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ASSESSING THE IMPACT OF SUBCLINICAL MASTITIS ON DAIRY CATTLE IN BALOCHISTAN

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Abstract

Subclinical mastitis is a common and costly disease affecting dairy cattle. This research aims to examine the impact of subclinical mastitis on dairy cattle production and evaluate methods for controlling and reducing its prevalence. The study will focus on the effects of subclinical mastitis on milk yield and quality, as well as its impact on animal health and welfare. The goal is to provide valuable insights for dairy farmers and industry professionals to improve management practices and reduce the economic losses associated with subclinical mastitis. A total of 144 cattle from 25 different farms were analyzed for subclinical mastitis, with 54.86% (n=79) of the cattle found to have subclinical mastitis. Results revealed a moderate association between the prevalence of subclinical mastitis as determined by the CMT test and the UdderCheck® test. The study also found that the most common bacteria causing subclinical mastitis were Coagulase Negative Staphylococci (CNS), Coliforms, Streptococcus spp. and Staphylococcus aureus (CPS). The results of the antibiotic sensitivity test showed that ciprofloxacin was the most effective in treating various pathogens associated with mastitis. The results of this study highlight the need for further investigation into the impact of subclinical mastitis on dairy cattle, particularly with respect to the various bacteria causing the condition and the effectiveness of different antibiotics in treating it.

Keywords: *Bacteria, California mastitis test, Dairy cattle, Subclinical mastitis, Udder check test*

INTRODUCTION

Subclinical mastitis is a major challenge for the dairy industry and can have a significant impact on animal welfare, milk production and quality, and economic losses for farmers (1). Despite its prevalence and impact, subclinical mastitis is often difficult to diagnose and monitor, as it does not cause obvious clinical signs (2). In order to better understand this condition and develop effective control and prevention strategies, epidemiological studies have been conducted in various regions around the world.

Epidemiological studies on subclinical mastitis can provide valuable information on the prevalence and risk factors for this condition in dairy cattle. For example, some studies have found that the prevalence of subclinical mastitis is higher in larger herds, and that this condition is more likely to occur in dairy cattle that are housed in more crowded and unsanitary conditions (3). Other studies have found that subclinical

mastitis is more likely to occur in the transition period, when the cow's immune system is undergoing changes (4).

In order to accurately assess the prevalence and risk factors for subclinical mastitis, it is important to use appropriate methods for sample collection and analysis. Milk samples collected from each quarter of the mammary gland are typically used to diagnose subclinical mastitis (5). Milk samples can be analyzed using a variety of methods, including bacterial culture, somatic cell count, and polymerase chain reaction (PCR) (6).

The results of epidemiological studies on subclinical mastitis can provide valuable information for dairy farmers, veterinarians, and researchers. For example, studies can provide insights into the most effective mastitis control strategies, such as the use of vaccines, antibiotics, and improved hygiene practices during milking (7). By reducing the incidence of subclinical mastitis, these strategies can help to improve animal welfare and reduce economic losses for dairy farmers.

In addition to providing information on the prevalence and risk factors for subclinical mastitis, epidemiological studies can also provide insights into the economic impact of this condition. For example, some studies have estimated that subclinical mastitis can result in a decrease in milk production of up to 30% (8). Additionally, the use of antibiotics to treat subclinical mastitis can result in increased costs for farmers, and may contribute to the development of antibiotic-resistant bacteria (9).

Epidemiological studies on subclinical mastitis in dairy cattle play a critical role in improving our understanding of this condition and in developing effective control and prevention strategies. By providing valuable information on the prevalence and risk factors for subclinical mastitis, as well as the economic impact of this condition, these studies can help to improve animal welfare and reduce economic losses for dairy farmers. By conducting further studies in this area, researchers can continue to advance our understanding of subclinical mastitis and develop more effective strategies for controlling and preventing this condition.

MATERIALS AND METHODS

AREA OF STUDY

A field survey to determine the prevalence of subclinical mastitis was carried out in the selected districts of north western province of Balochistan. All laboratory investigations were performed at the Molecular Biology & Biotechnology Laboratory of CASVAB, University of Balochistan, Quetta. The study was conducted over a one-year period, starting from June 2021 and concluding in August 2022.

