Milk Production on Smallholder Dairy Cattle Farms in Southern Vietnam

Management in relation to udder health

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Abstract

Dairy production is a rather new and not a traditional system in Vietnam. It is mainly based on smallholder dairy farms. The general aim of the studies in this thesis was to improve milk production on smallholder dairy farms in Southern Vietnam and also to create a foundation that could be used in the advisory service or/and in further research for better milking management routines. Studies were done to cover the specific objectives of this thesis. The studies were designed to identify the problems for dairy production on smallholder dairy farms, to investigate which are the management factors that influenced milk somatic cell count (SCC) in lactating cows, identify the prevalence of subclinical mastitis based on SCC and to study the protein degradation caused by *Streptococcus (Str.) agalactiae*.

The survey study indicated that the majority of the farmers kept between 2 to 17 cows (mean = 12). The main breed of dairy cow was Holstein Friesian (HF) crosses. This HF cows produced about 16 kg/day/cow. Around 35% of the farms provided fresh water *ad libitum* for the cows, while 51 % provided less than 30 L of water per cow per day. Moreover, milk SCC was high (1,300,000 cells/mL milk) in many of the studied farms. The second study found that limited to drinking water significantly increased herd SCC. *Str. agalactiae* was found to be a predominant species in infected udders. Further investigation showed that the prevalence of subclinical mastitis (SCC > 200,000 cells/mL milk) at quarter basis was 63.2% (285 out of 451) and at cow basis 88.6% (101 out 114). *Str. agalactiae* was found on 65% farms, 35.6% cows (41 out of 115) and 21% quarters (96 out of 458). Among 96 isolates of *Str. agalactiae*, 11 different strains were identified. The proteolysis of casein was higher (12-70%) compared with whey proteins (4-12%). The strains of *Str. agalactiae* in the same phylogenic group did not show the same degradation of casein and whey protein. *Str. aglactiae* caused proteolytic activity where the proteolysis of α_{S2}-casein was highest, up to 70%, compared with control milk. Proteolytic activity caused by different strains showed a large variation. The lowest breakdown of casein was found to be 30% compared with control milk.

Overall, the high milk SCC in this present study showed poor udder health of lactating cows on smallholder farms. The high milk SCC was mainly caused by the infection of udders with *Str. agalactiae.*

Keywords: smallholder dairy farm, somatic cell count, management factors, udder health, proteolysis, *Streptococcus agalactiae*

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Dedication

To my parents with my respectful gratitude,

To my darling Bui Phan Thu Hang,

and my lovely children:
Vo Huu Trong,
Vo Thuy Thuy Vy.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Lam, V., Wredle, E., Thao, N.T., Man, N.V., Svennersten-Sjaunja, K. (2010). Smallholder dairy production in Southern Vietnam: Production, management and milk quality problems. *African Journal of Agricultural Research* 5(19), 2668-2675.

II Lam, V., Östensson, K., Svennersten-Sjaunja, K., Norell, L. & Wredle, E. (2011). Management factors influencing milk somatic cell count and udder infection rate in smallholder dairy cows in Southern Vietnam. *Journal of Animal and Veterinary Advances* 10(7), 847-852.

III Östensson, K., Lam, V., Sjögren, N & Wredle, E. (2011). The prevalence of subclinical mastitis and isolated udder pathogens in dairy cows in Southern Vietnam. (*Submitted).*

IV Åkerstedt, M., Wredle, E., Lam, V. & Johansson, M. (2011). Protein degradation in bovine milk caused by *Streptococcus agalactiae.* (Manuscript).

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Abbreviations

AI	Artificial Insemination
CE	Capillary electrophoresis
CNS	Coagulase-negative Staphylococcus
^{o}C	Celsius degree
L	Litre
mL	Millilitre
mm	Millimetre
PFGE	Pulse-field gel electrophoresis
SCC	Somatic cell count
SVA	National Veterinary Institute, Sweden
UHT	Ultra-high temperature
HF	Holstein-Friesian

Introduction

The consumption of dairy products has grown dramatically in Asia over the last 25 years due to the fast economic growth in the region. The most rapid growth of milk consumption is seen in Southeast Asia, with a current consumption of 31 kg per capita (Moran, 2009). China, Thailand and Vietnam show the highest growth of dairy production in the region (Morgan, 2010). The increasing milk consumption stimulates the development of local producers to satisfy the domestic demand by replacing imported powder milk and it is noteworthy that over 80% of the milk is produced by smallholder farmers (Morgan, 2010).

Dairy production in Vietnam has grown significantly during the last two decades, but consumption still outpaces production. The average annual milk consumption *per capita* has increased from 0.5 kg in 1999 (Do & Hoang, 2001) to 9.4 kg in 2008 (Gautier, 2008). In 2009, the total milk consumption was about 430,000 tons, whereas total milk production was 278,000 tons (General Statistic Office, 2010). Due to the increasing demand for dairy products and motivation of government policies, the population of dairy cattle has increased from 40,000 in 2001 (NIAH, 2001) to 130,000 head in 2010 (General Statistic Office, 2010). Eighty percent of milk is produced by 20,000 smallholder dairy farmers, around 70% in and nearby Ho Chi Minh City (Gautier, 2008). Thus, smallholder dairying constitutes the majority of milk production systems in Vietnam.

The "Holsteinisation" program of crossbred Sindhi stock by using artificial insemination (AI) has been executed to accelerate the milk production of the country from the 90ies. Simultaneously live Holstein Friesian (HF) cows from temperate countries have also been imported. Today the Vietnamese dairy population consists of 14% pure HF, 80% of crossbred HF and the remaining 6% are crossbred Sindhi and other breeds (NIAH, 2010). The "Holsteinisation" has contributed to an increased milk yield, from 1200 kg/cow/lactation (Giang & Tuyen, 2001) to 3,400 kg/cow/ lactation (Gautier, 2008). However, cows with a high level of HF inheritance cannot exhibit their full genetic potential in the tropics due to poor management and feed quality and environmental stress factors (for reviews see Syrstad, 1996; Cunninghem & Syrstad, 1987; Kiwuwa, 1987). Moreover, although the increase of HF inheritance can increase milk yield (Luthi *et al.*, 2006), it can also result in high mortality and reduced fertility (Syrstad, 1996).

Dairy cattle production is a rather new farming system in Vietnam. Thus farmers probably have a lack of knowledge about management practices, especially relating to HF crosses that are needed to obtain a profitable and sustainable production. Therefore, problems with management practice in relation to milk production need to be addressed.

Background

Milking management

It is well established that the prerequisites for a sustainable and profitable dairy production are good management practices of the dairy cows and the replacement animals. Management includes several factors, including breeding, feeding, housing and milking. All factors have to be considered for a successful dairy production and several reports and theses have been published dealing with different types of management (for reviews see Rhone *et al.*, 2008; Luthi *et al.*, 2006; Suzuki, 2005). However, in this thesis we mainly address the problems related to milking management and their effect on milk composition and udder health.

According to Akers (2002), a well-known lactation physiologist, the investment in milking management at farms where feed, breed and care for animal obviously are wasted if milking procedures and milk handling are not satisfactory. This means that attention must be focused on milking practice to promote optimal milk production and good udder health. A good milking practice includes several steps. Milk ejection has to be stimulated in a proper way for a high milk flow and sufficient udder emptying. Pre-stimulation of milk ejection can be done either manually, by machine, or by letting the calf suckle before milking starts (Svennersten-Sjaunja *et al.*, 2004). If machine milking is practiced, milking equipment must be checked routinely for vacuum level, pulsation rate, and pulsation frequency and liner performance according to the recommendation of the manufacturer. Irrespective of whether milking is done by machine or by hand, hygiene must be considered, both to prevent udder health problems and to maintain a high hygienic quality of the raw milk (Eberhart *et al.*, 1968).

Milking in the tropical countries is done by hand or machine depending on the availability of services such as electricity, labor and technical support and level of production (Chantalakhana & Skunmun, 2002). However, both hand and machine milking may have negative impacts on udder health if milking practices are inappropriate. Hand milking was reported to cause injuries of the teats (Boonbrahm *et al.*, 2004b). Millogo *et al.* (2010) studied different types of hand milking and found that milk yield and composition were not affected by milking technique, but the milk yield varied between different milkers. No effect of milking technique on teat treatment was observed. Interestingly (Boonbrahm *et al.*, 2004a) reported a significantly higher milk SCC in cows that were bucket machine milked compared with hand milking, which is in line with what has been observed in dairy buffaloes (Thomas *et al.*, 2004).

