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Identification of *Escherichia coli* procured from bovine subclinical mastitis in Brij Region of Uttar Pradesh

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Abstract
Mastitis is defined as an inflammatory reaction of the mammary gland induced when pathogenic microorganisms in the udder produce toxins that are harmful to the mammary gland. Mastitis is primarily caused by bacterial infection and is a major cause of economic loss in dairy cattle production. In the present study a total of 180 quarter milk samples were obtained from 85 cows at different areas of Mathura districts. All the milk samples were tested for subclinical mastitis by California Mastitis Test (CMT), isolation of *E. coli* was confirmed by using selective media (EMB) and biochemical tests (IMViC). Overall prevalence of SCM in the studied area was 18.82% (16/85). Prevalence of *E. coli* in all positive isolates, were 37.5% (6/16). For the prevention and control of mastitis, regular screening of SCM and milking hygiene practices together with awareness of farmers should be implemented.

Keywords: *E. coli.,* Uttar Pradesh, cattle, prevalence, subclinical mastitis

Introduction
Environmental mastitis, a subclinical form of mastitis, is frequently caused by *Escherichia coli (E. coli*) in dairy cattle. Subclinical mastitis, also referred to as SCM is a serious issue that dairy animals face globally. Breeders suffer huge losses as a result, which has an impact on the nation's GDP (Ramachandrainh *et al.,* 1990) [1]. SCM has been described as the most difficult issue in dairy production, causing significant financial harm to the dairy sectors in developed as well as developing nations (Kovacevic *et al.,* 2021) [2]. *Escherichia coli* O157:H7, which produces verocytotoxins, has emerged as a significant food borne pathogen and is currently regarded as a serious public health concern as a result of these outbreaks (McKee *et al.,* 2003) [3] and responsible for producing major public health concern i.e. HC and HUS. Shiga toxins (stx) are secreted by O157:H7, which causes virulence by preventing the host cells from synthesising proteins and ultimately resulting in cell death. It is evidently implied that poor productivity and health issues in dairy animals are more common in economically disadvantaged and developing countries (Antanaitis *et al.,* 2021; Dabele *et al.,* 2021) [4, 5]. This might be caused by, among other things, inadequate dairy infrastructure, bad husbandry techniques, inadequate nutrition, and ignorance (Singh *et al.,* 2021) [6]. Mastitis is an inflammation of the mammary glands that affects the physical, chemica, and bacterial characteristics of the milk and mammary gland tissues of the afflicted animal (Kumari *et al.,* 2019; Bhakat *et al.,* 2020) [7, 8]. Clinical mastitis is easily recognized by its typical symptoms, which include udder enlargement and milk flakes. However, because subclinical mastitis exhibits no symptoms, it is typically overlooked. Milk supply is impacted by both subclinical and clinical mastitis; however, subclinical mastitis is comparatively more common than clinical mastitis (Mdegela *et al.,* 2009) [9]. Clinical and subclinical mastitis were found to reduce milk supply by an average of 50 and 17.5%, respectively (Joshi and Gokhale 2006) [10]. SCM has a negative impact on the health and milk production of dairy animals without visual signs in contrast to clinical mastitis cases where signs and symptoms are more severe and readily apparent to the unaided eye. In SCM cases, there is a huge decrease in milk yield as well as quality of milk (Khan *et al.,* 2022) [11]. Some of the most common pathogenic microorganisms are *Escherichia coli*, *Streptococcus dysgalactiae Staphylococcus aureus and Streptococcus uberis* and *Enterobactor spp.*, (Ibrahim, 2017) [12].

Materials and Methods

A total number of 180 milk samples from 85 dairy cattle were collected during the period from September 2024 to February, 2025 from different villages of brij region of Uttar-Pradesh. All the milk samples were collected in sterilized McCartney bottles directly from cattle teats then taken to the laboratory in ice-cooled and processed within 12 hours of collection. Confirmation of milk samples for SCM was done by CMT methods. CMT was done by CMT Reagent Kit. The OIE methodology for primary *E. coli* isolation was followed when processing the milk samples. 9 ml of Soyabean Casein Digest Medium (Trytone Soya Broth-TSB) was used to directly enrich 1 ml of milk sample and swab sticks of all kinds. The inoculum from MLA plates was selectively streaked on Eoisin Methylene Blue (EMB) Agar and incubated at 370°C for 24 hours for selective plating, while the loopful culture growth from TSB was streaked on Macconkey Lectose Agar (MLA) and incubated at 37°C for 24 hours for differential plating. On EMB agar, colonies have a distinctive metallic sheen. Additionally, the biochemical tests were conducted using the protocol outlined by Barrow and Feltham (1993) [13]. Each isolate's single colony was removed from the nutrient agar, inoculated in 5 mL of Brain Heart Infusion Broth (BHI), and then incubated for 4-6 hours at 37°C until the inoculum's turbidity reached 0.5 McFarland. In addition to IMViC, tests for indole, methyl red, Voges-proskauer, and citrate consumption were conducted.

Results and Discussion

In the present study, a total number of 85 dairy milch cattle were screened for subclinical mastitis, 16 animals found positive for SCM. The overall prevalence of subclinical mastitis was 18.82% (16/85). In India, subclinical mastitis prevalence ranged from 20.73 to 78.55 percent on cow basis and from 11.65 to 56.51% on quarter-basis. According to Cynthia (2005) [14], the prevalence of subclinical mastitis varies between 15 and 75% on a cow basis and 5 and 40% on a quarter basis. Abed *et al.* (2021) [15] found prevalence of subclinical mastitis 44.8% from SCC which is nearly similar to the findings of this study. Jena *et al.* (2015) [16] found 74.55% of prevalence of SCM from SCC which is higher to the finding of this study. Additionally, the estimate found in this study is larger than certain studies (Islam *et al*., 2011; Sarker *et al.*, 2013) [17, 18], although it is consistent with the findings provided by Rahman *et al*. (2010) [19]. In a present study the 06 *E. coli* isolates were found positive, out of 85 cow milk samples. The overall percent prevalence of *E.coli* from milk of dairy cattle was found to be 37.5% (06/16), which is nearby similar to the findings of Rajpoot (2013) [20] i.e. 28.75% and lower to the findings of Behera (2016) i.e., 51.66%. The overall percent of *E.coli* from SCM positive cattle milk was found to be 47.61% (30 out of 63), which is nearby similar to the findings of Ahmadi *et al.* (2020) [21] i.e. 43.25%, higher to the findings of Sheet *et al.* (2023) [22] i.e. 36.3% and lower to the findings of Chowdhury *et al.* (2024) [23] i.e. 50.54%.

Conclusion

In dairy cow subclinical mastitis continues to pose a serious threat to the health of the animals as well as the quality of the milk produced. To lessen its consequences, subclinical mastitis must be identified and treated with routine monitoring and diagnostic techniques. Antibiotic management and early molecular diagnosis are essential for lowering the prevalence of subclinical mastitis. *E. coli* has been found in milk samples from dairy calves with subclinical mastitis in studies, underscoring the necessity of good cleanliness, pasteurization, and milk processing methods to reduce this risk.

Conflict of interest: The authors declares no any conflict of interest

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