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PREVALENCE OF SUBCLINICAL MASTITIS IN LACTATING COWS AND EFFICACY OF INTRAMAMMARY INFUSION THERAPY

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ABSTRACT

The aim of the study was to estimate the prevalence of subclinical mastitis (SCM) in lactating cows of Mymensingh and Lakshmipur sadar upazilas and to evaluate the efficacy of intramammary infusion in subclinical mastitis affected cows. A cross-sectional study was carried out on 78 smallholder dairy farms using a pretested questionnaire to collect data from June 2011 to May 2012. Milk samples from 139 lactating cows were screened for SCM by California Mastitis Test. A total of 72 cows was found positive for SCM and treated with the Neomastipra-JR5® intramammary infusion tube (Hipra, Spain). The overall prevalence of subclinical mastitis in lactating cows was 51.8%, of which 51.4% at Mymensingh sadar and 52.2% at Lakshmipur sadar upazilas. The prevalence of subclinical mastitis was significantly increased with age and parity of cows. In left hind quarter, the prevalence of subclinical mastitis was significantly higher (33.1%) than those of other quarters. The efficacy of intramammary infusion with Neomastipra-JR5® in lactating cows was 66.7%.

Key words: Sub-clinical mastitis, lactating cows, California Mastitis Test, intramammary infusion

INTRODUCTION

Mastitis is an inflammatory condition of the mammary gland, characterized by the changes in the physical characteristics of the udder or milk (Nazifi *et al.*, 2011). The subclinical mastitis (SCM) is more serious and is responsible for much greater loss to the dairy industry in Bangladesh (Kader *et al.*, 2003). Subclinical mastitis is 15 to 40 times more prevalent than the clinical form, is of long duration and difficult to detect ((Almaw *et al.*, 2008; George *et al.*, 2008; Sarker *et al.*, 2013). In Bangladesh, the prevalence of SCM is recorded from 20 to 44% at cow level based on California Mastitis Test (CMT) (Rahman *et al.*, 2009; Islam *et al.*, 2010; Rabbani and Samad, 2010). The efficacy of antibiotic therapy for intramammary infections (IMIs) early in lactation is scarce and few, with the ones carried out reporting mixed results. The response to therapy with intramammary (IMM) cephapirin sodium on CMT positive quarters in lactating cows on cure rates and somatic cell count (Rosenberg *et al.*, 2002). It was determined that by the 4-week post-calving evaluation, quarters treated with cephapirin sodium had significantly increased cure rates, and SCC were significantly reduced. Wallace *et al*. (2004) also randomly assigning cows with CMT-positive quarters to receive either IMM cephapirin sodium or no treatment, found that there was no difference in cure rates for IMM antibiotic-treated quarters for major pathogens compared to the untreated cows. However, there was an advantage for cure rates using antibiotics against environmental streptococcal infections. Quarters with streptococci infections were 3.5 times more likely to cure if treated with cephapirin sodium. A field study has revealed that IMM antibiotic therapy was beneficial for Gram-positive organisms and coagulase-negative staphylococci, but ineffective for Gram-negative organism (Roberson *et al.*, 2004), because Gram-negative bacteria tend to have a more complex layering in their cell wall structure (Beveridge, 1999). In a clinical trial in three Californian dairies, bacteriologic cure assessed at 4 and 20 days after treatment with amoxicillin, cephapirin, or oxytocin (no antibacterial) did not differ for mild clinical mastitis cases caused by any pathogen, although antibacterial treatment resulted in better clinical cure rates for cases caused by pathogens other than streptococci and coliforms (Guterbock *et al.*, 1993). Proper milking procedure and hygiene may be the easiest and most economic way to control IMI (Hutton *et al.*, 1990).

