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Occurrence and diagnostic aspects of subclinical mastitis in goats in and around Jabalpur region

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Abstract

Subclinical mastitis (SCM) is considered more severe than clinical mastitis, as early detection is impossible without regular monitoring. In this study, a total of 668 lactating goats were tested using modified California mastitis test (MCMT), somatic cell count (SCC), milk pH and electrical conductivity (EC). The overall occurrence of infected animals was found to be 29.94 per cent on animal basis and 28.65 per cent on udder halves basis. The occurrence was higher in right udder halves and breed wise higher in Jamnapari goats. The age wise occurrence of SCM showed i.e. highest in goats of 4 years and above age. The lactation / parity wise occurrence of SCM showed i.e. highest in 4th and more lactation and early lactation stage of each parity. Apparently healthy lactating goats had mean value of SCC $5.66 \pm 0.33 \times 10^5$ cells/ml. The mean SCC in SCM ($15.16 \pm 0.31 \times 10^5$ cells/ml) was significantly ($p \leq 0.05$) increased as compared to control. Apparently healthy lactating goats had mean value of Milk pH 6.57 ± 0.04 . The mean milk pH in SCM (7.39 ± 0.04) was significantly ($p \leq 0.05$) increased as compared to control. Apparently healthy lactating goats had mean value of EC 4.36 ± 0.05 mS/cm. The mean EC in SCM (6.51 ± 0.04 mS/cm) was significantly ($p \leq 0.05$) increased as compared to control.

Keywords: Subclinical mastitis, MCMT tests, SCC, milk pH, EC, occurrence, goats

Introduction

Mastitis is the inflammation of the mammary glands, regardless of the cause. It is a multiple etiological disease complex, being most prevalent in high yielding dairy cattle, buffaloes, goats and sheep throughout the world (Watts, 1998) [33]. Mastitis is accompanied by abnormal alterations in physical, chemical and bacteriological composition of milk or clots, flakes, or watery milk is the clinical sign most consistently observed (Arshad, 1999) [2].

The interaction between the host, pathogen and environment, although stress and physical injuries may cause inflammation of mammary gland. This inflammation of the mammary gland (Mastitis) is known to be a complex and costly disease. The disease is associated with a decrease in milk production, an increase of veterinary services, treatment, labour costs and culling (Fthenakis, 1994) [9]. Mastitis is one of the most serious economic and health problems of small ruminates flocks worldwide.

An investigation about a disease helps to understand the distribution and epidemiology of the disease status. Mastitis pathogens have been divided into contagious and environmental organisms. Subclinical mastitis (SCM) denotes absence of apparent gross abnormalities in the mammary gland but presence of chemical and bacteriological changes in the milk. Detection of SCM is more difficult and continues to have adverse effects on quality and quantity of milk without any apparent sign of illness. The loss of milk production recorded is more in SCM.

Once mastitis is set-up clinically in animals, it is very difficult to treat because of milk nutritional contents available to pathogenic organisms. Therefore it is necessary to diagnose the disease when it is in subclinical phase. The California Mastitis Test (CMT) and Somatic Cell Counts (SCC) of the milk are useful monitoring tools to detect the presence of subclinical mastitis in the mammary glands of dairy goats. The other tests used for diagnosis of subclinical mastitis are milk electrical conductivity, pH, Bromocresol purple test, Bromothymol blue test and Hotis test (Radostits *et al.*, 2007) [23].

Therefore, present study has been planned to study the occurrence of subclinical mastitis in goat using modified California mastitis test and intensity of infection using Somatic cell count.

Materials and Methods

The present study was an attempt to determine the occurrence of SCM in goats. For this purpose lactating goats belonging to different private goat keepers, Instructional Livestock

Farm Complex (ILFC), Adhartal, Amanala goat farm, N.D.V.S.U., Jabalpur were screened for SCM. A total of 668 lactating goats were tested by using modified california mastitis test (MCMT), milk pH, electrical conductivity (EC) and somatic cell count (SCC).

