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REGULAR ARTICLES

Effects of clinical mastitis on reproductive and milk performance of Holstein cows in Morocco

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Abstract The aim of this study was to determine the influence of clinical mastitis and time of first mastitis occurrence on reproductive and milk performance of Holstein cows. Data were collected in a dairy farm from 2008 to 2012 on 1725 cows, among which 464 cows with mastitis. To determine the influence of clinical mastitis on reproductive and milk performance, models included fixed effects of parity, calving season, calving year, and group (cows with and with no mastitis). To determine the effect of time of 1st mastitis occurrence on reproductive performance, the mastitic cows group was further reclassified into three groups: prior to 60 days, between 60 and 90 days and greater than 90 days postpartum. For milk performance, the mastitic cows group was divided into two groups: before and after peak milk yield. Clinical mastitis had significant effects on calving to first AI interval, milk yield, and fat yield, but a non-significant effect on days open, number of inseminations per conception, and milk fat percentage. Mastitic cows had a calving to first AI interval 6.1 days longer and 549.6 kg milk and 20.4 kg fat per 305 days of lactation lower than those with no mastitis. Time of 1st mastitis occurrence did not have any significant effect on reproductive performance. Further, milk and fat yields of cows diseased before peak milk yield were 506 kg and 23.9 kg, respectively, lower than those of cows affected after peak milk yield. Extra attention needs to be paid to mastitis during the early postpartum period.

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Introduction

In 2008, Morocco started up a development plan for the dairy sector. Its main objective was to produce 4 to 5 billion liters of milk annually by the year 2020 (MAPM 2010). However, this plan was not accompanied by some management precautions resulting in the apparition of some health diseases, such as clinical mastitis. This inflammation of the mammary gland remains the most common disease affecting dairy cows and causing economical losses for the dairy sector. Cows with mastitis had a longer interval from calving to first AI, longer days open and higher number of AI per conception compared with cows with no mastitis (Barker et al. 1998; Ahmadzadeh et al. 2009; Nava-Trujillo et al. 2010). The timing of mastitis occurrence also appears to be important. If the disease occurred before the first AI after calving, the numbers of days to first AI and conception increase markedly, while mastitis occurred between first AI and conception, the days to conception and the number of AI per conception increased (Loeffler et al. 1999; Ahmadzadeh et al. 2009). Furthermore, several studies have found that clinical mastitis negatively affects milk yield (Lucey et al. 1986; Rajala-Schultz et al. 1999). The estimated lactational milk loss was found to vary between 0 to 11 % of the 305 days milk yield, and the fat yield loss was 0 to 12 % of the total yield (Hort and Seegers 1998; Seegers et al. 2003; Hagnestam et al. 2007). Moreover, effects of clinical mastitis on milk production varied according to the time of occurrence. Cows affected in early lactation produced less than those diseased late in lactation (Lucey et al. 1986; Hort and Seegers 1998; Hagnestam et al. 2007).

The aim of this study was to determine the influence of clinical mastitis and timing of the 1st mastitis occurrence on reproductive and milk performance of Holstein dairy cows in Morocco.

Material and methods

Herd management

Data were collected from 2008 to 2012 in an organized dairy farm located in North of Morocco on 1725 Holstein cows among which 464 cows had at least one mastitis case within 305 days of lactation. Average age at calving was 47.6± 15.8 months, ranging from 23 to 82 months, and average lactation number was 2.31±1.06, ranging from 1 to 4. During the study, mean milk production per cow was 8066± 1956 kg per 305 days of lactation.

All cows were housed in free-stall barns. They were observed for estrus two times daily, at 0900 h and 1500 h. Cows detected in estrus were inseminated within 1 h after detection of estrus. Pregnancy was confirmed by rectal palpation 55–65 days after artificial insemination. All lactating cows were fed the same diet that was formulated to meet the nutrient requirements for a lactating Holstein cow weighing 650 kg and producing 25–35 kg of milk/day.

The farm milked cows in fully automated milking parlors (Boumatic, USA) equipped with automatic milking machine. Cows were milked two to three times daily according to their lactation stage. Teat dipping was routinely performed at milking. Milking equipment was evaluated routinely and maintained per the recommendations of the manufacturer. At each milking, cows were examined for symptoms of clinical mastitis by the milking personnel. Clinical mastitis was characterized by visible abnormalities in the milk or inflammation, hardness, and redness of a quarter. Abnormal milk was detected using the strip cup test for the cow's udder quarters. Clinical mastitis was any first case of clinical mastitis detected by milking personnel on the basis of clinical symptoms, regardless of how many quarters were affected.