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Teat and udder skin should be healthy before milking and free of sores, wounds, or chapping where *S. aureus* could colonize the teat end and surrounding skin (Fox and Norell, 1994). Cleanliness at milking time is also important. Minimal use of water and premilking teat antisepsis may reduce new IMI. Additionally, the advent of post milking teat antisepsis has been important in contributing to decreasing contagious IMI. When teats were dipped after milking and cows were treated with penicillin-dihydrostreptomycin at dry-off, IMI caused by major mastitis pathogens decreased by 75% and 45%, respectively (Natzke *et al.*, 1972; Oliver and Mitchell, 1984). Post-dipping alone has been estimated to decrease the rate of new IMI by 50% (Nickerson and Boddie, 1997).

Antimicrobials are the most important tool in SCM control programs in cows. But there are not properly known by dairy farmers to infuse of intramammary antibiotics in SCM affected lactating cows. However, the reports on the efficacy of intramammary infusion in lactating cows are very limited (Siddiquee *et al.*, 2013) and the information on the prevalence of SCM in the area is lacking or very less (Rahman *et al.*, 2009; Rabbani and Samad, 2010; Rahman *et al.*, 2010; Islam *et al.*, 2011; Sarker *et al.*, 2013). Therefore, the objectives of this study were to estimate the prevalence of subclinical mastitis in lactating cows using (CMT) and to evaluate the efficacy of intramammary infusion against SCM in lactating dairy cows.

MATERIALS AND METHODS

Study sites and duration

The study was carried out on 78 smallholder dairy farms (at least two lactating cows must be present in the farm) from 6 villages of Mymensingh Sadar upazila (n = 42) and 9 villages of Lakshmipur Sadar upazila (n = 36). These farms were registered under Seed Bull Production Project of the Department of Genetics & Animal Breeding, Bangladesh Agricultural University, Mymensingh. A total of 139 lactating cows, 72 from Mymensingh sadar and 67 from Lakshmipur sadar upazilas were included in this study. The cows were aged between 5 to 12 years, at different lactation stages, parity and level of milk production. The study work was conducted during the period from June 2011 to May 2012.

Study design and data collection

A cross-sectional study was carried out by using a pretested questionnaire to collect cow and farm level data. The animal level variables were age, pregnancy, parity, milk yield, lactation stage, stimulation of milking and history of previous clinical mastitis. Data were collected by interviewing the owners as well as examining the cows in the farm. Cows with signs of clinical mastitis were excluded from the study.

Collection of milk samples

Milk samples were collected after antiseptic wash of teats with 70% ethanol and dried off by tissue paper. Two streams of milk were discarded and then 5 ml of milk were taken aseptically into labeled sterilized test tubes with rubber cap at the time of morning milking.

Detection of subclinical mastitis

Immediately after collection, the milk samples were subjected to CMT by using Leucocytest® (Synbiotics Corporation-2, France). A shallow half paddle having four cups was used. About 2 ml milk was taken into the cup and 2 ml reagent was added. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane for few seconds. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds. The results of CMT reaction were read immediately as per manufacturer's recommendation and were scored depending on the amount and thickness of gel formed.

Intramammary infusion of subclinical mastitis (SCM) affected cows

All the SCM affected cows (n = 72) received intramammary infusion with Neomastipra-JR5® (Benzylpnicillin procaine 100000 IU; Dihydrostreptomycin sulphate 62.4 mg; Neomycin sulphate 36 mg; Polymyxin B sulphate 50000 IU; Sulphadimidine sodium 250 mg; Sulphathiazole 250 mg; Hydrocortisone 20 mg, Hipra, Spain) at a dose of one syringe per affected quarter per cow twice daily after each morning and evening milking for 3 days.

Prior to infusion, the affected quarter was thoroughly milked out by hand and the teat end was cleaned using a cotton swab soaked with 70% alcohol. After 15 days of last dosing, milk samples were collected and tested for CMT.

Statistical analysis

Data were entered into Microsoft Excel 2003 and transferred to STATA®, version 2012 (Stata Corporation, Texas, USA) for analysis. The overall prevalence of SCM and its distribution in different categories of variables and their exact binomial 95% confidence intervals were calculated. Simple bivariable associations of independent variables with SCM were investigated by $\chi 2$ test.

