Teat Condition in Dairy Cows

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Francesca Neijenhuis

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promotor: Prof. Dr. J.P.T.M. Noordhuizen[1]

copromotoren: Dr. H. Hogeveen[1, 2]

Dr. H.W. Barkema[3]

[1]Department of Farm Animal Health

Faculty of Veterinary Medicine, Utrecht University

P.O. Box 80151, 3508 TD Utrecht, the Netherlands

[2]Business Economics Group

Wageningen University

P.O. Box 8130, 6700 EW Wageningen, the Netherlands

[3]Atlantic Veterinary College

University of Prince Edward Island

550 University Avenue, Charlottetown, PEI, C1A4P3, Canada

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Voor mijn ouders

Aan Martin

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

F. Neijenhuis

Wageningen University and Research Centre

Animal Sciences Group

Division Applied Research

P.O. Box 2176, 8203 AD Lelystad, the Netherlands

francesca.neijenhuis@wur.nl

INTRODUCTION

Many Dutch farmers pursue a large and modern dairy farm (Bergevoet et al., 2004). As a consequence, the farm size and milk production per cow have steadily increased over the past decades. In The Netherlands from 1980 to 2003, the average size of a farm enrolled in the dairy herd information program has increased from 35 to 59 cows and milk production has increased from 5,466 kg to 8,737 kg milk per lactation (NRS, 2004). Because of the larger farm size, the efficiency of milk harvesting has become a factor of increasing importance at the dairy farm.

As a consequence of these changes farmers spend less time per (high producing) cow, however it has been recommended that more time be spend at pre-milking procedures in order to ensure efficient milking and good teat hygiene in mastitis prevention (Pankey, 1989; Allore and Erb, 1998; Barkema et al., 1999). Moreover, higher milk production is associated with an increased risk of clinical mastitis (Schukken et al., 1990; Gröhn et al., 1995; Waage et al., 1998; Suriyasathaporn et al., 2000; Kornalijnslijper, 2003).

Mastitis

Efficient production of high quality milk is challenged when udder health problems occur. Classically, mastitis (from the Greek word *mastos* meaning breast and the suffix *itis* meaning inflammation) is defined as inflammation of the mammary gland (Kehrli and Shuster, 1994). According to the National Mastitis Council (Nickerson et al., 1996), mastitis is an inflammation of the mammary gland in response to injury for the purpose of destroying or neutralising the infectious agents and to prepare the way for healing and return to normal function. Inflammation can be caused by many types of injury including infectious agents and their toxins, physical trauma or chemical irritants. In the dairy cow, mastitis is nearly always caused by micro organisms, usually bacteria, that invade the udder, multiply in the milk-producing tissues, and produce toxins that are the immediate cause of injury.

The development of the disease starts with penetration of the teat canal by pathogens. Therefore, the teat is considered the first line of defence. The keratin in the teat canal can trap invading bacteria, and contains antimicrobial agents (Hibbit et al., 1969; Capuco et al., 1992). When pathogens enter the udder, a non-specific (innate) cellular defence and a specific immunity response of polymorphonuclear leucocytes (PMN) may be initiated. This constitutes the second line of defence.

In the udder, the first response to pathogens is phagocytosis and bactericidal activities of primarily neutrophils and macrophages; these responses may be followed rapidly by a massive influx of PMN's (Sordillo, 1997; Sordillo et al., 2003).

Despite huge efforts, and although progress has been made, udder health still is an important issue on the dairy farm. Mastitis remains a common and expensive disease for the individual farmer affecting dairy cattle throughout the world (De Graves and Fetrow, 1993; Miller et al., 1993; Houben et al., 1994; Hillerton, 1996; Østeras, 2000). Mastitis control and prevention schemes have been described since 1960 (Dodd et al, 1964; Neave et al., 1966). Current mastitis control programs are based on hygiene and include post-milking teat disinfection, antibiotic therapy and culling of chronically infected cows. The implementation of these programs has led to considerable progress in controlling mastitis caused by contagious mastitis pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus*. However, the proportion of mastitis cases caused by environmental pathogens such as *Escherichia coli* and *Streptococcus uberis* has increased markedly (Dodd, 1983; Smith, 1983; Oliver and Mitchell, 1984; Miltenburg et al., 1996). It is estimated that still 15-40% of dairy cows experience clinical mastitis during a lactation (Schukken, 1989;Miltenburg, et al.,1996;.Chassagne et al., 1997, Barkema et al., 1998; Sargeant et al., 1998; Beaudeau et al., 2002; Peeler et al., 2002; Sviland and Waage, 2002). Consequently, mastitis continues to be a significant factor in modern dairy production. Mastitis on herd level is reflected in bulk tank milk somatic cell count (SCC) and, bacterial count, and has adverse effects on milk composition, and quality and shelf life of dairy products (Eberhart et al., 1982; Harmon, 1994; Brolund, 1985; Allore et al., 1998; Auldist and Hubble, 1998; Jayarao and Wolfgang, 2003; Barbano, 2004).

Economic losses due to mastitis originate from a decrease in milk yield, higher probability of culling, treatment of clinical cases, increased probability of decreased milk price due to increased SCC, bacterial counts, or antibiotic residues, and a higher susceptibility for other diseases (Bartlett et al., 1990; Gill et al., 1990; Wilson et al., 1997; Wells et al., 1998; Ruegg and Tabone, 2000; Smith et al., 2000). In addition to economic consequences, mastitis causes great discomfort for the cow and, therefore, affects welfare of the dairy cow in a negative way (Eshraghi et al., 1999). Awareness of animal welfare is increasing, not only among the public or consumers, but also among the dairy producers (LTO, 2004). Finally, it is recognized that the need to control mastitis is more and more driven by the consumers who demand wholesome, nutritious and safe milk that is produced by healthy cows (Smith and Hogan, 1999; Council of the European Communities, 1993).

In general, the rate of intramammary infection (IMI) is established by a combination of exposure of the teat-end to pathogens and the effectiveness of the defence mechanisms of the cow. The susceptibility of cows to IMI is influenced by several factors which can be divided into factors influencing the penetration of the udder by pathogens and factors affecting the immune response of the cow when pathogens have entered the teat canal and/or the udder (Poutrel, 1982). Machine milking is a very relevant factor among these, since it may substantially affect the penetration risk of the udder by pathogens.

Table 1. Machine milking mechanisms potentially affecting new infection (NIR=New Infection Rate) (adapted from Hamann, 1987, p5 and Bramley et al., 1992, p355).

Mode of action	Main milking related mechanisms	General evidence for importance
I. Transfer of bacteria	Transfer of bacteria from: a) Environment to teat b) Cow to cow c) Teat to teat (same cow)	*Teat disinfection reduces bacterial numbers on the teat skin and orifice and decreases NIR *Experimental bacterial challenges increase NIR
II. Frequency and/or degree of udder evacuation	By changing: a) Susceptibility of gland to invading pathogens b) Concentrations of pathogens on the teat-end c) Duration of exposure to pathogens	*NIR is higher in dried off cows at the start of the dry period *Incomplete milking or omittance of milking tend to increase NIR
III. Increase bacterial penetration of teat duct	By causing impacts of bacteria-laden milk droplets & dispersing pathogens from: a) Teat canal to the teat sinus b) Teat sinus to the gland sinus and/or ducts	*Endotoxin, *Escherichia coli*, and dyes have been jetted through the teat canal *Shields or valves reduced NIR *High velocity air/liquid flows toward the teat-end increased NIR *Few IMI occur if bacteria placed within the teat sinus are carefully removed by stripping but bacteria placed within the gland sinus frequently cause NIR *In some experiments pre-stripping of teats before milking reduced NIR
IV. Resistance of teat to bacterial invasion	By affecting: a) Teat canal integrity b) Teat congestion and/or edema c) Increasing skin and/or orifice lesions	*NIR is increased by reaming keratin from the teat canal and by visible teat canal injuries *NIR is increased when pulsation is ineffective

Machine Milking and Mastitis

The basic prerequisites for milking cows are that milking be carried out quickly, cleanly, completely and gently. The main machine milking-related mechanisms of mastitis infection are described in Table 1.

The milking machine is a vector of bacteria, both between cows and from teat to teat within the cow (category I in Table 1). Virtually all infections enter the udder through the teat-end. A positive role of milking is found in milking out pathogens (category II in Table 1), but the milking machine can also play an active role in penetration of bacteria into the teat canal, teat sinus or gland sinus (category III in Table 1). The role of machine milking as a vector has been frequently studied in the past decades. For a review of these factors see Neijenhuis (2003). In addition to its role as a vector, the milking machine may cause trauma to the teat rendering it more susceptible to colonization and infection (category IV in Table 1). Knowledge of the impact of the milking machine on teat trauma and subsequent mastitis is limited.

Mechanical Forces on the Teat

Although there is great diversity in milking installations, milking machines work on the same basic principle: milk is sucked out of the udder by vacuum. The basic components of all milking machines are:

A vacuum system;

Pulsators that alter the vacuum level around the teat;

Milking units or cluster (the assembly of four teatcups with liners connected to a claw); and

A milk removal system that transports the milk away from the milking unit toward a storage unit.

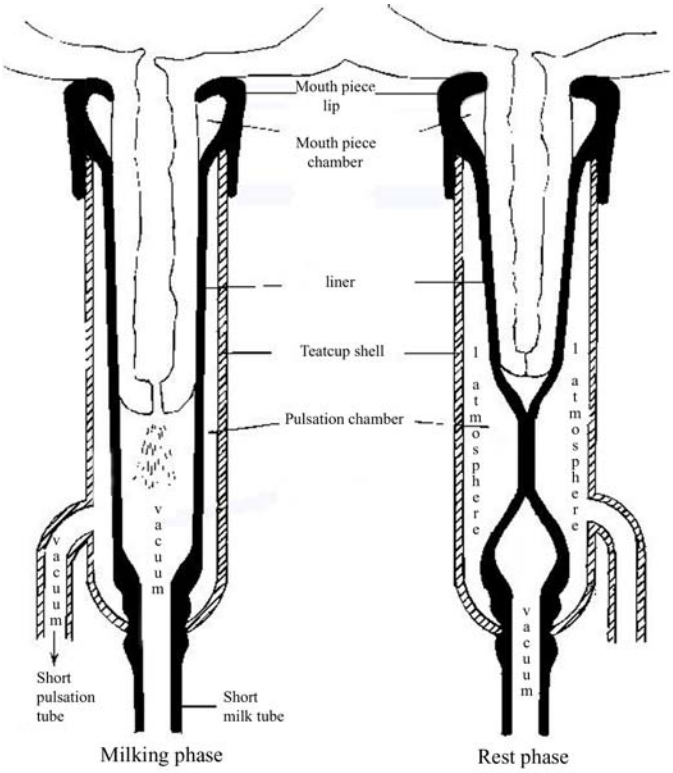

Figure 1. Pulsation of the teatcup liner (adapted from Van der Haven et al., 1996).

The teatcup liner is the only part of the milking machine that has direct contact with the cow. Pulsation of the teatcup liner consists of alternate collapse of the teatcup liner beneath the teat, when air at atmospheric pressure is admitted to the pulsation chamber of the teatcup, and reopening as the pulsation chamber is evacuated (Figure 1). The mechanical forces during machine milking result in changes in teat-end tissue. Milking vacuum aids in opening of the teat canal and milk flow, but also generates strain on the teat wall, induces dilation of blood vessels and expandable compartments in the perivascular tissue and draws blood and lymph to the teat-end (Bramley et al., 1992; Isaksson and Lind, 1992). The collapsing liner may exert a mechanical force on the teat-end, contributing to the teat canal closure and transport of blood and lymph back to the udder. When the liner is closed, there is still vacuum at the teat-end (O'Shea and O'Callaghan, 1980). The magnitude of the forces on the teat depends on milking and pulsation vacuum, pulsation rate and ratio, liner type, teat shape and teat size (Williams and Mein, 1980; Reitsma et al., 1981; Williams et al., 1981; Hamann and Mein, 1990; Hamann et al., 1993; Hamann et al., 1994; Hamann and Mein, 1996; Worstorff and Schatzl, 2000; Worstorff and Bilgery, 2002).

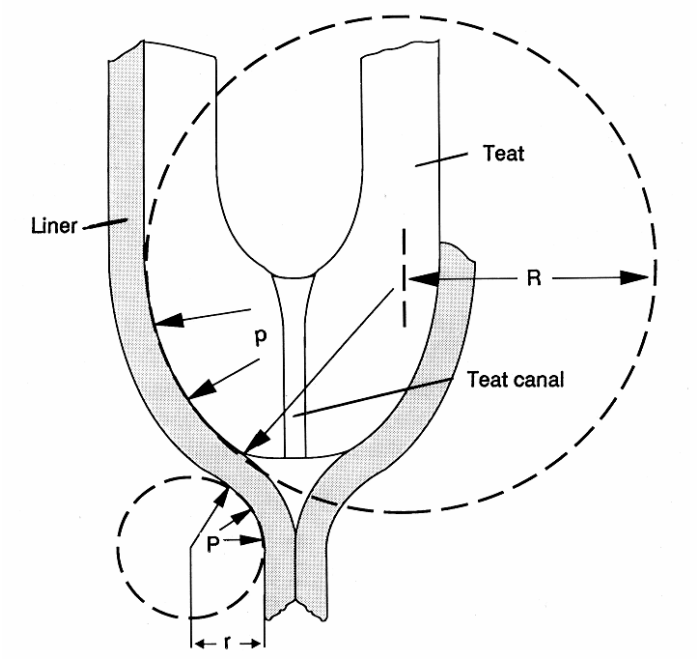

Figure 2. Sketch of section of the liner bent around the teat-end along the plane of collapse (Bramley et al., 1992).

The forces applied to the teat by the teat cup liner can be estimated from the shape of the curved liner (r:R ratio) and the pressure difference across the liner just underneath the teat (Figure 2; Williams and Mein, 1980). The compressive load applied by the liner on the teat should be 8 to 12 kPa, approximately the bovine arterial pressure of 10 kPa.

The teat as First Line of Defence

In order to meet the demands of high milk yield, milk efficiency and milk quality, the teats of the cow are important in terms of their function as first line of defence against mastitis. The physical-chemical defence of the teat is made up of the teat skin and the teat canal. The teat canal is a strong and important primary barrier against invasion of mastitis pathogens into the udder (Michel, 1974; Hamann, 1987). The sphincter muscle surrounding the teat duct is tightly closed between milkings and impedes bacterial intrusion from the teat opening into the interior of the gland (Nickerson, 1994).

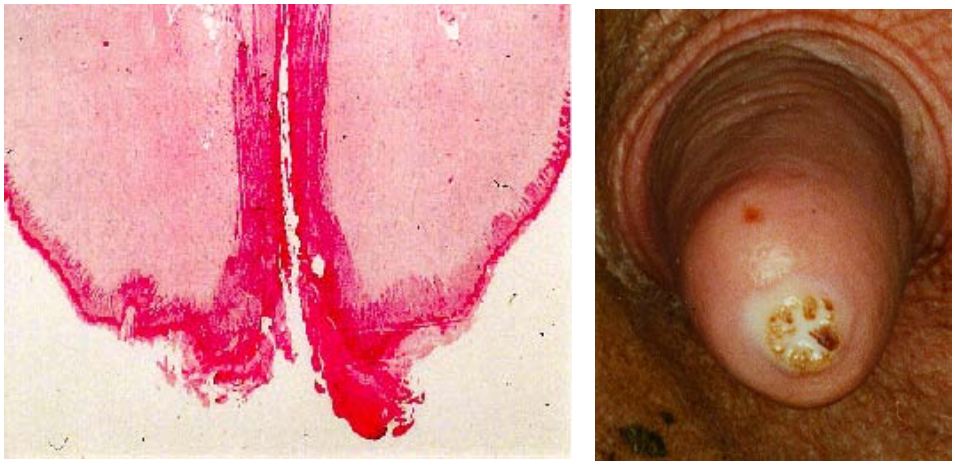

Figure 3. Microscopic view of a teat-end with a severe rough callosity ring (De Man, 1998) and the corresponding view from the outside.

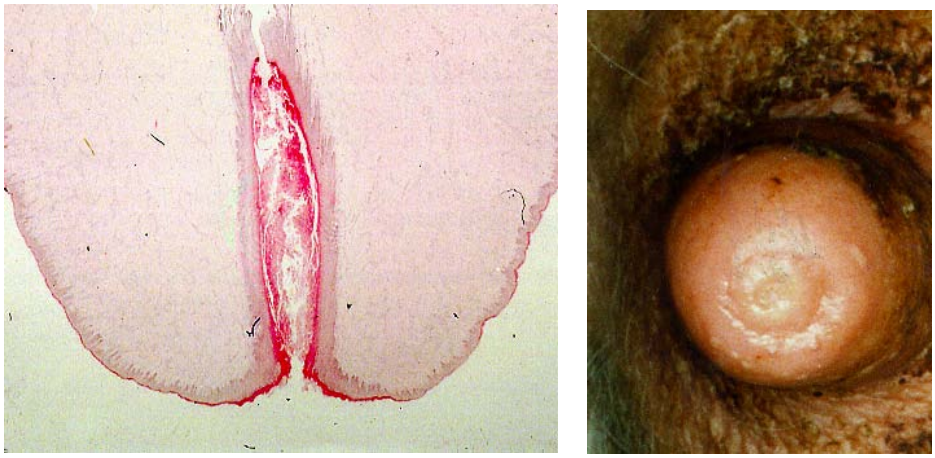

Figure 4. Microscopic view of a teat-end with a smooth and thin callosity ring (De Man, 1998) and the corresponding view from the outside.

Teat tissue changes by machine milking can be observed as teat swelling, teat flattening, colour changes, openness of the teat orifice, loss of keratin, vascular damage (haemorrhages), and teat-end callosity (**TEC**, Figure 3 and 4). Teats of cows suckled by their calves show less swelling and only a small increase in TEC compared to machine milked cows (Espe and Cannon, 1942; Sieber and Farnsworth, 1984; Hamann, 1991). Calves apply pressure and vacuum simultaneously to extract milk from the teat (Rasmussen and Mayntz, 1998).

Keratin

The duct of the teat canal is lined with keratin. Mature keratin occludes the closed canal and acts as a physical barrier to bacterial invasion. The keratin also has some anti-bacterial properties (Hibbitt et al., 1969). An essential mechanism of the ability of limiting bacterial growth is the desquamation of mature keratin largely by the speed or changing speed of milk flow that removes keratin and adhering bacteria. Production and loss of keratin by the teat canal should be in equilibrium (Woolford, 1997).

Teat Canal Closure

Any changes in duct closure due to milking, possibly caused by fatigue of the teat sphincter muscles, may increase the likelihood of invading pathogens. Pathogens of environmental origin are most likely to pass directly through the duct when it is not completely closed (Lacy-Hulbert and Hillerton, 1995). Increasing peak flow rates are associated with increasing penetrability of the teat canal and the probability of IMI (Duda, 1995). The risk of new IMI by contagious as well as environmental pathogens such as *Streptococcus uberis* is increased by machine-induced changes such as greater degree of openness of the teat canal orifice after milking (Mein et al., 2001). Reducing the machine-on time by adjusting the take-off level to a flow of 400 g/min instead of 200 g/min, improves teat condition without affecting milk yield or udder health (Rasmussen, 1993).

Teat-end Callosity

Machine milking can affect the extent of callus formation on the teat-end (Sieber, 1980; Hamann, 1987; O'Shea, 1987; Ebendorff and Ziesack, 1991). Therefore, TEC may be looked upon as one of the parameters by which the quality of machine milking can be evaluated. Seykora and McDaniel (1985) showed that SCC was influenced by teat-end lesion score defined by the degree of ulcerative and raw orifice. Also, Farnsworth (1995) found that teat-ends with severe erosions that were raw and ulcerated (broken skin) showed higher prevalence of mastitis. Moreover, teat-end lesions are frequently colonized by staphylococci and *Streptococcus dysgalactiae* (Kingwill et al., 1979). Therefore, they are a potential source of infection of deeper parts of the udder. Although never directly proven (Michel et al., 1974; Sieber and Farnsworth, 1981; Graf and Gedek, 1983), teat-ends with severe rough callus rings are often associated with IMI.

Teat Swelling

When teats are exposed to teat-cup liner conditions, some congestion and edema of the teat-end occurs at the end of peak milk flow even when using universally accepted milking machine settings (Bramley et al., 1992). Changes in the pliability of teat tissue caused by congestion or edema may change the resistance of the teat canal to bacterial invasion (O'Shea, 1987). Changes of the teat wall such as increased congestion and edema results in slower closure of the teat canal and/or hypoxia in teat tissues (Hamann et al., 1994). Fox and Cumming (1996) showed a relationship between thickness, chapping and *Staphylococcus aureus* colonization of bovine teat tissue. With different settings of the milking machine, such as vacuum level, flow rate at cluster removal and pulsation rate, machine-induced teat thickness or swelling varies (Hamann and Mein, 1990; Zecconi et al., 1992; Hamann et al., 1993; Rasmussen, 1993; Hamann and Mein, 1996). With increasing teat barrel thickness, the blood flow will be reduced however when only minor changes in teat thickness occur, the blood flow will increase immediately after milking (Hamann et al., 1994). Zecconi et al. (1992, 1996) showed that an increase of teat thickness of over 5% after milking is associated with an increased incidence of IMI (Figure 5).

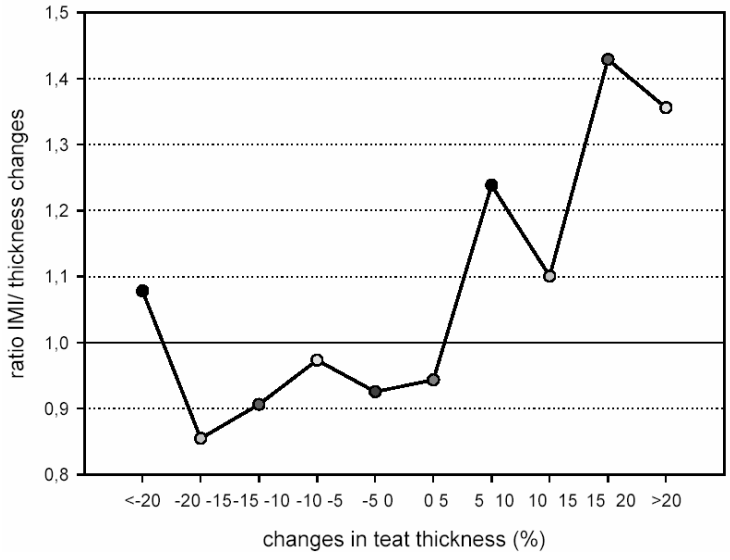

Figure 5. Ratio between levels of teat thickness changes and IMI frequencies (Zecconi et al., 1996).

Teat Condition Scoring in the Field

Teat condition scoring is nowadays a commonly pursued parameter in research. In the field, teat condition has been accepted as an indicator for quality of milking. For this reason, many different scoring systems for TEC have been developed (Michel et al., 1974; Sieber and Farnsworth, 1981; Graf and Gedek, 1983; Seykora and McDaniel, 1985; Ebendorff and Ziesack, 1991; Shearn and Hillerton, 1996). These systems differ in both their parameters and the number of parameter categories. It is, therefore, difficult to directly compare results obtained by different scoring systems.

Objective of this Thesis Research

The role of machine milking in mastitis occurrence may be quantified by analysing clinical mastitis cases. However, given the nature of mastitis occurrence it will take numerous cow milkings to establish a relationship between machine milking parameters and clinical mastitis. Teat condition can be readily scored on a daily basis. If these changes in teat condition have a sufficient strong relationship with the occurrence of clinical mastitis, quantifying the changes may serve as a method to predict the frequency of infection for different machine milking parameters. From previous research it is obvious that measurement of teat lesions, significant increases in teat thickness and openness of the teat canal after milking, can serve as a method to evaluate the milking process. When abnormalities occur, the milking machine can be functioning incorrect, causing trauma to the teat and rendering it more susceptible to IMI. Although the relationship between machine-induced teat swelling and mastitis is known, there is a lack of knowledge on how long teat swelling persists after milking.

The causal role of machine milking on the extent of TEC during lactation and its relationship with IMI is still not proven. To investigate this relationship there is no suitable scoring system of TEC available. The existing systems developed to classify teat-end condition either do not differentiate between smooth and rough callosity rings or include scabs or lesions. Moreover, the development of a complete classification system for TEC can be used to investigate the relationship between the categories of callosity and the rate of mastitis.

It is known that pulsation and vacuum on the teat during milking affects the teats. Changes in TEC will take several milkings to develop; conversely machine-induced teat swelling can be seen after one milking. When machine-induced teat swelling changes are precursory of TEC, machine-

induced teat swelling may be used in short-term experiments to evaluate different milking machine adjustments. For this reason, it is necessary to develop reliable a reproducible non-invasive methods to measure teat thickness.

The focus of this thesis is to address the relationship between teat-end condition, occurrence of mastitis and machine milking. The objectives are to:

I. design a classification system for TEC for use in research and field observations, and to obtain more insight in the influencing cow and machine milking factors affecting TEC (Chapters 2 and 6),
II. establish the relationship between the occurrence of mastitis and TEC and quantify the risk of clinical mastitis for both different degrees of TEC and for different mastitis causing pathogens (Chapters 3 and 5),
III. evaluate the use of a non-invasive method to measure machine-induced teat tissue changes and measure the recovery time of teat tissue after milking (Chapter 4),
IV. examine the relationship between machine-induced teat tissue changes and the development of TEC (Chapter 7).

The different findings and conclusions from part-research as reported in the various chapters will be discussed in the General Discussion at the end of this thesis.

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Chapter 2

CLASSIFICATION AND LONGITUDINAL EXAMINATION OF CALLUSED TEAT-ENDS IN DAIRY COWS

F. Neijenhuis,[1] H.W. Barkema,[2] H. Hogeveen,[1] and J.P.T.M. Noordhuizen[3]

[1] Research station for Cattle, Sheep and Horse Husbandry, P.O. Box 2176, 8203 AD Lelystad, The Netherlands

[2] Animal Health Service, P.O. Box 361, 9200 AJ Drachten, The Netherlands

[3] Utrecht University, Farm animal Health, Ruminant health unit, P.O. Box 80151, 3508 TD Utrecht, The Netherlands

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ABSTRACT

To examine the development of teat-end callosity thickness and roughness in early lactation and to quantify cow factors of interest, a system to classify teat-end condition was developed. A distinction was made between rough and smooth rings around the teat orifice. In addition, a classification of the degree of callosity was developed. Kappa coefficients for the repeatability of scoring by this classification system by different workers were 0.71 for teat-end callosity thickness and 0.86 for teat-end callosity roughness. The teat-end callosity classification system was used for a longitudinal study with 40 cows during the first 14 wk of lactation. Models were built to predict teat-end callosity thickness and roughness, machine-on time, and milk yield. For the response variables, teat-end callosity thickness, machine-on time, and milk yield, the consecutive measurements appeared to follow a lactation curve model with a subject-specific general slope and intercept. Teat-end callosity increased rapidly the first 8 wk. Cow factors such as days in milk, parity, machine-on time, and teat-end shape were associated with the degree of teat-end callosity, and the probability of the callosity ring to become rough. Teat-end callosity thickness did not decrease within the 14-wk trial period for most teats. Pointed or round teat-ends showed more callus than inverted teat-ends. Longer machine-on time resulted in a higher probability of the callosity ring to become rough. Rear teats showed less callosity than front teats in this study.

(**Key words**: teat-end condition, machine milking)

Abbreviation key: MT = machine-on time, **MY** = milk yield per day, **PAR** = parity, **TEC** = teat-end callosity, **TECR** = teat-end callosity roughness, **TECRday** = day of appearance of teat-end callosity roughness, **TECT** = teat-end callosity thickness, **TES** = teat-end shape.

