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Impact of hygiene training on dairy cows in northeast India

David Melin

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Impact of hygiene training on dairy cows in northeast India

En hygienthränings påverkan på mjölkkor i nordöstra Indien

David Melin

***Supervisor**: Johanna Lindahl, Department of Clinical Sciences, SLU*

***Assistant Supervisor**: Ram Pratim Deka, ILRI*

***Examiner**: Karin Persson Waller, Department of Clinical Sciences, SLU and Department of Animal Health and Antimicrobial Strategies, SVA*

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SUMMARY

Mastitis in dairy cows is an emerging and challenging disease in the tropics, including India. Nation-wide reports suggest that the incidence of clinical mastitis varies from 3.94% to 23.25%, and for subclinical mastitis from 15.78% to 81.60%. In Assam, a northeastern province of India, dairy is an essential part of the mixed farming system that exists in the state, but the milk yield is far below domestic standards. In 2009-2010, International Livestock Research Institute (ILRI) and local associates started to develop a training program for local farmers in Assam, in order to enhance the informal bovine dairy sector in and around Guwahati, India. The project continued until mid-2013. This study was conducted in order to investigate the prevalence of clinical and subclinical mastitis in dairy cows based on the California Mastitis Test (CMT) and clinical examination of the udder, in trained as well as untrained farms, to see if the hygiene training had led to lower disease burden. The objective also included getting an understanding of the pathogens involved, cow factors associated with higher CMT scores and comparing trained with untrained farms regarding milk production and hygiene routines. A questionnaire survey was conducted on 73 trained and 76 untrained farms. From these, 25 trained and 25 untrained farms were chosen for additional CMT and clinical examination of the udder, on 25% of the lactating cows on the farm. Noted parameters for each cow included stage of lactation, parity number and udder hygiene. In total 178 cows were screened. Cows with a CMT score of 3 or more were considered positive and subjected to a milk test for bacterial evaluation. The results were analyzed by descriptive statistics, χ^2–test and t-test. At cow level, 6.2 % suffered from clinical mastitis, while 50.6 % suffered from subclinical mastitis in at least one of the quarters (n=178). At quarter level, 1.6 % suffered from clinical mastitis, while 26.6 % suffered from subclinical mastitis (n=700). The mean CMT score among all cows was 2.08 (1-5). A near significant association existed (p=0.08) in CMT score in trained/untrained farms, indicating a lower CMT score in trained farms. The results from the bacterial analysis show that on quarter level for subclinical mastitis (n=184), coagulase-negative staphylococci (n=56) were the most common, followed by *Streptococcus agalactiae* (n=42), negative growth (n=42), *Staphylococcus aureus* (n=14), mixed growth (n=13), *Streptococcus dysgalactiae* (n=10) and 'streptococci, other' (n=7). On quarter level for clinical mastitis (n=11), S. agalactiae (n=4) and negative growth (n=4) were most common, followed by *S. aureus* (n=2) and coagulase-negative staphylococci (n=1). All *S. aureus* bacteria were tested for penicillinase production; all were negative. A near significant association (p=0.06) existed between CMT score and lactation stage, indicating a higher CMT score in later lactation stages. A near significant association (p=0.06) also existed between CMT score and parity number, indicating a general tendency against higher CMT scores for higher parity numbers. No significant association between udder hygiene and CMT score was found in this study. The average milk production in trained and untrained farms was 7.8 and 6.8 liters respectively. A two sample unpaired t-test showed that the difference is significant (p<0.01). The results show that the prevalence of clinical and subclinical mastitis is in harmony with other studies conducted in India and nearby countries. The bacterial testing saw exclusively gram-positive cultures. The significant increase in milk production and the tendency towards lower CMT score in trained farms indicate that the hygiene training has led to positive results for the dairy farmers.

SAMMANFATTNING

Mastit hos mjölkkor är en utmanande sjukdom i Asien, inklusive Indien. Landsomfattande rapporter tyder på att incidensen av klinisk mastit varierar från 3.94% till 23.25%, och för subklinisk mastit från 15.78% till 81.60%. I Assam, en provins i nordöstra Indien, är mjölkproduktion en viktig del av det blandade jordbruket som finns i staten. Jämfört med genomsnittet i landet är dock mjölkavkastningen långt under det normala. År 2009-2010, började International Livestock Research Institute (ILRI) och lokala samarbetspartners utveckla ett träningsprogram i hygien för lokala mjölkbönder i Assam, i syfte att förbättra den inofficiella mejerisektorn i och runt Guwahati. Projektet fortsatte fram tills mitten av 2013. En studie genomfördes för att undersöka prevalensen av klinisk och subklinisk mastit hos mjölkkor baserat på California Mastitis Test (CMT) och klinisk undersökning av juvret, i tränade samt otränade gårdar, för att undersöka om hygienutbildningen lett till lägre sjukdomsbörda. Målet omfattar även att kartlägga de patogener som är involverade, faktorer som är förknippade med högre CMT-poäng och att jämföra tränade med otränade gårdar avseende mjölkproduktion och hygienrutiner. En enkätundersökning genomfördes på 73 tränade och 76 otränade gårdar. Utifrån dessa valdes 25 tränade och 25 otränade gårdar för ytterligare bedömning med CMT och klinisk undersökning av juvret, på 25% av de lakterande korna på gården. Laktationsstadium, paritetsnummer och juverhygien noterades för varje undersökt ko. Sammanlagt undersöktes 178 kor. Kor med CMT-poäng på 3 eller mer ansågs positiva och utsattes för mjölkprovstagning för bakteriell analys. Resultaten analyserades med deskriptiv statistik, χ^2–test och t-test. Av alla kor led 6.2% av klinisk mastit och 50.6% av subklinisk mastit, i åtminstone en av juverfjärdedelarna (n=178). Av alla juverfjärdedelar var 1.6% påverkade av klinisk mastit, medan 26.6% var påverkade av subklinisk mastit (n=700). Medelvärdet för CMT bland alla kor var 2.08 (1-5). Ett nära signifikant samband fanns (p=0,08) mellan CMT-poäng och tränade/otränade gårdar; vilket indikerar lägre CMT-poäng i tränade gårdar. Resultaten från den bakteriella analysen visar att på juverfjärdedelsnivå för subklinisk mastit (n=184), var koagulas-negativa stafylokocker (n=56) vanligast förekommande, följt av *Streptococcus agalactiae* (n=42), negativ växt (n=42), *Staphylococcus aureus* (n=14), blandflora (n=13), *Streptococcus dysgalactiae* (n=10) och 'streptokocker, övriga' (n=7). På juverfjärdedelsnivå för klinisk mastit (n=11), var S. agalactiae (n=4) och negativ växt (n=4) vanligast förekommande, följt av *S. aureus* (n=2) och koagulas-negativa stafylokocker (n=1). Alla *S. aureus* testades för penicillinasproduktion; alla var negativa. Ett nära signifikant samband (p=0,06) fanns mellan CMT-poäng och laktationsstadium, vilket pekar på en högre CMT-poäng i senare laktationsstadier. Ett nära signifikant samband (p=0,06) fanns också mellan CMT-poäng och paritetsnummer, vilket pekar på en tendens mot högre CMT-poäng hos kor som kalvat fler gånger. Inget signifikant samband mellan juverhygien och CMT-poäng påvisades i denna studie. Den genomsnittliga mjölkproduktionen på tränade och otränade gårdar var 7,8 respektive 6,8 liter. Ett oparat t-test visade att skillnaden är signifikant (p<0,01), vilket indikerar att utbildningen har lett till en verklig ökning i mjölkproduktion. Resultaten visar att förekomsten av klinisk och subklinisk mastit är i överensstämmelse med andra studier som genomförts i Indien och i närliggande länder. I denna studie hittades enbart grampositiva bakterier. Den signifikanta ökningen i mjölkproduktion och tendensen mot lägre CMT-poäng i tränade gårdar indikerar att hygienutbildning har lett till positiva resultat för mjölkbönderna.

