*Indian J. Vet. Med. Vol. 41, No. 1, 2021 pp. 27-33*

# Comparison of occurrence of mastitis in indigenous and crossbred / exotic cows of organised cattle farm in Hisar district, Haryana

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**Abstract**

The present study was aimed to determine and to compare occurrence of mastitis in indigenous and exotic/ crossbred cows in an organized farm of LUVAS, Hisar, India along with determination of therapeutic efficacy of different antibiotics. A total of 117 quarter milk samples each from 30 lactating cows of indigenous dairy herd of Sahiwal and Hariana and 30 lactating crossbred/ exotic cows were collected. The overall occurrence rate based on CMT was 13.33 per cent animal wise and 9.4 per cent quarter wise in indigenous cows while in crossbred cows it was 26.66 per cent animal wise and 17.9 per cent quarter wise. Results of electrical conductivity indicated 20 per cent animals and 19.65 per cent quarters as positive in exotic/crossbred cow while 6.66 per cent animals and 6.83 per cent quarters in indigenous cows. Occurrence on basis of bacterial examination was 23.33 per cent animal wise and 18.8 per cent quarter wise in exotic/crossbred cows while 10 per cent animal wise and 6.83 per cent quarter wise in indigenous cows. The somatic cell count (SCC) above 500,000/ml of milk was recorded as 23.33 per cent animal wise and 21.36 per cent quarter wise in exotic/crossbred cow while 13.33 per cent animal wise and 9.4 per cent quarter wise in indigenous cows. Ten isolates of Staphylococci and eleven isolates of Streptococci and one quarter exhibited mixed infection of Staphylococci and Streptococci in exotic/crossbred cows while a total of three isolates of Staphylococci and five isolates of Streptococci were obtained from indigenous cows. Enrofloxacin was found to be the most sensitive drug for *Staphylococcus* and *Streptococcus* species isolates. Following treatment as per antibiotic sensitivity, all cows recovered.

**Keywords:** Indigenous**,** Mastitis, Prevalence, Antibiogram, Exotic

Bovine mastitis is a disease having multifaceted etio-pathogenesis and has remained one of the major constraints in growth of dairy industry in India and abroad (Constable *et al*., 2017). In India, annual losses due to mastitis in cows have been reported in crores (Jingar *et al.*, 2017). Milk yield in various states of India is very less due to low level of hygiene of dairies and milk handlers (Sharma *et al.*, 2017). The major challenge to dairy industry in India is the drastic increase in incidence of sub-clinical and clinical mastitis. Clinical mastitis is an individual animal health problem resulting in alterations of milk composition and appearance, which can be easily, detected with the help of cardinal signs of inflammation and abnormalities in milk, whereas sub-clinical mastitis is a herd problem and it may go unnoticed because no gross signs of inflammation or alterations in milk composition are readily apparent (Sinha *et al.*, 2014).

Mastitis is caused by a wide spectrum of pathogens and epidemiologically categorized in to contagious and environmental mastitis. Contagious pathogens may include: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma spp*. and *Corynebacterium bovis*. Environmental pathogens include *E. coli*, *Klebsiella spp*., *S. dysgalactiae* and *S. uberis* (Constable *et al.*, 2017). For the diagnosis of mastitis, various methods based on physical and chemical changes of milk are used (Hasan *et al.,* 2018). Therefore, dairy animals are required to be examined at regular intervals for early detection and treatment of clinical and sub-clinical mastitis in order to prevent such economic losses. After early diagnosis, the treatment of different forms of mastitis is generally attempted by administering antibiotics/antibacterial agents *via* intra-mammary and systemic routes and further losses can be prevented to the maximum extent. Indigenous cattle are reported to be more resistant to various diseases as compared to exotic and crossbred (Prajapati *et al.* 2017). But there is paucity of literature available regarding the status of mastitis in indigenous cattle in India, especially Haryana. Keeping these facts in view, the present study was planned with

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the objectives to determine prevalence and to compare mastitis in indigenous and exotic/crossbred cows in an organized farm located in LUVAS, Hisar, Haryana and to determine therapeutic efficacy of different antibiotics in treatment of mastitis.