INTRODUCTION

The teat canal is a strong and important primary barrier against invasion of mastitis pathogens into the udder (Hamann, 1987). The sphincter muscle surrounding the teat duct is tightly closed between milkings and impedes bacterial intrusion from the teat opening into the interior of the gland (Nickerson, 1994). A teat-end in good condition is an important resistance factor in the pathogenesis of bovine mastitis (Michel et al., 1974). Changes in teat tissue by milking, teat canal integrity, and teat tissue pliability may favor penetration of bacteria into the udder (O'Shea et al., 1987). Teat-end lesions are frequently colonized by staphylococci and *Streptococcus dysgalactiae* (Kingwill et al., 1979). Therefore, they are a potential source of infection of deeper parts of the udder. Although never directly proven (Graf and Gedek, 1983; Michel et al., 1974; Sieber and Farnsworth, 1981), teat-ends with severe rough callus rings are often associated with IMI. Seykora and McDaniel (1985) showed that SCC was influenced by lesion score using a system that included the parameter ulcerative and raw orifice. Also, Farnsworth (1995) found that teat-ends with severe erosions that were raw and ulcerated (broken skin) showed higher prevalence of mastitis. Because of the possible relation of teat-end callosity (**TEC**) and udder health, TEC was included in a large, nationwide mastitis system: the mastitis management planner (Lam et al., 1998).

After a single milking, teat length and tissue thickness change (Hamann et al., 1994). After repeated milkings, teat-end tissue shows changes that appear as a ring around the teat orifice. Hyperkeratosis of the teat duct orifice is the result of a normal physiological process of adaptation during the initiation of lactation. This process continues during lactation (Michel et al., 1974; Sieber and Farnsworth, 1981). Cow factors like teat-end shape (**TES**), teat position, teat length, milk production, DIM, and parity (**PAR**) show a relationship with callused teat-ends (Bakken, 1981; Graf, 1982; Johansson, 1957; Michel et al., 1974; Rathore, 1977; Sieber and Farnsworth, 1981).

Changes in teat-end tissue result from mechanical forces exerted by vacuum and the collapsing liner during machine milking. The magnitude of the force depends on milking vacuum, pulsation vacuum, liner type, and teat shape (Ebendorff and Ziesack, 1991; Hamann, 1987; Kingwill et al., 1979; Mein and Thompson, 1993). Callus type lesions do not progress from mild to severe or even to erosions or scabs as a natural process (Farnsworth, 1995). White rings can be found to a lesser extent in suckler cows and hand-milked cows, indicating that factors other than machine milking are also involved in the formation of these rings (Espe and Cannon, 1942; Sieber and Farnsworth,

1984). The prevalence of teat-ends without callosity in machine milked cows varied in several studies from 16 to 45% (Graf and Gedek, 1983; Rittenbach, 1973; Sieber and Farnsworth, 1981).

As early as 1942, everted teat sphincter in machine- milked teats, which became eroded, had been observed (Espe and Cannon, 1942). Sieber and Farnsworth (1981) defined teat-end lesion rings that appeared in three general forms: 1) smooth, 2) roughened raised ring of tissue around the orifice, and 3) formation of a scab. More recently, Shearn and Hillerton (1996) defined the rings as hyperkeratosis of the teat duct orifice and used a scoring system with six classes. No differentiation was made between a rough and a smooth raised ring. O'Shea et al. (1987) stated that the progressive state of teat orifice eversion is teat orifice erosion. Keratin is removed and tissue around the teat canal is destroyed.

Two aspects of teat-end callus can be distinguished: a smooth and a rough raised ring. The existing systems to classify teat-end condition either do not differentiate between smooth and rough callosity rings or do include scabs or lesions. The goal of this study was to examine longitudinal development of teat-end callosity thickness (**TECT**) and teat-end callosity roughness (**TECR**) in early lactation and to quantify cow factors of interest. Therefore, an adapted classification system for teat-end callosity (TEC), including both smoothness and thickness of the callosity ring was developed and tested.

MATERIALS AND METHODS

Classification of Teat-end Callosity and Teat-end Shape

A classification system was developed from 870 photographs of teat-ends from 174 cows made at 3-months intervals during 1.5 yr (Figure 1). All teats were photographed immediately after the milk cluster had been removed. The pictures showed marked differences in the thickness of the callosity ring, which were placed into five classes: 1) none [N], 2) slight [A], 3) moderate [B], 4) thick [C], and 5) extreme [D]. Average TECT of teats was calculated by using the unit scores from 1 to 5. Additionally, the ring was classified as 7smooth [1] or rough [2]. Rough teat-end callosity was parakeratosis of the epidermis and had a frayed appearance.

For examination of teat-ends on a farm, a reference sheet with full color pictures of a representative teat-end for each class of TEC was used. Two pictures of each teat were given: a view from below

and from the side. Because the callosity ring looked different on a black or a white skin, pictures for white as well as black teats were printed.

Teat-end shape was classified as round, flat, pointed or inverted.

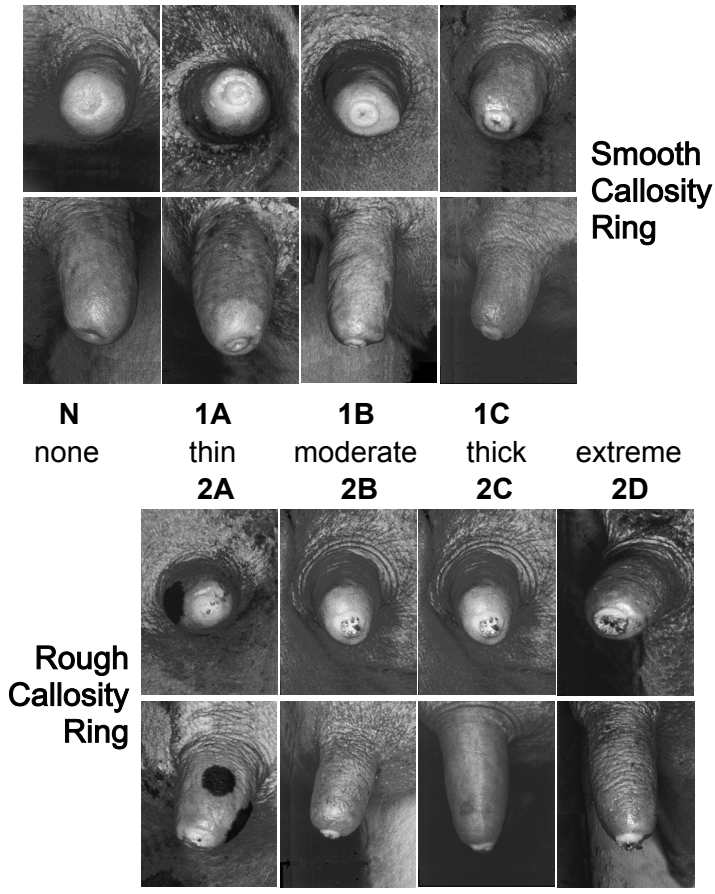

Figure 1. Teat-end callosity (TEC) classification system.

Study Design

A longitudinal study was carried out to examine the development of TEC during early lactation. On a research farm, 40 cows calved between January and April 1994 and were observed weekly during the first 14 wk of lactation. Twelve cows had calved for the first time, nine were in their second, and 19 in their third or higher lactation. Cows were milked in a double-eight herring- bone milking parlour with 47 kPa nominal vacuum, milk recorder jars, and automatic cluster removal. Premilking treatment consisted of dry wiping of the teats with paper towel during 15 s and immediate cluster attachment. Mean fat corrected milk yield in 305 d was 7515 kg/cow (range 5083 to 9607 kg) and milk yield per day (**MY**) was 29.5 kg (range 12.5 to 43 kg/day). Mean machine-on time per day (**MT**) was 14.4 min (range 6 to 32 min).

Once a week, photographs of teat-ends (side and bottom view) were taken from all teats of each cow just after cluster removal. The TEC was scored by one observer using the reference sheet (Figure 1). The same observer classified TES at 2 months of lactation. The TES classification was performed just before the start of cow preparation for milking.

Parameter Definition and Statistical Analysis

Before statistical analysis, observations were checked for unlikely values. No data were excluded for this reason. The distribution of teat scores across quarters was examined using chi-square statistics.

The repeatability of the classification system scores was determined using the repeated independent classification of 52 teats of 13 dairy cows after cluster removal by four different persons. Kappa coefficients were computed to determine the interobserver agreement (Tanner and Young, 1985).

For the response variables TECT, MT, and MY the consecutive measurements appeared to follow a lactation curve model with a subject-specific general slope and intercept. The general model is the Wood curve: $Y = A * DIM^{B} * e^{-C.DIM} * e$, where A is the intercept, B increasing slope and C the decreasing slope (Wood, 1976).

The response variable teat-end callosity roughness were split into two components: whether the ring became rough within the 14-wk period (TECR) and after how many days in lactation the callosity ring becomes rough (**TECRday**). The response variables TECT, TECR, TECRday, MT, and MY

were expected to be correlated (Kingwill et al., 1979). First, the complete random structure was tested; when not contributing to variance, terms were eliminated. Cow factors (PAR, TES, teat position, and DIM) were tested for their effects on the response variables as explanatory variables in the models. Backwards elimination was used ($P < 0.1$) for the interaction terms; main effects (DIM, teat position, PAR, and TES) were forced into the models. Individual traits (univariate approach) were modeled by random regression models using the method of REML (Genstat 5 Release 3.2, 1996; Genstat 5 Release 3 Reference Manual, 1996). So, these traits could also be examined for the relative importance of the individual-specific slope.

TECT and TECRday were tested using random regression models using the method of REML (Genstat 5 Release 3.2, 1996; Genstat 5 Release 3 Reference Manual, 1996). Data of TECR are binomial and were tested using generalized linear mixed regression with link logit using the procedure IRREML (Genstat 5 Release 3.2, 1996; Keen and Engel, 1996).

For TECT, TECR, TECRday, MT, and MY, random cow coefficients were estimated. The cow coefficients corresponded with the random effects fitted in the model. These cow coefficients were corrected if significant ($P < 0.05$) for intercept (A), decreasing slope of DIM (B), and for increasing slope of ln(DIM) (C). The TECR models are not on a time scale; variance between cows is only corrected on intercept. Correlations between the random cow coefficients from the five models were evaluated. A positive correlation indicated that parameters were correlated within cow. Correlation higher than 0.2 was regarded as a value of interest for discussion.

The final models were:

$$TECT_{ijklmno} = \mu + cow_i + cow(teat)_{ij} + cow(ln(DIM))_{ik} + DIM_k + ln(DIM)_k + PAR_l + TP_m + TES_n + (ln(DIM)*TP)_{km} + (DIM*TP)_{km} + (TP*TES)_{mn} + (DIM*TES)_{kn} + (DIM*PAR*TP)_{klm} + e_{ijklmno} \quad [1]$$

$$TECRday_{ilmno} = \mu + cow_i + PAR_l + TP_m + TES_n + (PAR*TP)_{lm} + (PAR*TES)_{ln} + e_{ilmno} \quad [2]$$

$$TECR_{ilmno} = \mu + cow_i + PAR_l + TP_m + TES_n + e_{ilmno} \quad [3]$$

Where

Response variable:

TECT = teat-end callosity thickness (1 = no ring, 2 = thin, 3 = moderate, 4 = thick, and 5 = extreme thick ring);

TECRday = interval between calving and the day the teat-end callosity ring became rough (days);

TECR = logit link function of prevalence of teat-end callosity roughness; and

μ = overall mean.

Random effects:

cow_i = cow i representing differences between herdmates;

$cow(teat)_{ij}$ = teat j within cow i representing differences between teats within cow; and

$cow(ln(DIM))_{ik}$ = lactation stage of cow i representing differences in decreasing slope after k DIM.

Fixed effects:

DIM_k = lactation stage expressed as k DIM;

$ln(DIM)_k$ = lactation stage expressed as natural logarithm of k DIM;

PAR_l = parity (l is 1, 2, or >2);

TP_m = teat position (m is 1 = front and 2 = rear);

TES_n = teat-end shape (n is 1 = pointed, 2 = round, 3 = flat, and 4 = inverted); and

$e_{ijklmno}$ = residual random error.

Models for MY and MT were also based on the Wood curve (Wood, 1976). In these models TES and PAR were included; TES was put into the models as an average TES per cow. For MT and MY, an inverse natural logarithm transformation was used.

RESULTS

General Descriptive Results

Round teat-ends were found in 43%, pointed teat-ends in 31%, and flat and inverted teat-ends both in 13% of the teats. Milk yield per day and MT increased until wk 6 (from 23.0 kg in 12.7 min in wk 1 to 31.8 in 14.7 min in wk 6).

The maximum of the curves for MY and MT was reached within wk 6 according to the models. The MY was associated with DIM, PAR, TES, and interactions between DIM and PAR, DIM and TES, and PAR and TES. The MT was associated with the same factors as MY except for the interaction between PAR and TES. On average, MY and MT increased from first to older parity cows. In wk 7, first parity cows produced 25.7 kg in 12.7 min, second parity cows produced 32.9 kg in 13.5 min, and older parity cows produced 34.6 kg in 14.6 min. Pointed teat-ends had longer MT than inverted teat-ends. In wk 7, MT for cows with on average pointed teat-ends was 16.1 min, 14.0 min with round teat-ends, 12.8 min with flat teat-ends, and 10.7 min with inverted teat-ends.

Repeatability of Classification

Thickness classifications by four observers were identical for 84% of the teats (Table 1). For TECR, 93% of teats were identically scored. The Kappa coefficients for the four observers for the thickness of the callosity ring were 0.71 (SE = 0.00477) and 0.86 for roughness (SE = 0.00356).

Table 1. Teat condition classification of roughness (TECR where 1 = smooth and 2 = rough callosity ring) and thickness (TECT where N = no ring, A = thin ring, B = moderate thick ring, C = thick ring and D = extreme thick callosity ring) of four observers on 52 teats of 13 cows.

		TECR		TECT				
teat	cow	1	2	N	A	B	C	D
1	1	4			4			
2	1	3	1		3	1		
3	1	3	1		3	1		
4	1	2	2		3	1		
5	2		4			4		
6	2		4			4		
7	2		4		3	1		
8	2		4		3	1		
9	3	4			4			
10	3	4			4			
11	3	3	1		4			
12	3	4			4			
13	4	1	3		4			
14	4		4		4			
15	4		4		4			
16	4		4		4			
17	5		4		4			
18	5		4		4			
19	5		4		4			
20	5		4		4			
21	6	4			4			
22	6	4			4			
23	6	4		2	2			
24	6	4		1	3			
25	7	4			4			
26	7	4			4			
27	7	4			4			
28	7	4			4			
29	8		4		1	3		
30	8		4				4	
31	8		4		4			
32	8		4		3	1		
33	9		4		2	2		
34	9		4			4		
35	9		4		1	3		
36	9		4		1	3		
37	10		4		3	1		
38	10		4			3	1	
39	10		4			3	1	
40	10		4			1	3	
41	11	3	1		3	1		
42	11	4			2	2		
43	11	4		3	1			
44	11	3	1		4			
45	12		4					4
46	12		4					4
47	12		4		1	3		
48	12		4		1	3		
49	13	4			4			
50	13	4			4			
51	13	4		4				
52	13	4		4				

Teat-end Callosity Thickness

On average, the TECT of the ring was 2.24 (Table 2). Thickness of the callosity rings increased during the first lactation weeks. Extremely thick callosity rings were rarely seen. At wk 13 after calving, 93.5% of the teat-ends had at least a thin callosity ring.

Table 3 shows the factors and variance components according to the model associated with TECT. In the model for TECT, 67% of the variance was accounted for by the differences between cows, 5% by differences between teats within cow, and 10% by the differences in increasing slope for DIM between cows.

Table 2. Distribution of teat-end callosity thickness (TECT) and roughness scores (TECR) of 40 dairy cows in wk 1, 7 and 13 of lactation, and as overall means (lactation wk 1 to 14) (255 missing values).

	Week 1	Week 7	Week 13	Total of 14 wk
Callosity thickness				
No ring [N][1]	55.7[2]	11.1	6.5	16.4
Thin ring [A]	41.4	56.9	52.3	52.1
Moderate ring [B]	2.9	26.1	27.5	22.9
Thick ring [C]	0.0	5.9	11.8	8.2
Extreme thick ring [D]	0.0	0.0	1.9	0.4
Average thickness Score	1.47	2.27	2.50	2.24
Roughness				
Smooth ring [1][1]	100.0[2]	70.6	66.7	76.0
Rough ring [2]	0.0	29.4	33.3	24.0
Number of quarters	140	153	153	142

[1][..] Refers to the classes in the system; see explanation in text.
[2] Percentage of udder quarters classified as such.

Front teats were more callused and had a higher level of thickness than rear teats (Table 3). Front teats of first- and second-parity cows showed more callus than rear teats during the complete observation period (Figures 2 and 3). During lactation, rear and front teats from older parity cows (PAR > 2) showed the same extent of callus (Figure 4). There was no difference in TECT between right and left teats ($P > 0.10$). The TECT did not decrease within the 14-wk trial period for most teats (Figure 2). Teats of first-lactation cows were less callused than those of older cows (PAR > 2). On teats with inverted teat-ends, TECT was less than on round teat-ends. These phenomena are best shown in Figures 2, 3, and 4 for round versus inverted teat-ends, and front versus rear teats.

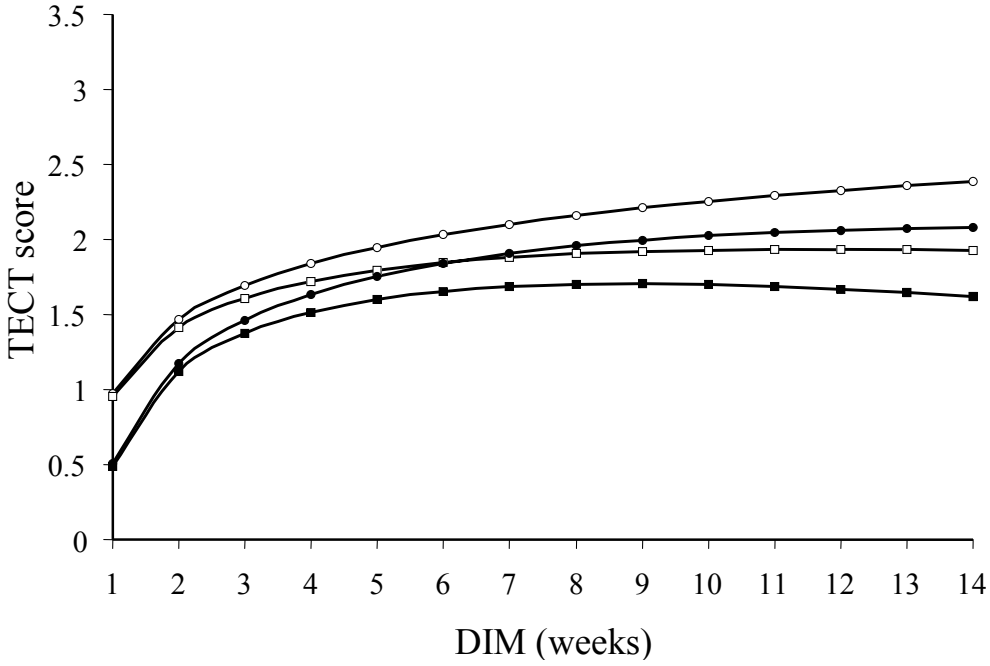

Figure 2. Teat-end callosity thickness score (TECT) during the first 14 wk of lactation of front and rear, inverted or round teat-ends of first parity cows according to the model (○ round, front teat; □ inverted, front teat; ● round, rear teat; ■ inverted, rear teat).

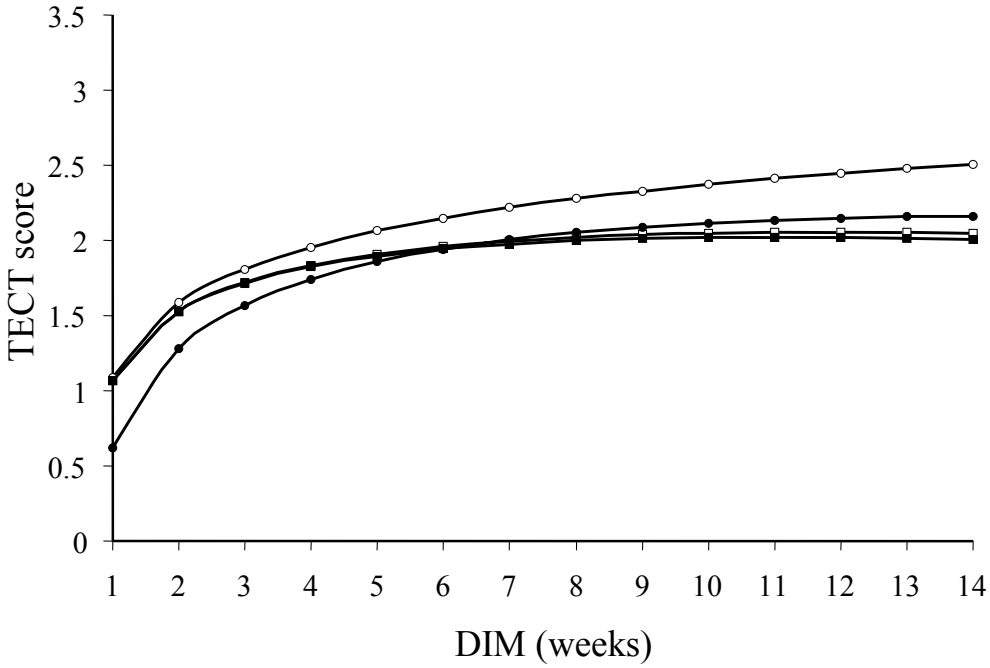

Figure 3. Teat-end callosity thickness score (TECT) during the first 14 wk of lactation of front and rear, inverted or round teat-ends of second parity cows according to the model (○ round, front teat; □ inverted, front teat; ● round, rear teat; ■ inverted, rear teat).

Table 3. Characteristics of final regression model on prevalence of teat-end callosity thickness (TECT) (first 14 lactation wk, 40 dairy cows).

Variable		Coefficient (ß)	SE (ß)	Variance comp.	SE	*P*-value[4]
Cow				0.672	0.1850	***
Cow(teat)				0.054	0.0092	***
Cow(ln(DIM))				0.098	0.0240	***
error				0.186	0.0062	***
Intercept		0.490	0.310			ns
DIM		-0.00350	0.0022			ns
ln(DIM)		0.456	0.0748			***
TP[1]	rear vs. front	-0.689	0.201			***
PAR[2]	Parity 2 vs. 1	0.113	0.426			ns
	Parity >2 vs. 1	0.423	0.357			†
	Parity 2 vs. >2	-0.310	0.393			ns
TES[3]	round vs. pointed	-0.0822	0.096			ns
	flat vs. pointed	-0.0642	0.145			ns
	inverted vs. pointed	-0.0860	0.184			ns
	round vs. flat	0.146	0.139			ns
	round vs. inverted	-0.00373	0.182			ns
	flat vs. inverted	0.150	0.184			ns
DIM*TP	rear vs. front teat	-0.00510	0.0023			*
ln(DIM)*TP	rear vs. front teat	0.1900	0.0791			*
TES*DIM	round vs. pointed	0.00249	0.0012			*
	flat vs. pointed	0.00107	0.0017			ns
	inverted vs. pointed	-0.00234	0.0020			ns
	round vs. flat	0.00142	0.0016			ns
	round vs. inverted	0.00483	0.0019			*
	flat vs. inverted	0.00341	0.0021			†
PAR*TP*DIM	Parity 2 vs. 1, front teat	0.000481	0.0025			ns
	Parity >2 vs. 1, front teat	0.00227	0.0021			ns
	Parity 2 vs. >2, front teat	0.00179	0.0024			ns
	Parity 2 vs. 1, rear teat	-0.00222	0.0025			ns
	Parity >2 vs. 1, rear teat	0.00540	0.0021			*
	Parity 2 vs. >2, rear teat	0.00762	0.0026			**

[1] TP = Teat position.
[2] PAR = Parity.
[3] TES = Teat-end shape.
[4] † P<0.10, *P< 0.05, **P< 0.01, ***P< 0.001, ns = Not significant

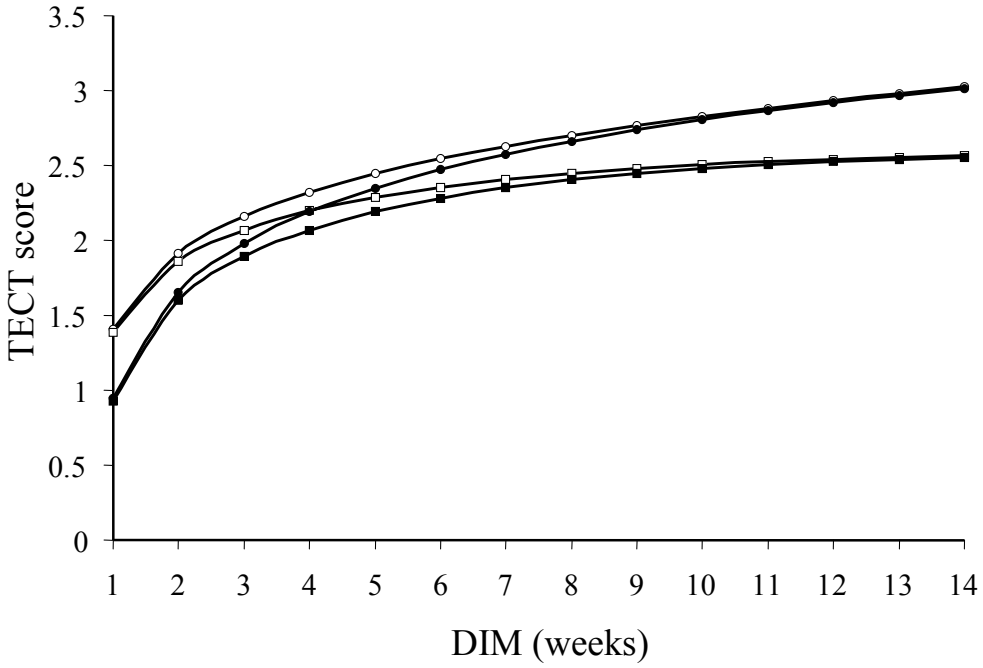

Figure 4. Teat-end callosity thickness score (TECT) during the first 14 wk of lactation of front and rear, inverted or round teat-ends of older parity cows (parity >2) according to the model (○ round, front teat; □ inverted, front teat; ● round, rear teat; ■ inverted, rear teat).

Day of Appearance of Teat-end Callosity Roughness

Table 4 shows the factors and variance components according to the final model describing TECRday. The TEC ring turned from smooth to rough at 56 DIM on average. Differences between cows explained 72% of the variance. Significant difference in TECRday could be found for TES, teat position, and PAR. On inverted rear teat-ends, the callosity ring turned rough later in lactation than on pointed or round teat-ends. Callosity rings on front teats with flat teat-ends became rough earlier in lactation than on rear teats with round teat-ends (Figure 5). The callosity rings on the teats of older parity cows (PAR > 2) became rough later in lactation than in second-parity cows.

Table 4. Characteristics of final regression model on day of appearance of teat-end callosity roughness (TECRday) and teat-end callosity roughness (TECR) (first 14 lactation wk, 40 dairy cows).

