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Original Article

A survey on bacterial mastitis in the cows from the traditional farms located in the suburb of Tabriz city, Iran

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Abstract
Mastitis is one of the most critical problems for dairy cattle worldwide. The purpose of this study was to determine whether mastitis is caused by bacteria in the dairy cows of traditional farms. In this randomized clinical trial, 54 Holstein cows with clinical mastitis (from October 2020 to September 2021) were selected from the traditional farms located in the suburbs of Tabriz city. All cows were subjected to sampling during four seasons according to the National Mastitis Council (NMC) guidelines and the collected samples were rapidly sent to the Veterinary faculty's microbiological laboratory for bacterial cultures. The distribution of mastitis was evaluated according to the stage of lactation, parity, season, and types of bedding system. The results were as follows: *E. coli* 39%, *Streptococcus uberis* 21%, *Streptococcus agalactia* 7%, and *Staphylococcus aureus* 33%. The results of this study showed that parturition, increased number of parity, wet and rainy seasons, as well as sawdust and cow manure bedding are among the risk factors for mastitis. In conclusion, in traditional farms, unlike industrial farms, environmental mastitis has more importance than contagious mastitis. Regarding the presence and shedding of *E. coli* microorganisms in the milk, this should be highlighted as a concern for public health.
Keywords: Cow, Mastitis, NMC, Tabriz, Traditional

Introduction

Mastitis is one of the most common and costly diseases in the dairy industry in the world that harms food safety and results in a reduction in milk production and loss in milk quality and quantity (Hogeveen, 2011; Reyher et al., 2011). The bacterial contamination of milk from the affected cows causes to transmit of diseases like tuberculosis, sore-throat, Q-fever, brucellosis, leptospirosis to humans and thus has zoonotic importance (Sharif et al., 2009). Mastitis is a complex multifactorial disease that affects dairy cows around the world (FAO, 2014). It is usually classified into three types, namely sub-clinical, clinical, and chronic mastitis. (Cobirka et al., 2020). Mastitis appears to be a major disease challenge and is still one of the most common diseases in dairy cows worldwide. It is responsible for approximately 40% of the cost of treating cows (Sharma et al., 2012). The subclinical type of mastitis causes a major loss of milk production and its detection is difficult due to the absence of any visible changes in the milk. Clinical mastitis characterized by swelling of the udder, clots in milk, and watery milk is observed grossly. The chronic form is rare but it can lead to persistent inflammation of the mammary gland (Argaw, 2016). Causative organisms of mastitis are divided into two groups: contagious and environmental factors. Major contagious pathogens include *Staphylococcus aureus*, *Mycoplasma spp.*, *Streptococcus agalactiae*, *Corynebacterium bovis*, and *Streptococcus dysgalactiae*, which usually live in the udder or teats skin. The organisms are transmitted from the carrier cow or quarter to the teats of non-infected cows or quarters during the milking process (Kibebew, 2017). On the other hand, environmental factors like *Enterobacter spp.*, *E. coli*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Streptococcus uberis*, yeasts, and molds survive in the environment and the bed is the first source of environmental factors: however, infected teats, intramammary injections, water used to wash the udder before milking, skin lesions, and flies are all considered sources of infection (Blowey and Edmondson, 2010). In affected animals, milk production is significantly reduced. In a study, the estimated losses after clinical mastitis were approximately 700 kg for cows in the first lactation period and 1200 kg for the second lactation period (Abdel-Rady and Sayed, 2009). The estimated annual economic losses due to mastitis were reported to be 2 billion US$ by the year 2009 in the United States of America (Viguier et al., 2009). Diagnosis of mastitis is based on clinical observations and various laboratory tests including California mastitis test (CMT), polymerase chain reaction (PCR), electrospray ionization mass spectrometry, and bacterial culture in specific media. Unlike industrial farms, there is not sufficient information about the causes and factors of mastitis in traditional farms, and unfortunately, the treatments performed are based on the protocols in industrial farms, which results in a lack of successful treatment in many cases (Zadoks and Fitzpatrick, 2009). Most studies on bovine mastitis have been performed on industrial farms, and therefore the recommendations for the treatment and prevention of bovine mastitis have been based more on industrial farms, such as post milking teat dipping, whereas, disinfection of teats and treatment of cattle during the drying period is often not done in traditional farms. Milking in traditional farms is usually done by hand and it seems that the type of mastitis and its causative factors are fundamentally different from industrial farms. So, the purpose of this study was to detect the causes of mastitis in traditional dairy farms.

