

# ***Staphylococcus aureus* in mastitic crossbreed cows and its associated risk factors in Addis Ababa City, Ethiopia**

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## **Abstract**

A cross sectional study was conducted in dairy farms of Addis Ababa city from November 2011 to April 2012 to estimate the prevalence of *Staphylococcus aureus* and its associated risk factors in mastitic dairy cows. Milk samples were collected from 153 (50 clinical and 103 subclinical) mastitic lactating crossbred cows and were subjected to laboratory examination for identification of *Staphylococcus aureus*. Of the 153 mastitic cows, 67 were Zebu-Holstein Frisian and 86 were Zebu-Jersey crossbred. Bacterial identification was based on colony morphology, gram staining characteristics, and enzymatic tests including catalase and coagulase. *Staphylococcus aureus* was identified in 74 (48.4%) milk samples. It was higher (n= 63, 61.1%) in subclinical cases as compared to clinical form of the disease (n= 11, 22%). The occurrence of bovine mastitis associated with *Staphylococcus aureus* varied significantly (p<0.05) between breed, lactation stage, parity and age. It was higher (n= 49, 56.9%) in Zebu-Jersey than Zebu-Holstein Frisian (n= 25, 37.3%) crossbred cows. *Staphylococcal mastitis* is a major health problem in dairy farm of Addis Ababa causing huge economic loss. Furthermore, *Staphylococcus aureus* can leads to public health hazard through consumption of raw milk or milk products from infected animals. Therefore, early diagnosis and screening of cows for mastitis is important to limit spread of the disease. Appropriate control and prevention measures should be in place to reduce *Staphylococcus aureus* mediated bovine mastitis. Routine pasteurization of milk should also be practiced to safeguard the public health.

**Keywords:** Addis Ababa, Crossbred Cows, Prevalence, Mastitis, *Staphylococcus aureus*

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## **Introduction**

Ethiopia holds large potential for dairy development due to its large cattle population and favorable climate for improved high yielding breeds. Cows represent the largest population of cattle of the country. However, the annual

consumption of milk in Ethiopian is low as compared to the average milk consumption of developed and developing countries. Local milk production does not satisfy the country's milk requirement due to low input husbandry practice and wide spread livestock diseases (Mohammed Ahmed *et al.*, 2004).

Mastitis, particularly the subclinical form, is a major bottle neck of the dairy sector (Demelash Biffa *et al.*, 2000). Likewise, there are reports from Ethiopia that indicated substantial economic loss in highland crossbred dairy cows due to subclinical mastitis. *Staphylococcus aureus* is the predominant cause of clinical and subclinical mastitis. Once established, a *Staphylococcus aureus* infection is difficult to cure (Roberson *et al.*, 1998).

In Ethiopia, currently there is a national interest to improve the dairy sector to enhance the benefits that would be gained from this sector. In line with this, animal disease researches that are directly related to dairy production help as an input for any intervention. But mastitis is insufficiently investigated and information related to its magnitude, distribution, and risk factors are scant in the country. This study was aimed at estimation of the prevalence of an important pathogen, *S. aureus* in mastitic crossbred cows and identification of its associated risk factors.

## **Materials and methods**

### **Study Area**

The study was conducted in small holder dairy farms of Addis Ababa city. Addis Ababa, the capital city of Ethiopia, is situated at a latitude of 9°3' North and 38°43' East and an altitude of 2408 meters above sea level. The estimated cattle population of the city is 58,568 of which the vast majority is cows (CSA, 2009). There are 5,167 small, medium, and large dairy farms in the city comprising 47% crossbreds and 53% local breeds (Azage Tegegn and Alemu G/Wold, 1998).

### **Study animals**

The study was conducted on lactating crossbred dairy cattle with 25-50% Holstein Friesian and Jersey breed blood level. The cows were randomly selected from small, medium and large dairy farms kept under intensive management system.

## Study design

A cross sectional study was undertaken from November 2011 to April 2012 on dairy farms in Addis Ababa to estimate the prevalence of *Staphylococcus aureus* and its associated risk factors in mastitic crossbred cows. By using single population proportion formula (Thrusfield, 2005) and 52.27% previous prevalence (Fufa Abunna *et al.*, 2013) in the study area, 400 crossbred cows was screened for mastitis.

## Ethical considerations

The study protocol was reviewed and approved by Institutional Review Board of Faculty of Veterinary Medicine, University of Gondar. Written informed consents were obtained from each participant after informing about the purpose of the study, the procedure, the risk, benefit and their right. All the information obtained from the study participants was kept confidential.

