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Screening of mastitis in dairy cattle's of district Khairpur, Sindh, Pakistan

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Abstract

Dairy cattle mastitis is disease that is usually caused by bacterial infections in udder tissue. When the milk production and quality are reduced, the disease causes considerable damage to cattle. Early detection and corrective actions may lead to early cure. Mastitis can be clinical or subclinical. Clinical mastitis can cause udder milk abnormalities and swelling of udder. A clinical mastitis case reaches 240 million annually, causing huge economic losses and other losses in Pakistan. Therefore, this study aimed to screen out mastitis in cattle present in different farms of district Khairpur. Out of 400 milk samples tested, 298samples were positive for mastitis as indicated by using world-recognized somatic cell counting test (SCC), California Mastitis Test (CMT). Bacteria were isolated and identified based on cultural, microscopic and biochemical tests. Our results show that among the animals reared in the backyard showed highest number ((34.5%), and subsequently surrounding areas (33.5%) and other farms (31.75%) cases of mastitis. In addition, one hundred eighty-three bacterial isolates belonging to 6 different genera i.e *Staphylococci* (37%), *Escherichia* (21%), *Streptococci* (17%), *Pseudomonas* (13%), *Klebsiella* (7%) and *Bacillus* (5%) were isolated. The study showed that routine testing of cattles and preventive measures are recommended to reduce the outbreaks of clinical and subclinical mastitis in Khairpur, Sindh, Pakistan.

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Introduction

The Milk consumed by us depend on its quality, but bacterial invasion to milk producing glands resultantly making this milk unfit for consumers, Mastitis is an infection caused by bacteria commonly known as Staphylococcus *aureus*. *S. aureus*is Gram positive bacteria capable to survive in high salt and high temperature range appearing as an opportunistic pathogen and frequent colonizer of the epithelial tissues cause devastating diseases in humans and domestic animals. The organism is of zoonotic importance that becomes more serious in its resistant form against antimicrobial agents (Barkema *et al*., 2009; Hiramatsu *et al*., 2011) Mastitis is one of the serous maladies in the cattles causing huge production losses to livestock industry in Pakistan (Ali *et al*., 2011). Bovine mastitis hence a major problem in dairy cattles of Khairpur, in those who are kept under various farming systems or domesticated both deserves attention due to its potential impact on milk production, safety and its security. Mastitis causes two third losses of the total milk production due to affected quarters of animal (Radostits *et al*., 2007). Most common pathogens isolated from mastitis milk are contagious (Bilal *et al*., 2004). According to a report 70-80% of all the bovine mastitis is generally caused by either infected with *Staphylococcus aureus* or *Streptococcus agalactiae* (Memon *et al*., 1994). Bovine mastitis is diagnosed by (CMT) California Mastitis Test, White side test (WST) or Surf field mastitis test and somatic cell counts (Muhammad *et al*., 2010). The current study observed mastitis in cattle's of three talukas named Gamabt, Theri bypass and Khairpur city of district Khairpur. Previously no any research work has been done on chemical analysis and microbial screening of isolates of mastitis at Khairpur district Sindh, Pakistan. Present study aimed to screened-out 400 cattle's from different farms of district Khairpur, through the world-recognized somatic cell counting test (SCC) / California Mastitis Test (CMT).

Material and methods

Selection of study area and study animals

Two hundred ninety-eight lactating cattle from the three areas (Gambat, Theri bypass and Khairpur city, Sindh, Pakistan) were observed. A total of 298 milk samples were collected in sterilized screw caped test tubes.

Physical examination of milk samples

Afterwards the sample collection, an on-farm screening was performed, where samples were subjected to physical examination with naked eyes for detection of any change in color of milk, order, atrophy, any swelling in nodes, consistency or presence of any blood clotting.

Diagnostic test used

Mastitis was detected by California mastitis test (CMT), which is most common type of the test used for diagnosis of clinical and sub clinical mastitis (Qunin *et al*., 1994).