## Materials and Methods

### *Source of milk samples and collection method*

A total of 117 quarters of cow milk samples from 30 indigenous cows and a total of 117 quarters of milk samples from 30 crossbred/exotic cows of organised farm, LUVAS Hisar were collected. All the samples of present study were examined in the College Central Laboratory (CCL), LUVAS, Hisar. The udders of animals were cleaned properly with diluted Potassium permanganate and allowed to dry. Then hands of sample collector were washed with soap and water and scrubbed with swab soaked in 70 percent ethanol. The first squirt of milk was discarded separately and the teat apices were thoroughly sanitized with swabs containing 70 percent ethanol. Each test tube was given the label number possessed by the animal and were also marked as right fore (RF), right hind (RH), left fore (LF) and left hind (LH) as marking of individual quarters. Sample collection was done first from near side and then from off side to avoid contamination of teat apices. Approximately 40 ml of milk from respective quarters each was collected aseptically into sterile milk samples tubes and transported to Veterinary College Central Laboratory (CCL), COVS, LUVAS, Hisar on ice packs and kept at 40°C until bacteriological examination was performed.

### *California Mastitis Test (CMT)*

CMT was performed using the modified reagent. To prepare CMT reagent, 3g of sodium lauryl sulfate was added to 100 ml distilled water and it was mixed thoroughly. The solution was heated at 500C in a water bath to make it clear. Then bromocresol purple was added to the solution to make the final concentration as 1:10000 and reagent pH was adjusted to 8. Plastic paddle having four shallow cups marked A, B, C and D was used for testing of RF, RH, LF and LH quarter milk samples, respectively. Equal amounts (2 ml) of milk and CMT reagent were mixed in the cup of paddle by gently rotating the paddle in circular motion in horizontal plane.

### *Electrical Conductivity of Milk*

Milk Checker (Eisai Co. Ltd., Tokyo) device available in CCL, LUVAS Hisar was used for assessing electric conductivity of milk. Electrical conductivity was determined by taking the milk in the cup of the mastitis detector up to the marked brim. After pressing the button on the detector, the reading appeared on the screen in mS/cm units was recorded. The milk samples having comparative electrical conductivity above 5 mS/cm were suspected for mastitis.

### *Somatic cell count (SCC)*

The somatic cells in milk were counted using Newman-Lampert stain. The milk sample was mixed thoroughly so as to obtain uniform distribution of cells. The sample was allowed to stand for two to five minutes to permit air bubbles and foam to disappear. A clean grease free slide was placed on a level area over a template to outline four 1.0 cm x 1.0 cm area. With a four mm diameter platinum loop, 0.01 ml of milk was spread evenly over the first template on the left side of the slide. This procedure was repeated with sample from each quarter. Slides were air dried and subjected to staining.

Slides were immersed for 30 seconds in Newman-Lampert stain. Excess stain was drained off and slides were air dried. The slides were then rinsed thrice in tap water, drained and rapidly air dried after gently blotting with a filter paper. Somatic cells were stained clearly with deep blue background.

The stained slides were examined under oil immersion objective and the cells in 25 fields were counted. A binocular microscope was used with 10X oculars and 1.8 mm oil immersion objective. The diameter of the field was measured with the help of a stage micrometer.

Diameter of microscopic field = 0.16 mm or 0.016 cm

Area of the field = $r^2$= 3.14 x $(0.008)^2$

= 0.0002 sq.cm

Since 0.01 ml of milk was spread in 1.0 $cm^2$, the possible number of fields counted in 1 sq. cm is 5000.

Milk volume represented by each field = 1/5000 x 1/100

= 1/500000 ml.

Hence, microscopic factor (MF) = 5,00,000

Working factor (WF) = No. of fields counted/ 20000 = 500000/25

No. of cells per ml = Total no. of cells counted in 25 fields x WF (20000)

Milk sample containing more than 5 lac cells per ml was considered positive.

*Bacteriological Examination*

For bacteriological examination, milk samples streaked on five percent sheep blood agar plates and MacConkey's lactose agar (MLA) plates separately with the help of 4.0 mm diameter sterile platinum loop. The plates were incubated aerobically at 37°C and examined after 24 hours. The resulting growth from respective plate of media was purified and provisionally identified on the basis of colony characteristics, morphology, Gram's reaction and hemolysis patterns.

*In-vitro drug sensitivity pattern*

Different strains of various organisms isolated from udder infections were subjected to *in-vitro* drug sensitivity testing using 12 antimicrobials by disc-diffusion method. The sensitivity was observed on the basis of zone size interpretation chart, provided by the manufacturer. The results were recorded as sensitive, intermediate and resistant.

