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The Incidence of Clinical Mastitis and Distribution of Microorganisms in Yangtze Dairy Farm

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Mastitis is a frequently occurring and economically important disease for dairy industries worldwide. This study was conducted to research the incidence of clinical mastitis and the distribution of organisms isolated from clinical cases in dairy cows of Yangtze Dairy Farm.
Study Design: 782 dairy cows were examined clinically from March to May 2017. The clinical mastitis milk samples were collected and the microorganisms were isolated and identified.
Place and Duration of Study: The study was conducted in the Yangtze Dairy Farm and the sample analysis was done at the Laboratory of Clinical Veterinary of Yangtze University.
Methodology: The animals were physically examined and the clinical mastitis milk samples were collected aseptically before antibiotic treatment. Milk samples were plated onto a blood agar and a MacConkey agar plate. The plates were examined for growth, morphology, pigmentation, hemolytic features, and the numbers of each colony type at 24, 48, and 72 h after inoculation. Identification of bacteria was done by Gram staining, inspection of the colony morphology, haemolytic reaction, and biochemical testing.
Results: The average incidence of clinical mastitis at cow and quarter levels in the study period were found to be 4.86% (38/782) and 1.63% (51/3128), respectively. The commonly recovered

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organisms were *Escherichia coli*, *Staphylococcus aureus*, *Yeast*, coagulase-negative staphylococci, coagulase-positive staphylococci (other than *S. aureus*), *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis, Proteus* spp., and *Corynebacterium* spp. Among the isolated pathogens, *E. coli*, *S. aureus* and *Yeast* were the most prevalent that accounted 31.25%, 18.75% and 10.42%, respectively.

Conclusion: The incidence of clinical mastitis in Yangtze Dairy Farm was 4.86% in cow and 1.63% in quarter level, respectively. The major isolated pathogens were *E. coli*, *S. aureus,* and *Yeast*.

Keywords: Bovine; mastitis; bacterial; Escherichia coli.

1. INTRODUCTION

Mastitis is a multietiologic disease of the mammary gland characterized mainly by a reduction in milk production and considered an economically important disease in the dairy farms in developed and developing countries [1]. Being a most economically damaging disease, mastitis severely reduces milk yield, profit margins and affects the quality of milk and milk products in all dairy-producing countries [2]. Mastitis is universally classified as clinical and subclinical mastitis [1,3]. Clinical mastitis is characterized mainly by appearances of changes in the milk such as lakes and clots and presence of signs of inflammation on the mammary glands such as swelling, heat, pain, and edema [1,4-6], as well as systemic signs on the animal including fever, rapid pulse, appetite loss, dehydration, and depression [7].

The incidence of clinical mastitis is an important indicator of animal health and welfare. The decrease in the incidence of clinical mastitis has a positive effect on animal health, animal welfare, antimicrobial use, and net return of the farm [8]. Furthermore, mastitis could be a danger to human health because milk from the mastitic udder of animal is contaminated with bacteria which could be a potential source of infection to consumers and many of them are responsible for diseases like tuberculosis, streptococcal intoxication, colibacillosis, streptococcal sore throat, and brucellosis in human [1,9-10].

Mastitis being a multietiologic disease, many microorganisms are implicated as causes. Majority of microorganisms that are responsible for mastitis include *Staphylococcus aureus*, *Streptococcus spp*, *Escherichia coli*, *Proteus* spp., environmental streptococci and *Enterobacter* spp. Some of the organisms are found in the environment of the cow; hence they can easily be contracted by the udder [7].

Bacteriological examination of mastitis milk is an important and helpful procedure for mastitis diagnosis and management. Timely and correct disease diagnosis along with identification helps to control major economic losses occurring due to mastitis in worldwide. Furthermore, control and prevention of mastitis in the dairy farms require a rigorous and systematic research and documentation of information on the status of the disease [1]. Therefore, the objectives of this study were to study the incidence of clinical mastitis in dairy cows of Yangtze Dairy Farm and the distribution of organisms isolated from clinical cases.