RESULTS AND DISCUSSION

Prevalence of subclinical mastitis

The overall prevalence of SCM in lactating cows was 51.8%. The prevalence of SCM in Mymensingh sadar and Lakshmipur sadar was 51.4% and 52.2%, respectively and there was no significant difference in the prevalence between two upazilas (Table 1).

The reported prevalence of SCM varied from 15.8% to 53.1% in crossbred cows in Bangladesh (Prodhan *et al.*, 1996; Kader *et al.*, 2002; Rahman *et al.*, 2009; Rabbani and Samad, 2010; Rahman *et al.*, 2010). These variations of prevalence rates of SCM might be due to difference of breed, management systems and test used for screening of milk samples.

Table 1. Prevalence of subclinical mastitis in lactating cows based on California Mastitis Test

Location	No. of cows tested	No. of positive cows	Prevalence (%)	95% confidence interval	p-value
Mymensingh	72	37	51.4	39.3, 63.3	0.92
Lakshmipur	67	35	52.2	39.6, 64.5	
Total	**139**	**72**	**51.8**	**43.2, 60.3**	

The highest prevalence of SCM was 69.6% in cow's age above 7 years (Table 2). The prevalence of SCM was significantly ($p<0.001$) increased with age of cows. This finding agree with Neelesh *et al.* (2012) who reported highest prevalence of SCM in lactating cows with advancement of age. This could be explained by the fact that the teat canal in older animals is more dilated or it remains partially open permanently due to years of repeated milking (Madut *et al.*, 2009). This encourages the introduction of environmental and skin-associated microorganisms into the teat canal, leading to SCM in cows (Karimuribo *et al.*, 2008).

In terms of parity, the prevalence of SCM was significantly ($p<0.001$) higher in cows of above second parity in comparison to 1st parity (Table 2). This observation supports with the reports of Joshi and Gokhale (2006), Byarugaba *et al.* (2008) and Rabbani and Samad (2010). The increase in the number of parity is associated with the corresponding increase in the prevalence of SCM (Sudhan *et al.*, 2005, Islam *et al.*, 2010). The higher prevalence of SCM in cows with the parity may be explained by the fact that resistance of cows might have lowered with advancement of parity (Byarugaba *et al.*, 2008).

The prevalence of SCM was highest (54.1%) in cows having more than 10 liter of milk production (Table 2), which is consistent with the report of Islam *et al.* (2011) who reported increased prevalence of SCM (42.85%) with the increased milk production. The prevalence of SCM was higher (60%) in pregnant cows than the non-pregnant cows (47.2%). However, there was no significant relationship between pregnancy of cows and SCM (Table 2).

The prevalence of SCM was the highest in cows having pendulous udder (61.4%) and cylindrical teat (58.1%) (Table 2). Pendulous type of udder is of bigger size and more likely to be injured than an udder held up closure to the body (Probric *et al.*, 1989; Sarker *et al.*, 2013). SCM has a strong association with pendulous udder and cylindrical shaped teats (Uddin *et al.*, 2009). The prevalence of SCM varied with the type of milking stimulation. Milking when stimulated by mustard oil, the prevalence was insignificantly higher (66.6%) than milking stimulation by calves (50.8%) (Table 2).

Table 2. Prevalence of subclinical mastitis in lactating cows based on age, parity, milk yield, pregnancy, udder type and teat type and ways of milking stimulation

