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Original article

Prevalence of Mastitis and Analysis of Risk factors among Dairy Buffaloes in Ubay, Bohol, Philippines

Emerson P. Tapdasan, DVM[1*], Lotis M. Balala, DVM, MS[2], Kenny Oriel A. Olana, DVM[2], Gundolino P. Bajenting, DVM[1], Caro B. Salces, MS, PhD[1]

[1]Philippine Carabao Center (PCC) at Ubay Stock Farm (USF), Ubay, Bohol
[2]College of Veterinary Medicine, Visayas State University (VSU), Baybay City, Leyte

ABSTRACT

Mastitis is an economically important disease of intensive dairy farming worldwide. Detection using reliable tests is important for its management and control. The purpose of this study was to estimate the prevalence and analyze the risk factors for mastitis in the dairy farm of Philippine Carabao Center at Ubay Stock Farm (PCC-USF) in Ubay, Bohol. Data on animal demographics, reproduction status, daily milk yield and Somatic Cell Count (SCC) were obtained from PCC-USF records. Data were analyzed using Chi-square test and Odds Ratio (OR) at 5% level of significance. Univariate analysis was used to assess the association between putative risk factors & elevated SCC (>200,000 cells/mL). The proportion of mastitis using SCC was 17.9%. The prevalence was lower than those reported in other studies. Specifically, mastitis prevalence was higher in the following groups of buffaloes: American Murrah, 6-7 years old, 1-90 days of lactation, non-pregnant and ≥2 parities (P>0.05). None of the factors investigated were associated with mastitis prevalence which could be attributed to the small sample size of the study. Future studies should be carried out involving large sample sizes and bacteriological examinations to determine animal-level risk factors and implicating microorganisms, respectively.

KEYWORDS: *mastitis, prevalence, risk factors, somatic cell count, water buffaloes*

1 INTRODUCTION

The production of milk of high quantity and quality is the primordial concern of any dairy farm operation. In 2016, the total dairy animal population in the Philippines grew by 6.34% from 44,432 heads in 2015. Specifically, dairy buffalo population grew by 2.91% in 2016 (PSA, 2018a). The Philippine government encourages dairy farms to exhibit standard practices of an ideal income generating enterprise for emulation by existing dairy buffalo raisers and promote dairying as a source of sustainable livelihood for the Filipino farmers. Although rising national demand for milk and dairy products has provided opportunities for local producers, quantity and quality of milk remain an issue. One potential deterrent to achieving high milk production volume is mastitis. Among the diseases in dairy animals, mastitis causes reduction in milk yield and quality, reduced cow sale value, culling and replacement, and rejection of milk due to antibiotic residues (Blosser, 1979; Huijps and Hagoveen, 2006). Monitoring of this inflammatory process is carried out using various tests such as SCC and alcohol test (AT). SCC is a useful index for detection of mastitis and milk quality as it measures the inflammatory response to an intramammary infection (Schukken *et al.*, 2003). AT on the other hand is a rapid test that measures increased level of acids in milk due to mastitis and other factors (FAO, 2004).

The prevalence of mastitis in dairy animals could vary due to individual animal characteristics, location, reproductive status of the animal, and farm management practices. Prevalence of mastitis in buffaloes was reported to be 51% in District Faisalabad, Pakistan (Sharif & Ahmad, 2007), 26.21% in Namakkal, India (Srinivasan *et al.*, 2013), and 27.36% in Tabriz Region, Iran (Beheshti *et al.*, 2011). In the Philippines, estimates of prevalence of mastitis in buffaloes are scarce. Villanada *et al.* (2012) reported a 37% prevalence of subclinical mastitis in a commercial dairy buffalo farm in Nueva Ecija, Philippines for a 19month observation period. The same farm also recorded a prevalence of 45.83% before 2012 (Villanada *et al.*, 2012). On the other hand, Salvador *et al.* (2012) reported a prevalence of 42.76% among lactating dairy buffaloes in the same commercial farm in Nueva Ecija. As a complex disease, mastitis is affected by stage of lactation, age, parity, seasonal and daily changes, anatomical abnormality of the udder, milking stress and management (Almaw *et al.*, 2008; Sharif *et al.*, 2009; Sharma *et al.*, 2011; Salvador *et al.*, 2012; Jingar *et al.*, 2014; Sharma & Sinhdu, 2017). Furthermore, only one study in the Philippines reported the association between buffalo mastitis and risk factors such as age and lactation length of buffaloes (Salvador *et al.*, 2012).

