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Impact of non-genetic factors on clinical mastitis incidence in Murrah buffaloes

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Abstract
Mastitis brings on economic losses, declined milk production, uplifted treatment costs and accelerated culling in buffaloes. Also, being multi-etiological in nature, control of mastitis is challenging in dairy animals. Hence, knowing the risk factors governing clinical mastitis incidence in buffalo might help in minimizing its occurrence. So, the present study was undertaken in 96 adult Murrah buffaloes to investigate the effect of parity, period of calving, season of calving and level of milk production on incidence of clinical mastitis using logistic regression in SAS v 9.3. The data of mastitis incidence was collected over a period of nineteen years (2000-2020) from Health record register of Livestock Research Centre of the institute. The incidence of mastitis was maximum in second parity (7.65%) followed by parity five and above (7.41%). Parity and period of calving had significant effects ($p<0.05$) on mastitis incidence. The odds ratio for incidence of mastitis of animals in parity (5 and above) was highest (3.832), in comparison to first lactation. The animals calving during the period (2004-2007), exhibited maximum incidence of clinical mastitis (14.75%). Higher mastitis incidence in higher parity animals may be due to the compromised immune system and widened teat canal. Therefore, proper management of animals especially for advanced pregnant animals is recommended for reducing incidence of mastitis.

Keywords: Mastitis, season, parity, odds ratio, mammary gland

Introduction

Mastitis is defined as an inflammatory reaction of the parenchyma of the mammary gland which brings huge economic loss to dairy farmers because of the reduced milk production, expenses of treatment, labour costs, discarding of milk following treatment due to the deleterious effects on the chemical and cytological composition of milk, premature culling and sometimes death (Yang *et al.*, 2012) [27]. Mastitis can occur in clinical and subclinical forms based on severity, nature of exudate, duration and primary cause. Clinical mastitis is the symptomatic form and characterized by physical, chemical and bacteriological changes in milk such as presence of blood, water, flakes and pus having clots as well as pathological changes evident in the glandular tissue of udder. According to its severity, rapid onset and duration, clinical form is further classified into peracute, acute, subacute and chronic forms (Fagiolo *et al.*, 2007) [8]. Progressive fibrosis accompanied by enlargement, and in severe cases, atrophy of mammary gland is seen in chronic form. While in subclinical mastitis, somatic cell count increases with normal appearance of udder and milk. So, it can be detected by screening tests such as California Mastitis test, detection of bacterial agents and electrical conductivity. Mastitis incidence is largely influenced by management practices, interaction between a variety of microbial infections and host responses in the udder (Fagiolo *et al.*, 2007) [8]. Moreover, mastitis being multifactorial in nature, no proper control measures are evident to contain the disease (Kavitha *et al.*, 2009) [13].

India is the highest milk producing country in the world with a total milk production of 221.06 million tonnes during 2021-22 (BAHS, 2022) and with a population of 109.85 million (Livestock Census, 2019), 45% of the total milk production of India is contributed by buffaloes (BAHS, 2022), and it is twice as rich in fat and other constituents of milk as compared to the cow milk (Kumar *et al.*, 2017) [16]. Bovine mastitis poses a significant impact on dairy farm economy. The first comprehensive report on economic loss caused by mastitis in India was published in 1962 reporting an annual loss of Rs. 52.9 Crores (Dandha & Sethi, 1962) [7]. However, later on with the upcoming of Operation flood milk yield of bovine population increased tremendously which came up with many fold increment in economic loss too. Reshi *et al.* (2015) [29] reported the annual economic losses due to bovine mastitis to be Rs. 7165.51 crores in India.