Clinical examination of the udder halves / milk

Each udder / teat was subjected to clinical examination by manual palpation for atrophy, consistency or variation in size of teat and teat position. The glands were palpated for indurations and asymmetry. Teat ends were observed for alterations such as scars, wounds, patent teat orifice and ease of milking. The udder was also examined to ascertain the abnormality (unilateral or bilateral) in the form of inflammatory swelling, fibrosis etc. Milk was examined for discoloration, clots or flakes, pus, blood staining and consistency.

Testing of milk samples

Modified california mastitis test (MCMT)

The MCMT was performed as per the method described by Schalm *et al.* (1971) [27]. The reagent was prepared by adding 2 ml stock solution B (Bromocresol purple reagent) to make volume 100 ml by adding remaining volume of stock solution A (Sodium lauryl sulfate reagent). A squirt of milk, about 5 ml from each udder half was placed in each of 2 shallow cups in the CMT plastic paddle. An equal amount of MCMT reagent was added to the milk. The paddle was rotated to mix the contents. Score was read in 10 seconds while continuing to rotate the paddle. The change in consistency of milk indicated the degree of severity of mastitis.

MCMT grading

The test is carried out with 5 ml of milk from each udder half into the CMT paddle. An equal amount of the above MCMT reagent was added and gently mixed in a horizontal plane with minimum agitation. The reaction was graded by intensity of gel formation and colour changes as follows:

Table: Type of MCMT grade

MCMT grade	Description
Negative	No change
Trace	Slime formation which disappeared with continuous movement of paddle
1+	Distinct slime but no gel formation
2+	Viscous with gel formation which adherent to the margin of the cup
3+	The gel formation with convex projection, the gel did not dislodge after swirling movement of the paddle

(Radostits *et al.*, 2010) [24]

Milk collection

Aseptic procedures for collecting udder halves milk samples as described by Sears *et al.* (1991) [29] and Quinn *et al.* (2004) [22] were followed with slight modification. Prior to milk collection udder half and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with potassium permanganate (0.01 per cent). A separate pledged of cotton was used for each teat. The first streams of milk were discarded and 10 ml of milk was collected into horizontally held fresh, sterile, labeled screw cap test tubes. After collection, the sample was placed in an ice box and transported to the laboratory for analysis.

Different laboratory tests

Somatic cell count (SCC)

The leukocyte count in the subclinical mastitis milk was performed to assess the degree of infection. The SCC was performed with the Pyronin Y Methyl Green (PYMG) staining as per the procedure described by Fitts and Gari (2006) [8] on day 0 (Pre treatment), 7 and 15 (post treatment).

Preparation of milk smear

The smear of milk for SCC was prepared within one hour of its collection to minimize disintegration of leukocyte. Firstly the slide was cleaned by soaking in cleaning solution and then rinsing it thoroughly in flowing water 10-15 seconds. There after the slide was heat dried with minimal exposure to dust and then stored dry. The smear of milk for SCC was prepared within one hour of its collection to minimize disintegration of leukocyte. The sample was uniformly mixed by 25 times gentle shaking of vials. Place 0.01 ml of milk sample on clean slide with the help of sterilized bacteriological loop, over one cm square rectangular area on a clean slide. After spreading MCMT positive milk, drying smears at 40-45°C within 5 minutes. Further in order to prevent smears from cracking and peeling from slide during staining, it was taken into consideration that heat should not be given rapidly. Excess milk was removed from exterior of tip by wiping tip with clean paper tissue. After this PYMG staining method was performed with the following procedure at the specified duration.

A drop of cedar wood oil was placed on the stained smear and examined under oil immersion. Examination of the milk smear was done at random. One cm square area of smear was divided in four equal parts by dividing it at the right angle. Cells were counted in five fields from each divided area. Thus, the cells were counted in total 20 fields. The average number of cells per square cm area was calculated. The average number of cells were multiplied by the multiplication factor of the microscope i.e. 497512 to obtain the number of cells per ml of the milk.

Derivation of common microscopic factor

The diameter of the field of 10X eye piece was predetermined by using stage micrometer. The lowest division of micrometer scale was 0.01 mm. Accordingly the diameter was measured and obtained as 0.016 cm. The area of the microscopic field was determined by the formula πr^2 . The calculations were made as under:

$$\begin{aligned} \text{Diameter} &= 0.0016 \text{ cm} \\ \text{Radius} &= 0.008 \text{ cm} \\ \text{Area} &= 3.14 \times (0.008)^2 \\ &= 0.000201 \\ \text{Microscopic factor} &= 100 \times (1/\text{Area}) \\ &= 100 \times (1/0.000201) \\ &= 497512.43 \text{ or } 497512 \end{aligned}$$

The same calibrated research microscope was used throughout the course of study (Shukla, 1980) [30].