Mastitis can be divided into clinical and subclinical

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forms based on signs, severity and duration of the disease (Sharif *et al.*, 2009). Clinical mastitis is manifested by secretion of abnormal milk such as presence of flakes or having watery consistency. Signs of inflammation like redness, swelling and hardness of the mammary gland are also observed (Mansour *et al.*, 2016). On the other hand, subclinical mastitis is characterized by a normal mammary gland and milk appearance but the SCC is elevated (Fagiolo and Lai, *et al.*, 2007). An SCC of 200,000/mL indicates that the animal is positive for mastitis (Salvador *et al.*, 2013).

Globally the losses due to mastitis amount to about \$53 billion (Sharif *et al.*, 2009). Specifically, the US dairy industry is losing approximately \$2 billion annually. Similar impact is observed in Europe and other countries (Salvador *et al.*, 2013). Losses due to mastitis may even be higher in developing countries because standard mastitis control and prevention practices such as pre and post milking antiseptic teat dipping and dry period antibiotic therapy are not being carried out in these countries (Fagiolo and Lai, *et al.*, 2007).

Milk quality monitoring such as SCC is particularly important to ensure food safety and guarantee that producers are able to sell their products at fair prices. This study investigated the prevalence and putative risk factors for bubaline mastitis. The information generated determines the extent of mastitis cases at PCC-USF institutional farm and provides valuable points in the refinement of the farm's mastitis control strategies. The study results could also be used as baseline information for other dairy farms struggling with mastitis.

2 MATERIALS AND METHODS

Study Site

PCC-USF is located in Ubay, Bohol in the Visayas (Fig. 1). The farm has a total land area of 547 hectares and lies in the northeastern part of Bohol province. Ubay has a total land area of 335 km^2 and a coastline of 61 km. The topography varies from level to steeply sloping, with land elevation ranging from 0 to near 900 m above sea level. Seventy three percent of the vegetation cover is grassland, coconut trees and forests (Provincial Government of Bohol [PGBh], 2006). It is the most populated municipality in the province, with a human population of 73,712 in 2015 (PSA, 2018b). The dry season starts in February and lasts through April sometimes extending to mid-May.

Animal Population

A total of 84 milking buffaloes during the period December 2016 to February 2017 were enrolled as the study units. The animals were managed under intensive grazing system with improved pasture grasses such as ruzi (*Brachiaria ruzizienses*) and humidicola (*Brachiaria humidicola*) interspersed with pintoi peanut (*Arachis pintoi*) and stylo (*Stylosanthes guyanensis*) as legumes. The Food and Agriculture Organization (FAO, 2009) describes intensive grazing as a production system characterized by high-quality grassland and fodder production to support large number of animals and are based mostly on individual ownership.

Detection of Mastitis

It was customary in PCC-USF that mastitis detection at PCC-USF is done through AT and SCC. Aside from mastitis detection, AT was carried out on a daily basis to test the quality of milk for processing into different dairy products. Somatic cell counting of milk was done once a month wherein samples were sent to PCC national headquarters in Muñoz, Nueva Ecija. Milk samples from all lactating buffaloes were tested for AT and SCC by PCC-USF milkers and PCC-national veterinary personnel, respectively. In AT, an equal volume of milk and 68% alcohol is mixed in a test tube and being inverted several times with the thumb pressed tightly over the open end of the tube. The tube is then examined whether the milk has coagulated. If it has, fine particles of curd will be visible. If the milk is of good quality, there will be no coagulation, clotting or precipitation (Tessema and Tibbo, 2009). On the other hand, SCC is done through the use of an automatic milk somatic cell counter (Fossomatic Minor®) wherein a value of >200,000 cells/mL was the cut-off level. Buffaloes were milked twice daily, however, if positive to AT, milk was either fed to the calves or used as substrate in making Lactic Acid Bacteria Solution (LABS) needed for silage production. Since conventionally, SCC is used as indicator of udder health and milk quality, SCC was used in this study to estimate prevalence of bubaline mastitis at PCC-USF.

Data Collection and Analysis

Data on animal demographic information, reproduction status, monthly SCC and daily milk yield were obtained from the records of PCC-USF. Data were encoded in MS Excel® spreadsheet. Descriptive statistics was carried out. Proportions and means were compared using Chi-square test at 5% level of significance. The association between the identified risk factors and elevated SCC was analyzed using Odds Ratio (OR) at 95% confidence intervals and P>0.05. Statistical analysis was performed using Epi Info® version 7.2.2.