Materials and methods

Location, animals, and experimental design

The experiment was conducted from October 2020 to September 2021 at the Specialized Hospital of the Faculty of Veterinary Medicine, University of Tabriz, Iran (38.0962° N, 46.2738° E). In this randomized clinical trial, a total number of 54 dairy cows belonging to traditional farms in Tabriz city that had been referred to the hospital of the Faculty of Veterinary Medicine with signs of mastitis were selected. At the time of admission, all information related to the type of farm, location, type of nutrition, type of bed, number of cows, milking method, farm hygiene conditions, history of mastitis occurrence, and number of parity were obtained and recorded in special forms. During the examination of the animal by the clinician, the rectal temperature, heart rate, respiration rate, stiffness, hardness, and the hot and painful nature of the affected teats, as well as the color and consistency of the milk were carefully examined and recorded. Before starting any treatment, regarding the health issues and according to the National Mastitis Council (NMC, 2004) Procedures for Collecting Milk Samples, sampling was done and sent to the microbiology laboratory of the Faculty of Veterinary Medicine in the shortest possible time.

Milk sampling method, culture, and incubation

After washing and disinfecting hands, the milk sampling was done according to the NMC Guidelines (NMC, 2004). Before culturing, the samples were kept at room temperature for half an hour to form a homogeneous milk solution. Milk samples were then cultured on the primary culture medium (nutrient agar or blood agar) using a swap.

After obtaining bacterial colonies, they were transferred to differential culture media (Middleton et al., 2014). For example, MacConkey and EMB (Eosin Methylene Blue) were used to isolate *E. coli*. The cultured plates were placed in an incubator at 37 °C and inspected after 24 and 48 hours (Tarr, 1995; Bewley, 2010;). Manitol Salt agar was used to isolate *Staphylococci*. The plates were then incubated at 37 °C for two days (48 hours) and after 48 hours, the colonies were identified according to Hoblet guidelines (Hoblet et al., 1986).

Statistical analysis

A one-way ANOVA was used for statistical analysis of the data using SPSS software (version 22, Inc., Chicago, IL, USA) and a P < 0.05 was considered significant.

Results

Isolated Bacteria

Out of 54 samples taken from cows with clinical mastitis, 21 cases of *Escherichia coli*, 11 cases of *Streptococcus uberis*, 18 cases of *Staphylococcus aureus*, and 4 cases of *Streptococcus agalactia* were isolated. In total, 60% of cases of clinical mastitis were caused by environmental pathogens and 40% caused by contagious agents (Figure 1). There was a significant difference among the examined groups (P < 0.05).

Pregnancy status of cows

In this experiment, 11 cows had less than seven months of pregnancy and one cow was in late pregnancy (over seven months), and 42 cows were in early post-partum period (Figure 2). There was a significant difference among the examined groups (P < 0.05).

Parity status of cows

Out of 54 cows with clinical mastitis, two cows were nulliparous and the rest were multiparous. Most of the infected cows had history of 4 or more parity (Figure 3). There was a significant difference among the examined groups (P < 0.05).

Seasonality of mastitis

The number of cows with mastitis was higher in the cold seasons than in the warm seasons (Figure 4). There was a significant difference among the examined groups (P < 0.05).

