumu et al., 2019; Romero et al., 2005). Furthermore, since the price of milk and its production cost vary among countries, it is crucial to be aware of the reproductive and productive losses a case of abortion can cause under tropical productive conditions like in Costa Rica and to estimate the economic value of these losses.

This study provides to Costa Rican and Latin American dairy industries evidence of the significant impact of abortion on their productive systems, from both a reproductive and productive perspective. Also, due to the large sample size, this study resembles a census of Costa Rican specialized dairy production systems. However, limitations of this study include the record collection by the farmers since they varied in periodicity and completeness of the data collection, particularly regarding the pregnancy diagnosis to be able to detect the non-observed abortions making the abortion dates less accurate. Other limitations include the pregnancy diagnosis being performed by various veterinarians introducing the inter-operator variability, and that the cows' vaccination and infectious state regarding *Brucella abortus*, IBR, BVD, and *Neospora caninum,* was unknown. Further research is needed to estimate the economic impact caused by bovine abortion in dairy herds under tropical productive conditions; it is also crucial to determine the main causes of these abortions.

The results obtained from this study illustrate the importance of regional-based studies to assess differences in the impact of health problems that might look generalizable between production systems, climatic and management conditions. Furthermore, the industry should be aware of the value of keeping complete and consistent records for research and to monitor their production system's profitability. Finally, this study strengthens the notion that the loss of abortion goes beyond a lost calf; it includes increased NSC and decreased DIM and milk production per lactation.

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6. Appendix

Table S1. Definitions of abortion used in studies.

Terminology	Definition	Reference
Fetal loss	After a pregnancy was diagnosed by palpation per rectum, fetal loss was considered to have occurred if the following events occurred before 260 days of gestation: 1) an expelled fetus or fetal membranes were observed, 2) the cow was observed in oestrus and was subsequently diagnosed nonpregnant or pregnant to a subsequent insemination or 3) the cow was diagnosed nonpregnant on a follow-up examination. Gestation period: 31-260 days.	(Forar et al., 1996)
Abortion	Any loss of pregnancy of a cow confirmed pregnant, in the period between 45 days and the minimum calving date (260 days of pregnancy). Includes all cows with observed abortions, and also cows found open or inseminated in the same lactation after pregnancy had been confirmed.	(Markusfeld-Nir, 1997)
Abortion	When the animal delivered a dead fetus before 260 days of pregnancy.	(Kornmatitsuk et al., 2003)
Abortion	An unsuccessful gestation occurring more than 200 days post-insemination (late term), excluding stillbirths, premature births, malformations and weak calves.	(Carpenter et al., 2006)
Abortion	Abortion at ≥152 d and ≤250 d (≤260 d for Brown Swiss) after conception or aborted at ≥200 DIM if no breeding date.	(Norman et al., 2012)
Abortion	Occurrence of either observed or inferred abortion.	
Observed abortion	An event corresponding with the interruption of gestation 42 days after conception, visualized and recorded by the farmer.	(Gädicke and Monti, 2013)
Inferred abortion	An abortion event inferred by the analysis of the differences between the dates of two consecutive AIs, which should be between 90 and 260 days.	
Abortion	Fetal death or return to oestrus after confirmed pregnancy between 63 and 252 days in pregnancy.	(Keshavarzi et al., 2017)
Abortion	Cows showing abortion signs before day 260 of gestation. Cows detected in oestrus by the pedometer system, and the date of abortion was recorded as 20 days before oestrus.	(Garcia-Ispierto and López-Gatius, 2019)
Rebreeding abortion	Abortion incidence between 60 and 260 days of gestation with no change in the lactation number.	(Keshavarzi et al., 2020)
New lactation abortion	Abortion incidence between 60 and 260 days in gestation that led to a new lactation.	
Fetal loss	If any of the following events occurred between 42 and 260 days of gestation (after pregnancy diagnosis): (1) an expelled fetus or fetal membrane, (2) the cow was observed in oestrus and examination verified that there was an abortion, (3) the cow was found non-pregnant on a follow-up examination.	(Rafati et al., 2010)
Pregnancy loss	The absence of an embryo or fetus on a given day that was previously detected by ultrasonography.	
Late embryonic loss	Up to days 41–44 of the gestation.	(Zobel et al., 2011)
Early fetal loss	After 41–44 until 86–88 days of gestation.	
Pregnancy loss	Includes embryonic and fetal loss.	(Lee and Kim, 2007)
Pregnancy loss	A negative pregnancy diagnosis on the second palpation per rectum undertaken between 90 and 96 days after insemination.	(López-Gatius et al., 2002)
Pregnancy attrition	When pregnancy diagnosis resulted negative in a second palpation carried out between 120 and 150 days following insemination.	(Labèrnia et al., 1996)

Table S2. Estimated general abortion/pregnancy loss frequency in dairy cattle.