During machine milking, too high vacuum levels can damage teat canals, which can result in negative effects on udder health (Hamann *et al.*, 1993; Bramley *et al.*, 1992). The teat canal acts as a primary defense mechanism to prevent new intramammary infections (Sandholm & Korhonen, 1995). One of the most common types of teat damage is hyperkeratosis, which is caused by overmilking, poor pulsation, too high vacuum level or milking with worn liners (Akers, 2002). Thus milking cows with a faulty machine that damages the teat end will increase the risk of new infection. A damaged teat skin provides an ideal environment for the growth of mastitis pathogens such as *Staphylococcus (S.) aureus*, *Streptococcus (Str.) agalactiae* and *Str. sdysagalactiae* (Blowey & Edmondson, 2010; Bramley *et al.*, 1992). Furthermore, during milking, vacuum fluctuations or vacuum slips with leakage of air around the teat cups that cause a retrograde movement of milk allow bacteria to pass from one teat to another and invade teat canals (Akers, 2002). Jungbluth & Grimm (2009) also listed some indirect factors related to aspects of poor management that influence udder health. Poor milking procedure, which might contribute to udder infections due to transmission of disease during milking time, poor installation or maintenance of milking equipment that causes tissue trauma, teat damage and poor milkout were some important factors. How milking management is working on smallholder dairy farm in Vietnam has not been fully evaluated, neither has its effect on udder health.

Milk synthesis and composition

Milk components are mainly synthesized in the secretory cells of the mammary gland, called alveoli cells. The alveoli are surrounded by muscle cells, called myoepithelial cells. The muscle cells will contract to squeeze milk from the alveoli into the ducts when the stimulus for milk let-down is introduced. Precursors needed for milk synthesis are provided by blood vessels. The basal end precursors of milk components are taken up from the blood and at the apical membrane milk components are secreted into the lumen of the alveoli. From there milk flows into the gland and teat cisterns. It is estimated that about 400-500 litres of blood pass through the mammary gland for production of 1 litre of milk. The main components of milk are water, lactose, fat and protein. In addition to these main components, there are many other elements and compounds in milk e.g. minerals , vitamins and enzymes (Walstra *et al.*, 2006; Akers, 2002).

Lactose is the major carbohydrate of milk. Lactose is synthesized in Golgi vesicles in the secretory cells. Glucose is produced in liver, primarily from propionate, a product of rumen fermentation. Glucose in blood is taken up to the udder and a part of the glucose is converted to galactose. Thereafter, one molecule of glucose binds with galactose to produced lactose. Lactose is the main osmotic determinant of milk. Fat is formed in the secretory cells when fatty acids are bound to glycerol, generating triglycerides, a neutral form of fat. More than 440 fatty acids have been identified in the milk originating from *de novo* synthesis in the udder, mainly from acetate, from dietary fat and especially during early lactation from mobilise adipose reserve. Short fatty acids, C_4-C_{14} are synthesized in the mammary gland, while C_{16} and C_{18} are derived from blood triglycerides (Walstra *et al.*, 2006; Akers, 2002).

Milk protein consists of casein and whey protein. Approximately 80% of the protein in milk is in the form of casein and 20% of whey protein. Amino acids are transported to the udder via the bloodstream and transformed into casein by the mammary alveolar cells. Casein is a mixture of α_{S1}-, α_{S2}-, β- and κ-caseins and γ-casein. Whey proteins are present in a dissolved form, consisting of α-lactalbumin and β-lactoglobulin (Walstra *et al.*, 2006; Akers, 2002).

Milk contains different types of enzymes. They include both indigenous enzymes, which are excreted by mammary gland and enzymes originating from microorganisms. Most of the indigenous enzymes are synthesized by the secretory cells, while others are derived from the blood, e.g. plasmin. Some of the enzymes are secreted by organisms such as protease and lipase. Most enzymes do not have a biological function in milk, but some have antimicrobial function, e.g. lactoperoxidase and lysozymes (Walstra *et al.*, 2006).

Holstein Friesian is a high-yielding dairy cow in temperate countries. With good management of feeding and milking, HF cows can yield more than 9,000 kg/cow/305 day lactation period (Chandan *et al.*, 2008). The milk lactose, fat and protein contents range from 4.6-4.8%, 3.8-4.9% and 3.0-3.6%, respectively (Blowey & Edmondson, 2010; Akers, 2002).

Mastitis

Mastitis is the most common and also most costly production disease in dairy production (Halasa *et al.*, 2007; Bradley, 2002). Mastitis can be present in both a clinical and a subclinical form and is primarily caused by bacterial infections of the mammary glands. Both mastitis forms are associated with increased SCC (Pandey *et al.*, 2005; Sandholm, 1995). Clinical mastitis is characterized by the presence of the external signs of udder inflammation such as heat, pain, swelling, tenderness and/or abnormal milk. Subclinical mastitis, on the other hand, exhibits no clinically visible signs and often remains undetected unless laboratory methods measuring milk SCC and bacteriological examination are used (Edmondson & Bramley, 2004). Subclinical mastitis is usually the most prevalent form on smallholder dairy farms (Byarugaba *et al.*, 2008). How prevalent subclinical mastitis is in dairy production in Vietnam has not been fully evaluated and neither have the risk factors for subclinical mastitis.

Normally, milk produced by healthy cows contains a very low concentration of micro-organisms, since the teat canal can act as an anatomical-mechanical and chemical-cellular barrier (Sandholm & Korhonen, 1995). In principle, when pathogenic bacteria enter the udder, the defense system of the udder sends a vast number of leucocytes into milk to remove the bacterial pathogens (Blowey & Edmondson, 2010; Sandholm & Korhonen, 1995). The sudden increase of SCC in milk is a primary feature of inflammation (Sandholm, 1995). If the inflammatory reaction cannot destroy bacteria, affected cows remain contagious.

Over 200 different organisms have been recorded today in scientific literature as being a cause of bovine mastitis (Blowey & Edmondson, 2010). They can be divided into two groups: contagious and environmental pathogens according to their origins (Pyörälä, 1995). Mastitis caused by contagious pathogens such as *S. aureus* or *Str. agalactiae* are widespread, usually causing subclinical infections and a large milk SCC increase (Blowey & Edmondson, 2010; Edmondson & Bramley, 2004). Environmental pathogens such as *Str. uberis* and *Str. dysagalactiae* cause considerably less SCC elevation (for reviews see Pyörälä, 1995; Smith & Hogan, 1993).

Thus the SCC level varies largely depending on the type of bacteria infecting the udder.

Causes of variation in milk somatic cell count

Milk somatic cell count is widely used to monitor udder health. As the definition of udder health refers to the inflammation status, SCC and bacteriological examination indicate the status of mammary gland health (Harmon, 1994). The SCC may be affected by several factors, such as bacterial infection, age and stage of lactation, environmental and management factors or a combination of these factors (Blowey & Edmondson, 2010; Harmon, 1994)

Cow age and stage of lactation

That milk SCC increase with advancing age comes with the exposure to previous infections (Harmon, 1994). This is due to the increased period of exposure of the udder experienced with infection over the lactations.

Milk SCC is often high in the first 7 to 10 days after calving and in late gestation (Blowey & Edmondson, 2010; Dohoo & Meek, 1982). High SCC in the first weeks after calving appears to be a part of the cow's natural immune system response in preparation for calving and enhances the mammary gland's defense at parturition time (Dohoo & Meek, 1982). Udder quarters with no infection have a rapid decline in SCC within a few weeks postpartum (Bartlett *et al.*, 1990). Towards the end of lactation, since the amount of milk produced is diminishing SCC increases in milk (Blowey & Laven, 2004).

Environmental factors

Stress of various types, such as oestrus, disease, vaccination and drug administration (Blowey & Laven, 2004; Barkema *et al.*, 1998; Harmon, 1994) and heat stress (Rhone *et al.*, 2008) may affect the SCC of individual cows. Stress may increase the number of leucocytes in blood (Blowey & Laven, 2004). The increased incidence of clinical mastitis in the summer in temperate countries is due to the warm and humid environment that increases the exposure of pathogenic agents (Hillerton, 2004). In addition, the cows that are susceptible to heat stress in the tropics may be at increased risk of developing new infections, which in turn give rise to higher SCC and reduced milk yield (Rhone *et al.*, 2008).

Milking frequency

It is generally known that milk SCC is higher in the afternoon milking than in the morning milking (Blowey & Laven, 2004; Hale *et al.*, 2003). This is due to the shorter milking interval and lower milk yield in the afternoon resulting in a concentration effect (Hale *et al.*, 2003). However, SCC varies from day to day due to the variety of previous factors listed, together with management factors such as hygienic conditions and/or milking machine function.

