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Prevalence, Risk Factors and Current Antibiogram Assay Associated with Subclinical *Escherichia coli* Mastitis in Goats

Brejesh Singh[1]*, Pramod Chandra Shukla[2], Rajesh Kumar Sharma[3], Arun Mourya[4], Ranvijay Singh[5] and Kajal Jadav[6]

[1]Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, INDIA
[2]Professor and Head, Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, INDIA
[3]Professor and Head, Department of Veterinary Pharmacology, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, INDIA
[4]Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, INDIA
[5]Associate Professor, Department of Veterinary Public Health & Epidemiology, College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, INDIA
[6]Assistant Professor, School of Wildlife Forensic & Health, NDVSU, Jabalpur, Madhya Pradesh, INDIA

*Corresponding Author: bsvet2209@gmail.com

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Abstract

The objective of this study was to isolate Escherichia coli spp. and to identify the risk factors associated with subclinical mastitis in goats. The overall animal wise prevalence of Escherichia coli SCM was 9.13 per cent and udder halves wise 6.02 per cent. Out of 1312 udder halves, 7.1 per cent udder halves were unilaterally affected and the prevalence was higher in right udder halves (6.38 per cent).The breed wise prevalence of Escherichia coli SCM was highest (25.0 per cent) in Black Bengal goats and the age wise prevalence of Escherichia coli SCM was found highest (23.80 per cent) in lactating goats in 3-4 years of age. The lactation / parity wise prevalence of Escherichia coli SCM was observed highest (18.08 per cent) in 3rd lactation. The lactation stage wise prevalence of SCM was found highest (17.43 per cent) in early lactation stage. The antibiogram assay revealed that Escherichia coli spp. was most susceptible to enrofloxacin with 23.25 mm zone of inhibition.

Keywords: Antibiogram, *Escherichia coli* spp., Goat, Risk Factors, Subclinical Mastitis

Introduction

India is an agricultural country with large number of domestic animals. Among domestic animals, Goat is one of the important members in milk and meat producing animals in both temperate and tropical agriculture (Haenlein, 2004). The current goat population in India is estimated to be around 135.17 million which is second largest 149.38 million after China (FAOSTAT, 2013). In India, goats produce 4.00 mMT of milk as per FAO (2013) report.

Various pathogenic microorganisms cause mastitis in goat resulting in decrease in milk yield, change in milk composition, reduces productive life and weight gain in lambs and incur huge economic loss to dairy farmer. Subclinical mastitis is one of the most important diseases in small ruminants and considered a constant risk of infection for the whole herds and their environment. Hence, udder infection must be prevented or detected at an early stage not only to protect the farmer, but also to protect human consumer (Zamin *et al*., 2010). The bedding used to house in animal is the primary source of environmental pathogens, but contaminated teat dips, intramammary infusions, water used for udder preparation before milking, water ponds or mud holes, skin lesions, teat trauma, and flies have all been incriminated as sources of infection. Various bacteria are commonly known to cause mastitis in goats including *Streptococcus* sp., *Staphylococcus* sp., *Pasteurella* sp., and coliforms, such as *E. coli*. The common coliform bacteria that cause mastitis *are E. coli*, *E. aerogenes, K. pneumonia* and *S. marcesans* and are associated with 30-40% of the clinical mastitis in farm. Among the coliform mastitis only *E. coli* cause 5.719% mastitis in goat. Besides, majority of coliform isolates from raw milk were *E. coli* 32%, *Enterobacter* spp. 29.2%, *Klebsiella* spp. 19.4%, *Serratia* spp. 11.1% and *Citrobacter* 1.0% (Salman and Hamad, 2011).

Today the greatest problem in the treatment and control of mastitis is the emergence of drug resistance due to indiscriminate and haphazard use of antibacterial agent. Antibiotic sensitivity tests can be performed to ensure adequate treatment. Therefore, regular studies on antibiotic sensitivity of bacterial isolates are mandatory for effective and economical treatment of the disease (Sanchez *et al.,* 2001) and to reduce the risk of zoonotic pathogens and antimicrobial drug residues (Bradely, 2002).

Keeping in view the above facts, the present study was therefore designed to isolate bacteria that cause subclinical mastitis in goats and evaluation of the bactericidal property of turmeric and garlic extract as alternative to synthetic antibiotics.