Type of bed and mastitis

The incidence of clinical mastitis in farms with sawdust or cow manure beds was higher than those were using mineral materials such as sand (Figure 5). There was a significant difference among the examined groups (P < 0.05).

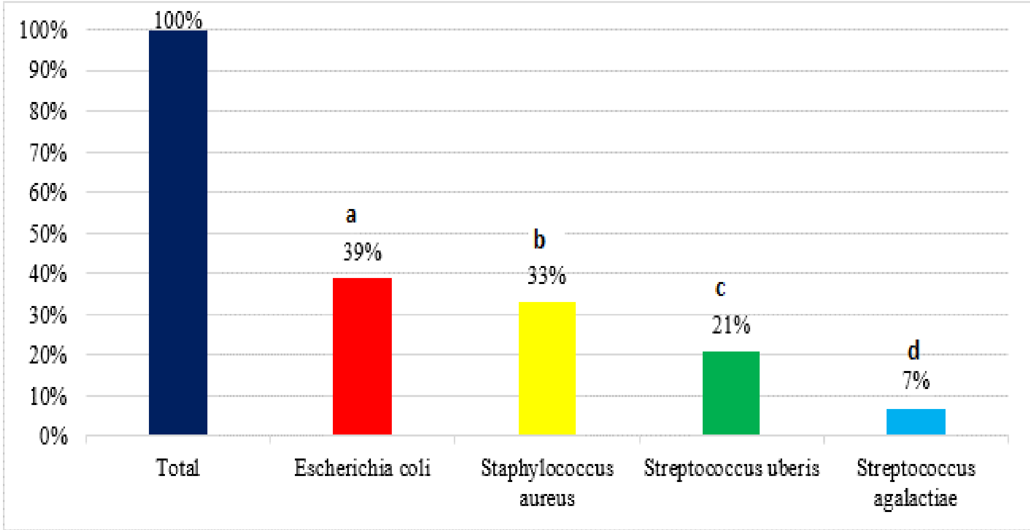

Fig. 1. The frequency of mastitis-causing bacteria in all samples taken from infected cows (Different letters indicate significant differences).

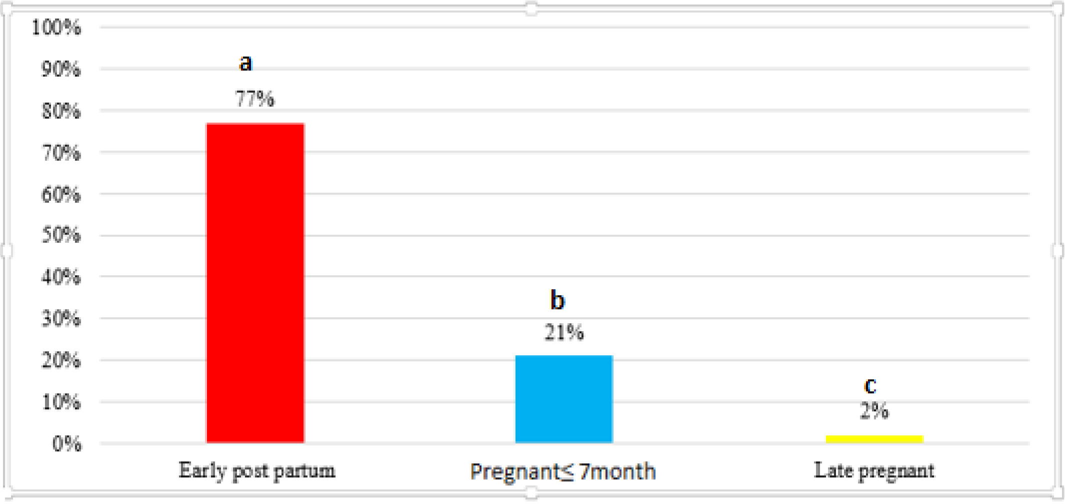

Fig. 2 . Pregnancy status of cows suffering from mastitis
(Different letters indicate significant differences).