Variable		Coefficient (ß)	SE (ß)	Variance comp.	SE	*P*-value[4]
TECRday						
Cow				380.6	138.1	**
Error				148.8	33.2	***
Intercept		56.12	8.857			***
TP[1]	Rear vs front	-4.136	5.279			ns
PAR[2]	Parity 2 vs 1	8.751	13.21			ns
	Parity >2 vs 1	-14.372	9.648			ns
	Parity 2 vs >2	-23.123	12.49			†
TES[3]	Round vs pointed	-7.92	6.062			ns
	Flat vs pointed	-0.89	7.830			ns
	Inverted vs pointed	-34.07	16.894			*
	Round vs flat	-7.03	7.21			ns
	Round vs inverted	26.15	16.66			ns
	Flat vs. inverted	33.18	17.45			†
TP*TES	Rear vs. front, round vs. pointed	-1.794	7.832			ns
	Rear vs. front, flat vs. pointed	19.173	12.407			ns
	Rear vs. front, inverted vs. pointed	50.153	23.568			*
	Rear vs. front, round vs. flat	-20.967	12.407			†
	Rear vs. front, round vs. inverted	-51.947	23.568			*
	Rear vs. front, flat vs. inverted	-30.98	23.568			ns
TECR						
Cow				3.399	1.288	**
Error				1.000	fixed	
Intercept		1.139	0.855			ns
TP	Rear vs. front	-0.773	0.471			†
PAR	Parity 2 vs. 1	-1.793	1.0694			†
	Parity >2 vs. 1	-0.0156	0.872			ns
	Parity 2 vs. >2	-1.777	1.004			†
TES	Round vs. pointed	-0.766	0.655			ns
	Flat vs. pointed	-0.196	0.998			ns
	Inverted vs. pointed	-2.474	1.135			*
	Round vs. flat	0.570	0.903			ns
	Round vs. inverted	1.708	1.100			ns
	Flat vs. inverted	2.279	1.238			†

[1] TP = Teat position. [2] PAR = Parity. [3] TES = Teat-end shape.
[4] †P<0.10, *P< 0.05, **P< 0.01, ***P< 0.001, ns = Not Significant.

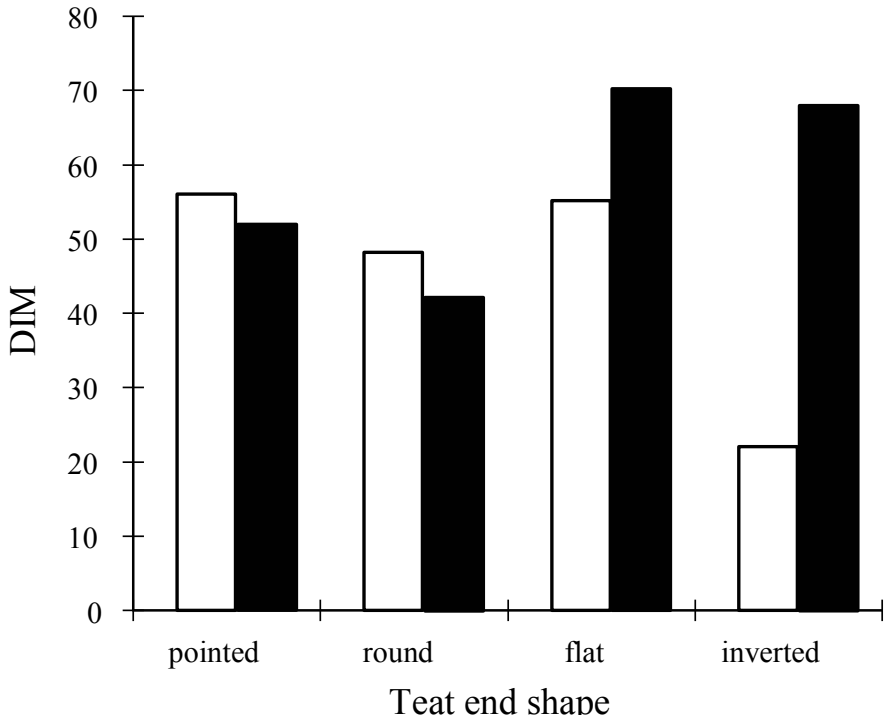

Figure 5. Moment in lactation teat-end callosity shifted to rough (TECRday) (DIM) stratified for different teat-end shape and teat position according to the model (□ front teat; ■ rear teat).

Percentage of the Teat-end Callosity Shifting to Rough

On average, 24% of the observations showed rough teat-end callosity. All cows came into lactation without rough callosity rings. Roughness of the callosity rings increased during the first lactation weeks (Table 2).

Table 4 shows the factors and variance components for the model describing TECR. Differences between cows explained 77% of variance. According to the model, callosity rings on front teats became rough more frequently than on rear teats. No significant differences were found between right and left teats. Inverted teat-ends became rough less frequently than pointed or flat teat-ends. The TEC rings of second parity cows became rough less frequently than those in first or older parity cows. These phenomena are best shown in Figure 6.

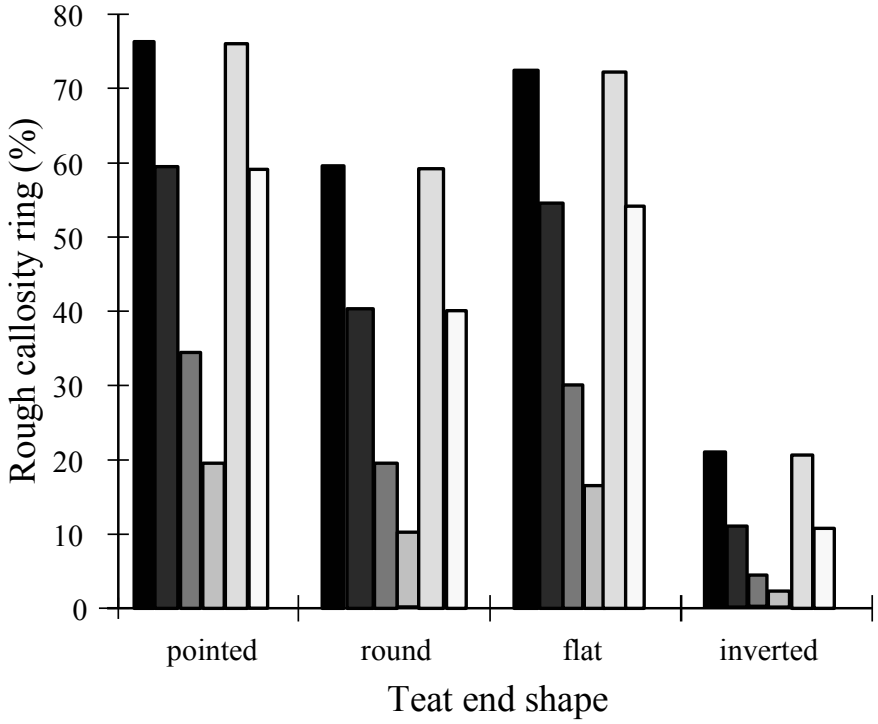

Figure 6. The chance the teat-end callosity becomes rough (TECR) for different teat-end shape, teat position and parity according to the model (first bars Parity 1, front teat; second bars Parity 1, rear teat; third bars Parity 2, front teat; fourth bars Parity 2, rear teat; fifth bars Parity > 2, front teat; sixth bars Parity >2, rear teat).

Relationship between Machine-on Time, Milk Yield, and Teat-end Callosity

A correlation between the random cow coefficients from the five final regression models showed that TECT and TECR level were correlated ($r=0.45$, $P=0.001$). When TECT became thicker, callosity rings became rough earlier in lactation ($r=-0.55$, $P<0.001$). When MT was longer, teat-end callosity became rough more frequently ($r=0.22$, $P=0.08$).

DISCUSSION

The repeatability of the TECT and TECR classification system between workers was high. Thus, the system can be used by different persons with good agreement regarding the scores. Unfortunately, it was not possible to collate the results on repeatability with other research. No statistical analyses were presented to show the repeatability of most of the reported systems used (Bakken, 1981; Ebendorff and Ziesack, 1991; Graf and Gedek, 1983; Johansson, 1957; Michel et al., 1974; Shearn and Hillerton, 1996; Sieber, 1980; Sieber and Farnsworth, 1981). Repeatability for TEC was 0.32 in the research of Seykora and McDaniel (1985), but the second lesion score was taken 6 months after the first. Although the repeatability between workers was high, in the study, only one observer was used. So, no variance caused by different workers was included in this study.

Teat-end callosity thickness and roughness, which were separately used in the system, were correlated ($r=0.45$, $P=0.001$). However, the correlation was far from complete. TECT and TECR were only partly exchangeable and were used as complementary parameters. Modeling TECT and TECR resulted in different explanatory factors in the models. Other described systems lack the ability to distinguish teat-end callosity thickness and roughness (Bakken, 1981; Graf and Gedek, 1983; Johansson, 1957; Michel et al., 1974; Rathore, 1977; Shearn and Hillerton, 1996). Both factors should be used independently in the system.

The TECT increased mainly during the first 6 to 8 wk of lactation. During the trial period covering the first 14 wk of lactation, decrease in TECT was only seen for certain combinations of TES and PAR. These results are similar to the increase in TEC during the first 4 months of lactation reported by Graf (1982) and by Shearn and Hillerton (1996). For inverted teat-ends from first parity cows, the TECT decreased within the first 14 wk of lactation. Farnsworth (1995) reported that first parity cows seemed to develop a degree of callus within the first 6 to 8 wk of lactation. Thereafter it was persistent and decreased to some degree during the dry period. An increase of TEC was reported by Francis (1984) for the first 120 DIM; thereafter the TEC decreased.

Increase of TECT goes parallel with the increase of MT and MY increases (first 6 to 8 wk of lactation). The moment of first appearance of TECR was 8 wk on average. A small positive relationship ($r=0.22$, $P=0.08$) was found between MT and TECR. Other studies reported a positive relationship between MY and the degree of erosions at the teat-end (Bakken, 1981; Farnsworth, 1995). Farnsworth (1995) reported that milking practices resulting in extended MT did increase the

degree of lesions. Sieber (1980) stated that increased MT indicated increased levels of overmilking. During overmilking, the teat is no longer filled with milk. Longer MT gives a higher probability of roughness of the TEC. Therefore, MT is an important cow factor with respect to TEC.

Round teat-ends showed more callus than inverted teat-ends. Other researchers also showed the influence of TES. Pointed or round teats ends were shown to have a higher degree of hyperkeratosis than flat teat-ends (Bakken, 1981; Johansson, 1957; Rathore, 1977). In the present study, front teats showed more callus than rear teats. However, a larger number of pointed teat-ends on rear than on front teats was found. Consequently, the influence of pointed teat-ends on TEC might have been underestimated. Pointed teat-ends became rough sooner as well as more frequently than inverted teat-ends.

The relationship between PAR and TEC was not quite clear in literature (Graf, 1982; Michel et al., 1974). In accordance with Sieber (1980), first parity cows showed less TEC than older parity cows (PAR>2). No significant effect on TECT was found between first- and second- or second- and older- parity cows (PAR>2). Effects of PAR were not consistent. With regard to TECR and TECRday, no differences were found between first and older parity cows (PAR>2). However, TEC of older cows became rough later but more frequently than in second-parity cows.

Teat position was associated with TEC. Front teats had increased TEC, which is in accordance to results of other studies (Sieber, 1980; Sieber and Farnsworth, 1981). Front teats give less milk so milking is finished earlier than in rear teats. Consequently, front teats tend to be overmilked for longer periods. In our study, the differences in TEC, when taking into account DIM, PAR, teat position, and TES, were approximately 30% due to differences within cows (teat position); the rest by differences between cows. In some studies, TEC was averaged per cow, thus ignoring differences between front and rear teats (Bakken, 1981; Shearn and Hillerton, 1996).

Machine milking can affect the extent of callus on the teat-end (Ebendorff and Ziesack, 1991; Hamann, 1987; O'Shea et al., 1987; Sieber, 1980). Therefore, TEC may be looked upon as an indicator of the quality of machine milking. Scoring of TEC can be a very useful addition to the set of parameters for assessing information on milking techniques. The research described in this paper showed that cows differ in the extent of TEC as their adaptive reaction on machine milking. These differences in cow factors must be taken into account before judging the milking machine or milking management. Cow factors as DIM, MT, PAR, and TES should be taken into account.

Currently, an adapted system as a part of the health planning system for udder health is used in the field to detect possible problems with the milking machine or management. However, the relationship milking machine, management, and TEC needs further investigation.

This research was done on one farm. Therefore, it is not known which proportion of the variation is caused by farm and farm management. However, although carried out on one farm, this research did show the effectiveness of a developed teat-end classification system and the importance of cow factors. Research at the larger population level is needed to get insight in farm and farm management variation. Differences in TEC between farms, taking into account known cow factors, may be associated with milking technique and management.

With the classification system developed, it should also be possible to show the presence of a relationship between TECR and TECT or both, and IMI. Known research on this subject was carried out with a classification system, which did not make distinction between smooth and rough callosity rings (Bakken, 1981; Ebendorff and Ziesack, 1991; Graf and Gedek, 1983; Johansson, 1957; Michel et al., 1974; Shearn and Hillerton, 1996), or included acute lesions (Sieber, 1980). Moreover, these studies were not longitudinal. It might be possible that the absolute level of TEC is less important than an increase or decrease for the relationship between IMI and TEC. Decreasing TEC might reduce the risk of IMI. Further research should be carried out on the relation between IMI and TEC. The system for TEC described in this paper can very well be used for such a study.

CONCLUSION

The developed two-factor TEC classification system, with the distinction between callosity thickness and roughness, can be used to establish a repeatable scoring of the teat-end callosity. The TECT and TECR are complementary parameters, and should both be used. Development of TEC is associated with cow factors such as TES, PAR, DIM, and MT. Therefore, cow factors should be taken into account when using teat-end condition. Population studies are needed to elaborate possible relationships between TEC and the risk of IMI.

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CHAPTER 3

RELATIONSHIP BETWEEN TEAT-END CALLOSITY AND OCCURRENCE OF CLINICAL MASTITIS

F. Neijenhuis,[1] H.W. Barkema,[2] H. Hogeveen,[1] and J.P.T.M. Noordhuizen[3]

[1] Research Institute for Animal Husbandry, P.O. Box 2176, 8203 AD Lelystad, The Netherlands

[2] Animal Health Service, P.O. Box 361, 9200 AJ Drachten, The Netherlands

[3] Utrecht University, Department of Farm animal Health, Ruminant health unit, P.O. Box 80151, 3508 TD Utrecht, The Netherlands

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ABSTRACT

A longitudinal study in 15 herds, with a total of 2157 cows, was conducted to examine the relationship between teat-end callosity (TEC) and the incidence of clinical mastitis. During the 1.5-yr study period, clinical mastitis was diagnosed by the farmers based on clinical signs. Teat-end callosity was scored every month according to a teat-end callosity classification system, which discriminates between teat-end callosity thickness (TECT) and roughness (TECR). Differences in TECT between healthy and clinical mastitis quarters within infected cows were small but significant 3 months before (0.13 higher), in the month during which the clinical mastitis occurred (0.08 higher), and in the following 2 months (0.06 and 0.05 higher). To compare TECT and TECR between cows with and without clinical mastitis, 199 cows with clinical mastitis were paired with control cows based on herd, days in milk, and parity. Clinical mastitis cows had more TEC than their healthy herd mates, particularly when clinical mastitis occurred between the second and fifth months of lactation. Clinical *Escherichia coli* mastitis in the second or third month of lactation occurred in cows with less TEC than in cows with clinical mastitis caused by other pathogens. Clinical culture-negative, yeast, *Klebsiella pneumoniae*, and *Enterobacter aerogenes* mastitis cows had more TECT and TECR than other cows with clinical mastitis in the same month of lactation. Pointed teat-ends had higher TECT and TECR than flat or inverted teat-ends. Teat-end callosity thickness increased with a higher milk yield at peak production.

(**Key words**: clinical mastitis, teat condition, teat- end callosity)

Abbreviation key: **TEC** = teat-end callosity, **TECR** = teat-end callosity roughness, **TECT** = teat-end callosity thickness.

INTRODUCTION

Mastitis continues to be one of the economically most important diseases in dairy farming. The incidence rate of clinical mastitis ranges from 12.7 to 30% per cow-year at risk (Barkema et al., 1998; Chassagne et al., 1997; Miltenburg et al., 1996; Sargeant et al., 1998; Schukken, 1989). Bacteria that cause clinical mastitis usually enter the udder through the teat canal. The first line of defence against clinical mastitis is therefore the teat canal, and changes in teat tissue around the teat canal may favor penetration of bacteria into the udder (O'Shea, 1987).

Mechanical forces during machine milking may induce changes in teat-end tissue. Teat-end callosity (**TEC**) builds up until approximately 4 months of lactation and decreases thereafter (Neijenhuis et al., 2000). Cow factors like teat-end shape, teat position, teat length, milk yield, stage of lactation, and parity are associated with the degree of TEC (Bakken, 1981; Graf, 1982; Johansson, 1957; Michel et al., 1974; Neijenhuis et al., 2000; Rathore, 1977; Sieber, 1980; Sieber and Farnsworth, 1981).

An association between teat-end condition and clinical mastitis is often assumed in the field. Severe teat-end lesions (erosions or scabs) are positively associated with the prevalence of subclinical mastitis (Sieber and Farnsworth, 1981; Jackson, 1970). At the farm level, no relationship between SCC and degree of TEC was found (Shearn and Hillerton, 1996). Michel et al. (1974) stated that TEC is not a criterion for udder health based on SCC and bacteriology. In a cross-sectional study on 3982 teats, no positive correlation was found between TEC and the incidence of clinical mastitis (Thompson and Sieber, 1980). However, a relationship between TEC and the incidence of clinical mastitis was never examined in a longitudinal study, the month that mastitis occurred was not taken into account, and inadequate TEC classification systems were used.

The goal of this study was to examine the relationship between the occurrence of clinical mastitis and TEC in more detail. First, we determined differences in TEC between quarters within clinical mastitis cows. Second, we investigated, in a longitudinal study, the differences in TEC between clinical mastitis cows and paired herd mates without clinical mastitis, taking into account DIM and the lactation month in which clinical mastitis occurred. Third, we evaluated differences between pathogens causing the clinical mastitis with respect to TEC. Fourth, we examined the association between TEC and clinical mastitis while accounting for cow parameters (teat length, teat-end shape, and milk production).

MATERIALS AND METHODS

Study Population and Data Collection

Fifteen farms throughout The Netherlands were selected that a) participated in a three or four weekly milk recording scheme (Royal Dutch Dairy Syndicate, Arnhem, The Netherlands), b) had good cow identification in the milking parlour, and c) had milkers with known ability to detect clinical mastitis. Farms were either experimental (farm 1 to 9) or commercial (farms 10 to 15).

Between the 15 farms, 305-d milk yield ranged from 6682 to 10,069 kg. Average milk yield per cow per day ranged from 21 to 35 kg (Table 1). These ranges reflect the differences throughout The Netherlands (CR Delta, 1999).

Farms were visited monthly from August 1995 until February 1997. On average, 18 visits were made per farm. During the visit, TEC of the four teats of all cows was scored according to a previously described TEC classification system (Neijenhuis et al., 2000). In this classification system, TEC thickness (**TECT**) is scored from 1 to 5 (where 1 = no callosity ring to 5 = extremely thick callosity ring), and TEC roughness (**TECR**) is scored 0 or 1 (where 0 = smooth or no callosity ring, and 1 = rough callosity ring). Average TECT of teats was calculated by using the unit scores 1 to 5; TECR is expressed as proportion of the teats showing rough callosity rings. A total of 88,672 quarter records of TECT and TECR were obtained. Teat scoring was done by well-trained staff, and the accuracy of scoring was checked every 6 months to make scoring results as comparable as possible.

During the study period, the farmers took aseptic milk samples before treatment from all udder quarters that had clinical signs of mastitis before treatment. After collection, the milk samples were immediately frozen at approximately −20°C. During farm visits, the frozen milk samples were collected and taken to the laboratory for bacteriological examination. Bacteriological examination was carried out according to National Mastitis Council standards (Hogan et al., 1999).

Teat length and teat-end shape were scored twice a year. Teat-end shape was classified as round, flat, pointed, or inverted. Teat length was measured just before premilking treatment, from base to tip, with a handmade device consisting of a pipe with a diameter of 4.5 cm and length of 11 cm.

Data Analyses

Before statistical analysis, observations were checked for unlikely values. No data were excluded for this reason. The analyses were carried out in two steps: 1) relationship between TEC and occurrence of clinical mastitis within cows, and 2) differences in TEC between cows with clinical mastitis and paired cows without clinical mastitis. The final regression model of step 2 was used to examine the association of specific pathogens with TEC. Effects of teat length, teat-end shape, and peak production on TEC were analyzed by including these factors in the same basic regression model in which clinical mastitis was included.

Within-Cow Analysis

Only first occurrences of clinical mastitis were eligible for inclusion into the analyses. Differences in TEC between the clinical mastitis quarter and the lateral quarter without clinical mastitis (T_DIFF) were calculated. Data from up to 4 months before the occurrence of clinical mastitis until 4 months after were used. Only records of cows in lactation were used. For the response variable T_DIFF, the following random regression model was fitted using the method of residual maximum likelihood (REML) (Genstat 5, 1996, Genstat 5, 1993):

$\Delta_{t=} Y_{1t}\ Y_{2t} = \mu_0$ + fixed effects + random effects + residual

$$= \mu_0 + \text{PER} + \text{Farm.Cow.Udder half} + \text{Farm.Cow.Udder half.Period} + \varepsilon_{rt}[1]$$

where

Δ_t = difference in TECT or TECR between clinical mastitis quarter and the lateral quarter without clinical mastitis, where t indicates month from up to 4 months before the occurrence of clinical mastitis until 4 months after (t =–4,-3,. . 4),

Y_{1t} = TECT or TECR of clinical mastitis quarter,

Y_{2t} = TECT or TECR of lateral quarter without clinical mastitis,

μ_0 = overall mean,

PER = effect of month compared with the moment of clinical mastitis (-4 to 4),

Farm.Cow.Udder Half = random effect of udder halve within cow within farm,

Farm.Cow.Udder Half.Period = random effect of month within udder half within cow within farm, and

ε_{rt} = residual random error.

Table 1. Descriptive characteristics of herds during the study period.

Farm #	Average number lactating cows	Mean parity	Average 305-d milk yield (kg/cow)	Average daily milk yield (kg/cow)	% clinical mastitis[1]	Average TECT score[2]	Average TECR score[3]
1	114	2.8	7936	26	13	2.14	0.34
2	91	2.9	7843	25	25	2.16	0.30
3	40	2.4	9672	30	48	2.33	0.41
4	118	3.0	7743	24	16	2.20	0.41
5	72	2.8	7951	25	20	2.17	0.28
6	70	3.2	7897	25	11	2.12	0.24
7	71	2.4	7763	25	25	2.22	0.33
8	68	3.0	8135	26	38	2.24	0.44
9	37	2.8	7968	28	15	2.24	0.28
10	99	2.1	10,069	35	57	2.33	0.58
11	96	3.2	7048	23	23	2.11	0.28
12	55	3.5	7617	25	45	2.19	0.45
13	85	3.3	7980	25	22	2.09	0.22
14	110	2.7	6682	21	35	2.12	0.31
15	71	3.3	7803	26	27	2.07	0.25
Average	80	2.9	8000	26	28	2.18	0.34

[1]Calculated as quarter cases per 100 cows present per year.
[2]TECT = Teat-end callosity thickness
[3]TECR = Teat-end callosity roughness

Between-Cow Analysis

Cows that had clinical mastitis were eligible for inclusion as cases. Only data from the lactation in which the clinical mastitis occurred were used. The clinical mastitis cows were paired with control cows from the same farm and with the same parity (1, 2, 3, 4, and 5 or ≥ 6) and calving date (within 30 d), that did not have clinical mastitis during the study period. Only the scoring periods where both the clinical mastitis cow and her healthy herd mate had TEC data were used.

The response variables TECT and TECR were averaged per cow per month and analyzed by random regression models using the method of REML (Genstat 5, 1996; Genstat 5, 1993). For the response variables the consecutive measurements were shown to follow a lactation curve with a subject-specific slope and intercept (Neijenhuis et al., 2000; Wood, 1976):

$$Y = C_0 + C_1 DIM + C_2 \ln(DIM) + \varepsilon_r \quad [2]$$

where

Y = average TEC per cow per month,

C_0 = level,

$C_1 DIM$ = decreasing slope per DIM,

$C_2 \ln(DIM)$ = increasing slope per DIM, and

ε_r = residual random error.

Where level is modeled as:

$$C_0 = \beta_0 + PAR + MAST + Farm + Farm.CC + Farm.CC.Cow + Farm.CC.Per + Farm.CC.Cow.Per \quad [2a]$$

where

β_0 = overall mean,

PAR = effect of parity (PAR 1 = parity 1, 2 = 2, 3 = 3 and 4 and, 4 ≥ 5),

$MAST$ = effect of whether the cow belongs to the paired animals without clinical mastitis or to the clinical mastitis group, and if so, in which month the clinical mastitis occurred (MAST is 0 = no clinical mastitis during the lactation and clinical mastitis did occur in 1 = first month of lactation, 2 = second month of lactation, 3 = third month of lactation, 4 = fourth and fifth months of lactation and 5 = later than 5th months of lactation), *Farm*= random effect of farm (where farm range from 1 to 15),

Farm.CC = random effect of couples of paired animals within farm (where couples range from 1 to 199),

Farm.CC.Cow = random effect of cow within couples of paired animals within farm (where animal is 1 or 2),

Farm.CC.Per = random effect of month within couples of paired animals within farm (where month ranges from 1 to 18) and,

Farm.CC.Cow.Per = random effect of month within cow within couples of paired animals within farm where decreasing slope is modeled as:

$$\underline{C}_1 DIM = \{\beta_1 + PAR + MAST + Farm.CC + Farm.CC.Cow\}\ DIM \quad [2b]$$

where parameters are as in model [2a], and increasing slope is modeled as:

$$\underline{C}_2 \ln DIM = \{\beta_2 + PAR + MAST + Farm.CC + Farm.CC.Cow\} \ln(DIM) \quad [2c]$$

where parameters are as in model [2a].

Table 2. Number of clinical mastitis cases by pathogen and lactation month

	Lactation stage (month)					
	1	2	3	4 & 5	≥6	total
Escherichia coli	13	6	10	11	8	48
Staphylococcus aureus	17	8	0	8	2	35
Coagulase-negative staphylococci	13	1	1	3	4	22
Streptococcus dysgalactiae	12	5	1	4	2	24
Culture-negative	8	3	2	6	2	21
Streptococcus uberis	6	1	2	1	1	11
Other	10	4	6	4	1	25
Not sampled	6	1	3	0	3	13
Total	85	29	25	37	23	199

Pathogens

Model 2 was also a basis to examine whether TEC of cows that had clinical mastitis caused by a specific pathogen differed from the average TEC of cows with clinical mastitis. A pathogen was

studied if that specific pathogen was identified in more than 10 mastitis cases during the study period. The remainder pathogens were grouped as 'other.' Because specific pathogen cases were not orthogonally spread over the lactation months in which clinical mastitis occurred (Table 2), only TEC curves were fitted if more than two cows had clinical mastitis with a specific pathogen in a specific lactation month. *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, coagulase-negative staphylococci, and *Streptococcus uberis* were the major pathogens involved. For each pathogen, a separate model was built. These models consisted of model 2, extended with terms for level, decrease, and increase of the pathogen of interest. The use of level, decrease, and increase allows for a subject-specific slope and intercept (Neijenhuis et al., 2000; Wood, 1976). For example, the *E. coli* model looks as follows:

$$Y = C_0 + C_1 DIM + C_2 \ln(DIM) + ECO + ECODIM + ECO\ln(DIM) + \varepsilon_r \quad [3]$$

where parameters are as in model [2] and *ECO* is a binary variable with value 1 when a cow had *E. coli* mastitis in a month or 0 when a cow had no *E. coli* mastitis in that specific month.

The differences between the effect estimates for TEC as given by the pathogen-specific models (such as model 3) and general mastitis model (model 2) were calculated. These differences were used to evaluate the effect of a specific mastitis pathogen on TEC.