Materials and Methods

For this study, a total of 668 lactating goats belonging to Sirohi, Barbari, Black Bengal, Jamnapari and non-descript breeds were screened from February 2017 to December 2018 by MCMT.

The isolation and identification of bacterial pathogens were performed according to the procedure described by Quinn *et al*. (2004). In brief, 01 ml milk sample was inoculated into the test tube containing 05 ml of MacConkey's broth and incubated aerobically at 37ºC for 18-24 hours. The enriched inoculum was streaked onto eosin methylene blue (EMB) agar plate and metallic sheen colony was tested for gram's staining and biochemical tests like indole, methyl red, Voges proskauer and citrate utilization test (Cruikshank *et al.,* 1975)**.**

Extraction of Plant Material

Two different extracts were prepared i.e. aqueous and methonolic.

Preparation of *Allium sativum* Extract

Aqueous Extract of *Allium sativum* (Garlic)

The fresh cloves of *Allium sativum* were separated and peeled to obtain the edible portion. The 100 grams of edible portion was chopped and dried in the shed at room temperature after dividing into small pieces. The dried pieces were then powdered in the grinder and sieved through muslin cloth. *Allium sativum* powder was weighed and soaked in one liter of distilled water at room temperature for 5 days with daily intermittent shaking. After 5 days the solution was filtered using clean, sterile, muslin cloth and re-filtered again using Whatman's filter paper No. 1. The extract was transferred to previously weighed petri dish for the evaporation of distilled water with the help of water bath.

The concentrated extract (18.5 grams) obtained was kept under refrigeration till used.

Methanolic Extract of *Allium sativum* (Garlic)

The fresh cloves of *Allium sativum* were separated and peeled to obtain the edible portion. The 100 grams of edible portion was chopped and dried in the shed at room temperature after dividing into small pieces. The dried pieces were then powdered in the grinder and sieved through muslin cloth. Thimble of 100 grams of powder *Allium sativum* was prepared and kept in soxhlet apparatus for preparation of methanolic extract for 5 days. The extract was then transferred to previously weighed petri dish for the evaporation of distilled water in order to get a good concentrate with the help of water bath. The concentrated extract (8.6 grams) obtained was kept under refrigeration till used.

Preparation of *Curcuma longa* Extract

Aqueous Extract of *Curcuma longa* (Haldi)

Fresh rhizomes of *Curcuma longa* were dried in the shed at room temperature after dividing into small pieces. The dried pieces were then powdered in the grinder and sieved through muslin cloth. *Curcuma longa* powder (100 grams) was weighed and soaked in one liter of distilled water at room temperature for 5 days with daily intermittent shaking. After 5 days the solution was filtered using clean, sterile, muslin cloth and re-filtered again using Whatman's filter paper No. 1. The extract was then transferred to previously weighed petri dish for the evaporation of distilled water in order to get a good concentrate with the help of water bath. The concentrated extract (21 grams) obtained was kept under refrigeration till used.

Methanolic Extract of *Curcuma longa*

Fresh rhizomes of *Curcuma longa* were dried in the shed at room temperature after dividing into small pieces. The dried pieces were then powdered in the grinder and sieved through muslin cloth. Thimble of 100 grams of powdered *Curcuma longa* was prepared and kept in soxhlet apparatus for preparation of methanolic extract for 5 days. The extract was then transferred to previously weighed petri dish for the evaporation of methanol in order to get a good concentrate with the help of water bath (Gupta *et al.*, 2015). The concentrated extract (7.83 grams) obtained was kept under refrigeration till used.

Disc Preparation

The extracts were prepared at 100 per cent concentration as the stock solution. Sterile discs of 6 mm diameter were allowed to suck up the extract filtrate and maintained and impregnated with 100 mg of respective solvent extract per ml and left dry under the laminar air flow. The produced discs (each one) have the ability to absorb about 0.01 ml.