2. MATERIALS AND METHODS

2.1 Animals

In this study, 782 Holstein dairy cows were detected clinical mastitis (CM) in Yangtze Dairy Farm between March and May 2017, which located in Huanggang of Hubei Province. The CM cases were detected routinely by herd supervisors at milking time and confirmed by a veterinarian. The diets consisted of hay, straw, and silage. All lactating cows were milked twice daily in a double-14 fishbone milking parlor.

2.2 Protocol Design and Method

In this study physical examination of the udder was conducted following the standard procedure. Briefly, udders or teats were physically examined first by visualization and then by palpation to detect the presence of a gross lesion. Clinical mastitis was diagnosed on the basis of a manifestation of visible signs of inflammation and abnormal milk. A quarter, which was warm and swollen and had pain upon palpation, were considered to have acute clinical mastitis. Viscosity and appearance of the milk secretion from each quarter were examined for the presence of clots, lakes, blood, and watery

secretion. Besides, rectal temperature was taken for acute mastitis cases to check systemic involvement of the infection. On the other hand, atrophied, misshaped, and any blind, hard, and fibrotic quarters were considered to have chronic mastitis [1].

2.3 Sampling

Participating producers were asked to collect milk samples aseptically from CM cases into 20-ml sterile centrifuge tubes before antibiotic treatment was initiated. Milk samples were stored in a cool box containing ice pack with a temperature of 4°C and transported to the Clinical Veterinary Laboratory of Yangtze University for bacteria isolation and identification.

2.4 Microbiological Culture and Identification

Microbiologic procedures were conducted according to the guidelines of NMC (1999) [11]. Briefly, a wire loop (about 10 µl) of milk from each sample was plated onto a blood agar and a MacConkey agar plate, and the plates were incubated aerobically at 37°C for up to 72 h. The plates were examined for growth, morphology, pigmentation, hemolytic features, and the numbers of each colony type at 24, 48, and 72 h after inoculation. Samples were considered culture-positive if 1 or more colonies were observed (≥100 cfu/ml) [12]. Milk samples with 2 pathogens identified on culture was categorised as mixed growth. Milk samples with 3 or more pathogens identified on culture were considered contaminated, unless *S. aureus* or *Str. agalactiae* were isolated [13].

Identification of bacteria was done by Gram staining, inspection of the colony morphology, haemolytic reaction, and biochemical testing. Gram stain was used to distinguish between gram positive and negative bacteria and to study the microscopical features of the isolated bacteria. For Gram-positive cocci, catalase tests were performed to distinguish catalase-negative *Streptococcus* spp. from catalase-positive *Staphylococcus* spp. *S. aureus* was identified by α- and β-hemolysis on blood agar, a positive catalase test, a positive tube coagulase test, mannitol reaction, and a positive DNase test. Coagulase-negative staphylococci were differentiated from *Micrococcus* by furazolidone sensibility test and were classified as CNS. Streptococci were differentiated as esculin-positive (*Str. uberis* and other esculin-positive cocci) or esculin-negative cocci (*Str. dysgalactiae* and *Str. agalactiae*). Christie, Atkins, and Munch-Petersen (CAMP) tests were used to distinguish *Str. dysgalactiae* (CAMP-negative) from *Str. agalactiae* (CAMP-positive). Gram-positive rod-shaped bacteria, which were catalase-negative and oxidase-negative, were identified as *Trueperella pyogenes*. Gram-positive rod-shaped bacteria, with catalase-positive and urease-positive tests, were identified as *Corynebacterium* spp. Gram-negative bacteria were identified by colony morphology, lactose fermentation on MacConkey agar, incubation in sulfide-indole-motility (SIM) medium, and oxidase, triple sugar iron (TSI), citrate and urease testing. Yeasts were identified by visual assessment of colony morphology and microscopic examination at 400× magnification. Species that could not be classified using the above mentioned test procedures were subjected to 16S rDNA sequencing [14].