Other Cow Parameters

On top of model [2] teat-end shape, teat length, and peak milk yield were examined for their relationship with TEC.

$$Y = C_0 + \underline{C_1} DIM + \underline{C_2} \ln(DIM) + TES + TL + KG + \varepsilon_r \quad [4]$$

where

Y = average TEC, C_0 = level,

$\underline{C_1} DIM$ = decreasing slope, per DIM,

$\underline{C_2} \ln(DIM)$ = increasing slope, per DIM,

TES = effect of teat-end shape (where 1 = pointed, 2 = round, 3 = flat, and 4 = inverted);

TL = effect of teat length (where 1 < 4.5 cm, 2 = 4.5 to 5.0 cm, 3 = 5.0 to 5.5 cm, and 4 = > 5.5 cm);

KG = effect of peak milk production per day between 10 and 20 wk of lactation (where milk yield ranges from 14 to 63 kg), and

ε_r = residual random error.

RESULTS

Study Population and Descriptive Results

During the 1.5-yr study period, 2157 different animals were observed during the farm visits. Teat-end callosity of 2051 animals was scored; 123 animals were only scored during the dry period or as non-lactating pregnant heifers. From the 1928 lactating animals, 103 had only one TEC score during lactation. On average, TEC was scored 8.6 times per animal during lactation. Average TECT score was 2.18, ranging per farm from 2.07 to 2.33 (Table 1). For lactating cows, the average score was 2.22, ranging from 2.10 to 2.41 per farm. On average, 34% of the udder quarters had rough callous rings, ranging from 22 to 58% among farms. On average, 38% of the udder quarters of lactating cows had rough callous rings, ranging from 24 to 65% among farms.

Table 3. Differences in teat-end callosity thickness (TECT) and roughness (TECR) of clinical mastitis quarters and lateral quarters without clinical mastitis on average (μ), and 4 months before the clinical mastitis occurred to 4 months after the clinical mastitis (X[-4] – X[4] and X[0] = month of clinical mastitis), calculated from the final regression model.

Variable	TECT			TECR		
	Coefficient	SE(β)	*P*-value	Coefficient	SE(β)	*P*-value
μ	0.03188	0.02107	0.065	0.007861	0.01370	0.28
X[-4]	0.05667	0.05133	0.13	0.02719	0.03619	0.23
X[-3]	0.1347	0.04383	0.0011	0.02311	0.03086	0.23
X[-2]	0.008781	0.03917	0.41	-0.02623	0.02757	0.17
X[-1]	0.01991	0.03556	0.29	0.02869	0.02501	0.13
X[0]	0.07543	0.03108	0.0077	0.007737	0.02187	0.36
X[1]	0.05976	0.03163	0.030	-0.02640	0.02230	0.12
X[2]	0.05826	0.03256	0.037	0.004219	0.02301	0.43
X[3]	0.04913	0.03405	0.075	-0.02522	0.02411	0.15
X[4]	0.02560	0.03666	0.24	0.02493	0.02601	0.17

Within-Cow Analysis

In the within-cow analysis, 491 cows and 505 udder halves were included. Teat-end callosity thickness of the clinical mastitis quarters was on average 0.03 ($P = 0.07$) higher than lateral quarters without clinical mastitis (Table 3). Clinical mastitis quarters had 0.8% ($P = 0.28$) more rough callosity rings than healthy quarters. Before and after the clinical mastitis occurred, TECT was higher on the clinical mastitis quarters than in lateral quarters without clinical mastitis. Three months before the clinical mastitis occurred, TECT was 0.13 higher ($P < 0.05$). In the month clinical mastitis occurred, TECT was 0.08 higher ($P = 0.008$), and 1 months after the clinical mastitis, TECT was 0.06 higher ($P = 0.03$). Two months after clinical mastitis, the TECT was 0.06 higher ($P = 0.04$). Clinical mastitis quarters and lateral quarters without clinical mastitis did not differ significantly in TECR in any of the months during the period of up to 4 months before to 4 months after clinical mastitis occurred.

Between-Cow Analysis

Between farms, the incidence of clinical mastitis ranged from 11 to 57% per 100 cows per year (Table 1). During the study period, 827 quarter cases of clinical mastitis were diagnosed. These clinical mastitis cases involved 674 different quarters of 509 different animals. From these clinical mastitis cases, 42 animals had no or just one record on TEC. From the cows with clinical mastitis, 467 were eligible for inclusion into the analyses with their first clinical mastitis case. All of them had TEC scores during lactation. On average, 10.8 records on TEC during lactation were recorded for the animals that had clinical mastitis. The 2570 records of TEC from 199 couples of paired animals were brought into the analysis.

Days in milk, parity, and whether and when clinical mastitis occurred were associated with the course of TECT and TECR. Teat-end callosity increased after parturition until approximately 120 DIM and decreased thereafter. Clinical mastitis cows had thicker, and more frequently rough, callous rings on their teat-ends than cows that did not have clinical mastitis, both before and after the clinical mastitis occurred, at least if the clinical mastitis occurred after the first and before the sixth month of lactation (Figures 1 and 2). On the other hand, cows with clinical mastitis in the first month of lactation showed less TECT and TECR during lactation than other cows.

First-parity cows had the least TECT followed by the higher-parity cows (parity ≥ 5). Cows with parities 3 and 4 had the highest TECT followed by parity 2 cows (Figure 3). First-parity cows had the least TECR followed by the second-parity cows. Cows with parities 3 and 4 had the highest TECR followed by higher-parity cows (parity ≥ 5) (Figure 4). Within-parity groups, clinical mastitis cows had more TEC than nonmastitis cows.

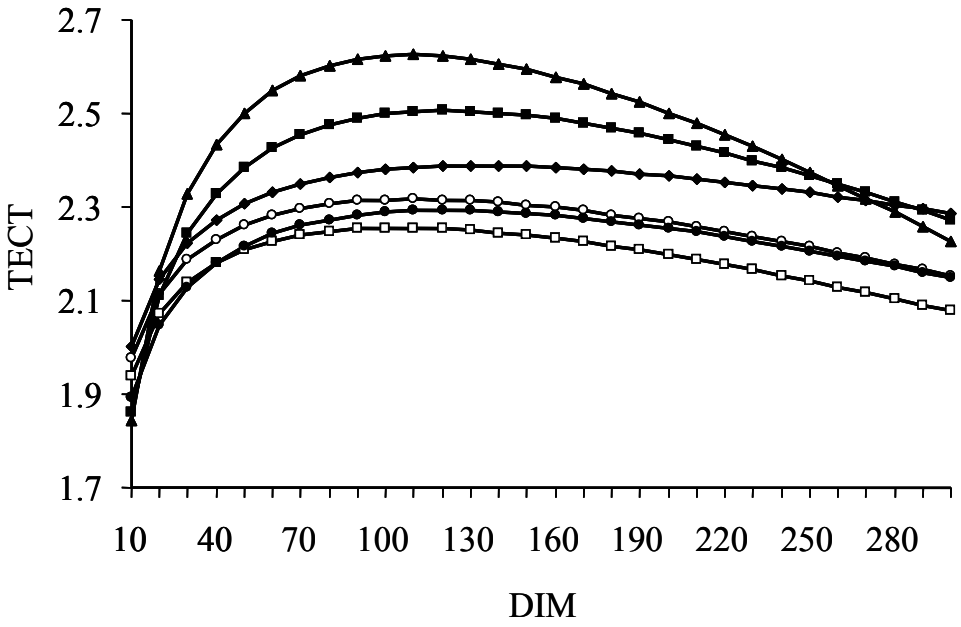

Figure 1. Teat-end callosity thickness (TECT) during lactation for second-parity cows without clinical mastitis (—O—), or with clinical mastitis in the first (—□—), second (—▲—), third (—■—), fourth and fifth (—◆—), and ≥ 6(—●—) months of lactation, calculated from the final regression model.

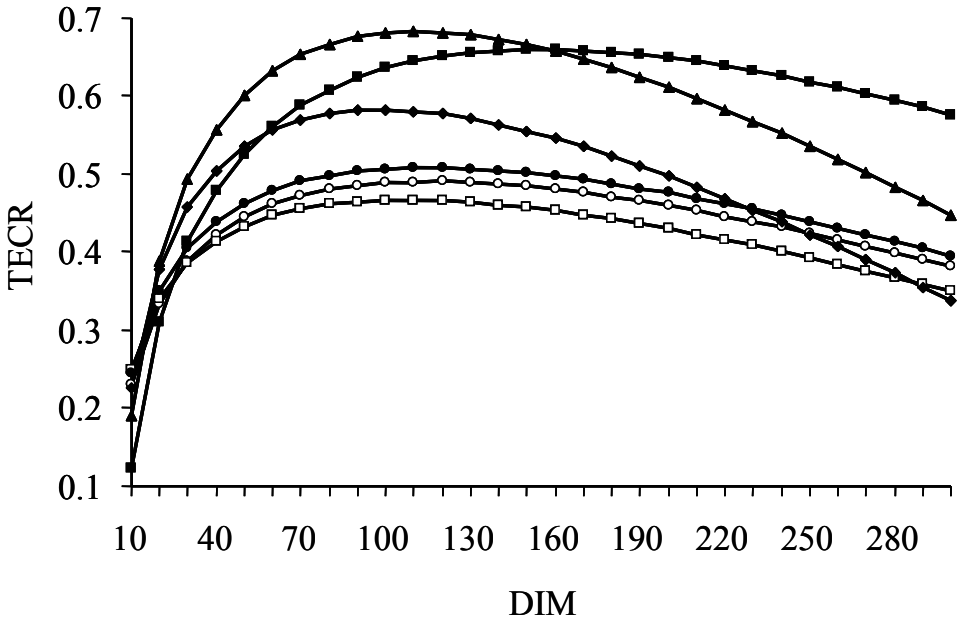

Figure 2. Teat-end callosity roughness (TECR) during lactation for second-parity cows without clinical mastitis (—○—), or with clinical mastitis in the first (—□—), second (—▲—), third (—■—), fourth and fifth (—◆—), and ≥ 6 (—●—) months of lactation, calculated from the final regression model.

Pathogens

The pathogens most frequently isolated from the 827 quarter cases of clinical mastitis were *Staph. aureus* (24%), *E. coli* (21%), *Strep. dysgalactiae* (11%), coagulase-negative staphylococci (11%), and *Strep. uberis*(7%). From 11% of the milk samples no pathogen was isolated.

From the 199 clinical mastitis cases selected for the analysis, 186 where examined bacteriologically. Pathogens most frequently isolated in this subset were *E. coli* (26%), *Staph. aureus* (19%), *Strep. dysgalactiae* (13%), coagulase-negative staphylococci (12%), and *Strep. uberis* (6%). From 11% of the milk samples, no pathogen was isolated.

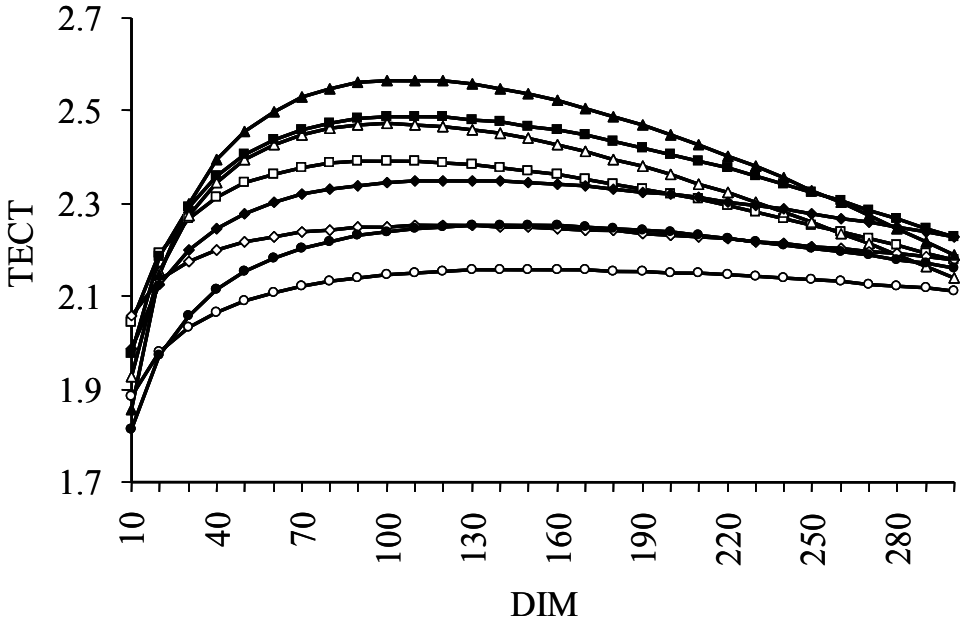

Figure 3. Teat-end callosity thickness (TECT) during lactation for cows with (solid) and without clinical mastitis (open) per parity (● = parity 1, ■ = parity 2, ▲ = parity 3 and 4, and ◆ = parity ≥ 5), calculated from the final regression model.

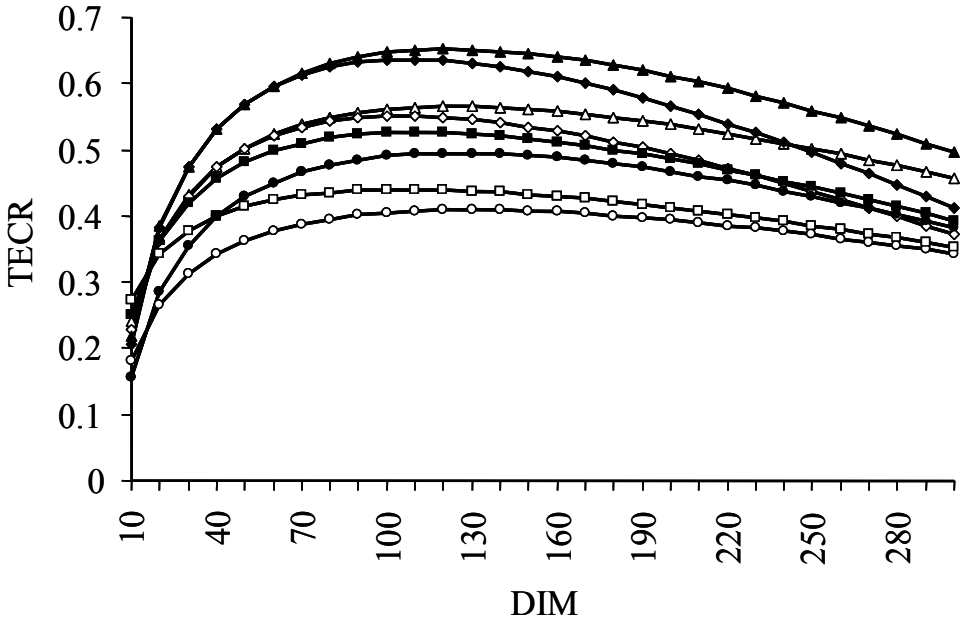

Figure 4. Teat-end callosity roughness (TECR) during lactation for cows with (solid) and without clinical mastitis (open) per parity (● = parity 1, ■ = parity 2, ▲ = parity 3 and 4, and ◆ = parity ≥ 5), calculated from the final regression model.

Compared with cases of clinical mastitis in the same month of lactation, some pathogens were associated with a small difference in TECT or TECR. Clinical *E. coli* mastitis in the second- (level $P = 0.023$) or third- lactation month (level $P = 0.014$ and increase $P < 0.001$) occurred in cows with less TECT during the complete lactation. Cows with clinical mastitis caused by other pathogens in the second (increase $P = 0.0073$) or third lactation month (increase $P = 0.001$) had more TECT, the TECR was also more severe in cows with clinical mastitis in the third months of lactation (level $P = 0.014$). The clinical mastitis cases that were culture negative had more TECT if clinical mastitis occurred in the second-lactation month (level $P = 0.032$), fourth- or fifth- lactation month (decrease $P = 0.0065$) as did the cases after the fifth month (decrease $P = 0.012$), the TECR was also more severe in cows with clinical mastitis in the fourth-or fifth-lactation month (decrease $P = 0.018$).

Teat-End Shape and Length and Milk Production

Cows with clinical mastitis more frequently had pointed teat-ends than nonmastitis cows (6 versus 2%; $P = 0.019$). In both groups, most of the teat-ends were round (58%). No difference in teat length between the clinical mastitis and the nonmastitis cows was found ($P = 0.23$); teat length was on average 4.8 cm. Milk yield at top lactation was on average 33.5 kg/d and did not differ between the clinical mastitis and the nonmastitis cows ($P = 0.28$).

Table 4. Association between teat-end shape and maximum milk yield per day between 10 to 20 weeks in lactation, and teat-end callosity thickness (TECT), and teat-end callosity roughness (TECR) on top of the complete model (199 couples of matched cows).

		TECT		TECR	
		coefficient	*P*-value	coefficient	*P*-value
Teat-end shape:					
	Round vs. pointed	-0.1120	0.16	-0.1197	0.048
	Flat vs. pointed	-0.2433	0.018	-0.2941	<0.001
	Inverted vs. pointed	-0.4069	<0.001	-0.4513	<0.001
	Flat vs. round	-0.1313	0.0050	-0.1744	<0.001
	Inverted vs. round	-0.2949	0.0089	-0.3316	<0.001
	Inverted vs. flat	-0.1636	0.099	-0.1572	0.020
Peak milk yield (per 10 kg)		0.1043	<0.001		

[1]Empty cells indicate that factor is not significant and left out of the model.

When adding the cow factors, teat length, teat-end shape, and peak yield to model [2], teat length did not account for any variance and was left out of the models for TECT and TECR. Peak yield did also not account for any variance in the model for TECR and was left out. The addition of teat-end

shape and peak yield resulted in almost the same estimations for the coefficients of the other variables in the basic model [2]. Pointed teat-ends had higher TECT and TECR than flat or inverted teat-ends (Table 4). Round teats had more TECT and TECR than inverted teat-ends. Flat teats had more TECR than inverted teat-ends. Pointed teats had more TECR than round teat-ends. With increasing peak yield, TECT increased. For each 10-kg increase in milk yield, the TECT was 0.1 higher ($P < 0.001$; Table 4).

DISCUSSION

Forty percent of the quarters had clinical mastitis during the 1.5-yr study period; on average, 28% quarter cases per 100 cows were present per year. This falls within the range of other research (Barkema et al., 1998; Miltenburg et al., 1996; Schukken, 1989; Chassagne et al., 1997; Sargeant et al., 1998).

In contrast to previously published work (Sieber and Farnsworth, 1981; Thompson and Sieber, 1980), this study has shown a small but consistent and significant relationship between TEC and clinical mastitis within mastitis cows, and between clinical mastitis cows and paired herd mates without clinical mastitis. Before, and particularly after, the clinical mastitis occurred, TECT was higher in the clinical mastitis quarters than in lateral quarters without clinical mastitis of the same cow. It is assumed that clinical mastitis causes milk flow changes in the affected quarter. These changes may lead to more stress on the teat by milking, and may result in more TECT after the clinical mastitis had occurred. Higher levels of TECT before clinical mastitis indicate a relationship between TECT and risk of clinical mastitis. The difference of TECT 3 months before the clinical mastitis occurred, between the clinical mastitis quarters compared with the healthy lateral quarter, was 0.13-unit scores. On a scale of 5, this is not a large difference and the biological importance of this finding may be questioned. However, the difference of TECT between farms is 0.26-unit scores, which shows that the difference between mastitis and nonmastitis udder quarters is relatively high. Moreover, this difference in TECT between farms may be part of an explanation for the large differences in mastitis incidence between farms. However, this should be confirmed by further research. The small difference in TECT between quarters with and without mastitis can therefore be considered as biologically important. TECR did not differ significantly between the clinical mastitis quarters compared with the lateral quarters without clinical mastitis of the same cow. This may be

evidence that the development of TECT and TECR are not the same process and should be used as complementary parameters (Neijenhuis et al., 2000).

Differences were found in TEC between clinical mastitis cases and paired healthy cows. Part of the observed differences in TEC between cows could be explained by differences in stage of lactation, milk yield, parity, and teat-end shape. This is in accordance with other research (Graf, 1982; Johansson, 1957; Michel et al., 1974; Neijenhuis et al., 2000; Rathore, 1977; Sieber, 1980; Sieber and Farnsworth, 1981).

Cows that presented with clinical mastitis in the second to the fifth month of lactation showed more TEC than the paired healthy cows. Teat-end callosity develops in the first month up to fourth month in lactation and decreases thereafter. The association with clinical mastitis and TEC was found within this period, suggesting a biological relationship between development of TEC and clinical mastitis.

Cows with clinical mastitis cases in the first month of lactation showed no difference in TEC compared with paired cows without clinical mastitis. The distribution of cases of clinical mastitis during lactation is skewed, with most cases in the beginning of lactation (43%), which is similar to other research (Miltenburg et al., 1996; Rajala-Schultz et al., 1999). The cause of these cases may be the depressed immune system at parturition and smooth muscle contraction, which is vital to closure of the teat sphincter after milking, and is suspected to be impaired by hypocalcaemia (Goff and Horst, 1997).

Specific pathogens may differ in opportune use of TEC to multiply or to enter the teat canal. The only groups of pathogens of clinical mastitis cases occurring in the second or third months of lactation that had an association with increased TECT were the group ‘other’. Because of the small incidence, the group ‘other’ contained 10 different pathogens. Within this group clinical mastitis cases with yeast (two cows), *Klebsiella pneumoniae* (one cow), and *Enterobacter aerogenes* (two cows) had higher TEC than other cows. Barkema et al. (1999) reported an association between milking machine factors and clinical *E. coli* mastitis. Some of these milking machine factors are associated with increasing TEC. In contrast, in this research, an association of clinical *E. coli* mastitis with less TECT was found. Teat-end shape was mostly round, similar to that found in other research (Chrystal et al., 1999; Bakken, 1981; Johansson, 1957). The finding that round or rather pointed teat-ends were more prone to have higher TECT and TECR than flat or inverted teat-ends

was in agreement with other research (Bakken, 1981; Johansson, 1957; Neijenhuis et al., 2000; Rathore, 1977). Cows with clinical mastitis frequently had more pointed teat-ends than their paired healthy herd mates. Natzke et al. (1978) found that cows with pointed teat-ends had the highest rate of new infections. In contrast, other studies (Hodgson and Murdock, 1980; Seykora and McDaniel, 1985) reported that as teat-end shape varied from pointed toward flat and inverted, SCC increased. However, a more recent study (Chrystal et al., 1999) showed no relationship between teat-end shape and SCC. Adding teat-end shape to the model in this study did not alter the relationship between clinical mastitis and TEC.

The average teat length found was 4.8 cm, within the range of other research (Hamann, 1987; Bakken, 1981). Teat length did not account for differences in TEC, similar as found by Johansson (1957). With increasing peak yield, TECT increased, similar to that found in other research (Bakken, 1981; Sieber, 1980). Higher milk yield will be accompanied by longer machine-on time, which results in more TEC (Neijenhuis et al, 2000). When milkflow rate was adjusted for milk yield, Seykora and McDaniel (1985) found a negative association with TEC. Dohoo and Martin (1984) stated that the level of milk production was not significantly related to the risk of mastitis. Woolford (1985) found that there is milk loss around the occurrence of clinical mastitis but compensation takes place in older cows. Therefore, the influence of peak milk yield can be underestimated within first-parity cows. Adding peak yield to the model in this study did not alter the relationship between clinical mastitis and TEC.

CONCLUSIONS

The level of TECT was higher in the clinical mastitis quarter than in the lateral healthy quarter within a cow, before and particularly during and after occurrence of clinical mastitis. Quarters with or without clinical mastitis did not differ significantly in roughness of the callosity ring.

Teat-end callosity of cows with clinical mastitis in the second to fourth months of lactation was higher before and after the occurrence of clinical mastitis occurred compared with paired healthy herd mates. However, cows with clinical mastitis in the first or after the fifth months of lactation, had less TECT and TECR. Clinical mastitis cases which were culture-negative or caused by less frequently found pathogens like yeast, *Kl. pneumoniae* and *E. aerogenes* were associated with higher TEC. Clinical *E. coli* mastitis was associated with less TECT.

Teat-end shape was associated with the degree of TECT and TECR. With increasing peak yield, TECT increased. Teat length was not associated with TEC. From this study, it is clear that clinical mastitis does have a relationship with TEC. Further research should focus on TEC as risk condition for mastitis. If TEC is a risk factor for clinical mastitis, it could be used as a predictor for clinical mastitis in milking machine research.

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CHAPTER 4

RECOVERY OF COW TEATS AFTER MILKING AS DETERMINED BY ULTRASONOGRAPHIC SCANNING

F. Neijenhuis, G.H. Klungel, and H. Hogeveen

Research Institute for Animal Husbandry, P.O. Box 2176, 8203 AD Lelystad, The Netherlands

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ABSTRACT

Recovery time of teat tissue after milking was determined by ultrasonographic scanning. Teat-canal length, teat-end width, teat-wall thickness, and teat- cistern width of 18 cows varying in parity and lactation stage were measured in duplicate before and directly after milking and every hour for 8 h. The ratio between teat-wall thickness and teat-cistern width was calculated. The effects of time after milking and teat position on the teat parameters were estimated using REML models. The mean differences of ultrasound measurements of teat-end width and teat-canal length between duplicate measurements were 3.6 and 8.2%, and between days 4.4 and 7.8%, respectively. Teat-wall thickness and teat-cistern width were more variable (15.3 and 22% between duplicates, and 13.6 and 19.9% between days, respectively).

Teat recovery after milking took a considerable amount of time: teat-wall thickness, 6 h; teat-end width, >8 h; teat-canal length, >8 h; teat-cistern width for rear teats, 3 h, and front teat 8 h, and the ratio of teat-wall thickness and teat-cistern width, 6 h.

Ultrasonographic scanning of teat parameters was a useful tool to study teat changes caused by milking. Teat recovery took more time than expected, which makes caution necessary when increasing the milking frequency.

(**Key words:** ultrasonographic scanning, teat condition)

INTRODUCTION

The teat is an important part of the cow's defence mechanisms against IMI (O'Shea, 1987). Therefore, the teat is also referred to as first-line defence against IMI. Functioning of the teats as barrier against IMI can be negatively influenced through husbandry circumstances. It is obvious that injury and disease of the bovine teat should be prevented as much as possible. Moreover, when teats are exposed to teat-cup liner conditions, some congestion and edema of the teat-end occurs at the end of peak milk flow even when using universally accepted milking machine settings (Bramley et al., 1992). Milking vacuum generates strain on the teat wall, which induces dilation of blood vessels and expandable compartments in the perivascular tissue (Bramley et al., 1992). Changes in the pliability of teat tissue, caused by congestion or edema may change the resistance of the teat canal to bacterial invasion (O'Shea, 1987).

When teat thickness increases more than 5% (caliper measurement), teat duct microbial colonization is greater (Zecconi et al., 1992). With different settings of the milking machine, such as vacuum level, level of cluster removal, and pulsation rate, teat thickness varies (Hamann and Mein, 1990, 1996; Hamann et al., 1993; Rasmussen, 1993; Zecconi et al., 1992). Besides the teat tissue reactions directly measurable after machine milking, recovery time of the teats is also an important issue. Closure time of the teat canal is important because of the increased penetrability of teats after milking. Penetrability of teats and the teat- canal diameter are recovered within 2 h after milking (McDonald, 1975; Schultze and Bright, 1983). Also the teat-end thickness, as measured by the cutimeter, was the lowest 2 h after milking (Hamann, 1987). The recovery time of the teat is important to determine a minimum milking interval. When a cow is milked when the teat tissue is not yet recovered, irreversible chronic changes of the teat tissue may occur (Hamann and Østeras, 1994).