Variables		No. of cows tested	No. of positive cows	Prevalence (%)	95% confidence interval	p-value
Age (year)	3.5-7	83	33	39.8	29.2, 51.1	0.001
	>7	56	39	69.6	55.9, 81.2	
Parity	1-2	69	26	37.7	26.3, 50.2	0.001
	>2	70	46	65.7	53.4, 76.7	
Milk yield (litre)	1-5	15	6	40.0	16.3, 67.7	0.59
	5-10	39	20	51.3	34.8, 67.6	
	>10	85	46	54.1	42.9, 64.9	
Pregnancy	Absent	89	42	47.2	36.5, 58.1	0.15
	Present	50	30	60.0	45.2, 73.6	
Udder type	Round	82	37	45.1	34.1, 56.5	0.06
	Pendulous	57	35	61.4	47.6, 74	
Teat type	Conical	77	36	46.8	35.3, 58.5	0.18
	Cylindrical	62	36	58.1	44.8, 70.5	
Milking stimulation	Calf	130	66	50.8	41.9, 59.6	0.36
	Mustard oil	9	6	66.6	29.9, 92.5	

Quarter-wise prevalence of subclinical mastitis in lactating cows

Quarter-wise prevalence of SCM was 28.1% in Left Front (LF), 33.1% in Left Hind (LH), 24.5% in Right Front (RF), and 17.3% in Right Hind quarters by CMT (Table 3). The prevalence of SCM in LH quarter (33.1%) was significantly higher than RH quarter. These findings are in agreement with Sudhan *et al.* (2005) who reported that the hind quarter was the most affected quarter. Sharma *et al.* (2007) also reported that single and hind quarter involvement was maximum in case of SCM. This could be explained by the fact that the hind quarters are more exposed to dung and urine (Chakrabarti, 2007).

Table 3. Quarter-wise prevalence of subclinical mastitis in lactating cows

Quarters	No. of tested cows	CMT positive, No. (%)				95% confidence interval
		1+	2+	3+	Total	
LF	139	23 (16.5)	13 (9.4)	3 (2.2)	39 (28.1)	20.8, 36.3
LH	139	24 (17.3)	16 (11.5)	6 (4.3)	46 (33.1)	25.4, 41.6**
RF	139	24 (17.3)	8 (5.8)	2 (1.4)	34 (24.5)	17.6, 32.5
RH	139	15 (10.8)	6 (4.3)	3 (2.2)	24 (17.3)	11.4, 24.6**
Total	556	86 (15.5)	43 (7.7)	14 (2.5)	143 (25.7)	22.1, 29.6

LF = Left Front, LH = Left Hind, RF = Right Front and RH = Right Hind.
**Significant at $p<0.001$.

Efficacy of intramammary infusion in lactating cows

The efficacy of intramammary infusion in the treatment of IMIs in lactating cows was 66.7% in this study. The efficacy was insignificantly higher at Mymensingh sadar upazila (70.2%) than the Lakshmipur sadar upazila (62.9%) (Table 4). The efficacy of intramammary infusion with penicillin G or penethamate has been shown similar result (McDougall *et al.,* 2007). In this study, the efficacy of IMIs in lactating cows have shown better due to the proper infusion of IMM tube at every 12 hours interval on affected quarter and maintain udder hygienic environment. The combinations of penicillin and aminoglycosides have shown a better efficacy for SCM in cows (Taponen *et al.*, 2002). Owens and Nickerson (2001) showed similar result with cephapirin benzathine, penicillin-streptomycin, penicillin-novobiocin and tilmicosin infused intramammary infection in lactating cows for SCM.

Table 4. Efficacy of intramammary infusion with Neomastipra-JR5® in lactating cows affected with subclinical mastitis

Area	No. of SCM affected cows treated	No. of cows recovered after IMM infusion (CMT negative cows)	Percentage of recovered cows	95% CI
Mymensingh	37	26	70.3%	53.0, 84.1
Lakshmipur	35	22	62.9%	44.9, 78.5
Total	72	48	66.7%	54.6, 77.3

In conclusion, the overall prevalence of SCM in lactating cows of smallholder dairy farms from Mymensingh and Lakshmipur sadar upazila are found similar result. The higher prevalence was found advancement of age and parity. The prevalence was also high due to increasing of milk production of cows. Hind quarters were more susceptible to SCM than fore quarters in lactating cows. The efficacy of intramammary infections of SCM affected cows was found effective treatment with Neomastipra-JR5® intramammary infusion tube.

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