3 RESULTS AND DISCUSSION

Animal Demographics

A total of 84 lactating buffalo cows were included in the study with a median age of 6.9 years. Majority of the buffaloes were Bulgarian Murrah and were at ≥181 days of lactation. During the study period, 59.2% buffaloes were pregnant and had a parity of ≥3 (38.1%) (Table 1). The average daily milk yield per buffalo was 5.6 (±2.4) liters.

There are two types of buffaloes in the world: the swamp-type and the river-type. The Philippine carabao

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is a classic example of a swamp-type buffalo that is primarily used as a draft animal and as source of meat and seldom for milk production. Riverine buffaloes produce much more milk than swamp buffaloes. They are the dairy type water buffalo (Mingala and Gundran, 2008; Bondoc, 2010).

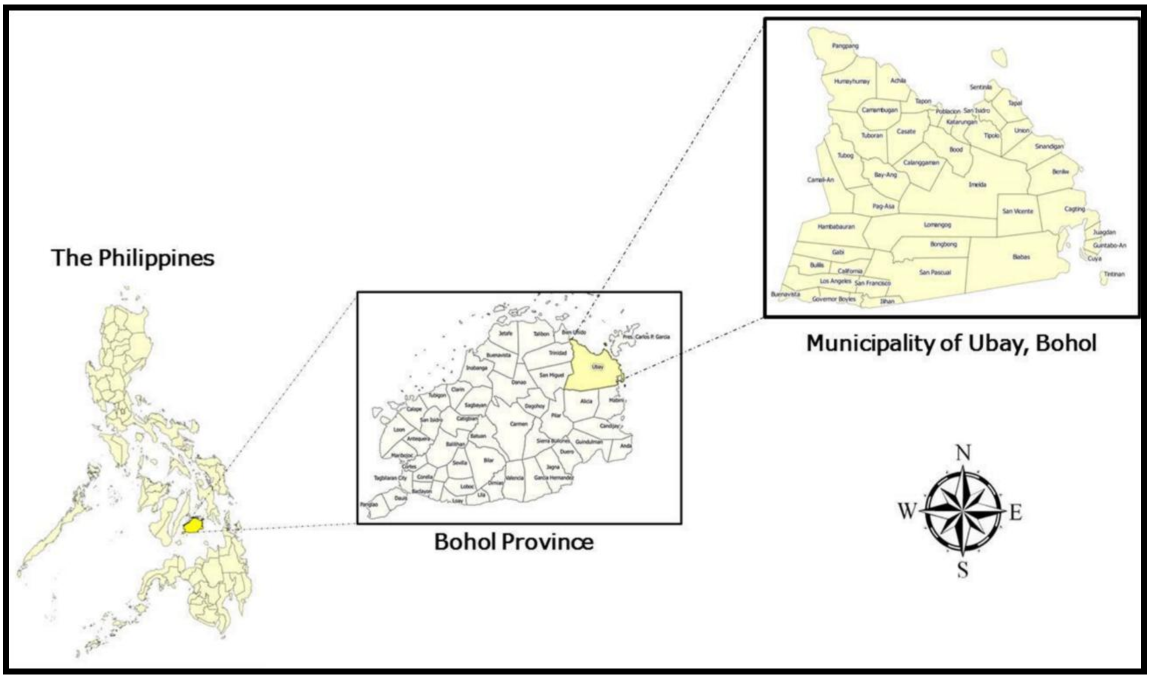

Figure 1. The map of Ubay, Bohol where the Institutional Farm of PCC at USF is located

Table 1. Demographics and mean milk SCC of 84 dairy buffaloes at the institutional herd of PCC at USF for the period December 2016 to February 2017

Variable	Category	N (n = 84)	%	*P*- value	Mean SCC (cells/mL)
Breed				0.0001	
	Bulgarian Murrah	63	75		172,063.5
	American Murrah	21	25		189,714.3
Age (years)				0.0295	
	<6	23	27.4		89,000
	6-7	39	46.4		236,300
	>7	22	26.2		153,727.3
Location (days)				<0.0001	
	1-90	6	7.1		222,333.3
	91-180	21	25		232,809.5
	≥181	57	67.9		150,894.7
Pregnancy Status				1.0	
	Non-pregnant	34	40.5		152,235.3
	Pregnant	50	59.2		192,960.0
Parity				0.6056	
	1	24	28.6		97,083.3
	2	28	33.3		26,1035.7
	≥3	32	38.1		162,031.3