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INTRODUCTION

Mastitis in dairy cows is an emerging and challenging disease, associated with substantial economic losses. In India, it is estimated that the average national loss in milk yield due to clinical mastitis is approximately 50%. In Assam, a northeastern state of India, dairy is an essential part of the mixed farming system that exists in the state. Compared to the Indian national average however, the milk yield is far below domestic standards. According to FAO (2002), accumulated milk consumption in India grew by more than 50% between the early 1980s to the late 1990s. While many other states in the country enjoyed an upswing in milk production, the production in Assam had stagnated. In 2009-2010, International Livestock Research Institute (ILRI) and local associates started to develop a training program for local farmers in Assam, in order to enhance the informal bovine dairy sector in and around Guwahati, India. The program focused on 'safe milk' and hygiene practices in milk production, as well as general information on bacteria; how it spreads and how it cause disease.

OBJECTIVES

The objective of this study is to investigate the prevalence of clinical and subclinical mastitis in dairy cows based on the California Mastitis Test (CMT) and clinical examination of the udder, in trained as well as untrained farms in Guwahati, Assam, to see if the hygiene training given by ILRI has led to lower disease burden. The objective also includes getting an understanding of the pathogens involved, cow factors associated with higher CMT scores and comparing trained with untrained farms regarding milk production and hygiene routines. The project is a Minor Field studies – 'MFS' – program sponsored by the Swedish governmental organization SIDA.

LITTERATURE REVIEW

Livestock revolution

Between the early 1970s and early 1990s, meat, milk and egg consumption in developing countries increased by approximately 50 percent in per capita consumption. This upswing was caused by income growth, urbanization, population growth and demands for more diversified diets (Delgado *et al,* 1999). According to FAO (2002), accumulated milk consumption in India grew by more than 50% between the early 1980s to the late 1990s. In the late 1990s, India was responsible for 13 percent of the total world consumption, and 31 percent of milk consumption in developing countries. In 2020, it is projected that over half of the world's milk consumption will come from developing countries (FAO, 2002). However, the increased livestock production has raised concerns regarding the accumulation of both antibiotics and pesticides in the food-chain. Food safety risks from microbial contamination are also causing worry (Delgado *et al,* 1999).

Dairy sector in India

India, located in South Asia, is the seventh largest country by land, and the second largest country population-wise with its 1.2 billion people. It is also considered the world largest democracy (National Port of India, 2014). India is one of the top five milk-producing countries in the world; responsible for approximately 8% of global milk production from cows. It is estimated that around 70 million households in India are involved in milk production. Most dairy farms in India are small producers that are rural based. A typical Indian dairy farm comprise of only two milking cows. The farms are run by family members that use their own milking utensils. In India, dairy foods play an important role among the countries' poor as it provides a vital source of calcium and animal protein (Douphrate *et al,* 2013).

In Assam, a northeastern province of India, bovine dairy is an essential part of the mixed farming system that exists in the state. Compared to the Indian national average however, the milk yield is far below domestic standards. Studies have shown that approximately 80% of rural households in Assam keep either cattle or buffalo. Keeping cattle is by far more common that keeping buffalo. Like in other parts of India, dairying in the state consists of small-scale producers using indigenous animals. The organized milk sector (milk networks that pasteurize the milk) in Assam is almost non-existent. It only encompasses around 3% of all the produced milk in the state. This means that most milk in Assam is not properly pasteurized at the time of sale to the consumer. For small-holder producers there is normally no other option than the traditional market (Kumar & Staal, 2010).

Sarma *et al* (2011) conducted a study to assess the knowledge level of dairy farmers in the Kamrup district of Assam. They concluded that the knowledge level among the dairy farmers was generally "medium to high level", although there were large variations in different zones of the district. However, it is not possible to conclude which questions were actually asked in this study. The authors stressed that farmers should be provided with "more exposures to the dairy development programs" and that knowledge level plays an important role in increasing milk production.

Mastitis

Mastitis – defined as an inflammation in the mammary gland – can either be of infectious or non-infectious origin (Radostits *et al*, 2007). Infectious origin is most common and caused by micro-organisms overcoming the physical-anatomical barrier of the teat canal. Consequently, the pathogens duplicate and multiply in the mammary gland, more easily so when the immune-system response is insufficient. The immune system can be suppressed during the time around early lactation and parturition. The causative agents also influence the immune response in the mammary gland (Wellnitz & Bruckmaier, 2011).

Mastitis can present itself in a number of different ways. The mildest form is called subclinical mastitis which shows no clinical signs of infection. The most severe form is the per-acute clinical mastitis which may result in death for the animal. In between these extremes are many varieties of the disease. The clinical form is characterized by inflammatory symptoms such as redness, swelling and heat, changes in milk composition, general illness and often long-term negative influence on milk production. The subclinical form shows no obvious symptoms but is characterized by an increase in somatic cell count (SCC) and reduced milk production (Radostits *et al*, 2007). An episode of acute clinical mastitis given the proper treatment might last for only a few days whereas the duration of a chronic or subclinical infection can last for weeks, even months. Clinical mastitis is primarily an individual's problem, while subclinical mastitis is considered a herd problem, as individuals who show no symptoms might function as reservoirs of the disease (Radostits *et al*, 2007). Therefore, mastitis control schemes have shifted from individual to herd-level; including observation, treatment and prevention methods (Keefe, 1997).

