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With special focus on animal welfare, milk yield and brucellosis

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Kan hygienträning av bönder i nordöstra Indien resultera i friskare kor?

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SUMMARY

In the years of 2009-2013 a hygiene training program was developed in the north eastern state of Assam, India, by the International Livestock Research Institute (ILRI) together with local partners. The goal was to improve the hygiene and quality throughout the process of producing and selling milk. This study aimed to follow up the training and to critically evaluate the impact the training had on the overall health and welfare of the animals as well as the milk production. Seroprevalence of brucellosis in the area was also investigated. The study was carried out as a Minor Field Study financed by the Swedish international development cooperation agency (SIDA). A comparison was made between 74 farms where the farmer previously had engaged in hygiene training ("trained farm") and 76 farms where nobody had been trained ("non-trained farm") regarding overall health status, hygiene routines and seroprevalence of brucellosis. Clinical examinations were used to check body condition, hygiene status and hoof status of the cows. Milk sampling took place to investigate milk seroprevalence of brucellosis, using the Brucella milk ring test (MRT) and a questionnaire was used to interview farmers about hygiene routines, health status, milk yield etc. The results showed that the ILRI hygiene training had positive effects in some areas and foremost was associated with a significant increase in the milk yield (p=0.003), with a mean increase of 0.67 liters of milk per cow per day in trained farms. Because milk production is the main income for 90% of the farmers, this increase should have a large impact on their economy. No difference was seen between trained and non-trained farms regarding body condition, hygiene and hoof status. However, a majority of the trained farmers had experienced an improvement in the overall health status of the animals.

The MRT showed that at least half of the tested cows in Assam were positive for brucellosis. No difference was seen between trained and non-trained farmers which was not surprising since the hygiene training did not focus on disease control. Six areas were visited and significant differences were seen in seroprevalence with the area of 8th Mile having the most brucellosis and Amsing Jorabat having the least. One important factor in the spreading of brucellosis is through infected semen, and it is recommended that routines for breeding are studied further. Increasing the general knowledge about the ways of infection is important in trying to limit the spread of the disease, especially in a country like India where slaughter of cows is prohibited.

The conclusions of the study were that hygiene training is associated with a positive effect in milk yield thus having an important impact in improving the economy of the farmers. It also seems to have improved the over-all health status among dairy cows based on experience of the farmers.

SAMMANFATTNING

Under åren 2009-2013 påbörjades ett program för att träna lokala mjölkbönder i hygienrutiner i Assam, nordöstra Indien. Programmet utvecklades av International Livestock Research Institute (ILRI) i ett arbete att förbättra hygien och kvalitet kring mjölkproduktion och försäljning. Den här studien hade som mål att kritiskt utvärdera sagda hygienprogram för att se om det fanns några effekter av träningen inom områden som inte direkt hade berörts, genom att utvärdera kornas allmänna hälsa och välfärd, mjölkavkastning, och seroprevalens av brucellos. Studien var en del i en Minor Field Study (MFS) finansierat av SIDA (styrelsen för internationellt samarbete). En jämförelse gjordes mellan 74 gårdar, vars ägare deltagit i hygienträningen ("tränad gård") och 76 gårdar där ingen hade deltagit i träning (icke-tränad gård") avseende hälsostatus på djuren, hygienrutiner samt seroprevalens av brucellos. För att utvärdera djurens hälso- och välfärdsstatus utfördes hullbedömning (BCS), klövbedömning samt hygienbedömning genom klinisk undersökning. Mjölkprover togs för att undersöka seroprevalens av *Brucella abortus* i mjölk genom användning av agglutinationstest, s.k. Brucella milk ring test (MRT). Ett frågeformulär togs fram för intervjuer med bönderna och bestod av frågor gällande hygienrutiner vid mjölkning, hälsostatus på djuren, mjölkavkastning etc. Resultaten visade att ILRI:s hygienträning haft positiva effekter på framförallt mjölkavkastningen där produktionen ökat signifikant (p=0.003) med i snitt 0.67 liter per ko per dag på tränade gårdar. Eftersom mjölkproduktionen hos 90% av bönderna står för den huvudsakliga inkomsten bör en sådan ökning ha en stor betydelse för ekonomin på gården. Vid jämförelse av tränade och icke-tränade gårdar sågs ingen signifikant skillnad med avseende på BCS, hygien och klövstatus. Dock upplevde en klar majoritet av de tränade bönderna en allmän förbättring gällande djurens hälsa liksom tidigare nämnda ökade mjölkavkastning.

Provtagning och test för *Brucella abortus* visade att mer än hälften av de provtagna korna i Assam var positiva för brucellos. Ingen skillnad sågs mellan den tränade och den otränade gruppen, dock fokuserade inte hygienprogrammet på smittkontroll och resultatet är därför ej förvånande. Sex områden besöktes och en signifikant skillnad i seroprevalens av brucellos sågs, "8th mile" var det område med högst andel brucellos och Amsing Jorabat det område med minst. En viktig smittväg för brucellos är via infekterad sperma och det rekommenderas att betydelsen av denna smittväg studeras vidare. Att öka allmän kännedom och kunskap om hur sjukdomen sprids är viktigt för att förhindra ytterligare spridning, något som är av yttersta vikt i ett land som Indien där slakt av kor inte är tillåtet i alla stater.

De slutsatser som kan dras av studien är att hygienträning haft en positiv effekt på mjölkavkastning och därmed spelar en viktig roll i att öka inkomsten hos mjölkbönder i Assam. Träningen tycks även ha lett till en allmän förbättring av hälsostatus bland korna, något som dock ej kunnat konstateras utifrån kliniska parametrar utan baseras på böndernas egen uppfattning.

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INTRODUCTION

With 17% of the total global milk production, India is today the number one milk-producing country in the world. The dairy production is mainly a small-scale, family-run industry and the milk production is mainly carried out by small, rural-based farmers and laborers with no own land (Douphrate *et al.*, 2013).

Milk is contributing to the Indian food security, and is an important source of animal source proteins for the large vegetarian population, but there are still food safety issues. It is widely known that milk can be an important source of many food-borne pathogens and therefore impose a risk to human health. This risk is affected by many factors including farm management practices and hygiene. The farm environment can serve as an important reservoir of microorganisms that could contaminate milk directly through contact with contaminated surfaces, equipment or tools, or indirectly through poor udder and milking hygiene, which can cause mastitis leading to excretion of bacteria in the milk (Oliver e*t al.*, 2005). One such food-borne pathogen is *Brucella abortus* the bacteria causing bovine brucellosis, which is an important zoonotic disease and public health hazard (Corbel, 2006; Radostits *et al.*, 2007).

Hygiene training

In the years of 2009-2010, a hygiene training program was developed in the north eastern state of Assam by the International Livestock Research Institute (ILRI) together with local partners, in order to improve the hygiene and quality throughout the process of producing and selling milk. The overall objectives were:

"…to improve the hygiene and quality of milk produced and marketed by informal dairy market actors, to reduce the risk of zoonotic (eg. brucellosis, tuberculosis etc.), and milk borne diseases, to make the informal dairy market actors competitive in the emerging open retail market lead by big corporate houses, to increase self-esteem, self-satisfaction and social status of the informal dairy market actors, to bring the informal sector dairy market actors under the ambit of some sort of regulation".

The first training was taking place during 2009-2011 and included 471 farmers from different parts of the state, both male and female (ILRI, 2013). The farmers took part in a 5-day training course with daily lectures and practical exercises concerning good husbandry, hygienic milking routines and milk handling etc. (Table 1). Due to continued demand from farmers more training was performed during 2012-2013. All farmers that displayed an interest were allowed to participate.