*Treatment of the animals*

Animals which were found culturally positive were given antimicrobial treatment by systemic route as per the antibiotic sensitivity test report (s). After treatment, the milk samples from affected animals were collected 72-96 hours post treatment. Milk samples from all farms which were found culturally positive were collected aseptically after 72-96 hours post treatment. Milk samples were analyzed by different conventional tests viz. California mastitis test (CMT), Electrical conductivity test by Milk Checker (Eisai Co. Ltd., Tokyo) device available in CCL, LUVAS and the somatic cells in milk. Then, milk samples were subjected for cultural examination and subjected to antimicrobial sensitivity test.

## Results and Discussion

*Occurrence of mastitis in indigenous cows*

A total of 117-quarter milk samples from 30 lactating cows of indigenous dairy herd of Sahiwal and Hariana, at Cattle farm, LUVAS Hisar were screened for mastitis. Results of California mastitis test (CMT), Electrical conductivity test (ECT), somatic cell count (SCC), cultural examination (CE) are presented in Table 1, Table 2, Table 3 and Fig. 1. The overall occurrence rate based on CMT was 13.33 per cent animal wise and 9.4 per cent quarter wise. The somatic cell count (SCC) above 500,000/ml of milk was recorded as 13.33 per cent animal wise and 9.4 per cent quarter wise. Results of electrical conductivity indicated 6.66 per cent animals and 6.83 per cent quarter wise as positive. Occurrence on basis of bacterial examination was 10 per cent animal wise and 6.83 per cent quarter wise

*Occurrence of mastitis in crossbred/ exotic cows*

A total of 117-quarter milk samples from 30 lactating crossbred/ exotic cows at LUVAS farm, Hisar were screened for mastitis. Results of California mastitis test (CMT), Electrical conductivity test (ECT), somatic cell count (SCC), cultural examination (CE) are presented in Table 1, Table 2, Table 3 and Fig. 1. The overall occurrence rate based on CMT was 26.66 per cent animal wise and 17.9 per cent quarter wise. Results of electrical

**Table 1: Occurrence of mastitis in indigenous and crossbred/ exotic cows of LUVAS farm, Hisar, Haryana**

| | Indigenous cows (30) of LUVAS | Crossbred/ Exotic cows (30) of LUVAS |
|---|---|---|
| Total quarters | 120 | 120 |
| Quarters examined | 117 | 117 |
| Quarters CMT.(+ ve) | 11 (9.4 per cent ) | 21 (17.9 per cent ) |
| Quarters Cult.(+ ve) | 8 (6.83 per cent ) | 22 (18.8 per cent ) |

**Table 2: Comparison of Occurrence of mastitis in Indigenous cows and Crossbred/ Exotic cows of LUVAS farm, Hisar, Haryana, Animal wise**

| No of animals/ Quarters | Animals culturally positive | Animals showing SCC > 5 lac/ml | Animal Electrical conductivity positive | Animal CMT positive |
|---|---|---|---|---|
| Indigenous cows | 3 (10.0%) | 4 (13.33%) | 2 (6.66) | 4 (13.33%) |
| Crossbred/ exoticcows | 7 (23.33%) | 7 (23.33%) | 6 (20 %) | 8 (26.66%) |
| Chi square test value | 1.92 | 1.00 | 2.31 | 1.67 |

**Table 3: Comparison of Occurrence of mastitis in indigenous cows and Crossbred/ Exotic cows of LUVAS farm, Hisar, Haryana, Quarter wise**

| No of animals/ Quarters | Quarter culturally positive | Quarter showing electric conduc-tivity>5 | Quarter showing CMT positive | Quarter showing SCC > 5 lac/ml | Quarters showing | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | SCC > 5 lac/ml | SCC > 5 lac/ml and culturally positive | SCC < 5 lac/ml and culturally positive | SCC > 5 lac/ml and culturally negative |
| Indigenous cows | 8 (6.83) | 8 (6.83%) | 11 (9.4%) | 11 (9.4%) | 11 (9.4%) | 7 (5.98%) | 1 (0.85%) | 2 (1.70%) |
| Cross/ exotic cows | 22 (18.8%) | 23 (19.65%) | 21 (17.9) | 25 (21.36%) | 25 (21.36%) | 22 (18.8%) | 0 (0%) | 2 (1.70%) |
| Chi square test value | 7.49** | 8.37** | 3.62 | 6.43* | | | | |

*Significant at 5% level; ** Significant at 1% level.

conductivity indicated 20 per cent animals and 19.65 per cent quarter wise as positive. Occurrence on basis of bacterial examination was 23.33 per cent animal wise and 18.8 per cent quarter wise. The somatic cell count (SCC) above 500,000/ml of milk was recorded as 23.33 per cent animal wise and 21.36 per cent quarter wise.