Antibacterial Activity of *Escherichia coli* specie Isolates

Antibiotic sensitivity profiles of *E. coli* isolates were studied against six different antibiotics according to methods of Bauer *et al.* (1966). Briefly, bacteria grown in nutrient broth for 4-6 hours was smeared on Mueller-Hinton Agar (Hi-media). Different antibiotic discs namely cefepime (30 mcg/disc), ampicillin+ sulbactum (10/10 mcg/disc), enrofloxacin (10 mcg/disc), ciprofloxacin (5 mcg/disc), Allium sativum (100 mg/ml solution/disc) and Curcuma longa (100 mg/ml solution/disc) was placed on Mueller-Hinton agar plates at equal distance and incubated at 37°C for 24 hours. Diameter of bacterial inhibition zone around each disc was measured. Bacterial isolates showing no inhibition zone around the antibiotic disc used and well-developed colonies within the zone were rechecked against same drug for confirmation of resistance based on CLSI, 2015.

Statistical Analysis

Analysis of data of prevalence study was done by using Chi square test. (Snedecor and Cochran, 1994).

Results and Discussion

Total of 1312 milk samples from functional halves of lactating goats were screened for udder health status. Milk

samples were collected for bacterial isolation and identification on primary and selective media, respectively, from 376 udder halves found positive on Modified California Mastitis Test (MCMT). Out of 376 milk samples collected from mastitic udder halves, 79 (21.01 per cent) isolates were found *E. coli* positive as revealed by culture on selective media and biochemical tests (IMViC test).

Prevalence of *Escherichia coli* SCM

The overall prevalence of *E. coli* SCM from February 2017 to December 2018 was 11.82 per cent. However, the prevalence of *E. coli* spp. among the lactating goats suffering from SCM was reported to be 39.50 per cent. Details are shown in Table 1. The udder halves wise prevalence of *E. coli* spp. in SCM was 6.02 per cent and prevalence among SCM was found to be 21.01 per cent. Significant variation ($p \leq 0.05$) was observed in prevalence of *Escherichia coli* SCM in lactating goats. Details are shown in Table 2. These findings are closely similar with Sarker and Samed (2011) and Rudra and Dutta (2018) who reported that *E. coli* isolated from SCM milk samples were 27.12 per cent and 27.6 per cent, respectively.

Table 1: Animal wise prevalence of *Escherichia coli* SCM in goats

S. No.	Particulars	Number examined	Number positive	Per cent (%)
1	Total number of animals	668	79	11.82
2	SCM	200	79	39.5
$X^2 = 40.04$		df = 01	p = 0.00001	

Table 2: Udder halves wise prevalence of *Escherichia coli* SCM in goats

S. No.	Udder halves	Number examined	Number positive	Per cent (%)
1	Total number of functional halves	1312	79	6.02
2	SCM	376	79	21.01
$X^2 = 59.79$		df = 01	p = 0.00001	

Individual Udder Halves Wise

The udder halves wise prevalence of *E. coli* SCM showed non-significant variation among lactating goats. Out of 1312 udder halves, 7.1 per cent udder halves were unilaterally affected whereas, 5.95 per cent udder halves had bilateral affection. In the present study prevalence was higher in right udder halves *i.e.* 6.38 per cent followed by left udder halves involvement i.e. 4.34 per cent, respectively. The details are outlined in Table 3.

Table 3: Individual udder halves wise of *Escherichia coli* SCM in goats

S. No.	Udder halves		Number examined (1312)	Number positive (79)	Prevalence (%)
1	Unilateral udder halves	Right	47	4	6.38
		Left	23	1	4.34
2	Bilateral udder halves		1242	74	5.95
$X^2 = 0.55$			df = 02	p = 0.75	

Breed Wise

The breed wise prevalence of *E. coli* SCM in lactating goats revealed a highest prevalence i.e. 25.0 per cent in Black Bengal goats followed by in Jamnapari, Barbari, Sirohi and non-descript breed. The breed wise prevalence of *E. coli* SCM showed significant variation ($p \leq 0.05$) among various breeds of lactating goats. The results are outlined in Table 4. These reports did not tally with the reports of Rudra and Dutta (2018) who reported that 50 per cent prevalence was recorded in cross breed goats.

Table 4: Breed wise prevalence of *Escherichia coli* SCM in goats

S. No.	Breeds	Number examined (668)	Number positive (79)	Prevalence (%)
1	Sirohi	58	5	8.62
2	Barbari	109	19	17.43
3	Black Bengal	20	5	25
4	Jamnapari	72	16	22.22
5	Non-descript	409	34	8.31
$X^2 = 14.60$ df = 04 p = 0.005				

Age wise

The age wise prevalence of *E. coli* SCM in lactating goats revealed a highest i.e. 23.80 per cent prevalence in lactating goats of in 3-4 years of age followed in 1-2 years, 4 years and above of age and 2-3 years of age, respectively. The age wise prevalence of *E. coli* SCM showed significant variation ($p \leq 0.05$) among various age groups of lactating goats. The results are outlined in Table 5.