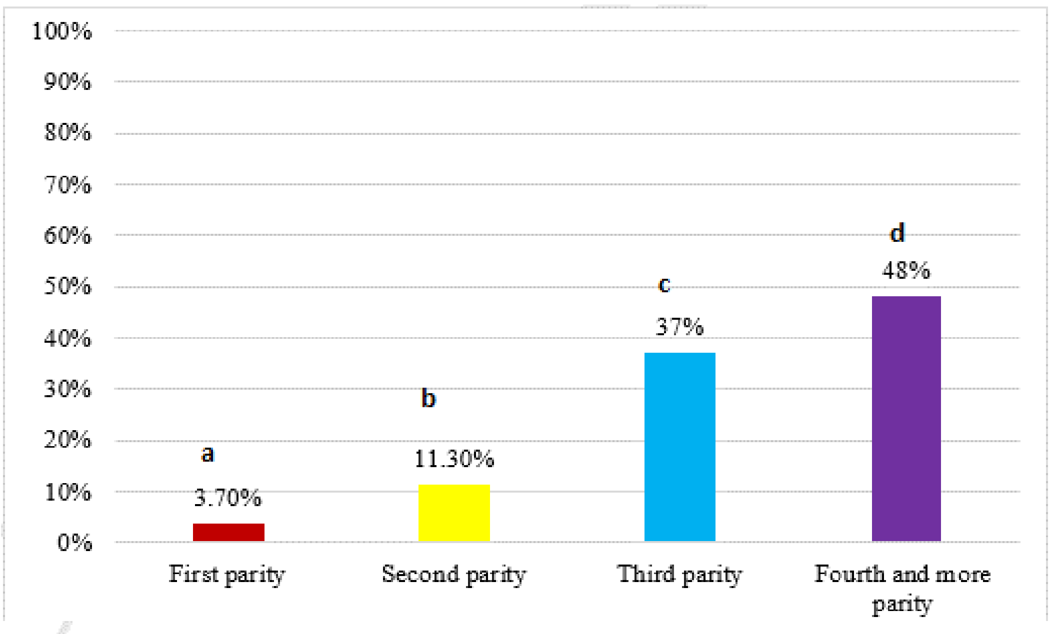

Fig. 3. Parity status of cows suffering from clinical mastitis
(Different letters indicate significant differences).

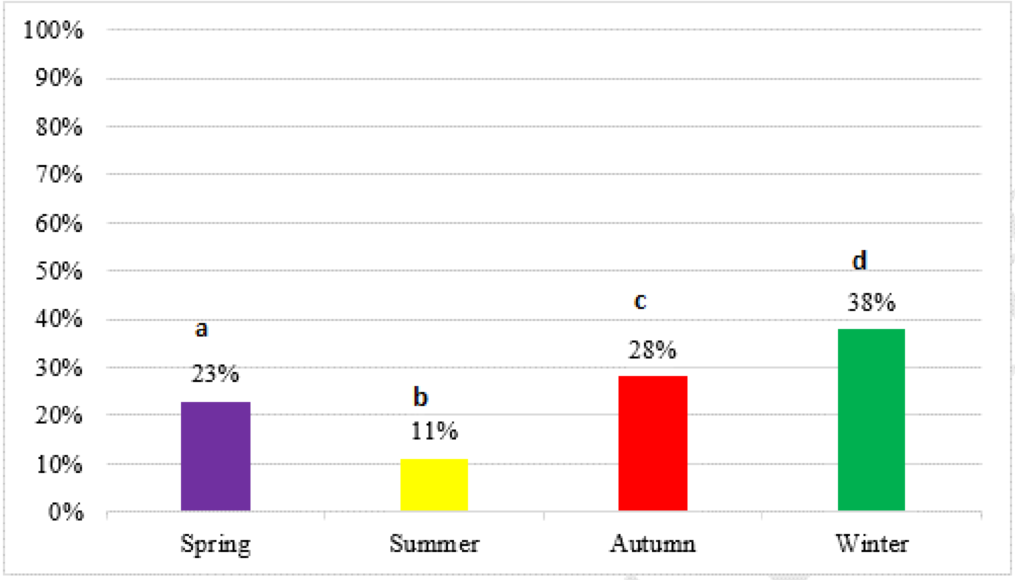

Fig. 4. Distribution of clinical mastitis in different seasons of the year (Different letters indicate significant differences).