Table 1. *Contents of ILRI hygiene training* (ILRI, 2013)

Day 1	Introduction **-** Overview and objective of the training, group work on the advantages of clean, good quality milk, group work on germs, what hygiene is
Day 2	Knowledge of what germs are, sources of germ and how they spread, myths about food safety, correct hand wash
Day 3	Dairy types and breeds, what to think about when buying cattle, how to keep your cow healthy, preventing mastitis, good husbandry including milking routines and routines on farm
Day 4	Rules and equipment for hygienic milking and milk handling, hand milking, teat dipping, practical exercise
Day 5	Milk handling, cleaning agents and disinfectants, cleaning of milk containers and cloths for washing and drying teats, practical exercises

Aim of the study

The aim of this study was to investigate whether the mentioned hygiene training project affected the health status of the animals and the economy of the farmers. Although one of the goals with the training program had been to reduce milk-borne diseases such as brucellosis, the program did not include training on how to reduce the prevalence of the diseases in the animals. This study also investigated seroprevalence of brucellosis in the milking cows in order to see if the increased awareness about zoonotic diseases would have been associated with reduced prevalence.

This study also aimed to critically evaluate if a hygienic training leads to a better animal health and welfare with increased milk production and therefore in the end a better economic situation for the farmers. As with most interventions, it is important to evaluate the impact in order to be able to give advice for future investments.

LITTERATURE REVIEW

Indian dairy production

For many small farmers the dairy production is primarily carried out for their families' own consumption but also play an important role as a supplementary income source (Rajendran & Mohanty, 2004). Across the country there are 38.5 million dairy cows (Douphrate *et al.*, 2013), most commonly kept in small herds (Renukaradhya *et al.,* 2002) with the average herd size being around two milking cows (Douphrate *et al.,* 2013). In Assam, a Northeastern state of India, 82% of households have a cow or a buffalo. The milk production is mainly carried out by rural-based smallholders using local cattle, or by peri-urban/urban farmers more specialized in dairy farming, mainly using cross-breeds. Most commonly, the animals are kept in small herds consisting of two to eight animals which usually are kept in tied stalls. The milking is done by hand (Kumar & Staal, 2010). Feed can consist of mainly agricultural bi-products with only some concentrate or compound feed.

The milk is to a large part consumed or used on-farm, but for many farmers also selling a part of the milk creates an opportunity to earn a market-based income (NDDB, 2014). The organized milk market however is fairly insignificant in Assam, with only a small part (about three percent) of the milk being sold via the official market of pasteurized milk- and dairy products (ILRI, 2007). Compared to the dairy industry in the rest of India, the development in Assam has been slow (NDDB, 2014).

In Assam indigenous cattle are dominating in the dairy sector. The milk yield of these cows can be very low, with a mean production of one liter per cow and day. A slightly higher, but still very low, milk yield of about 3.6 L per cow and day is observed in the small cross-bred population, (ILRI, 2007). One of the most important factors resulting in a low milk production is disease (Diskhita & Birthalb, 2010). Zoonotic bacterial reproductive diseases are known to affect not only animal production but also human health.

Brucellosis

Brucellosis is one of the most common zoonotic diseases in the world (Radostits *et al.,* 2007; Mantur and Amarnath, 2008). It is an important disease amongst cattle and a cause for reproductive problems such as delayed heat, abortions, and infertility as well as interruption of lactation and loss of milk production. This makes brucellosis an economically important disease resulting in loss of income, especially in developing countries without national control programs for brucellosis (Renukaradhya *et al.,* 2002; Radostits *et al.,* 2007).

Brucellosis is caused by bacteria from the *Brucella spp*, which are gram negative, facultative intracellular coccobacilli or short rods. There are several different species where *Brucella abortus* mainly causes disease in cattle, *B. melitensis* affects sheep and goats, *B. suis* is the main cause of brucellosis in pigs and *B. canis* in dogs. Humans can get infected with all of the above species, with the *B. melitensis* being the most common (Radostits *et al.,* 2007; Mantur & Amarnath, 2008). The disease is easy to miss in humans since it may present itself with symptoms similar to for example influenza, malaria, typhoid and paratyphoid (Reukaradhya *et al.* 2002).

In female animals the bacterium is concentrated in the uterus, fetus, fetal membranes and in the udder, causing large amounts of bacteria to be shed through the placenta, fetus, uterine discharges and the milk. In males infection can lead to temporary or permanent infertility following orchitis or epididymitis (Radostits *et al.,* 2007; Gwida *et al.,* 2010). The bacteria can be transmitted in the semen, and artificial insemination is causing the highest risk (Radostits *et al.,* 2007), since one infected male can spread the disease to many females. Movement of animals, unrestricted trade and poor farm hygiene are some parameters that are associated with transmission of bovine brucellosis between animals (Gwida *et al.,* 2010).

Infection in humans can occur through inhalation, ingestion, entrance through skin lesions or conjunctiva. Transmission of the disease from animals to humans is mostly through infected domestic animals, with dairy products posing an important risk since the concentration of bacteria can become very high in products made from unpasteurized milk (Radostits *et al.,* 2007; Mantur & Amarnath, 2008). The bacteria can survive for a long time in the environment, aborted fetuses, meat and dairy products. People working with animals or meat such as farmers, veterinarians or butchers run a higher risk of exposure and therefore a higher risk of being infected (Radostits e*t al.* 2007).

Increasing public awareness about disease control and reducing disease in cattle are the most important factors to prevent zoonotic infection, since there is no vaccine available for humans (Mantur & Amarnath, 2008). The spread of infection from animals to humans has however been reduced, mainly due to the practice of boiling the milk before consumption (Reukaradhya *et al.,* 2002). Disease prevalence in cattle can be reduced through vaccination. The most common vaccine used is called *Brucella abortus* S19. Vaccination is usually done in female calves at the age of 3 to 6 months, but a reduced dose can be used in adult animals, administrated subcutaneously or through the conjunctiva (OIE, 2015).

Treating brucellosis is difficult since it is caused by an intracellular bacterium, and is therefore not possible to reach with most antibiotics, even though most of the strains actually are sensitive to many antimicrobials (Radostits *et al*., 2007; Mantur & Amarnath, 2008). To reduce spreading of the disease, control programs have been developed in many countries, using vaccination of healthy animals and slaughter of exposed or infected animals (Radostits *et al*., 2007).

Detecting Brucella abortus

There are a number of different laboratory techniques used to detect *Brucella abortus* in animals. Isolation and identification can be made from different clinical samples, such as milk, semen or serum, and the testing can aim to detect antigens, genome and antibodies. The tests used to detect antibodies against brucellosis can be divided into agglutination tests, complement fixation tests, precipitation tests and primary binding tests (Nielsen, 2002; Radostits *et al.,* 2007). The most frequently used test globally for serum is the serum agglutination test (SAT), a test that measures total quantity of agglutinating antibodies (Mantur & Amarnath, 2008).

The Brucella Milk Ring Test

The milk ring test is a type of agglutination test that detects brucella antibodies in milk samples and is recommended as a screening test for bovine brucellosis by OIE (OIE, 2015). It consists of *Brucella abortus* cells stained with hematoxylin, which are added to a sample of 1 to 2 ml of milk and incubated at 37°C for one hour (Huddleson & Carillo, 1949; OIE, 2015). If antibodies for *Brucella abortus* are present in the milk the antigen agglutinate with the cells, and float to the surface where it forms a sharp blue or purple colored cream layer. The milk column underneath will remain white. The milk ring test can be used on individual samples but also in entire herds by using pooled milk samples or bulk milk (Huddleson & Carillo, 1949). It is a relatively insensitive test, especially in large herds, since changes in the milk due to for example mastitis or colostrum can affect the interpretation of the test. However, it is considered to be a useful alternative if an ELISA test is not available (OIE, 2015). Especially in low-income countries, the low costs of the test and the fact that it does not require any expensive equipment or highly skilled laboratory staff makes it very valuable.

As with other serological tests, vaccinated animals can also be positive, and thus the results may be higher than what the prevalence really is.

