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Research paper

Occurrence of Caprine Mastitis and its Etiological Agents and Associated Selected Risk in Mid Lactating Goats in the Oodi Extension Area of Kgatleng District, Botswana

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ABSTRACT

The negative impacts of mastitis on milk production pose a challenge to the profitability and sustainability of small scale dairy enterprises, thereby affecting the social welfare of farmers. The prevalence and epidemiological profile of Caprine mastitis in Botswana remains undocumented. The objective of this study was to determine the prevalence of Caprine mastitis in mid lactating goats in the Oodi extension area of Kgatleng District, Botswana. A cross-sectional study was conducted in which a total of one hundred and sixty-three (163) lactating goats from 17 flocks were purposefully selected based on the use of goat milk and stage of lactation (45 to 60 days post-partum). All goats were subjected to clinical examination and Somatic Cell Count Test (SCC). Samples with SCC above the threshold of 0.5×10^6 cells/ml were classified to be infected with sub-clinical mastitis. Each milk sample (0.5 ml) declared positive from Nucleo-counter SCC was further subjected to bacteriological tests to isolate for bacteria. The overall prevalence rate of mastitis was 17.8% (95% CI), with a significantly ($P<0.05$) higher prevalence rate for sub-clinical (13.5%) than clinical mastitis (4.29%). Most (93%) of the cases of mastitis were due to Coagulase Negative Staphylococci (CNS). Positive cases were also isolated for *Staphylococcus aureus* (83%), *Streptococcus species* (62%), *Escherichia coli* (48%), *Bacillus species* (41%) and unidentified gram negative bacteria (51%). Caprine mastitis was significantly ($P>0.05$) associated to risk factors; parity class, breed, housing floors, flock size and suckling litter number. Sub-clinical mastitis was four times more prevalent than the clinical form. The current study showed that the probabilities of the disease occurring was high in goat under poor management where the environmental and host risk factors are common sources for Coagulase Negative Staphylococci (CNS) and *S. aureus*. There is need to strengthen capacity building in employing basic mastitis control and prevention measures, especially in the informal milk value chain where milk does not follow any standard of formal hygiene and safety testing.

Key words: Caprine mastitis, occurrence, prevalence, risk factors, somatic cell count.

INTRODUCTION

Mastitis is an inflammatory reaction of parenchyma of the mammary glands characterized by physical, chemical and bacteriological changes in milk and pathological changes in glandular tissues (Radostits et al., 2000). Mastitis is cited as an important economic disease in dairy farming systems of sub-Saharan Africa where milk is mainly from small scale farmers (FAO, 2014). The negative impacts of mastitis on milk production pose a challenge to the profitability and

sustainability of small scale dairy enterprises which in turn affect the social welfare of farmers (Girma et al., 2012).

Javed et al. (2009) reported that the losses due to Caprine mastitis might be greater in developing countries where disease prevention, control and reporting systems are not well established. To date, many countries including Botswana continue to overlook Caprine mastitis since general results obtained from Bovine mastitis studies are used to infer on Caprine mastitis despite the difference in the species (Contreras et al., 2007). The prevalence and epidemiological profile of Caprine mastitis in Botswana remains undocumented due to limited research efforts, poor and/lack of farm reporting and documentation systems and unreliable statistics. This information gap impacts negatively on how Caprine mastitis management programs are designed and implemented. Furthermore, food borne pathogen residing in milk with mastitis may be overlooked especially in the informal milk value chain where milk does not follow any standard of formal hygiene and safety testing. This study was therefore, designed to investigate the prevalence rate of Caprine mastitis and its etiological agents and risk factors in mid lactating goats in the Oodi extension area of Kgatleng District, Botswana.

MATERIALS AND METHODS

Study design and sampling method

A cross-sectional study, methodology involving seventeen (17) goat flocks in the Oodi extension area was adopted. The empirical data was based on on-farm experiments carried out from August to October, 2014**.** The sample size was determined by the epidemiological formula $n = \frac{1.96^2 \, P_{exp} \, (1-P_{exp})}{d^2}$ where n =required sample size, 1.96^2=95% confidence interval, P_{exp}= expected/assumed prevalence and d^2 = desired absolute precision (Thrusfield, 2005).

For the current study, the assumption was expected prevalence rate of 50% while the statistical confidence level and precision was 95 and 10% respectively. Since the prevalence of Caprine mastitis in Botswana is not known nor documented, a prior 50% prevalence was assumed (Thrusfield, 2005).