Effect of mastitis on milk composition

Mastitis may cause an alternation in fat, lactose and protein content in milk (Nielsen *et al.*, 2005; Urech *et al.*, 1999; Auldist & Hubble, 1998). Declining fat content during mastitis is due to the reduced synthetic and secretory capacity of the mammary gland. Free fatty acids in mastitis milk may increase as a consequence of inflammation, probably caused by increased activity of the enzyme lipase. Lactose decreases as a consequence of reduced synthetic capacity and losses to circulation, but also as a way to maintain the osmolic pressure, since mastitis causes an increase in ion content (Auldist & Hubble, 1998; Kitchen, 1981). Protein composition changes towards increased whey protein content, while content of casein proteins declines (Walstra *et al.*, 2006)

It is established that mastitis bacteria can affect the quality of milk. Ma *et al*. (2000) looked at the relationship between high SCC and quality of pasteurized fluid milk by infusing *Str. agalactiae* to elevated SCC. Their work confirmed that mastitis caused by *Str. agalactiae* adversely affected the quality of pasteurized fluid milk (Ma *et al.*, 2000). With regard to the infection, proteolytic activity of milk decreased after infections were cured but remained significantly higher than the pre-infection activity (Saeman *et al.*, 1988). Larsen *et al.* (2004) found that, in high SCC milk from *S. uberis* infected quarters, proteases apart from the plasmin contribute significantly to the proteolysis. Grieve & Kitchen (1985) found that proteinases from leukocytes and from psychrotrophic microorganisms are not important in proteolysis of milk. Moreover, the proteolytic and lipolytic enzyme activities produced by psychrotrophic microorganisms showed increased activity after 2 to 3 days at 10^{o}C (Burdová *et al.*, 2002)

Objectives

The general aim of this study was to generate information that could lead to improved milk production on smallholder dairy farms in Southern Vietnam. The aim was also to create a foundation that could be used in the advisory service or/and in further research for better milking management routines which in turn will improve milk quality.

Therefore, the specific objectives were:

- To identify the problems of dairy production on smallholder farms in Southern Vietnam.
- To investigate the management factors influencing milk SCC in lactating cows on smallholder dairy farms.
- To identify the prevalence of subclinical mastitis based on SCC.
- To study the protein degradation caused by *Str. agalactiae*.

Materials and methods

Study sites

The southern part of Vietnam has a typical tropical monsoonal climate characterized by only two different seasons, dry (December to March) and wet (April to November). The annual rainfall ranges from 1,500 to 2,000 mm. The peak rainfall occurs in July to August. The temperature is quite warm and stable all year-round (Sterling *et al.*, 2006).

The survey (Paper I) was carried out in peri-urban areas of Ho Chi Minh City (Fig. 1) with an air temperature that ranged from 25.9 to 33.3 ^{o}C while the mean maximum and minimum relative humidity was 81 and 68%, respectively. Annual rainfall varies from 1,500 to 1,600 mm and the rainy season is between May and October. The study was done during May to June, 2006. Around 54% of all dairy cattle in Vietnam are found in this area.

The studies on factors influencing milk SCC and on the prevalence of subclinical mastitis (Paper II and Paper III) were carried out in Long Thanh district, Dong Nai province to the west of Ho Chi Minh City (Fig.1). The studies were conducted at the onset of the rainy season (March to June, 2008).

Farms, cows and designs

In the survey study (Paper I), 120 farms representing approximately 6% of smallholder dairy farms in the two districts were randomly selected. The study was done by direct interviews with the smallholder dairy farmers based on a questionnaire to obtain data on milk production and farm management and a protocol for field observation of on-farm practices. The questionnaire was pretested in the field and modified before being used to guide the official interviews with representatives of each household. Each interview lasted for about 3 hours. The interviewers also performed an additional farm visit to take field observations, and milk and feed samples for analysis. Composite milk of 360 cows, 20% of clinically healthy cows on each studied farm, was sampled for analysis of milk composition and SCC. Administrative maps as well as secondary data of socio-economic and dairy production in the area were collected in local offices.

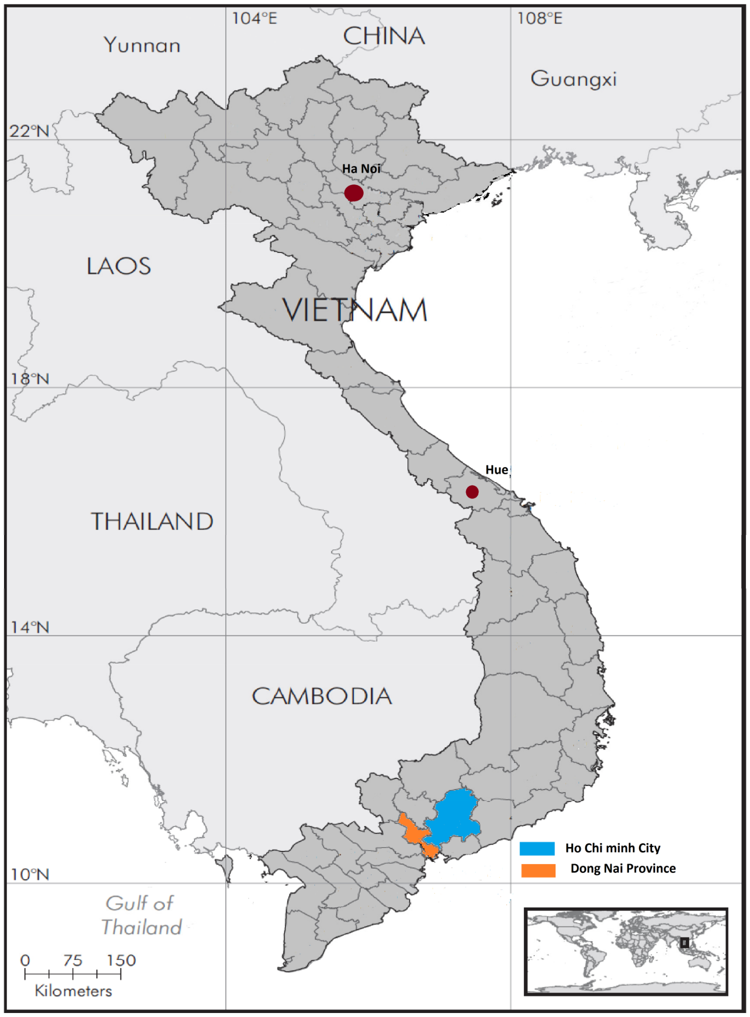

Figure 1. Administrative map of Vietnam with study sites: Ho Chi Minh City and Dong Nai Province. Adapted from “Vietnam, a natural history” (Sterling *et al.*, 2006)

For the second and the third papers, twenty farms were selected. Inclusion criteria were at least 6 lactating cows and use of the bucket machine milking system. Only cows that according to farm records were clinically healthy and without mastitis episodes were selected for sampling. All farms were visited during morning or evening milking by the same team of two persons. Milk samples were collected and the farmers were interviewed about their management routines, including, housing, feeding, milking practices, and hygiene. Milking practices were observed during the entire milking to record the performing of milking, milking times, teat cleaning, teat cup cleaning, cow hygiene, use of water and feed hygiene, and housing system.

Milk sampling and analysis

In Paper I, individual cow milk samples were taken in one afternoon milking and preserved with bronopol. The samples were then analysed for fat, protein, lactose, dry matter, and solid non-fat according to the mid-infrared spectroscopy method (Farm Milk Analyser, Mirris AB, Uppsala, Sweden). Milk SCC was determined on the farms, directly following sampling, by a fluorescent method, using a DeLaval cell counter (DCC) (DeLaval, Tumba, Sweden). The respiration rate and rectal temperature of selected cows were measured twice a day, at 08:00 and 14:00, on the same day as milk sampling took place, to determine the animal's state of heat stress. Air temperature and relative humidity were recorded at the same time.

In Paper II and Paper III, quarter strip milk samples were taken at one morning or afternoon milking. Mastistrips cassettes (Mastistrip©, SVA, Uppsala, Sweden) were used to collect the milk samples. The cassettes were then sent to the Mastitis laboratory, SVA, for identification of bacterial species according to the laboratory's accredited methods. Twenty-five mL of strip milk was concurrently collected in a plastic bottle for analysis of quarter milk SCC. Somatic cell count was analysed by a fluorescent method described above. In total, 458 quarter milk samples of 115 lactating cows were analysed.

Genotyping the strains of *Str. agalactiae* isolates was done at SVA and analyses of proteolytic activity was done at the laboratory of the Food Science Department, SLU. Pulse-field gel electrophoresis (PFGE) was employed to genotype the strains of *Str. agalactiae* (Fasola *et al.*, 1993), while Capillary electrophoresis (CE) was used in the analysis of proteolysis (Heck *et al.*, 2008) (see Paper IV).

Statistical analysis

Detailed descriptions of statistical methods and models used are shown in the individual research papers. Briefly, in Paper I, SPSS for Windows version 14.02 (SPSS Inc., ® 1989-2005) was employed to analyse categorical data and quantitative variables were compared by using the t-test for significant differences at $P < 0.05$ and Chi-squared tests were used for categorical variables. Procedures of SAS (SAS Institute Inc., 2008) were used to investigate and describe that factors that influence milk SCC (Paper II).

Results

Milk production and management system

Table 1 describes a profile of dairy farms in the study area. On average, dairy farms included 4,700 m^2 of land, including land for pasture and crops. Of the farmers operating the farms, 60.8% had 10 to 20 years of experience, but there was a wide variation in dairy farming experience among the surveyed farmers, ranging from 2 to 30 years. Dairy farmers living near the city center had significantly ($P < 0.001$) longer experience compared with farmers who were living far from the city center, 13 and 9 years, respectively. The number of animals in the herds ranged from 2 to 50 cows with a majority of households owning between 2 to 17 cows (mean = 12).