Category	Abortion Frequency	Frequency measure calculation	Sample size	Year(s) of study	Country	Reference
Overall	10.8%	Cumulative proportion of pregnancies lost from days 31-260 of pregnancy	255 pregnancies 10 herds	1991-1992	USA	(Forar et al., 1996)
Overall	9.6%	Cumulative proportion of pregnancies lost from days 42-260 of pregnancy				
Overall	4.7 fetal losses per 10,000 cow-days at risk	Number of losses on days 31-260 of pregnancy divided by the total number of cow-days at-risk in each interval				
Overall	7.9%	Number of cows with pregnancy loss/ number of pregnant cows	4,212 pregnancies 9 herds	1987-1994	Spain	(Labèrnia et al., 1996)
Overall	3.9 per 10,000 cow-days-at risk	NG	58,048 pregnancies 111 herds	1995	Israel	(Markusfeld-Nir, 1997)
	5.5%	Number of abortions/ number of pregnant cows				
Overall	10.6%	Number of cows with pregnancy loss/ number of pregnant cows	601 pregnancies 1 herd	1998-2000	Spain	(López-Gatius et al., 2002)
Overall	12.5%	Number of cows with pregnancy loss/ number of pregnant cows	1,393 cows 3 herds	1999-2001	USA	(Chebel et al., 2004)
Overall	6.9%	Number of cows with pregnancy loss/ number of cows pregnant	1,001 cows 7 herds	2000-2006	Korea	(Lee and Kim, 2007)
Overall	9.6%	Number of cows with pregnancy loss/ number of pregnant cows	766 cows 1442 pregnancies 1 herd	1997-2001	Spain	(López-Gatius et al., 2004)
Overall	7.8%	Number of cows with pregnancy loss/ number of cows pregnant	860 cows 2 herds	2006-2011	Croatia	(Zobel et al., 2011)
Overall	1.3%	NG	8,496,291 lactations	2001-2009	USA	(Norman et al., 2012)
General	1.7 per 100 cow-months at risk	Number of cases of abortion/100 cow-months at risk	44,959 lactations 20,977 cows 77 herds	2001-2005	Chile	(Gädicke and Monti, 2013)
Observed	0.3 per 100 cow-months at risk					
Inferred	1.4 per 100 cow-months at risk					
Overall	15.4%	Number of aborted cows/ number of pregnant cows	26,289 cows 6 herds	2005-2014	Iran	(Keshavarzi et al., 2017)
Repeated	1.9%	Number of cows with an abortion after a preceding abortion in either present or previous lactation/ number of pregnant cows				
Overall	12.5%	Percentage of cows that had at least one abortion (fetal death during 60–260 days in pregnancy) during their lactations	4,552 cows 5 herds	2005-2014	Iran	(Keshavarzi et al., 2020)
New lactation	1.9%	Percentage of cows that initiated a new lactation due to abortion (fetal death up to 260 days in pregnancy)				

Abbreviations: NG: not given.

Table S3. Abortion/pregnancy loss frequency according to different pregnancy characteristics in dairy cattle.

