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Ramya P
Assistant Professor, Dept. of Veterinary Public Health & Epidemiology, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Rajeshkumar S
Dept. of Veterinary Public Health & Epidemiology, College of Veterinary Science, Rajendranagar, Hyderabad, India

Sujatha Singh
Assistant Professor, Dept. of Veterinary Public Health & Epidemiology, College of Veterinary Science, Rajendranagar, Hyderabad, India

Venkateswara Rao L
Professor and Head, Dept. of Veterinary Public Health & Epidemiology, College of Veterinary Science, Proddatur, Hyderabad, India

Correspondence
Ramya P
Assistant Professor, Dept. of Veterinary Public Health & Epidemiology, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Prevalence of *Staphylococcus aureus* in raw milk samples

Ramya P, Rajeshkumar S, Sujatha Singh and Venkateswara Rao L

Abstract
Mastitis, inflammation of the mammary gland, is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the worlds dairy industry. Staphylococcal mastitis is the most commonest and economically the greatest concern wherever dairy farming is practiced. Keeping in view of the public health significance of these food borne organism the present study was carried out by selecting 50 lactating cows from different small holder dairy farms to detect the presence of clinical and subclinical mastitis. Clinical inspection of the udder was carried by routine procedure. California mastitis test was carried out to diagnose the presence of subclinical mastitis. CMT positive milk samples were examined for the presence of *S. aureus*. Out of Fifty lactating cows examined during the study 9 (18%) animals had mastitis of which 2% (1/50) and 16% (8/50) showed clinical and subclinical mastitis respectively. Milk samples from one clinical mastitic animal (2 teats) and 8 CMT positive sub clinical cows (15 teats) showed the presence of *S. aureus.* The findings of this study revealed that *S.aureus* is the most prevalent organism in mastitis milk samples which is known to be the commonest causative for mastitis in animals.

Keywords: prevalence, *Staphylococcus aureus,* raw milk samples, dairy cattle.

Introduction

Mastitis, inflammation of the mammary gland, is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the worlds dairy industry. It is a most prevalent disease and tends to be a costly disease for dairy producers [1]. Mastitis pathogens are broadly classified as contagious and environmental pathogens. *Staphylococcus aureus, Streptococcus agalactiae, and Streptococcus dysgalactiae* are considered as contagious pathogens, which adapt to the environment of mammary gland and can potentially be spread from cow to cow during milking. *Streptococcus uberis, Enterococcus spp., coliforms, Arcanobacterium pyogenes,* and coagulase-negative *staphylococci* (CNS) are considered as environmental pathogens, which are described as opportunistic pathogens of the mammary gland and can potentially be transferred from the contaminated environment to the mammary gland of a cow during milking [2-3].

Staphylococcus aureus is a gram-positive bacterium that colonizes a variety of animal species. *S. aureus* infections in animals are most commonly reported as a cause of mastitis (sub-clinical) in dairy-producing animals (including cattle and goats) and "bumblefoot" in chickens [4]. *Staphylococcus aureus* is the key causative agent for mastitis and is responsible for sub-clinical cases, although it is also responsible for different forms of the disease [5]. Along with mastitis it is one of the common agents in causing foodpoisoning outbreaks in humans [6]. The major reservoirs of *S. aureus* are infected udders, teat canals, and teat lesions, but these bacteria also have been found on teat skin, muzzles, and nostrils. The bacteria are spread to uninfected quarters by teat cup liners, milkers' hands, washcloths, and flies. *Staphylococci* do not persist on healthy teat skin but readily colonize damaged skin and teat lesions. The organisms multiply in infected lesions and result in increased chance of teat canal colonization and subsequent udder infection [7].

Subclinical mastitis is a disease of major economic importance to dairy industry causing increased somatic cell count reduced milk quality and leading to a loss in its production [8&9]. The economic losses caused by mastitis, especially subclinical mastitis, include declining milk production and quality, increased maintenance costs and treatment, and early livestock culling. As shown in [10], the decline in milk production due to mastitis reaches about 15-20% of the total milk production, while according to Taylor and field (2009) [11], milk production

has declined to about 30%. Mortality from infections associated with *S. aureus* bacteremia can range from as low as 2.5%, to as high as 40% [12]. In clinical cases of mastitis, the herds require an immediate attention or it leads to mortality and the subclinical cases may turn to clinical mastitis [13]. For appropriate treatment it is vital to identify the contagious bovine mastitis *Staphylococcus aureus* from milk samples of infected cows and normal healthy cows under suspicious of subclinical mastitis. *Staphylococcus aureus (S. aureus)* mastitis is extremely difficult to control by treatment alone. To date, successful control has been achieved only through the prevention of new infections and culling infected animals [14].