When averaged over the survey data (Paper I), the cows were fed between 20 to 40 kg of roughage, fresh matter, depending on the availability of green grasses, rice straw, stage of lactation and amount of concentrates. Brewery by-products and commercial concentrates were mixed with water and were given as protein supplementation. Of the observed farms, feed in 45% (54 farms) of the troughs had fermented. Only 35.8% (43 farms) of the farms provided fresh water *ad libitum* in separate trough for the cows and 51.7% (62 farms) of farmers provided less than 30 L of water per cow per day (Paper I).

Hand milking was practiced on 90.4% of the farms, whereas 9.6% of farmers used milking machines. Laborers were employed for milking in 34% of the farms, while in 66% of the farms milkings were managed by family members. Different hand milking techniques were used: 78.3% used full-hand grip, 20% thumb-in and 1.7% used pull down (Fig. 2). Farmers usually cleaned the cow's udder with water before milking, although a few of the observed farmers used solutions for cleaning the teats. They did not perform post-dipping of teats after milking, except in cases of mastitis. On those farms where machine milking was practiced, teat cups were dipped into a solution of sodium hypochloride (NaClO) after each milking in order to clean and sanitize the equipment (Paper I).

Table 1. Description of dairy farm profile in the survey study (n = 120 farms)

Categories	Frequency (%)
Dairy farming system	
Dairy cattle only	77.5
Dairy cattle and crops	20.0
Cattle and other animal	2.5
Type of dairy farmer	
Full-time	90.8
Local officials, teachers and retailers	9.2
Farmer's education	
Primary school	41.7
Junior high school	35.8
Senior high school	20.0
Vocational	0.8
College or university	1.6
Herd size	
1 – 5 cows	25.0
6 – 10 cows	39.0
>10 cows	36.0
Dairy breed	
HF crosses	95.8
Crossbred Sindhi	4.2

In the survey data (Paper I), 84% of the farmers reported that their lactating cows were sensitive to an increase in temperature during the day. Artificial insemination was used for both heifers and cows. The AI success rate for cows was lower than for heifers. On average, heifers were artificially inseminated 1.5 times, whereas 47.5% of lactating cows were inseminated 3 to 4 times and 43.4% were inseminated 5 to 7 times per pregnancy. Consequently, 27.5% cows lactated for more than 12 months, 50% lactated up to 10 months and 14.2% only produced milk for 7 to 8 months.

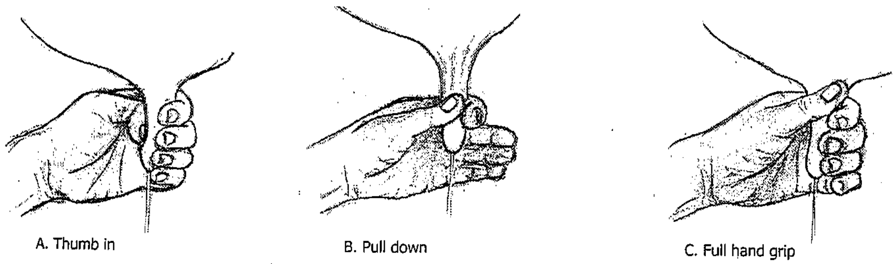

Figure 2. Katarina Cvek-Hopkins (2009) illustrated the three hand-milking techniques. Adapted from Milk production of hand-milked dairy cattle in Burkina Faso (Millogo, 2010).

Milk yield, composition and somatic cell count

The average daily milk yield was 16 kg/day/cow (n = 360 cows). The average fat, protein and lactose contents were 4.1% (SD = 0.54), 3.2 (SD = 0.15) and 4.7% (SD = 0.25), respectively. Fat content was an important consideration for 88.9% of the farmers, whereas 11.1% did not consider milk composition to be important (Paper I).

Milk SCC was high on most studied farms (n = 120). The average was 1,300,000 cells/mL milk (SD = 900,000 cells/mL). Sixty-nine percent of the cows had SCC > 400,000 cells/mL milk, while 31% had SCC < 400,000 cells/mL milk. Herd size did not significantly influence SCC, while a numerical difference was observed in SCC due to the age of the cows (Paper I).

Factors causing elevated SCC

The access to drinking water was found to significantly influence milk herd SCC (P = 0.008). In herds providing drinking water *ad libitum* the measured herd milk SCC was lower (403,000 cells/mL milk) than in herds where the cows were offered drinking water restrictedly (860,000 cells/mL milk) (Paper II).

The method of teat cup cleaning had a tendency to influence herd milk SCC (P = 0.078). Farms using water and detergent to clean the teat cups after each milking had a numerally lower herd milk SCC (179,000 cells/mL milk) compared with farms where the teat cups were cleaned with only water after each milking (546,000 cells/mL milk) and farms where the teat cups were additionally cleaned with detergent twice a week (774,000 cells/mL milk). Vacuum pressure, housing system, type of milker and method of udder cleaning were not found to significantly influence herd milk SCC (Paper II).

Bacterial prevalence

Str.agalactiae was the predominant species in infected quarters at all management routines practiced (Paper II). Herds where drinking water was provided *ad libitum* showed higher percentage of quarters with *Str. agalactiae* infection (26%) compared with herds where water for the cows was restricted (16%). The percentage of udder quarters with *Str. agalactiae* infection was lower (3%) in herds where teat cups were cleaned with water and detergent after each milking compared with herds where teat cups were cleaned only with water (18%) and those cleaning with water and additionally with detergent twice a week (27%). The percentage of quarter infection with *Str. agalctiae* was higher (21%) in herds where cows were milked with a milking vacuum of > 45 kPa compared with herds where cows were milked with a milking vacuum of 37-45 kPa (13%) (Paper II).

Farms where water and dry towels were commonly used for cleaning teats showed the highest percentage of quarters with *Str. agalactiae* infection (31%) compared with other pre-milking cleaning practices (Paper II).

The routines for cleaning teat cups without using a detergent after each milking as well as cleaning udders/teats pre-milking and cooling the cows using a water hose were found to be associated with high frequencies of quarter milk samples with growth of CNS and *Str.uberis*, respectively (Paper II).

In Paper III, the prevalence of subclinical mastitis (SCC > 200,000 cells/mL milk) at quarter basis was 63.2% (n = 285 out of 451) and at cow basis 88.6% (101 out of 114). Of quarters with subclinical mastitis, bacteria were isolated from 51.9% (148 out of 285), while from the quarters with SCC < 200,000 cell/mL milk, only 10.8 % (18 out of 166) were bacteriologically positive. Twenty-two percent of the quarters (99 out of 451) had a SCC ≤ 100,000 cells/mL milk. There were 36.0% (165 out of 458) of the quarter milk samples that were bacteriologically positive. In total, there were 40% of cows that were bacteriologically positive in all udder quarters.

The most commonly bacteria species was *Str. agalactiae*, which was isolated from 21.0% (n = 96) of 35.7% of cows (n = 41) on 65% (n = 13) of farms. *S.aureus* and *Str. dysagalactiae* were noticeably low, with 4.2 and 1.8% of all bacterial isolates, respectively (Paper III).

Streptococcus agalactiae strains

Pulse-field gel electrophoresis was performed to identify different strains of *Str. agalactiae*. Among 96 isolates collected from 41 cows on 12 farms, 11 different profiles were generated. One to five different strains of *Str. agalactiae* was usually found on individual farms, but on two farms only one strain was found (Paper IV).

Proteolysis activities

The strains of *Str. agalactiae* did not show the same degradation of casein and whey protein. The proteolysis of casein was higher (12-70%) compared with whey proteins (4-12%). The highest proteolysis was observed for α_{S2}-casein, where some strains degraded more than 70% compared with bacteria-free control milk, and the lowest break down for α_{S2}-casein was found to be 30% (Paper IV).

General discussion

Milk production and management in smallholder systems

An important feature of dairy cattle production in southern Vietnam is that the smallholder models are common in peri-urban areas, where good markets and production services are found (Tam, 2004). This is in agreement with Devendra (2002) who found that the expansion of smallholder dairying was mainly based on integrated systems of crop-animal in the peri-urban areas where the dairy production is essentially driven by urban demand. However, the major constraints to production in these dairy systems are feed resources, reproduction and animal health care (Devendra, 2001), milking management and milk quality (Rhone *et al.*, 2008; Alejandrino *et al.*, 1999).

According to our findings, dairy farmers in the studied area began their dairying with a small number of cows from their own investment and they obtained knowledge of dairying from annual short training courses provided by extension service centers. Thereafter, experiences in dairy production have been transferred within the local communities. One of the findings in Paper I was that dairy farmers' experience in dairy production in areas near the city center was significantly ($P< 0.001$) longer compared with those famers who were far from the city center. Three factors largely explain the rapidly increasing dairy production in the area namely: (1) high population, (2) relatively high *per capita* income, (3) concentration of most services for production and processing factories (Tam, 2004). However, due to the rapid urbanization that has encroached on agricultural land during the last 25 years, dairy production has moved to more remote areas. This could be a possible reason for the large variation in experience of dairy production (ranging from 2-30 years) found in the area (Paper I). The production systems have also shifted from integrated subsistence to new systems based on milk production (Luthi *et al.*, 2006) due to the impact of urbanization and competition. Therefore, the results in Paper I showed that 77.5% of smallholder farmers kept dairy cattle only and 90.8% of the farmers actually worked on the farm.