Variable	Category	Abortion Frequency	P-value	Frequency measure calculation	Sample size	Country	Reference
Gestation days	46-90	8.3 per 10,000 cow-days-at-risk	a	NG	58,048 pregnancies 111 herds	Israel	(Markusfeld-Nir, 1997)
	91-180	3.8 per 10,000 cow-days-at-risk	bc				
	181-260	5.2 per 10,000 cow-days-at-risk	bd				
	31-55	8.8 fetal losses per 10,000 cow-days at risk	NG	Number of losses divided by the total number of cow-days at-risk in each interval	255 pregnancies 10 herds	USA	(Forar et al., 1996)
	201-230	1.9 fetal losses per 10,000 cow-days at risk					
	231-260	2.4 fetal losses per 10,000 cow-days at risk					
	≤90	1.39 per 100 cow-months at risk	0.05	Number of cases of abortion/100 cow-months at risk	44,959 lactations 20,977 cows 77 herds	Chile	(Gädicke and Monti, 2013)
	91-180	0.36 per 100 cow-months at risk					
	≥181	0.81 per 100 cow-months at risk					
	46-90	1.3%	<0.01	Number of cows with pregnancy loss/ number of cows pregnant	1,001 cows 7 herds	Korea	(Lee and Kim, 2007)
	91-180	3.4%					
	181-260	2.3%	NS				
	152-175	4.4%	<0.001	NG	8,496,291 lactations	USA	(Norman et al., 2012)
	176-200	3.3%					
	201-225	1.2%					
	226-250	0.6%					
	135-154	17.4%	<0.0001	Number of cows with abortion/ number of cows with twin pregnancy	1,194 twin pregnancies	Spain	(Garcia-Ispierto and López-Gatius, 2019)
	155–174	7.7%	0.6				
	175–194	9.6%	0.1				
	195–214	7.8%	0.5				
	>214	6.6%	REF				
Type of pregnancy	Single	8.2%	0.0002	Number of cows with abortion/ number of cows with single pregnancy	601 pregnancies 1 herd	Spain	(López-Gatius et al., 2002)
	Twin	21.4%		Number of cows with abortion/ number of cows with twin pregnancy			
	Single	7.7%	NG	Number of cows with abortion/ number of cows with single pregnancy	766 cows 1442 pregnancies 1 herd	Spain	(López-Gatius et al., 2004)
	Twin	28.8%		Number of cows with abortion/ number of cows with twin pregnancy			
	Twin	23.3%	NA	Number of cows with abortion/ number of cows with twin pregnancy	1,194 twin pregnancies	Spain	(Garcia-Ispierto and López-Gatius, 2019)
Insemina-tion protocol	TAI	10.4%	NS	Number of cows with pregnancy loss/ number of pregnant cows	1,393 cows 3 herds	USA	(Chebel et al., 2004)
	ED	13.2%					

Abbreviations: NG: not given; NS: not significant; NA: not applicable; REF: reference; TAI: timed artificial insemination; ED: oestrus detection.

[a,b,c,d] Different letters indicate a statistical difference of the values (p < 0.001) within that variable.

Table S4. Abortion/pregnancy loss frequency according to cow characteristics in dairy cattle.

Variable	Category	Abortion Frequency	P-value	Frequency measure calculation	Sample size	Country	Reference
Breed	Holstein	15.0%	NG	NG	5,167 lactations, 4,426 cows, 42 herds	Chile	(Gädicke and Monti, 2013)
	Black Frison	11.0%					
	Black-Pied Holstein	13.7%					
	Red-Pied Holstein	3.7%					
	Other	3.7					
	Holstein	1.3%	0.01	NG	8,496,291 lactations	USA	(Norman et al., 2012)
	Jersey	1.1%					
	Other	1.3%	NS				
	Holstein (H)	5.3%	<0.05	NG	1,343 cows, 1 herd	Egypt	(El-Tarabany, 2015)
	Brown Swiss (BS)	2.2%					
	Crossbred (HxBS)	3.6%	NS				
Parity	Heifers	2.8%	0.0001	Number of cows with pregnancy loss/ number of pregnant cows	4,212 pregnancies 9 herds	Spain	(Labèrnia et al., 1996)
	Parous	9.6%					
	Primiparous	12.4%	NS	Number of cows with pregnancy loss/ number of pregnant cows	1,393 cows 3 herds	USA	(Chebel et al., 2004)
	Multiparous	12.6%					
	Primiparous	9.1%	NG	Number of cows with pregnancy loss/ number of primiparous pregnant cows	766 cows 1442 pregnancies 1 herd	Spain	(López-Gatius et al., 2004)
	Multiparous	10.0%		Number of cows with pregnancy loss/ number of multiparous pregnant cows			
	Heifers	9.2%	NG	Number of cows with pregnancy loss/ number of pregnant cows	44,629 pregnancies 9 herds	Iran	(Rafati et al., 2010)
	Parous	16.0%					
	Heifers	3.0 per 10,000 cow-days-at-risk	c	NG	58,048 pregnancies 111 herds	Israel	(Markusfeld-Nir, 1997)
	1	4.7 per 10,000 cow-days-at-risk	d				
	2	5.3 per 10,000 cow-days-at-risk	ad				
	≥3	4.5 per 10,000 cow-days-at-risk	bd				
Lactation	1	1.9 per 100 cow-months at risk	a	Number of cases of abortion/100 cow-months at risk	44,959 lactations 20,977 cows 77 herds	Chile	(Gädicke and Monti, 2013)
	2	1.7 per 100 cow-months at risk	a				
	3	1.7 per 100 cow-months at risk	b				
	≥4	1.6 per 100 cow-months at risk	b				

Abbreviations: NG: not given; NS: not significant.

