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Prevalence and Risk Factors of Subclinical Mastitis in Iranian Holstein Cows

Research Article

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ABSTRACT

The present study aimed to estimate the effects of parity, calving season and year of calving on the prevalence of subclinical mastitis in Holstein cows. A total of 2682 records from 869 Holstein cows in a large dairy farm (Azarbaijan province, Iran), respecting the period from 2006 to 2009, were collected. Data was analyzed using Proc Mixed of SAS software by MIVQUE method. Subclinical mastitis was also studied based on three different models including: 1) considering each udder quarter as a separate unit (Udder quarter model), 2) considering all the four udder quarters of a cow as one overall unit (Subclinm model) and 3) considering the sum of positive subclinical mastitis scores in all udder quarters of an animal (Episode model). Diagnosis of subclinical mastitis was based on California Mastitis Test. Results showed that the prevalence of subclinical mastitis was 20.83%. The prevalence of cows with only one udder quarter affected by subclinical mastitis was 23.71%. Parity and year of calving significantly affected the prevalence of subclinical mastitis (P<0.001). Older cows with higher parity number had increased prevalence of subclinical mastitis. Therefore, the highest prevalence of subclinical mastitis was observed in cows having number of parities between 5 and 11. The lowest subclinical mastitis prevalence (using three mastitis models) was recorded in 2010, whilst its highest prevalence was observed in 2008. Season of calving also significantly influenced subclinical mastitis prevalence (P<0.05): cows calving in autumn had higher prevalence of subclinical mastitis than those calving in the other seasons. Concluding, data from the present study demonstrated that parity, calving season and year of calving influenced the prevalence of subclinical mastitis on the three mentioned models.

KEY WORDS calving season, Holstein cows, parity, risk factors, subclinical mastitis.

INTRODUCTION

Major animal breeding objectives focus on increasing dairy cattle productivity, which may lead to lower resistance to diseases (Van Dorp *et al.* 1998). Mastitis is one of the diseases with high prevalence as a result of animal breeding (Oltenacu and Broom 2010). Mastitis can be described as clinical and subclinical (Harmon and Reneau, 1993) according to existing symptoms. Subclinical mastitis is the most economically important disease of dairy industries around the world (Schultz *et al.* 1990) due to its effect on reducing milk yield and quality and also by reducing fertility in cows, while increasing abortion risk and the costs with treatments (Phuektes *et al.* 2001; Meiri-Bendek *et al.* 2002). Fisher (1979) reported up to 45% reduction in milk production in the udder quarters affected by subclinical mastitis. *Staphylococcus aureus* is one of the most common causes of mastitis in dairy farms, and this pathogen may prevail in 50-100% of herds (Fisher, 1979). The majority of infections due to *Staphylococcus aureus* are subclinical mastitis and usually its response to treatment is poor (Sutra and Poutrel, 1990). Subclinical mastitis can be diagnosed by two methods including somatic cell counts (SCC) in milk, either by direct counting or by an indirect method called California mastitis test (CMT) (Harmon and Reneau, 1993). Previous studies showed that mastitis is under the influence of parity, year of calving, calving season, age and milk yield (Emanuelson *et al.* 1988; Banos and Shook, 1990; Schultz *et al.* 1990; Hansen, 1992; Rahman *et al.* 2009). Detilleux *et al.* (2012) studied the relationship between subclinical and clinical mastitis with some risk factors of herd condition, feeding practices and control strategies of mastitis. Braund and Schultz (1963) reported the existence of significant effects of parity on subclinical mastitis, so that cows in the first lactation had lower number of udder quarters affected by subclinical mastitis than older cows. In another study, Lindström and Syväjärvi (1978) showed an increase in subclinical mastitis score (using CMT) with increasing lactation numbers. Besides, it was also evidenced that the month of calving influences the prevalence of subclinical mastitis. Miller *et al.* (1976) reported that cows calving between February and July had higher prevalence of subclinical mastitis than those calving between August and January. However, there is no available report regarding the effects of inbreeding on mastitis prevalence in Azarbaijan province of Iran. Therefore, the present study was to evaluate the prevalence and risk factors of subclinical mastitis in Holstein herds in the northwest of Iran.