The progress of dairy production, the widespread urbanization that occurs in the area and increased competition among dairy farmers changes dairy production management. Results presented in Paper I show that the number of dairy cattle per farm in this area tends to increase with time. The average herd size appears to have increased markedly during the last few years, with an average of 12 cows in the present study compared with 2 to 5 cows per farm reported by Tam (2004) and a range of 1 to 4 cows that was found in the north (Suzuki *et al.*, 2006).The herd size correlated positively with farmers' experience of dairy production (Paper I), indicating the development of dairy production in the studied area.

The majority of the farmers had primary and junior school education only (Paper I). Instead of relevant education, they rely on their own experiences or by learning from neighbors. Most of the farmers attended a yearly short training course as a way to improve practical knowledge for dairy production and management. This indicates that there are prerequisites for technical transfer and continued education for dairy farmers in the area. This is in agreement with Falvey & Chantalakhana (2001), who concluded that future smallholder dairy development will rely on continued education.

Feeding management and water supply

Cows were generally fed with green grasses, rice straw and industrial by-products, which are available in the area, supplemented with commercial concentrate. Farmers estimated that around 0.5 kg of concentrate was required to produce 1 L milk per cow per day. They often fed cows rice straw at night to improve the fat content in milk. An improvement of feeding systems is an important prerequisite for increased profitability of dairy production in this region since the cost of feeding accounts for 40-60% of the total cost of milk production (Devendra, 2002; Man, 2001).

Since many farmers have only limited experience of dairy husbandry, they are likely to underestimate the amount of water, especially that required by HF crossbred dairy cattle, which are poorly adapted to a hot environment. Only 36% of dairy farmers in the present survey provided drinking water for their cows *ad libitum,* while 52% provided less than 30 L (Paper I). This finding is in agreement with that of Suzuki *et al.*(2006), who found that only 3 out of 99 studied farmers provided their cows with water *ad libitum*. According to Radostits (2001), inadequate water supply results in reduced dry matter intake and decreased milk production of dairy cows and a consequential loss of body weight. Interestingly, access to drinking water significantly influenced herd milk SCC. The highest herd milk SCC was noted in farms where drinking water was provided restrictedly. However, restricted drinking water was not related to reduced milk yield (Paper II). Lactating cows in the tropics obviously need more water to alleviate heat stress (Beede & Collier, 1986). Restricted water gives rise to dehydration reflected by increased blood packed cell volume and osmolality, as reported by Chase (1988). Therefore, providing adequate drinking for the cow to ameliorate the effect of heat is recommended by many authors (for reviews see Suzuki, 2005; Fielding & Mathewman, 2004).

Surprisingly, a numerally higher frequency of quarters with *Str. agalactiae* infection was observed on farms where water was provided *ad libitum* (Paper II). It is unlikely that drinking water influences *Str. agalactiae* infection rate. The actual reason was not known in this study. Among each group of studied farms with different management routines, such as milking cows with high vacuum pressure, cleaning teats only with water and cleaning teat cups only with water was related to the prevalence of *Str. agalactiae*.

Dairy breeds on smallholder farms

The use of AI and knowledge of genetic upgrading and crossbreeding on smallholder dairy farms has led to increased milk production during the last two decades. Holstein Friesian crosses dominates in smallholder dairy farms, mostly at F2 (75% HF inheritance), F3 (87.5% HF inheritance) or more HF blood (Gautier, 2008; NIAH, 2010; Cai, 2002). As shown in Paper I, the average milk yield was 16 kg/day/cow, and HF crossbred cows constituted on average 96% of the herds. The yield was higher compared to the 13 kg/day/cow reported by Luthi *et al.* (2006) and Suzuki (2005). However, the milk yield was found to be 13.4 kg/cow/day in Paper II, which is in agreement with Luthi *et al.* (2006) and Suzuki (2005). The increase in milk yield observed in Paper I may depend on the fact that the F3 generation was dominant in the studied farms. In addition, the results of the survey study (Paper I) showed that repeated breeding is a problem i.e. three or more services often were required before conception in the lactating cows. This result is in agreement with Alejandrino *et al.*(1999), who explained that these breeding problems on smallholder farms due to poor breeding management that causes poor ovarian function, which in turn is reflected by a low progesterone level. Wolfenson *et al.*(2000) confirmed that lower progesterone secretion, led to an increase in embryo mortality. Cavestany *et* *al.*(1985) reported that high environmental temperatures are associated with low breeding efficiencies. Therefore, it is reasonable to assume that cows with high HF inheritance do not exhibit their full genetic potential in the hot and humid environment of the tropics. It is a challenge for smallholder farmers to be successful with the breeding management of HF crossbred cows. Such management is vital for profitable production.

Effect of management on milk composition

The average milk fat, protein and lactose contents reported in Paper I are in agreement with those reported by Aiumlamai (2010) and Luthi *et al.* (2006). However, a large variation in milk components among studied farms is noteworthy.

In the survey study (Paper I), the majority of farmers considered fat content as important and they claimed that it was possible to improve the fat content by feeding rice straw during the night time. The low fat content among many of the studied farms indicates that feeding may be problem. According Davis & Brown (1970), low fat content could be a result of a low proportion of roughages in the diet. Rice straw has a low concentration of crude protein and the farmers did not generally treat rice straw in order to improve the nutrient value. Urea-treated fresh rice straw, for example, markedly improves the nutritional value of the feed (Man, 2001). In practice, to maintain optimum milk yield, farmers supplemented their cows with commercial concentrates, but they did not consider the fibre level in the diet. It is generally accepted that low fibre - high concentrate diets have a negative impact on milk fat content.

The survey (Paper I) found that the only 8% of farmers supplemented their cows with minerals *ad libitum*. Bouraoui *et al.*(2002) also reported that summer heat stress reduced milk yield and lowered milk fat and protein contents. It is an important aspect that 83% of the smallholder dairy farmers in the study area reported that their cows experienced heat stress during the day time.

Results of the survey study (Paper I), indicated that farmers supplemented their cows with commercial concentrates, usually mixed with brewery by-products, in order to improve milk yield. Protein content is not considered in the payment system for milk in the area and it is, therefore, not important for the farmers take measures to increase protein content in milk. Climate and temperature can influence the protein content of milk due to their effect on animal metabolism. A high temperature was reported to affect both protein and energy metabolism of ruminants. Several authors have noted a reduction in milk protein content at temperatures above 27°C (Kadzere *et al.*, 2002; Kirchgessner *et al.*, 1967; Collier & Zimbelman).

Compared with fat and protein contents, the lactose content is less affected by nutrition and breed, but more dependent external factors. Low lactose levels are usually related to clinical mastitis (Kitchen, 1981; Linzell & Peaker, 1972), and moderately increased levels of milk SCC (Berglund *et al.*, 2007). The low lactose that was observed in some of the sampled milks in Paper I may be related to udder infection (Pyörälä, 2003; Kitchen, 1981).

Milk somatic cell count

Somatic cell counts are general indicators of udder health (Dohoo & Meek, 1982). The results in the survey study (Paper I) showed that the average milk SCC was high, 1,300,000 cells/mL milk, in almost all of studied farms. Although there was a wide variation among farms, most of the cows had high SCC. Cell count in composite samples taken from cows with all four quarters free of infection have been reported to average from about 100,000 to more than 200,000 cells/mL, depending on cow's age (Dohoo & Leslie, 1991; Dohoo & Meek, 1982). Thus the above finding indicates a considerable risk of infection. The most important factor affecting milk SCC is infection status of quarters (Dohoo & Meek, 1982), while other factors have only minor effects (Pyörälä, 2003). High SCC affect both milk quality and milk yield (Harmon, 1994). This is in agreement with the observed negative correlation between milk SCC and milk yield reported in Paper I.

Regular use of teat dip has consistently been associated with lower SCC (for reviews see Dohoo & Meek, 1982; Moxley *et al.*, 1978; Schultz, 1977). Many farmers in the present study did not apply udder hygiene practices, such as udder preparation before milking, cleaning teat pre-milking and post-milking teat dip. Post dipping teats after every milking is one of the most important practices in mastitis control in European countries (Blowey & Edmondson, 2010). These listed factors may have contributed to the infection rate that induced elevated SCC among studied cows, and, therefore, consequently on the farms. Unexpectedly, the method of udder cleaning was not found to influence herd SCC (Paper II), which might due to a relatively low number of studied farms.