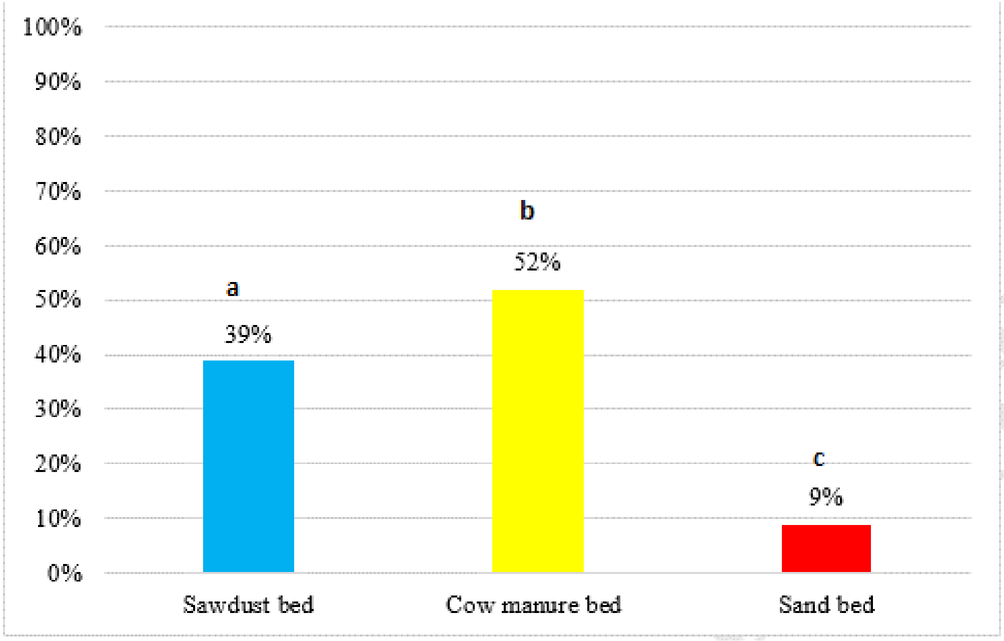

Fig. 5. The percent of cows with mastitis and the type of material used on the bed (Different letters indicate significant differences).

Discussion

Mastitis is a multifactorial disease, as a result, knowing the risk factors is important to understand the complexity of this disease and making the right decision for its control and treatment (Zigo et al., 2021). Overall, mastitis is a mutual result between infectious agents and management practices that stress the host's defenses and allow pathogens to invade the mammary gland. Invading pathogens and their toxins cause inflammatory reactions in the mammary gland to destroy or neutralize the infectious agents for recovery and return to normal function (Harmon, 1995). The outcome of microbial invasion to mammary gland depends on the complex interaction between the pathogens. Host responses are required to eliminate infectious agents, as well as various risk factors that affect the infectivity of pathogens and mammary gland defense mechanisms. The factors required for the onset and severity of mastitis are divided into three main groups: host factors, pathogenic factors, and environmental factors (Tomazi et al., 2018).