Costs, risk factors and treatment of mastitis

Mastitis is one of the most common diseases among dairy cattle (Radostits *et al*, 2007). World-wide, the incidence of clinical mastitis per cow year lies between 20-40 cases per 100 cows. (Heringstad *et al*, 1999). Mastitis is also one of the most costly diseases associated with dairy production. The costs related to mastitis include treatment and veterinary expenditures, reduced production of milk during the rest of the lactation, loss of milk during antibiotics treatment; additional work associated with the disease, reduced milk quality and culling (Heikkilä *et al*, 2012).

Risk factors for mastitis fall into three major categories: exposure to bacteria, defense mechanisms of the cow, and environmental and managerial factors (Neave *et al,* 1969). According to Bhutto *et al* (2010), risk factors associated with mastitis – both clinical and subclinical – include big herd size, inadequate farm management procedures, poor udder and teat cleanliness, poor hygiene practices and diet. Other risk factors include teat position, lactation number, breed, previous mastitis episodes and disease around partition (Leelahapongsathon *et al*, 2014).

Mastitis is the most common disease associated with the use of antibiotics in cattle (Guterbock *et al*, 1993). Antibiotics are a common treatment method for clinical mastitis, and sometimes subclinical mastitis. Factors that affect the cure rate of antibiotics include individual parameters (age, lactation stage and length of infection), as well as managerial factors (detection-rate of mastitis and interval between detection and treatment). Other factors that affect the cure rate include choosing a suitable type of antibiotic, duration of treatment and administering the medicine in a correct manner. Lastly, bacteriological factors such as antibiotic resistance also affect the cure rate of these medicines (McDougall *et al* 2013).

Mastitis in and around India

In India, the average loss in milk yield due to clinical mastitis is approximately 50%. The same number for subclinical mastitis is 17.5%. Nation-wide reports suggest that the incidence of clinical mastitis varies from 3.94% to 23.25%, and for subclinical mastitis from 15.8% to 81.6% (Joshi & Gokale, 2006). Joshi & Gokale (2006) found in their study of 250 lactating cows from peri-urban farms in Maharashtra, that the incidence of subclinical mastitis in Holstein-Friesian crossbred cows was 46%. Comparatively, Rabbani & Samad (2010) reported a prevalence of 43.75% when studying 96 Holstein-Friesian crossbred cows in Rajbari district of neighboring country Bangladesh. Hegde *et al* (2012) studied the prevalence of subclinical mastitis in the organized (milk networks that pasteurize the milk) and the unorganized sector (private farmers who directly sell to milk to vendors) in and around Bangalore, India. They found the prevalence of subclinical mastitis to be 45% and 62% for the organized and unorganized sector respectively. Bangar *et al* (2014) conducted a meta-analysis of pooled data from 28 studies conducted in India, and found the overall prevalence of subclinical mastitis to be 46.6%. However, they reported a large heterogeneity between different studies. These authors concluded that the high prevalence of mastitis needs to be controlled by "adopting scientific, managerial, and therapeutic measures".

Somatic cell count

Somatic cells in milk are mainly immune system cells, part of the innate defense system in the udder. The somatic cells consist of macrophages, lymphocytes, polymorph-nuclear (e.g. neutrophils) cells and a limited amount of epithelial cells. In the uninfected quarter of the udder, these immune cells comprise around 80% of the total amount of cells. In the infected quarter however, more than 99% of the somatic cells are white blood cells, and the numbers can increase exponentially. Therefore, one can say that the somatic cells give an assessment of the inflammatory processes in the respective quarter, and therefore SCC can be used to determine the inflammatory state of the udder (Schukken *et al*, 2003).

According to Schukken *et al* (2003), research in Europe and North American has concluded that healthy quarters have a mean SCC of around 70 000 cells/ml, although this is individual and rises with age and reduced milk production. When infection occurs, leucocytes in that quarter signal to a pool of dormant white blood cells in the circulatory system. This results in an inflow of polymorph-nuclear cells (mostly neutrophils) to the milk in that quarter, in order to eliminate the bacteria. When this fails, it results in a chronic infection, and a constant activation of the immune system. This results in a long-term rise in SCC (Schukken *et al*, 2003). In many parts of the world, the price of the milk at the farm is linked to SCC in bulk milk (Whyte *et al*, 2004).

The California Mastitis test (CMT) is acknowledged as a simple field test to give an approximation of the SCC (Bhutto *et al*, 2010). When the CMT solution is mixed with milk in equivalent volumes, the CMT reagent disturbs both the cell wall and the nuclear cell wall of cells present in the milk (mainly leucocytes). This results in DNA being released from these cells into the milk. Together with the CMT reagent, the DNA forms a sticky gel. The gel formation is proportionate to total number of cells in the milk (Schalm & Noorlander, 1957). Mellenberger (2001) ranks the gel formation into five categories; N (negative, approx. 100.000 in SCC), T (trace, approx. 300 000 in SCC), 1 (approx. 900 000 in SCC), 2 (approx. 2.7 million in SCC) and 3 (approx. 8.1 million in SCC). Scores 1-3 are considered positives. Other ways of scoring CMT is also possible, with 1 being equal to the N in Mellenberg's (2010) scoring system, 2 equals T, and 3-5 equals 1-3 respectively (Persson & Olofsson, 2011). Of all indirect tests available for mastitis diagnosis, Joshi & Gokale (2006) found the CMT test to be most efficient, and therefore recommended it for field use.

Mastitis causing pathogens

Harmon (1994) classifies the significant mastitis-causing bacteria into *major* and *minor* pathogens. Major pathogens consist of *Staphylococcus aureus* and *Streptococcus agalactiae* (both of which are transmittable between individuals); and coliforms, streptococci and enterococci (bacteria that originate from the environment). The major pathogens often result in clinical illness, accompanied by changes in milk composition and increased somatic cell count. The minor pathogens include *Corynebacterium bovis* and coagulase-negative staphylococci. Infections caused by these bacteria usually lead to increased cell count and modest inflammation of the udder, but they only rarely lead to alterations of the milk composition (Harmon, 1994). There are indications however, that the spectrum of agents causing mastitis is varying over time and that geographic variation exist (Heringstad *et al*, 1999). In order to develop effective control methods of mastitis, one must understand the epidemiology of the pathogens involved (Joshi & Gokale, 2006).