Milk SCC is also, as mentioned previously, used to monitor the occurrence of subclinical mastitis (Pandey *et al.*, 2005; Dohoo & Leslie, 1991). The most important source affecting SCC of milk is from individual infected quarters, which consequently will affect the SCC at cow and herd levels (Dohoo & Leslie, 1991). Only 22% (99 out of 451) of the quarters had SCC < 100,000 cells/mL milk (Paper III). According to Harman (2002) and

Hillerton (1999) the healthy udder has SCC less than 100,000 cells/mL. Brolund (1985) confirmed that an udder SCC greater than 200,000 cells/mL milk is a considerable risk of infection. Thus the high frequency of udders with SCC greater than 200,000 cells/mL in this study indicates a high risk of udder infection among the studied cows.

Cows with clinical signs of mastitis were excluded from the investigations. Therefore, subclinical mastitis presumably was a major cause of elevated SCC in the survey area. Later investigations (Paper II & III) showed a high percentage of udder quarters infected with *Str. agalactiae*, which certainly contributed to the high milk SCC reported in Paper I.

Prevalence of mastitis pathogens

Infection with *Str. agalactiae* usually cause subclinical mastitis and is associated with elevated SCC in dairy cows (Keefe, 1997). *Str. agalactiae* was a major pathogen causing subclinical mastitis in the pre-antibiotic era, according to Jain (1979). Still, it is also today a serious cause of subclinical mastitis in tropical countries (Cheng *et al.*, 2010; Souza *et al.*, 2005). *Str. agalactiae* is an obligate parasite of the bovine mammary gland and it can multiply in milk and adhere on the mammary epithelium (Keefe, 1997; Jain, 1979).

The infection rate both at cow and udder quarter levels was higher in Vietnamese systems (Paper III) than in European countries and in the United States (for reviews see Keefe, 1997; Wilson *et al.*, 1997; Oliver & Mitchell, 1984; Jain, 1979). The rate was also higher than in other areas of Southeast Asia (Yang *et al.*, 2011; Cheng *et al.*, 2010). Moreover, the infection rate of *Str. agalactiae* was significantly higher than that of *S. aureus* (Paper II & III). This is in contrast with the situation other tropical areas (Almaw *et al.*, 2008; Getahun *et al.*, 2008; Lafi *et al.*, 1994). *Str. agalactiae* does not survive in the environment surrounding the cow and may be erased by both antibiotics and suitable management routines (Keefe, 1997; Jain, 1979). It is, therefore, reasonable to conclude that the milking hygiene was poor in the studied farms. It was observed that farmers in the studied area replaced culled cows by purchasing new cows within the community (Paper I & II). Presumably infected cows may also be a source to spread *Str. agalactiae*.

Str. agalactiae was found to have a heterogeneous genetic background, with 11 different strains (Paper IV). It was also found that there was an intra-herd prevalence, since infected cows within a herd usually shared the same pulsotype *of Str. agalactiae*. It is known that when a herd gets infected with *Str. agalactiae*, the prevalence within the herd will be high (Gonzalez *et al.*, 1986). The virulence of the various strains of *Str. agalactiae* is related to their ability to adhere to the mammary surfaces (Keefe, 1997; Jain, 1979).

Both herd SCC and level of infection of *Str. agalactiae* were lower in herds where teat cups were washed with water and detergent after each milking compared with the other teat cup cleaning practices (Paper II). According to Bramley (1992) bacteria can transmit from cow to cow or within cow by quarter to quarter, if cows are milked with a contaminated machine. Blowey & Edmondson (2010) confirmed that careless cleaning of teat cups resulted in milk residue and bacterial build-up within the teat cup. Milking cows with unstable, and moderately cyclic fluctuation are known to negatively affect udder health and increase mastitis occurrence (for reviews see Hamann *et al.*, 1993; Bramley *et al.*, 1992). In the present study (Paper II), farmers milked cows at an average of 49 kPa, but with a wide variation among the studied farms. In practice, air pressure fluctuated during the milking course since the vacuum gauges were purchased from the local market and the electric supply was unstable during the milking course. Milking cows with too high vacuum level will induce teat orifice damage, leading to increased mastitis occurrence and consequently high milk SCC. However, this study did not find that high vacuum pressure was related to herd milk SCC, but it was associated with a higher percentage of quarters infected with *Str. agalactiae*.

Proteolysis in milk

The results in Paper IV show that *Str. agalactiae* mostly degraded casein protein rather than whey protein and α_{S2}-casein was particularly found to be damaged by the different strains of *Str. agalactiae*. This result is in agreement with a previous study by Haddadi *et al.* (2005) with *Escherichia* (*E.*) *coli*. According to Fajardo-Lira & Nielsen (1998), bacterial proteases can break down the casein micelles and release enzymes, but the degradation sensitivity of casein varied with type of casein exposed to proteolysis and with incubation time (Haddadi *et al.*, 2005). In addition, the relationship between bacterial protease in milk and the plasmin system was not clearly known. Grieve and Kitchen (1985) reported that the results for the casein degradation by leucocyte proteases was in the order α_{S1}-> β->> κ-casein. By examining the effect of *E. coli* on casein degradation, Haddadi *et al.*(2005) showed that *E. coli* protease has a direct effect on casein. β-casein is shown to be slightly more resistant to enzymatic degradation than α_{S1}-casein and κ-casein (Haddadi *et al.*, 2005). β-casein and κ-casein can be degraded by bacterial protease (Fairbairn & Law, 1986). According to Haddadi *et al.* (2005) the casein hydrolysis by bovine plasmin showed that β-casein was hydrolysed at a faster rate than α_S-casein, while κ-casein was relatively resistant to proteolysis by proteases. The result in this study could be explained by the fact that the peptides generated by hydrolysis of κ-casein can inhibit *Str. agalactiae* enzymes (Haddadi *et al.*, 2005).

The three-dimensional structure of the proteins may affect the differences in degradation susceptibility but also the accessibility of proteolytic enzymes to the amount of protein (Fajardo-Lira & Nielsen, 1998; Fairbairn & Law, 1986). The differences in protein degradation caused by different strains of *Str. agalactiae* could be observed in our study. More investigations are needed, including further studies on the characterization of peptides and amino acids formed after proteolysis to evaluate the origin of protein degradation.

Conclusions

The main constraint to milk production on smallholder dairy farms was found to be the relatively high somatic cell count in milk from almost all individual cows and at all stages of lactation. The high somatic cell count indicates poor udder health. This indicates a need to improve udder health of lactating cows in order in turn to improve milk quality.

Management practices, especially restricted drinking water, show the limitation of dairy husbandry practices among smallholder dairy farmers. It is suggested that water should be provided for dairy cows *ad libitum* in the environmental conditions of hot and humid Southern Vietnam.

Str. agalactiae was found to be the predominant species of subclinical mastitis. This indicates that infection with *Str. agalactiae* contributes to the high somatic cell count and influences milk quality. The careless milking hygiene practices contribute to the high prevalence of subclinical mastitis at cow and herd levels.

When *Str. agalactiae* was added to the milk, protein degradation was observed in comparison to control milk without bacteria. The caseins were degraded to a large extent, but degradation was also observed for the whey protein.

Implementation and future research

From the findings of the studies in this thesis study, it appears that the udder health of smallholder lactating cows needs to be improved in order to develop a profitable and sustainable dairy production. Improved milking hygiene, with careful cleaning of teats twice a day, pre- and post-dipping and cleaning teat cups with detergent/sanitized solution after each milking should be promoted. Such practical techniques should be combined with an eradication program of *Str. agalactiae*.

The future of smallholder dairy production in Vietnam will rely on continued education of smallholder dairy farmers and relevant research.

- Education should focus on improving dairy husbandry and on on-farm milking management routines that will be affordable for the farmers.
- Research must acknowledge the role of bovine udder health in profitable and sustainable milk production in smallholder dairy farms, such as establishment of on-farm trials so that the results can be directly transferred to the producers. Development of a recording system and an SCC monitoring program is the long-term technical goal for sustainable milk production on smallholder dairy farms in Southern Vietnam.

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References

Aiumlamai, S. (2010). *Dairy management, health and production in Thailand*. Khon Kaen: Khon Kean University. Nutrition based on local feedstuffs in ruminant production and health.

Akers, R.M. (2002). *Lactation and the mammary gland*. Iowa: Wiley-Blackwell.

Alejandrino, A.L., Asaad, C.O., Malabayabas, B., De Vera, A.C., Herrera, M.S., Deocaris, C.C., Ignacio, L.M. & Palo, L.P. (1999). Constraints on dairy cattle productivity at the smallholder level in the Philippines. *Prev. Vet. Med.* 38(2-3), 167-178.

Almaw, G., Zerihun, A. & Asfaw, Y. (2008). Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Barhir Dar, Ethiopia. *Trop Anim Health Pro* 40(6), 427-432.

Auldist, M.J. & Hubble, I.B. (1998). Effects of mastitis on raw milk and dairy products. *Aust. J. Dairy Technol.* 53(1), 28-36.