In a study to determine the causative agents and the prevalence of pathogens causing clinical mastitis in 59 traditional and industrial farms in Canada, the most common agents isolated included coagulase-negative staphylococci, *Bacillus spp.*, *Streptococcus*, *Staphylococcus aureus*, and *E. coli*; also, it was observed that the incidence rate of clinical mastitis in traditional farms was higher than in industrial farms (23.7 vs. 13.2 cases per 100 cow-years; Levison et al., 2016). In the current study, *E. coli*, *Staphylococcus aureus*, and *Streptococcus* were the most isolated bacteria from the cows with clinical mastitis (Fig. 1). Therefore, our results are in accordance with the results obtained by Levison et al. (2016). In a study on 16,000 bacterial culture samples isolated from cows with clinical mastitis in Canada, the most isolated bacterial agents were *Staphylococcus spp*. (coagulase-negative), *Staphylococcus aureus*, *Streptococcus spp.*, and *E. coli*, respectively, which shows that, unlike the present study, *staphylococci spp.* were the most abundant (Dufour et al., 2019).

The risk of clinical mastitis is influenced by the stage of lactation and parity (Mungube et al., 2004). It has been reported that the possibility of clinical mastitis is more in the early stage of lactation. Clinical mastitis most often happens around parturition. Two weeks before and after parturition, the risk of intramammary infection is very high (Kerro Dego and Tareke, 2003; Valde et al., 2004). The blood concentrations of cortisol increases near parturition; thus, the cow's immune system is suppressed around calving. Consequently, the cross-talk between the neuroendocrine and immune systems of cows during the periparturient period is associated with a higher incidence of severe clinical mastitis (Burton et al., 2005; Vangroenweghe et al., 2005; Pyörälä, 2008). In the present study, the most cases of clinical mastitis were in the early post-partum period (77%), which is similar to the results of previous studies (Vangroenweghe et al., 2005; Pyörälä, 2008).

The prevalence of infected quarters was increased with age, being higher at 7–year old. The higher incidence of clinical mastitis in multiparous cows as compared with heifers over all stages of lactation period has been reported in previous study (Riekerink et al., 2007).Similarly in the recent study, the probability of clinical mastitis was also increased with the increase in the number of parities consequently 85% of clinical mastitis cases were in cows with third and more parity (Figure 3).

The relation between the incidence of mastitis and season of the year is variable, depending on geographic and climatic conditions. In subtropical and tropical areas, the incidence may be higher during winter or spring calving due to increase in humidity and growth of pathogenic agents (Elghafghuf et al., 2014). Deficiencies in some nutrients such as selenium and vitamin E result in an increased incidence of clinical mastitis, which can routinely happen during late pregnancy and early lactation (Weiss et al., 1990). In the case of seasonality, the result of the present study is also similar to the results of the previous studies; in other words, the prevalence rate of clinical mastitis in the wet season such as winter, spring, and autumn was higher than in summer (Figure 4).

Growth of bacteria is dependent on the presence of food, warmth, moisture, and mid-range pH and if one of these factors is not present, the growth of bacteria will be limited (Blowey and Edmondson, 2010). The best type of bed to reduce the growth of bacterial agents is sand. Sawdust and cow manure that used as bedding are contaminated with *E. coli*, and *Streptococcous uberis*, which are major risk factors for environmental mastitis (Radostits et al., 2007). In conclusion, our data showed that the incidence of mastitis was highest in cows kept on manure bed (52%), followed by sawdust bed (39%), and lowest on sand bed (9%) (Figure 5).

Conclusion

The main causes of mastitis in traditional farms are different from those in industrial farms. According to research in the field of mastitis, it is in agreement that in industrial farms, the causative agents of clinical mastitis in most cases belong to the contagious group, although environmental factors could be infective in some environmental conditions. In other word, in traditional farms due to the ways of milking and the type of bedding used, the prevalence of the environmental mastitis caused by *E. coli* and *Streptococcus uberis* is higher than that of contagious mastitis. Therefore, it is possible to effectively reduce the incidence of clinical mastitis in traditional farms by better managing cows around parturition, adjusting the time of insemination and, subsequently the season of parturition, and using suitable bedding.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Ethical approval

Not applicable.

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