Streptococcus

Streptococcal infections have been long known as causes of mastitis. The most common agents include *Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis* (Watts, 1988).

Streptococcus dysgalactiae is a gram-positive coccus (Radostits *et al*, 2007), regularly isolated from mastitis infections, both in the lactating and non-lactating period (Calvinho *et al*, 1997). In a New Zealand study on antimicrobial resistance of mastitis pathogens, McDougall *et al* (2013) found that *S. dysgalactiae* was fully susceptible to penicillin. The bacterium is contagious both through the environment as well as between individuals. Reservoirs include manure, bedding and the udder. Poor stable management and reduced individual hygiene increase risk of infection. *S. dysgalactiae* is also involved in the multi-complex type of mastitis which is known as 'summer mastitis'. The bacterium has been isolated from the fly *Hydrotaea irritans* which has been shown to be involved in the etiology of the above described clinical state. *S. dysgalactiae* uses structural mechanisms, enzymes and toxins in order to overthrow the natural defense system of the host (Calvinho *et al*, 1997).

Streptococcus agalactiae – a gram-positive coccus – is a central mastitis-causing pathogen. Before antibiotics were introduced as a treatment method for mastitis, *S. agalactiae* was an important cause of chronic mastitis. The pathogen remains a causative agent today but measures for diagnostics and treatment have now been implemented in many farms. *S. agalactiae* is highly contagious and normally leads to low-grade infections that are long-term. Non-symptomatic individuals may function as reservoirs. The bacteria cannot survive outside the mammary gland for long periods, and is often sensitive to treatment with penicillin. Therefore, suppression of the pathogen is possible in a closed herd through isolation and treatment or removal of infected animals. *S. agalactiae* has the capability to adhere to the mammary gland epithelium and different strains of the bacteria vary in virulence according to their adherence ability. The pathogen requires the typical micro-environment of the udder in order to multiply (Keefe, 1997).

Streptococcus uberis is also a common mastitis-causing agent. The bacterium is usually considered environmental (Radostits *et al*, 2007), however, some studies suggest that some strains of *S. uberis* can be transferred from cow to cow (Tassi *et al*, 2013). The virulence factors of *S. uberis* are poorly understood which makes it difficult to develop control strategies for treatment (Shome *et al*, 2012). However, McDougall *et al* (2013) found in a New Zealand antimicrobial sensitivity study that *S. uberis* is sensitive to penicillin.

Staphylococcus

Staphylococcus aureus is another gram-positive coccal bacterium that causes bovine mastitis, along with a range of other diseases in animals (Radostits *et al*, 2007). It is one of the most central mastitis-causing pathogens worldwide, although its prevalence differs from country to country. The bacterium causes significant losses to dairy production due to decreased milk quality and diminished milk yields. *S. aureus* is also a possible human health threat as it produces enterotoxin which may lead to food poisoning (Peton & Le Loir, 2013). Infections often cause subclinical mastitis with increased SCC, but may also cause severe clinical disease (Roy & Keefe, 2012). The bacteria produce toxins which disable neutrophil cells. Furthermore, between 40-60% of the different strains within the *S. aureus* genera can produce biofilms *in vitro*. Formation of biofilm *in vivo* may lead to decreased antimicrobial sensitivity, and diminishing cure rates. The main reservoir of the bacteria is the infected udder and the skin of the teats. It spreads during milking via the milking equipment or the farmers hands (Peton & Le Loir, 2013). The only treatment method available for *S. aureus* infection is the use of antibiotics. Unfortunately, the extensive use of antibiotics has led to resistant strains (Li & Zhang, 2013). Control measures against the bacteria include hygiene during milking, disinfecting the milking equipment and segregation of infected animals (Roy & Keefe, 2012).

Coagulase-negative staphylococci (CNS) constitute a group of many different bacteria. CNS are common causative agents of mastitis – in some countries the most prevalent. The most common bacteria isolated are *Staphylococcus chromogens* and *Staphylococcus simulans.* Under field conditions, the specific bacteria amongst CNS are usually not identified. Rather, they are treated as a common group. Infection with CNS results in most cases in a mild or subclinical infection characterized by an increased SCC. However, these infections may be persistent. Treatment methods differ in different countries and regions. Some treatment strategies include antimicrobial therapy even for mild infections, while other management plans suggest frequent milking. Recent studies show that CNS are susceptible to penicillin treatment, with a few exceptions (Pyörälä & Taponen, 2009). According to Pyörälä & Taponen (2009), the cure rate of CNS infection is high even without the use of antibiotics. However, there might be geographic differences to this, but local studies from India are difficult to find.

Escherichia coli

Mastitis caused by *Escherichia coli* is a frequent clinical state and often linked with an onset of acute symptoms (Radostits *et al*, 2007). In lactating dairy cows the outcome can be fatal. Endotoxinemia and disseminated intra-vascular coagulation (DIC) caused by the bacteremia are documented to be the cause of these fatalities. Individual cow factors such as age and lactation stage may influence the severity of infection (Hagiwara *et al*, 2014). Blum *et al* (2014) found that mammary infection with *E.coli* is associated with long-term reductions in milk production. The central virulence factor in *E. coli* is lipopolysaccharide (LPS), which is released when the bacteria dies or multiply. Broad-spectrum antibiotics are often used for treatment of *E.coli* mastitis. However, fallouts from treatment studies have been inconclusive. The only treatment methods that have showed scientific evidence are the use of flouroquinolones or cephalosporins. Alternative non-antibiotic treatment methods – for mild to moderate infections – have been suggested which include the use of fluid therapy, glucocorticoids and/or non-steroid anti-inflammatory drugs (NSAID) and repeated milking of the infected quarter (Suojala *et al*, 2013).

ILRI training

In 2009-2010, International Livestock Research Institute (ILRI) and local associates started to develop a training program for local farmers in order to enhance the informal dairy sector in and around Guwahati, India. More specifically, the initiative sought to "improve the hygiene and quality of milk produced and marketed by informal dairy market actors". Additional objectives included reducing risk of zoonotic and milk-borne diseases (e.g. brucellosis, tuberculosis) and to "make the informal dairy market actors competitive in the emerging open retail market lead by big corporate houses". With help from local associations (governmental as well as non-governmental), and funding from the World Bank, the training went underway in 2010-2013.