Various methods have been described to measure teat tissue changes. Radiography techniques have been used for many years (Pier et al., 1956; McDonald, 1975; Mein et al., 1973) and have provided good results. To use radiography, a contrast medium needs to be infused into the cow's udder, making this a complicated and nonphysiologic method. Impedance measurement has been used to measure teat-end congestion indirectly by measuring the flow of blood through teat tissue (Mayntz and Almgren, 1985). The method is also fairly complex. Another indirect method to monitor teat tissue reaction via blood flow is the use of infrared to measure the temperature of teat tissue. Skin

temperature is used as a parameter that relates to the intensity of the circulation of blood (Hamann and Duck, 1984). The laser Doppler flowmetry method is also an indirect method for measuring the congestion of teat tissue by continuous evaluation of blood flow (Hamann et al., 1994b). Hamann et al. (1994b) stated that a decreased blood flow period after milking can be associated with an increase in teat thickness.

A spring-loaded caliper device, the cutimeter, is described as a simple, noninvasive tool for the measurement of teat thickness (Hamann and Mein, 1988). The cutimeter should be used with care, since results are dependent on the time and application of pressure on the teat. Moreover, since pressure is applied to the teats, this method may not be representative of recovery results of teats after milking.

Ultrasound has been used to study the mammary gland as a whole (Caruolo and Mochrie, 1967), to diagnose abnormalities (Cartee et al., 1986; Jenniger, 1989), to picture gland and mammary cisterns (Bruckmaier and Blum, 1992) and to study the relation between machine milking and teat-wall thickness (Spencer, 1995; Worstorff et al., 1986). Because of its noninvasive nature, ultrasound seems to be a good, direct, method to monitor the recovery of teats after milking. Studies on the recovery of teats after milking published to date have not used ultrasound. Moreover, studies using ultrasound only measured once directly after milking. The goals of this study were to evaluate both the use of ultrasound to measure teat tissue changes in relation to machine milking and the recovery time of teat tissue after milking.

MATERIALS AND METHODS

Cows

Eighteen Holstein cows from one herd were selected for the study. To be selected, cows had to be free of clinical mastitis for the last 4 wk before the start of the experiment and needed to have an udder without abnormalities such as nonlactating quarters or teat injuries. Moreover, because cows needed to stand up frequently during the experiment, selected cows needed to be free of leg problems. Average DIM was 170 (ranging from 21 to 306). Of the selected cows, five were in first, four in third, four in fourth, and five in fifth or higher lactation. Teat length was on average 5.1 cm for front teats (ranging form 3.8 to 7.8 cm) and 4.4 cm for rear teats (ranging from 3.2 to 6.2 cm).

Teat-end shape was round (74%), pointed or flat (11 and 13%) and, sometimes disk-shaped (3%). The cows were housed in a free-stall barn and milked in a 2 X 5 side-open milking parlour. A milking machine (Gascoigne Melotte, Emmeloord, The Netherlands) with 43-kPa milking vacuum was used with a Gascoigne Melotte cluster (liner type D381988; mouthpiece bore 24 mm), operating at a pulsation rate of 55 pulsations/min, and the pulsator ratio was 65:35. Milk yield and machine on time were recorded with a GM 2000 milk meter (Gascoigne Melotte, Emmeloord, The Netherlands), which sends a signal per 100 g. Take-off took place when milk flow, according to the milk meter, was less then 0.2 kg/min. Average milk flow was calculated by dividing milk yield by machine on time.

Premilking treatment consisted of cleaning with a dry paper towel followed by stripping. The total preparation time until unit application lasted 15 s. The milking intervals were 14 h before the morning milking and 10 h before the evening milking. Average milk yield of the cows during the experiment was 10.8 kg (ranging from 2.7 to 20.4 kg) during morning milkings and 11.7 kg (ranging from 5.5 to 15.4 kg) during afternoon milkings. Average machine-on time during the morning milking (when teat scans were performed) was 6.8 min (ranging from 4.2 to 12 min). Average milk flow rate during the morning milking was 1.9 kg/min (ranging from 0.9 to 3.1 kg/min).

Ultrasonographic Scanning

The ultrasonographic scans were carried out with a 200 VET scanner with a linear array 7.5 MHz probe (Pie Medical, Maastricht, The Netherlands). During scanning, direct contact between the head of the probe and the teat must be prevented because that will deform the teat image. Therefore, during ultrasound scanning, the teat was immersed in water (35°C) in a latex bag. The probe with sufficient contact jelly was held against the bag lateral to the teat. Teats are not completely circular, therefore the position of the probe on the teat during the experimental period was held equal.

Measurements

Because of the intensive nature of the measurements, only five cows per day could be scanned. Therefore, teat scans of the 18 cows were made during four different days (d 1, 2, 8, and 9). Of two cows, teat scans were made for 2 days (1 and 7 days apart). In the milking parlour during the morning milking, teat scans of the left front and the right rear teat were made directly after routine

premilking treatment (t_{-1}) and directly after removal of the milking cluster (t_0). Then the cows were moved to a tie-stall barn and repeated scans of the same teats were taken at 1, 2, 3, 4, 5, 6, 7, and 8 h after milking (t_1 to t_8).

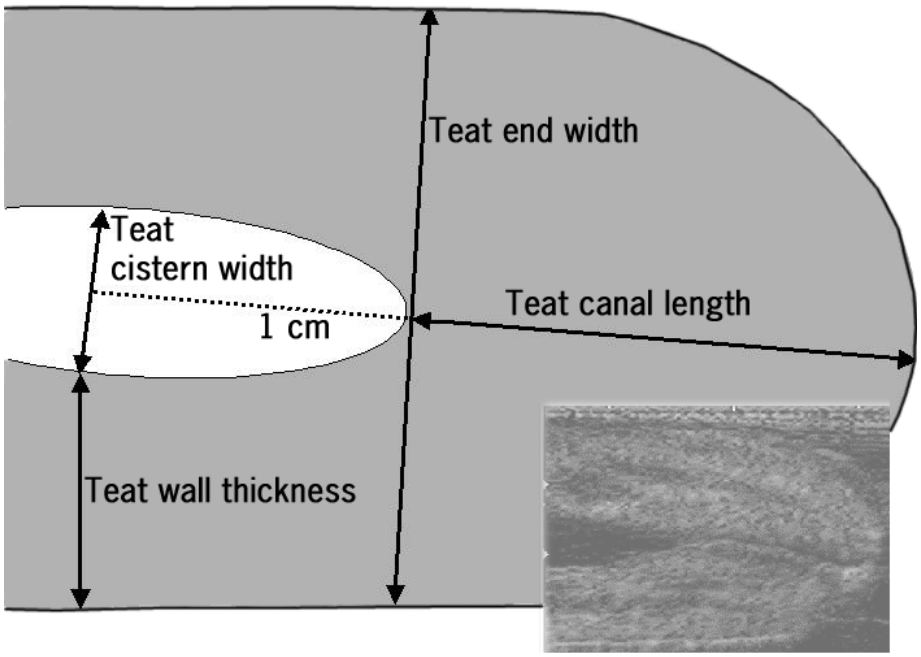

Figure 1. Measurements on ultrasonographic scan: teat-canal length, teat-end width at the top of the teat canal, teat-wall thickness at 1 cm above the end of the teat canal and teat-cistern width at 1 cm above the end of the teat canal.

The real-time scanning was shown on the terminal of the scanning device. When the picture of the teat had a satisfactory sharpness, it was stored in the memory of the scanning device. When the pictures of both front and rear teat were stored, they were transmitted to a connected personal computer. Thereafter, the same teats were scanned again (duplicate). Image transfer was carried out using the OdtCom communication software (version 0.9, Pie Medical, Maastricht, The Netherlands). The same person made all scans. Measurements of four different properties of the teat were taken by one person, using the Eview software (version 0.9, Pie Medical, Maastricht, the Netherlands): teat-canal length, teat-end width at the top of the teat canal, teat-wall thickness at 1 cm above the end of the teat canal, and teat-cistern width at 1 cm above the end of the teat canal (Figure 1). Based on the measurements, the ratio between teat-wall thickness and teat- cistern width was calculated as fifth parameter.

Statistical Analyses

Duplicate scans of the same teats (at t_{-1}, t_0, t_1 to t_8) were used to analyze the repeatability of teat scanning for the various parameters. All teat scans where both measurements were available were used to calculate the mean value and the standard error of the duplicates. Teat scans of the same cows approximately on the same time but at two different days (1 and 7 days apart) were used to analyze the repeatability of teat scanning from measurements over days.

To analyze differences in time compared with the measurement just before milking, we built a model. Teat parameter measurements taken just before milking, just after milking, and every consecutive hour after milking (Tm) were put into a statistical model as response variables. The measurements of second duplicate day of the two cows were left out of the data for this analysis. Because of the structure of the experiment, date, cow, and duplicates were put in the random structure of the model. The time of measurement and teat position were put into the model as predictive variable. Random regression models using REML (Lawes Agricultural Trust, 1996; Genstat 5 Committee, 1993) were used. The model fitted was:

$$Y_{ijklm} = \mu + ((Date_i + Cow) * TP) * (Time/Duplo))_{ijklm} + Time_l + TP_k + (Time * TP)_{lk} + e_{ijklm}$$

where:

Y_{ijklm}= teat-tissue parameter (teat-wall thickness, teat-cistern width, teat-canal length, teat-end width and the teat-wall thickness divided by the teat-cistern width),

μ = overall mean,

$((Date + Cow) * TP) * (Time/Duplo))_{ijklm}$ = random effect representing nested teat k (k = rear or front teats) within cows j (j = 1 . . . 18) crossed by duplicate measurement m (m = 1 or 2) nested within time l (l = –1, 0, 1 . . ., 8), within date i (i = 1 . . . 4),

$Time_l$ = time of measurement; just before milking (l = –1), just after milking (l = 0) and each consecutive hour after milking (l = 1, 2, . . ., 8),

TP_k = teat position; k = 1 for rear teats, and k = 2 for front teats, and

e_{ijklm} = residual random error

For the ratio between the teat-wall thickness and teat-cistern width, an inverse natural logarithm transformation was used.

RESULTS

Of the theoretically possible 800 measurements (on 4 days, five cows per day, 10 time points, two teats, and duplicate scans per teat), 700 measurements were actually done. Ninety-two measurements were missing because on day 2 measurements were made until 4 h after milking (two cows) and 3 h after milking (three cows) instead of 8 h. Moreover, eight duplicate measurements were missing. On six scans, teat-wall thickness and teat-wall cistern width could not be measured.

Table 1. Differences in duplicate measurements of teat parameters (mm) of 18 cows.

	#	Mean	Mean duplicate1	Mean duplicate 2	Mean difference	SED	%
Teat-end width	416	21.59	21.54	21.64	0.84	0.78	3.6
Teat cistern width	412	9.27	9.22	9.31	2.18	2.04	22.0
Teat canal length	416	11.09	11.05	11.14	0.88	0.91	8.2
Teat wall thickness	412	7.67	7.66	7.68	1.12	1.17	15.3

Repeatability of Method

The mean difference of duplicate ultrasound measurements of the various teat parameters varied from 3.6% for teat-end width (416 duplicates) to 22.0% for teat-cistern width (412 duplicates; Table 1). These values were 4.4 and 19.9%, respectively, for teat-end width and teat-cistern width between days (22 duplicates; Table 2). Differences between days were comparable with differences between duplicate measurements.

Table 2. Differences in measurements of teat parameters (mm) of the same two cows on two different days.

	#	Mean	Mean duo 1	Mean duo 2	Mean difference	SED	%
Teat-end width	22	19.15	18.87	19.43	1.02	0.84	4.4
Teat cistern width	22	7.08	6.93	7.23	1.80	1.41	19.9
Teat canal length	22	9.74	9.68	9.79	0.95	0.76	7.8
Teat wall thickness	22	7.39	7.40	7.38	1.06	1.01	13.6

Recovery of Cow Teats After Milking

To analyze the recovery of teat parameters, 640 of the theoretically possible 720 measurements (18 cows on 4 d, 10 time points, two teats and duplicate scans per teat) were actually done. Eighty measurements were missing because of missing time points on day 2 (72 missing), and missing duplicates (8 missing). Moreover, because of unclear scans on which teat-wall thickness and teat-wall cistern width could not be measured, six measurements were missing.

Recovery of teat-end width, teat-cistern width, teat- canal length, teat-wall thickness, and the ratio between teat-wall thickness and teat-cistern width according to the fitted models is given in Table 3. Date did account for negligible variance and was therefore left out of the model. For teat-end width, teat-cistern width and teat- canal length, cow was the random factor accounting for the largest proportion of variance (70, 50, and 60%, respectively). For teat-wall thickness and the ratio between teat-wall thickness and teat-cistern width, the cow within date random factor explained a smaller part of the variation (38 and 37%, respectively), the series of duplicate measurements did account for 40 and 34% of the variation.

All teat parameters were significantly associated with time of measurement after milking, and teat position had only a significant effect on teat-cistern width. The changes of the teat-end width, teat-wall thickness, and teat-canal length, relative to the situation before milking, are graphically presented in Figure 2. The length of the teat canal was on average 10 mm before milking (Table 4) and it increased to 11.2 mm directly after milking. Two hours after milking length of the teat canal was at its maximum (11.8 mm). Teat-canal length decreased thereafter, but 8 h after milking, it was still significantly longer than before milking (11.0 mm). Changes in teat-end width were relatively small compared with the other teat parameters. The average teat-end width increased from 21.2 mm before milking to 21.7 mm after milking. The maximum teat-end width was 22.0 mm at 6 h after milking and did not reach the value before milking within the 8 h after milking trial period. Teat-wall thickness increased from 6.8 mm before milking to 9.1 mm just after milking. It declined rapidly, and there was no significant difference any more 6 h after milking.

Table 3. Variance components, standard error, and P-values of factors affecting teat parameters according to the complete model.

	Teat-end width		Teat cistern width		Teat canal length		Teat wall thickness		Teat wall / teat cistern	
Random effects	Comp.	SE	Comp.	SE	Comp.	SE	Comp.	SE	Comp.	SE
Cow	3.84	1.47	7.67	2.90	2.46	0.89	1.24	0.50	0.14	0.05
Cow(teat)	0.86	0.31	0.91	0.44	0.51	0.18	0.27	0.13	0.01	0.009
Cow(time)	0.03	0.05	1.03	0.43	0.23	0.07	0.17	0.11	0.02	0.02
Time(duplo)	0.003	0.01	0.21	0.16	0	0.02	0.01	0.02	0.004	0.004
Cow(teat(time))	0.14	0.07	1.19	0.47	0.05	0.07	0.28	0.14	0.07	0.02
Cow(time(duplo))	0	0.05	0.15	0.35	0	0.07	0.02	0.11	0.008	0.01
Cow(teat(time (duplo)))	0.65	0.07	4.11	0.47	0.83	0.09	1.30	0.15	0.13	0.02
Fixed effects	Comp.	*P*-value	Comp.	*P*-value	Comp.	*P*-value	Comp.	*P*-value	Comp.	*P*-value
Intercept	21.7		9.4		11.3		7.7		-0.16	
Time[1]		***		***		***		***		***
Teat										
Rear	21.8	[2]	9.9	***	11.1		7.6		-0.22	
Front	21.6		8.8		11.4		7.7		-0.10	
Teat * Time				***[1]						

[1]See Table 4 for components
[2]Empty cells indicate that factors are not significant
*P≤0.05, **P≤0.01, ***P≤0.001.

The ratio of teat-wall thickness to the teat-cistern width was 0.6 before milking and increased to 1.2 directly after milking (Table 4 and Figure 3). The ratio recovered within 6 h after milking. Width of the teat cistern was larger in front teats than rear teats before milking and decreased more than 50% during milking (Table 4 and Figure 4). There was no significant difference in the cistern width of rear teats 3 h after milking and of front teats 8 h after milking with the value before milking.

Table 4. Teat parameters (mm) just after milking (t_0), and 1 to 8 h after milking ($t_{1..8}$), compared to the value just before attachment of the milking cluster (t_{-1}) according to the model.

	t_{-1}	t_0	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
Teat-end width	21.2	21.7[1]	21.7[1]	21.8[1]	21.7[1]	21.8[1]	21.8[1]	22.0[1]	21.8[1]	21.7[1]
Teat cistern width, rear teat	11.1	5.6[1]	5.9[1]	9.1[1]	10.4[1]	11.3[1]	10.2[1]	11.4[1]	11.6	12.5
Teat cistern width, front teat	11.8	5.4[1]	7.1[1]	8.0[1]	8.8[1]	9.4	8.8[1]	8.9[1]	9.8[1]	10.5
Teat canal length	10.0	11.2[1]	11.6[1]	11.8[1]	11.6[1]	11.4[1]	11.2[1]	11.3[1]	11.3[1]	11.0[1]
Teat wall thickness	6.8	9.1[1]	8.7[1]	8.1[1]	7.7[1]	7.2	7.8[1]	7.4	7.3	6.9
Ratio wall/cistern	0.6	1.2[1]	1.3[1]	1.0[1]	0.8[1]	0.7	0.8[1]	0.8[1]	0.7	0.6

[1]Difference is significant (P<0.05) compared to before milking (t_{-1}).

DISCUSSION

The repeatability of ultrasound scanning of teat-end width and teat-canal length was good. This could be based on the fact that differences between duplicate measurements as well as differences between days were relatively small (3.6 and 8.2% between duplicates and 4.4 and 7.8% between days, respectively). Differences between days were based on only two cows and 22 duplicates, so this should be looked on as an indication. Teat- wall thickness and teat-cistern width were more variable (15.3 and 22% between duplicates and 13.6 and 19.9% between days, respectively). Moreover, experiences during the experiment showed that the scanning equipment was relatively easy to use. Together with the non-invasive nature, this makes the ultrasound scanning technique a good tool to study the teat. It can be used, for instance, to evaluate the effects of different milking techniques, liners, and milking machine set- tings on the reaction of the teat (Spencer, 1995).

In most literature, recovery time of teats is interpreted as the time that it takes for the teats to decrease the penetrability of the teat canal to endotoxin or teat- canal diameter after milking (Hamann and Burvenich, 1994; Schultze and Bright, 1983; McDonald, 1975). Teat-canal penetrability is an important parameter for management aspects. It is advised to keep cows standing for 1 to 2 h after they have been milked. When teats have an increased penetrability, they should not be in contact with possible infection sources, such as bedding material of cubicles. The teat penetrability is dependent on the biochemical composition of the teat tissue, the opening of the sphincter, and the changes in teat tissue. Biochemical composition and opening of the teat sphincter are very difficult to measure. Either intensive analysis or invasive methods are required. As with other studies in this field, teat-canal penetrability by ultrasound measurements cannot be measured directly.

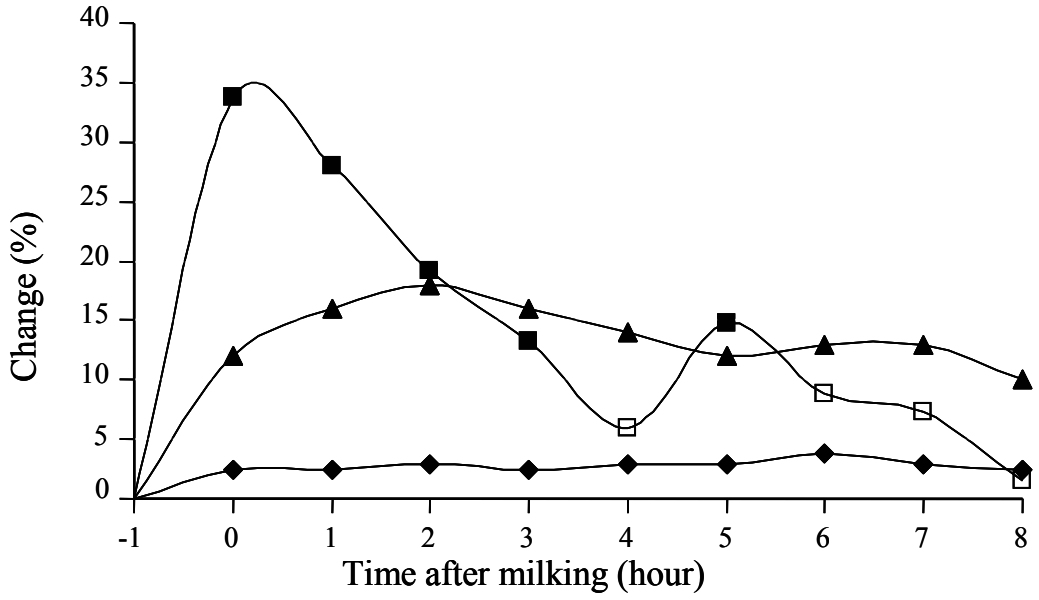

Figure 2. Relative changes (%) of teat-end width (—◆—), teat-canal length (—▲—), and teat-wall thickness (—■—) measured just after milking (0), and at each consecutive hour after milking (1 to 8) compared to the measurement before milking (-1) (filled symbols different from measurement before milking).

However, it is assumed that teat tissue changes reflect teat-canal penetrability. Therefore, parameters that estimate the changes in teat tissue are the best available parameters to estimate teat-canal penetrability under practical circumstances. However, good studies to validate this assumption are not available. Hamann et al. (1994a) compared three different studies and reported a similar pattern after milking of changes in penetrability (inflammatory response to implantation of endotoxin), teat-canal diameter and teat thickness (cutimeter measurements) after milking. Studies to prove the association between teat-canal penetrability and changes in teat thickness measured with ultrasound should be done.

In this study, teat-wall thickness and teat-cistern width showed the highest relative changes after milking. At 6 and 8 h after milking, respectively, teat-wall thickness and teat-cistern width had recovered. Teat-wall thickness was not significant from the value before milking at 4 h after milking, but the difference in- creased again. This fluctuation could also be seen with the ratio between teat-wall thickness and teat-cistern width. Although no explanation can be given, others (Schultze and Bright, 1983; McDonald, 1975) using endotoxin penetrability or radiography, also found this pattern. Teat-wall thickness and teat-cistern width change through the milking process in

the opposite direction. The milk is withdrawn from the teat cistern during milking. As the cistern decreases, the dimension of the teat-wall increases.

Recovery of teat-end width and teat-canal length after milking had a different pattern. Changes were relatively smaller compared with teat-wall thickness and teat-cistern width (especially for teat-end width) and were almost constant during the 8-h period after milking. Teat-end width and teat-canal length were measured in the less pliable teat tip area. Increase was less and more consistent than the increase of teat-wall thickness. Teat-canal length increased relatively more than teat-end width, which indicates that the teat tip is more pliable or more under stress during milking in length than width.

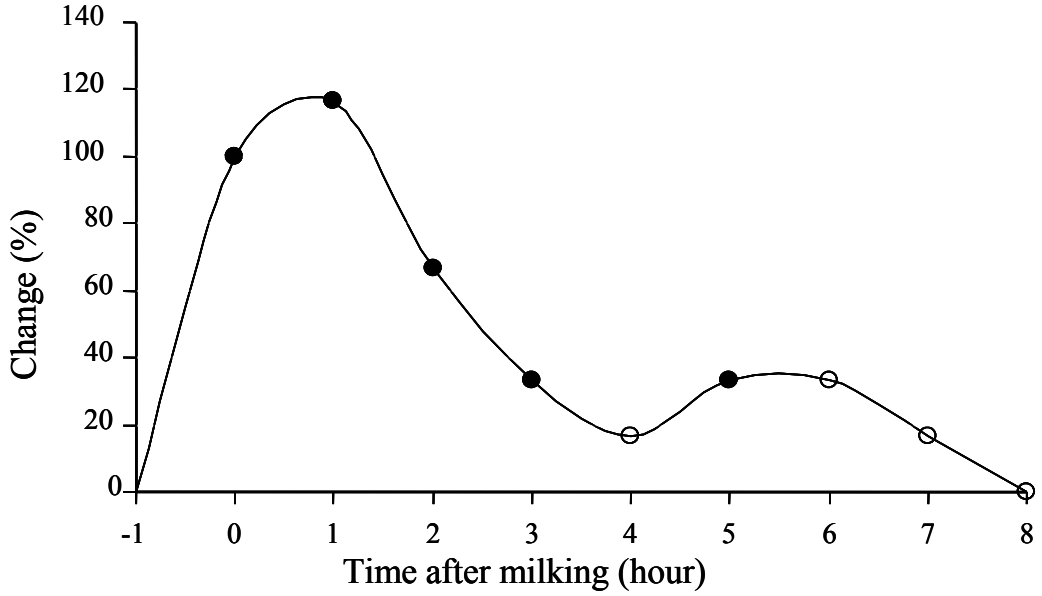

Figure 3. Relative change (%) of the ratio of teat-wall thickness divided by the teat-cistern width, just after milking (0), and at each consecutive hour after milking (1 to 8) compared with the measurement before milking (-1) (filled symbols different from measurement before milking).

Although teat-wall thickness and teat-cistern width were more or less recovered within 4 to 8 h after milking, the complete recovery of teats took more than 8 h. At 8 h after milking, teat-end width as well as teat- canal length still differed significantly from the values before milking. Because the daytime milking interval on the farm was 10 h, it is doubtful whether these parameters would have recovered before the next milking. This is a strange phenomenon, and it may partly be explained by the influence of pretreatment on the teat parameters measured. In this study, teat parameters were compared with their values after pretreatment. Another possible explanation could be the difference

in length of milking intervals between morning and evening milkings (14 vs. 10 h). Moreover, due to lower temperatures and a different activity pattern of cows, the dimensions of the teats may be smaller during night time.

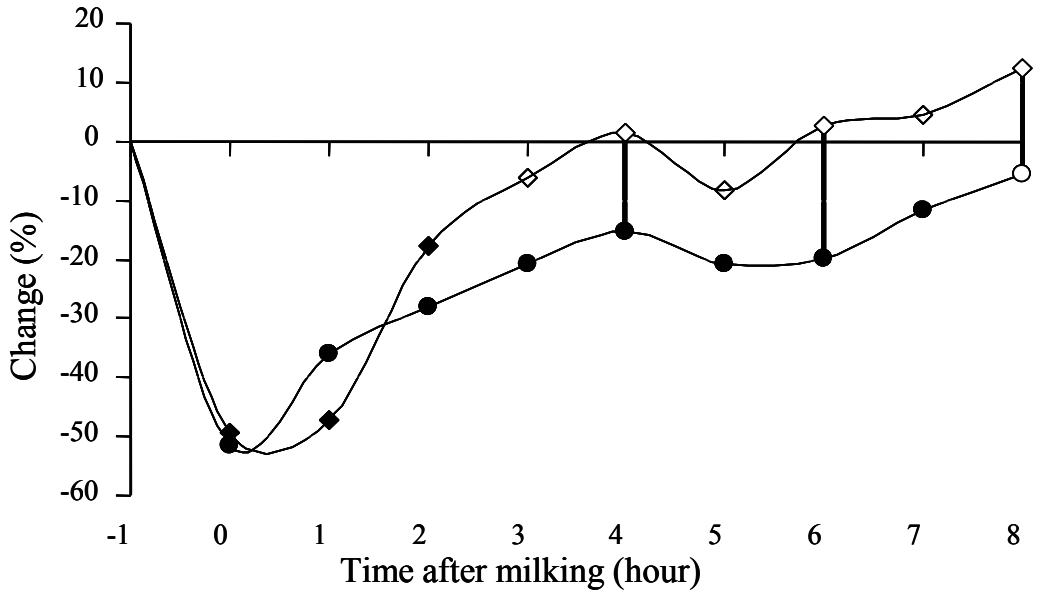

Figure 4. Relative change (%) of the teat-cistern width for rear (■) and front (●) teats, just after milking (0), and at each consecutive hour after milking (1 to 8) compared with the measurement before milking (-1) (filled symbols different from measurement before milking). Differences between front and rear teats are indicated with a line.

The fact that teat recovery can take up to more than 8 h is important when discussing preferred milking intervals. Milking with shorter intervals may lead to incomplete recovery of teats (Hamann and Østeras,1994). This can lead to an accumulation of teat damage. Especially when applying automatic milking, milking intervals may be short. Data of one farm with an automatic milking system showed that there was a large variation in milking intervals (De Koning and Ouweltjes, 2000). With a median milking interval of 8 h, 9.7 and 0.5% of the milkings had a preceding milking interval shorter than, respectively, 6 and 4 h (Hogeveen et al., 2001).