PYMG staining method

In the beaker fresh Carnoy's fixative reagent (30 per cent chloroform + 10 per cent glacial acetic acid + 60 per cent ethyl alcohol) was taken and the smear was dipped for 5 minutes then excess reagent was drained. Then the smear was dipped in 50 per cent ethanol for 1 minute again the excess amount of reagent was drained. In the next step smear was dipped in 30 per cent ethanol for 1 minute. After that slide

was washed with water for 1 minute. Then smear was stained with PYMG stain for 6 minutes and dried completely. After that smear was flushed briefly with the N-Butyl alcohol followed by xylene. Then the slide was allowed to dry. Later on, put a drop of immersion oil on the smear and examined under oil immersion lens.

Milk pH

It was estimated by digital pH meter standardized by non buffer solution thereafter, the pH reading of the normal and mastitic milk sample was recorded on day 0 (pre-treatment) and on day 7 and 15 (post-treatment).

Electrical conductivity (EC)

It was estimated by the digital Electrical conductivity meter standardized by non buffer solution thereafter, the EC reading of the normal and mastitis milk sample was recorded on day 0 (Pre treatment), 7 and 15 (post treatment).

Results and Discussion

Modified california mastitis test (MCMT)

In the present study 668 lactating goats were screened for SCM and 29.94 per cent (200 out of 668 lactating goats) showed positive results by formation of gel or viscous mass on CMT paddle. The above findings are similar to the Bawaskar (2000) [3]; Abdel Rady and Sayed (2008) [1], who have also stated MCMT as the most accurate and reliable diagnostic test in the field conditions.

MCMT grading

MCMT score in lactating goats with SCM are given in table 01. A score of +1, +2 and +3 in MCMT was noticed in 59.37 per cent (779 out of 1312 halves), 22.25 per cent (292 out of 1312 halves) and 18.36 per cent (241 out of 1312 halves) in lactating goats affected with SCM, respectively.

Table 1: MCMT Grading in SCM

Score	No. of positive udder halves for SCM (n= 1312)	Per cent
+1	779	59.37
+2	292	22.25
+3	241	18.36

Clinical examination of the udder halves / milk

Temperature, respiration and pulse were normal in apparently healthy and subclinical cases of SCM. Clinical examination of

udder halves, supra mammary lymph nodes showed that they were apparently normal in goats with SCM. Colour, consistency and odour of milk samples from all positive cases of SCM were found to be apparently normal udder halves whereas reduction in milk yield was recorded in SCM.

Similar, observations were made by Schalm *et al.* (1971) [27], Chakraborti (2004) [6], Saravanan *et al.* (2009) [26] and Hafaty *et al.* (2016) [11] who have reported that no clinical signs associated with SCM as well as no physical abnormalities were found in the milk.

Different laboratory tests

Somatic cell count (SCC)

Apparently healthy lactating goats had a mean value of SCC $5.66 \pm 0.33 \times 10^5$ cells/ml. The mean SCC in SCM ($15.16 \pm 0.31 \times 10^5$ cells/ml) was significantly ($p \leq 0.05$) increased as compared to control (Table 02). Similar, observations were made by Poutrel and Lerondelle (1983) [21], Kalogridou-Vassliadou *et al.* (1992) [14] and Zeng (1999) [34] who have reported that in the milk from infected udders, mean Fossomatic SCC's were found to vary from as low as 550 000 to as high as 4 800 000 cells/ml.

Milk pH

Apparently healthy lactating goats had a mean value of Milk pH 6.57 ± 0.04 . The mean milk pH in SCM (7.39 ± 0.04) was significantly ($p \leq 0.05$) increased as compared to control (Table 02).