Brucellosis in India

Bovine brucellosis was discovered in India in early 1940s and is now endemic in all the states (Renukaradhya *et al.*, 2002; Radostits *et al.*, 2007). The species of biggest concern are *B. melitensis* and *B. abortus* (Radostits *et al.*, 2007; Mantur & Amarnath, 2008). An increase in prevalence of the disease seemed to have taken place during the early years of 2000. The cause of this is not fully known, but one reason could be the increased trade and movement of livestock. Another potential cause may be the use of natural bull service and artificial insemination, which could be an important part of spreading the disease. The fact that slaughter of cows is prohibited in many states in India makes it difficult to limit the spreading of the disease. As mentioned earlier, a possible way of controlling brucellosis is through vaccination (Renukaradhya *et al.*, 2002). Cattle are usually vaccinated in their calf hood, preferably at the age of three to eight months, but can also be done in adult animals (Radostits *et al.*, 2007; Pacheco *et al., 2012*). Chand *et al.* (2014) conducted a study in the areas of Punjab, Haryana and Uttrakand, using a reduced dose of *Brucella abortus* S19 vaccine in adult dairy cows. When administrating the vaccine through the conjunctiva, negative effects as abortions and persistent antibody titers were not seen in that study. Conclusions were made that conjunctival vaccination in adult dairy cattle, with the S19 vaccine, can possibly be used to control brucellosis in endemic areas.

Investigations to assess the prevalence of brucellosis have been carried out at different times in different parts of the country using different test methods, which complicates comparisons. A serological survey of brucellosis in cattle and buffalo was performed by Isloor *et al.* (1998) in 23 states of India, with more than 30 000 bovine samples screened. The rose bengal plate test (RBPT) and serum tube agglutination test were used. The prevalence of antibodies for brucellosis at the time was 1.9% in cattle and 1.8% in buffalo. In organized farms with previous reproductive problems the prevalence rate was higher at 17%.

Trangadia *et al.* (2010) carried out a study in four farms in western, southern, central and northern regions of India in order to investigate seroprevalence of bovine brucellosis in organized dairy farms with a history of abortion. ELISA and RBPT were used in all four farms and MRT in one farm. The results indicated that 22.18% of the animals were seropositive by ELISA, 13.78% by RBPT and 12.82% of the cows that were tested with MRT.

RBPT and ELISA were used by Chand and Chhabra (2013) to investigate prevalence of brucellosis in dairy farms in 22 districts in Haryana and Punjab. The results showed an individual animal prevalence of brucellosis of 26.50% and an overall herd prevalence of 65.54%.

Brucellosis is often considered a neglected tropical disease, and is frequently underdiagnosed and underreported. In spite of seroprevalence studies showing its presence, there was no human clinical cases diagnosed in Assam until 2014 (Deka, personal communication).

Animal health and welfare

Animal welfare is a complex matter and measuring it is even more difficult. It can be viewed as three main areas: the natural-living conception (ability to express natural behavior), the functioning-based conception (being in good health and having normal behavior and physiological function), and the feeling-based conception (being free from pain, fear etc.) (Fraser *et al*., 1997). These three areas often overlap. Lameness, for example, may cause pain which affects the animal's feelings, may cause limited mobility affecting its natural behavior, and may affect the physiological function by causing a reduced milk production (Fraser, 2003).

An evaluation system has been developed by the European Welfare Quality® project, with the aim to evaluate the overall animal welfare on the farm and in slaughterhouses. The multi-criteria protocol includes pigs, poultry and cattle. It is set up from four animal welfare principles with measures designed specifically to each animal species. The measures for dairy cows are: good feeding (body condition score, access to clean water), good housing (behavior around resting, cleanliness, presence of tethering, access to exercise), good health (skin alteration, lameness, diseases of the respiratory, digestive or reproductive system, dehorning, tail docking) and appropriate behavior (aggressive behavior, access to pasture, avoiding human contact, qualitative assessment of behavior) (Botreau *et al.*, 2009).

Body condition

It is difficult to find scientifically proven methods to measure animal health and welfare since it is quite complex with many factors having an impact on the way an animal feels. One method used is to score the animals body condition (BCS), a way of measuring the proportion of body fat of the animal (Roche *et al.*, 2009). This is likely to be associated with the health of dairy cattle, with health problems related to both obesity and emaciation (Green *et al.*, 2013). In order to evaluate the body condition, different scales have been developed all over the world, from a 4-point scale, 5-point scale, 6-point scale, 8-point scale and a 10-point scale. Although the scales differ, a low score always means thin or emaciated animals and a high score is set for fat or obese animals (Roche *et al*., 2009). Some techniques include palpating the animals in order to assess the thickness of the tissue, whereas other methods are only visual and therefore not requiring the animals being under restraint (Edmonson *et al*., 1989). In a large literature review, Roche *et al.* (2009) discusses the complexness of animal welfare and the correlation between welfare and changes in BCS. The conclusions are that although there are indications of BCS having an impact on animal welfare, more research is needed.

The subjectivity of the method can lead to inconsistencies in judgment between different assessors as shown by Kristensen *et al.* (2006). Significant differences were seen between 51 practicing dairy veterinarians. These differences were reduced as the participants underwent some training increasing the accuracy and precision of their judgment. However, after evaluating the accuracy of BCS when used by different assessors, Edmonson *et al.* (1989) showed no significant differences between the assessors. The authors developed a scoring chart for free ranging Holstein dairy cows, with a scale ranging from 1 to 5, though the .25 unit increments turned it into a 17 point scale, where 1 meant severe under-conditioning or emaciation, and 5 meant severe obesity. The anatomical points and angles evaluated in the chart were as follows: the spinous processes, the spinous to transverse processes, the transverse processes, the overhanging shelf, tuber coxae and tuber ischia, area between pins and hooks, area between the hooks and finally the tail head to pins. The authors came to the conclusion that their scoring chart would work as a precise tool in measuring body fat in Holstein cows and reducing subjectivity of the method.

The BCS changes during the lactation cycle and the management of this is believed to have an impact of milk production, health and reproduction (Roche *et al.,* 2009). Several studies indicate a likely relation between BCS and milk yield and BCS and reproduction (Edmonson *et al.,* 1989; Gearhart *et al.,* 1990; Domecq *et al.,* 1997; Hoedemaker *et al.,* 2009). In a study by Hoedemaker *et al.* (2009), German Holstein cows with a BCS <3.0 (5-point scale) at time for calving and early lactation, had a higher culling rate and incidence of lameness and reproductive problems. Loker *et al.* (2012) showed a genetic association with low BCS and mastitis and metabolic disease. In a study by Green *et al.* (2013), both low and high BCS affected the milk yield negatively, although no strong association between BCS and milk yield was seen over the whole lactation. They also showed that cows with a BCS <2.5 (5-point scale) were exposed to a higher risk of lameness, which would have a negative impact on the animal welfare and lead to reduced milk production.

Hoof status

The status of the hoofs has a large impact on the welfare of the animal. Foot disorders can lead to lameness (Bruijnis *et al*., 2012) and can also lead to limitations in the natural behavior for the cow; such as the ability to rest, problems rising up or laying down thus causing a negative impact on the welfare of the animal (Bruijnis *et al.,* 2012). According to Bruijnis *et al.* (2012), 46% of the welfare impact is caused by clinical hoof disorders.

In addition to the impact on animal welfare, which is important in itself, this may also have secondary effects on milk production and economy. Studies of herds in New York (Warnick *et al.,* 2001) and the UK (Green *et al.,* 2013) have shown a significant decrease in milk production in cows diagnosed with lameness. Hoof and leg disorders were the third most important factor affecting the farmer’s economy, after mastitis and fertility problems. It is however difficult to assess the effect of foot disorders on milk yield since there are many influencing factors such as feeding routines and nutrition (Sogstad *et al.*, 2007).

In order to keep the hoofs in a good condition, regular trimming is important. Studies have shown that trimming improved the shape of the hoofs and prevented lesions for up to eight months (Manske *et al.*, 2002). Furthermore, it was associated with reduced stress levels and maintaining a good BCS (Ando *et al.*, 2008). Fjeldaas *et al.* (2005) showed that the best preventive effect was seen if the trimmings were done routinely, with only minor positive effects when the trimming was done occasionally. Sogstad *et al.* (2007) showed an increase in milk yield after hoof trimming compared to before. Nishimori *et al.* (2005) saw no change in milk yield after hoof trimming, but a significant increase in the milk fat and milk protein composition.