Properties of the teat parameters change as a result of milking. The liner is the only part of the milking machine that has direct contact with the teat. The liner must exert pressure or compressive load on the teat to decrease fluid accumulation in the teat tissue. The total pressure generated is determined by the pressure difference acting across the opposing liner walls and by the size of the airspace within the collapsed liner directly below the teat apex (Mein et al., 1987). Therefore, the liner has to fit nicely to the teat tip as well as higher up on the teat to avoid congestion. In this study

cow was the random factor accounting for a large proportion of variance, which indicates that the reaction of cows on milking varies largely from cow to cow. The teat dimensions relative to the liner dimensions might cause part of this variation. The liner used during this study, fits best on average teat shape.

Differences in milking technique influence the reaction of teats on milking and the rate of recovery after milking (Hamann et al., 1994a; Hamann and Mein,1988, 1990; Hamann, 1989). For instance, positive pressure in the pulsation chamber of the teat cups gives a decrease in teat thickness as measured by the cutimeter (Hamann et al., 1994a). Moreover, the observation that milk flow rate differs between cows gives reason to believe that optimal machine milking parameters may differ from cow to cow. Modern milking technology makes it possible to apply specific milking parameters such as vacuum, pulsator ratio and pulsation rate, to individual cows (Lind et al., 2000). In the future, this may lead to a decreased teat-end penetrability of cows after milking, which is beneficial for udder health. However, case-control research will be needed to prove differences in reaction of teats to milking between and within cows due to milk flow. Evaluation of teat parameters by ultrasound measurement can be a good help in this research.

CONCLUSIONS

Ultrasound measurement of teat parameters has shown to be a useful tool for studying changes in teat properties caused by milking. Machine milking had a large effect on the length of the teat-canal, the width of the teat-cistern, width of teat-end, and the thickness of the teat-wall. Maximum deviations for the teat-wall thickness and teat-cistern width were found directly after milking. Teat-end width and teat-canal length deviated most compared with before milking at 6 or 2 h after milking, respectively. At 8 h after milking, teat- end width and teat-canal length still differed from before milking. Teat-wall thickness and teat-cistern width were recovered after 6 and 8 h. The results indicate that caution is necessary when milking more frequently, and that cow-specific milking might be beneficial to teat condition.

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CHAPTER 5

QUANTIFICATION OF THE INCIDENCE OF CLINICAL MASTITIS WITH DIFFERENT TEAT-END CALLOSITY

F. Neijenhuis,[1] G. André, [1] H. Hogeveen,[2,3] and J.P.T.M. Noordhuizen[3]

[1] Applied Research, Animal Sciences Group, Wageningen UR, P.O. Box 2176, 8203 AD Lelystad, The Netherlands

[2] Business Economics, Wageningen University, P.O. Box 8130, 6700 EW Wageningen, The Netherlands

[3] Department of Farm Animal Health, Utrecht University, P.O. Box 80151, 3508 TD Utrecht, The Netherlands

Submitted

ABSTRACT

Clinical mastitis is, from an economic and welfare point of view, an important disease on dairy farms, given reported average annual incidences of 13% to 30% per cow year at risk. Data from a longitudinal study in 15 herds for 18 months were collected to quantify the incidence risk of clinical mastitis in cows with different teat-end callosity thickness (TECT) and roughness (TECR) classes. A generalized linear model with a logit link was used to analyse binomial data. The model produced incidence risk and odds ratios for clinical mastitis one month after the observation attributable to parity, stage of lactation, teat-end shape, TECT and TECR category respectively.

A teat with a thin TECT ring was less prone to show clinical mastitis. Rough teat-end callosity had an odds ratio for increased incidence risk of clinical mastitis the following month of 1.37. Moderate or thick TECT increased the incidence risk of clinical mastitis the following month compared to a thin TECT with odds ratios of 1.29 and 1.58, respectively. Teats without any callosity ring also showed an increased risk of clinical mastitis (OR 2.52).

Increase in TECT and TECR can be used as an early warning signal for increased risk of clinical mastitis.

(**Key words**: clinical mastitis, teat-end callosity, teat condition)

Abbreviation key: **PAR** = parity, **TES** = teat-end shape, **SOL** = stage of lactation; **TEC** = teat-end callosity, **TECR** = teat-end callosity roughness, **TECT** = teat-end callosity thickness.

INTRODUCTION

Clinical mastitis is, from an economic and welfare point of view, an important disease on dairy farms. In spite of recommended udder health management incidence of clinical mastitis remains high. Estimates for the incidence rate of clinical mastitis in The Netherlands range from 13% to 30% per cow-year at risk (Schukken et al., 1989; Miltenburg et al., 1996; Barkema et al., 1998). The primary pathway of intramammary infections (**IMI**) is through the teat orifice. Therefore, the teat acts as the first line of defence against pathogens, and changes in teat tissue around the teat canal may favor penetration of bacteria into the udder (Paape, 1979; O'Shea, 1987). Severe teat-end lesions (erosions or scabs) are positively associated with the prevalence of subclinical mastitis or bacterial colonization (Jackson, 1970; Sieber and Farnsworth, 1981; Fox and Cumming, 1996).

Machine milking can affect the extent of callus formation on the teat-end (Sieber, 1980; Hamann, 1987; O'Shea, 1987; Ebendorff and Ziesack, 1991). Therefore, teat-end callosity (TEC) may be looked upon as one of the mechanisms through which the quality of machine milking can affect the udder health in a herd. Until recently, no statistically significant association between the presence of chronic smooth or rough rings on teat-ends and the risk of mastitis could be demonstrated (Michel et al., 1974; Thompson and Sieber, 1980; Sieber and Farnsworth, 1981; Shearn and Hillerton, 1996). However, results of a recent study provided a different perspective (Neijenhuis et al., 2001). This study described the appearance of TEC during the lactation of cows with clinical mastitis and cows of the same herds without clinical mastitis. Unlike other studies, data were collected longitudinally, the month of lactation the mastitis occurred was taken into account, and an adequate TEC classification system was used. Cows suffering from clinical mastitis did show more TEC than their healthy herd mates, particularly when clinical mastitis occurred between the second and fifth month of lactation. However, no quantification of the risk of the different categories of TEC on clinical mastitis was made. Moreover, the found relation was only an association and no conclusions could be drawn regarding the cause and effects.

The purpose of this study was to quantify the incidence risk (defined as the incidence rate of clinical mastitis per cow month at risk) of clinical mastitis for different teat-end callosity thickness (TECT) and teat-end callosity roughness (TECR) classes observed one month preceding the clinical mastitis.

MATERIAL AND METHODS

Data Collection

Fifteen dairy farms throughout The Netherlands were purposely selected to participate in the study. Nine herds were located at research/teaching institutes and 6 were commercial dairy herds. The farms were visited monthly from August 1995 until February 1997 with each teat of each cow being scored for callosity thickness and roughness using a previously described scoring system (Neijenhuis et al., 2000). Callosity thickness (**TECT**) was scored as N=no ring, A=thin, B=moderate, C=thick, and D=extremely thick callosity ring. Roughness (**TECR**) was scored as 1=smooth (or no ring present) and 2=rough. In addition, at approximately 6-month intervals, the teat shape and length were assessed. Teat-end shape categories (**TES**) were 1= round, 2=concave, 3=flat and 4=pointed. For each lactation record, the parity (**PAR**) of the cow was recorded as 1^{st}, 2^{nd} or 3^{rd}+, and the stage of lactation (**SOL**) as begin, mid or end of lactation. Details of the data and their collection process have been previously described (Neijenhuis et al., 2001).

Statistical Analysis

Since TEC observations were made monthly and mastitis observations by the dairy farmer could be made daily, the mastitis cases were related to TEC on a monthly basis. When one or more quarters of a cow showed signs of clinical mastitis, all udder quarters of that cow were taken into the analyses as a mastitis case. Only the first mastitis case per cow per lactation was taken into the analysis. Quarter observations after the mastitis case, were again taken into the analyses as non-mastitis quarters.

The incidence risk of clinical mastitis attributable to TEC is defined as the number of quarter observations of the cows experiencing a clinical mastitis case, relative to the total number of observations (month t). SOL, PAR and TES were taken into account in order to examine interactions with TEC. Observations on quarter level of TEC the month preceding the clinical mastitis case (month t-1) were only taken into the analyses when SOL, PAR, and TES were all known. The model was built in three steps. In the first step, the influence of the single explanatory variables in month t-1 SOL, PAR and, TES were screened by entering these variables as only explanatory variable in the model. In the second step, SOL, PAR and TES were put in the model in

the order of importance and TECT and TECR were added. At step three the interactions between SOL, PAR, TES and TECT or TECR were tested.

Since the distribution of the incidence risk of clinical mastitis is binomial, the data were analyzed using a generalized linear model with a logit link. Analyses of deviance were carried out using the quasi likelihood method (Lawes Agricultural Trust, 2002; McCullagh and Nelder, 1989). The model used for the incidence risk of clinical mastitis was as follows:

$\text{Logit}(\Pi_{ijklm}) = \mu + SOL_i + PAR_j + TES_k + TECT_l + TECR_m + e_{ijklmn}$

With

$$\Pi_{ijklm} = E\left(\frac{Y_{ijklm}}{N_{ijklm}}\right)$$

and

$e_{ijklmn} \sim N(0;\Phi)$

Where

Π = incidence risk of clinical mastitis in month t,

SOL_i = stage of lactation in month t-1 (i is 1=begin lactation 1 to 3 months, 2= mid-lactation 4 to 7 months, and 3= end lactation 8 to 10 months),

PAR_j = parity in month t-1 (j is 1=first, 2=second, and 3= older than second parity),

TES_k = teat-end shape in month t-1 (k is 1=pointed, 2=round, 3=flat, and 4=inverted),

$TECT_l$ = teat-end callosity thickness in month t-1 (l is 1=no ring, 2=thin, 3= moderate, 4=thick, and 5=extreme thick ring),

$TECR_m$ = teat-end callosity roughness in month t-1 (m is 0=smooth or no ring, and 1=rough ring),

e_{ijklmn} = residual random error which is based on a normalized distribution at the scale of the predictor where Φ is dispersion factor,

Y = number of quarter observations of cows with clinical mastitis in month t , and

N = the total number of quarter observations in month t.

In addition to this model, interaction terms were tested.

The model produced incidence risk of clinical mastitis attributable to PAR, SOL, TES, TECT and TECR categories respectively. An accumulated analysis of deviance was used to test the significance of the terms (P<0.05). T-probabilities of pair wise differences were used to test for significant differences within the fitted terms (P<0.05). Odds ratios were calculated to get more insight into the weight of the influence of the different categories within the factors.

Table 1: Distributions (%) of total observations on quarter level and quarter observations with clinical mastitis one month after the observation per category of teat-end callosity thickness, teat-end callosity roughness, teat-end shape, parity and, stage of lactation.

Category:	Total quarter observations	Observations one month preceding clinical mastitis
Stage of lactation		
Beginning	29.7[a]	46.7[c]
Mid	41.1[a]	38.7[a]
End	29.2[a]	14.6[c]
Parity		
1	28.1[a]	15.0[c]
2	23.2[a]	20.1[b]
>2	48.8[a]	64.9[c]
Teat-end shape		
Pointed	7.6[a]	10.0 [b]
Round	60.4[a]	60.5 [a]
Flat	29.7[a]	28.2[ab]
Inverted	2.4[a]	1.3[ab]
Teat-end callosity thickness		
None	3.1[a]	6.7[c]
Thin	74.4[a]	64.3[c]
Moderate thick	18.5[a]	22.5[b]
Thick	3.2[a]	5.2[b]
Extreme thick	1.0[a]	1.3[ab]
Teat-end callosity roughness		
Smooth	60.2[a]	50.8[b]
Rough	39.8[a]	49.2[c]
Total		
Observations	57762	974
Percentage	100	1.7

Different superscripts indicate significant differences (P ≤ 0.05) between groups of a factor.

RESULTS

Descriptive Results

A total of 57,762 TEC quarter observations were made on 1,757 different cows with 2,671 lactations during the 18 monthly farm visits (Table 1). From the TEC observations, 9,051 originated from the teats of 414 cows, which had clinical mastitis in a total of 417 lactations. In the month the mastitis case occurred, 21% of these cows were first parity, 19% second, and 60% older parity. Also, 47% were in the first 3 months of lactation, 43% in mid lactation and, 10% at the end of lactation. Teat-end callosity observations one month before the clinical mastitis occurred were available on 248 cows and comprised 974 quarter observations. Of these cows 15% were in first, 20% second, and 65% later parity, while 31% of the clinical mastitis cases occurred in the first 3 months of lactation, 48% mid lactation and 20% in the last 3 months of lactation.

Quarter observations in the data set were most frequently rounded teat-ends with thin smooth TEC (Table 1). Cows with clinical mastitis had on average less frequently thin and smooth callosity rings, and TES was more frequently pointed. Moreover, these cows were more often older and in the beginning of lactation or in mid lactation. More detailed, overall descriptive statistics can be found in an earlier paper (Neijenhuis et al., 2001).

Table 2: Accumulated analysis of deviance of the incidence risk of clinical mastitis the month following the quarter observation by stage of lactation, parity, teat-end shape, teat-end callosity thickness, and teat-end callosity roughness.

Parameter	d.f.	Deviance	Mean Deviance	*F*-value	*T*-prob.[1]
Stage of lactation	2	172.1	86.1	50.6	***
Parity	2	117.1	58.5	34.6	***
Teat-end shape	3	19.2	6.4	3.8	*
Teat-end callosity thickness	4	49.4	12.3	7.2	***
Teat-end callosity roughness	1	19.3	19.3	11.4	***
residual	241	419.7	1.7		
Total	253	796.8			

[1] *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

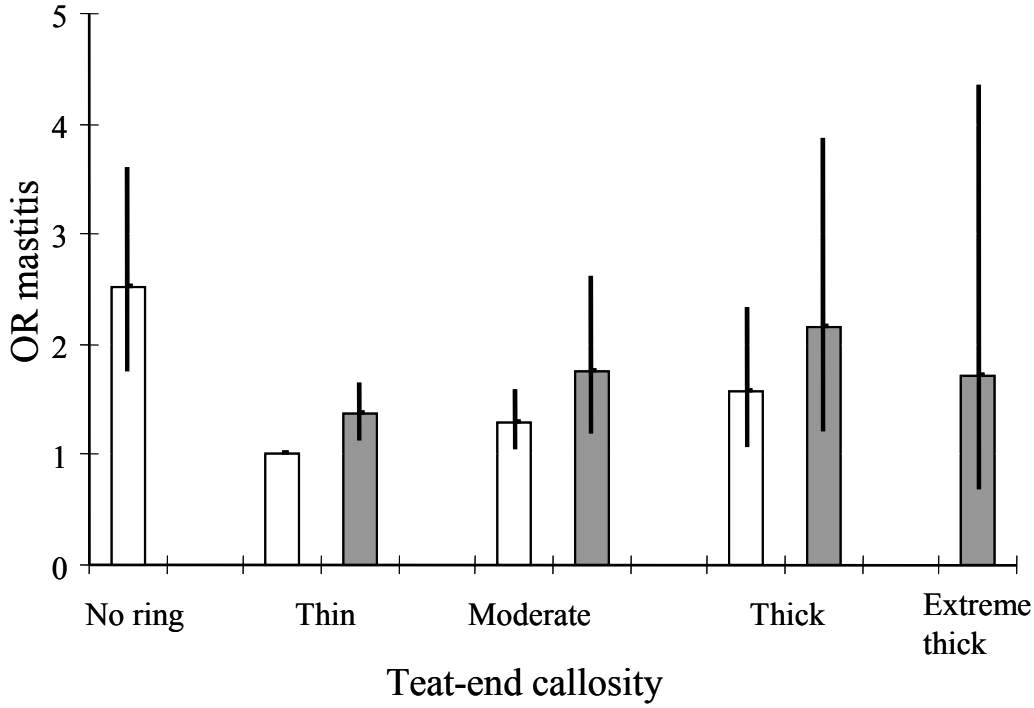

Figure 1. Odds ratio (OR) with the 95% confidence interval of the incidence risk of clinical mastitis during the month following the TEC score per TEC category (Chapter 5), according to the model. Teat-end callosity was scored as N=no ring, A=thin, B=moderate, C=thick, and D=extremely thick callosity ring and additional teat-end callosity roughness was scored as 1=smooth (or no ring present) and 2=rough (filled bars).

Model results

The accumulated analysis of deviance is presented in Table 2. The adjusted R^2 for the model was 0.45. Teat-end callosity accounted for 9% of the variance in the incidence risk of clinical mastitis. The stage of lactation accounted for the greatest proportion of the variance (21 %) followed by parity (14%) and teat-end shape explained a small amount (2%).

Estimates of the parameters are shown in Table 3. Older parity cows in the first 6 months of lactation with pointed TES had an increased incidence risk of clinical mastitis. Rough TEC had an increased odds ratio for the incidence risk of clinical mastitis of 1.37 compared with teats with a smooth callosity ring. A teat with a thin TEC ring was less prone to show clinical mastitis. Moderate or thick TEC had an increased odds ratio for the incidence risk of clinical mastitis compared to a thin TEC of 1.29, and 1.58, respectively. Teats without any callosity ring had an increased odds ratio for the incidence risk of clinical mastitis of 2.52 compared to teats with a thin

callosity ring. Teats with an extreme thick and rough callosity ring did not show a significant increase of in the incidence risk of clinical mastitis the following month. The results of this analysis revealed a broad confidence interval. These results are illustrated in Figure 1 which represents the odds ratios with 95% confidence intervals for increased risk of clinical mastitis the following month per category of TEC.

Table 3: Per factor category the number of quarters preceding a clinical mastitis case (Y), number of total quarter observations (N), incidence risk (IR), estimate with standard error (Est. and Se), and odds ratio with 95% with confidence interval (OR and 95% CI) of clinical mastitis in the month following the quarter observation according to the model.

	IR	Y	N	Est.	Se	T-prob.[1]	OR	95% CI
Constant								
	1.6	974	57762	-4.554	0.129			
Stage of lactation								
Begin	2.6	455	17156	0.5524	0.0948	***	1.74	1.44-2.10
Mid	1.5	377	16842			Ref.	1.00	
End	0.8	142	23764	-0.589	0.131	***	0.55	0.43-0.72
Parity								
First	0.9	146	28170	-0.461	0.146	**	0.63	0.47-0.85
Second	1.5	196	13375			Ref.	1.00	
Older	2.1	632	16217	0.385	0.110	***	1.47	1.18-1.83
Teat-end shape								
Pointed	2.2	97	4368	0.329	0.151	*	1.39	1.03-1.88
Round	1.6	589	34866			Ref.	1.00	
Flat	1.6	275	17147	-0.115	0.0993	NS	0.99	0.81-1.21
Inverted	1.0	13	1361	-0.461	0.376	NS	0.63	0.30-1.34
Teat-end callosity thickness								
None	3.2	65	1782	0.923	0.183	***	2.52	1.75-3.63
Thin	1.5	626	42966			Ref.	1.00	
Moderate	1.9	219	10660	0.251	0.108	*	1.29	1.04-1.60
Thick	2.3	51	1808	0.457	0.201	*	1.58	1.06-2.36
Extreme	2.3	13	546	0.227	0.380	NS	1.25	0.59-2.68
Teat-end callosity roughness								
Smooth	1.5	495	34761			Ref.	1.00	
Rough	1.9	479	23001	0.315		***	1.37	1.13-1.66

[1]T-probability refers to pair wise differences with the reference value.
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, NS = not significant.

Two interaction terms accounted for significant proportion of variance in the incidence risk of clinical mastitis: interaction SOL*TECT and TES*TECT accounted for 2.5% and 4.1% of the

variance. However, these interactions were a result of only a few significant combinations, and did not affect the coefficient of the main parameters. The interaction between SOL and TECT was only found to be significant for the combination of end of lactation cows without any callosity ring (OR=1.78). The interaction between TES and TECT was found to be significant for pointed teat-ends without any callosity ring (OR=2.00), moderate thick callosity rings (OR=1.37) and extreme thick callosity rings (OR=2.36), and for inverted teat-ends with thick callosity rings (OR=4.14). For reasons of readability, interaction terms were therefore left out of the presented model.

DISCUSSION

Most of the clinical mastitis cases occurred during the first 3 months of lactation. This is in accordance with other research (e.g., Barkema et al., 1998). However, when a clinical mastitis case occurred in this study during the first month of lactation, no preceding quarter observation will have been considered in the analysis. Therefore, the true distribution will be even more skewed. This did not become a problem during the analysis because the model corrected for the stage of lactation.

We were interested in the incidence risk of clinical mastitis following the quarter observations; the time at risk was not of interest. Therefore we did not use survival analysis. Moreover, using survival analysis, many records would be censored on the left side of our time span, because of cows that have calved before the start of data collection period. We assumed that all quarters of a cow with a clinical mastitis case (in one or more quarters) were mastitis quarters. This may have caused bias in the estimates of the incidence risk of clinical mastitis. Therefore we presented the outcome of the incidence risk as odds ratios. Further research needs to be conducted to obtain more insight into the longitudinal aspects of the relation between TEC and incidence rate of clinical mastitis on quarter basis. The data could be analysed in more detail with GLMM or survival models (Dohoo et al., 2003).

The goal of the analysis was not to look at differences between farms in incidence risk of clinical mastitis. The interdependence between dates within cows, and cows within farms was not modeled in detail. This clustering has leaded to overdispersion, and therefore we used quasi likelihood instead of maximum likelihood to analyse the deviance (McCullagh and Nelder, 1989).

The effect of previous TEC on the incidence risk of clinical mastitis in the first month of lactation is not included in the analyses. TEC in the first month of lactation will, however, probably not contribute to the risk of mastitis. Cows with clinical mastitis cases in the first month of lactation showed no difference in TEC compared with paired cows without clinical mastitis in previous research (Neijenhuis et al., 2001). These early clinical mastitis cases may be not related to TEC, but caused by the generally depressed immune system due to severe negative energy balance or fatty liver after parturition (Suriyasathaporn et al., 2000; Thanasak, 2003).

The model included PAR, TES and SOL since from previous research, it was known that these factors accounted for a proportion of the differences in TEC, and we were interested in the interactions (Neijenhuis et al., 2001). Nonetheless, TECT and TECR accounted for a significant proportion of variance in the incidence risk of clinical mastitis. The proportion of TEC explains 8.6% of the variance in incidence risk of clinical mastitis in the following month.

In this study, we found the lowest incidence risk of clinical mastitis for teats with a thin smooth TEC ring. A low grade of TEC does not appear to increase the risk of IMI in the lactating dairy cow, and may be considered as a beneficial, physiological response of the teat to machine milking. Teats without any TEC had a higher risk of clinical mastitis. This is in agreement with Hamann (1987) who stated that there were no data to suggest that TEC per se increases new IMI rate but a slight increase in the degree of TEC could decrease susceptibility to pathogens. Moreover, thicker and rough callosity also increases the incidence risk of clinical mastitis. There might be two different mechanisms behind the increased risk of clinical mastitis.

A thick TEC around the teat orifice negatively influences the ability of the teat to close and the time needed to close after machine milking. Moreover, rough callosity may harbour bacteria, which are not easily removed during pre-treatment of the teats, and may enter the teat canal more easily (Fox and Cumming, 1996). Hence, SCC and incidence risk of mastitis will increase.

The most probable mechanism behind the fact that teats without TEC show an increase in risk on clinical mastitis is the following. The rate of keratin regeneration within the teat canal must be in balance with its removal during milking (Lacy-Hulbert et al., 1996). The production of keratin is promoted by the action of the teat-cup liner on the teat during milking. The keratin content is consistent with epithelial hyperplasia induced by milking (Capuco et al., 2000). Therefore, teats without any TEC will have also less keratin. Tested interactions confirmed this hypothesis since

cows particularly at the end of lactation without any callosity ring exhibit a significantly higher incidence risk of clinical mastitis.

Neijenhuis et al. (2001) found that there was a relationship between incidence of clinical mastitis and TEC. However, the association between TECT and the incidence of clinical mastitis was not quantified and the found relation was not more than an association. The results of the current study showed that cows with rough or thicker TEC had a higher incidence of clinical mastitis the following month (OR = 1.3 - 1.6). Not only the TEC observations of cows not experiencing a clinical mastitis the next month, but also the TEC observations following a clinical mastitis were taken into account as the reference. Thus, this supports the hypothesis that a higher proportion of TEC is not only a result of clinical mastitis, from which cows may have more problems with machine milking, but also an indicator for increased incidence risk of clinical mastitis. An increase in TECT and TECR can therefore be used as an early warning signal for increased risk of clinical mastitis.

CONCLUSIONS

The incidence of clinical mastitis was quantified for different severities of TEC. A thin and smooth TEC ring showed the lowest incidence risk of clinical mastitis. Increasing TECT or TECR increased the incidence risk of clinical mastitis. But quarters without any callosity ring also showed an increased incidence risk of clinical mastitis the next month. An increase in TECT and TECR can be used as an early warning signal for enhanced risk of clinical mastitis.

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CHAPTER 6

MACHINE MILKING RISK FACTORS FOR TEAT-END CALLOSITY IN DAIRY COWS ON HERD LEVEL

F. Neijenhuis,[1] G.H. Klungel,[1,*] H. Hogeveen,[2,3] and J.P.T.M. Noordhuizen[3]

[1]Applied Research, Animal Sciences Group, Wageningen UR, P.O. Box 2176, 8203 AD Lelystad, The Netherlands

[2] Business Economics, Wageningen University, P.O. Box 8130, 6700 EW Wageningen, The Netherlands

[3] Department of Farm Animal Health, Utrecht University, P.O. Box 80151, 3508 TD Utrecht, The Netherlands

[*] Current address: Insentec B.V., Repelweg 10, 8316 PV Marknesse, The Netherlands

Submitted

ABSTRACT

The objectives of this study were to evaluate the effect of milking on teat-end callosity (TEC) at the herd level. In total, 192 farms were visited once to collect data on the milking management, teat condition and milking machine. Teat-end callosity was grouped in four categories: no callosity, smooth callosity, rough callosity, and very rough callosity. Teats in the rough and very rough callosity category were regarded as exhibiting a higher risk for clinical mastitis. Therefore, the percentage of teats within a herd with rough and very rough callosity (%ROUGH) was used as the dependent variable in a basic generalized linear model with a logit link in which the effects of single independent variables were analyzed. Farms with milking parlours using a milk meter had on average higher %ROUGH than farms with high mounted milk recorder jars or without milk measurement. Longer machine-on time resulted in higher %ROUGH. Higher vacuum differences in the short milk tube were associated with lower %ROUGH as were increasing diameters of the short milk tube. Central feeding of concentrates in the milking parlour was associated with lower %ROUGH than individual feeding. There was an association between teat cup liner brand and %ROUGH. More concave and flat teat-ends showed lower %ROUGH. Post-milking teat disinfecting was associated with higher %ROUGH compared to no post-milking teat disinfection.

Increases in rough and very rough teat-end callosity are associated with increased incidence of clinical mastitis, and therefore teat-end callosity can be used as a monitoring tool for assessing the quality of milking.