Although buffaloes are less susceptible to mastitis than cattle because of thicker streak canal epithelium (Uppal *et al.*, 1994) [24] but a high mortality rate of the calves is seen in the first three months of life due to maternal mastitis, which further leads to decline in buffalo productivity (Akhtar and Ali, 1994) [2]. The incidence of clinical mastitis varies as per the physiological status of animal and more predominantly with the environment. The environment factors include the non-genetic factors such as season of calving, period of calving, parity, feeding regime, milk production levels, etc. that significantly affect the incidence of mastitis in dairy herds. A number of researchers have reported significant effect of different non-genetic factors like season of calving (Joshi and Shrestha, 1995; Taraphder *et al.*, 2006) [2, 23], period of calving (Chand *et al.*, 1995) [5], parity (Chand *et al.*, 1995; Joshi and Shrestha, 1995; Taraphder *et al.*, 2006; Sharma *et al.*, 2013; Jingar *et al.*, 2014) [5, 2, 23, 18, 11] on incidence of clinical mastitis in buffaloes. Taraphder *et al.* (2006) [23] reported 8.75% incidence of clinical mastitis in winter season while 16.28% in rainy season and they also observed a consistent increase in mastitis incidence with increase in parity. Jingar *et al.* (2014) [11] reported that incidence of mastitis increased from 22.78% (First parity) to 32.89% (fourth parity) during different parities. For bringing out increased milk production, the phenotypic selection is done which predisposes the milch animals for environmental influences subsequently causing mastitis infection (Sinha *et al.*, 2021) [20]. So, the present study was carried out to investigate the effect of season of calving, period of calving, parity and levels of milk production on the incidence of clinical mastitis in Murrah buffaloes.

Materials and methods

Experimental station and data sources

Adult Murrah buffaloes (N=96) reared at Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal (Haryana), India were undertaken for carrying out the present study. The experimental animals were housed under loose housing system with an open paddock having proper drainage system with an adequate slope. The mastitis incidence data was recorded over a period of twenty years (2000-2020) from the Mastitis register kept at Animal Health Complex of Livestock Research Centre (LRC) of ICAR-National Dairy Research Institute (ICAR-NDRI). The mastitis incidence was recorded such that the animals who got affected with mastitis even once throughout their lifetime were given the code 1 (Affected) and those who never got infected even once till date were given the code 0 (not affected). Out of 96 animals, 59 cases and 37 controls (controls were identified as animals which did not suffer mastitis even once in their lifetime till then) were recorded. The recording for affected was based on the clinical symptoms being recorded in the Mastitis register which were udder swelling, flakes in milk, hardening of udder and based on the treatment given to the affected ones along with the date of recovery. The final dataset contained information on 387 lactations of 96 multiparous buffaloes of different parities. The multiple mastitis cases occurring in same parity of the same animal were included in analysis. A new case of mastitis was defined for the same buffalo when a different quarter was affected or a gap period of at least 21 days exists since the previous diagnosis. Hence, a total of 409 records were available for analysis, of which 288 entries matched the category of "not affected" (coded as 0), and 117 records matched the category of "affected" (Coded as 1). The data for other variables like season and period of calving and parity was recorded from the birth register and history-cum-pedigree sheets kept in the record room of Animal Genetics and Breeding division of ICAR-NDRI, Karnal.

Data classification

The data classification for different variables (Supplementary file) like season of calving, period of calving, parity and level of milk production was done according to the variation(s) observed.

Based on the climate at experimental region and fodder resources available at the farm, the season was classified into four groups which are: December–March (season 1 = Winter), April–June (2 = Summer), July–August (season 3 = Rainy), September–November (season 4 = Autumn). For period of calving, data was classified into five groups, taking four years in each group which were coded from 1 to 5 as: 2000-2003 (code 1), 2004-2007 (code 2), 2008-2011 (Code 3), 2012-2015 (code 4) and 2016-2020 (code 5). Parity was classified into five groups based on number of animals in each parity and the classification was as follows: First Parity (Code 1), Second Parity (code 2), Third Parity (code 3), Fourth Parity (code 4) and Fifth and above (code 5). Animals of parity 5 and above were taken in one group as very few animals belonged to the fifth parity and above in the herd. On the basis of level of milk production (kg) in 305 days for the first lactation of each animal, the data was classified into three production groups as: low (<1500 kg) producers coded as 1, medium (1500-2500 kg) producers coded as 2 and the high (>2500 kg) coded as 3.

Statistical analysis

The effect of non-genetic factors *viz*. season of calving, period of calving, parity and level of milk production on the incidence of clinical mastitis was investigated by using the maximum likelihood method of the LOGISTIC procedure of SAS Version 9.3.