From the formula, the minimum sample size required was 96. A total of one hundred and sixty-three (163) milking goats from the compiled list of flocks and animals were eventually sampled using a purposeful and simple random sampling technique. In this case, lactating animals that had not reached the 45 to 60 days post-partum phase were also included in the list but later sampled.

Risk factors

All animals were examined from their respective flock location. Information on risk factors such as breed, age, parity, kidding month, body score condition, suckling litter size, tick on udder and teats, teat or udder injuries, previous history of mastitis, type of housing floor, production system and flock size were collected through a self-administered questionnaire.

Field milk sampling

An aseptic procedure for collecting milk samples was employed during sampling. Prior to milk collection, each teat end was cleaned and disinfected with 70% ethanol swabs and allowed to dry. Milk samples were taken after the first streams (3 to 4 strips) of milk were discarded (Hogan et al., 1999). Since mastitis was defined at animal level, milk from the two halves was pooled into a composite sample from each doe. Approximately, 5 to 10 ml of milk was aseptically collected into a horizontally held sterile vial with an identity number. The samples were transported from the field to the laboratory in an ice-cooled box and transferred to a refrigerator at 4°C for somatic cell counting. Laboratory analysis was performed within 2 to 3 h post sampling

Classification of type of mastitis

The classification of mastitis was based on clinical examinations, milk bacteriological testing and somatic cell counts (Nucleo-counter SCC-100) in milk samples. ASCC>500 000 cell/ml and non-existence general symptoms in milk and host were considered in sub-clinical mastitis. Visible milk abnormalities and/or mammary gland lesions gland and SCC>500 000 cells/ml of milk indicated clinical mastitis ((BOBS, 2011; Bochniarz et al., 2013; Moon et al., 2007).

Somatic cell count (SCC) test

The Nucleo-counter SCC-100 laboratory instrument and procedures by Chemometec (2006) were employed in the analysis of the milk samples for sub-clinical mastitis. A stained lysate solution was prepared by dispensing 50 µl of the milk sample into a sample vial and adding 50 µl of reagent C (1:1 dilution). The lysate solution was loaded into a SCC-Cassette and then into the nucleo-counter instrument for counting of dyed nucleus from lysed cells.

Bacteriological analysis

Milk samples were examined using culture technique as described by Quinn et al. (1999). Around 10 µl of each milk sample (n=29) declared positive of mastitis by the Nucleo-counter SCC was plated onto nutrient agar, blood agar and McConkey's. Plates were incubated at 37°C for 24 h and observed for bacterial growth using a colony counter and

Table 1: Mastitis prevalence forms ad SCC ranges for positive cases.

Mastitis forms	No cases	Positive cases (%)	χ^2	Pr >X^2
No infected	134	0	-	-
Clinical mastitis	7	4.29	-	-
Sub-clinical mastitis	22	13.5	-	-
Overall mastitis	29	17.8	7.76	0.0001
SCC range(cell/ml)				
< 0.5×10^6	134	0.0	-	-
0.5-1.0 $\times10^6$ sub-clinical	17	58.6	-	-
1.0-1.5$\times10^6$ sub-clinical	5	17.2	-	-
1.5 - 2.0$\times10^6$ clinical	3	10.3	-	-
>2.0$\times10^6$ clinical	4	13.8	114.56	0.0001

microscopic morphology observation post staining by gram's technique. Colony morphological features, hemolytic properties on blood agar and catalase tests were used in tentatively bacteria identification; in the meantime, isolate bacteria were categorized biochemically using selectivemedia and fermentation of different sugars (Quinn et al., 1999).

Statistical analysis

All data were analyzed using SAS procedures (SAS Institute, 2011). To analyze the association of risk factors with mastitis occurrence the means were separated using the Chi-square (X^2) test. Independent variables having an association with mastitis at P< 0.05 were analyzed in multiple logistic regression model in order to establish mastitis Odds ratio (OR) in levels of categories under each risk factor. The SAS procedure of PROC LOGISTIC was employed by fitting the following model:

$$\text{Logit}\,(Y_{ijk....n}) = \alpha + \beta_i(x_i) + \beta_j(x_j) + \ldots + \beta_n(x_n) + \varepsilon_{ijk....n}$$

Where: Yijk= Probability of occurrence of mastitis, α = intercept, α = intercept, $X_1 \ldots X_n$ = independent variables (risk factors), $\beta_1 \ldots \beta_n$ = slope and $\varepsilon_{ijk...n}$= random variation. Differences among means were accepted as significant at P < 0.05.