The above findings are in conformity to the studies of Hassan (2013) [12] who investigated variations in milk composition of some farm animals resulted by SCM. The pH was significantly ($p < 0.05$) higher in the infected animals milk (cows, ewes and goats) than uninfected animals milk (6.94 ± 0.06 , 6.71 ± 0.04 , 6.73 ± 0.01), respectively

Electrical conductivity (EC)

Apparently healthy lactating goats had a mean value of EC 4.36 ± 0.05 mS/cm. The mean EC in SCM (6.51 ± 0.04 mS/cm) was significantly ($p \leq 0.05$) increased as compared to control (Table 02).

The above findings are similar to the Roukbi *et al.* (2015) [25] who reported the value of EC for negative, suspected and positive (+1, +2 and +3) milk samples were 3.93 ± 0.64 mS/cm, 4.47 ± 0.61 mS/cm, 4.68 ± 0.72 mS/cm, 4.81 ± 0.76 mS/cm and 6.56 ± 0.85 mS/cm, respectively.

Table 2: Milk profile in SCM

S. No.	Parameters	Apparently healthy control (n= 6)	SCM (n= 200)
1.	SCC (10^5 cells/ml)	5.66 ± 0.33	15.16 ± 0.31
2.	Milk pH	6.57 ± 0.04	7.39 ± 0.040
3.	EC (mS/cm)	4.36 ± 0.05	6.51 ± 0.04

Occurrence

The overall occurrence of infected animals was found to be

29.94 per cent (200 out of 668) on animal basis and 28.65 per cent (376 out of 1312) on halves basis, respectively.

Table 3: Occurrence of SCM in goats

S. No.	Particulars	Number examined	Number positive	Occurrence (%)
1.	Total number of animals	668	200	29.94
2.	Total number of halves	1312 (24 blind teats)	376	28.65

Individual udder halves wise

The occurrence of SCM in different udder halves wise in goats is illustrated in table 04. Out of 376 affected udder

halves, 50 per cent (35 out of 70; Right =26 and Left= 9) udder halves were unilaterally affected whereas 27.45 per cent (341 out of 1242) udder halves had bilateral affection. In the

present study occurrence was higher in right udder halves i.e. 55.31 per cent (26 out of 47) followed by left udder halves involvement i.e. 39.13 per cent (9 out of 23), respectively. The udder halves wise occurrence of SCM in lactating goats revealed significant ($p \leq 0.05$) variation. These findings are in agreement with the findings of Pirazada *et al.* (2016)^[20] and Sree Priya *et al.* (2016)^[32] who have reported that 56.58 per

cent and 55.56 per cent infection in right udder halves followed by 43.42 per cent and 44.44 per cent in left udder halves.

These reports are not in agreement with the reports of Kumar *et al.* (2016)^[15] who reported that 52.70 per cent right udder halves and 47.30 per cent left udder halves were affected.

Table 4: Individual udder halves wise

S. No.	Udder halves		Number examined	Number positive	Occurrence (%)
1.	Unilateral udder halves	Right	47	26	55.31
		Left	23	9	39.13
2.	Bilateral udder halves		1242	341	27.45
$X^2 = 8.62$ df = 02 p = 0.013					

Breed wise

The breed wise occurrence of SCM in lactating goats revealed a highest occurrence i.e. 41.66 per cent (30 out of 72) in Jamnapari goats followed by 30 percent (6 out of 20) in Black Bengal, 29.82 per cent (122 out of 409) in non descript, 25.68 per cent (28 out of 109) in Barbari and 24.13 per cent (14 out of 58) in Sirohi breed. Among the breeds Jamnapari breeds found to have the higher occurrence of SCM. Breed wise occurrence revealed the non significant effect amongst different breeds on occurrence of SCM (Table 05).

These findings are in agreement with the findings of Kumhar and Shukla (2017)^[16] who have been reported that among the different breeds Jamnapari breed have the higher (40 per cent) incidence of SCM and which was due to large pendulus halves, which always predisposed to teat injury by trauma.