Hygiene

Evaluating hygiene in dairy cows is another method to assess welfare as it is an indicator on the life quality of the animals as well as the quality of the farm facilities. The level of contamination on foremost the udder and the hind legs are also influencing the somatic cell count, thus being one of the risk factors in causing mastitis (Schreiner & Ruegg., 2003; Sant'anna & Paranhos da Costa., 2011). Poor hygiene is also an important factor in the risk of developing foot disorders (Hultgren & Bergsten., 2001). The level of contamination may vary throughout the year, with a higher percentage of dirty cows during rainy seasons, probably as a result of more mud in the facilities (Sant'anna & Paranhos da Costa., 2011). Housing, feeding and management are also factors influencing the level of cleanliness in dairy cows (Hauge *et al*., 2012).

MATERIALS AND METHODS

This study was carried out as a Minor Field Study (MFS), a project financed by the Swedish international development cooperation agency (SIDA), comparing trained farmers with untrained farmers around Guwahati, in the north eastern state of Assam, India. The survey was done through interviews with the farmers, milk sampling for laboratory testing and clinical examinations of the animals.

The project took place in the months of September through November 2014, starting at the end of the rain season. Six different areas were visited (8^{th} mile, 11^{th} mile, 14^{th} mile, Ramkhinagar, Ganesh Mandir and Amsing Jorabat). These areas were selected by local persons, and in all areas there were farmers previously taking part in the hygiene training as well as non-trained farmers. The original goal was to visit 100 trained and 100 non-trained farmers. Due to time limitation and problem with the delivery of materials that goal was not possible to reach. Eventually, 56 farmers, 29 trained and 27 non-trained, were visited for the full survey (interviews, milk sampling and clinical examination). In order to increase the total number of farmers participating in the study, a sub-survey excluding clinical examinations was run parallel to the full survey. This made it possible to interview a larger number of farmers and to get more milk samples for laboratory testing. An extra 94 farms could hereby be included in the study, making a total of 150 farmers being interviewed (74 trained and 76 non-trained) and 487 cows sampled for detection of antibodies for *Brucella abortus* in the milk. Most of the cattle were cross-breeds (local and western breeds) and generally of a smaller size than western cows, although with exceptions.

In order to assess different aspects of welfare, farmers were asked about their perception of animal health and how this had been changing. In addition, the body condition was measured and hoof health was assessed. These parameters can be found within the Welfare Quality® assessment protocol.

Selection of farmers and animals

The trained farmers were selected from a list of participants in the previous ILRI hygiene training program. Five farmers, from each of the six geographical areas were randomly chosen by computer, to take the full survey. In the same way eight farmers were chosen for the sub-survey. The non-trained farmers were selected from a list set up by a local informer in the field. From that list five farmers were chosen for the full survey and eight for the partial survey by random selection by us through systematic picking for example number 2, 4, 6 on the list. In total 74 trained and 76 non-trained farmers were included.

For milk sampling, the aim was to include 25% of lactating cows at the farm, with a minimum of three lactating cows. If farms had less than three lactating animals, all were included. If there were more than three lactating animals, 25% were selected by random systematic picking using different start numbers. A total of 487 milk samples were collected.

To evaluate BCS, hygiene and hoof status, the aim was to score all cows and heifers on each farm according to the description below. The final total number of animals evaluated was 1040 animals checked for hygiene, 1023 for BCS and 1033 for hoofs, due to missed observations by the observer.

Questionnaire

Because of the language-barrier, we could not communicate directly with the farmers. The interviews were therefore done in the local language with the help of our co-workers. The interviews were documented through a questionnaire developed together with two other students. The questionnaire consisted of 32 questions concerning farm size, milking and hygiene routines, the overall health status of the animals, milk yield before training and at time of visit, and for non-trained farms, at time of visit and two years previously etc. The questions relevant for the aim of this thesis were included and analyzed in the present study (Table 2).

Table 2. *The questions from the questionnaire that was included and analyzed in this thesis*

- What is your total number of cattle? o Lactating o Dry o Heifers
- How much milk is produced per day?
- What is the price per liter?
- How important is dairy (milk and calf and cow sales) as your income for the household?
- Are the udders being cleaned or disinfected before milking? Yes/ no. If yes, with what do you use to clean the udder?
- Are the udders cleaned or disinfected after milking? Yes/no. If yes, with what do you use to clean the udder?
- Have you ever received hygiene training by ILRI? Yes/no
- If yes, have you experienced any change in the health status amongst the animals?
- If yes, what are the three most important benefits you have observed because of those changes?
- If yes, has there been a change in milk yield since the hygiene training? If no training, please compare to 2 years ago.
- What are presently the main diseases/health issues for your cows? Please pick the three most common out of the following o Inappetence/emaciation o Mastitis o Diarrhea o Reduced milk production o Abortion o Sick calves o Fertility problems o Foot and mouth disease (FMD) o Respiratory problems o Other (specify)
- Against what diseases are your cows vaccinated?
- Are you aware that Brucellosis may be transmitted from dairy cows to humans through milk or other sources? Yes/no
- Are the hoofs being trimmed? Yes/no. If yes, how often?

Brucella milk ring test

To test for presence of antibodies for brucellosis in milk, 25% of the lactating cows in each farm were randomly selected for milk sampling, with a minimum of three cows per farm. In farms with only one or two lactating cows at the time for the visit, all lactating cows were sampled. The samples were kept in a cooling bag and taken to the laboratory for testing using the Brucella Milk Ring Test (MRT). If testing could not be done immediately, samples were kept frozen. Two drops of pre-stained *Brucella abortus* antigen was added to each sample of 2 ml of milk and then incubated in 37°C for 45-60 minutes. The samples were then checked visually and given a score from 0 to 3 (Table 3, figure 1).

Table 3. *Interpretation of the Brucella milk ring test (Genest et al., 1956)*

0	**Negative test. A blue colored milk column with white or no cream layer.**
1	Doubtful/weak positive. Slightly blue colored cream layer and blue colored milk column.
2	Moderately positive. A clear, deep blue cream layer and slightly blue colored milk column
3	Strong positive test. A distinct, deep blue cream layer on top of a white colored milk column.

According to OIE (2015) all samples showing a blue layer at the interface of the milk and cream (i.e 1 to 3) should be considered positive and samples when the color of the milk column is stronger than the cream layer should be considered negative. These criteria were used for classification of samples here. Results are not reliable when the milk is changed due to mastitis, and therefore an analysis was done both including all results, and one analysis excluding all results with weak positives (scored 1) and with visually changed milk.

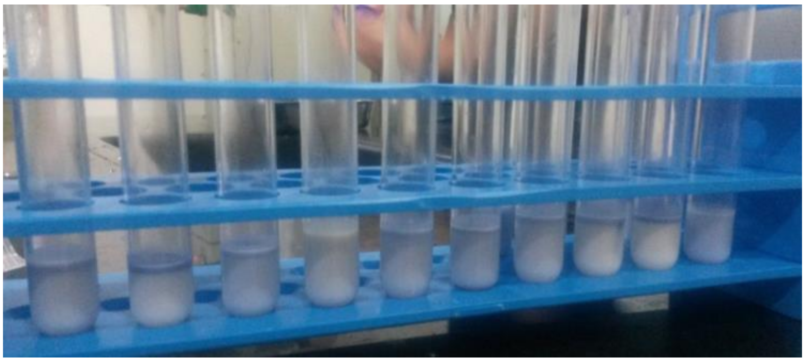


Figure 1. *Brucella Milk Ring Test. Milk samples after incubation*

Body condition score

In order to evaluate the overall health status in the animals their body condition score (BCS) was scored on a scale of 1 to 5; 1 meant the animal being very thin or emaciated and 5 meant severe overweight. A simplified version of the scale developed by Edmonson *et al*. (1989) was used. The following checkpoints were evaluated: the spinous processes, the angle from the spinous to the transverse processes, the transverse processes, tuber coxae and tuber ischii and the angle between the two, and finally the cavity formed around the tail head (figure 2). The evaluation was visual and did not include palpation of the animals.