## Data collection

Each selected lactating cow was screened for mastitis based on clinical examinations and California Mastitis Test (CMT). Mastitic cows were subjected to bacteriological examination to identify *Staphylococcus aureus*. Bacterial isolation and identification was carried out based on standard bacteriological techniques previously established (Quinn *et al.*, 2004). In addition, data on potential risk factors including breed, age, parity, lactation stage, and previous history of mastitis treatment were collected from farm record and from interview of farm owners whenever farm records were not complete. The hygiene of the house was observed during the farm visit.

## Clinical examination and California mastitis test

Clinical examination of the udder was based on the method previously indicated (Radostitis *et al.*, 2007). The clinical findings considered include abnormalities of the secretion, abnormalities of the udder and teat, and systemic reaction. The California Mastitis Test was performed according to previously established method (Quinn *et al.*, 2004). In summary: about 2ml of milk from each quarter was placed in each of the four shallow cups of the CMT paddle. An equal amount of CMT reagent was added to each cup. A gentle circular motion to mix at a horizontal plane was applied for 15 seconds. The result was recorded as positive when gel formation is observed in at least one quarter.

## **Milk sample collection**

Milk samples were collected according to earlier protocol (Quinn *et al.*, 2004). Briefly: quarters were washed with tap water and dried. The teat ends were then cleaned with cotton soaked with 70% ethyl alcohol and 10-15ml milk was collected aseptically into a sterile screw-capped test tube after discarding the first three streams of milk. Finally, milk samples were transported with ice box to Shola Veterinary Laboratory Center, Addis Ababa, for bacteriological investigation.

## **Bacteriological examination of milk samples**

Bacteriological examination of milk sample was done according to the National Mastitis Council (NMC) guideline (NMC, 1990). In short: milk samples from clinical mastitic and CMT positive cows were cultured on the plate count agar (Oxoid, England). The plate was examined for growth, colony morphology features such as golden yellow color, and  $\beta$ -hemolysis in blood agar within 24- 48 hours. Gram staining characteristics of the pure colonies was recorded. Suspected colonies of *S. aureus* were selected and subcultured on MacConkey (Oxoid, England) and mannitol salt agars (Oxoid, England) and incubated aerobically at 37°C for 24-48 hours. Finally, presumably *Staphylococcus aureus* colonies were subjected to catalase, and slide and tube coagulase tests (Quinn *et al.*, 2004).

## **Data collection and analysis**

The collected data about the risk factors including breed, age, parity, lactation stage, housing and previous history of mastitis treatment and the laboratory results were coded and entered into Microsoft excel spread sheet. Statistical analysis was performed using SPSS version 20. The prevalence was calculated by dividing the number of positive animals for *S. aureus* to the total number of mastitic animals examined times 100%. Chi- square was used to test the existence of association between *S. aureus* mastitis positivity and the risk factors. In all the cases, 95% confidence level and  $p < 0.05$  was used to determine statistical significance.

## **Results**

A total of 153 lactating crossbred cows with either clinical or subclinical (CMT positive) mastitis were examined for the involvement of *Staphylococcus au-*

*reus. Staphylococcus aureus* was isolated from 37.3% (N=25) and 56.9% (N=49) of the clinical and sub-clinical cases, respectively. The overall prevalence of *Staphylococcus aureus* was 48.3% (N=74) as indicated in table 1.

**Table 1: Prevalence of *Staphylococcus aureus* in clinical and subclinical mastitis**

Form of mastitis	No of cows examined	No of <i>S. aureus</i> isolated	Prevalence
Clinical	50	25	37.3%
Subclinical	103	49	56.9%
Total	153	74	48.3%

In the present study, 67 of the examined cows were Zebu-Holstein Frisian crossbred and the remaining 86 were Zebu-Jersey crossbred kept under small dairy farms. The prevalence of mastitis associated with *Staphylococcus aureus* was found statistically significantly higher in Zebu-Jersey crossbred than the Zebu-Holestein Frisian crossbred ( $p < 0.05$ ). The involvement of *Staphylococcus aureus* in mastitic cows was increasing as the age and number of parity increases. Statistically significant ( $p < 0.05$ ) prevalence of *Staphylococcus aureus* was also observed in mastitic cows at late stage of lactation than cows in early and mid-stage of lactation. However, housing and previous history of mastitis treatment were not statistically significantly associated with *Staphylococcus aureus* involving mastitis ( $p > 0.05$ ) as tabulated in table 2.