STUDY DESIGN AND POPULATION

In this cross-sectional study, the study animals were randomly selected, and the inclusion criteria were that all apparently healthy lactating dairy cattle were eligible to participate. The study involved 144 dairy cattle from private and household cattle farms in five districts (Quetta, Pishin, Loralai, Sibi and Lasbella) of Balochistan. Of all 144 dairy cattle, Lohani, Bhag Nari, Holstein-Friesian and Kundhi were included, as 23 Lohani, 14 Bhag Nari, 65 Holstein-Friesian and 42 Kundhi cattle were included.

DATA COLLECTION

The data were collected through a questionnaire specifically created for the study. Before its final use, the questionnaire underwent a pre-testing process. It was given to the participating farmers who provided answers related to farm management, animal husbandry practices, and factors that influence the incidence of mastitis, such as host and environmental factors.

MILK COLLECTION

Milk samples were collected from 198 goat udder halves. Prior to sample collection, each teat was thoroughly disinfected using cotton swabs and 70% alcohol, with extra care taken for the teat ends. Color-coded sterile sample tubes, specific to each udder half, were used. After disinfection, a small amount of milk

from each udder half was obtained and placed into the corresponding sample tube, ensuring no contact between the stream of milk and the milker's hands. To minimize the entry of dust, the tubes were kept as horizontal as possible and the sterile rubber stoppers were handled to prevent contamination. Upon collecting samples from each udder half, the goat identification number was marked on each tube with a waterproof marker pen. The tubes were transported in an ice box to the Molecular Biology & Biotechnology Laboratory of CASVAB, University of Balochistan, Quetta, where they were stored at -20°C until cultured on standard bacteriological media.

PHYSICAL EXAMINATION OF MILK SAMPLES

Immediately after collection, the milk samples underwent physical examination to identify any abnormalities in terms of color, odor, consistency, and presence of blood, clots, flakes, or any other noticeable anomalies.

SUB CLINICAL MASTITIS DIAGNOSTIC TESTS

A veterinarian examined the cattle and found no signs of disease, so they were considered healthy. The udders and teats were examined physically to rule out clinical mastitis. The age of the cattle ranged from 3 to 7 years. No diseases were tested for but the presence of subclinical mastitis was tested using California Mastitis Test (CMT) and UdderCheck®. Before collecting milk samples, the udders and teats were visually and physically checked for fibrosis, inflammation, swelling, injury, abscesses, tick bites, and tissue atrophy. The udders and teats were cleaned, dried, and disinfected before milking. The first milk was tested for clinical mastitis, then each teat was subjected to the four tests. Results were interpreted based on quarter variations. Before collecting samples for bacterial examination, the milk was checked for visible issues and screened using the CMT method described by Quinn *et al.* (1999) (10). A squirt of milk from each udder half was placed in cups on the CMT paddle and mixed with an equal amount of 3% CMT reagent. Results were classified into four scores based on the reaction: Negative (no change), Trace (slight positive), 1 (mild positive), 2 (moderate positive), and 3 (highly positive). The scores reflect the degree of gel formation, which is proportional to the severity of infection (11).

BACTERIOLOGICAL TEST

Under aseptic conditions, each milk sample that tested positive with the CMT was separated and placed in labeled, sterile screw-capped bottles. These samples were promptly sent to the laboratory for standard culturing techniques. The milk was cultured on 10% sheep blood agar and MacConkey agar plates. The suspected colonies were identified using morphological, microscopic, and biochemical methods as described by Farooq *et al.* (2008) (12).

ANTIBIOTIC SUSCEPTIBILITY TEST

The antibiotic susceptibility test was conducted using the Kirby-Bauer disc diffusion method (13). This test is based on the presence or absence of an inhibition zone around the discs containing antibiotics. The test and interpretation were done following the guidelines of the National Committee for Clinical and Laboratory Standards (NCCLS, 2001) using Oxide discs impregnated with specific antibiotics. Statistical analysis was performed on the experimental data.

STATISTICAL ANALYSIS

The factors that commonly influence the occurrence of subclinical mastitis were recorded and analyzed using SPSS version 1.0.0.1406 software. The statistical significance of the associations was determined through the chi-square test.

RESULTS

PREVALENCE OF SUBCLINICAL MASTITIS IN CATTLE

All cattle (n=144) from 25 different farms included in the study were analyzed for subclinical mastitis and it was observed that 54.86% (n=79) of the cattle had subclinical mastitis (Table I). Our findings revealed a moderate association (Cramer's V = 0.522) with statistical significance (P = 0.004) between the prevalence of subclinical mastitis as determined by the CMT test and the UdderCheck® test.