The Dichotomous Logistic model is as follows:

$$ln\left[\frac{p}{1-p}\right] = \beta_o + \sum_{j=1}^{c} \beta_j X_j$$

Marginal one unit increase in X_j brings about an increase in Log $[p_i/1 - p_i]$ by β_1

P = Probability of mastitis resistant animals

β_0 = Intercept

β_j = Partial regression coefficients

X_j = Non-genetic factor

Results and Discussion

Effect of non-genetic factors on incidence of clinical mastitis

The effect of non-genetic factors on the incidence of clinical mastitis was investigated taking four factors which are season of calving, period of calving, parity and milk production levels. Based on the results obtained from maximum likelihood method of the Logistic procedure of SAS Version 9.3, it was found that parity and period of calving had significant effects while season of calving and milk production levels did not have any major influence on the incidence of clinical mastitis in the present study (Table 1).

The implicated factors like parity (Sinha *et al.*, 2021; Abo-Gamil *et al.*, 2021) [20, 1] milk production levels (Sinha *et al.*, 2021), lactation stage and season of calving (Oliveira *et al.*, 2015; Abo-Gamil *et al.*, 2021) [17, 1], percentage of grass silage in diet, age and herd incidence rate (Waage *et al.*, 1998) [25] have been reported to have significant influence on clinical mastitis incidence. This difference in significant risk factors may be due to lesser number of samples in the present study due to which it might not have achieved the significance threshold ($p<0.05$). Ghosh *et al.* (2004) [9] investigated effect of milk yield on mastitis incidence which was not found significant, similar to the findings obtained in the present study. The study conducted by Sinha *et al.* (2021) [20] on two different breeds *viz*. Sahiwal and Karan Fries showed that level of milk production had significant effect on mastitis incidence in Sahiwal while it was not so in Karan Fries animals, the possible reason for that was reported to be because of presence of less variability in milk production among the animals of Karan Fries animals. The present study also shows that level of milk production did not have much animals in category of high level of milk production.

The percentage of incidence of mastitis was maximum in second parity (7.65%) as during earlier lactations, the animals are not much adapted to the milking methods, thereby udder tissues are more pressurized and weaning associated behavior might affect immune response at early stages of lactations predisposing the animal to mastitis incidence (Jingar *et al.*, 2014; Singh *et al.*, 2001) [11, 19]. Early lactational milk production that is raised further weakens the immune system because of increased metabolic stress, hyperketonemia, and a negative energy balance (Suriyasathaporn *et al.*, 2000; Janosi *et al.*, 2003) [22, 10]. Moreover, dairy cows and buffaloes are more vulnerable to infections at this time due to weakened host defence systems, which may be directly caused by a variety of physiological and environmental variables. Reactive oxygen species (ROS), which are generally referred to as increased oxygen-derived reactants and increased oxygen demand, harm the phagocytic cells' cell membranes and impair an animal's immune system. As a result, the high prevalence of mastitis shown in second parity (7.65%) in this study was caused by increased udder stress and a poor energy balance (Sori *et al.*, 2005) [21]. On the contrary, the present study shows higher mastitis incidence during parity fifth and above (7.41%), similar results have been earlier reported by Kavitha *et al.* (2009) [13] and Chishty *et al.* (2007) [6] in buffaloes and crossbred cows. The reason for higher incidence towards increasing parities might be because buffaloes achieve peak yield at fifth parity, therefore, more energy is directed towards the udder thus supressing the immune response of the host. The capability to fight infection decreases substantially as parity increases owing to reduced effectiveness of streak canal as barrier (Wilson *et al.* 2004; Khate and Yadav, 2010) [26, 14]. Yu *et al.* (2011) [28] reported that as the parity advances somatic cell count also increases indicating subclinical mastitis. The incidence of mastitis was comparatively lower in third (5.68%) and fourth parity (3.70%) to second and higher parities (5 and above). It might be because upto mid-lactational stages, the udder tissues are adapted for milk production as well negative energy balance is far more convenient, hence, the mid-parities might show lesser incidence of clinical mastitis. Wald chi-square analysis revealed that parity had significant effect for incidence of clinical mastitis ($p<0.05$). The odds ratio of parity fifth and above was highest (3.832) which indicates that fifth and higher parity was having the maximum odds of being associated with mastitis (Table 2). This shows that the incidence of clinical mastitis for parity fifth and above, was 383.2% more as compared to the first parity, with a 95% confidence interval lying in the range of 1.495-9.825 (Table 2). The animals who calved during second period of calving (2004-2007) had maximum incidence of mastitis (14.75%) for which the compromised management conditions might be a possible reason. The odds ratio revealed that clinical mastitis incidence was 447.8% and 326.1% more in second (2004-2007) and fifth period (2016-2020) of calving with confidence interval of (1.738-11.539) and (1.091-9.752) respectively in comparison to first period of calving (2000-2003) as shown in Table 2.