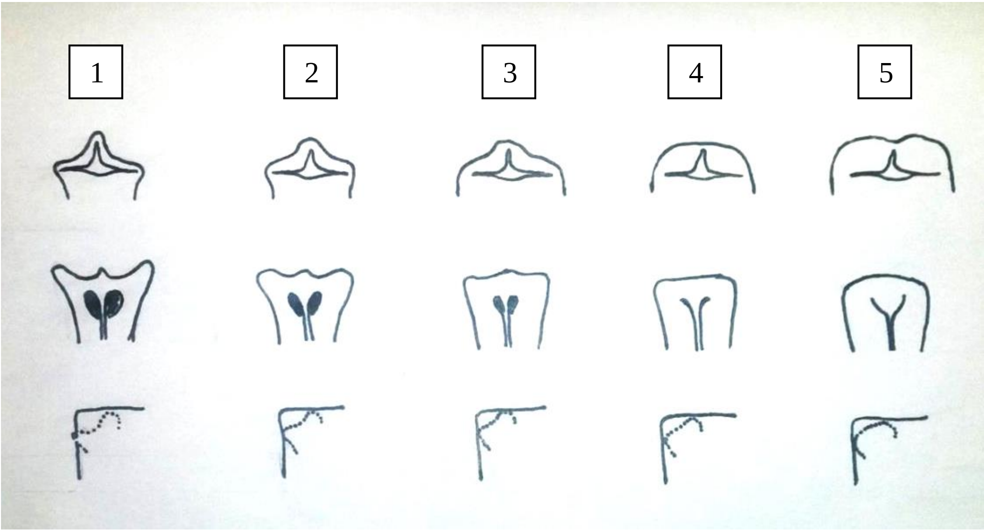


Figure 2. *Body condition score 1-5, redrawn from Edmonson et al. (1989). 1=emaciation, 2=under-conditioned, 3=good condition, 4=over-conditioned, 5=obese*

Hoof scoring

For the hoof scoring a scoring chart from 1-4 was developed and adapted to the local conditions, using clinical experience and pictures of hoofs of different length and shape. All hoofs were judged and a cow was given one overall score. A score of 1 meant that the cow had hoofs of a normal length and shape, 2 meant that one or more hoofs were slightly overgrown, 3 represented cows with one or more hoofs affecting the stance with/without scissor claws. Cows with a score of 4, had one or more hoofs that were too long, cork screwed or seriously affected the stance. (Figure 3)

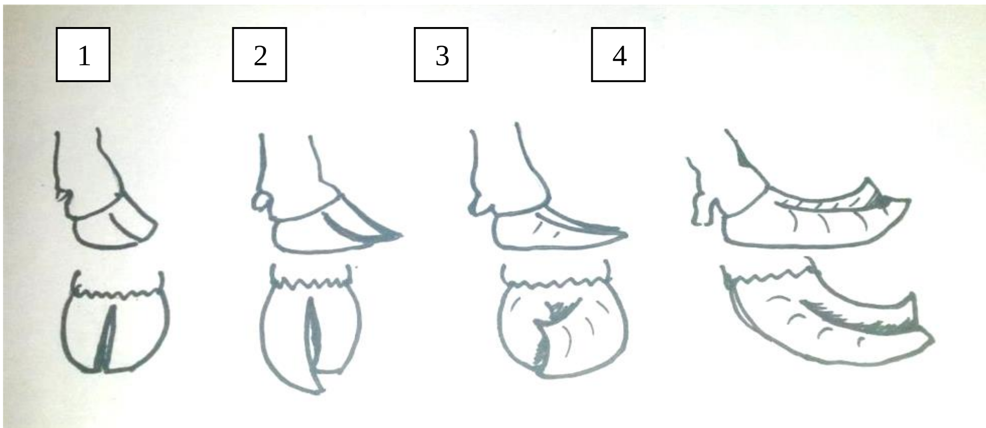


Figure 3. *Hoof scoring scale 1-4, 1=normal length and shape, 2=slightly overgrown, 3=too long, affecting the stance and/or scissor claws, 4=too long, cork screwed or seriously affecting the stance*

Hygiene scoring

To measure the hygienic conditions in the farms, the animal's hind legs were inspected and each animal was given a hygiene score on a scale from 1 to 4. A score of 1 meant no contamination, 2 meant contamination of feces along the hind legs, 3 stood for heavy contamination with hair still visible through the feces and 4 meant heavy contamination with the build-up of an armor of feces up along the hind legs, with no hairs visible through (figure 4). Level of contamination of the udder was not included in the evaluation.

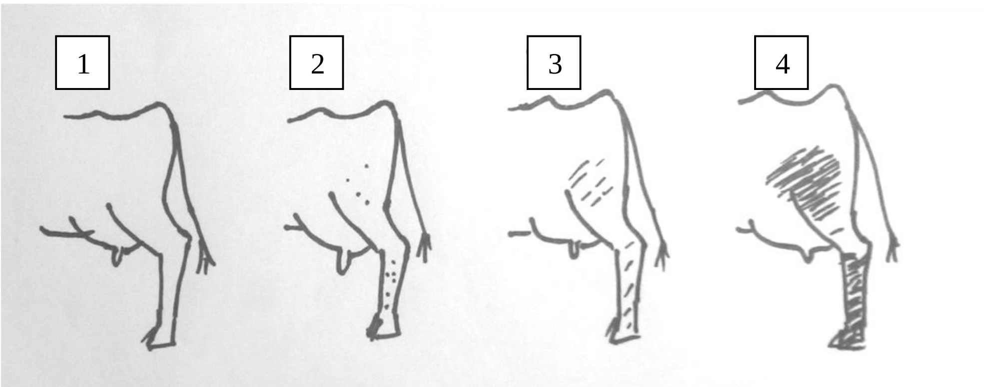


Figure 4. *Hygiene scoring scale 1-4, redrawn from University of Wisconsin School of Veterinary Medicine (n.d.): 1=clean, 2=slight contamination along the legs, 3=heavy contamination with hairs still visible through, 4=build-up of an armor of feces with no hairs visible through*

Statistical analysis

The results from the questionnaire were analyzed at farm level, and clinical evaluation and the brucellosis data at cow level. Descriptive analyses were done for number of cattle in the farm, hoof scores, BCS, hygienic score and serological results for MRT.

To analyze the association of hoof scores, BCS, hygienic scores and MRT results with training status of the farm, χ^2-test was used. To account for clustering at farm level, farm was included as a random effect using a Glimmix model in SAS (2011), to see that the results did not change because of clustering. Cow seropositivity for brucellosis was also analyzed for association with the area using χ^2-test. To account for clustering at farm level, the same test was performed with farm included as a random effect using a Xtmelogit model in Stata 14. Similarly, χ^2-test was used to analyze the association between training and the answers to the knowledge questions in the questionnaire, how health problems were perceived, and which problems were most important. The difference in milk yield, both previous and present, between trained and non-trained farms was assessed using two-sample t-test.
A total of 487 milk samples were collected for analysis of seroprevalence of *Brucella abortus*. 456 of these milk samples were included in the statistical analysis. The samples not included in the analysis were removed because of poor milk quality, due to storage problems, or because of previous vaccination of the herd. Including these samples in the analysis would have posed a risk for false positive results.

RESULT

The average herd size, excluding calves, was 20 cows and heifers (median 18.5) within the range from four to 68. The average number of lactating cows was 12. The number of lactating animals ranged from two to 46 (median 11).

Brucellosis

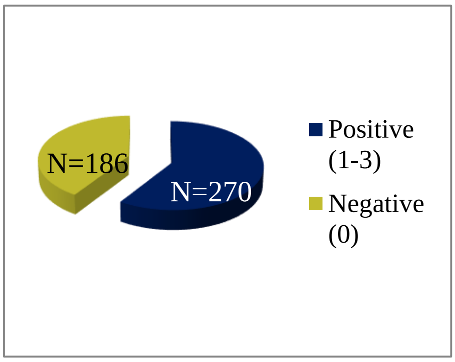
In total 456 cows from 133 farms were included. In 117 (88%) of these farms one or more cows were positive or brucellosis. In positive farms between 20 and 100 % of the animals were positive. Of all cows more than half tested positive for Brucella (Table 4). 186 of the 456 milk samples (40.8%) had been given a score of 0 (negative test) and 270 (59.2%) scored 1-3 (positive test) (figure 5a).