**Table 2: Risk factors associated with the occurrence of *Staphylococcus aureus* in mastitic cows**

Risk factor		Total animals examined	<i>S. aureus</i> positives No (%)	$X^2$	p-value
Breed	Zebu-Holstein	67	25(37.3)	8.214	0.018
	Frisian				
	Zebu-Jersey	86	49(56.9)		
Age (years)	3-5	43	20(46.5)	7.284	0.026
	6-9	65	25(38.4)		
	>9	45	29(64.4)		
Parity	1-2	27	4(14.8)	19.12	0.001
	3-4	73	34(46.5)		
	>4	53	36(67.9)		
Lactation stage	<3	23	3(13.0)	23.52	0.001
	3-6	73	30(41.0)		
	>9	57	41(55.4)		
Housing	Muddy	82	42(51.2)	0.338	0.561
	Concrete	71	32(45.0)		
History of mastitis treatment	No	68	36(52.9)	4.291	0.068
	Yes	85	38(44.7)		

## Discussion

The current study revealed 48.3% overall prevalence of *Staphylococcus aureus* in the mastitic crossbred lactating cows in the study area which is in agreement with 47% prevalence reported from dairy farms of Holeta town, central Ethiopia (Birhanu Mekibib *et al.*, 2010). However; other studies conducted in different parts of the country at different times indicated lower prevalence than the current study (Girma Sisay *et al.*, 2012; Mekonnen Hailemariam *et al.*, 2005; Teshale Sori *et al.*, 2011). On contrary, higher prevalence than the present result was reported in recent times from Ethiopia (Getaneh Alemu, 2010; Lidet G/Michael *et al.*, 2013). The variability in the occurrence of *Staphylococcus aureus* in mastitic cows among different reports may be attributed to differences in farm management practices and environmental inconsistency.

In this study, *Staphylococcus aureus* was more frequently identified in sub-clinical mastitis than the clinical cases. This is almost similar with previous studies that proved *Staphylococcus aureus* is the principal causative agent of subclinical mastitis (Radostitis et al., 2007; Andrew et al., 2004).

In general the incidence of mastitis varies from breed to breed. Previous research findings indicated that mastitis was greater in Holstein-Friesian than in Jersey breeds (Radostitis et al., 2007; Compton et al., 2007). As opposed to this, our study showed higher prevalence of *Staphylococcus aureus* in Zebu-Jersey crossbreed than the Zebu-Holstein Friesian crossbreed. This may be ascribed to variation in the level of exotic blood in the two groups and differences in the management practices.

Our investigation revealed that the prevalence of *Staphylococcus aureus* associated mastitis significantly varied by stage of lactation in which it was highest in late stage of lactation followed by mid and early stages of lactations in that order. This result was found to be consistent with the research findings of previous researches in Adama and Hawassa towns, Ethiopia (Mesele Abera et al., 2010 and Nibret Moges et al., 2012). However; other reports from Southern part of Ethiopia indicated higher prevalence in early stage of lactation which disagrees with our study result (Demelash Biffa et al., 2000; Oudessa Kerro and Fisseha Tereke, 2003).

In the current study, the prevalence of *Staphylococcus aureus* associated mastitis was found to be increasing as the age and parity of the cows increases. This finding agreed with different studies in different times and places in Ethiopia (Demelash Biffa et al., 2000; Oudessa Kerro and Fisseha Tereke, 2003; Busato et al., 2000; Rahman et al., 2009) that reported the risk of *Staphylococcus aureus* associated clinical and subclinical mastitis increases significantly with advancing age and parity of the cow. These two variables are closely related since whenever the age of the cow increases, the number of parity also increases. The increasing prevalence of mastitis along with age and parity could be partially explained by the possibility of exposure to the infectious agents with increasing age and parity. Besides preparturient cows have more effective defense mechanism than multiparous cows (Erskine et al., 2002).

The present investigation indicated that the prevalence of *Staphylococcus aureus* is not associated with housing conditions. This finding was in disagreement with the report from Adama, Ethiopia, (Mesele Abera et al., 2010) that reported the prevalence of *Staphylococcus aureus* mastitis in houses with mud-

dy floor was higher compared to concrete floor houses. This variation may be due to differences in management since *Staphylococcus aureus* is a contagious pathogen that can be transmitted from cow to cow easily during unhygienic milking procedures including using contaminated milking utensils and milkers' hands, through flies, and cross-suckling (Radostitis *et al.*, 2007).

## Conclusion

The prevalence of *Staphylococcus aureus* in mastitic cows was found to be significant. Zebu-Jersey crossbreed, increasing age, increased number of parity, and late stages of lactation were associated factors with the occurrence of *Staphylococcus aureus* implicated bovine mastitis in the present research. Isolation of this organism in milk indicates a potential risk for public health hazard. Therefore, regular checking of udder health and milk quality should be in place for early detection and prompt action should be planned and implemented to control staphylococcal mastitis in dairy cows. Awareness creation should be practiced about the importance of pasteurization in order to protect the community from *Staphylococcus aureus* infection. Further in-depth and wider antimicrobial susceptibility profile and molecular characteristic study is recommended at the national level.

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