RESULTS AND DISCUSSION

Prevalence of mastitis based on SCC

An overall prevalence rate of mastitis was 17.8%, however, a significantly (P<0.05) higher prevalence rate was reported for sub-clinical (13.5%) than clinical mastitis cases (4.29%) (Table 1). Majority (58.6%) of the sub-clinical cases had an SSC range of 0.5 to 1.0 $\times10^6$ cells/ml; meanwhile, the highest number (13.8%) of clinical cases exhibited SSC range of >2.0$\times10^6$ cells/ml. The results of the current study were close consent with the summarized results by Contreras et al. (2007) who indicated a 5 and 30% international prevalence rate for clinical and sub-clinical mastitis respectively. A greater variability in overall prevalence rates of Caprine mastitis was globally reported. This variability maybe explained by the difference in management of the farms, milking management practices, breed considered or technical knowledge of the investigators (Amin et al., 2011).

Prevalence of bacteria isolated from milk of goats with sub-clinical mastitis

Coagulase-negative Staphylococci was the most frequent (93%) bacteria isolated, occurring in both clinical (100%) and sub-clinical (86%) cases. The other isolated bacteria from positive cases include *Staphylococcus aureus* (83%), *Streptococcus species* (62%), *Escherichia coli* (48%), *Bacillus species* (41%) and unidentified gram negative bacteria (51%) (Table 2). The results of the current study were in close consent with findings of Islam et al. (2011); Ali et al. (2010) reported coagulase negative Staphylococci and *S. aureus* to be major etiological agents of caprine mastitis in Bangladesh and Pakistan respectively.

Flock size and housing floor based prevalence

The highest significant (P<0.05) prevalence of mastitis was revealed in goats raised on concrete floor housing (40.9%) and in flock sizes >60 animals (13.7%), while the lowest was recorded in earth floor housing (12.5%) and in flocks with less than 30 animals (6.26%) (Table 3). There was a significant difference in the odds of mastitis occurrence among the flock sizes and housing floor. In this case, the odds increased with the increase in the flock sizes and animal density per unit area. Furthermore, the probability of mastitis occurrence was 0.83 and 3.87 times as likely in flock sizes of 31 to 60 and >60 animals as compared to those in flocks with less than 30 animals (Table 3).
In relation to flock size, the results of the current study

Table 2: Frequency of micro-organisms isolated from milk samples positive for mastitis.

Bacterial isolates	Frequency of isolates		
	Clinical mastitis	Sub-clinical mastitis	Total
Staphylococcus aureus	5 (71)	19 (86)	24(83)
Escherichia coli	4 (57)	10 (44)	14(48)
Coagulase-negative staphylococci	7 (100)	19 (86)	27(93)
Streptococcus species	6 (85)	12 (56)	18(62)
Bacillus species	7 (100)	5 (23)	12(41)
Unidentified gram negative bacteria	7 (100)	8 (36)	15(51)

Table 3: Association of flock size and housing floors to the occurrence of caprine mastitis in Oodi extension area of Kgatleng District, Botswana.

	Mastitis prevalence						
Risk factors	**N**	**+No**	**Rate (%)**	**Odds**	**OR**	**95% CI**	**Pr>χ^2**
Flock size							
<30	48	3	6.26	0.07	RF		
31-60	39	8	20.5	0.26	3.87	0.95- 15.8	0.05**
>60	76	18	13.7	0.31	4.66	1.29- 16.8	0.02**
Housing floors							
Concrete	22	9	40.9	0.69	RF	-	-
Earth concrete combination	53	9	16.9	0.21	0.3	0.10-0.90	0.03**
Earth floor	88	11	12.5	0.14	0.21	0.07 -0.60	0.004**

N= sample size; + No= number of positive; OR = Odd ratio; CI= Confidence of OR; RF = Reference factor ** were significant at (P<0.05).

Table 4: Association of breeds and breed types to the occurrence of caprine mastitis in Oodi extension area of Kgatleng District, Botswana.

