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Prevalence and influence of clinical and subclinical mastitis in the service period of dairy cows in Tarumirim (MG)

Prevalência e influência da mastite clínica e subclínica no período de serviço de vacas leiteiras em Tarumirim (MG)

João Vitor Gonçalves Biscotto[1], Matheus Machado Amaral[1], Adriano França da Cunha[1*]

ABSTRACT: The objective was to assess the prevalence and influence of clinical and subclinical mastitis during the service period of cows in a herd in Tarumirim (MG), from October 2019 to May 2020. Around 65 Gir x Holstein and Guzerá x Holstein lactating crossbred cows were evaluated. Subclinical mastitis was diagnosed by means of the California Mastitis Test (CMT), and clinical mastitis, by means of the strip cup test and the animals' clinical signs. Pregnancy after calving was diagnosed by the rectal palpation method, with the aid of ultrasound. The time from calving to conception was considered as the service time. The prevalence rates of clinical and subclinical mastitis were 2.9 and 63.7%, respectively, throughout the experimental period. The mean service period was shorter (p<0.05) in healthy cows (63.1 days) than in cows with subclinical mastitis (88.5 days) and clinical mastitis (111.2 days). The service period increased by 30.9 days for each mammary quarter with clinical mastitis (p<0.05). With each increase in the degree and days of duration of clinical and subclinical mastitis, the service period rose by 25.4 and 6.5 days, and 8.5 and 0.6 days, respectively (p<0.05). Therefore, mastitis increases the service period of dairy cows. Good milking hygiene and animal handling practices should be adopted in order to reduce the prevalence of mastitis in the herd.

KEYWORDS: Pregnancy; mastitis; postpartum; reproduction.

RESUMO: Objetivou-se avaliar a prevalência e influência da mastite clínica e subclínica no período de serviço de vacas em um rebanho de Tarumirim (MG), ao longo de outubro de 2019 a maio de 2020. Em torno de 65 vacas mestiças Gir x Holandês e Guzerá x Holandês em lactação foram avaliadas. A mastite subclínica foi diagnosticada por meio de *California Mastitis Test* (CMT) e a mastite clínica por meio do teste da caneca e sinais clínicos dos animais. O diagnóstico de gestação após o parto foi realizado pelo método de palpação retal, com auxílio de ultrassom. O tempo do parto até a concepção foi considerado como o tempo de serviço. As prevalências de mastite clínica e subclínica foram de 2,9 e 63,7%, respectivamente, ao longo do período experimental. O período de serviço médio foi menor (p<0,05) em vacas sadias (63,1 dias) que em vacas com mastite subclínica (88,5 dias) e clínica (111,2 dias). O período de serviço aumentou 30,9 dias a cada quarto mamário com mastite clínica (p<0,05). A cada aumento do grau e dias de duração da mastite clínica e subclínica, o período de serviço aumentou 25,4 e 6,5 dias e 8,5 e 0,6 dias, respectivamente (p<0,05). Portanto, a mastite aumenta o período de serviço de vacas leiteiras. Boas práticas de higiene de ordenha e manejo dos animais devem ser adotadas para reduzir a prevalência de mastite no rebanho.

PALAVRAS-CHAVE: Gestação; mamite; pós-parto; reprodução.

INTRODUCTION

When it comes to cattle raising, Brazil stands out among the largest milk-producing countries. With a herd of 16.3 million milked cows, the country produces more than 33 billion liters of milk, which accounts for 5% of world production. Minas Gerais is the largest producing state, participating with 26.4% of national production (EMBRAPA, 2020). However, a disease that reduces milk production is mastitis (COSTA et al., 2017).

Mastitis is defined as the inflammation of the mammary gland, regardless of its cause. The main causes are ascending infections via teat canal, by bacteria, fungi, yeasts and algae, the former being the most common (SANTOS; MENDONÇA; MUNIZ, 2020; LAFFRANCHI et al., 2001). A reduction in the prevalence of mastitis in the herd increases the production and levels of milk solids, favoring a higher yield of dairy products (RODRIGUES et al., 2018).