Table 5: Breed wise occurrence of SCM in goats

S. No.	Breeds	Number examined	Number positive	Occurrence (%)
1.	Sirohi	58	14	24.13
2.	Barbari	109	28	25.68
3.	Black Bengal	20	6	30
4.	Jamnapari	72	30	41.66
5.	Non descript	409	122	29.82
$X^2 = 3.38$ df = 04 p = 0.49				

Age wise

The age wise occurrence of SCM in lactating goats revealed a highest i.e. 38.88 per cent (98 out of 252) occurrence in goats of 4 years and above age followed 31.97 per cent (47 out of 147) in 3-4 years, 21.62 per cent (40 out of 185) in 2-3 years of age and 17.85 per cent (15 out of 84) in 1-2 years of age, respectively. The age wise occurrence revealed a significant variation ($p \leq 0.05$) among various age groups. The details are outlined in table 06.

SCM prevalence has progressively increased with age of the lactating goats and are in agreement with other studies

(Gebrewahid *et al.*, 2012^[10], Haftay *et al.*, 2016^[11], Ferdous *et al.*, 2018^[7] and Mahlangu *et al.*, 2018)^[18]. Age is the most significant factor in determining the prevalence of mastitis in goats.

Table 6: Age wise occurrence of SCM in goats

S. No.	Age group	Number examined	Number positive	Occurrence (%)
1.	1-2 years	84	15	17.85
2.	2-3 years	185	40	21.62
3.	3-4 years	147	47	31.97
4.	4 and above years	252	98	38.88
$X^2 = 11.99$ df = 3 p = 0.007				

Lactation / Parity wise

Lactation / parity wise occurrence of SCM was also recorded. The lactation number was taken from 1st to 4th and more lactation number. The lactation wise occurrence revealed a significant variation ($p \leq 0.05$) among various lactation groups. The highest occurrence of SCM was observed in 4th and more lactation i.e. 38.42 per cent (83 out of 216) followed by 31.91 per cent (60 out of 188) in 3rd lactation, 23.45 per cent (38 out of 162) in 2nd lactation and 18.62 per cent (19 out of 102) in 1st lactation. The results are outlined in table 07.

An increased occurrence related to lactation / parity has been reported in goats by many researchers (Boscos *et al.*, 1996^[5], Bergonier *et al.*, 2003^[4], Kumar *et al.*, 2016^[15] and Mahlangu *et al.*, 2018)^[18]. When the duration of exposure to infection is long and spontaneous cure rate is low, prevalence increases. The possible explanation is that the increasing age and number of parity there by, resulted in widening of teat canal which may prone to infection by entry of the microorganism via contamination.

However, these observations are in contrast with those of Singh (2009)^[31], Gebrewahid (2012)^[10] and Hafaty (2016)^[11], who have reported the occurrence as 11.25 per cent, 17.8 per cent and 27.8 per cent, respectively in 4th parity.

Table 7: Lactation / Parity wise occurrence of SCM in goats

S. No.	Lactation number	Number examined	Number positive	Occurrence (%)
1.	1 st	102	19	18.62
2.	2 nd	162	38	23.45
3.	3 rd	188	60	31.91
4.	4 th and more	216	83	38.42
$X^2 = 9.45$ df = 3 p = 0.02				

Lactation stage wise

The lactation stage was divided in to 3 classes i.e. early lactation (1 to 2 months), mid lactation (2 to 3 months) and

late lactation (3 months and onwards till drying off). The overall occurrence of SCM according to lactation stage was observed as 38.89 per cent (85 out of 218), 29.62 per cent (72

out of 243) and 20.77 per cent (43 out of 207) in early, mid and late lactation stage, respectively (Table 08). The lactation stage wise occurrence of SCM showed significant variation ($p \leq 0.05$) among different lactation stage.

These findings are in agreement with the findings of Islam *et al.*, (2011) [13] and Ferdous *et al.*, (2018) [7] who have reported that highest occurrence of SCM in the does in the early lactation stage followed by mid lactation and late lactation stage. This may be due to the increase in the SCC in initial stage of lactation, as also reported by Scott *et al.* (2002) [28].

Table 8: Lactation stage wise occurrence of SCM in goats

S. No.	Lactation stage	Number examined	Number positive	Occurrence (%)
1.	Early (1-2 months)	218	85	38.99
2.	Mid (2-3 months)	243	72	29.62
3.	Late (>3 months)	207	43	20.77
$X^2 = 9.10$ $df = 2$ $p = 0.01$				

Litter Size wise

Litter size wise occurrence of SCM was also recorded. The litter size was taken from lactating goats those were having one to four numbers of kids. The litter size occurrence revealed a significant variation ($p \leq 0.05$) among various groups. The occurrence of SCM was higher i.e. 41.66 per cent (5 out of 12) followed by 40 per cent (36 out of 90), 35.35 per cent (105 out of 297) and 20.07 per cent (54 out of 269) in lactating goats having four kids, three kids, two kids and one kid, respectively. The results are outlined in table 09.