MATERIALS AND METHODS

Data collection

Data concerning 869 Holstein cows represented 2682 records retrieved from the database of one large dairy farm in Azarbaijan province, Iran for the period of 2006-2009. Milk was collected and information recorded three times per day and twice a month (except for the first 20 days after calving, when cows were milked four times per day). Data related to parity, calving season and year of calving were also recorded. Cows' parities were categorized as 1st, 2nd, 3rd, 4th, and 5th-11th. In addition, calving season was categorized as spring (from middle of March to the end of June), summer (from the end of Jun to the end of September), autumn (from the end of September to the end of December) and winter (from the end of December to the end of March). Inbreeding coefficient (F) of the population was calculated using the Pedig software.

Detection of subclinical mastitis

Subclinical mastitis was diagnosed by CMT; in this test, the nature of coagulation and the viscosity of the mixture are indicators for presence and severity of the inflammation, respectively (Harmon, 1994). California mastitis test was performed twice a month, in the morning before milking according to the veterinary recommendations. In this test, after pouring milk samples of each udder quarters inside the CMT containers, the intensity of subclinical mastitis was coded as follows: 1) if the mixture inside the container remained liquid, homogeneous, with no coagulation, corresponding to a healthy record; 2) if the mixture inside the container started coagulating, but this coagulation disappeared after rotation of the paddle; 3) if the mixture inside the container coagulated, but did not stick and form jelly and 4) if the mixture inside the container coagulated, sticks, and tended to form jelly, and this form was considered as the most severe kind of subclinical mastitis.

Designing three models for evaluating subclinical mastitis

In the present study, the prevalence of subclinical mastitis was studied according to the following models:

1) Udder quarter model: study of subclinical mastitis in each udder quarter separately including: left rear quarter, right rear quarter, left front quarter, and right front quarter.

2) Subclinm model: study of subclinical mastitis in all the udder quarters of a cow. In this model, if at least one quarter was affected by subclinical mastitis (CMT scores of 2, 3 or 4), cow's mammary gland was coded as 1. While, in a condition that all udder quarters were healthy (CMT scores of 1), cow's mammary gland was coded as 0.

3) Episode model: in this model the sum of CMT scores of all udder quarters of an animal was considered as an episode.

Statistical analysis

Prevalence of subclinical mastitis is dependent on some specific risk factors. The prevalence of subclinical mastitis was calculated as the percentage of subclinical mastitis affected cows out of the total lactating cows. In order to see the effects of individual risk factors on subclinical mastitis, all analyses were carried out using Proc Mixed of SAS software according to Minimum Variance Quadratic Unbiased Estimation (MIVQUE) method, SAS (2004). The Mixed model used for analysis of Episode, Subclinm and Udder quarter models is described as:

$$Y_{ijkmn} = \mu + Y_i + S_j + PAR_k + F_m + S_n + (PAR_k \times F_m) + e_{ijkmn}$$

Where:

Y_{ijkmn}: observation of subclinical mastitis based on Episode, Subclinm and Udder quarter models.

μ: population mean.

Y_i: fixed effect of calving year.

S_j: fixed effect of calving season.

PAR_k: fixed effect of Parity.

F_m: fixed effect of inbreeding coefficient.
S_n: random effect of sire.
$PAR_k \times F_m$: interaction among parity and inbreeding coefficient.
e_{ijkmn}: experimental error for i^{th} (i=2007, 2008, 2009 and 2010) year of calving, j^{th} (j=spring, summer, autumn and winter) calving season, k^{th} (k=1, 2, 3, 4 and 5) parity, m^{th} (1,2,3…) cow and the n^{th} (1, 2, 3,…) sire.