Barkema, H., Schukken, Y., Lam, T., Beiboer, M., Benedictus, G. & Brand, A. (1998). Management practices associated with low, medium, and high somatic cell counts in bulk milk. *J. Dairy Sci.* 81(7), 1917-1927.

Bartlett, P.C., Miller, G.Y., Anderson, C.R. & Kirk, J.H. (1990). Milk production and somatic cell count in Michigan dairy herds. *J. Dairy Sci.* 73(10), 2794-2800.

Beede, D.K. & Collier, R.J. (1986). Potential nutritional strategies for intensively managed cattle during thermal stress. *J. Anim. Sci.* 62, 543-554.

Berglund, I., Pettersson, G., Östensson, K. & Svennersten-Sjaunja, K. (2007). Quarter milking for improved detection of increased SCC. *Reprod. Domest. Anim.* 42, 427-432.

Blowey, R.W. & Edmondson, P.W. (2010). *Mastitis control in dairy herds*. 2nd. ed: CABI.

Blowey, R.W. & Laven, R.A. (2004). Factors affecting milk quality. In: Andrews, A.H., *et al.* (Eds.) *Bovine medicine diseases and husbandry of cattle*. pp. 373-378 Blackwell Science.

Boonbrahm, N., Peters, K.J. & Iintisang, W. (2004a). The influence of calf rearing methods and milking methods on performance traits of crossbred dairy cattle in Thailand: Milk yield and udder health. *Arch. Tierz.* 47, 211-224

Boonbrahm, N., Peters, K.J. & Iintisang, W. (2004b). The influence of calf rearing methods and milking methods on performance traits of crossbred dairy cattle in

Thailand: Reproductive performance and cow body weight. *Arch. Tierz.* 47, 303-312
Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M. & Belyea, R. (2002). The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *Anim. Res.* 51(6), 479-491.
Bradley, A.J. (2002). Bovine Mastitis: An evolving disease. *The Veterinary Journal* 164(2), 116-128.
Bramley, A.J., Dodd, F.H., Mein, G.A. & Bramley, J.A. (1992). *Machine milking and lactation*. Newbury, Berkshire; Huntington: Insight Books
Brolund, L. (1985). Causes of variation and applicability for diagnosis of subclinical mastitis. *Acta Vet. Scand. Suppl.* 80, 1-123.
Burdová , O., Baranová, M., Lauková, A., Rózanska, H. & Rola, J.G. (2002). Hygiene of pasteurized milk depending on psychrotropic microorganisms. *Bull. Vet. Inst. Pulawy* 46, 325-329.
Byarugaba, D.K., Nakavuma, J.L., Vaarst, M. & Laker, C. (2008). Mastitis occurrence and constraints to mastitis control in smallholder dairy farming systems in Uganda. *LRRD* 20(1), 2005.
Cai, D.V. (2002). *Report on dairy breeding development: Setting and solutions*. Ho Chi Minh, Vietnam: The Institute of Agricultural Technologis in the South of Vietnam.
Cavestany, D., El-Wishy, A.B. & Foote, R.H. (1985). Effect of season and high environmental temperature on fertility of Holstein cattle. *J. Dairy Sci.* 68, 1471-1478.
Chandan, R.C., Kilara, A. & Shah, N.P. (2008). *Dairy processing & quality assurance*: Wiley-Blackwell.
Chantalakhana, C. & Skunmun, P. (2002). *Sustainable smallholder animal systems in the tropics*. Bangkok, Thailand: Kasetsart University Press.
Chase, L.E. (1988). Water needs of dairy cattle. *Agri-Practice* 9, 23-24.
Cheng, D.R., Zhu, S.Y., Yin, Z.H., Ding, W.W., Mu, Z.X., Su, Z.R. & Sun, H.C. (2010). Prevalence of bacterial infection responsible for bovine mastitis. *Afr. J. Microbiol. Res.* 4(11), 1110-1116.
Collier, R.J. & Zimbelman, R.B. (2007). Heat stress effects on cattle: What we know and what we don't know. In: *Proceedings of 22nd Annual Southwest Nutrition & Management Conference*, The University of Arizona.
Cunninghem, E.P. & Syrstad, O. (1987). *Crossbreeding Bos indicus and Bos taurus for milk production in the tropics*. Rome: FAO. Animal Production and Health; 68.
Davis, C.L. & Brown, R.E. (1970). Low-fat milk syndrome. In: Philips, A.T. (Ed.) *Physiology and metabolism in the ruminant*. Newcastle: Oriel Press.
Devendra, C. (2001). Smallholder dairy production systems in developing countries: Characteristics, potential and opportinuties for improvement *Asian-Aust. J. Anim. Sci.* 14, 104-113.
Devendra, C. (2002). Crop-animal systems in Asia: future perspectives. *Agric. Syst.* 71(1-2), 179-186.
Do, K.T. & Hoang, K.G. (2001). *Dairy production in Vietnam and development plan for 2002-2010*. Ha Noi, Vietnam: Ministry of Agriculture and Rural Development.

Dohoo, I.R. & Leslie, K.E. (1991). Evaluation of changes in somatic cell counts as indicators of new intramammary infections. *Prev. Vet. Med.* 10, 225-237.

Dohoo, I.R. & Meek, A.H. (1982). Somatic cell counts in bovine milk. *Can. vet. J.* 23(4), 119-125.

Eberhart, R.J., Cloninger, W.H. & Card, C.S. (1968). Effects of unstable milking vacuum on some measures of udder health. *J. Dairy Sci.* 51(7), 1026-1030.

Edmondson, P.W. & Bramley, A.J. (2004). Mastitis. In: Andrew, A.H.*, et al.* (Eds.) *Bovine medicine diseases and husbandry of cattle*. pp. 326-336 Backwell Science.

Fairbairn, D.J. & Law, B.A. (1986). Proteinase of psychrotrophic bacteria: their production, properties, effects and control. *J. Dairy Res.* 53(1), 139-177.

Fajardo-Lira, C. & Nielsen, S.S. (1998). Effect of psychrotrophic microorganisms on the plasmin system in milk. *J. Dairy Sci.* 75, 1402-1410.

Falvey, L. & Chantalakhana, C. (2001). Supporting smallholder darying in Asia. *Asia-Pacific Dev. J.* 89-99(2).

Fasola, E., Livdahl, C. & Ferrieri, P. (1993). Molecular analysis of multiple isolates of the major serotypes of group B streptococci. *J. Clin. Microbiol.* 31(10), 2616-2620.

Fielding, R.D. & Mathewman, R.W. (2004). Tropical cattle management. In: Andrews, A.H.*, et al.* (Eds.) *Bovine medicine diseases and husbandry of cattle*. pp. 68-82 Blackwell Scince.

Gautier, P. (2008). Smallholder dairy development in Vietnam. In: Morgan, N. (Ed.) *Proceedings of developing an Asia regional strategy for sustainable small holder dairy development*, Chiang Mai, Thailand 2008. pp. 18-21: FAO/APHCA/CFC.

General Statistic Office (2010). *Statistic of dairy population and milk production to August, 2010*. Hanoi, Vietnam: General Statistics Department.

Getahun, K., Kelay, B., Bekana, M. & Lobago, F. (2008). Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Trop Anim Health Pro* 40, 261-268.

Giang, V.D. & Tuyen, N.T. Proportion of ingredents and nutrition levels in the rations of dairy cows in the Red River Delta. In: *Proceedings of a South-South workshop* Anand, India 2001: ILRI.

Gonzalez, R.N., Jasper, D.E., Bushnell, R.B. & Farver, T.B. (1986). Relationship between mastitis pathogen numbers in bulk tank milk and bovine udder infections in California dairy herds. *J. Am. Vet. Med. Assoc.* 198, 442-445.

Grieve, P.A. & Kitchen, B.J. (1985). Proteolysis in milk: the significance of proteinases originating from milk leucocytes and a comparison of the action of leucocyte, bacterial and natural milk proteinases on casein. *J. Dairy Res.* 52, 101-112.

Haddadi, K., Moussaoui, F., Hebia, I., Laurent, F. & Le Roux, Y. (2005). *E. coli* proteolytic activity in milk and casein breakdown. *Reprod. Nutr. Dev.* 45(4), 485-496.

Halasa, T., Huijps, K., Østerås, O. & Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: A review. In: *Vet. Q.* pp. 18-31.

Hale, S.A., Capuco, A.V. & Erdman, R.A. (2003). Milk yield and mammary growth effects due to increased milking frequency during early lactation. *J. Dairy Sci.* 86, 2061–2071.

Hamann, J., Mein, G.A. & Wetzel, S. (1993). Teat tissue reactions to milking: Effects of vacuum level. *J. Dairy Sci.* 76(4), 1040-1046.

Harmann, J. (2002). Relationships between somatic cell count and milk composition. *IDF Bulletin* 37, 256-259.

Harmon, R.J. (1994). Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci.* 77, 2103-2112.