Transportation of milk samples

Strongly positive results (+++) in CMT were transferred to Microbiological laboratory of Shah Abdu latif University Khairpur in thermopile box.

Bacterial isolation from Milk

Before incubation, the sample was allowed in normal temperature, then 100ul of milk sample was mixed with an equal volume of nutrient broth with the help of pipette and allowed for incubation on 37°**C** for 24hrs.After incubation, one loop of incubated sample was streaked on nutrient agar and again incubated on37°C for 24hrs. as described by (Lafi and Hailat, 1998). The bacterial colonies were then identified based on cultural and morphological characters (Jhon., 2000).

The prevalence

The prevalence was expressed in percent by using the following formula:

Prevalence (%) = No. of Animals Positive / No. of Animals Tested×100

Results and discussion

Mastitis is an Inflammatory Infections (IMI) usually caused *by Staphylococcus aureus,* which has a wide range host spectrum and can cause serious infection and economic loss (Kenar *et al.,* 2017*)*. In this study, then countered bacterial genera including

Staphylococci (37%), *Escherichia* (21%), *Streptococci* (17%), *Pseudomonas* (13%), *Klebsiella* (7%) and *Bacillus* (5%) (Giraudo., *et al.*, 1997). The bovine mastitis and its control may be depending on detection of various species found during study and elimination of different host which are associated with the risk factor in mastitis. Amongst multiple reasons of mastitis, sustained level of wide variety of microbes, particularly different bacterial species is the most vital one; it is observed that *Staphylococcus aureus*, is the most dominating microbial bacteria casing bovine mastitis. A cell count of somatic cells usually in milk is called somatic cell count (SCC), The greater the SCC, the higher level of inflammation in udder tissue. It is because diapedesis of leukocytes is localized, then only udder tissue quarter that is infected will have significant increase in the concentration of leukocytes (SCC). Table 1 shows the prevalence of mastitis in cattle's of different cities of district Khairpur.

Table 1. Prevalence of mastitis screened by (CMT) Test.

Area	No of positive sample (CMT)	Positive Bacterial Growth	Percentage (100%)	No. of Isolated Bacteria
Kairpur city	108	61	36.2	6
Their bypass	92	55	30.8	4
Gambat	98	59	32.8	5
Total	298		100	

Table 2. Above table Showed the prevalence of mastitis screened by California mastitis test (CMT) out of 298 samples majorly, Khairpur city possess highest no 108CMT positive and 92isolated bacteria, followed by Their bypass with 92 positive CMT and Gambat taluka possess 98 positive samples.

Table 3. Form wise prevalence of mastitis in cattles screened by (CMT) Test.

Type of form	Total no of cattles	Khairpur	Thehri bypass	Gambat	Prevalence Percent
Backyard	134	48	43	51	(34.5%)
Surrounding areas	133	44	46	44	(33.5%)
Other farms	133	44	44	39	(31.75%)
Total	400				

Table 3 in this table the forms which were under observation specially Backyard showed highest number positive samples in cattle's that were about 34.5%, followed by surround areas that was 33.5 positive cattle's for mastitis it was 31.75%other forms were positive for mastitis.

Table 4. Bacterial genus wise prevalence of mastitis from three areas of Khairpur district.

Bacterial spp.	No	(%)
Staphylococci	37	(37%),
Escherichia	21	(21%),
Streptococci	17	(17%)
Pseudomonas	13	(13%),
Klebsiella	7	(7%)
Bacillus	5	(5%)