The local farmers association informed all farmers about the training, and as soon as enough farmers signed up, training was availed to all that were interested in the area. The program consisted of a 5 days course. The first day included an introduction focusing on information on the importance of safe milk and hygiene practices in milk production. The second day handled information regarding bacteria; its sources, how it spreads, how it can get into the milk and the importance of hand hygiene. The third day consisted of practical information on how to clean the farm house, its drainage and surroundings etc. The fourth day involved hygiene practices during milking (e.g. teat dipping, disinfection of teats) as well as cleaning routines regarding milking utensils. The last day focused on information regarding the most common cattle diseases, predisposing factors of disease as well as preventive measures.

The program went underway using discussions with the help of handouts and training manuals. Role play, group exercises and milk quality tests were also used as tools to deliver the training. The farmers were given printed handouts to take home for self-studies. Post training evaluation revealed that farmers gave the training a score of 8.9 out of 10. ILRI:s enumerators also reported that many farmers were pleased with the training program and that the training had produced positive fallouts. Hence, many untrained farmers also requested to avail the training. ILRI then decided to invest another USD 20 000 in order to train additional farmers and to bring a coherent end to the training program started in 2010. The project continued until mid-2013.

After the training ended, there were several informal reports from farmers who stated that the general health among their cattle had improved. Reports indicated that the frequency of mastitis had declined. However, a comparative study between trained and untrained farmers was never implemented until this study was performed.

AIMS OF THE STUDY

The aims of this study is to investigate the prevalence of clinical and subclinical mastitis in dairy cows based on the California Mastitis Test (CMT) and clinical examination of the udder, in trained as well as untrained farms in Guwahati, Assam, to see if the hygiene training given by ILRI has led to lower disease burden. The objective also includes getting an understanding of the pathogens involved, cow factors associated with higher CMT scores and comparing trained with untrained farms regarding milk production and hygiene routines. The study is part of a larger project led by Dr. Ram Pratim Deka involving two more SLU students, whose objectives include investigating prevalence of Brucella and aflatoxins, as well as evaluation of clinical parameters (e.g. hoof health and body condition score).

MATERIAL AND METHODS

Study area

Located in the north-eastern part of India, Guwahati is the largest commercial, educational and industrial hub in the region and the capital city of the state of Assam (City development plan, Guwahati, 2006). The study was conducted from September 1st to November 15th in the hilly areas around the city where the dairy farmers are settled. Elevated 55.5 meters above sea level, Guwahati has a humid subtropical climate. During September to November, the average low is 21.1 degrees and the average high is 29.7 degrees. The studied was conducted during the end of the rain period, and the average rainfall during this period is around 100 mm per month.

Study animals

A questionnaire survey was conducted on 73 trained and 76 untrained farms. From these, 25 trained and 25 untrained farms were chosen for additional CMT and clinical examination of the udder, on 25% of the lactating cows on the farm, with a minimum of 3 cows examined. Only a fourth of the lactating cows were examined because of time constraint and consideration of the objectives of the Brucella and aflatoxin study. The number of cows examined on each farm varied from 3 to 6. The trained farms were randomly selected from a list consisting of all trained farmers in each region. In total, five regions around Guwahati were visited. For the untrained farms, a list of untrained farms was created in each region with the help of a local guide. From this list, untrained farms to include in the study were chosen randomly. All cows on the farms were hand-milked twice daily. A total number of 178 cows were examined during the study. In the questionnaire, farmers were asked to give the average milk production per day today, as well the average milk production before training/two years ago.

Study design

Milk and data collection from farms were acquired from September 15th to November 1th, 2014. 25% of the lactating cows (with or without signs of clinical mastitis) on each farm were chosen for a clinical examination of the udder and evaluation of the milk using the California Mastitis test. Criteria for clinical mastitis included swollen, hard or warm udder together with abnormal milk composition. Criteria for subclinical mastitis included a raise in CMT without clinical signs of inflammation. A basic clinical sheet was also completed for each cow. The clinical sheet included information on parity number, stage of lactation, presence of teat lesions and udder hygiene. Udder hygiene score was ranked on a scale from 1 to 4. A score of 1 indicates that the udder is free of dirt, a score of 2 indicates that the udder is slightly dirty (dirt on 2-10 % of surface area), a score of 3 indicates that the udder is moderately dirty (dirt on 10-30 % of surface area) and a score of 4 indicates that the udder is very dirty (dirt on >30% of surface area). The animals were chosen according to randomization; for example, if the farm had 20 lactating cows, every fourth lactating cow were chosen for examination, with varying start numbers.

CMT and sample collection

Milk was collected after the first and before the second milking of the day. Initially, the first three sprays of milk were discarded. Subsequently, equal amounts of milk and CMT were added in each cup of a CMT paddle. Next, the CMT paddle was gently rotated. CMT score 1-5 was determined according to the method described by Schalm & Noorlander (1957) and Mellenberger (2001), with 1 being normal milk. The CMT paddle was rinsed with water after each examined individual. Cows with a CMT score of 3 or more were considered positive and subjected to milk sampling for bacteriological examination. Milk collection was performed aseptically. Before sampling, the teat was cleaned with water followed by a change of plastic gloves. Afterwards the teat was disinfected with a 70% alcohol solution and let dry for approximately 30 seconds. Sterile test tubes were opened faced down to prevent contamination, and the milk collection was completed by holding the test tube in a 45 degree angle while milking only one milk stream into the tube. Once sampled, the test tubes were put in a cool bag.

Bacterial examination

Bacterial examination was carried out in the North Eastern Region Disease Diagnostic Laboratory (NERDDL), a Government of India funded laboratory in Guwahati, Assam. The bacterial culturing was always carried out the same day as sampling except on Fridays when the samples were put in a refrigerator over the weekend. All handling of the milk samples were carried out in a fume cabinet.

Initially, all milk samples were spread out with a 10 µl sterile plastic loop on a 5% sheep blood agar plate. The plate was immediately put in the incubator at 37 degrees for 24 hours. If there was no growth after 24 hours, the plate was put into the incubator for another 24 hours. If positive growth after 24 hours (or 48 hours), 3-5 colonies were spread out on a McConkey and Mannitole-Salt agar plate and put in the incubator for 24 hours. Afterwards, the McConkey and Mannitole-Salt agar plates were examined for growth. All colonies were judged from mild, moderate to severe growth. The colonies were also inspected for presence of alpha- or beta hemolysis.