Table 4. *Results of brucellosis testing with milk ring test in 456 dairy cows from 150 farms in Assam*

Seroprevalence of *Brucella abortus* in cow's milk					
	0	**1**	**2**	**3**	
Trained	97 (42.5%)	45 (19.7%)	61 (26.8%)	25 (11.0%)	228
Non-trained	89 (39.0%)	33 (14.5%)	71 (31.1%)	35 (15.4%)	228

All	186 (40.8%)	78 (17.1%)	132 (28.9%)	60 (13.2%)	456

After excluding samples with a score of 1, meaning weak positive samples, 84 out of 178 samples (47.2%) in trained farms were positive for brucellosis. In the non-trained farms 96 out of 183 samples (52.5%) were positive. In total 180 out of 361 samples (49.9%) were positive, i.e. with a score of 2 to 3 (figure 5b). The difference between the two groups was not statistically significant.



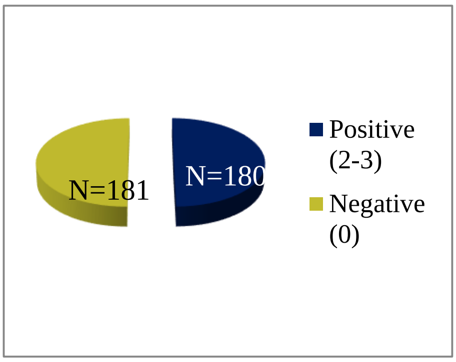


Figure 5a (left): *Samples positive for Brucella abortus, all scores included (i.e 1 to 3)* Figure 5b (right): *Samples positive for Brucella abortus after removing week/doubtful positive samples (i.e. samples with score 1)*

Differences between areas

When comparing seroprevalence of brucellosis between different areas, counting both weak and strong positives, a significant difference could be seen (p-value=0.017). The area with the most brucellosis, 8th Mile, had significantly higher levels than Amsing Jorabat, 14th Mile and Ramkhinagar. (Table 5).

Table 5. *Seroprevalence of brucellosis in cows from 133 farms in different areas in the state of Assam*

	11th mile	**14th mile**	**8th mile**	**Amsing Jorabat**	**Ganesh Mandir**	**Ramkhinagar**
Negative (0)	34 (35.4%)	38 (50%)	24 (28.9%)	43 (51.2%)	29 (36.3%)	18 (47.4%)
Positive (1-3)	62 (64.6%)	38 (50%)	59 (71.1%)	41 (48.8%)	51 (63.7%)	20 (52.6%)

Total	96 (100%)	76 (100%)	83 (100%)	84 (100%)	80 (100%)	38 (100%)

BCS

A majority of the cows and heifers, 55.3% (n=1024) were given a body condition score of 3, and just over a third (36.4%) scored 2. No significant difference could be seen between the trained and non-trained group (Table 6).

Table 6. *Distribution of body condition scores (BCS) among cows and heifers in 74 farms where hygiene training had taken place, and 76 non-trained farms in the state of Assam*

Body condition score						
	BCS 1	**BCS 2**	**BCS 3**	**BCS 4**	**BCS 5**	
Trained	14 (2.5%)	204 (35.9%)	325 (57.1%)	26 (4.6%)	0 (0%)	569
Non-trained	11 (2.4%)	169 (37.1%)	241 (53.0%)	33 (7.3%)	1 (0.2%)	455
Total	25 (2.4%)	373 (36.4%)	566 (55.3%)	59 (5.8%)	1 (0.1%)	1024

Hoofs

The most common hoof score among cows and heifers was 2, slightly elongated, which was found among 37.1% (n=1033) of the animals, followed by 26.5% scoring 1. 36.4% of the animals scored 3 to 4, meaning they had too long or cork screwed hoofs. No significant difference could be seen between the trained and the non-trained farms (Table 7).

Table 7. *Distribution of hoof scores among cows and heifers in 74 farms where hygiene training had taken place, and 76 non-trained farms in the state of Assam*

Hoof score					
	Score 1	**Score 2**	**Score 3**	**Score 4**	

Trained	155 (26.7%)	210 (36.2%)	125 (21.6%)	90 (15.5%)	580
Non-trained	119 (26.3%)	173 (38.2%)	91 (20.1%)	70 (15.5%)	453
Total	274 (26.5%)	383 (37.1%)	216 (20.9%)	160 (15.5%)	1033

Hygiene

The majority of the cows and heifers, 84.3% (n=1040), were given a hygiene score of 2 to 3. When comparing the two groups, no significant difference could be seen (Table 8).

Table 8. *Distribution of hygiene scores among cows and heifers in 74 farms where hygiene training had taken place, and 76 non-trained farms in the state of Assam*

Hygiene score					
	Score 1	**Score 2**	**Score 3**	**Score 4**	
Trained	34 (5.9%)	247 (42.5%)	246 (42.3%)	54 (9.3%)	581
Non-trained	36 (7.8%)	209 (45.5%)	175 (38.2%)	39 (8.5%)	459
Total	70 (6.7%)	456 (43.8%)	421 (40.5%)	93 (9.3%)	1040

Interviews

General health of animals

Of the trained farmers, 63 out of 72 (87.5%) answered that the cows were generally healthier after the training compared to before, and nine (12.5%) stated that there was no change in health status. No one stated that the animals were less healthy after the training. In the non-trained group, 52 of 76 (68.4%) farmers saw no change in health status over the last two years, eleven (14.5%) stated that disease incidence increased or that the cows were less healthy and four (5%) answered that the disease incidence was reduced.

For the question of what the three most important benefits of the training had been, 37 (50%) of the trained farmers (n=74) stated that the milk production had increased., 41 (55.4%) answered that the disease incidence had been reduced or that the over-all health status had improved and 21 (28.4%) experienced a decrease in mastitis. Four farmers (5.4%) did not experience any improvements since the hygiene training.

When asked what diseases/health issues was the biggest cause of concern in the farms at present 87 farmers (n=150) answered no to all the options, including “other”. The five most common problems were mastitis (12.8%), followed by fertility problems (8.7%), laminitis (7.3%), inappetence/emaciation (6%) and diarrhea (6%) (Figure 6). Respiratory problems and sick calves were only stated by one farmer (0.7% each) as the largest cause of concern. No one claimed that abortions or FMD was the main problem. No significant differences were seen between trained and non-trained farms.

General knowledge of diseases

On the question whether farmers were aware that brucellosis may be transmitted from dairy cows to humans through milk or other sources, 47 (31.3%) answered yes, 84 (56%) answered no and 18 (12.7%) did not reply (n=149). Comparing the two groups, the following results could be seen: 31 out of 74 trained farmers (41.9%) answered yes, 35 (47.3%) said no and eight (10.8%) did not reply. In the non-trained group 16 out of 75 answered yes (21.3%), 49 (65.3%) said no and ten (13.3%) did not answer. The difference between the two groups was statistically significant (p-value=0.008).

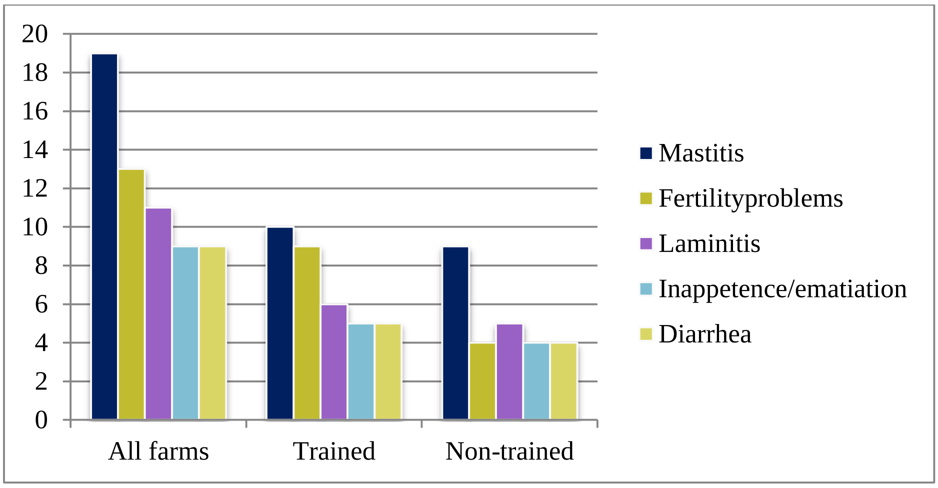


Figure 6. *Most common health problems in dairy cows in Assam, according to farmers in 74 farms where hygiene training had taken place, and 76 non-trained farms*

Vaccination

A majority, 142 (94.7%) of the farmers (n=150) stated that they had vaccinated against foot and mouth disease. 28 (18.6%) vaccinated against haemorrhagic septicaemia (HS) and seven (4.7%) against brucellosis. Five (3.3%) farmers stated they had not vaccinated at all and four (2.7%) did not reply. Four out of the seven farmers (57%) vaccinating against brucellosis were located in 8^{th} mile and three (43%) were located in the area of Ganesh Mandir. From the farms where vaccination against brucellosis had occurred, 27 milk samples were analyzed. Eight out of the 27 (29.6%) tested negative with the MRT and 19 (70.4%) were positive with a score from 1 to 3.