Studied variables and statistical analyses

Detailed information on clinical mastitis occurrence, calvings, reproductive performance, and milk production was available. Reproductive performance included days to first AI (number of days from calving to the first insemination), days open (number of days from calving to conception), and the number of AI per conception. Milk production data included milk yield, percentage of milk fat, and fat yield per 305 days of lactation. During the study period, only the observations of one lactation of a cow were used for analysis.

Data were analyzed by least-squares method using the GLM procedure (SAS 2002). To determine the effect of clinical mastitis on reproductive and milk performance, models included fixed effects of parity (four levels: 1, 2, 3, 4), season of calving (three levels: February–June, July–September, October–January), year of calving (five levels: 2008, 2009, 2010, 2011, 2012), and group of cows (two levels: cows with mastitis and cows with no mastitis). Furthermore, the influence of timing of first clinical mastitis on reproductive and milk performance was studied using the data of 464 cows with mastitis. To determine the effect of time of 1st mastitis occurrence on reproductive performance, the statistical model included fixed effects of parity, calving season, calving year, and time of 1st mastitis occurrence (three levels: prior to 60 days postpartum, between 60 and 90 days postpartum, and after 90 days postpartum). Likewise, the model used to test the effect of time of 1st mastitis occurrence on milk performance included fixed effects of parity, calving season, calving year, and time of 1st mastitis occurrence (two levels: before peak milk yield and after peak milk yield). The peak milk yield was reached 41.4 days after calving (Boujenane and Hilal 2012). Interactions between the fixed effects were assumed negligible and were not tested. Significant differences among least-squares means were examined by Tukey method for multiple comparisons.

Results and discussion

Within 305 days of lactation, clinical mastitis was observed in 26.9 % of cows. At the beginning of the study, cows with and with no mastitis had similar lactation numbers, ages at calving, and multiparous cows had similar previous lactation milk yields.

Effects of clinical mastitis and timing on reproductive performance

The influence of clinical mastitis on reproductive performance was studied through the comparison of cows with at least one case of mastitis with cows without any case of mastitis within 305 days of lactation. Clinical mastitis is associated with significantly more days to 1st AI, but without a significant effect on the days open and the number of AI per conception. The interval from calving to first AI of mastitic cows was 6.1 days longer than that of cows not exhibiting clinical mastitis (Table 1). These results are in agreement with those of Nava-Trujillo et al. (2010) who reported that cows with at least one mastitis case had an interval from calving to first AI 37.8 days longer than that of uninfected cows. Huszenicza et al. (1998) reported that the increased interval from calving to first AI for mastitic cows is due to gram-negative bacteria that delay the first ovulation and the first estrus, which in turn might increase days to first AI. Gunay and Gunay (2008) showed that clinical mastitis didn't have significant effects

Table 1 Least-squares means (LSM)±standard errors (SE) for reproductive performance of cows with mastitis and with no mastitis[1]

Group	Number	Interval from calving to first AI (days) LSM±SE	Days open LSM±SE	Number of AI per conception LSM±SE
Cows with no mastitis	1261	92.5±1.79[a]	185.5±3.92	2.48±0.60
Cows with mastitis	464	98.6±2.41[b]	189.6±5.26	2.50±0.08
Prob.		0.0064	0.4008	0.8667

[1] Cows with no mastitis: Cows without any clinical mastitis case within 305 days of lactation

Cows with mastitis: Cows with at least one clinical mastitis case within 305 days of lactation

Least-squares means within a column that do not have a common superscript (a and b) are significantly different ($P<0.05$)

on calving to first AI interval and number of AI per conception, but had a significant effect on days open. Klaas et al. (2004) concluded that reproductive performances were not influenced by clinical mastitis.