[1]Centro Universitário de Viçosa/UNIVIÇOSA, Av. Maria de Paula Santana, n.3815, Silvestre, Viçosa, Minas Gerais, Brasil
*Corresponding author: adrianofcunha@hotmail.com.br
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Clinical mastitis is characterized by clinical manifestations in the animal, such as milk changes, edema and systemic changes (CUNHA et al., 2016). Animals with subclinical mastitis do not have clinical symptoms, but there is a recruitment of immune system cells to the mammary gland, which, along with the squamous cells of the milk-secreting epithelium, constitute the somatic cells of milk (ASHRAF; IMRAN, 2020; CUNHA et al., 2015).

Mastitis can have negative effects on the reproductive performance of animals due to oxidative stress. The increase in pro-inflammatory cytokines in the bloodstream, due to mastitis, affects the composition of the follicular fluid, impairs the development of the ovarian follicle and, consequently, affects ovulation. This causes delays in ovulation and waste with reproductive management (JÓŹWIK et al., 2012; MOUSSAVI; MESGARAN; GILBERT, 2012; LAVON et al., 2010).

Longer service periods and, consequently, longer calving intervals are related to luteolysis due to prostaglandins produced as a result of the inflammatory reaction in the animal (HOCKETT et al., 2000). A prolonged service period can cause productive and economic losses for the property (PRATA et al., 2014). Therefore, the present study aimed to assess the prevalence and influence of clinical and subclinical mastitis during the service period of dairy cows in a herd in Tarumirim (MG).

MATERIAL AND METHODS

The research work was conducted on a dairy farm in Tarumirim (MG) between October 2019 and May 2020. The property had monthly fluctuations of around 65 Gir x Holstein and Guzerá x Holstein lactating crossbred cows, with an average production of 2,600 liters of milk per day, and an average number of 3.7 deliveries. The property was selected due to its history of high prevalence of clinical and subclinical mastitis among the animals.

The type of animal husbandry on the property was characterized by being semi-intensive, and the animals were milked twice a day mechanically. Feeding consisted of corn silage and grass in the dry season, and grazing in the rainy season. Protein concentrate was supplied in both periods of the year. The animals' breeding system was set by natural mating, artificial insemination and embryo transfer.

All animals in the herd were evaluated monthly throughout the experiment, with fluctuations occurring in the number of lactating animals throughout the period due to new births and the animals being dried off. Therefore, eight months were obtained for mastitis detection. Factors that may interfere with the occurrence of mastitis and the service period of the animals, such as breed and breeding system, were treated randomly.

Subclinical mastitis was detected weekly using the CMT (California Mastitis Test). To this end, aliquots of milk were ejected from each mammary quarter into corresponding wells of an appropriate tray, together with neutral anionic detergent. The test result was evaluated as a function of the degree of gelatinization, being expressed in five scores: negative (0), trace (1), one cross (2), two crosses (3) and three crosses (4). Animals with crosses in any mammary quarter were considered to have subclinical mastitis (CUNHA et al., 2015; RIBEIRO et al., 2008).

Clinical mastitis was diagnosed daily by means of the strip cup test throughout the animals' service period. Three or four jets of milk were ejected from each mammary quarter into the cup, in order to check for the presence of milk lumps, pus and blood. In addition, clinical symptoms were verified, such as hyperthermia, swollen and hyperemic mammary gland, and increased volume of supramammary lymph nodes (ASHRAF; IMRAN, 2020; CUNHA et al., 2016). The quarters were classified into five scores: healthy (0); mild mastitis (1); moderate mastitis (2); and severe mastitis (3) (SANTOS; FONSECA, 2019).