Table I. Mastitis prevalence using different tests

Diagnostic test	No. of farms	No. of animals tested	No. of mastitis +ve cattle	% mastitis +ve
UdderCheck®	25	144	80	55.55
CMT	25	144	78	54.16

PREVALENCE OF SUBCLINICAL MASTITIS IN DIFFERENT BREEDS OF CATTLE

Our findings showed that subclinical mastitis prevalence in Lohani, Kundhi, Holstein-Friesian and Bhag Nari cattle, determined using the CMT test, was 56.52%, 52.38%, 53.84% and 57.14% at cattle level, respectively. At udder teat level, the prevalence was 25.00% in Lohani, 30.53% in Kundhi, 34.23 in Holstein-Friesian and 17.85% in Bhag Nari cattle. The results indicated that the breed of cattle had a significant ($p<0.05$) effect on the prevalence of subclinical mastitis at both test levels (Table II).

Table II. Prevalence of subclinical mastitis in different breeds of cattle

Breeds	Cattle level			Udder teats level			Chi-square Value	Level of significance
	Total cattle	Infected cattle	% infected	Total udder teats	Infected udder teats	% infected		
Lohani	23	13	56.52	92	23	25.00		
Kundhi	42	22	52.38	168	51	30.53		
Holstein-Friesian	65	35	53.84	260	89	34.23		
Bhag Nari	14	8	57.14	56	10	17.85		
Total	144	78	54.16	576	173	30.03	10.23	**

**significant at 1% level ($p<0.01$)

BACTERIAL ISOLATES OF SUBCLINICAL MASTITIS INFECTED CATTLET MILK

Of the 200 milk samples collected from udder teats of 78 lactating cattle, 95.5% (n=191) samples were positive for bacteria while 4.5% (09 samples) showed no bacterial growth. The most common bacteria found among the positive samples were: Coagulase Negative *Staphylococci* (CNS) 32.98% (n=63) samples, *Coliforms* 24.60% (n=47) samples, *Streptococcus spp.* 14.65% (n=28) samples, *Staphylococcus aureus* (CPS) 11.51% (n=22) samples, *Micrococcus spp.* 7.85% (n=15) samples, *Pseudomonas aeruginosa* 6.28% (n=12) samples and *Bacillus cereus* 2.09% (n=04) samples. The majority of subclinical cases were caused by CNS, *Coliforms*, *Streptococcus spp*. and *Staphylococcus aureus* (CPS).

Table III. Distribution of bacterial isolates in milk samples from goats with subclinical mastitis

S. no	Bacterial species	No. of samples	% (Frequency)
1	Coagulase Negative *Staphylococci* (CNS)	63	32.98
2	*Coliforms*	47	24.60%
3	*Streptococcus spp.*	28	14.65
4	*Staphylococcus aureus*	22	11.51
5	*Micrococcus spp*	15	7.85
6	*Pseudomonas aeruginosa*	12	6.28
7	*Bacillus cereus*	04	2.09
8	No growth	09	4.50
	Total	200	100

ANTIBIOTIC SENSITIVITY PATTERN OF ISOLATED BACTERIA

The results of the antibiotic sensitivity test on bacterial isolates are shown in Table IV, using six widely utilized antibiotics. The table demonstrates that the average sensitivity of the isolates was highest for ciprofloxacin followed by gentamicin, amoxicillin, ampicillin, erythromycin and penicillin. The study found that gentamicin was the most effective and penicillin the least effective in treating various pathogens associated with mastitis.

Table IV. *In-vitro* antibiotic sensitivity test results of the bacterial isolates

Bacterial isolates	Percentage of sensitivity of isolates against					
	Ciprofloxacin	Gentamicin	Amoxicillin	Ampicillin	Erythromycin	Penicillin
Staphylococcus aureus	93.3	93.3	75	73.3	85	50
Coagulase -Ve *Staphylococci*	93.3	80	73.3	50	85	35
Streptococcus spp.	95	85	75	65	80	40
Coliforms	85	80	60	40	15	10
Pseudomonas aeruginosa	85	75	75	45	10	0
Bacillus cereus	95	90	60	40	15	0
Proteus spp.	85	70	60	40	15	0

DISCUSSION

The results of this study provide valuable insights into the prevalence and impact of subclinical mastitis in dairy cattle. The study found that 54.86% of the 144 cattle included from 25 different farms had subclinical mastitis, which is consistent with previous studies that have reported a high prevalence of subclinical mastitis in dairy cattle populations worldwide (14). A study by Bangar *et al.* (2015) reported a prevalence of subclinical mastitis in dairy cattle ranging from 33.1% to 64.8% across different countries (15). The prevalence of subclinical mastitis observed in this study is higher than the findings of a study conducted in Pakistan (31.6%) (16). However, the prevalence of subclinical mastitis reported in this study is lower compared to a study conducted in Turkey (62.7%) (17).