[a,b] Different letters indicate a statistical difference of the values ($p < 0.05$) within that variable.

[c,d] Different letters indicate a statistical difference of the values ($p < 0.001$) within that variable.

Table S5. Abortion/pregnancy loss frequency according to infectious causes in cattle.

Cause	Frequency (%)	Infectious agents identified	Number of cases analyzed	Country	Reference
Bacteria	16.0	*Actinomyces pyogenes*, *Streptococcus* spp., *Leptospira* spp., *Salmonella* spp., *Escherichia coli*, *Bacillus* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus* spp., *Yersinia pseudotuberculosis*, *Pseudomonas aeruginosa*, *Pasteurella haemolytica*, *Serratia marcescens*	468	USA	(Anderson et al., 1990)
Virus	5.6	IBR, BVD, PI3			
Protozoa	3.2				
EBA	1.7				
Fungi	1.1				
NI	70.5				
Bacteria	14.5		8,962	USA	(Kirkbride, 1992)
Virus	10.6	IBR, BVD			
Fungi	5.3				
NI	67.2				
Bacteria	18.0	*Actinomyces* spp., *Bacillus* spp., *Brucella abortus*, *Campylobacter fetus* spp., *Campylobacter jejuni*, *Escherichia coli*, *Haemophilus somnus*, *Leptospira pomona*, *Listeria monocytogenes*, *Listeria ivanovii*, *Pasteurella* spp., *Pseudomonas* sp., *Rhodococcus equi*, *Salmonella* spp., *Serratia marcescens*, *Staphylococcus* sp., *Streptococcus* sp., *Ureoplasma* sp., *Yersinia pseudotuberculosis*	595	USA	(Jamaluddin et al., 1996)
Virus	3.2	IBR, BVD, PI3			
Protozoa	14.6	*Neosporum*-like, *Sarcocystis*			
Fungi	1.3	*Aspergillus fumigatus*, *Candida parapsilosis*, *Torulopsis glabrata*			
NI	57.3				
Bacteria	22.6	*Brucella abortus*, *Campylobacter fetus* spp., *Escherichia coli*, *Acinetobacter* spp., *Actinomyces pyogenes*, *Aeromona hydrophila*, *Bordetella parapertusis*, *Cardiobacterium* spp., *Klebsiella pneumoniae*, *Enterobacter hafniae*, *Pasteurella haemolytica*, *Staphylococcus* spp., *Streptococcus uberis*, *Yersinia paratuberculosis*	354	Argentina	(Campero et al., 2003)
Virus	4.2	Bovine herpesvirus, BVD			
Protozoa	7.3	*Neospora caninum*			
Fungi	0.3	*Aspergillus alutaceus*			
NI	54.5				
Bacteria	24.4	*Arcanobacterium pyogenes*, *Listeria monocytogenes*, *Campylobacter* spp., *Histophilus somni*, *Escherichia coli*, *Salmonella* spp., and *Pasteurella multocida*	234	Canada	(Khodakaram-Tafti and Ikede, 2005)
Virus	6.0	IBR, BVD			
Protozoa	2.1	*Neospora* spp.			
Fungi	6.8	*Aspergillus* spp., *Mucor* spp.			
NI	59.0				
Bacteria	14.7	*Trueperella pyogenes*, *Escherichia coli*, *Streptococcus bovis* complex, *Mannheimia haemolytica*, *Pasteurella multocida*, *Bacillus* sp., *Staphylococcus aureus*, *Nocardia* sp., *Klebsiella pneumoniae*, *Aerococcus urinae*, *Enterobacter* sp., *Listeria monocytogenes*, *Listeria ivanovii*, *Campylobacter jejuni* subsp. *jejuni*, *Campylobacter fetus* subsp. *fetus*, *Campylobacter fetus* subsp. *venerealis*, *Salmonella enterica*, *Coxiella burnetii*	709	USA	(Clothier and Anderson, 2016)
Virus	5.2	Bovine herpesvirus I, BVD			
Protozoa	9.3	*Neospora caninum*			
EBA	16.2				
Fungi	1.8				
NI	52.8				

Abbreviations: IBR: Infectious bovine rhinotracheitis; BVD: Bovine viral diarrhea virus; PI3: Parainfluenza-3 virus; EBA: Epizootic bovine abortion; NI: not identified; NG: not given. Table continued on the next page.