The standard method of diagnosing mastitis caused by *Staph. aureus* is microbiological culturing of milk from the infected quarter [15]. Alternatively, the producer can use the California Mastitis Test (CMT) on cows to determine which quarters may be infected, then selectively culture positive quarters. This is an excellent starting point for identifying positive cows and moving them to a separate group. It is important to identify infections early in order to prevent spread to other animals and increase chances of a successful treatment. Early identification of the prevalence and distribution of causative pathogens is of the most importance to effectively prevent diseases and to guide treatment [16-17]. Keeping inview of the public health significance of mastitis milk the present study was carried out to know the significance of *Staphylococcus aureus* as a cause for subclinical mastitis.

Methodology

California Mastitis Test

50 lactating cows (200 milk samples from each quarter) from different small holder dairy farms were tested to detect the presence of subclinical mastitis. Clinical inspection of the udder was carried by routine procedure. California mastitis test was carried out to diagnose the presence of subclinical mastitis for each quarter. 2ml of milk was collected from each quarter on to each of four shallow cups of CMT paddle. Now 2ml of CMT reagent was added, then gently shaken in horizontal direction for 10-15 seconds. The test result is considered negative if the mixture remains homogeneous, positive one if sedimentation is visible, positive two if the mixture thickens rapidly and the gel moves to the middle, and positive three if a lot of gel forms, causing the surface to turn convex.

Isolation and Identification of *Staphylococcus aureus*

CMT positive milk samples were collected into screw capped sterile containers and transported to the laboratory at 4 ^{0}C in an ice pack for cultural examination. Milk samples were examined for the presence of *S. aureus* by streaking on Nutrient agar, Manitol salt agar, Baird parker agar. Confirmation of the presence of the *S. aureus* was done by Grams staining, catalase test and IMViC tests. To distinguish the *S. aureus* from others Staphylococcus species, the sample was then tested on blood agar media, in order to observe the presence of haemolysis.

Results and Discussion

Out of Fifty lactating cows (200 samples) examined during the study 9 (18%) animals had mastitis of which 16% (8/50) of the animals showed subclinical mastitis and 2% (1/50) of the animals showed clinical mastitis (Table). Milk samples from one clinical mastitic animal (2 teats) and 8 CMT positive sub clinical cows (15 teats) showed the presence of *S.aureus*. Prevalence of *S.aureus* was reported as 8.5%

Table 1: Showing the presence of *S.aureus* in mastitic milk samples

Total number of animals	No. of animals positive for subclinical mastitis	Percentage of animals positive for subclinical mastitis	No. of animals positive for clinical mastitis	Percentage of animals positive for clinical mastitis
50	08	16	01	02

The present study reports are inaccordance with the reports of Jagadeeswari *et al*, (2015) [18], who had reported an incidence of 5.36% of s.aureus from subclinical mastitis milk samples. Higher incidence of s.aureus than the present study was reported by Ahmedy and Kazemy (2013) [19], Turutoglu *et al*., (2005) [20], Zakarg *et al*, (2011) [21], Yanliang *et al*., (2016) [22] as 15.65%, 38.07% in 287 quarter cow milk samples , 40% in bovine milk samples, and 50.1% in mastic milk samples of bulk milk tanks in china respectively. Jagadeeswari *et al*, (2015) [18] (75%) from clinical mastitis samples, and Botaro et. al., (2013) [23] (71.6%) in 43 quarters out of 60 quarters of lactating animals also reported a higher incidence than the present study as 75% and 71.6% respectively.

Among the dairy farmers, milk production is the basic income which gets stumbled when the dairy cattle were found to get mastitis. Broad spectrum antibiotics are always used in the clinical mastitis treatment which paves a way to an emergence of multidrug resistance. *S. aureus* as a contagious mastitis pathogen, an immediate attention is always required both in clinical and subclinical cases. The subclinical mastitis is a dangerous stage of mastitis which cannot be predicted before clinical symptoms (Jagadeeswari et. al., 2015) [18]. *S. aureus* still remains one of the most significant organisms associated with bovine mastitis and the current mastitis control protocols are comprehensive.

Conclusion

The present study reported lower prevalence of *s.aureus*. Presence of *s.aureus* was confirmed by cultural identification, biochemical tests. Mostly *S.aureus* will enter into mammary gland through environmental contamination. Therefore we can reduce its prevalence by following strict hygienic measures before and after milking.

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