The study also found a moderate and statistically significant association between the prevalence of subclinical mastitis as determined by the California Mastitis Test (CMT) and the UdderCheck® test (15). This supports previous research that suggests the CMT test is a reliable method for detecting subclinical mastitis in dairy cattle [3]. The moderate association between the prevalence of subclinical mastitis as determined by the CMT test and the UdderCheck® test is a significant finding. The CMT test is widely used to screen for subclinical mastitis, and the results of this study demonstrate its validity in detecting subclinical mastitis. The results also support the use of the UdderCheck® test, a rapid and convenient test, in detecting subclinical mastitis.

The results of this study show that Coagulase Negative *Staphylococci* (CNS), *Coliforms, Streptococcus spp.,* and *Staphylococcus aureus* (CPS) were the most common bacteria found in positive samples collected from the udder teats of lactating cattle. This is in agreement with previous studies (16, 17) that reported CNS as the leading cause of subclinical mastitis in dairy cattle. The findings of this study highlight the

importance of detecting and controlling CNS and other bacterial pathogens that are prevalent in dairy cattle, as they can have a significant impact on animal health and productivity.

The results of the in vitro antibiotic sensitivity test on bacterial isolates showed that ciprofloxacin was the most effective, followed by gentamicin, amoxicillin, ampicillin, erythromycin, and penicillin (18). These findings are in line with previous studies that have reported ciprofloxacin to be an effective treatment for mastitis caused by various pathogens (19). These findings are consistent with previous studies that have reported ciprofloxacin as a highly effective antibiotic for treating mastitis caused by various bacterial pathogens (20, 21). The study also found that gentamicin was the most effective and penicillin the least effective in treating various pathogens associated with mastitis, similar to the findings of previous studies (16, 17). However, it is important to note that the results of *in vitro* sensitivity tests may not always reflect the effectiveness of antibiotics in vivo (22), and that other factors such as the presence of antibiotic-resistant strains and the variability in response among individual cattle should be taken into account when selecting antibiotics for treatment (23).

Moreover, the results of the study contribute to the growing body of literature on the impact of subclinical mastitis on dairy cattle (24). The findings highlight the need for regular screening and effective treatment of subclinical mastitis in dairy cattle populations (25). Further research is needed to fully understand the mechanisms of subclinical mastitis and to develop more effective control programs to reduce its impact on dairy cattle health and productivity (26).

To conclude, the findings of this study highlight the importance of implementing effective measures to detect and control subclinical mastitis in dairy cattle. The high prevalence of subclinical mastitis observed in this study, along with the significant association between the CMT test and the UdderCheck® test, underscores the need for regular screening and early detection of subclinical mastitis in dairy cattle. The results of the antibiotic sensitivity test suggest that ciprofloxacin and gentamicin may be effective in treating various bacterial pathogens associated with mastitis. Further research is needed to validate these findings and to determine the long-term impact of subclinical mastitis on dairy cattle health and productivity.

CONCLUSION

The conclusion of the study can be summarized as follows:

1. The study found that subclinical mastitis was prevalent in 54.86% (79 out of 144) of the cattle examined.
2. A moderate association was observed between the prevalence of subclinical mastitis as determined by the CMT test and the UdderCheck® test, with statistical significance (P = 0.004).
3. The study revealed that the most common bacteria causing subclinical mastitis were Coagulase Negative *Staphylococci* (CNS), *Coliforms, Streptococcus spp.* and *Staphylococcus aureus* (CPS).
4. The results of the antibiotic sensitivity test demonstrated that ciprofloxacin was the most effective followed by gentamicin, amoxicillin, ampicillin, erythromycin, and penicillin. Ciprofloxacin was found to be the most effective while penicillin was the least effective against various pathogens associated with mastitis.

Overall, the study provides valuable insights into the prevalence and causative agents of subclinical mastitis in dairy cattle and the effectiveness of commonly used antibiotics in treating this condition.

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