Risk factor	Categories	N	Mastitis prevalence					Pr > χ^2
			+No	Rate (%)	Odds	OR	95% CI	
Breed type	Meat breeds	139	19	13.7	0.16	RF	-	-
	Dairy breeds	24	10	41.7	0.71	0.22	0.09 -0.57	0.02**
Breeds	Tswana	95	13	13.7	0.16	RF		
	Boer	24	4	16.7	0.2	0.79	0.23 -2.69	0.71ns
	Kalahari	12	2	16.7	0.2	0.79	0.16 -4.03	0.78ns
	Saanen	24	10	41.7	0.71	0.22	0.08- 0.60	0.00***
	Tswana*Boer	8	0	0	0	0	0	0
Milking status	Milking	110	20	18.2				
	non-milking	53	9	17.0	n.a	n.a	n.a	0.85ns

N= sample size, + No= number of positive; OR = Odd ratio; CI= Confidence of OR; RF = Reference factor; n.a. = Not applicable (model did not converge); ns = Not significant (superscript indicates no significant level of categorical variable; P-Values with *** were significant at (P<0.001).

are in line with the study by Jegede and Tekdek (2000) who reported a significant association (P<0.05) between flock size and occurrence of mastitis in Nigerian states of Zamfara, Kebbi and Kaduna. Megersa et al. (2010) reported no significant association between the flock size and mastitis prevalence in Borana, Southern Ethiopia.

The observed housing floor prevalence is a consent to the results obtained by Begum et al. (2012) who reported a higher significant (P<0.05) prevalence in goats reared on earth floor (62.2%) as compared to those reared in raised floors (35.8%). The high incidences of mastitis in concrete floors can be attributed to intensive production systems characterized by high stocking densities per unit area and prolonged durations of contact between pathogens residing in the bedding and other host animals.

Breed and breed types based prevalence

Highest prevalence rate was observed in dairy type (41.7 %). The Saanen breed (13.7%) had significant the highest prevalence rate (P<0.05) (Table 4). The probabilities of

Table 5: Association of parity, litter, and age and body condition to the occurrence of caprine mastitis in Oodi extension area of Kgatleng District, Botswana.

Risk factor	Categories	Mastitis prevalence						Pr > χ^2
		N	+No	Rate (%)	Odds	OR	95% CI	
Parity	Primiparous	40	3	7.5	0.08	RF	-	-
	Multiparous	123	26	21.1	0.27	0.3	0.09-1.06	0.05**
Litter size	Single	95	12	12.6	0.15	RF	-	-
	Multiple	68	17	25	0.33	2.31	1.02-5.22	0.04**
Age	<2	42	4	9.52	-	-	-	-
	2-5	93	18	19.4	-	-	-	-
	>5	28	7	25	n.a	n.a	n.a	0.23ns
Body condition	Malnourished	84	16	19.1	-	-	-	-
	Normal	62	10	16.1	-	-	-	-
	Obese	17	3	17.65	n.a	n.a	n.a	0.90ns

N= number examined; + No= number of positive infections; OR = Odd ratio; CI= Confidence of OR; RF = Reference factor; n.a. = Not applicable (model did not converge); ** Categorical levels significant at P<0.05.

mastitis occurrence were 0.16 and 0.71 times for meat and dairy breeds respectively. Among the meat breeds, the Kalahari Red and the Boer breeds had no significant variation in their occurrence rate (16.7%) and likelihood of contracting mastitis (0.67). Meanwhile, the Tswana and Tswana* Boer crosses reported the least odds of prevalence at 0.16 and 0 respectively (Table 4).

Further observations in the current study revealed that 13 does had pendulous asymmetric udder conformation of which 7 were diagnosed with mastitis. The Saanen breed (85.7%) and Kalahari red breed (14.3%) constituted the group of the infected does. No significant (P>0.05) difference was observed for milking status, but does milked exhibited a high (18.2%) prevalence rate as compared to non-milking does (17.0%) (Table 4).

The results observed in the current study are in consent with observations by Ameh and Tari (2000), Begum et al. (2012) and Gebrewahid et al. (2012) who revealed that mastitis prevalence was significantly (P<0.05) influenced by the breed of goats.

Anzuino et al. (2010) highlighted that risks of infections and injuries to the udder and teats increased with the decrease in distances between teats and the floor. In the meantime, Watts (1988) pointed out that high-yielding goats are at high risk of contracting mastitis. Even though the aspect of teat and floor distances was not covered in the current study it can be inferred that the dairy type breed being the Saanen was more susceptible as compared to the other breeds due to its high milk yield, large sized udder and short teat end to floor distance.