The time of 1st mastitis occurrence did not have any significant effect on days to first AI, days open, and number of AI per conception ($P>0.05$) (Table 2). The absence of significant effect of time of 1st mastitis occurrence on the calving to first AI interval is not consistent with the observations reported by Barker et al. (1998), Santos et al. (2004) and Nava-Trujillo et al. (2010), who indicated that clinical mastitis before the first AI increased the days to first AI. Likewise, the lack of timing of 1st mastitis infection effect on days open is not in agreement with the results of previous studies (Barker et al. 1998; Santos et al. 2004; Ahmadzadeh et al. 2009), where days open and number of AI per conception were higher when cows exhibited mastitis between first AI and conception. Moreover, Barker et al. (1998) reported that increased days to first AI for cows with clinical mastitis prior to first AI resulted from the loss of behavioral estrus due to insufficient follicular development. Additionally, delay in follicle maturation and resumption of ovarian cycles were due to the influence of clinical mastitis early in lactation on energy balance by increasing body weight losses and extending the period of energy deficit (Butler 2000). Chebel et al. (2002), in a study investigating embryonic mortality between 31 and 45 days after AI, reported an increase in the incidence of pregnancy loss when clinical mastitis occurred after conception. Hansen et al. (2004) showed that mastitis can cause embryonic mortality through the release of molecules of bacterial origin that activate inflammatory and immune responses. As a result, there is an increase in cytokine synthesis from the mammary gland that disrupts function of the hypothalamus, pituitary, ovary and uterus, leading to embryonic mortality (Hansen et al. 2004). Also, the increase in number of AI per conception caused by mastitis is associated with anovulation at oestrus, fertilization failure, and embryonic mortality (Moore et al. 1991; Huszenicza et al. 2005). The difference between the current study and the previous studies (Barker et al. 1998; Santos et al. 2004; Gunay and Gunay 2008) regarding the effect of the time of 1st mastitis occurrence on reproductive performance may be due to the definition of the time frame where mastitis could occur. In the present study, classes of the time of 1st mastitis occurrence were defined as fixed time intervals (prior to 60 days, between 60 and 90 days, and after 90 days postpartum), whereas in the previous studies, classes were defined as "prior to first AI, between first AI and conception, and after conception". This latter definition is not adequate since the longer the interval is, the more likely the cow will experience mastitis within this interval. In other words, in the first group (prior to 60 days postpartum) e.g., one cow has 60 days at risk for mastitis, no matter when it is inseminated, whereas when the interval is defined as "prior to 1st AI", the cow may have few days at risk for mastitis if it was inseminated early, but more days at risk for mastitis if, for whatever reason, it was inseminated late. Therefore, since the risk of mastitis per cow is more or less constant, the longer the interval prior to 1st AI is, the more likely a cow will have a case of mastitis before 1st AI. The same reasoning may apply for mastitis occurrence between 1st

Table 2 Least-squares means (LSM)±standard errors (SE) for reproductive performance of cows with mastitis according to the time of 1st mastitis occurrence

Time of 1st mastitis occurrence	Number	Interval from calving to first AI (days) LSM±SE	Days open LSM±SE	Number of AI per conception LSM±SE
Prior to 60 days postpartum	261	99.1±5.17	156.9±10.9	2.02±0.17
Between 60 and 90 days postpartum	73	101.8±6.42	163.5±13.6	2.17±0.21
After 90 days postpartum	130	98.0±5.59	177.1±11.8	2.22±0.19
Prob.		0.8228	0.1101	0.3728

Table 3 Least-squares means (LSM)±standard errors (SE) for milk production of cows with mastitis and with no mastitis[1]

Group	Number	Milk yield (kg) LSM±SE	% fat LSM±SE	Fat yield (kg) LSM±SE
Cows with no mastitis	1255	8274.9±79.6[a]	4.21±0.02	348.3±3.65[a]
Cows with mastitis	462	7725.3±106.7[b]	4.26±0.02	327.9±4.89[b]
Prob.		<0.0001	0.0508	<0.0001

[1] Cows with no mastitis: Cows without any clinical mastitis case within 305 days of lactation

Cows with mastitis: Cows with at least one clinical mastitis case within 305 days of lactation

Least-squares means within a column that do not have a common superscript (a and b) are significantly different ($P<0.05$)

AI and conception interval and for interval after conception. Therefore, it seems that the time of 1st mastitis occurrence in Holstein cows does not affect significantly the reproductive performance. Klaas et al. (2004) have also pointed out the importance of definition of time of disease occurrence.