If both the McConkey and Mannitole-Salt agar plates showed negative growth, the bacteria was considered *Streptococcal* and an agglutination test (Himedia Histrept identification kit) was carried out in order to classify the Streptococcal bacteria into Lancefield groups.

If there was growth on the Mannitole-Salt agar but not the McConkey agar plate, the bacteria were considered to be *Staphylococcal.* If the Mannitole-Salt agar showed a yellowish color-change, the bacteria was considered to be *Staphylococcus aureus*. A Cefinase patch was then put in the area of the color change and the Mannitole-Salt agar was once more put into the incubator, this time for 30 minutes. If a strong purple color change was observable on the Cefinase patch, the grown *Staphylococcus* strain was considered being able to produce penicillinase. If growth but no observable color-change on the Mannitole-Salt agar plate, the bacteria was considered to be coagulase-negative Staphylococcus.

If there was growth on the McConkey agar plate but not the Mannitole-Salt agar plate, the bacteria was considered gram-negative.

Statistical analysis

Based on the CMT results and clinical findings, prevalence for clinical and subclinical mastitis was calculated on cow and quarter level for all cows, cows on trained farms and cows on untrained farms. CMT scores were tabulated by descriptive statistics and analyzed with a χ^2–test in order to see if there exists a significant difference between trained and untrained farmers. χ^2–tests were also performed to see correlation between CMT and parity, lactation stage and udder hygiene score.

Milk production was compared with descriptive statistics and with a two sample unpaired t-test in order to see if there existed a significant difference.

A multivariable analysis using xtmelogit was performed with training status, parity, body condition score, lactation status as independent variables, subclinical mastitis as dependent variable and farm as random effect.

Error sources

The CMT solution order did not arrive in time. Therefore, we had to prepare a CMT-reagent using a recipe of Sodium hydroxide - 1.5 g, Teepol - 0.5ml, Bromothymol blue - 0.01 g and distilled water - 100 ml for producing 100 ml reagent. This CMT solution was used for approximately two weeks before the original order arrived. Once the original order arrived, the two solutions were compared using around 10 milk samples. The CMT score from the two different solutions were similar. However it might be difficult to extrapolate these results, which might lead to a small skew in CMT score. In the analyses below, the results are treated as if the CMT solutions were equal.

The agglutination test for Lancefield-grouping resulted in a number of negative tests (non-Lancefield-classable streptococci). It is likely that these 'negative' streptococci are in fact *Streptococcus uberis*, since this bacteria is not classable by Lancefield grouping, but a common udder pathogen. Another explanation could be that these bacteria might not be streptococcal whatsoever. All McConkey agar plates showed negative growth, and were therefore suspected to be malfunctioned. However, gram-negative bacteria are less common in subclinical disease, and it might also be that all samples were negative.

The bacteria were tested under 'field conditions'; meaning no confirmatory testing was done of the bacteria. It is therefore possible the bacterial outcome is not entirely accurate.

RESULTS

Prevalence clinical and subclinical mastitis

A total number of 178 cows – on untrained and untrained farms – were screened with the California Mastitis Test and clinical examination of the udder. A total of 712 quarters were examined. Of these, 12 quarters were blind. All blind quarters came from individual cows. Prevalence for clinical and subclinical mastitis on cow and quarter level is presented in table 1.

Table 1. The prevalence of clinical mastitis and subclinical mastitis on cow and quarter level based on CMT and clinical examination of the udder, and mean CMT score for all cows, cows on trained farms and cows on untrained farms in Assam, India.

	Cow level: Prevalence clinical mastitis	**Cow level: Prevalence subclinical mastitis**	**Quarter level: Prevalence clinical mastitis**	**Quarter level: Prevalence subclinical mastitis**	**Mean CMT score**
All cows (n=178)	6.2 %	50.6 %	1.6 %	26.6 %	2.08
Cows on trained farms (n=92)	7.6 %	44.6 %	1.9 %	23.9 %	1.98
Cows on untrained farms (n=86)	4.6 %	57.0 %	1.2 %	29.5 %	2.18

Distribution of CMT scores

The distribution of CMT scores is shown in table 2. A χ^2–test was performed in order to see if there exists a significant difference in CMT distribution between trained and untrained farms. The test shows a near significant association (p=0.08), indicating a lower CMT score in trained farms. Another χ^2–test was run to see if there exists a correlation between CMT≥3 (clinical/subclinical mastitis) and trained/untrained farms. However, this test did not result in significant or near significant results.

Table 2. Distribution of CMT scores for cows sampled on trained farms and untrained farms in Assam, India.

	CMT 1	CMT 2	CMT 3	CMT 4	CMT 5
Percentage of quarters (all cows)	36.9%	34.9%	15.2%	9.7%	3.3%
Percentage of quarters (trained farms)	41.9%	32.2%	14.7%	8.3%	2.8%
Percentage of quarters (untrained farms)	31.6%	37.8%	15.6%	11.2%	3.8%

CMT score on the basis of stage of lactation, parity and udder hygiene

Prevalence of CMT≥3 on the basis of stage of lactation

The results indicate a higher CMT score in later lactation stages (see table 3). A χ^2–test was performed to test significance. The results show a close to significant correlation (p=0.06).

Prevalence of CMT≥3 on the basis of parity

The results indicate a general tendency against higher CMT scores for higher parity numbers (see table 3). A χ^2–test was performed to see test significance. The results show a near significant correlation (p=0.06).

Prevalence of CMT≥3 on the basis of udder hygiene

(See table 3). A χ^2–test was performed to test significance. However, no significant correlation was found. Results from the clinical examinations of the udders show that trained farms had an average udder hygiene score of 2.23 while untrained farms had an average udder hygiene score of 1.99, actually indicating dirtier udders in trained farms.

A multivariable analysis was performed but did not result in any variable coming out as significant or near significant.