According to Weigl (2005), the maximum lifespan of buffaloes in captivity is believed to be 34.9 years; however, the oldest living buffalo at PCC-USF was only 17.5 years old. The standard lactation period observed in the farm was 305 days. This is consistent with the statement of Syrstad (1993) who claimed that 305 days is approximately the normal lactation length of buffaloes with calving at intervals of 12 months. On the other hand, Perera (2011) reported that lactation period lasts until 200–300 days, when the yield drops to uneconomical amounts.

A large proportion of heifers attain puberty at 3-5 years of age (Nanda *et al.*, 2003). It is at puberty when heifers become sexually mature, are receptive to bulls and eventually become pregnant. The age at puberty is influenced by genotype, nutrition, management and climate, and under favorable conditions occurs at 15-24 months in swamp buffalo (Perera, 2011).

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Prevalence of Mastitis

Overall prevalence based on SCC was 17.9% which is lower than those reported by Villanada *et al.* (2012) and Salvador *et al.* (2012) at 37% and 42.76%, respectively. Prevalence of mastitis in various categories are presented in Table 2. Prevalence was higher in the following groups: American Murrah, 6-7 years old, 1-90 days of lactation, nonpregnant and ≥2 parities (P>0.05).

In this study, higher prevalence was seen in American Murrah breed. Though a high probability exists that breed could be an identified risk factor observed among dairy buffaloes, its degree of influence is still unknown. Srinivasan *et al.* (2013) reported that between breeds, mastitis was higher in graded Murrah (15.33%) than it was in graded Suruti (5.83%) and non-descript (4.85%) breeds in India. As explained by Kavitha *et al.* (2009), the difference could be due to their milk production potential. High milk yielding (4-7 L) animals were more prone to mastitis when compared to low milk yielding animals (Kavitha *et al.*, 2009). Moreover, high-producing cattle breeds such as Brown Swiss (423.31×103 cells/mL) and Black Holstein (310.36× 103 cells/mL) were reported to have higher presence of SCC/mL in milk than low milk-producing breeds (Sharma *et al.*, 2011). Whether or not the breed of the animal is prone to mastitis can be attributed to the anatomic features of the udder and teats, mammary gland immunology and milk composition (Sahin *et al.*, 2017).

In this study, mastitis was higher in buffaloes within 6-7 years than in other age group. Various studies reported that the prevalence of mastitis in animals increases as they age. It has been shown that the higher prevalence of mastitis in older animals is due to increased potency of teats and increased degree and frequency of previous exposure in multiparous old cows (Sharma *et al.*, 2011; FAO, 2014). Moreover, the linear relationship of advancing age and mastitis prevalence was linked to the gradual suppression of the immune system of the body and structural changes in the udder and teats (Salvador *et al.*, 2012).

This study reported that most cases of mastitis were noted in buffaloes within 1-90 days of lactation. This finding is confirmed in the study of Jingar *et al.* (2014) wherein the highest incidence of mastitis was detected in early lactation (up to 90 days) in indigenous cows and buffaloes and was lowest in late stage of lactation. At early lactation, elevation of somatic cells is probably due to non-adaptation of animals to the milking methods, more udder pressure and weaning associated milking behavior and may be directly due to numerous physiological and environmental factors during the transition period (Gitto *et al.*, 2002 as cited by Sharma *et al.*, 2011). Furthermore, SCC increases with progressing lactation (late lactation) regardless of whether the cow is infected or not (Dohoo & Meek, 1982 as cited by Sharma *et al.*, 2011). At this point, SCC elevation has been linked with an animal's innate immune response in preparation for calving and to enhance the mammary gland defense mechanism at this critical calving time.