RESULTS AND DISCUSSION

The Subclinm model results showed that, in the farm enrolled in the present study, the prevalence of subclinical mastitis was 20.83% based, lower than the reported by Abaineh (1997) in Fiche (65%), by Abaineh *et al.* (2002) in Modjjo farms in central Ethiopia (47.5%) and by Rafyi-Barzoki (1998) in the Semnan Iranian province (33.2%). Busato *et al.* (2010) mentioned a prevalence of subclinical mastitis at the quarter level of 21.2% for a period of 7 ± 100 days post-partum. Mastitis is a complex disease, under the influence of multiple factors, including the environment, management, factors related to animal and causative organisms. Because the effects of these factors on SCC differ among herds, lactation phases and cattle breeds, prevalence of mastitis is expected to be different from place to place (Monardes and Hayes, 1984). In the present study, in 23.71% of CMT records only one udder quarter was affected by subclinical mastitis, while the proportion of cows with more than one affected udder quarter was lower (17.81%). Moreover, the results also showed that 58.5% of CMT records were counted as healthy based on the Episode model (Figure 1). Accordingly, several other studies showed that subclinical mastitis often occurs in just one udder quarter (Barkema *et al.* 1997; Berglund *et al.* 2004; Forsbäck *et al.* 2009). The effects of parity, year of calving and calving season on subclinical mastitis prevalence according to the Episode, Subclinm and Udder quarter models are presented in Table 1. Parity significantly influenced the prevalence of subclinical mastitis based on the three mentioned models (P<0.001). First parity cows showed the lowest prevalence, whilst the highest prevalence for subclinical mastitis was recorded in cows with more or equal to 5 parities (5^{th} to 11^{th}); exceptionally, the highest prevalence of subclinical mastitis arising in the right rear quarter was observed at the 4^{th} parity. The results presented herein showed that high yield cows with increasing parity number were predisposed to subclinical mastitis. Higher parity cows have higher production, so the presence of high CMT scores among these cows may be related to the existence of physiological stress associated to the high production, resulting in an increase in the somatic cells counts in milk.

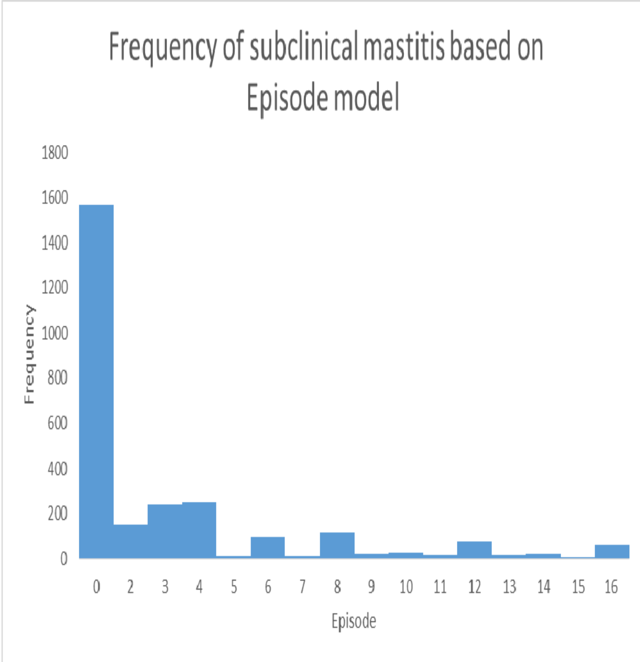

Figure 1 Frequency of subclinical mastitis based on the Episode model

Thus, higher SCC associated to subclinical mastitis may eventually cause a reduction in milk production. Slettbakk *et al.* (1995) showed that mammary glandular tissues in high yielding cows were more susceptible to mastitis. This could be originated by a weakness of the local defense mechanism in older cows compared with younger cows (Dulin *et al.* 1988). Several other studies reported similar findings (Slettbakk *et al.* 1995; Quaderi, 2005; Rahman *et al.* 2009).

Also, Biffa *et al.* (2005) found a significant influence of parity on prevalence of subclinical mastitis (P<0.001) referring that cows with more than seven calves were exposed to a 13-times greater risk of SCC than those with less than 3 calves.

This could be associated to a higher activity of leukocytes in primiparous cows compared to multiparous cows (Dulin *et al.* 1988). Miller *et al.* (1976) showed that mastitis-affected cows had usually higher milk yield than the healthy cows.

It was also showed that an increase in milk production from 5000 to 15000 kg caused a considerable increase (from 0.33 to 0.48%; P<0.001) in the prevalence of mastitis (Noshahr Ala and Shadparvar, 2011).

Batra (1980) reported a significant (P<0.01) effect of parity on the prevalence of mastitis in all four quarters of cows and showed a rapid increase in CMT score from the first to the second lactation; furthermore, a slight increase was observed from the second to the fourth lactations (Batra, 1980).

