



ASSOCIATION BETWEEN SUBCLINICAL MASTITIS WITH EARLY LOSS OF GESTATION IN A DAIRY COW HERD

ASOCIACIÓN ENTRE LA MASTITIS SUBCLÍNICA CON LA PÉRDIDA TEMPRANA DE GESTACIÓN EN UN HATO DE VACAS LECHERAS

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Resumen

El objetivo del presente estudio fue evaluar la asociación entre los diferentes grados de mastitis subclínica con la pérdida temprana de gestación durante los primeros 90 días posteriores al servicio en vacas lecheras Holstein. La investigación se realizó en una explotación lechera ubicada en Ecuador, provincia de Pichincha, cantón Mejía. Para el estudio se analizaron los datos de 619 vacas durante el período de octubre de 2015 hasta octubre de 2016. Se utilizaron tres grupos experimentales clasificados por la severidad de mastitis subclínica diagnosticada por California Mastitis Test (CMT). El Grupo control (CMT 0) vacas que no presentaron mastitis subclínica hasta los 90 días posteriores a la inseminación artificial (IA). El grupo CMT T-1, vacas diagnosticadas con mastitis subclínica grado trazas y grado 1 hasta los 90 días posteriores a la IA y el grupo CMT 2-3, vacas que presentaron mastitis subclínica grado 2 y 3 hasta los 90 días posteriores a la IA. El diagnóstico de gestación se realizó por ultrasonografía transrectal entre los 28 y 35 días posteriores a la IA y se realizó un seguimiento ecográfico a los 60 y 90 días de gestación. Se encontró una pérdida de gestación entre los 30 a 60 días del 12% y entre los 60 a 90 días del 5%. Con este resultado se evidenció una asociación entre mastitis subclínica grado 2 y 3 con la pérdida temprana de gestación (OR 2,6; p <0,01). Se postula que un proceso infeccioso en la ubre posterior a la IA desencadenaría la liberación de mediadores inflamatorios como la prostaglandina $F2\alpha$ que ocasionaría lisis del cuerpo lúteo y pérdida de la gestación. En conclusión, las vacas que tienen mastitis subclínica de grado 2 y 3 tienen mayor riesgo de pérdida de gestación durante los primeros 90 días posteriores al servicio.

Palabras clave: Bovinos, gestación, luteólisis, mastitis, subclínica.

Abstract

The objective of this research was to evaluate the relationship between different levels of subclinical mastitis with the early gestation lost during the first 90 post-insemination days in Holstein dairy cattle. The research was made on a dairy farm located in Mejía, Pichincha Province, Ecuador. This research analyzed data from a sample of 619 cows during one year since October 2015 until 2016. Three experimental groups graded by the severity of subclinical mastitis and diagnosed by California Mastitis Test (CMT) were used. The control group (CMT 0), formed by cows that did not present any subclinical mastitis until 90 post-insemination days. The CMT T-1 group, formed by cows diagnosed with subclinical mastitis grade 1 up to 90 post-insemination days and the CMT 2-3 group, cows that presented subclinical mastitis grade 2 and 3 up to 90 post-insemination days. The pregnancy diagnosis was made by transrectal ultrasonography between 28 and 35 post-insemination days and a follow up ultrasound was performed at 60 and 90 days of gestation. A pregnancy loss of 12% was found between 30 to 60 and 5% between 60 to 90 days. With this result a relationship between subclinical mastitis grade 2 and 3 with early pregnancy loss was evidenced (OR 2.6, $p < 0.01$). It is postulated that an infectious process in the udder after the insemination can induce the release of inflammatory mediators such as prostaglandin $F2\alpha$ that would cause lysis of the corpus luteum and loss of gestation. In conclusion, dairy cattle presenting subclinical mastitis grade 2 and 3 have a higher risk of pregnancy loss during the first 90 post-insemination days.

Keywords: Cattle, gestation, luteolysis, mastitis, subclinical.

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1 Introduction

Mastitis in cattle is a contagious infected disease of the udder, in which an inflammatory process by invasion occurs through the nipple channel of different types of bacteria, mycoplasmas, fungi, yeasts and some viruses. Bacteria of the genera *Streptococcus*, *Staphylococcus*, *Corynebacterium* and some Gram - germs are responsible for more than 90% of clinical and subclinical infections. It is the most common and expensive disease affecting dairy cattle; there are numerous studies indicating the importance of the disease, mainly for causing economic losses and risks in public health (Philpot and Nickerson, 2002).

Its impact is mainly on the volume and quality of production, animal welfare and herd reproduction (Hillerton and Berry, 2005). The profitability in the dairy industry depends on several factors of reproductive efficiency. The conception rate, the rate of estrus detection and gestation loss are among the main factors that determine reproductive efficiency in dairy herds. However, the loss of gestation can have detrimental effects on the economic success of dairy herds. It is estimated that there is an average loss of USD \$640 for each gestation loss (Thurmond et al., 1990).