Table 2. Prevalence of mastitis in 84 dairy buffaloes at the institutional herd of PCC at USF from December 2016 to February 2017

Variable		No. with mastitis (SCC >200,000)	%
Breed			
	Bulgarian Murrah	11	17.5 (11/63)
	American Murrah	4	19.1 (4/21)
Age (years)			
	<6	2	8.7 (2/23)
	6-7	10	25.6 (10/39)
	>7	3	13.6 (3/22)
Lactation (day)			
	1-90	2	33.3 (2/6)
	91-180	6	28.6 (6/21)
	>181	7	12.3 (7/57)
Pregnancy status			
	Non-pregnant	7	20.6 (7/34)
	Pregnant	8	16.0 (8/50)
Parity			
	1	3	12.5 (3/24)
	2	8	28.6 (8/28)
	≥3	4	12.5 (4/32)
	Total	15	**17.86 (15/84)**

It was also observed that mastitis was higher in buffaloes with ≥2 parities. According to Cerón-Muñoz *et al.* (2002), increasing days of milking and parturition are associated with high SCC. Fagiolo and Lai (2007) observed that the presence of an inflammatory status of the udder is frequent after the first trimester of lactation and in buffaloes with two or more parturitions. In dairy cattle, Hagnestam (2006) reported that first-parity cows developed mastitis earlier in lactation than older cows. The lactational incidence risk of mastitis was 19.8% in primiparous cows and 28.7% in multiparous cows. In the study of Salvador *et al.* (2012) parity was not found

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a significant factor causing subclinical mastitis, however, a major difference in their study is that they used California Mastitis Test (CMT) in detecting subclinical mastitis as compared to SCC used by many researchers.

Limited studies relate mastitis with reproductive state of buffaloes. However, a study by Ahmadzadeh *et al.* (2009) on the effect of clinical mastitis and other diseases on the reproductive performance of Holstein cows revealed that cows with mastitis remained non-pregnant by 224 days postpartum. They concluded that reproductive efficiency was decreased by the presence of clinical mastitis and further exacerbated when cows experienced both clinical mastitis and other diseases. It could also be that subclinical mastitis may be responsible for the non-pregnant state of the animal. McDougall *et al.* (2005) reported that higher rates of pregnancy loss, usually at early gestation, were associated with the occurrence of clinical mastitis (hazards ratio =1.57; P=0.071). Intramammary infection has been associated with increased uterine sensitivity to prostaglandin F2α and increased prostaglandin metabolite levels in blood following exposure to oxytocin (McDougall *et al.*, 2015), which is a possible mechanism by which mastitis may induce pregnancy loss.

Univariate Analysis

Using univariate analysis (Table 3), none of the possible risk factors investigated were associated with mastitis prevalence. However, a number of studies found an association of mastitis prevalence with age, lactation and parity of animals. The absence of association could be due to the smaller number of animals in this study.

Table 3. Univariate analysis of potential risk factors for mastitis (milk SCC >200,000 cells/mL) in buffaloes of PCC, Ubay, Bohol for the period December 2016 to February 2017

Variable	Category	Mastitis No.	%	*P*-value	OR (95% CI)
Breed				1.0	
	Bulgarian Murrah	11	17.5		1
	American Murrah	4	19.1		0.9 (0.2 – 4.4)
Age (yrs.)				0.18	
	>7	3	13.6		1
	<6	2	8.7		0.6(0.1-4.0)
	6-7	10	25.6		2.2(0.5-8.9)
Lacatation (day)				0.08	
	1-90	2	33.3		1.3(0.2 – 8.7)
	91-180	6	28.6		1
	≥181	7	12.3		0.4(0.1–1.2)
Pregnancy status				0.59	
	Non-pregnant	7	20.6		1
	Pregnant	8	16.0		1.4(0.4 – 4.2)
Parity				0.12	
	1	3	12.5		1.0 (0.2 – 5)
	2	8	28.6		2.8 (0.7 – 10.6)
	≥3	4	12.5		1

4 SUMMARY AND CONCLUSION

The lower prevalence of mastitis (17.9%) at PCC-USF compared to the results of other studies in the country could indicate a good management system in place at the farm to control mastitis incidence. Specifically, mastitis prevalence was higher in the following categories of buffaloes: American Murrah, 6-7 years old, 1-90 days of lactation, non-pregnant and ≥2 parities (P>0.05). However, SCC should be used as basis for mastitis detection rather than AT since the former directly measures the inflammatory response to an intra-mammary infection whereas the latter only measures the level of acid in milk due to several factors (including mastitis). It is also recommended that regular SCC testing should be done more than once a month to check on the incidence of mastitis at the shortest observation period possible. Furthermore, future studies should be carried out involving large sample sizes and bacteriological examinations to determine individual animal risk factors and implicating microorganisms, respectively.

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