Milk yield

Among the trained farmers the mean milk yield reported before training was 7.07 liters per cow per day, compared to the non-trained group where the mean milk yield two years ago was reported to be 7.20 liters per cow per day thus showing no significant difference. After the hygiene training the mean milk yield in the trained group was reported to be 7.74 compared to 6.79 in the non-trained farms, a statistically significant difference (p-value=0.003) between the groups. This means an increase in milk production in the trained farms with a mean 0.67 liters per cow per day, compared to the non-trained group where the milk yield over the last two years was reduced with a mean 0.41 liters per cow per day.

The average amount of milk sold in total, was a mean 78.7 liters per farm per day with the average selling price of 39 INR per liter.

Milking hygiene

Comparing milking hygiene, 73 (98.6%) trained (n=74), and 73 (96%) non-trained farmers (n=76) stated that they did clean/disinfect the udder before milking, showing little difference between the two groups. However, a difference could be seen in the cleaning of the udder after milking. Seventythree percent of the trained farmers stated that they cleaned the udder after milking compared to 47% of the non-trained. This difference was statistically significant (p-value=0.001). All farmers cleaning the udder either before or after milking stated they did so with water. No one claimed to use soap or disinfectant (Table 9).

Table 9. *Milking hygiene in trained and non-trained dairy farms in Assam*

	Udder cleaned after milking	**Udder not cleaned after milking**	**Total**
Hygiene training	54 (73.0%)	20 (27.0%)	74
No hygiene training	36 (47.4%)	40 (52.6%)	76
Total	90	60	150

Hoof trimming

As shown above, a majority of the cows (73.3%) scored 2 or more in the hoof grading, i.e. had hoofs that were slightly overgrown, long, corkscrewed and/or affected the stance of the cow. Two trained farmers gave duplicate, compromising answers when asked about hoof trimming routines and were therefore excluded from the analysis. Of all the farmers (n=149), 119 (79.9%) trimmed the hoofs. When comparing the two groups, 62 out of 72 (86.1%) trained farmers trimmed the hoofs and 57 (75%) out of the 76 non-trained, a non-significant difference. Regarding the frequency of trimming, 1.7% stated that trimming was done every six months, 61.3% trimmed yearly and 37% answered that the trimming was done when needed or necessary. Very little difference was seen between the trained and non-trained group. No one trimmed the hoofs as often as 1 to 2 times per month (Table 10).

Table 10. *Hoof trimming routines in dairy farms in Assam*

	Every 6 months	Yearly	When needed/ necessary	Total
Farms with hygiene training	1	39	22	62
Farms with no hygiene training	1	34	22	57
Total	2 (1.7%)	73 (61.3%)	44 (37.0%)	119

DISCUSSION

In this study an assessment of general health and welfare in Assamese cows were attempted. There are few studies published regarding overall health and welfare in dairy cattle in developing regions such as Assam. It is therefore difficult to compare the results in this study at a global level.

The methods used to evaluate health and welfare in cattle, are often based on subjective evaluation which could affect the results. This was also the case in this study.

Fraser (2008) lists three improvements that can be done in order to increase animal welfare in less industrialized countries. One argument is through economic incentive to reduce stress, injury and malnutrition, thus reducing losses. The same reasoning is behind this study; an intervention aiming to have a positive economic impact may have secondary effects on animal welfare. It might be primarily the economic gain that makes farmers continue with the changed routines, even though they may appreciate the secondary effects as well.

BCS was one of the parameters chosen to evaluate whether improved hygiene and knowledge among farmers would improve the overall health of the animals and indirectly improve the animal welfare, which might have been reflected as a change in BCS. No clear difference could be seen between farms that took part in hygiene training and farms without such training. The majority of the cows were scored between BCS 2 and BCS 3 (scale 1-5). This is slightly lower than the recommended BCS of 3 to 3.5 (Oliver *et al.* 2005). It is worth mentioning that the BCS of heifers was generally a little higher than the BCS of the cows, many of the heifers scoring BCS 4, which could influence the result leading to a higher general score in farms with a large number of heifers. No consideration was taken to this in the study and it should therefore be noted as a possible source of bias, showing a false high BCS in the area. To exclude the heifers from the evaluation, or to assess them as a separate group, would alleviate this problem. The scale used for evaluating BCS was developed for Holstein dairy cows, while the cattle in Assam mostly were cross-breeds. This could have made it more difficult to correctly assess the BCS of the animals in the area. The optimal BCS varies with the lactation cycle (Roche *et al.*, 2009), but no consideration was taken to stage in the lactation cycle when the BCS was evaluated. To correlate lactation cycle and BCS might have given a more precise result as well.

BCS is believed to be associated with the general health (Hoedemaker *et al.*, 2009, Loker *et al.*, 2012) in animals and possibly also the milk yield (Green *et al.* 2013). Since it is foremost the calving BCS that is considered to have the greatest impact on health and milk yield, keeping the heifers in a slightly higher body condition may not be a cause of concern. As mentioned before, the optimal BCS in cows varies with the lactation cycle and in western dairy farming it is an important tool in maximizing the milk yield. Loker *et al.* (2012) discussed the possibility of using BCS as a predictor of breeding values for different health traits. This is a knowledge that also could be used in an area like Assam in order to improve the productivity and possibly the animal welfare. Informing and educating farmers about the impact of BCS on milk yield and in reducing metabolic diseases should therefore be recommended.

Another parameter evaluated in order to measure the animal welfare was the hoofs of the cows. Studies (Warnick *et al.*, 2001, Bruijnis *et al.*, 2012) have shown that hoof disorders can lead to lameness and a loss of milk production, which in the end could cause not only suffering for the animals but also a loss of income for the farmers. In this study a majority of the cows had slightly long, very long or cork screwed hoofs and a large part scored the higher grades of 3 to 4. This is not ideal and clearly indicates the need for better and more regular hoof trimming. Interestingly, a majority of the farmers stated that they trim the hoofs yearly or when needed. However, in many of the cows the hoofs had clearly been trimmed at a score of 3 to 4, meaning they had reached a point where the stance was already affected. Trimming the hoofs at that point would probably ave helped the cow during rising up and lying down, but it would not have had much effect on the stance of the cow since the angles of the joints already would have been changed. The change of stance is likely to be causing the animal pain and therefore have a negative effect on the animal welfare. Informing the farmers to trim the hoofs more often, or at the point when hoof length is just slightly too long, i.e. score 2 would be advisable. In this study there was no data collected on who was doing the trimming. However, in order to improve hoof health, a more in-depth study of hoof trimming routines and practices may be warranted.

No clear difference could be seen comparing the trained and non-trained groups, but since hoof trimming was not a part of the ILRI hygiene training that result is not surprising. The regularity of trimming seems to be a key point as shown by Fjeldaas *et al.* (2005), which may cause a problem in an area like Assam since the farmers do not have the same tools and resources as farmers in the western part of the world. Those resources being for example access to experienced hoof trimmers and possibilities to restrain the animals properly. According to Fjeldaas *et al.* (2005), the impact of trimming on milk yield was not significant in herds with a low milk yield. Also, in most studies the cows evaluated are high producing, making the comparison with low-producing animals difficult. The positive effect on productivity in herds like those in Assam may therefore be questionable, but the possible improvement on individual cow level is not negligible. Even though it is not possible to say the impact of the hoof health on milk production in this study, the elongated hooves are likely to have an impact on animal welfare and should not be ignored. Raising general knowledge amongst farmers about the effect of hoof status on general health, welfare, and possibly milk production is recommendable.