After identification, isolates were transferred to brain-heart infusion broth and incubated at 37°C until turbidity (indicating bacterial growth) was visible approximately 4 h later. 800 µL of the broth was then mixed with 200 µL of glycerol and stored at −70°C.

3. RESULTS AND DISCUSSION

A total of 3128 quarters belonging to 782 dairy cows were examined during the study period clinically, 77 (2.46%) quarters were with blind teat, which is in agreement with the reports by Kivaria et al. [15] (2.1%) and Getahun et al. [16] (2.3%), it is lower than the reports by Mungube et al. [17] (3.7%), Almaw et al. [18] (3.8%), Tesfaye et al. [19] (6%), Sarba and Tola [20] (5.5%), and Zeryehun and Abera [1] (6.6%).

The average incidence of clinical mastitis at cow and quarter levels in the study period of 3 months were found to be 4.86% (38/782) and 1.63% (51/3128), respectively. It is in agreement with the reports by Tesfaye et al. [19] (1.2% in quarter level), while, they are lower than the reports by Yang et al. [21] (8.7% and 3.7%), Sarba and Tola [20] (9.9% and 9.3%), and Bhat et al. [22] (11.5% and 5.76%). The discrepancies in these studies could be attributed to the difference in the breed, management system, season, and the epidemiological status.

Table 1. The bacterial distribution of clinical mastitis milk samples

Bacterial species	No. of milk samples (%)	Total no. of bacteria isolates (%)
Single growth	42 (82.35)	**-**
E. coli	13 (25.49)	15 (31.25)
S. aureus	8 (15.69)	9 (18.75)
CNS	3 (5.88)	4 (8.33)
CPS	4 (7.84)	4 (8.33)
Str. agalactiae	4 (7.84)	4 (8.33)
Str. dysgalactiae	3 (5.88)	3 (6.25)
Str. uberis	2 (3.92)	2 (4.17)
Yeast	3 (5.88)	5 (10.42)
Proteus spp.	1 (1.96)	1 (2.08)
Corynebacterium spp.	1 (1.96)	1 (2.08))
Mixed growths	3 (5.88)	-
S. aureus + Yeast	1 (1.96)	-
Yeast + E. coli	1 (1.96)	-
E. coli + CNS	1 (1.96)	-
No growth	6 (11.76)	
Total	51 (100)	48 (100)

CNS: Coagulase-negative staphylococci; CPS: Coagulase-positive staphylococci (other than S. aureus).

Out of 51 clinical mastitis samples, 42 (82.35%) were single growth, and 3 (5.88%) were mixed growth (Table 1 above). Based on the culture growth, the most common isolates were recorded for *E. coli* was the most frequently isolated single pathogen in our study which is consistent with the previous studies by [23]. The high prevalence of staphylococci species may contribute to the presence of these agents on the skin and mucus membranes of various parts of the animal body and their contagious nature, especially *S. aureus* and *Str. agalactiae* [24].

Staphylococcus species and coliforms accounted for about 66.67% of the total isolates. The high prevalence of staphylococci species and coliforms in this study is in accordance with other studies [24-25,23]. The mixed growths in clinical mastitis was 5.88%, it is lower than the reports by Yang et al. [21] (8.7%) and (7.46%). Miscellaneous mastitis pathogens are associated with poor and unhygienic housing and milking, unsanitary intramammary infusion practices, indiscriminate use of antibiotics, and non-implementation of mastitis control program.

4. CONCLUSIONS

Bovine mastitis has long been considered to be a disease of economic importance in the dairy industry. The results of our investigation from March to May 2017 revealed that clinical mastitis is a common disease in Yangtze Dairy Farm with a prevalence of 4.86% in cow and 1.63% in quarter level. The major isolated pathogens were *E. coli*, *S. aureus*, and *Yeast*, which accounted 31.25%, 18.75%, 10.42%, respectively.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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