Pregnancy diagnosis was performed using the rectal palpation method, with the aid of ultrasound equipment (Mindray® DP-20, Shenzhen Mindray Bio-Medical Electronics, China). The parameters verified included: size and consistency of the structures present in the ovaries (follicles, corpus luteum and cyst); consistency, tonicity and thickness of the uterine wall; symmetry of the uterine horns; evaluation of the cervix; and evaluation of the body of the uterus. The service period was determined after the diagnosis of pregnancy, by calculating the number of days between calving and conception of the animals.

To assess the occurrence of clinical and subclinical mastitis in the herd throughout the period, the results of all animals were used, regardless of the moment they were in lactation. To assess the influence of clinical and subclinical mastitis during the service period of the animals, only those that had mastitis during this period were considered. Mastitis length (days), number of quarters (0 to 4) with mastitis, and degree of subclinical (0 to 4) and clinical (0 to 3) mastitis were associated with the service period.

The data obtained were subjected to Analysis of Variance (ANOVA), and the means of the service period among the animals that presented and did not present clinical and subclinical mastitis were compared using Tukey's test. The results were also subjected to linear regression analysis, and the association strength coefficient (r-value) was obtained. All data were analyzed using the SigmaPlot 12.0 software (Systat Software Inc., San Jose, USA), at a 5% significance level. The research was approved by the Research and Extension Center [*Núcleo de Pesquisa e Extensão*] (NUPEX) of the University Center of Viçosa [*Centro Universitário de Viçosa*] (UNIVIÇOSA) under protocol number 579.2019.02.01.015.03.

RESULTS AND DISCUSSION

Throughout the experimental period, the prevalence of clinical mastitis in the herd fluctuated monthly between zero and 5.2%, with a prevalence of 2.9% at the end of the assessed period (Table 1). As for the animals selected for the experiment, the prevalence of clinical mastitis ranged from zero to 50%, with a prevalence of 8.5% at the end of the period. According to SANTOS; FONSECA (2019), the maximum prevalence of clinical mastitis should be 5% in the herd; therefore, only in January the prevalence was considered high, with 5.2%.

It was observed that the prevalence of subclinical mastitis in the herd throughout the period fluctuated monthly between 52.8 and 78.8%, with a prevalence of 63.7% at the end of the assessed period (Table 2). As for the animals selected for the experiment, the prevalence of subclinical mastitis ranged from 36.8 to 80.0%, with a prevalence of 47.5% at the end of the period. The prevalence rates of subclinical mastitis in the herd were high, because according to SANTOS; FONSECA (2019), the maximum prevalence of the disease should be 15% in the herd.

Different prevalence rates were found in Brazilian herds, ranging from 6.6 to 76.9% (PINTO et al., 2021; OLIVEIRA et al., 2020; CUNHA et al., 2015; BRITO et al., 2014; VIEIRA et al., 2013; FERREIRA et al., 2007). Mastitis is a multifactorial disease and, therefore, its prevalence in different production systems and times of the year may vary (QUINTÃO et al., 2017).

Hygiene during the milking process, water quality, herd health, and nutritional and environmental management can influence the occurrence of mastitis in herds. However, factors inherent to production can also predispose animals to this disease, such as volume of milk produced, yield, breed and number of lactating animals (CUNHA et al., 2015; CUNHA et al., 2016).

The beginning of lactation, which culminates in the service period, is a moment when the risk of mastitis is high due to the animals' transition period. The total energy consumption by the animals is usually lower than their energy requirements. A negative energy balance is a risk factor for mastitis (COLLARD et al., 2000). The probability becomes higher in herds with inadequate hygienic and sanitary management (CUNHA et al. 2016).

The mean lengths of service period for animals with both clinical and subclinical mastitis were higher ($p<0.05$) than those for healthy animals (Table 3). Together, animals with clinical and subclinical mastitis also had a longer mean service period ($p<0.05$) than healthy ones did.