(**Key-words**: dairy, milking; teat; callosity)

Abbreviation key: **%ROUGH**= percentage of rough callosity rings on a farm; **TEC** = Teat-end callosity

INTRODUCTION

The teat of a cow is the first line of defence against mastitis. The ability of the teat to prevent infections is associated with the condition of the teat-end (Michel et al., 1974; O'Shea et al., 1987). Therefore, teat-end condition, often measured in terms of teat-end callosity (TEC), is an important physiological parameter in order to gain more insight into the first line of udder defence. Moreover, a direct relationship between TEC and the incidence of clinical mastitis was found in a large study of 15 dairy farms. Roughness and thickness of the callosity ring are indicators for increased risk of clinical mastitis (Neijenhuis et al., 2001; Neijenhuis et al., submitted). Teats with a smooth thin callosity ring had the lowest risk of clinical mastitis. Teats without any callosity ring had a higher risk of clinical mastitis compared to teats with a thin callosity ring. On the other hand, thicker and rough callosity also increased the incidence of clinical mastitis. Mein et al. (2001) simplified the TEC scoring system on basis of the relationship with mastitis into four categories in order to standardize classification of TEC in the field.

Cow factors (parity, lactation stage, udder anatomy) influence TEC (Michel et al., 1974; Sieber and Farnsworth, 1981; Neijenhuis et al., 2000). It is expected that besides cow factors, the functioning of the milking machine, and milking methods applied by the milker (milking technique) also influence TEC (Hamann, 1987; Ziesack et al., 1989; Rasmussen, 1993; Shearn and Hillerton, 1996). Changes in TEC may result from mechanical forces exerted by vacuum and the collapsing liner during machine milking (Mein et al., 2003). If there is a direct relation between the functioning of the milking machine and TEC, an increase in TEC can be used as an early warning signal of milking machine failures.

Until now, the relationship between different settings of the milking machines or different milking systems and TEC have only been studied in relatively small experimental trials (Hamann, 1987, Ziesack et al., 1989). These field trials fail to provide sufficient information on the relation between the level of TEC at the herd level and machine milking. Therefore, the objectives of this study were to evaluate the association between TEC and machine milking parameters at the herd level.

MATERIALS AND METHODS

Farms

A total of 200 dairy farms, spread throughout The Netherlands were asked to participate in this study. Inclusion criteria were a minimal herd size of 35 lactating cows, and the use of a milking parlour. Farms with a tie-stall or an automatic milking system were excluded from participation. Of the 200 dairy farms that were asked to participate, 192 farms entered the study.

Table 1: Variables taken into account in the statistical analysis to identify their association with rough and very rough teat-end callosity (%ROUGH).

Category	Variables
General farm description	Herd size (lactating dairy cows), type and size of milking parlour, feeding of concentrate during milking, automatic cluster removal, diameters short and long milk tube, length long milk tube, long milk tube support
Liners	Brand, material and replacement strategy
Measurements during one evening milking	Milk production, pre-treatment strategy and time, air sucking, cluster attachment, machine on-time, moment cluster detachment, post-milking teat disinfection, hygiene during milking, milking high SCC cows last, proportion of teat-ends classified as pointed, proportion of teats classified as thick or long.
Dynamic measurements	Average, minimum, maximum, and difference in vacuum in the short and long milk tube, difference in vacuum between long and short milk tube, vacuum decrease in the short milk tube at the beginning and half way milking
Maintenance report milking machine	Air usage and vacuum measurements
Pulsator maintenance report	Pulsation rate and ratio, and phases

Data Collection

From January to June 1998, each farm was visited once. During each farm visit, photographs of all four teats were taken of, on average, 35 randomly selected cows (range from 18 to 42 cows per farm) immediately after milking. The teat photographs were interpreted by one trained technician who determined TEC in terms of TEC thickness and TEC roughness using a standardized

classification system (Neijenhuis et al., 2000). In order to standardize the scoring to the international standard for field studies (Mein et al., 2001), the results were grouped in 4 categories: N = no callosity (N), S = smooth callosity (1A, 1B and 2A), R = rough callosity (1C and 2B), and VR = very rough callosity (2C and 2D). Additionally, teat-end shape was scored as round, pointed, flat or concave.

Besides the TEC-measurements, farm and machine milking data were collected (Table 1). Descriptive statistics can be found in Tables 2 to 8. The most recent milking machine maintenance report available on the farm is the result of a yearly check of the milking machine by the dealer and is a standardized report (DeKoning and Huijsmans, 2001). Measurements in this report are not carried out during milking itself and include parameters such as vacuum level, capacity, pulsation rate, and pulsator ratio (Anonymous, 1996). Information on milking techniques and the milking machine were obtained by a questionnaire and observations in the milking parlour. Furthermore, the functioning of the milking machine was evaluated with dynamic measurements during the evening milking. The questionnaire and observations in the milking parlour included information about pre-milking treatment, the method of milking cluster attachment and detachment (improper detachment was defined as detachment later than 30 sec. after ceasing of milk flow), the method and materials used during post-milking teat disinfection, hygiene during milking (scored as very clean, normal or very dirty), and maintenance of the milking machine (e.g. time between replacement of liners). Replacement of liners was considered to be on time when they were replaced before 2500 and 7500 milkings for rubber or silicone liners respectively.

Table 2. Descriptive results and characteristics of the two variables milk measurement and machine-on time forced into the generalized linear regression model of teat-end callosity roughness (%ROUGH). Data from 184 farms.

Basic model	Average or category	#	ß	SE	Deviance Ratio	F-prob	Observed %ROUGH
Constant			-2.118	0.343			0.17
Milk Measurement					86	0.009	
	Milk meter	40					0.21
	Jars high	75	-0.307	0.121			0.16
	Jars low	31	-0.134	0.148			0.18
	None	38	-0.428	0.148			0.14
Machine-on time	383 s	184	0.002	0.0008	39	0.021	

The duration of pre-milking treatment, the intervals between pre-milking treatment and cluster attachment, the machine-on time, and milk production were measured for two batches of cows during milking. Depending on the size of the milking parlour, these two batches of cows comprised between 6 to 40 cows. Additionally, the vacuum level under the teat in the short milk tube and vacuum level in the long milk tube was measured with two vacuum gauges connected to a recorder at one milking unit during a complete milking of all cows milked with that unit.

All data were collected by four technicians who were specifically trained to make the photographs of the teats, fill out the questionnaire, and to carry out this standardized short dynamic milking evaluation.

Table 3. Descriptive results and characteristics of general farm description variables offered to the generalized linear regression model in addition to the variable milk measurement and machine-on time (maximal 184 farms) in association with teat-end callosity Roughness (%ROUGH) with deviance ratio (Dev. Rat.) given when F-probability < 0.25.

Variable	Average or category	#	# missing	ß	SE	Dev. Rat.[1]	F-prob	Observed %ROUGH
Number of lactating cows	65	184	0	-0.001	0.016		0.463	
Type milking parlour	Rotary	4	0			11	0.199	0.20
	Tandem	23		-0.061	0.331			0.18
	Herringbone	152		-0.160	0.308			0.17
	Side-by-side	5		-0.770	0.447			0.11
Size of milking parlour	10 milking units	184	0	-0.022	0.017	12	0.200	
Concentrate in parlour	No	52	9			47	0.037	0.17
	Central	63		-0.101	0.121			0.15
	Individual	60		0.191	0.119			0.19
Automatic cluster removal	Yes	146	3				0.442	0.17
	No	35		-0.010	0.132			0.16
Diameter short milk tube	10.6 mm	184	0	-0.072	0.025	59	0.004	
Diameter long milk tube	16.4 mm	184	0	-0.044	0.023	27	0.052	
Length long milk tube	2.8 m	172	12	-0.006	0.005	14	0.168	
Long milk tube support	Yes	116	4			10	0.247	0.18
	No	64		-0.121	0.104			0.16

[1]Deviance ratio is given for all variables with F<0.25.

Statistical Analyses

As a descriptive analysis, the frequency of occurrence (categorical variables) or average value (continuous variables) was calculated for all variables. Because TEC categories R and VR as having a higher risk for clinical mastitis (Neijenhuis et al., submitted), as well as showing a correlation with machine milking per farm the following dependent variable was calculated: percentage teats with TEC category R or VR (%ROUGH). Before statistical analyses were carried out, observations were checked for unlikely values. No data were excluded for this reason.

A generalized basic linear model with a logit link function for the binomial distributed outcome variable %ROUGH was formed with type of milk measurement and average machine-on time (McCullagh and Nelder, 1989). The type of milk measurements included in this study were: milk recorder jars with or without a milk lift from the cluster to the milk recorder jars, milk meter with low line plant or no milk recording system with low line plant. In addition to this basic model, added single effects of independent variables were analyzed. Analyses of deviance were carried out using Genstat's quasi-likelihood method (Lawes Agricultural Trust, 2002).

Independent variables which contributed to the dependent variable with a level of significance $P < 0.25$ in the basic model were selected for multivariable analysis (Hosmer and Lemeshow, 1989). Forward stepwise selection of these variables was performed ($P<0.05$). The variable that accounted for the greatest variance, as measured by the deviance ratio, was added to the model. The goodness of fit of the final model was assessed by residual analysis and ratio size of the deviance to the degrees of freedom (McCullagh and Nelder, 1989). The estimated regression parameters were converted into odds ratios, representing indications for ether an increase or a decrease in the risk of percentage of teats with %ROUGH on farm level.

RESULTS

Descriptive Statistics

From the initial 192 farms, 184 farms were used in the analysis. Six farms were eliminated because the breed was not Holstein Friesian or Holstein Friesian cross breed. Another two farms were dropped because the milking frequency was 3 times a day instead of twice daily. The remaining farms had on average 65 lactating cows (range 31 to 350). The recorded cows produced during the visit on average 13 kg milk (range 8 to 19 kg milk) in 383 seconds (range 258 to 611). The mean percentage of teats within a category of TEC on a farm was: 1.7% N, 81.4% S, 16.8% R and 0.1% VR. On average 17% (range from 0 to 51%) of the teats were scored as %ROUGH. The distribution of %ROUGH is shown in Figure 1.

The tables containing the modeling results (Tables 2-8) include the descriptive statistics (frequencies or average value) for the variables. Table 2 includes the variables offered to the basic model and Tables 3 to 8 report the descriptive results of the variables of the 184 farms offered during modeling in addition to the basic model. Not all variables were available for all farms; missing values occurred in the milking machine maintenance report (Tables 7 and 8), farm visits (Tables 3, 4 and 5), and vacuum recordings (Table 6).

Model Results

Milk measurement type and machine-on time were always forced into the regression model (Table 2). Tables 3 to 8 summarize the results of the fit of all variables in addition to the two forced variables for %ROUGH during the first step of modeling. Of the 70 variables offered to the model, 23 variables were associated with %ROUGH ($P < 0.25$). After forward stepwise introduction of the 23 selected variables of the first step, 6 remained significant ($P < 0.05$) and were added to the final model (Table 9). The final model contained information for 163 farms.

Based on the fitted model, the average %ROUGH was 16.8%. The machine-on time was 6.4 minutes, the percentage of concave and flat teat-ends was 11%, the difference in the short milk tube during milking was 10.9 kPa and the diameter of the short milk tube was 10.6 mm. The final model had 144 df and a deviance of 789.

Farms with milking parlours using a milk meter had an increase %ROUGH than farms with high mounted milk recorder jars or without milk measurement. Higher vacuum differences in the short milk tube were associated with lower %ROUGH as was an increasing diameter of short milk tube. Central feeding of concentrates in the milking parlour was associated with lower %ROUGH compared to individual feeding. Farms using teat cup liners brand A had on average more %ROUGH than farms using brand F or H. Farms with a higher proportion of concave and flat teat-ends had less %ROUGH. Practicing post-milking teat disinfection was associated with a higher %ROUGH compared to no application of teat disinfectant after milking.

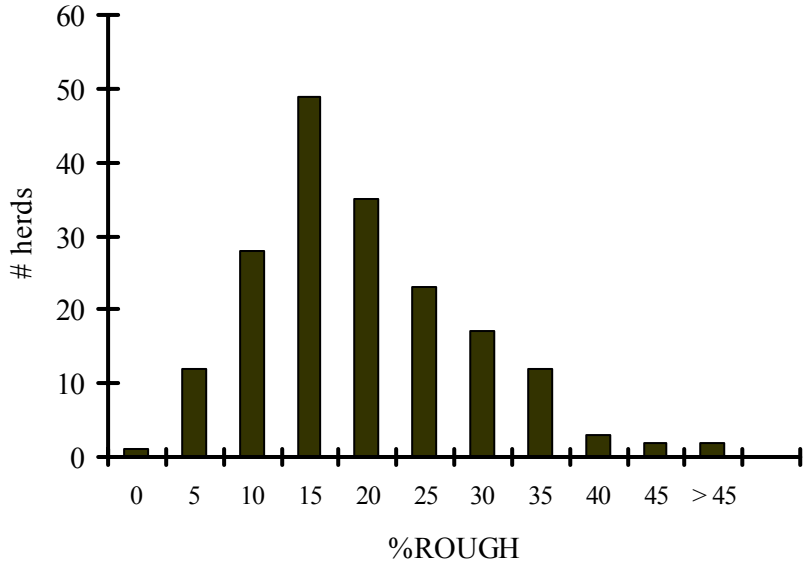

Figure 1. Frequency distribution of rough and very rough teat-ends (%ROUGH) per farm.

Table 4. Descriptive results and characteristics of liner variables offered to the generalized linear regression model in addition to the variable milk measurement and machine-on time (maximal 184 farms) in association with teat-end callosity Roughness (%ROUGH) with deviance ratio (Dev. Rat.) given when F-probability < 0.25.

Variable	Category	#	# missing	ß	SE	Dev. Rat.[1]	F-prob	Observed %ROUGH
Brand teat cup liner	A	37	0	Ref		29	<0.001	0.19
	B	4		0.051	0.297			0.22
	C	23		-0.032	0.157			0.19
	D	21		-0.017	0.162			0.19
	E	30		-0.265	0.150			0.15
	F	12		-0.805	0.233			0.11
	G	33		-0.005	0.140			0.19
	H	24		-0.631	0.179			0.13
Teat cup liner material	Rubber	178	0	Ref		30	0.043	0.17
	Silicone	6		-0.601	0.316			0.11
Replacement of liners	On time	49	0	Ref			0.377	0.16
	Too late	135		0.097	0.110			0.17

[1]Deviance ratio is given for all variables with F<0.25.

Table 5. Descriptive results and characteristics of observations and measurements during one evening milking variables offered to the generalized linear regression model in addition to the variable milk measurement and machine-on time (maximal 184 farms) in association with teat-end callosity Roughness (%ROUGH) with deviance ratio (Dev. Rat.) given when F-probability < 0.25.

Variable	Average or category	#	# missing	ß	SE	Dev. Rat.[1]	F-prob	Observed %ROUGH
Milk yield	13 kg/milking	147	37	0.019	0.027		0.485	
Pre-treatment	Dry	173	2	Ref			0.588	
method	Wet	4		-0.069	0.328			0.16
	Dirty udders wet	5		-0.324	0.329			0.14
Cloth	Cotton	108	0	Ref			0.373	0.16
	Thick paper	60		0.081	0.105			0.18
	Thin paper	16		0.228	0.171			0.19
Cows per cloth	5.5 cows /cloth	182	2	-0.004	0.006		0.536	
Duration pre-treatment	14 sec	184	0	0.005	0.007		0.477	
Fore-stripping	Yes	86	0	Ref			0.687	0.17
	No	98		0.039	0.096			0.17
Cluster attachment	0%	10	17	Ref			0.321	0.17
(% air suckage)	10%	31		-0.061	0.246			0.16
	-25%	71		0.054	0.223			0.18
	>25%	55		-0.168	0.233			0.15
Moment cluster	On time	131	15	Ref		14	0.153	0.17
detachment	Not on time	38		0.168	0.116			0.20
Post-milking	Not	18	0	Ref		25	0.032	0.15
disinfection	Dip	78		0.321	0.173			0.19
	Spray	88		0.089	0.174			0.15
High SCC cows	Yes	37	1	Ref			0.987	0.16
milked last	No	146		0.002	0.122			0.17
Hygiene during	Good	80	14	Ref		11	0.220	0.16
milking	Normal	87		0.171	0.098			0.18
	Bad	3		0.040	0.370			0.17
Ratio flat and concave to round and pointed teats	3%	184	0	-0.374	0.232	26	0.006	

[1]Deviance ratio is given for all variables with F<0.25.

Table 6. Descriptive results and characteristics of measurements obtained during dynamic testing of the milking machine offered to the generalized linear regression model in addition to the variable milk measurement and machine-on time (maximal 184 farms) in association with teat-end callosity Roughness (%ROUGH) with deviance ratio (Dev. Rat.) given when F-probability < 0.25.

Variable	Average or category	#	# missing	ß	SE	Dev. Rat.[1]	F-prob	Observed %ROUGH
Vacuum decrease begin milking	Yes	100	15				0.814	0.17
	No	69		0.027	0.1160			0.17
Vacuum decrease mid milking	Yes	81	20				0.566	0.16
	No	87		0.058	0.1010			0.18
Average vacuum short milk tube	35.8 kPa	174	10	-0.009	0.0118		0.429	
Minimum vacuum short milk tube	29.3 kPa	166	18	0.002	0.0097		0.799	
Maximum vacuum short milk tube	40.0 kPa	166	18	-0.015	0.0106	14	0.167	
Vacuum difference short milk tube	10.7 kPa	171	13	-0.025	0.0112	37	0.024	
Average vacuum long milk tube	40.4 kPa	174	10	0.020	0.0177		0.266	
Minimum vacuum long milk tube	37.6 kPa	167	17	0.019	0.0124	17	0.114	
Maximum vacuum long milk tube	41.4 kPa	167	17	0.002	0.0175	10	0.231	
Vacuum difference long milk tube	3.7 kPa	164	20	-0.014	0.0151		0.356	
Vacuum difference long vs short milk tube	4.6 kPa	174	10	0.026	0.0133	27	0.052	

[1]Deviance ratio is given for all variables with F<0.25.

Table 7. Descriptive results and characteristics of measurements obtained during dry testing of the milking machine offered to the generalized linear regression model in addition to the variable milk measurement and machine-on time (maximal 184 farms) in association with teat-end callosity Roughness (%ROUGH) with deviance ratio given when F-probability < 0.25.

Variable	Average	#	# missing	ß	SE	Dev. Rat.[1]	F-prob	%[2]
1.0 Vacuum one cluster	43.3 kPa	162	22	0.0261	0.0302		0.391	
1.1 Vacuum all clusters	43.3 kPa	168	14	0.0147	0.0219		0.503	
1.0 Vacuum gage	43.1 kPa	174	10	0.0243	0.0289		0.404	
1.2 Regulator response	0.03 kPa	169	15	-0.1800	0.4640		0.697	
Operating vacuum	41.6 kPa	174	10	0.0214	0.0253		0.400	
2.0 Reserve capacity	1036 l/min	167	17	0.0000	0.0001		0.818	
2.0 Minimum reserve capacity	481 l/min	162	22	-0.0002	0.0003		0.630	
2.1 Air used by regulator(s)	9 l/min	171	13	-0.0015	0.0031		0.620	
2.1 Maximum air used by regulator(s)	103 l/min	148	36	-0.0001	0.0004		0.895	
2.2 Manual reserve capacity	1047 l/min	171	13	0.0000	0.0001		0.987	
2.3 Air flow pulsator system	286 l/min	173	11	-0.0001	0.0004		0.752	
2.4 Capacity without pulsator(s)	1336 l/min	169	15	-0.0000	0.0001		0.838	
2.5 Air flow clusters	101 l/min	165	19	-0.0004	0.0013		0.756	
2.5 Max air flow clusters	108 l/min	176	8	-0.0022	0.0013	21	0.082	
2.5 Standard air flow clusters	122 l/min	184	0	-0.0018	0.0014	12	0.200	
2.5 Meet standard air flow clusters	7 l/min	165	19	-0.0022	0.0014	18	0.115	
2.6 Air use ancillary equipment	30 l/min	164	20	0.0000	0.0009		0.995	
2.7 Capacity installation	1462 l/min	164	20	-0.0000	0.0001		0.685	
2.8 Leakage milk line	10 l/min	162	22	0.0024	0.0024		0.343	
2.8 Length milk line	125 m	20	164	-0.0020	0.0076		0.796	
2.8 Max air usage milk line	25 l/min	25	159	-0.0073	0.0047	21	0.083	
2.9 Sealed milk line	1433 l/min	176	8	-0.0001	0.0001		0.651	
2.10 Air usage pulsator air line	19 l/min	126	58	0.0000	0.0016		0.982	
2.10 Length pulsator air line	27 m	116	68	-0.0006	0.0059		0.921	
2.10 Maximum air usage pulsator air line	67 l/min	92	92	-0.0001	0.0019		0.959	
2.11 Air displacement vacuum pump	1483 l/min	135	49	-0.0001	0.0001		0.274	

[1] Deviance ratio is given for all variables with F<0.25.
[2] % is observed %ROUGH.

Table 8. Descriptive results and characteristics of measurements obtained during dry testing of the pulsation system offered to the generalized linear regression model in addition to the variable milk measurement and machine-on time (maximal 184 farms) in association with teat-end callosity Roughness (%ROUGH) with deviance ratio (Dev. Rat.) given when F-probability < 0.25.

Variable	Average	#	# missing	ß	SE	Dev. Rat.[1]	F-prob	Observed %ROUGH
Pulsation rate	59 p/min	161	23	-0.038	0.0179	28	0.040	
Limping	0.3	138	46	-0.168	0.2010		0.401	
A-phase (%)	<15	68	17	Ref			0.492	.16
	15.1-20	83		0.118	0.108			.18
	>20	16		-0.023	0.186			.15
B-phase (%)	≤45	42	17	Ref			0.759	.16
	45.1-50	87		0.089	0.123			.17
	>50	38		0.044	0.146			.17
C-phase (%)	≤15	116	21	Ref			0.413	.17
	15.1-17.5	40		-0.052	0.118			.16
	>17.5	7		0.278	0.229			.21
D-phase (%)	≤20	21	21	Ref			0.732	.18
	20.1-25	104		-0.063	0.152			.17
	>25	38		-0.131	0.172			.16
AB-phase (%)	63	163	21	0.012	0.0201		0.544	
CD-phase (%)	37	155	29	-0.017	0.0211		0.419	

[1]Deviance ratio is given for all variables with F<0.25.

Table 9: Results of final generalized linear regression analysis with prevalence of variables present on farms (#), deviance (dev.), β value with standard error, T-value and F-value, Odds ratio, and prediction (Pred. %) with standard error (total 163 farms) in association with teat-end callosity Roughness (%ROUGH).

Variable	Average or category	#	Dev.	ß	SE	T	F	OR	Pred. %	SE
Overall mean		163		-0.835	0.527	-1.59			0.168	0.006
Milk	Meter	36	65.8		Ref		0.009	1.00	0.201	0.015
measurement	Jars high	69		-0.380	0.121	-3.14		0.68	0.155	0.009
	Jars low	27		-0.280	0.151	-1.86		0.76	0.169	0.016
	None	31		-0.455	0.141	-3.22		0.63	0.146	0.013
Machine-on time	385 Sec	163	13.3	0.002	0.001	2.14	0.121	1.00		
Diameter short milk tube	10.6 mm	163	55.3	-0.064	0.032	-2.02	0.002	0.94		
Concentrate	No	46	51.6		Ref		0.010	1.00	0.164	0.012
feeding	Central	60		-0.137	0.118	-1.16		0.87	0.147	0.009
	Individual	57		0.206	0.115	-1.79		1.23	0.194	0.011
Vacuum difference short milk tube	10.9 kPa	163	50.2	-0.025	0.010	-2.40	0.003	0.98		
Brand liner	A	32	116.0		Ref		0.005	1.00	0.200	0.016
	B	3		-0.230	0.325	-0.71		0.79	0.166	0.042
	C	21		-0.126	0.174	-0.72		0.89	0.181	0.020
	D	21		-0.150	0.157	-0.95		0.86	0.177	0.018
	E	25		-0.354	0.158	-2.24		0.70	0.150	0.016
	F	11		-0.477	0.237	-2.01		0.63	0.135	0.026
	G	28		-0.125	0.149	-0.84		0.89	0.181	0.016
	H	22		-0.604	0.181	-3.34		0.55	0.121	0.016
Ratio flat and concave teats to round and pointed teats	11.3%	163	56.8	-0.022	0.007	-3.03	0.002	0.98		
Post-milking teat	No	14	40.6		Ref		0.027	1.00	0.142	0.019
disinfection	Dipping	69		0.333	0.176	1.89		1.40	0.188	0.010
	Spraying	80		0.103	0.174	0.59		1.11	0.155	0.009
Residual		144	789.0							

DISCUSSION

During 1997, the farms in this study all voluntarily participated in a field evaluation of a mastitis management program. The program evaluation was carried out on 263 farms spread geographically over The Netherlands (Hogeveen et al., 1997; Lam et al., 1998). Because farms voluntarily entered both the field evaluation of the mastitis management program and this study, the 192 farms in this study may differ in their attitude towards clinical mastitis from the average farm in The Netherlands. However, the distribution of both bulk milk SCC and proportion of high SCC cows was not different from the average in Dutch DHI participating farms (Hogeveen, personal communication).

Milk meters were associated with a higher %ROUGH than farms with low mounted milk jars or low milk line without milk measuring. An important factor in the milking machine is the nominal vacuum and the linked vacuum in the short milk tube. Nominal vacuum is set higher when milk has to travel a vertical distance from the claw to the milkline or milk meter, or when using a milk recorder jar. Differences in milk measurement will therefore have a large influence on all kinds of vacuum and airflow measurements and was, for that reason, forced into the model. Farms using milk meters had on average more cows and higher milk production. This may be the cause of the association of milk meters with %ROUGH.

In accordance with other research, the machine-on time appeared to be positively correlated (F=0.12) to %ROUGH in the final model of this study (Rasmussen, 1993; Shearn and Hillerton, 1996). Mein et al. (2003) stated that TEC is induced by the over-pressure applied by the liner on the teat. Over-pressure is also known as compressive load, the load that is applied on the teat by the closing or closed liner. Longer periods of pressure on the teat, by longer machine-on time, or longer periods of low flow period during milking, will increase %ROUGH (Rasmussen, 1993).

Larger diameters of the short milk tube were associated with less %ROUGH. A more stable vacuum near teat-end may be established with a larger diameter of the short milk tube due to a decrease in the slugs of milk in the milk line. However, in our data, vacuum differences in the short milk tube were also associated with a lower %ROUGH. The vacuum differences in the short milk tube in this study were calculated over several pulsation cycles and will contain regular and irregular fluctuations. Irregular fluctuations occur when the teatcup liners slip or fall from the teats or air enters when milking units are changed carelessly. Cyclic (regular) fluctuations are caused by the

cyclic movements of the liner in each pulsation cycle by increasing and decreasing the volume of the liner under the teat. Milk flow can cause marked changes in the vacuum below the teat. This can be reduced by wide bore short milk tubes. The reason for why large vacuum differences in the short milk tube in this research are associated with higher TEC scores may lie in the irregular fluctuations or in the relationship between high yielding cows, longer machine-on times, higher milk flows and high TEC scores. Machine-on time and milk production showed a correlation of 0.5 in this study.

Individual feeding of concentrate in the milking parlour is associated with lower TEC scores than central feeding which may have been caused by a better pre-stimulating effect and therefore shorter machine-on times. Individual feeding can be closely tuned to make the pre-stimulation effective (Samuelsson et al., 1992).

The association between higher TEC scores and the brand of liners may be due to differences in the liner dimension and through different pulsation and other milking characteristics between the brands. Mein et al. (2003) found that different liners apply a range of different over-pressures on the teat and that, with increasing vacuum levels and decreasing length of the C-phase of pulsation over-pressure will be increased. In this study, no direct relationship between vacuum level or pulsator phases and callosity was found. However, variance component analysis for differences between the brand with the highest TEC scores compared to the brands with significantly lower TEC scores revealed a higher operating vacuum (42.8 versus 41.1 kPa) and a shorter A-and C-phase of the pulsation (14.6 vs 17.3% and 10.6 vs 13.4%). The reason for increasing over-pressure and increasing %ROUGH may lay in the more rapidly compressed teat-end (Mein et al., 1987).