The costs of mastitis are mainly associated with milk loss, increased discard rates and treatment costs. However, this disease also indirectly affects reproductive performance in dairy cows by altering interstellar intervals, shortening the luteum phase (premature luteolysis) and gestation losses (Moore et al., 1991).

Several studies have found that bacterial multiplication, the release of endotoxins and exotoxins are involved in the release of inflammatory mediators, which could lead to luteolysis (Riollet et al., 2001). Inflammatory mediators such as prostaglandins, histamine leukotriene and serotonin have been shown to increase cases of experimentally induced mastitis through intravenous infusions of lipopolysaccharides endotoxins (LPS) or intramammary infusions of endotoxin of *Escherichia coli* or *Salmonella typhimurium* (Blum et al., 2000; Waller et al., 2003). In addition, there are studies that have shown the synthesis of luteolytic prostaglandin concentrations after an infusion of endotoxins or septicaemia by gram-negative. *Salmonella enteritidis* intravenous endotoxin causes abortion in mice. Risco et al. (1999) concluded that the use of bacterial endotoxins causes dose-dependent clinical signs, ranging from transient fevers to abortions 24 to 48 hours after the intravenous injection.

Mastitis caused by Gram negative bacteria can cause bacteremia in more than 30% of cases in affected cows (Wenz et al., 2001). With regard to Gram positive bacteria, their cell wall is composed of many layers of peptidogly-

can mucopeptide, these do not have endotoxin, but their presence in the mammary gland cause an inflammatory response that is identical to that caused by the endotoxins of Gram negative bacteria (Salyers and Whitt, 1994). Therefore, it is clear the information that shows the influence of mastitis on the rates of conception, early embryonic mortality and abortions (Risco et al., 1999; Barker et al., 1988).

2 Materials and methods

The experiments were conducted in a private livestock farming that is committed to the production of milk under a semi-intensive production system. It is located in Ecuador, in the province of Pichincha, Canton Mejía, Tambillo Parish; the farm is at an altitude of 2827 masl, there is an average temperature between 10 and 25 °C and a rainfall of 1157 mm per year.

2.1 Factors under study

The livestock farm has an average of 500 Holstein milking cows, with an average production of 24 liters cow/day. Data on 619 cows were analyzed for the test. The study period was carried out during the months of October 2015 to December 2016. Lactating cows and cattle that passed the 60-day voluntary waiting period and were in a position to be inseminated were considered. Cows that had clinical mastitis through the CMT test were not considered in this study because they received anti-inflammatory and antibiotic treatment to control udder infection.

The diagnosis of subclinical mastitis was made by the Veterinary Physician of the livestock farm, using the field test CMT to the group of cows in production once a month during the length of the research. The protocol for applying the test was as follows:

To avoid false positives, the first milk jets of each nipple were discarded, then milked in the 2 ml milk palette, an equivalent amount of reagent was added, stirred to mix the milk with the reagent and the reading was made: negative, trace, one, two, three (Zurita, 1982). The CMT test is a diagnostic field tool based on the detergent breaking down cells (lysator) and letting their DNA out of the cell membrane, these DNA filaments tend to form gel-like structures when they bind with others (Philpot and Nickerson, 2002). The highest degree of inflammation is characterized by releasing a higher concentration of DNA; therefore, the result and interpretation will be a gelatin reaction (Smith, 1990; Saran and Chaffer, 2000; Medina and Montaldo, 2003).

The classification made by Ruiz (1996) was used for the interpretation of the degree of subclinical mastitis:

- Negative: There is no precipitate, therefore there is no infection.
- Traces (T): There is slight precipitation that disappears when shaking, a veil forms in the well of the paddle.
- Grade 1: There is a slight agitation with some lumpy filaments, when moving the paddle for about 20 seconds the lumps tend to disappear, it has a light gelatin appearance.
- Grade 2: It has the appearance of an egg white, it takes mucus consistency and drops the precipitate into the well slowly, this grade corresponds to a serious infection.
- Grade 3: The reaction has a consistency of clot that sticks to the paddle, falls very slowly and it does not lose its shape despite the agitation, this degree corresponds to a serious infection.

2.2 Diagnosis of gestation

The diagnosis of gestation was made by a gynecologist veterinary doctor using transrectal ultrasound (Mindray DP-10 VET with 8.5 mHZ transducer) between 28 and

35 days post-insemination. The gestation was monitored twice during 60 and 90 days gestation.