Table 5: Age wise prevalence of *Escherichia coli* SCM in goats

S. No.	Age group	Number examined (668)	Number positive (79)	Prevalence (%)
1	1-2 years	84	17	20.23
2	2-3 years	185	9	4.86
3	3-4 years	147	35	23.8
4	4 years and above	252	18	7.14
$X^2 = 30.18$ df = 03 p = 0.00001				

Lactation / Parity Wise

The lactation / parity wise prevalence of *E. coli* SCM was also recorded. The lactation number was taken from 1st to 4th and more lactation number. The highest prevalence of *E. coli* SCM was observed in 3rd lactation i.e. 18.08 per cent followed in 1st lactation, 4th and more lactation and 2nd lactation. The lactation / parity wise prevalence of *E. coli* SCM showed significant variation ($p \leq 0.05$) among various lactations / parity in goats. The results are outlined in Table 6.

Table 6: Lactation / Parity wise prevalence of *Escherichia coli* SCM in goats

S. No.	Lactation number	Number examined (668)	Number positive (79)	Prevalence (%)
1	1st	102	18	17.64
2	2nd	162	8	4.93
3	3rd	188	34	18.08
4	4th and more	216	19	8.79
$X^2 = 15.49$ df = 03 p = 0.001				

Lactation Stage Wise

The overall prevalence of *E. coli* SCM according to lactation stage was observed highest i.e. 17.43 per cent in early followed by in mid and late lactation stage, respectively. The lactation stage wise prevalence of SCM showed significant variation ($p \leq 0.05$) among different lactation stages. The results are outlined in Table 7.

Table 7: Lactation stage wise prevalence of *Escherichia coli* SCM in goats

S. No.	Lactation stage	Number examined (668)	Number positive (79)	Prevalence (%)
1	Early (1-2 months)	218	38	17.43
2	Mid (2-3 months)	243	23	9.46
3	Late (>3 months)	207	18	8.69

Litter Size Wise

The litter size wise prevalence of *E. coli* SCM was also recorded. The prevalence of *E. coli* SCM was higher i.e. 16.66 per cent followed by in lactating goats having four kids, three kids, two kids and one kid, respectively. The litter size wise prevalence of SCM showed significant variation ($p \leq 0.05$) among different lactation stages. The results are outlined in Table 8.

Table 8: Litter size wise prevalence of *Escherichia coli* SCM in goats

S. No.	Litter size	Number examined (668)	Number positive (79)	Prevalence (%)
1	One	269	14	5.2
2	Two	297	49	16.49
3	Three	90	15	16.66
4	Four	12	1	8.33
$X^2 = 15.85$ df = 03 p = 0.001				

Season wise

The season wise prevalence of *E. coli* SCM was also recorded. The overall prevalence of *E. coli* SCM according to season was observed highest i.e. 18.13 per cent in rainy season followed by in winter season and summer season, respectively. The season wise prevalence of *E. coli* SCM showed significant variation ($p \leq 0.05$) among different seasons. The results are outlined in Table 9.

Table 9: Season wise prevalence of *Escherichia coli* SCM in goats

S. No.	Season	Month	Number screened (668)	Number positive (79)	Prevalence (%)
1	Rainy season	July – October	193	35	18.13
2	Winter season	November – February	241	29	12.03
3	Summer season	March – June	234	15	6.41
$X^2 = 4.93$ df = 02 p = 0.004					

Organized and Unorganized Sector Wise

Prevalence of *E. coli* SCM in organized and unorganized sector was observed as 12.86 per cent and 6.89 per cent. No significant variation was noticed in the prevalence of *E. coli* SCM with respect of rearing pattern of goats. Results are represented in Table 10.