Cause	Frequency (%)	Infectious agents identified	Number of cases analyzed	Country	Reference
Bacteria	19.6	*Escherichia coli*, *Streptococcus* sp., *Staphylococcus* sp., *Mannheimia* sp., *Trueperella pyogenes*, *Providencia stuartii, Coxiella burnetii, Campylobacter fetus* subsp. *venerealis, Leptospira interrogans, Salmonella enterica* serovar Newport	102	Uruguay	(Macías-Rioseco et al., 2020)
Virus	1.0	PI3			
Protozoa	29.4	*Neospora caninum*			
NI	47.0				
Bacteria	11.7	*Trueperella pyogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus licheniformis*, *Streptococcus* spp., *Klebsiella pneumoniae*, *Aeromonas* spp., *Lactococcus garvieae*	162	Denmark	(Wolf-Jäckel et al., 2020)
Virus	0.6	BVD			
Protozoa	19.1	*Neospora caninum*			
Fungi	1.2				
NI	19.8				

Abbreviations: IBR: Infectious bovine rhinotracheitis; BVD: Bovine viral diarrhea virus; PI3: Parainfluenza-3 virus; EBA: Epizootic bovine abortion; NI: not identified; NG: not given.

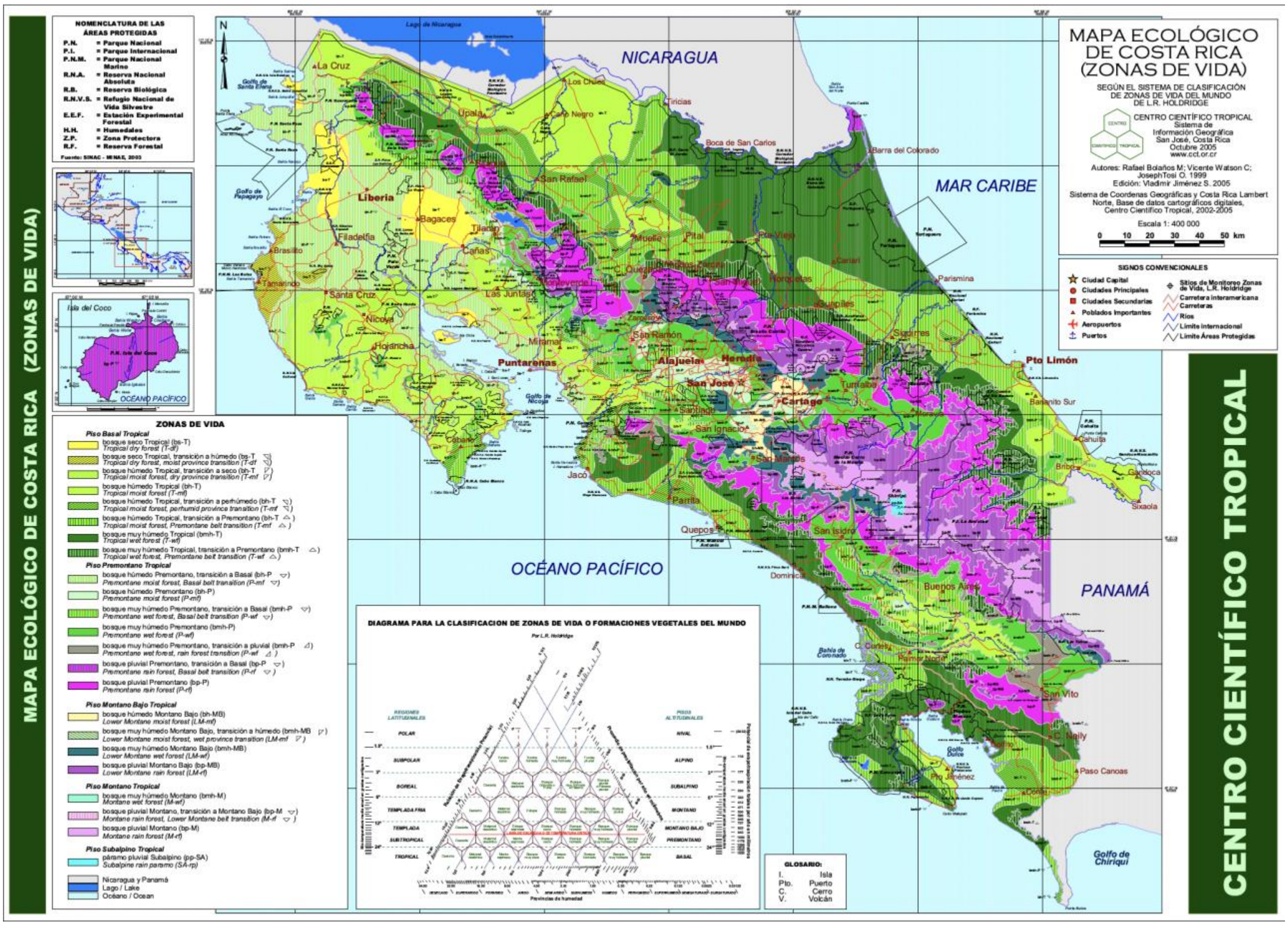

Figure S1. Map of Costa Rica's ecological zones based on Holdridge's classification of life areas (Bolaños et al., 2005).

Table S6. Summary of statistical methods and final models used to analyze the effects of abortion on the different reproductive and productive response variables.

Response variable	Statistical method (distribution)	Fixed variables	Interaction terms (P-value)	Random effect
Number of services per conception	Linear mixed model (Poisson)	Abortion (yes, no), cow breed, lactation number, ecozone	Abortion during the lactation and cow breed (P<0.0001), abortion during the lactation and lactation number (P<0.0001), abortion during the lactation and ecozone (P<0.0001), cow breed and lactation number (P<0.0001), cow breed and ecozone (P<0.0001), lactation number and ecozone (P<0.0001)	Cow identification Farm
Days in milk per lactation	Generalized linear mixed model (normal)	Abortion (yes, no), cow breed, lactation number, ecozone	Cow breed and lactation number (P<0.0001), lactation number and ecozone (P<0.0001)	Cow identification Farm