*In-vitro antimicrobial sensitivity testing*

A total of three isolates of Staphylococci and five isolates of Streptococci were obtained from 117 milk quarters of Indigenous cattle of LUVAS Farm, Hisar. Ten isolates of Staphylococci and eleven isolates of Streptococci were obtained from 117 milk quarters of exotic /cross bred cows of LUVAS Farm, Hisar. One quarter exhibited mixed infection of staphylococci and streptococci (figure 2).

Results of *in-vitro* antimicrobial sensitivity of all bacterial isolates are shown in Table 4. Enrofloxacin was found to be the most sensitive drug followed by Moxifloxacin for *Staphylococcus* spp. and *Streptococcus* spp in Indigenous as well as Exotic/Crossbred Cattle.

All culturally positive animals were treated with Ceftrioxone @ 10mg per kg body weight and Enrofloxacin @ 5-10 mg/kg b.wt. as per the antibiotic sensitivity test results. After treatment, the milk samples from affected animals were collected 72-96 hours post treatment and were analyzed by different conventional tests viz. CMT, Electrical conductivity test and the somatic cells count. Then, Milk samples were subjected for cultural examination and subjected to antimicrobial sensitivity test. Results of post treatment are shown in Table 5. It was found that all the cows recovered and none was found culturally positive and SCC was within range.

In present study, a total of 117 quarter milk samples each from 30 lactating cows of indigenous and crossbred/exotic dairy herd of Cattle were screened for mastitis. Routine cow side tests and laboratory

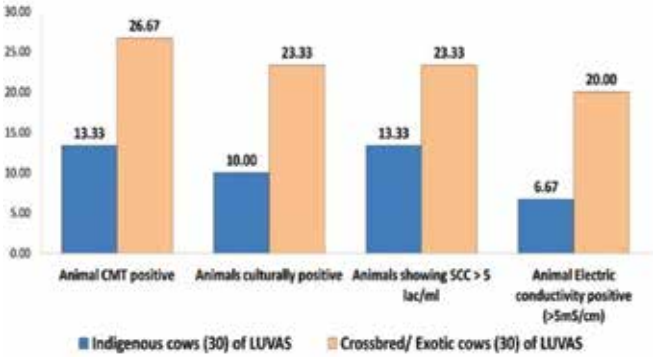


Fig. 1: Comparison of occurrence of mastitis in indigenous and crossbred/ exotic cows of LUVAS farm, Hisar, Haryana

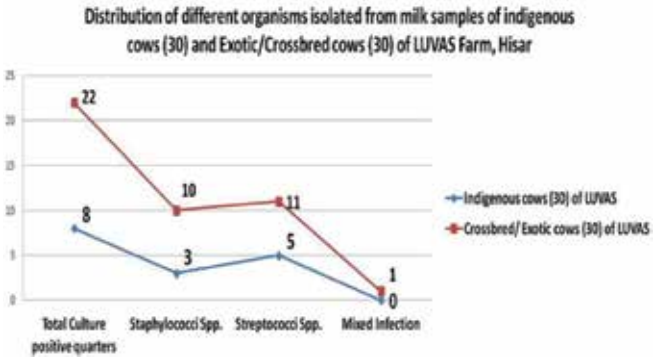


Fig. 2: Distribution of different organisms isolated from milk samples of indigenous and exotic/ crossbred cows of LUVAS Farm hisar