Table 3. Mean service period of healthy cows and cows with clinical and subclinical mastitis in a herd from Tarumirim (MG)

Mastitis	N	Mean (Days)	CV (%)
Healthy	26	63.1c	37.1
Clinical	5	111.2a	16.2
Subclinical	28	88.5b	43,1
Clinical + Subclinical	33	91.2ab	47.3

Means followed by different lowercase letters differ significantly by Tukey's test ($p<0.05$).

Table 1. Prevalence of clinical mastitis in the herd and in cows selected for experimentation on a property in Tarumirim (MG)

Month	Herd			Experiment		
	N	Positive	%	N	Positive	%
October	43	2	4.7	5	1	20.0
November	52	1	1.9	7	0	0.0
December	76	3	3.9	19	1	5.3
January	77	4	5.2	19	2	10.5
February	62	3	4.8	7	0	0.0
March	72	0	0.0	2	1	50.0
April	76	2	2.6	0	0	-
May	65	0	0.0	0	0	-
Total	523	15	2.9	59	5	8.5

Table 2. Prevalence of subclinical mastitis in the herd and in cows selected for experimentation on a property in Tarumirim (MG)

Month	Herd			Experiment		
	N	Positive	%	N	Positive	%
October	43	33	76.7	5	4	80.0
November	52	41	78.8	7	3	42.9
December	76	43	56.6	19	7	36.8
January	77	53	68.8	19	9	47.4
February	62	40	64.5	7	4	57.1
March	72	38	52.8	2	1	50.0
April	76	44	57.9	0	0	-
May	65	41	63.1	0	0	-
Total	523	333	63.7	59	28	47.5

Animals with clinical and subclinical mastitis had an average of 48.1 and 25.4 days more of service period than healthy animals did, respectively. Thus, it is observed that clinical mastitis compromises the service period of the animals more than subclinical mastitis does, which causes greater disturbances, such as increase in the calving intervals and dry period of the animals (PRATA et al., 2014).

SCHRICK et al. (2001) observed a mean difference in days of service similar to that found in the present study. Cows with subclinical mastitis before the first service had a mean increase in the service period from 85.4 days to 107.7 days, that is, an increase of 24.6 days. PINEDO et al. (2009) observed superior results, as cows with subclinical mastitis had an increase of 48.7 days in the service period.

An increase in the dry period can cause a decrease in milk production and lactations during the life of the animals, in addition to lower replacements of heifers and animals for sale, which leads to lower financial returns for the dairy activity (PRATA et al., 2014). An increase in the postpartum anestrus period causes the animal not to return to estrus in a maximum of 75 to 90 days after calving (LEITE; MORAES, PIMENTEL, 2001; NEVES; GONÇALVES; OLIVEIRA, 1999), which was observed in the group of animals with clinical mastitis (111.2 days).

Based on the service periods observed, healthy animals and those with clinical or subclinical mastitis would have an expected calving interval of around 347, 395 and 372 days, respectively. The ideal calving interval to maximize reproductive and productive efficiency should not exceed 365 days (PEREIRA et al., 2013; LEITE; MORAES, PIMENTEL, 2001).

It was observed that the number of quarters, as well as the degree and duration of clinical and subclinical mastitis cases increased ($p<0.05$) the animals' service period (Table 4). Only the number of isolated cases of subclinical mastitis was not associated with the length of service period ($p>0.05$).

The negative effect of clinical and subclinical mastitis on the animals' service period is related to the stress caused by the disease. Released cortisol can trigger a blockage in the secretion of luteinizing hormone (LH) and ovulation impairment. A reduction in preovulatory estradiol concentration interferes with the induction of gonadotropin-releasing hormone (GnRH) and reduces the pulsatile secretion of luteinizing hormone, which can cause delays in ovulation (LAVON et al., 2010).