Parity and litter size based prevalence

In the present study, highly significantly different (P<0.05) prevalence rates and odds were reported in does which are multiparous (21.1 %: 0.27) and suckling more than one kid (25.0%: 0.33 (Table 5). Moreover, the probability of mastitis occurrence was significantly lower in does which were primiparous (0.08) as well as those suckling a single kid (0.15) compared to multiparous does suckling (0.27) multiple kids (0.33) (Table 5). No significant (P>0.05) differences were observed for body condition score and age. Despite the lack of significance the highest mastitis cases were reported in does that were malnourished (19.1 %) and medium aged does between 2 to 5 years (19.4%).

In terms of the litter size, results of the current study are in agreement with findings by Amin et al. (2011) who reported higher significant (P<0.05) prevalence in does suckling multiple litter (43.0%) and low rates for single litter does (16.7%). In contrast with the current study, Ndegwa et al. (2010) reported no significant association (P>0.05) between litter size to mastitis occurrence. Despite the lack of significance (P>0.05) association age to mastitis in the current study, Ameh and Tari (2000) found a high significant association of mastitis in goats between the age of 2±5 than in goats between 1 and 2 years of age.

In association to parity, the current study was in contrast with findings by Begum et al. (2012) who reported no significant (P>0.05) association to mastitis. In relation to body condition of goats, the results obtained were in contrast to findings by Begum et al. (2012) who reported significantly (P<0.05) low sub-clinical mastitis in goats with good body condition (39.5%) as compared to poor body condition group (69.1%).

The role of suckling kids as reservoir and carriers of mastitis causing micro-organisms (*S. aureus)* to lactating does was reported by Contreras et al. (2007).

None associated risk factors

The current study reported no significant association (P>0.05) to the occurrence of mastitis for cluster, age, body condition score and month of kidding (Table 6). Despite the lack of significance, high prevalence rates were reported for

Table 6: risk factors not associated with mastitis occurrences in Oodi extension area of Kgatleng District, Botswana.

Risk Factors	Categories	N	Mastitis prevalence			
			+No	Rate%	X^2	P-value
Cluster	Matebeleng	42	12	28.6	5.46	0.07^{ns}
	Oodi	30	6	20.0	-	-
	Boladu	91	11	12.1	-	-
Age	<2	42	4	9.52	2.96	0.23^{ns}
	3-5	93	18	19.4	-	-
	>5	28	7	25	-	-
Body condition	Malnourished	84	16	19.1	0.21	0.90^{ns}
	Normal	62	10	16.1	-	-
	Obese	17	3	17.7	-	-
Month of kidding	July	52	5	9.62	3.49	0.06^{ns}
	August	111	24	21.6	-	-
Type of doe	Milking	110	20	18.2	0.04	0.85^{ns}
	Suckling	53	9	16.9	-	-
Tick infestation	Present	21	4	19.1	0.03	0.87^{ns}
	Absent	142	25	17.6	-	-
Floor type	Dung heap	95	13	13.7	3.88	0.14^{ns}
	Earth floor	35	10	28.6	-	-
	Concrete	33	6	18.2	-	-

N= sample size; + No= number of positive; OR = Odd ratio; CI= Confidence of OR; RF = Reference factor; ns = not significant (superscript indicates no significant level of categorical variable.

does in the Matebeleng cluster. Furthermore, categorical levels of earth floors, tick infestations, milking and underfeeding reported higher incidences of mastitis.

Conclusion

Caprine mastitis in the Oodi extension area of Kgatleng District, Botswana was prevalent and within the international range. Its sub-clinical form was four times more prevalent than the clinical form. Mastitis prevalence was associated to multiple risk factors acting simultaneously and the disease generally involves interplay between management practice, animal physiology and environment. The current study revealed that the probabilities of the disease occurring increased in high producing animals as well as in poorly managed animal. Early detection and treatment of the disease could prove worthy in reducing the risks of permanent udder damage and loss in milking stock. Stakeholder involved in animal health and husbandry need to strengthen capacity building in employing basic mastitis detection and prevention measures, especially, in the informal milk value chain where milk does not follow any standards of formal hygiene and safety testing.

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