These findings are in agreement with Islam *et al.* (2011) [13] who have reported that prevalence of SCM was higher does having four kids followed by three kids, two kids and one kid,

respectively. Mastitis prevalence has progressively increased due to prolonged exposure to pathogens in multiparous animals compared to primiparous.

Table 9: Litter size wise occurrence of SCM in goats

S. No.	Litter size	Number examined	Number positive	Occurrence (%)
1.	One	269	54	20.07
2.	Two	297	105	35.35
3.	Three	90	36	40
4.	Four	12	5	41.66
$X^2 = 11.99$ $df = 3$ $p = 0.007$				

Season wise

Season wise occurrence of SCM was also recorded. The season was divided in 3 classes i.e. rainy season (July - October months), winter season (November – February months) and summer season (March – June months). The overall occurrence of SCM according to season was observed as 39.83 per cent (96 out of 241), 30.56 per cent (59 out of 193) and 19.23 per cent (45 out of 234) in winter season, rainy season and summer season, respectively (Table 10). The season wise occurrence revealed a significant variation ($p \leq 0.05$) among different seasons.

These findings are in agreement with Kumar *et al.*, (2016) [15] who reported that mastitis prevalence was significantly higher in lactating goats sampled during the period from November to December than the periods from September to October. The reason of increased rate of prevalence during November to December months could be higher number of kidding in these months which leads to increased susceptibility to udder infection.

Table 10: Season wise occurrence of SCM in goats

S. No.	Season	Month	Number screened	Number positive	Occurrence (%)
1.	Rainy season	July – October	193	59	30.56
2.	Winter season	November – February	241	96	39.83
3.	Summer season	March – June	234	45	19.23
$X^2 = 13.17$ $df = 2$ $p = 0.001$					

Organized and unorganized sector wise

Occurrence of SCM in organized and unorganized sector was observed as 25 per cent (29 out of 116) and 30.97 per cent (171 out of 552). No significant variation was noticed in the occurrence with respect of rearing pattern of goats. Results are represented in table 11.

These findings are in agreement with Mahlangu *et al.* (2018)

[18] who reported that higher prevalence of SCM was observed in goats residing in house cleaned at least once a fortnight. The occurrence of SCM was found to be higher in unorganized sector as compared to organized sector might be due to poor hygienic management, lack of awareness, method of milking, type of housing, bedding and weather.

Table 11: Organized and unorganized sector wise occurrence of SCM in goats

S. No.	Sector / Rearing pattern	Number screened	Number positive	Occurrence (%)
1.	Organized sector	116	29	25
2.	Unorganized sector	552	171	30.97
$X^2 = 0.90$ $df = 01$ $p = 0.34$				

The higher occurrence of Mastitis could be attributed to expansion of cross breeding programme in the country have lead to an increase in the occurrence of SCM. The variation in occurrence of SCM may be due to more susceptibility to the infection because of the injured halves, poor management, selection of number of animals, breeds etc.

Conclusion

In our study, overall occurrence of SCM was found to be 29.94 per cent on animal basis and 28.65 per cent on udder

halves basis. The occurrence was higher in right udder halves and breed wise highest in Jamnapari goats. The age wise occurrence of SCM showed i.e. highest in goats of 4 years and above age. The lactation / parity wise occurrence of SCM showed i.e. highest in 4th and more lactation and early lactation stage of each parity. Apparently healthy lactating goats had mean value of SCC $5.66 \pm 0.33 \times 10^5$ cells/ml. The mean SCC in SCM was significantly increased as compared to control. Apparently healthy lactating goats had mean value of Milk pH 6.57 ± 0.04 . The mean milk pH in SCM was

significantly increased as compared to control. Apparently healthy lactating goats had mean value of EC 4.36 ± 0.05 mS/cm. The mean EC in SCM was significantly increased as compared to control.

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