2.3 Experimental groups

2.3.1 Control group

Cows that were diagnosed as pregnant within 30 days of service and did not have mastitis by testing CMT until 90 days after artificial insemination (AI).

2.3.2 Group 1

Cows that were diagnosed as pregnant within 30 days of service and who had trace-grade subclinical mastitis and grade 1 subclinical mastitis through the CMT test up to 90 days after AI.

2.3.3 Group 2

Cows that were diagnosed as pregnant within 30 days of service and who had grade 2 subclinical mastitis and grade 3 subclinical mastitis through the CMT test up to 90 days after AI.

Table 1. Odds Ratio (OR) and p value of the different degrees of subclinical mastitis.

Degree of mastitis	N	Cows with gestation loss	Pregnant cows	OR	p value
0	309	44	265	reference	
T	130	14	116	0,72	>0,01
1	77	14	63	1,38	>0,01
2	48	13	35	2,24	<0,05
3	55	18	37	2,93	<0,01

The degrees of mastitis are indicated by 0: Negative, T: Traces, 1: Subclinical mastitis degree 1, 2: Subclinical mastitis degree 2, 3: Subclinical mastitis degree 3. OR Represents the reproductive rate.

2.4 Statistical analysis

To determine the association between the embryonic and fetal loss variable in Holstein dairy cows during the first 90 days of gestation with the exposure factor presence of subclinical mastitis, the Odds Ratio (OR) test was performed in group 1 and group 2, and the significance level was analyzed using the Square Chi test. The reason for opportunities or probability ratio is a statistical measure used in cross-sectional epidemiological studies, case and control studies, as well as meta-analysis. It is defined as the possibility of one health condition or disease occurring in one animal population group versus the risk of it occurring in another animal.

3 Results

The OR for group 1 was 0.94, and the test of Chi square concluded that there was no significant association between the presence of trace-grade subclinical mastitis and grade 1 with gestation loss. The OR for group 2 was 2.6 and the test of Chi square concluded that there was a highly significant association between the presence of grade 2 and grade 3 subclinical mastitis with the gestation loss ($P < 0.01$).

Table 1 shows the score results of the different degrees of subclinical mastitis with the results of the OR and Chi square test, which concludes that cows with grade 2 mastitis have a risk of 2.24 times more of having a loss of ges-

tation compared to cows that do not have mastitis during the first 90 days following insemination; while cows with grade 3 mastitis have a 2.93 times higher risk of having a loss of gestation compared to cows that do not have mastitis during the first 90 days post insemination.

3.1 Rate of gestation loss

Embryonic mortality in cattle refers to losses that occur during the first 45 days of gestation that agrees with the end of the embryo differentiation period. Embryonic losses in turn can be classified as early embryo mortality when occurring within 25 days, and late embryo mortality between 25 and 45 days (Humblot, 2001). The terms stillbirth or abortion refer to losses that occur between 45 and 260 days of gestation. Table 2 shows the results of the number of cows who lost gestation and who had subclinical mastitis during the first 90 days post-insemination. Out of a total of 103 cows that lost gestation, 31 cows had

some degree of udder infection, possibly causing embryonic and fetal death. The highest percentage of gestation loss was present in cows with grade 3 subclinical mastitis.

The highest number of embryonic and fetal deaths occurred between day 30 and 60 post-insemination with a percentage of 12%, while between days 61 and 90 days, 5% stillbirth was recorded. There is a total embryonic and fetal loss of 17% during the first 90 days post-insemination, as shown in Figure 1.

Within the group of cows who lost gestation caused by subclinical mastitis grade 2 between 30 and 60 days, 89% of cows lost gestation in this period, while cows that had grade 2 subclinical mastitis between 61 and 90 days, 50% of cows lost gestation. In the group of cows who lost gestation caused by grade 3 subclinical mastitis between 30 and 60 days, 67% of cows lost gestation in this period, while cows that had grade 3 subclinical mastitis between 61 and 90 days, 17% of cows lost on gestation.

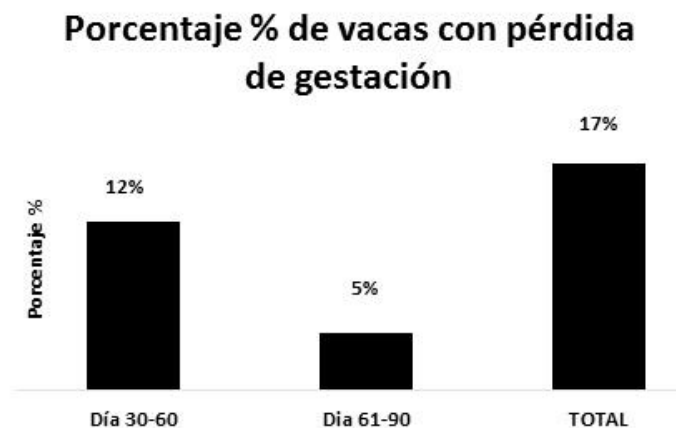


Figure 1. Percentage (%) of embryonic and fetal losses between days 30-60 and 61-90 post-insemination in Holstein dairy cows.