Heck, J.M.L., Olieman, C., Schennink, A., van Valenberg, H.J.F., M.H.P.W., V., Meuldijk, R.C.R. & van Hooijdonk, A.C.M. (2008). Estimation of variation in concentration, phosphorylation and genetic polymorphism of milk proteins using capillary zone electrophoresis. *Int. Dairy J.* 18(5), 548-555.

Hillerton, J.E. (1999). Redefining mastitis based on somatic cell count. *IDF Bulletin* 345, 4-6.

Hillerton, J.E. (2004). Summer mastitis. In: Andrews, A.H.*, et al.* (Eds.) *Bovine medicine diseases and husbandry of cattle*. pp. 337-340 Blackwell Science.

Jain, N.C. (1979). Common mammary pathogens and factors in infection and mastitis. *J. Dairy Sci.* 62(1), 128-134.

Jungbluth, T. & Grimm, H. (2009). Milking and milking technology - need for future research. *ART-Schriftenreihe* (9), 1-8.

Kadzere, C.T., Murphya, M.R., Silanikove, N. & Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Lives. Prod. Sci.* 77 59-91.

Keefe, G.P. (1997). *Streptococcus agalactiae* mastitis: A review. *Can. vet. J.* 38, 429-437.

Kirchgessner, M., Friesecke, H. & Koch, G. (1967). *Nutrition and the composition of milk*. London: Crosby Lockwood & Son.

Kitchen, B.J. (1981). Review of the progress of dairy science: Bovine mastitis: Milk compositional changes and related diagnostic tests. *J. Dairy Res.* 48, 167-188.

Kiwuwa, G.H. (1987). Choice between dairy cattle breeds for the tropics. *Quarterly Journal of International Agriculture* 26(1), 72-85.

Lafi, S.Q., Al-Rawashdeh, O.F., Ereifej, K.I. & Hailat, N.Q. (1994). Incidence of clinical mastitis and prevalence of subclinical udder infections in Jordanian dairy cattle. *Pre. Vet. Med.* 18(2), 89-98.

Larsen, L.B., Rasmussen, M.D., Bjerring, M. & Nielsen, J.H. (2004). Proteases and protein degradation in milk from cows infected with *Streptococcus uberis*. *Int. Dairy J.* 14(10), 899-907.

Linzell, J.L. & Peaker, M. (1972). Day-to-day variations in milk composition in the goat and cow as a guide to the detection of subclinical mastitis. *Br. vet. J.* 128(6), 284-295.

Luthi, N.B., Fabozzi, L., Gutier, P., Trung, P.Q. & Smith, D. (2006). *Review, analysis and dissemination of experiences in dairy production in Viet Nam*: FAO. A living from livestock.

Ma, Y., Ryan, C., Barbano, D.M., Galton, D.M., Rudan, M.A. & Boor, K.J. (2000). Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. *J. Dairy Sci.* 83(2), 264-274.

Man, N.V. (2001). *Better use of local forages for dairy cattle in Vietnam: Improving grasses, rice straw and protein rich forages.* Diss. Uppsala: Swedish University of Agricultural Sciences

Millogo, V. (2010). *Milk production of hand-milked dairy cattle in Burkina Faso*. Diss. Uppsala: Swedish University of Agricultural Sciences.

Moran, J. (2009). *Business management for tropical dairy farmers*: Land Links.

Morgan, N. (2010). *Smallholder dairy development: Lessons learned in Asia*. Bangkok, Thailand: FAO.

Moxley, J., Kennedy, B., Downey, B. & Bowman, J. (1978). Survey of milking hygiene practices and their relationships to somatic cell counts and milk production. *J. Dairy Sci.* 61(11), 1637-1644.

NIAH (2001). *Livestock production in Vietnam from 1990-2001*. Hanoi, Vietnam: National Institute for Animal Husbandry.

NIAH (2010). *Report on dairy production in Vietnam in 2010*. Hanoi, Vietnam: National Institute for Animal Husbandry.

Nielsen, N., Larsen, T., Bjerring, M. & Ingvartsen, K. (2005). Quarter health, milking interval, and sampling time during milking affect the concentration of milk constituents. *J. Dairy Sci.* 88(9), 3186-3200.

Oliver, S.P. & Mitchell, B.A. (1984). Prevalence of mastitis pathogens in herds participating in a mastitis control program. *J. Dairy Sci.* 67(10), 2436-2440.

Pandey, P., Pandey, V. & Singh, J. (2005). Somatic cell count in relation to milk production, milk composition and subclinical mastitis: A review. *Internl. J. of Cow Sci.* 1(1), 27-32.

Pyörälä, S. (1995). Staphylococcal and streptococcal mastitis. In: Sandholm, M.*, et al.* (Eds.) *The bovine udder and mastitis*. pp. 143-148. Helsinki, Finland: University of Helsinki.

Pyörälä, S. (2003). Indicators of inflammation in the diagnosis of mastitis. *Vet. Res.* 34(5), 565-578.

Radostits, O.M. (2001). *Herd health: Food animal production medicine*. 3rd. ed. Philadelphia: Saunders.

Rhone, J., Koonawootrittriron, S. & Elzo, M. (2008). Factors affecting milk yield, milk fat, bacterial score, and bulk tank somatic cell count of dairy farms in the Central region of Thailand. *Trop Anim Health Pro* 40(2), 147-153.

Saeman, A., Verdi, R., Galton, D. & Barbano, D. (1988). Effect of mastitis on proteolytic activity in bovine milk. *J. Dairy Sci.* 71(2), 505-512.

Sandholm, M. (1995). Detection of inflammatory changes in milk. In: Sandholm, M.*, et al.* (Eds.) *The bovine udder and mastitis*. pp. 89-104. Helsinki, Finland: University of Helsinki.

Sandholm, M. & Korhonen, H. (1995). Mastitis. In: Sandholm, M.*, et al.* (Eds.) *The bovine udder and mastitis*. pp. 37-48. Helsinki, Finland: University of Helsinki.

SAS Institute Inc. (2008). SAS/STAT® User's guide, version 9.2. In. Cary, NC, USA.

Schultz, L. (1977). Somatic cell counting of milk in production testing programs as a mastitis control technique. *J. Am. vet. med. Ass.* 170, 1244-1246.

Smith, K. & Hogan, J. (1993). Environmental mastitis. *Vet. Clinics N. Amer.* 69(3), 489-498.

Souza, G.N., Brito, J.R.F., Brito, M.A.V.P., Moreira, É.C. & Silva, M.V.G.B.d. (2005). Factors affecting somatic cell counts in Brazillian cows. *ISAH 2005 - Warsaw, Poland* 1, 237-240.

Sterling, E.J., Hurley, M.M. & Minh, L.D. (2006). *Vietnam: A natural history*: Yale University Press.

Suzuki, K. (2005). *Investigation into the constraints to dairy cattle health and production in Northern Vietnam.* Diss. London: The Royal Veterinary College.

Suzuki, K., Kanameda, M., Ogawa, T., Nguyen, T.T.D., Dang, T.T.S., Luu, Q.H. & Pfeiffer, D.U. (2006). Productivity and socio-economic profile of dairy cattle farmers amongst rural smallholder communities in northern Vietnam. *Livest. Sci.* 101, 242-250.

Svennersten-Sjaunja, K., Bruckmaier, R., Hamann, J. & Woolford, M. (2004). Stimulation of milk ejection: a basic need or just a time-consuming routine. *IDF Bulletin* (388), 11-19.

Syrstad, O. (1996). Dairy cattle crossbreeding in the tropics: Choice of crossbreeding strategy. *Trop Anim Health Pro* 28, 223-229.

Tam, P.G. (2004). *Securing small producer participation in restructured national and regional agri-food systems: The case of Vietnam*. Ho Chi Minh: Nong-Lam University, Vietnam. Regoverning markets.

Thomas, S.C., Nimmervoll, H., Boije, C., Svennersten-Sjaunja, K., Lundeheim, N. & Östensson, K. (2004). Occurrence of subclinical mastitis in buffaloes in different herd sizes and milking management systems. *Buff. J.* 3, 289-306.

Urech, E., Puhan, Z. & Schällibaum, M. (1999). Changes in milk protein fraction as affected by subclinical mastitis. *J. Dairy Sci.* 82(11), 2402-2411.

Walstra, P., Wouters, J.T.M. & Geurts, T.J. (2006). *Dairy science and technology*: Taylor & Francis group.

Wilson, D.J., Gonzalez, R.N. & Das, H.H. (1997). Bovine mastitis pathogens in New York and Pennsylvania: Prevalence and effects on somatic cell count and milk production. *J. Dairy Sci.* 80(10), 2592-2598.

Wolfenson, D., Roth, Z. & Meidan, R. (2000). Impaired reproduction in heat-stressed cattle: basic and applied aspects. *Anim. Reprod. Sci.* 60/61, 535-547.

Yang, F.-L., Li, X.-S., He, B.-X., Du, Y.-L., Li, G.-H., Yang, B.-B. & Qin-Hua, H. (2011). Bovine mastitis in subtropical dairy farms, 2005-2009 *J. Anim. Vet. Adv.* 10(1), 68-72.