Table 1 Effects of parity, and calving year or season on prevalence of subclinical mastitis in Iranian milking cows based on Episode, Subclinm, and Udder quarter (right rear quarter, left rear quarter, right front quarter and left front quarter) models

Risk Factors	Level	Right rear quarter	Left rear quarter	Right front quarter	Left front quarter	Subclinm	Episode
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Parity	1	0.08±0.01^{d}	0.14±0.01^{d}	0.06±0.02^{c}	0.20±0.02^{d}	0.12±0.01^{d}	0.53±0.06^{d}
	2	0.12±0.01^{c}	0.21±0.02^{c}	0.31±0.02^{b}	0.57±0.02^{c}	0.14±0.01^{c}	0.94±0.06^{c}
	3	0.21±0.02^{b}	0.31±0.01^{b}	0.49±0.04^{a}	0.69±0.02^{b}	0.23±0.02^{b}	1.62±0.06^{b}
	4	0.49±0.04^{a}	0.48±0.02^{a}	0.51±0.04^{a}	0.85±02^{a}	0.30±0.01^{a}	2.25±0.06^{a}
	5-11	0.44±0.04^{b}	0.52±0.02^{a}	0.62±0.04^{a}	0.90±0.02^{a}	0.33±0.01^{a}	2.3±0.06^{a}
		NS	NS	NS	NS	P<0.05	P<0.05
Season of calving	Spring	0.06±0.02	0.22±0.02	0.34±0.02	0.62±0.02	0.14±0.01^{c}	1.22±0.05^{c}
	Summer	0.04±0.02	0.21±0.02	0.32±0.02	0.61±0.02	0.15±0.01^{c}	1.10±0.05^{c}
	Autumn	0.11±0.02	0.23±0.02	0.39±0.02	0.67±0.02	0.21±0.01^{a}	1.55±0.05^{a}
	Winter	0.09±0.02	0.23±0.02	0.31±0.02	0.67±0.02	0.18±0.01^{b}	1.39±0.05^{b}
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Year of calving	2007	0.12±0.02^{b}	0.23±0.02^{b}	0.39±0.02^{b}	0.67±0.02^{b}	0.13±0.01^{c}	1.41±0.05^{b}
	2008	0.33±0.02^{a}	0.57±0.02^{a}	0.62±0.02^{a}	0.88±0.04^{a}	0.36±0.01^{a}	2.41±0.06^{a}
	2009	0.25±0.02^{b}	0.31±0.02^{b}	0.45±0.02^{b}	0.72±0.04^{b}	0.23±0.01^{b}	1.62±0.06^{b}
	2010	0.09±0.02^{c}	0.14±0.02^{c}	0.012±0.02^{c}	0.31±0.02^{c}	0.03±0.01^{d}	0.17±0.05^{c}

[*] Data are presented as least square means ± SE.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
NS: non significant.

The present study showed that the year of calving significantly affected the prevalence of subclinical mastitis based on Episode, Subclinm and Udder quarter models (P<0.001). The lowest prevalence of subclinical mastitis was identified in all the three models in 2010 while the highest was observed in 2008.

The improvement found in 2010 could be associated to the genetic improvement of the herd for milk yield, health situation, and management; it is possible that the genetic improvement might be a major reason for reducing subclinical mastitis occurrences. In similar studies, Wilton *et al.* (1972) and Alrawi *et al.* (1979) reported that variation in prevalence of mastitis was associated with year-season of calving.

The present study showed that the calving season significantly affect the prevalence of subclinical mastitis according to Subclinm and Episode models (P<0.05). Cows calving in autumn showed higher prevalence of subclinical mastitis compared to cows calving in other seasons. However, the calving season had no effect on the prevalence of subclinical mastitis based on Udder quarter model. Likewise, Noshahr Ala and Shadparvar (2011) reported that cows calving in winter had higher prevalence of subclinical mastitis than those calving in spring (P<0.05). Independent studies, by Batra (1980) and Salsberg *et al.* (1984), found that cows calving from June to August were more susceptible to subclinical mastitis than cows calving in other months.

They concluded that heat stress could be a reason for high occurrences of subclinical mastitis in summer months (Batra, 1980; Salsberg *et al.* 1984), thereby concluding that high or low environmental temperatures related to seasons were able to increase the risk of subclinical mastitis in cows.

Future studies using milk somatic cell counts and its bacteriological spectrum are necessary to confirm the preliminary data concerning the prevalence and causative agents of subclinical mastitis reported herein.

CONCLUSION

This study showed that one fifth of the cows had subclinical mastitis, which usually occurred only in one of the udder quarters. In addition, it was showed that older cows with higher milk yield were more susceptible for subclinical mastitis. The year of calving and calving season both influenced the prevalence of subclinical mastitis (based on the mentioned models) such as the highest prevalence of subclinical mastitis was observed in 2008 and in autumn, respectively.

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