Table 4 showed prevalence of bacteria as *Staphylococci* 37%, *Escherichia* 21%, *Streptococci* 17% *Pseudomonas* (13%), *Klebsiella* (7%) *Bacillus* (5%) at district Khairpur. Prevalence of mastitis was assessed from population of cattles raised under forms of district Khairpur (CMT) test. The prevalence was higher in Khairpur followed Gambat (%) and Theri bypass 45(%) forms. It shows difference due to poor hygienic and management conditions. Cumulative percentage of mastitis in cattles observed was 44% which is much lower than 92% recorded by (Lafi and hailat., 1998) Similarly, an incidence of 54.7, 32.85 and 23.18% has been reported by (Getahun *et al.*, 2007; Pitkalae *et al.*, 2004; Iqbal, *et al.*, 2004)respectively. Variation in prevalence of mastitis may be due the region climatic condition, environment and the management condition. The area wise prevalence of bovine mastitis is presented in Table 2. The results showed highest prevalence in Gambat city. Prevalence recorded was the highest (36%) at Gambat followed by their bypass (55%) and Kairpur city (45%) least no of mastitis found in Khairpur. It may be due to availability of veterinary hospitals in Khairpur city and quality medicine provided to different forms. Mastitis is caused by different bacterial genera as *Staphylococcus*, *Streptococcus* and *Escherichia* bovine mastitis (Allore, 1993; Ahmad, 2001). Bacterial genera found during this research work were *Staphylococci* 37%, *Escherichia* 21%, *Streptococci* 17% *Pseudomonas* 13%, *Klebsiella* 7%, *Bacillus* 5%. The data of bacterial isolates is presented in Table 3. In the recent study, bacterial growth was observed in milk samples. These identified Bacteria were of seven different genera.

(Pitkala *et al*., 2004) reported microbial growth in 21-33% of milk samples, whereas, (Iqbal *et al*., 2004) reported only 15.16% in dairy buffaloes. This variation may be due to season, management conditions at the farm, area, transportation conditions, difference in sample handling in the laboratory and use of antibiotic.

In present study, S. *aureus* (37%) was isolated as top-ranking pathogen from positive cases for mastitis. In previous studies, it was also reported as major pathogen (Kapur *et al*., 1992; Allore., 1993; Rabello *et al*,.2005; Arshad *et al*., 2006; Ebrahimi *et al*., 2007; Ali *et al*., 2008; Botrel *et al*., 2009; Ebrahimi *et al*., 2007) reported 8.33% *Streptococcus agalactiae* and 9.44% *E. coli* isolates from subclinical bovine mastitis milk samples while Ali *et al*. (2008) obtained 30% growth of *Strep. agalactiae* and *Strep. dysgalactiae* and 42.6% *Staph. aureus*. Contaminated environment of farm is a main source of coliforms and mostly cause clinical infections. (Ebrahimi *et al*., 2007) obtained 3.88% Coagulase-negative *Staphylococci* (CNS), 8.33% *Streptococci* other than *agalactiae* and 9.44% *E. coli*. (Botrel *et al*., 2009) isolated 30.2% CNS, 13.7% coagulase negative *Staphylococci* and 9.3% *Strep. dysgalactiae* from subclinical mastitis milk sample. The table 1. Displays three areas variation in which Gambat had a highest no of positive mastitis cases including 61 isolated bacteria. It showed prevalence of mastitis in three areas of Gambat, Theri bypass and Khairpur city. Table 2. Showed mastitis screened by California mastitis test (CMT) at Khairpur city, where no. of 108 mastitis positive cases gave 61 no. samples yielded bacterial growth. While Gambat bypass 98 no. positive sample yielded 80 bacterial growth. Table 3 no. of positive mastitis animals according to the different forms visited during research work. Backyard showed 133 mastitis positive its about 54%. While surrounding areas were 42% and other forms 32% respectfully. Bacterial species wise prevalence of mastitis from three area of Khairpur district the *Staphylococci* was 37%, *Bacillus* was lowest no. of 5% species found in above areas. There should be mastitis control programs, which can help to control and to evaluate the forming system in the district Khairpur. Keeping in view the animal and human health hazard anchored with this pathogen, the current study was designed to estimate the outbreak of mastitis in the regions of Khairpur district.

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