4 Discussion

The results obtained in this research confirm the hypothesis raised, i.e., that there is an association between grade 2 and 3 subclinical mastitis with early gestation loss during the first 90 days of gestation in Holstein dairy cows.

In the CMT 2-3 group, an association with gestation loss was found during the first 90 days (OR = 2.6). The

The association results of cows of the CMT 2-3 group with gestation loss agree with the Moore et al. (2005), in which the association between subclinical mastitis and

gestation loss shown in this group could be caused by the luteal regression induced by the release of cytokines such as prostaglandin $F2\alpha$, $TNF-\alpha$, $INF-\gamma$ and/or the effect of endotoxins, such as LPS (lipopolysaccharide) and bacterial exotoxins on the ovary, uterus and/or embryo (Hansen et al., 2004; Hertl et al., 2014). Embryonic mortality is considered the leading cause of the increase in the interval between deliveries in cattle (Thatcher et al., 1994; Vanroose et al., 2000; Sreenan et al., 2001).

gestation maintenance was analyzed. Cows that experienced subclinical mastitis immediately (LSCC >4.5) before AI had 2.4 times higher risk of losing gestation

Table 2. Percentage (%) of cows with gestation loss according to mastitis grades in Holstein dairy cows during the first 90 days post insemination.

Degree of mastitis	N	Cows with gestation losses
0	309	44 (14%)
T	130	14 (11%)
1	77	14(18%)
2	48	13 (27%)
3	55	18 (33%)
Total	619	103 (17%)

The degrees of mastitis are indicated by 0: Negative, T: Traces, 1: Subclinical mastitis degree 1, 2: Subclinical mastitis degree 2, 3: Subclinical mastitis degree 3. OR represents the base reproductive rate.

between 28 and 35 days after AI, compared to cows that had a linear somatic cell count less than 4.5 (LSCC <4.5, (Moore et al., 2005)).

6000-8000 kg of milk per year in Ireland (Silke et al., 2002) to 42,7% in high-production cows under heat stress (Cartmill et al., 2001).

In this study, the overall loss of gestation between 30 and 60 days was 12% and between 61 and 90 days was 5%. Reproductive losses in lactating dairy cows have increased in recent years (Lucy, 2001). Several researchers have been able to characterize the timing and extent of late embryonic losses in livestock through ultrasonography and other methods for early diagnosis of pregnancy. Humblot (2001) assessed embryonic losses on Holstein cows in 44 herds in France and noted that early and late embryonic death after the first AI was 31.6 and 14,7%, respectively. Late embryonic death after day 27 of gestation ranged from 3,2% in dairy cows, producing

These results agree with the conducted by McDougall et al. (2005), which showed the preponderance and risk factors related to gestation losses in lactating dairy cows fed with pasture in New Zealand. A total of 2004 pregnant cows participated in the study and 128 animals (6,4%) lost their pregnancy. The rate of pregnancy loss was higher between weeks 6 to 10 of gestation than between weeks 10 to 14. Likewise, Santos et al. (2004) summarized information from several studies and concluded that the risk of pregnancy loss was much higher at the beginning of pregnancy than towards the end.

Table 3. Number of cows losing gestation between days 30-60 and 61-90 post-insemination with varying degrees of subclinical mastitis and absence of mastitis in dairy cows.

Degree of mastitis	Day 30-60	Percentage	Day 61-90	Percentage
0	34/309	11.00%	10/275	4.00%
T	11/130	9.00%	3/119	3.00%
1	Nov-77	14.00%	March-66	5.00%
2	Sept-48	19.00%	April-39	10.00%
3	Dec-55	22.00%	June-43	14.00%

The degrees of mastitis are indicated by 0: Negative, T: Traces, 1: Subclinical mastitis degree 1, 2: Subclinical mastitis degree 2, 3: Subclinical mastitis degree 3. OR represents the base reproductive rate.

5 Conclusions

The results of the experiments confirm the association between grade 2 and grade 3 subclinical mastitis with early gestation loss during the first 90 days of gestation in Holstein lactating dairy cows. Gestation loss was greater

between 30 and 60 days than between 61 and 90 days of gestation.

Cows with grade 2 and 3 subclinical mastitis were found to be 2.24 and 2.93, respectively, more likely to lose gestation during the first 90 days post-insemination. Ove-

rall gestation loss between 30 and 60 days was 12% and 5% between 61 and 90 days.

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