Effects of clinical mastitis and timing on milk production

The comparison between cows with and with no mastitis showed that milk and fat yields were influenced by clinical mastitis ($P<0.001$), while percentage of milk fat was not affected ($P>0.05$). Milk and fat yields of cows with mastitis were 549.6 and 20.4 kg per 305 days of lactation, respectively, lower than those of cows with no mastitis (Table 3). These values are consistent with those of other researchers (Seegers et al. 2003; Santos et al. 2004; Hagnestam et al. 2007), those found the same milk and fat losses. Hagnestam et al. (2007) showed that the magnitude of yield loss varied from 0 to 902 kg (0 to 11 % of the 305 days milk yield) and the fat yield loss was 0 to 41 kg (0 to 12 % of the total yield). Rajala-Schultz et al. (1999) indicated that cows diagnosed with clinical mastitis had an estimated milk yield loss of 110–552 kg milk when contrasted with production prior to the mastitis event. Hagnestam et al. (2007) showed that daily milk yield started to decline 2 to 4 weeks before diagnosis, and on the day of clinical onset, the milk yield of mastitic cows was reduced by 1 to 8 kg. Moreover, after the occurrence of mastitis, the daily loss of Holstein cows during the first 2 weeks varied from 5 to 9 kg/day, and the total loss over the entire lactation varied from 570 to 690 kg (Rajala-Schultz et al. 1999). However, comparisons of milk loss between studies are difficult to make because there is considerable variation in the data collected with regard to period of collection, parity, time of mastitis occurrence, yield level of cows, pathogens involved, and analytical methods used (Gröhn et al. 2004; Hagnestam et al. 2007). Moreover, since cows with no mastitis are usually poorer producers, the use of their yield as a reference for the production level of healthy cows certainly results in an underestimation of the effect of clinical mastitis on milk yield. Further, cows with mastitis can be forced drying off and therefore shortened lactation. When this is the case, the yield losses will probably be even more severe than those reported (Gröhn et al. 2004; Hagnestam et al. 2007). Several researchers (Santos et al. 2004; Hagnestam et al. 2007) reported that clinical mastitis significantly decrease production and alters milk composition. The negative effect of clinical mastitis on milk yield may be explained by the ability of several species of bacteria to invade the mammary gland, multiply there, and produce toxins that result in an inflammatory response and reduction of milk yield. However, the decrease of fat yield in cows with mastitis seems to be caused by reduced milk production and not by changes in the fat content of the milk, as shown by the results of the present study.

The effect of timing of first mastitis occurrence was significant on milk and fat yields, but not significant on percentage of milk fat (Table 4). This result is in agreement with those of Lucey et al. (1986) and Rajala-Schultz et al. (1999), those showed that the magnitude of the 305-day losses varied according to when in lactation the cow is diseased. In the present study, cows affected before peak milk yield produced 506 kg milk and 23.9 kg fat per 305 days of lactation less than cows exhibiting mastitis after peak milk yield, indicating that

Table 4 Least-squares means (LSM)±standard errors (SE) for milk production of cows with mastitis according to the time of 1st mastitis occurrence[1]

Time of 1st mastitis occurrence	Number	Milk yield (kg) LSM±SE	% fat LSM±SE	Fat yield (kg) LSM±SE
Before peak milk yield	191	7265.3±262.7[a]	4.21±0.06	302.6±12.1[a]
After peak milk yield	271	7771.3±240.1[b]	4.22±0.06	326.5±11.0[b]
Prob.		0.0054	0.7624	0.0041

[1] Peak milk yield was reached 41.4 days after calving (Boujenane and Hilal 2012)

Least-squares means within a column that do not have a common superscript (a and b) are significantly different ($P<0.05$)

clinical mastitis causes more severe losses in early lactation. This is in agreement with Santos et al. (2004) who concluded that clinical mastitis either prior to or immediately after the first postpartum AI had marked effects on yields of milk and milk components in lactating dairy cows. It is noteworthy that regardless of the time of occurrence during the lactation, mastitis had a long-lasting effect on milk yield; cows with mastitis did not reach their premastitis milk yields during the remainder of the lactation after onset of the disease (Rajala-Schultz et al. 1999; Gröhn et al. 20044).

Conclusion

In conclusion, the present study demonstrated that clinical mastitis in Holstein cows, especially during early lactation, had a detrimental impact on their reproductive and milk performance. Therefore, dairy farmers should examine their cows for mastitis during the early postpartum period in order to avoid the negative effects of mastitis on reproductive and milk performance.

Conflict of interest The authors have no conflict of interests regarding the research reported in this manuscript.

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