The increase in pro-inflammatory cytokines in the bloodstream due to mastitis affects the composition of the follicular fluid and impairs oocyte quality. Reactive oxygen microbial species and tumor necrosis factor (TNF-α) can inhibit progesterone and estrogen synthesis. Endotoxins, prostaglandin (PGF2-α), interleukin (IL-1β, IL-2), interferon (INF-γ) and tumor necrosis factor (TNF-α, TNF-β) can induce luteal cell death, luteolysis, changes in the progesterone profile, and induce embryonic or fetal death (HERTL et al., 2010; IRELAND et al., 2010; LAVON et al., 2010; HANSEN; SOTO; NATZKE, 2004; HOCKETT et al., 2000).

For every mammary quarter affected by clinical mastitis, the service period increased by 30.9 days. The service period increased by 25.4 and 6.5 days with each increase in the degree of clinical and subclinical mastitis, respectively. With each additional day in the duration of clinical and subclinical mastitis cases, the service period increased by 8.5 and 0.6 days, respectively.

The service period increased by 31.7 days when the same animal had a quarter affected by clinical mastitis and another by subclinical mastitis. It is clear that the number of quarters, and the degree and duration of clinical mastitis cases caused a greater delay in the service period. This affects the reproductive and productive efficiency of the herd, generating greater economic losses (PRATA et al., 2014).

In addition to mastitis, other factors can influence the length of the service period. Age, number of births, breed and individuality are among the intrinsic factors, but the season of the year and nutritional management can prolong the period and

Table 4. Effect of number of quarters, degree and duration of clinical and subclinical mastitis on service period (days) in a herd from Tarumirim (MG)

Mastitis	Parameter	p*	r*	Regression
Clinical	Quarters (0 to 4)	0.004	0.520	SP = 69.670 + (30.937 * QClin)
	Degree (0 to 3)	0.004	0.521	SP = 70.120 + (25.440 * DClin)
	Length (days)	0.003	0.529	SP = 69.795 + (8.518 * LClin)
Subclinical	Quarters (0 to 4)	0.174	0.324	SP = 67.416 + (9.095 * QSubclin)
	Degree (0 to 4)	0.046	0.260	SP = 68.585 + (6.527 * GSubclin)
	Length (days)	0.002	0.555	SP = 60.128 + (0.646 * LSubclin)
Clinical + Subclinical	Quarters (0 to 4)	0.012	0.537	SP = 66.052 + (4.139 * QSubclin) + (27.595 * QClin)
	Degree (0 to 4)	0.013	0.534	SP = 66.515 + (3.108 * GSubclin) + (23.746 * GClin)
	Length (days)	0.004	0.586	SP = 62.333 + (0.419 * LSubclin) + (4.357 * LClin)

* "p" value lower than 0.050 indicates significant association between mastitis parameter and days of service period (SP); **Coefficient $r \geq 0.7$ indicates strong association, $0.3 \geq r < 0.7$ indicates moderate association, and $r<0.3$ indicates weak association; Q = number of quarters; D = degree of clinical or subclinical mastitis; L = length of occurrence of clinical or subclinical mastitis.

compromise the reproductive efficiency of the herd (BEZERRA et al., 2011; BERGAMASCHI et al., 2010; RANGEL et al., 2009). In the present study, such factors were treated randomly.

In addition to causing the reproductive harms observed in the present study, mastitis can also mean direct losses due to the costs of treating the cases, milk discard, reduced milk production, loss of bonuses given by milk quality, death of cows, and costs generated by control measures (COSTA et al., 2017; LOPES et al., 2012).

CONCLUSIONS

The prevalence rates of clinical and subclinical mastitis in the herd of Tarumirim (MG) are 2.9 and 63.7%, respectively. Mastitis increases the service period of dairy cows. The number of affected mammary quarters, as well as the degree and duration of clinical mastitis cases delay the service period more than subclinical mastitis cases do. Good milking hygiene and animal handling practices should be adopted in order to reduce the prevalence of mastitis in the herd.

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