Table 3. Number of cows and udder quarters with CMT≥3 and possible risk factors

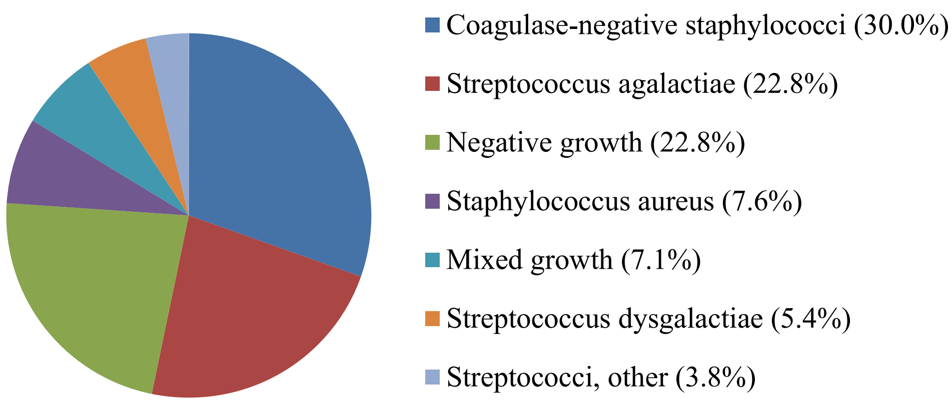
Factors	Type	Number of tested cows	Number of CMT ≥3 (% of cows)	Number of udder quarters tested	Number of CMT ≥3 (% of udder quarters)
Stage of lactation	<30 days	34	16 (47.1%)	133	28 (21.1%)
	31-120 days	59	34 (57.6%)	233	63 (27.0%)
	>120 days	85	51 (60.0%)	334	106 (31.7%)
		Total: 178	Total: 101	Total: 700	Total 197
Parity	1	34	18 (52.9%)	133	31 (23.3%)
	2	28	13 (46.4%)	111	23 (20.7%)
	3	52	30 (57.7%)	205	62 (30.2%)
	≥4	64	40 (62.5%)	251	81 (32.3%)
		Total: 178	Total: 101	Total: 700	Total: 197
Udder hygiene	1	45	25 (55.6%)	179	48 (26.8%)
	2	82	47 (57.3%)	320	100 (31.3%)
	3	40	21 (52.5%)	158	37 (23.4%)
	4	11	8 (72.2%)	43	12 (27.9%)
		Total: 178	Total: 101	Total: 700	Total: 197

Bacterial analysis

On quarter level for subclinical mastitis (n=184), coagulase-negative staphylococci (n=56) were most common, followed by *S. agalactiae* (n=42), negative growth (n=42), *S. aureus* (n=14), mixed growth (n=13), *S. dysgalactiae* (n=10) and 'streptococci, other' (n=7) (See figure 1).

On quarter level for clinical mastitis (n=11), S. agalactiae (n=4) and negative growth (n=4) were most common, followed *by S. aureus* (n=2) and coagulase-negative staphylococci (n=1). (See figure 2).

The streptococci were divided into Lancefield groups using an agglutination test (Himedia). Some streptococci were not separable into Lancefield groups. These streptococci are described as 'streptococci, other'.



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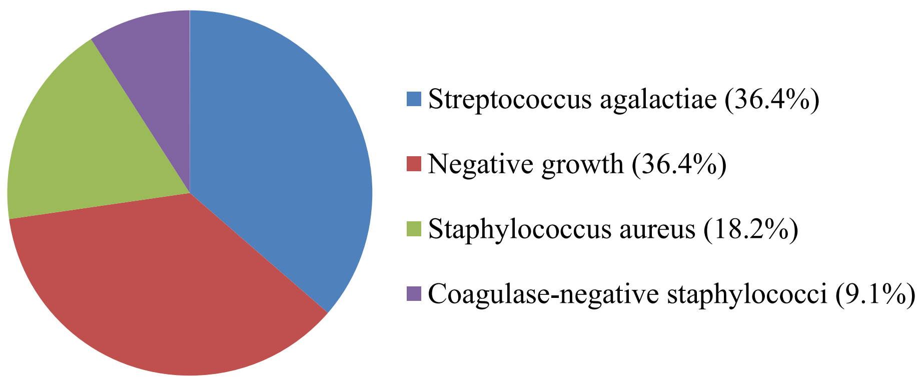
untrained farms in Assam, India. The figures after each pathogen represent the percentage of positive



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farms in Assam, India. The figures after each pathogen represent the percentage of positive cultures.

Cefinase test for penicillinase production in *Staphylococcus aureus*

All *S. aureus* isolates with color change on the mannitole-salt agar plates were tested for penicillinase production using a Cefinase-test (manufactured by bioMérieux). All tested *S. aureus* cultures (n=16) were negative for penicillinase production.

Milk production on trained and untrained farms

Information on average milk production per cow and day – before and after the training – was attained from the questionnaire. Milk production for each examined cow was not recorded. Among untrained farmers, average milk production per cow and day was 6.8 liters. Among trained farmers, the average milk production was 7.8 liters (See table 4). Hence, trained farmers showed a 14.6% increase in milk production compared to untrained farmers.

Trained farmers also reported an increased milk production since availing the training. Before the hygiene training, the average milk production among trained farmers was reported to be 7.0 liters per cow and day. For trained farmers, after availing the training, milk production increased by 11.7 %. Untrained farmers on the other hand, saw a 6.9% decrease in milk production when compared to milk production two years ago.

A two sample unpaired t-test comparing trained with untrained farmers on milk productions shows that the difference is significant ($p<0.01$), indicating that the training has led to a real increase in milk production.

Table 4. Average milk production in trained and untrained farms, before the hygiene training/two years ago and today, based on data given by the farmers in Assam, India.

	Average milk production in liters per cow and day 2 years ago/before ILRI training	**Average milk production in liters per cow and day now**
Trained farmers	7.0 (range 2.5-10)	7.8 (range 3-15)
Untrained farmers	7.3 (range 2.5-14)	6.8 (range 2.5-14)

Milking hygiene on trained and untrained farms

When asked whether the udder is being cleaned/disinfected before milking, 98.6 % of the trained and 96.2 % of the untrained answered “yes”. When asked whether the udder is cleaned/disinfected after milking, 74.0 % of the trained farmers and 53.2 % of the untrained farmers answered “yes”, indicating improved hygiene procedures in trained farms. Among these farmers, all used simply water to clean before and/or after milking. All farmers – both trained and untrained – answered yes when asked whether the milking utensils are cleaned after each milking session.