Table 10: Organized and unorganized sector wise prevalence of *Escherichia coli* SCM in goats

S. No.	Sector / Rearing pattern	Number screened (668)	Number positive (79)	Prevalence (%)
1	Organized sector	116	8	6.89
2	Unorganized sector	552	71	12.86
$X^2 = 2.67$ df = 01 p = 0.10				

Antibiogram for *Escherichia coli* isolates

In the present study *in vitro* antimicrobial activity of different chemotherapeutic agents against the isolates of *Escherichia coli* spp. from 24 SCM infected lactating goats was investigated. Among the different chemotherapeutic

agents, zone of inhibition was higher in enrofloxacin (23.25 mm) followed ciprofloxacin, cefepime and ampicillin+ sulbactum, respectively (as shown in Table 11). These findings are in agreement with Bhojne *et al*. (2000), who reported that the *E. coli* spp. showed sensitivity to enrofloxacillin, gentamicin and ceftriaxone while, it was resistant to norfloxacin, oxytetracycline, ampicillin, cloxacillin and chloramphenicol. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against bacterial isolates. Tras *et al.,* (2007) also reported that antibiotic resistance may be due to misuse of antibiotics because frequently use of the same antibiotics may lead to such resistance.

Table 11: Antibiogram for *Escherichia coli* isolates as per the zone of inhibition

S. No.	Antibiotic	Zone of inhibition (Out of 24 isolates)
1	Cefepime (30 mcg)	13.41 mm
2	Ampicillin+ sulbactum (10/10 mcg)	10.25 mm
3	Enrofloxacin (10 mcg)	23.25 mm
4	Ciprofloxacin (5 mcg)	20.5 mm
5	*Allium sativum* (100 mg/ml, aqueous extract)	18.58 mm
6	*Allium sativum* (100 mg/ml, methanolic extract)	16.54 mm
7	*Curcuma longa* (100 mg/ml, aqueous extract)	17.41 mm
8	*Curcuma longa* (100 mg/ml, methanolic extract)	19.70 mm

Antibacterial susceptibility tests of aqueous and methanolic extract of *Allium sativum* showed the zone of inhibition as 18.58 mm and 16.54 mm, respective. The results are closely similar with the findings of Iwalokun *et al.* (2004) who reported the significant antibacterial effect of garlic extract against several multi-resistant bacterial strains including *E. coli* and *S. aureus*. The diameters of the inhibition zones were 21.5 mm and 24.6 mm, respectively.

Similarly, antibacterial susceptibility tests of aqueous and methanolic extract of *Curcuma longa* showed the zone of inhibition 17.41 mm and 19.70 mm, respectively. It was noted that the results found in this work was quite similar as the work done by Gul and Bakht (2015) which is remarkable and it reveals that different extracts of turmeric had antimicrobial activity against *E. coli* produced different zones of inhibition. As per Gupta *et al*. (2015) who reported the antimicrobial susceptibility tests of different fractions of *Curcuma longa* rhizome extract against *S. aureus* ATCC 6571 and clinical isolates showed that all fractions of *Curcuma longa* rhizome are highly active against standard and clinical isolates of *S. aureus* showing zone of inhibition ranges between 9 mm and 21 mm.

Conclusion

The overall prevalence of *E. coli* in SCM from February 2017 to December 2018 was 11.82 per cent. Among the lactating goats suffered from SCM was reported as 39.50 per cent. The udder halves wise prevalence of *E. coli* spp. in SCM was 6.02 per cent and prevalence among SCM was found to be 21.01 per cent. Out of 1312 udder halves, 7.1 per cent udder halves were unilaterally affected. The prevalence was higher in right udder halves. Breed and age wise analysis of prevalence of *E. coli* SCM reveals that it is found highest in Black Bengal goats and lactating goats of 3-4 years. Further, it was also found that the prevalence of *E. coli* SCM showed highest in 3rd lactation and was remarkably high in early lactation stage. The litter size wise prevalence of *E.coli* SCM showed higher in goats had four kids. The overall season wise prevalence of *E. coli* SCM highest in rainy season. Prevalence of *E. coli* SCM was higher in organized sector. Results of antibiogram against *E. coli* spp. revealed that most effective antimicrobial agent was enrofloxacin followed ciprofloxacin, cefepime and ampicillin+ sulbactum. Aqueous and methanolic extract of *Allium sativum* showed the zone of inhibition 18.58 mm and 16.54 mm. Similarly, aqueous and methanolic extract of *Curcuma longa* showed the zone of inhibition 17.41 mm and 19.70 mm.

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Conflict of Interests

There is no conflict of interest.

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