Table 1: Analysis of effects on clinical mastitis incidence

Effect	Wald chi-square	p-value
Parity	10.1979	0.0372*
Season of Calving	3.1627	0.3672
Period of Calving	13.7061	0.0083**
Milk Production Level	2.3214	0.3133

*Significant at $p<0.05$, **Significant at $p<0.01$

Table 2: Incidence of clinical mastitis under different categories of non-genetic factors and their effect on incidence of clinical mastitiss

Effect	No. of animals (n)	Incidence (%)	Estimate±SE	Wald Chi square	p-value	Odds Ratio	95% CI
Intercept			-2.3886±0.5988	15.9097	0.0001		
Parity							
Parity 1 (1st)		4.44%					
Parity 2 (2nd)	11	7.65%	0.3356±0.3687	0.8286	0.3627	1.399	0.679-2.881
Parity 3 (3rd)	35	5.68%	0.0251±0.3956	0.0040	0.9494	1.025	0.472-2.227
Parity 4 (4th)	21	3.70%	0.0277±0.4619	0.0036	0.9521	1.028	0.416-2.542
Parity 5 (5 and above)	29	7.41%	1.3434±0.4804	7.8213	0.0052**	3.832	1.495-9.825
Season							
Season 1 (Dec to Mar)	14	8.58%					
Season 2 (Apr to Jun)	24	4.56%	-0.5181±0.3871	1.7918	0.1807	0.596	0.279-1.272
Season 3 (Jul to Aug)	36	4.02%	-0.5521±0.3920	1.9839	0.1590	0.576	0.267-1.241
Season 4 (Sept to Nov)	22	10.46%	-0.1123±0.3118	0.1297	0.7188	0.894	0.485-1.647
Period							
POC 1 (2000-2003)	33	1.61%					
POC 2 (2004-2007)	26	14.75%	1.4993±0.4829	9.6392	0.0019**	4.478	1.738-11.539
POC 3 (2008-2011)	14	2.14%	0.3206±0.6819	0.2210	0.6383	1.378	0.362-5.244
POC 4 (2012-2015)	13	3.75%	1.0088±0.5731	3.0984	0.0784	2.742	0.892-8.432
POC 5 (2016-2020)	10	5.36%	1.1820±0.5589	4.4732	0.0344*	3.261	1.091-9.752

Level of production							
MP 1 (<1500 Kg)	20	6.41%					
MP 2 (1500-2500 Kg)	58	18.72%	0.4132±0.3263	1.6035	0.2054	1.512	0.797-2.865
MP 3 (>2500 Kg)	18	3.33%	0.00977±0.4717	0.0004	0.9835	1.010	0.401-2.546

Table 3: Classification of non-genetic factors and their coding

Season of calving		**Period of calving**		**Parity**		**Level of milk production**	
Duration	Code	Period	Code	Parity number	Code	Milk yield (kg) in 305 days	Code
December to March (Winter)	1	2000-2003	1	First parity	1	Low (<1500)	1
April to June (Summer)	2	2004-2007	2	Second parity	2	Medium (1500-2500)	2
July to August (Rainy)	3	2008-2011	3	Third parity	3	High (>2500)	3
September to November (Autumn)	4	2012-2015	4	Fourth parity	4		
				Fifth parity and above	5 and above		

Table 4: Distribution of total records available for analysis on the basis of parity

Parity	No. of records (n)
1	100
2	101
3	90
4	53
5 and above	65
Total records	409

Conclusion

Mastitis being one of the common and devastating diseases affecting the bovine industry across the world. Environmental factors influence the occurrence of mastitis to a great extent in dairy animals. The present study revealed that the non-genetic factors (parity and period of calving) should be considered while performing evaluation of dairy animals. The incidence of mastitis was maximum in second parity (7.65%) while the second period of calving (2004-2007) had maximum incidence of mastitis (14.75%). The compromised immune system and widened teat canal and elongated teat shape are the primary predisposing factors for mastitis infection with the increase in parity. Therefore, for animals in earlier as well as advanced parity as compared to those in mid-locational stages, proper management and care is recommended to prevent mastitis infection.

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