**Table 4: Antibiotic sensitivity (%) among Indigenous Cattle and Exotic/Crossbred Cattle of LUVAS Farm, Hisar**

| Antimicrobial drug | Indigenous Cattle | | Exotic/Crossbred Cattle | |
|---|---|---|---|---|
| | ***Staphylococcus* Spp. (03)** | ***Streptococcus* Spp. (05)** | ***Staphylococcus* Spp. (11)** | ***Streptococcus* Spp. (12)** |
| Ceftriaxone | 0 | 20 | 100 | 100 |
| Chloramphenicol | 100 | 20 | 18.18 | 58.33 |
| Gentamicin | 100 | 0 | 72.72 | 66.67 |
| Cefoperazone | 0 | 20 | 100 | 100 |
| Amoxicillin | 0 | 0 | 9.09 | 83.33 |
| Enrofloxacin | 100 | 100 | 81.81 | 100 |
| Cefpodoxime | 100 | 100 | 18.18 | 66.61 |
| Moxifloxacin | 100 | 100 | 72.72 | 100 |
| Levofloxacin | 100 | 0 | 100 | 73 |
| Kanamycin | 0 | 0 | 0 | 25 |
| Neomycin | 0 | 0 | 27.27 | 41.66 |
| Amikacin | 100 | 0 | 9.09 | 25 |

tests (California mastitis test, Electrical conductivity test, somatic cell count and cultural examination) were conducted.

For further validation, laboratory tests viz. Somatic cell count and cultural examination for diagnosis of mastitis was performed animal wise and quarter wise and it was found that quarter wise occurrence was almost two times in crossbred/exotic cows than in Indigenous cows and significant difference was observed statistically. Almost similar rate of occurrence has been reported by Joshi *et al.* (2013), Birhanu *et al.* (2017), Sarba and Tola (2017), Bhatt *et al.* (2017), Amer *et al.* (2018) but differs from findings of Abbe *et al.* (2016) and Kabir *et al.* (2017). The differences in the occurrence rates of mastitis as reported by different workers are perhaps, due to different managemental and hygienic practices, adopted in different dairy herds. The lower rate of prevalence as observed in present investigation, might be attributed to proper intensive management, hygiene, sanitation and control measures adopted by professionally trained veterinarians and paravet staff in both farms.

On cultural examination of milk samples, Staphylococci and Streptococci were found to be most prevalent organisms in milk samples from both Indigenous as well as exotic/crossbred cows. The mixed infection of both Staphylococci and Streptococci was observed in higher percentage in crossbred cows than indigenous cows of LUVAS farms. *Staphylococcus* spp. was the most prevalent among isolates. The findings of current study in reference to cultural isolates was in confirmation to the findings of Sindhu *et al.* (2010), Harini and Sumathi (2011) and Mittal *et al.* (2018) whereas it differs with the findings of Birhanu *et al.* (2017).

On Antimicrobial sensitivity testing of cultural isolates, Enrofloxacin drug was found to be most sensitive followed by Moxifloxacin whereas Kanamycin and Neomycin were least sensitive. The study complies with the findings of Mohanty *et al.* (2013) and Bhat *et al.* (2017).

**Table 5: Occurrence of mastitis in indigenous cows and cross/ exotic cows of cattle farm, LUVAS animal wise post treatment**

| No of animals/ Quarters | Animals culturally positive | Animals showing SCC > 5 lac/ml | Animal Electrical conductivity positive | Animal CMT positive |
|---|---|---|---|---|
| Indigenous and Crossbred/ exoticcows | Nil | Nil | Nil | Nil |

All culturally positive animals in the study were treated with Ceftrioxone @ 10mg per kg body weight and Enrofloxacin @ 5-10 mg/kg b.wt. as per the antibiotic sensitivity test results and revaluated 72-96 hours post treatment. In current study, all the quarters of indigenous and crossbred/exotic cows recovered fully with negative results in CMT, electrical conductivity, somatic cell count and cultural examination. The findings of the current study are in accordance with the studies reported in different countries across the globe (Moges *et al.,* 2012).

Further, it is recommended that the traits responsible for higher resistance in indigenous animals in comparison to exotic/cross bred may be identified so that they can be genetically explored for breed development which are resistant to various diseases including mastitis. It is also strongly recommended that framing of any breeding policy contrary to natural selection of breeds should be avoided and due preservation of indigenous breeds both *in situ* and *ex situ* may be ensured to conserve precious indigenous germplasm.

## Conclusions

Present study inferred that mastitis was more prevalent in cross bred/exotic cows than Indigenous cows. Staphylococci and Streptococci were found to be more prevalent isolates along with ceftriaxone and enrofloxacin as most sensitive drug based on antibiogram.

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*Received : 12.02.2021*
*Accepted : 10.06.2021*