Total days in milk per cow	Generalized linear mixed model (normal)	Abortion per lactation number, cow breed, number of calving, ecozone	Abortion per lactation and cow breed (P<0.0001), abortion per lactation and number of calving (P<0.0001), cow breed and number of calving (P<0.0001), cow breed and ecozone (P=0.01), number of calving and ecozone (P=0.01)	Farm
Total days in milk per cow	Generalized linear mixed model (normal)	Number of lactations with abortions, cow breed, number of calving, ecozone	Number of lactations with abortions and cow breed (P<0.0001), number of lactations with abortions and number of calving (P<0.0001), cow breed and number of calving (P<0.0001), cow breed and ecozone (P=0.01), number of calving and ecozone (P=0.01)	Farm
305-day milk production per lactation	Generalized linear mixed model (normal)	Abortion (yes, no), cow breed, lactation number, ecozone	Cow breed and lactation number (P<0.0001), lactation number and ecozone (P<0.0001)	Cow identification Farm
Total milk production per lactation	Generalized linear mixed model (normal)	Abortion (yes, no), cow breed, lactation number, ecozone	Lactation number and ecozone (P<0.0001)	Cow identification Farm
Total life milk production per cow	Generalized linear mixed model (normal)	Abortion per lactation, cow breed, number of calving, ecozone	Abortion per lactation and cow breed (P<0.0001), abortion per lactation and number of calving (P=0.02), abortion per lactation and ecozone (P=0.03), cow breed and number of calving (P<0.0001), number of calving and ecozone (P=0.002), number of calving and ecozone (P<0.0001)	Farm
Total life milk production per cow	Generalized linear mixed model (normal)	Number of lactations with abortion, cow breed, number of calving, ecozone	Number of lactations with abortion and cow breed (P<0.0001), number of lactations with abortion and number of calving (P=0.002), cow breed and number of calving (P<0.0001), cow breed and ecozone (P=0.002), number of calving and ecozone (P<0.0001)	Farm