When asking the farmers about the health in the herds, a vast majority of the trained group stated that their animals were healthier after hygiene training. In the non-trained group only a few percent said that their animals were healthier compared to two years earlier. Possible reasons for this could be an increased knowledge about spreading of diseases and ways to prevent it (e.g. mastitis and brucellosis) thus reducing the risk for infections entering the herd. There are however many factors affecting animal health and judging health and welfare of animals is a complex task. The results indicate that hygiene training can play a part in improving health and welfare of dairy cattle. As mentioned before, further studies are needed in order to evaluate the methods for clinically measuring health and welfare. Although the design of this study aimed mainly to compare the two groups of farmers, there are no previous studies available on neither BCS, hygiene nor hoof health, and this study may therefore provide a first picture of the present animal welfare situation in the state of Assam.

When comparing milk yield, a clear difference between the two groups was seen, with an increase in the trained farms while the non-trained farms had experienced a decrease over the last two years. This difference was statistically significant and shows that hygiene training could have a positive effect on both health and milk yield. Although the increase might seem small, it is an increase corresponding to about 10% of the daily production. With the price being on average 39 INR per liter, an increase of 0.67 liters per cow per day would make a difference in the economy for the farmers, especially in farms with bigger herds. For an average Assam farm with 12 lactating cows this increase in milk production would mean an additional 8 liters of milk per day, resulting in an extra 312 INR per day. In a week the additional income would be 2184 INR and an extra 8736 INR per month. For an average Indian herd with two dairy cows per farm (Douphrate *et al.,* 2013), this increase would correspond to about 1463 INR per month. Since milk production is the main source of income in over 90% of the farms, an increase in milk production would have a large impact on the economy of the farmer. The experienced improvement in the overall health together with a significant increase in milk yield indicates that hygiene training indeed does have an effect in health and milk production and is likely to increase the income for the farmers.

The overall hygiene amongst the cows did not differ between the two groups despite hygiene training. One reason for this could be that many of the non-trained farms are located near trained farms and could therefore have been influenced by their neighbors. Another reason might be the way the farms were set up. The building materials and environment could prove hard to keep clean and hygienic. Another reason of course, could be a low compliance and there is also the influence of the climate. The first weeks of this study were carried out at the end of the rain period, when it is more difficult to keep the farms clean.

When it comes to milking hygiene there was a clear difference in the hygiene routines i.e. the cleaning of the udder after milking. A majority of the trained farmers stated they cleaned the udder after milking which could have had a positive effect mainly in decreasing the frequency of mastitis. This would be conclusive with the fact that the farmers that took part in hygiene training experienced a decrease in occurrence of clinical mastitis in their herds (Melin, 2015). These results indicate that although no clear difference was seen in the over-all hygiene of the cows, the ILRI hygiene training has been positive in improving the milking hygiene. A better milking hygiene would decrease the risk of contaminating the milk as well as possibly reducing the risk for the cows to develop clinical mastitis. Minimizing the risk of contaminating the milk was one of the main goals with the hygiene training, a goal that appears to successfully have been reached.

The results in this study indicated that at least half of the dairy cows tested in and around Guwahati, Assam were positive for antibodies against *Brucella abortus,* implying that they have been infected previously. In this analysis, care was taken to exclude milk samples of doubtful quality and samples from vaccinated herds which might have given false positives. One analysis was also run excluding the weak positive samples in order to remove the risk of getting false negative results. This together with the fact that the Brucella milk ring test is not a very sensitive test makes it likely that the proportion of positive cows in this area is even higher. In future studies, it would however be good to include other diagnostics as well for confirmation.

Our results shows a remarkably higher seroprevalence of brucellosis than previously seen in studies carried out in other parts of the country (Isloor *et al.,*1998; Trangadia *et al., 2*009; Chand & Chhabra., 2013), where the prevalence varied from a few percent to an individual animal prevalence of up to 26.5% and an over-all herd prevalence of over 65.54% . One can only speculate why the prevalence seems to be much higher in this area than in others. Perhaps there has been a parallel increase in prevalence across the country, and similar high proportions may be detected if studies were to be remade in areas previously tested.

As mentioned a few farms reported that they had vaccinated their herds, however the data did not reveal when the vaccination had been done, nor if non-vaccinated animals had been introduced to the herd after vaccination. Almost a third of the animals in these farms had milk samples that tested negative for brucellosis. However, as previously shown by Pacheco *et al.* (2012) the excretion of *Brucella abortus* S19 in milk is not constant but intermittent throughout the lactation. In their study over 90% of the samples in vaccinated herd were negative when tested with the milk ring test, compared to 30% in our study. This difference could perhaps be explained by the fact that the excretion is intermittent and varies through the lactation cycle (Pacheco *et al.,* 2012). Information about stage of the lactation of sampled cows was not collected in our study. Since the antibody response after vaccination is the same as after an infection, the antibodies gained cannot be differed. The results are therefore difficult to evaluate and these tests were excluded from the analysis. It is also possible that some farms could not remember that they had vaccinated their animals, were unaware of what a vaccination was for, or had purchased vaccinated animals, thereby increaseing the seroprevalence.

The milk ring test uses *Brucella abortus* antigen, and thus positive reactions are likely to be *B.abortus,* although cows theoretically also could have *B. melitensis,* though this is less common. No attempts to identify the bacteria was done, but would be recommended in future studies. Since *Brucella abortus* is the most common type causing brucellosis in cattle it is likely to be the strain that was found in this area.

One important mode of transmission of brucellosis between animals is through the use of semen from infected bulls (Renukaradhya *et al.*, 2002). The survey did not include questions about use of natural bull services or artificial insemination. However, after talking to local farmers the apprehension was that artificial insemination was most common in the area, but there are no statistics in this study supporting that claim. It would be recommended to follow up with studies regarding the breeding system in the region, and if possible implement screening regimes for bulls.

When comparing the different areas visited, significant differences could be seen. In this analysis all positive samples were included (i.e. score 1 to 3). It is difficult to interpret the weak positive samples (score 1), and to confirm a positive result, re-testing of these cows would be needed. However since OIE recommend that all samples with slight color changes should be considered positive, it was decided to include these in the analysis to make certain not to get false low negative results. The area with the highest percentage of animals positive for brucellosis was 8th mile. Difference between the areas are difficult to explain. A key point could be the possible use of the same bull within one area. Another reason could be location. In some areas the farms are situated more closely to each other than in others, and therefore increasing the risk of spreading infection. Increasing the general knowledge about transmission of brucellosis with emphasis on insemination is one way to try stopping the disease from spreading. If farmers know about the risks of using infected semen they have a chance to question the holder of the bull and if possible, only use bulls that are tested free from brucellosis.

Although the objective of the hygiene training included reduction of zoonotic diseases, such as brucellosis, no significant difference could be seen between the trained and non-trained farms. However, the hygiene training did not focus on disease control, and information on biosecurity were not given. Nevertheless could an increased hygiene and general knowledge amongst trained farmers reduce the risk of transmission of brucellosis from the animals to the farmers. This study showed that the trained farmers were more aware of risks with zoonotic transmission from milk, and thus it may have an impact on human health as the result of increased awareness. Given the importance of milk for food security, and the importance of zoonotic pathogens for food safety, this warrants continued training of farmers, but with future inclusions of more disease control measures.

CONCLUSIONS

The results of this study shows that the hygiene training has led to some benefits. A significant increase in milk yield was seen in trained farms, which should lead to an increased income for the farmers. Another benefit was the experience of an increase in the overall health of the animals, possibly leading to decreased treatment costs as well as a possible improvement in the animal welfare. However, an improvement in overall hygiene was not detected, and there were no significant differences in body condition and hoof status, leaving room for improvement in the training. Finally, this study implies that at least half of the cows in the visited area could have been exposed to *Brucella* spp. a result that suggests that brucellosis may be an underdiagnosed public health problem in the area as well. This highlights the importance of an increased awareness amongst farmers about the spreading of brucellosis as well as the zoonotic risks. A continued work to reduce the occurrence of brucellosis in the area is recommended, perhaps through vaccination campaigns.

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