DISCUSSION

A prevalence of 50.3 % for subclinical mastitis on cow level and 26.6 % for subclinical mastitis on quarter level is in harmony with other studies conducted in India and in nearby countries. Bangar *et al* (2014) found, during their meta-analysis of 6344 cows in India, the prevalence of subclinical mastitis on cow level to be 46.4 %, and on quarter level 23.3%. Similarly, Joshi & Gokale (2006) found in their study the incidence of subclinical mastitis on cow level to be 46%, and on quarter level between 19.8% and 27.0%. Hedge *et al* (2012) found the prevalence of subclinical mastitis in the organized and unorganized sector to be 45% and 62% respectively. Rabbani & Samad (2010) found the prevalence of subclinical mastitis to be 43.8% on cow level and 19.2% on quarter level in a study conducted in neighboring country Bangladesh. In a Chinese study of subclinical mastitis, the prevalence was 52.3% and 20.3% on cow and quarter level respectively (Memon *et al,* 2012). For clinical mastitis, this study recorded a prevalence of 6.2% on cow level and 1.6% on quarter level. Other reports in the locale suggest similar findings. Joshi & Gokale (2006) report a nation-wide incidence on clinical mastitis on cow level between 1-10%. Bhatt *et al* (2011) found in a mastitis study in western India the prevalence of clinical mastitis to be 5.5%. However, a further comparison of clinical mastitis is problematic since most studies in and around India only includes data on subclinical mastitis.

Joshi & Gokale (2006) found the monsoon season more likely to cause increased prevalence of subclinical mastitis. This could offer an explanation to the slightly higher prevalence of subclinical mastitis found in this study, when comparing pooled data from the study conducted by Bangar *et al* (2014).

A near significant correlation (p=0.08) exists between CMT score and trained/untrained farms, indicating lower CMT score from milk in trained farms. This is an encouraging result, suggesting that the hygiene training has led to desirable results. Trained farms had 12.4% and 5.6% lower prevalence of subclinical mastitis on cow and quarter level respectively. This difference however was not found to be statistically significant.

The overall bacterial outcome saw exclusively gram-positive cultures. For subclinical mastitis, coagulase-negative staphylococci (30.0%) were the most prominent group of bacteria, followed by *S. agalactiae* (22.8%), negative growth (22.8%), *S. aureus* (7.6%), mixed growth (7.1%), *S. dysgalactiae* (5.4%) and 'streptococci, other' (3.8%). This result is not in accordance with other studies conducted in the locale. Joshi & Gokale (2006) found in their subclinical mastitis study that mixed infection (37.4%) was the most prominent bacterial outcome, followed by gram-positive bacteria (35.7%) and gram-negative bacteria (27.0%). In this study however, there was no further grouping of the bacteria and no mention of negative growth. A large number of results with multiple bacteria in subclinical mastitis may also be indicative of problems with sample contamination. Hegde *et al* (2012) found in their subclinical mastitis study that staphylococci were the most common bacteriological outcome, followed by streptococci and *E.coli*. However, in this particular study there was also no mention of negative growth. For clinical mastitis, a comparison to other studies is more problematic since this study only recorded a few clinical mastitis cases. Also, it is difficult to find studies on clinical mastitis in the locale. When studying the role of *E. coli* in clinical mastitis cases in eastern China, Memon *et al* (2012) concluded that more than one third of the cultures yielded the bacteria. This is not in accordance with this study where no gram-negative bacteria were found whatsoever. It is suspicious that this study could show no gram-negative bacteria, and the possibility of defective agar plates may be a source of error.

The fact that all *S. aureus* were negative on the test for penicillinase production is a positive and unexpected outcome. It would have been intriguing to further study antibiotic resistance in other groups of bacteria as well, however time and material constraint made it unmanageable.

This study recorded a tendency of higher CMT scores in later lactation stages and in higher parity numbers. This is in line with the findings of Joshi & Gokale (2006) who found the incidence of subclinical mastitis to increase in later lactation stages and higher parity. Memon *et al* (2012) also found similar results in their study; a tendency towards higher prevalence of subclinical mastitis in later lactation stages and in higher parity numbers.

A significant difference exists between trained and untrained farms in regards to milk production. This is a welcoming fact since it indicates that the hygiene training has led to some valuable results for the farmers. Milk production must be considered the most important factor when comparing trained with untrained farms, since it directly affects the economy and the livelihood of the farmer. Since it is difficult for farmers to perceive improved milk quality as an outcome of changed behavior, it is important with an additional outcome with direct positive effects that might make improved hygiene more likely to be sustained. In this study, milk production was only recorded on farm level, not on individual level. Looking back, it would have been better to record milk production for each examined cow in order to compare it to various other parameters such as CMT score and udder hygiene. It is possible that farmers participating in the training were more motivated than farmers who did not partake. Therefore, the increase in milk production could be the result of simply more motivated and hardworking farmers. In order to account for this, recall data about earlier milk production was collected, and interestingly, the average milk production was then lower for the group that was trained, indicating that this group of farmers was not more productive than the other from the beginning. This study cannot demonstrate which aspect of the training that might have caused the increased milk production, but improved udder health might constitute part of the explanation.

It seems that trained farmers have better hygiene practices when it comes to cleaning the teats after milking. However this is contradictory since the results from the clinical examinations show that cows in trained farms generally had dirtier udders. While it is positive that the udder is being cleaned before and/or after milking, it would have been even better if some form of disinfectant was being used instead of just water. Possible reasons for not using disinfectant after milking might be lack of information or economic restraints. In future ILRI trainings, more focus on information regarding disinfection of the teats after milking could prove beneficial. One observation made during discussions with the farmers regarding mastitis, is that farmers in general were unaware of grouping of sick individuals and milking them last in the milking order. Perhaps these specifics could also be added in future training made by ILRI; grouping of mastitis-positive cows, keeping them last in the milking order and teach the farmers to pay extra attention to the hygiene routines around milking in order to prevent spread of bacteria to other cows in the herd. This could possibly decrease the mastitis prevalence in the region, preventing bacteria like *S. agalactiae* (a significant bacterium in this study) to spread between cows.

The primary purpose of this training was not to reduce mastitis, but to improve milk hygiene. However, perceived reduced risk of mastitis and increased milk yield seem to be the main reasons why farmers are happy with the training and continue. It would have been intriguing to also investigate what force drives some farmers to attend the training, while other farmers choose not to participate. However, time constraints made such inquiries unmanageable. It is possible that the ones who attended the ILRI training were the most motivated farmers and this fact could affect the results of this study.

CONCLUSION

This is the first study showing how hygiene training can have an impact on mastitis and milk production in India. From this study – as well as other studies conducted in nearby areas – it is clear that subclinical and clinical mastitis pose a major challenge for farmers. The comparative results in milk production indicate that the hygiene training has led to some beneficial results, although it is not possible to show an objective reduction in cases of mastitis. Additional training to more farmers could increase the milk production in the region, making it more competitive on a national level, and increasing the livelihood of the farmers. There are however further improvements that need to be done; especially when it comes to cleaning and disinfection of the udders and segregation of sick animals, and future training made by ILRI should focus more on these aspects.

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