The proportion of concave and flat teat-ends on a farm had a large influence on the average proportion of %ROUGH. This is in accordance to earlier work where the relationship was shown within cows on a single farm (Neijenhuis et al., 2000). During milking, the massage pressure of the teatcup liner will be divided onto a smaller area when the teat-end is pointed teat than when the teat-ends are concave or flat.

Post-milking teat disinfection was associated with an increased %ROUGH. Other research showed an increase in risk of clinical mastitis when post-milking teat disinfection was used (Barkema et al., 1999; Elbers et al., 1998, Lam et al., 1997, Peeler et al., 2000). Post-milking teat disinfection might be significantly associated with %ROUGH due to a cause and effect reversal. Farms with a higher risk of mastitis may be applying post-milking teat disinfection more often. But there might also be a

real effect. Post-milking teat disinfection may increase TEC through poor teat conditioning properties of the teat dip composition (Hemling, 2002). More-over, the increasing effect on TEC of disinfection applied with spray was less than dipping the teats, which may be explained by the fact that with spraying less disinfection fluid is used.

In contrast to Ziesack et al. (1989), we did not find a decrease in the effect of lower operating vacuum on %ROUGH. However, in our study, a decrease in the effect on severe callosity of teat-ends was found when decreasing the milking vacuum from 50 to 40 kPa in a high pipeline milking parlour. In this study all farms used low pipeline milking parlours with an average operating vacuum of 42 kPa.

The residual mean deviance of the final model is high (mean deviance 5.5) which may reflect the heterogeneity or lack of fit in the data. Upon removal of two farms from the dataset that exhibited a large influence on the estimation (high measures of leverage), the model was not significantly altered and the mean deviance ratio did not decrease in order to obtain lower overdispersion. The dispersion parameter for the variance of the response is estimated from the residual mean square of the fitted model thus the standard errors of the fitted variables are correct. Perhaps a deficiency in the fit of the data is obtained since all cow effects were not included in the model. It is known from previous work that lactation stage, parity, and teat-end shape influence the proportion of TEC (Neijenhuis et al., 2000). Moreover, machine milking parameters did account for the 36% of the variance in %ROUGH between farms in the model. The percentage of concave and flat teats is included in the model and did account for a high proportion of the declared variation. However, no data about lactation stage and parity were recorded in this study. Farms were visited between January and June and, although the calving pattern in The Netherlands is spread over the year, lactation stages may still have differed between farms. On the other hand, other management or milking machine variables not taken into account in this study may have an association with %ROUGH.

Earlier research showed that TEC is a risk factor for clinical mastitis (Neijenhuis et al., 2001; Neijenhuis et al., submitted). Furthermore this research showed that %ROUGH is influenced by the milking machine and milking management on the herd level. Given this circumstantial evidence, TEC, and more specifically %ROUGH, can be used as an early warning signal for an increased risk of clinical mastitis in milking machine research and as a monitoring tool for the quality of milking, including the machine and management, in the field.

CONCLUSIONS

Milking machine and milking management influence %ROUGH. Variation in %ROUGH between farms is explained by cow factors such as teat-end shape and machine-on time and milking machine factors such as the liner and the vacuum. Cows with more TEC have also an increased risk of clinical mastitis, and because of this relationship, %ROUGH can be used as an early warning signal for increased risk of clinical mastitis, and therefore can be used as a monitoring tool to assess the quality of machine milking.

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CHAPTER 7

GENERAL DISCUSSION

F. Neijenhuis

Animal Sciences Group

Wageningen University and Research Centre

Division Applied Research

P.O. Box 2176, 8203 AD, Lelystad, The Netherlands

Francesca.neijenhuis@wur.nl

I INTRODUCTION

It is general knowledge that machine milking affects the rate of intramammary infection (**IMI**). The motivation of the research described in this thesis lies in the change of resistance of the teat barrier to bacterial invasion due to machine milking and its effects on the rate of new IMI (General Introduction: Table 1, point IV). The objectives of this study were to gain further insight into the formation of teat-end callosity (**TEC**), its association with clinical mastitis, the recovery time of teats after milking and the relation between TEC and machine-induced teat swelling. Research has been carried out on the development of TEC during the first months of lactation and cow factors affecting it (Chapter 2), differences in TEC within and between cows with clinical mastitis and healthy cows (Chapter 3), and the association between TEC and the incidence of clinical mastitis (Chapter 5). Also, machine milking and management factors influencing TEC were explored (Chapter 6). Recovery of teats after milking was graphically sketched (Chapter 4).

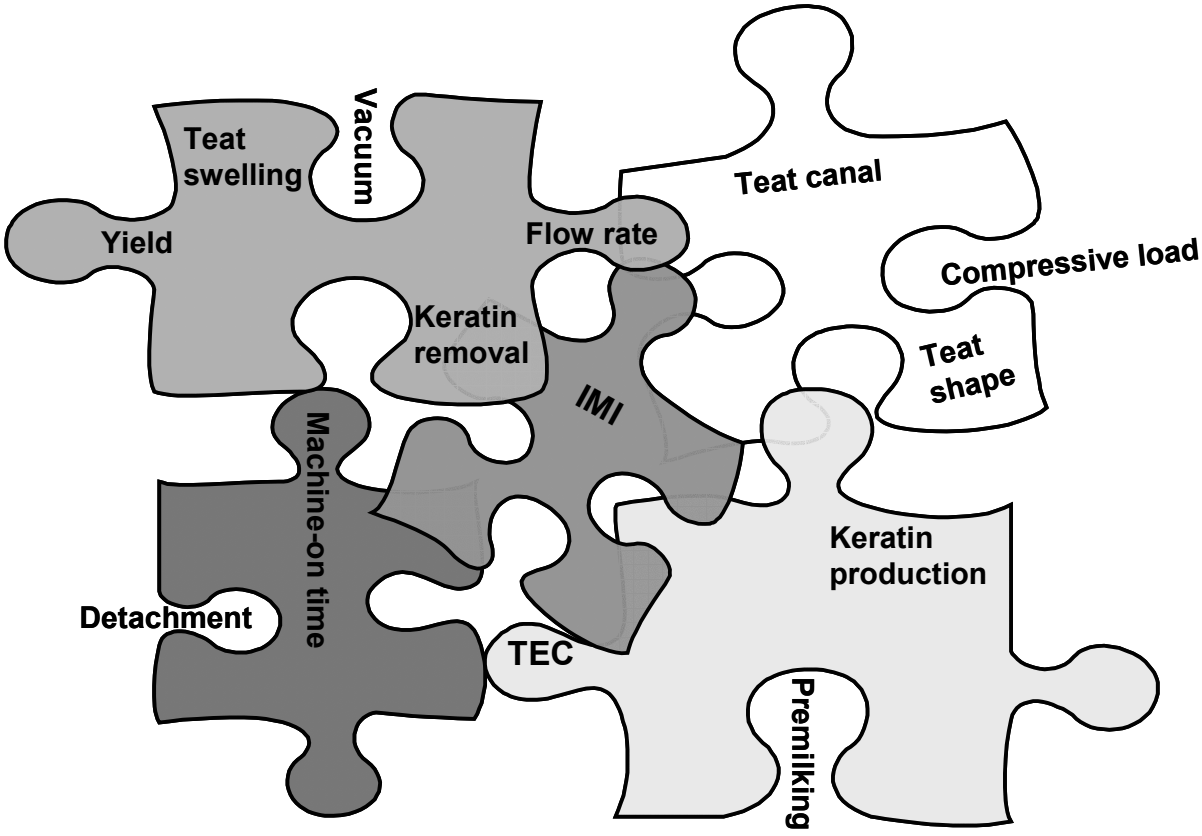


Figure 1. Pieces of the mechanisms through which machine milking may increase the rate of new intramammary infection (IMI) by changing the resistance of the teat to bacterial invasion as discussed in this thesis.

This discussion mainly deals with the relationships between IMI and teat condition, presented here as a jigsaw puzzle in which many relationships are not quite clear (Figure 1). In the second section factors influencing TEC and clinical mastitis as found in this thesis are discussed and compared with the literature on IMI and factors affecting it. The third section provides an overview of the mechanism by which machine milking influences the teat condition and how TEC and machine-induced swelling are related. Subsequently, the implications of the relationships found in the realms of machine milking and mastitis management are explored as well as current and future use of the TEC classification system subsequently. The conclusions outline the relevance of this thesis, and finally, further research to fill in the missing spots on the knowledge of teat condition in relation to IMI is proposed.

II. IMI, TEC AND INFLUENCING FACTORS

The teat is the first line of defence against invading mastitis pathogens into the udder. The white ring around the teat orifice is callus and is induced by milking. Teat end callosity (TEC) is a teat condition state that influences the resistance of the teat canal. In the TEC classification system used in this research the condition of the tissue around the teat orifice is first categorized as no ring, smooth or rough rings (**TECR**; Chapter 2). In addition, the rings are classified according to their thickness (**TECT**).

TEC and IMI

Callus results from hyperkeratosis or hypertrophy of the corneous layer or *stratum corneum* of the skin. It is a physiological response of the skin to chronic pressure or friction. In humans callosity is known as a hardened, thickened region of skin in an area subject to recurrent pressure or trauma. Common sites for callosity are the palmar surface of the hand and the plantar surface of the foot. Although callus formation is a normal physiological response, on teat ends it may become pathological when the functioning of the teat as the first line of defence against invading pathogens or as the pathway for milk removal is jeopardised. In this thesis evidence is presented that TEC indeed can cross the border from physiological to pathological (Chapters 3 and 5; Figure 2). Cows with thin smooth TEC rings proved to have the lowest risk of clinical mastitis (Chapter 5; Figure 2). This is in agreement with Hamann (1987) who stated that there were no data to suggest that TEC per se increases new IMI rate rather a slight increase in the degree of TEC could decrease

susceptibility to pathogens. Therefore, a low grade of TEC can be considered as a beneficial physiological response of the teat to machine milking.

Cows with no callosity rings seem to lack this beneficial response to machine milking and are at higher risk of clinical mastitis (Chapter 5). The mechanism underlying this phenomenon may be found in its coherence with a low keratin regeneration rate in the teat canal, as discussed hereafter.

In contrast, teats with thicker callosity rings have a less tightly closed teat canal that can be penetrated more easily by micro-organisms. In these callused teats, cells of the *stratum corneum* maintain a nucleus, a condition called parakeratosis. Teats with higher scores for rough TEC show a perivascular reaction: infiltration of lymphocytes, granulocytes or erythrocytes (De Man, 1998). Also, severe TEC may harbour pathogens (Kingwill et al., 1979). Note that teat lesions however, are not an extreme form of callosity since they have other causes, such as injury.

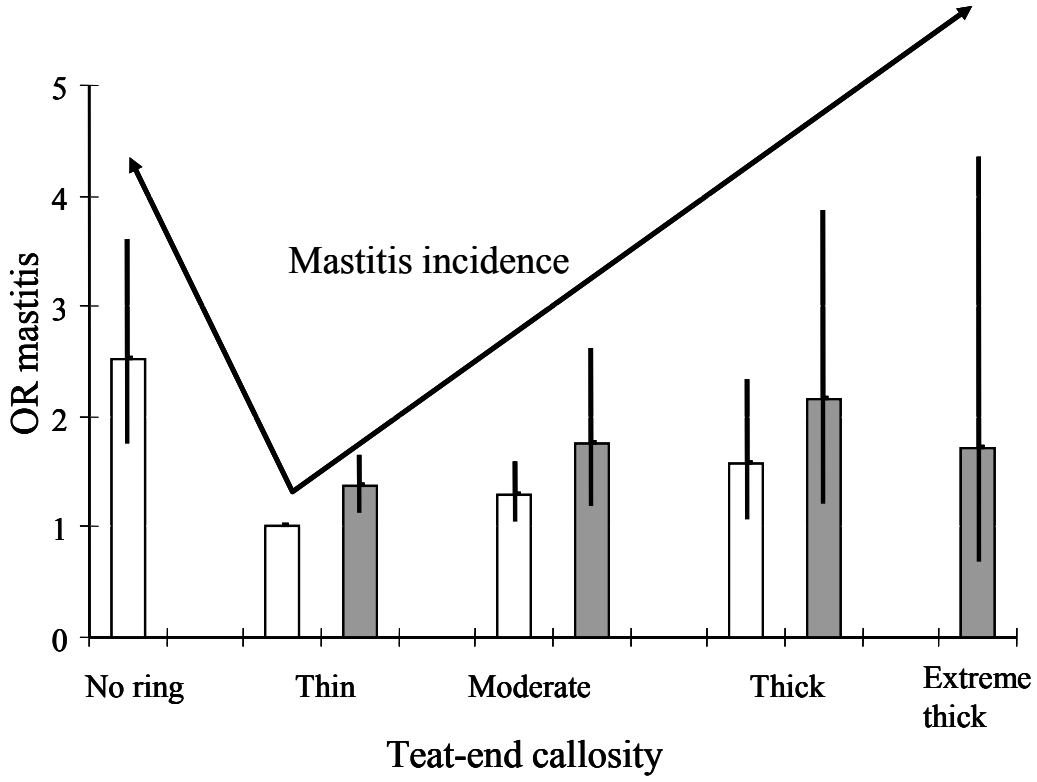


Figure 2. The Odds Ratio (OR) of the incidence risk of clinical mastitis during the month following the TEC score per TEC category (Chapter 5), where the open bars represent the category no ring and smooth rings and the filled bars represent the rough callosity rings (with 95% CI). The line represents the expectation on the direction of the mastitis incidence (Chapter 5).

Keratin and TEC

The presence of a smooth TEC ring is associated with a mild thickening of the keratin seal and underlying germinal layers of skin in the teat canal (Capuco et al, 2000). Keratin is a waxy substance that serves as a plug between milkings and aids in reducing penetration by micro organisms (NMC Glossary). The epithelium covering the longitudinally folded teat canal provides keratin and is very similar to normal skin on the outside of the teat. Furthermore, keratin has a high turnover; during milking, the action of pulsation of the liner on the teat, and the shear forces of milk flow remove significant amounts of keratin which is then expelled as cellular detritus together with trapped bacteria. The dynamic state of the keratin lining seems to be important, as it aids in preventing bacteria from colonizing the teat canal and in limiting bacteria from entering the gland. The rate of production and loss of keratin of the teat canal should be in equilibrium. It is estimated that 40% of the keratin should be washed out during milking to maintain a fresh and healthy layer in the teat canal (Woolford, 1997). With respect to milking, a smooth TEC ring may reflect a healthy balance between the degree of desquamation per milking and the rate of regeneration of the keratin seal within the teat canal (Mein et al., 2000; Williams and Mein, 1985). As stated, teats without callosity rings may exhibit a keratin regeneration rate that is too low. On the other hand, high TEC scores may reflect a too high turnover rate (Figure 3).

SCC and TEC

Analysis of data from a study on 15 farms in The Netherlands, where teats were observed before, during and after transition to an automatic milking system, showed that increasing cow somatic cell count (SCC) were associated with higher scores for TEC. SCC reflects clinical but moreover, subclinical mastitis. A study in the UK revealed a similar increased risk of subclinical mastitis with higher TEC although the results were not statistically significant (Lewis et al., 2000).

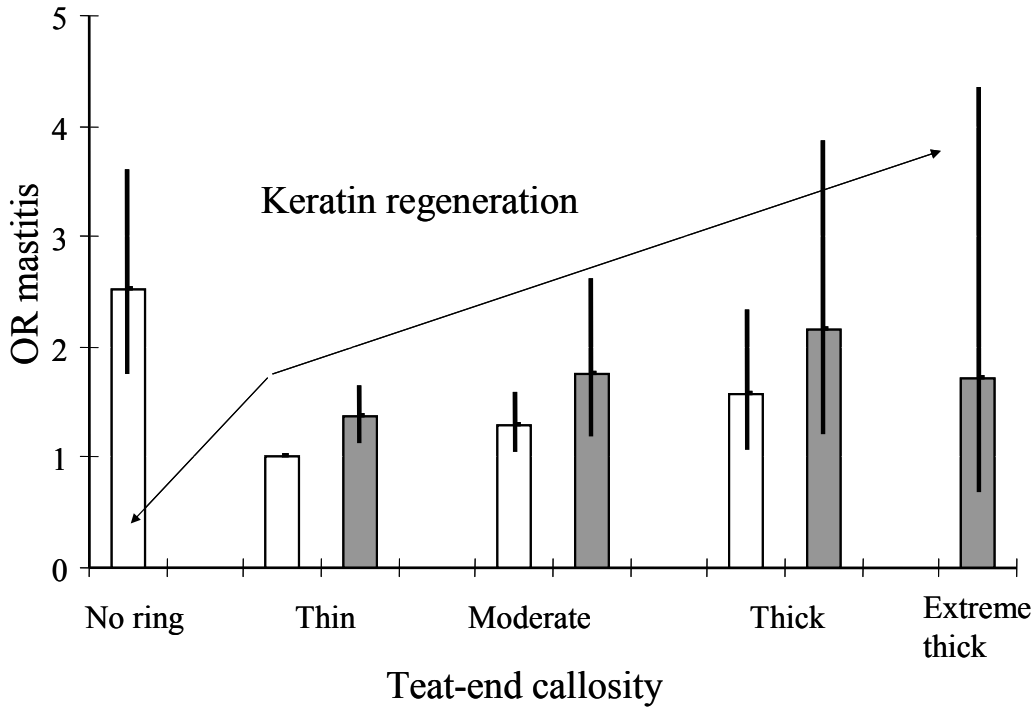


Figure 3. The Odds Ratio (OR) of the incidence risk of clinical mastitis during the month following the TEC score per TEC category (Chapter 5), where the open bars represent the category no ring and smooth rings and the filled bars represent the rough callosity rings (with 95% CI). The line represents the hypothesis on the amount of keratin regeneration.

So, teats with smooth and thin callosity rings are physiologically normal teats. An increase in the risk of IMI is observed when thickness and/or roughness of TEC increase. This is caused by less tightly closed teat canals after milking, a too high turnover of keratin, or the harbouring of pathogens in TEC. The higher risk of IMI in teats without a callosity ring may be caused by a decreased rate of keratin regeneration in the teat canal.

Table 1. Influence of factors found in this thesis on teat-end callosity thickness (TECT) and roughness (TECR) and on clinical mastitis (an empty cell indicates that the relationship was not studied, + indicates a positive relationship, - negative relationship, and • a lack of relationship), the influence of the same factors on IMI as found in literature, and the underlying mechanisms supposed.

	This thesis:				Literature:		
Factor	TECT	TECR	Clinical mastitis	Chapter	IMI	Reference	Mechanism[1]
First 8 weeks of lactation	+	+	+	2,5	+	Van de Geer et al, 1988; Barkema et al., 1999; Miltenburg et al., 1996; Rajala-Schultz et al., 1999	immune response, teat canal closure (Goff & Horst, 1997)
>120 DIM	-	-	-	3	-	Barkema et al., 1999	machine-on time
Older parity	+	+	+	2,5	+	Verhoeff et al., 1981	longer teats, stretchability teat canal (McDonald, 1968; Rathore & Sheldrake, 1977)
Rear versus front quarter	-	-		2	+	Verhoeff et al., 1981; Van de Geer et al, 1988	milk yield (Rothenanger et al., 1995), flow rate, milking empty teats, teat canal diameter (McDonald, 1968; Seykora & McDaniel, 1985)
Pointed teat end	+	+	+	2,3,5	+	Natzke et al., 1978	milk yield (Pander & Chopra, 1986), machine-on time, compressive load (Williams & Mein, 1980) migration macrophages (Zecconi & Hamann, 2004)
Inverted teat end	-	-	•	2,3	+	Hodgson & Murdock, 1980; Seykora & McDaniel, 1985; Jorstad et al., 1989	teat canal diameter, teat diameter (Chrystal, et al. 1999; Jorstad et al., 1989), milk flow rate (Pander & Chopra, 1986)
Long teats	•	•	•	3	+	Rupp & Boichard, 1999, Nash et al., 2003	compressive load, parity (Hebel et al., 1979); liner slip (Rogers & Spencer, 1991)
Milk yield	+	•	•	3	+	Schukken et el., 1990; Rogers et al., 1998; Fleischer et al., 2001; Kornalijnslijper, 2003	flow rate (Rupp & Boichard, 1999), machine-on time, TEC (Farnsworth, 1995; Bakken, 1981; Mein, 1998),
Machine-on time	+	+		2,6	•	Rasmussen, 1993	milk yield

[1] Likely mechanism underlying the relation between the factor and IMI found in this thesis, or in literature, or indirect evidence of a mechanism found in additional references (given between brackets).

Influencing Factors

In this thesis we found several factors that increase the amount of TEC and the incidence of clinical mastitis. In Table 1 these findings are compared with the relationships between these factors and IMI as found in the literature. However, not all IMI originate from increased TEC. There are also other ways by which machine milking affects the teat barrier. Therefore, the mechanisms likely to cause the increase of IMI are indicated in Table 1. Thereafter, based upon Table 1, the main factors affecting the teat barrier against IMI are discussed.

In Table 1 it is stated, for example, that in this thesis (Chapters 2 and 3) inverted teat-ends are found to be associated with lower TECT and TECR and pointed teat-ends with higher amounts of TECT and TECR. Furthermore, these two rows in Table 1 show that the results of this thesis reveal that pointed teat-ends have a higher risk of clinical mastitis (Chapters 3 and 5). Only Natzke et al. (1978) found a positive relationship between pointed teat-ends and IMI. Several other studies (Hodgson and Murdock, 1980; Seykora and McDaniel, 1985; Jorstad et al., 1989) found a positive relationship between IMI and inverted teat-ends. The likely mechanisms underlying these phenomena are found in larger teat canal diameters in cows with inverted rather than with pointed teat-ends (Jorstad et al., 1989), which make bacterial penetration into the udder easier (Seykora and McDaniel, 1985). Larger teat canal diameters may correspond to larger teat diameters (Chrystal et al., 1999; Jorstad et al., 1989). Cows with pointed teat-ends have a higher milk yield (Pander and Chopra; 1986). This "high milk yield factor" is more extensively discussed in the next section. Also, cows with pointed teat-ends have a longer machine-on time compared to cows with inverted teat-ends (Chapter 2) which will induce more TEC. The relationship between high amounts of TEC and IMI is explained elsewhere in this chapter (section on TEC and IMI). Pointed teat ends will experience higher compressive loads by the liner on the teat end than inverted teat-ends (Williams and Mein, 1980), which, in addition, may lead to higher TEC scores and to decreased migration of macrophages (Zecconi and Hamann, 2004). On the other hand, quarters with inverted teat-ends tend to have higher milk flow rates compared to pointed teat-ends (Pander and Chopra, 1986) which may cohere with insufficient keratin regeneration rate, thus making penetration by pathogens easier (more information on flow rates in the section on Milk Yield and Flow Rate).

Based on the data presented in Table 1, two factors affecting the teat barrier draw attention: milk yield and flow rate. The paramount role of milk yield and flow rate will be discussed in the next

section. Machine milking effects on teat condition will be discussed in the section on Machine milking and teat condition.

Milk Yield and Flow Rate

Discussion is ongoing as to whether very high yielding cows have an increased risk of IMI (Schukken et al., 1990; Gröhn et al., 1995; Rogers et al., 1998; Waage et al., 1998; Suriyasathaporn et al., 2000; Fleischer et al., 2001; Kornalijnslijper, 2003). A relationship between peak flow rate and IMI has been found in several studies (Van de Geer et al., 1988; Grindal et al., 1991; Grindal and Hillerton, 1991; Duda, 1995; Lacy-Hulbert and Hillerton, 1995; Dodenhof et al., 1999; Rupp and Boichard, 1999). Part of the relationships between IMI, high peak flow rate and high milk yield do not involve an increase in TEC. Capuco et al. (1990) hypothesized that, as milk production increases, more keratin is lost during milking. Cows with higher milk yield often exhibit higher peak flow rates (Miller et al., 1976; Wellnitz et al., 1999; Wagner and Ruegg, 2002; Inderwies et al., 2003). In high yielding cows intramammary pressure is higher, probably due to larger vertical milk columns in the udder cisterns (Bruckmaier, personal communication). Quarter peak flow rate of >1.6 kg/min (compared to < 0.8 kg/min) was associated with a 12 fold increase in IMI (Grindal and Hillerton, 1991) and udder peak flow rate as well as mean flow rate of 3.5-4.0 kg/min was associated with the lowest IMI rate (Roth et al., 1998). The shear forces of high peak flow rate (Williams and Mein, 1986) may remove keratin above the physiologically preferred amounts. Lacy-Hulbert et al. (1996) stated, however, that keratin loss during milking appears to be controlled by liner compression rather than by the rate of milk flowing through the teat canal. Higher flow rates and higher milk yield are associated with higher stretchability of the teat canal (Rathore and Sheldrake, 1977), and are caused by a combination of thinner teat walls and the fact that the teat canal is surrounded by a small volume of elastic tissue (rear versus front quarters, Ledu et al., 1994). This may increase the penetrability of the teat canal. Teats with high milk flow rate show less machine-induced thickening of the teat-end (Naumann and Fahr, 2000). A high peak flow rate is positively correlated to a higher milk yield and a longer milk flow decline phase (Tancin et al., 2002). A longer quarter milk flow decline phase (> 80s) increased SCC. The decline phase may be longer because high milk flow rates at early milking are limiting the milk flow towards the end of milking (Bruckmaier, 2000). High milk flow rate is associated with shorter teat canals (Naumann and Fahr, 2000) and a shorter teat canal increases the probability of IMI (Lacy-Hulbert and Hillerton, 1995; Gulyas and Ivancsics, 2001) Also, higher milk yield induces more machine-

induced teat canal lengthening (Gleeson et al., 2002), which may influence teat canal closure after milking. Higher milk yields coinciding with larger teat canal diameters and higher intramammary pressure, may lead to milk leakage, and therefore be associated with increased IMI (Jorstad et al., 1989; Waage et al., 1998).

Thus, in addition to an increase in TEC, high milk flow rate, as well as short and wide teat canals may be predisposing factors for an increased likelihood of IMI.

Pathogens and Teat Condition

The ability of bacteria to invade through the teat duct varies with the type of pathogen. Specific types of pathogens may differ in their opportunistic use of orifice dysfunction to enter or multiply in the teat canal. *Escherichia coli* is a so-called environmental pathogen. Its primary reservoir is the environment of the dairy cow. Bacteria of environmental origin are most likely to pass directly through the duct when it is not completely closed (Lacy-Hulbert and Hillerton, 1995). In this study (Chapter 3), clinical *E. coli* mastitis is associated with lower levels of hyperkeratosis on the teat-end compared with clinical mastitis caused by other pathogens. *E. coli* mastitis is also more common in rear than in front quarters and less common on teats with lesions (Verhoeff et al., 1981). Barkema et al. (1999) found that a short d-phase (<150 ms) in the pulsation cycle had the strongest association with clinical *E. coli* mastitis. Short d-phases will increase machine-induced teat swelling because of limited time of compressive load and this swelling may be a predisposing factor for teat duct colonization by environmental pathogens (Zecconi et al., 1992). Teats without a visibly beneficial, physiological response (no callosity ring) and/or with high level of machine-induced swelling may be more easily penetrated by environmental pathogens during the intermilking period due to insufficient regeneration of keratin and/or less (tightly) closed teat canals.

Contagious pathogens, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, are associated with the cow. These pathogens find their transmission largely limited to the milking process (Fox and Gay, 1993). Contagious pathogens first colonize the duct where they grow. Skin lesions are often colonized by *Staphylococcus aureus* (Myllys et al., 1994; Fox and Cumming, 1996; Burmeister et al, 1998; Larsen et al., 2000). Teats with high scores of TEC have an increased risk of *Staphylococcus aureus* mastitis compared with teats with less TEC (Zadoks et al., 2001), which supports the findings in Chapter 3. Schukken et al. (1991) reported an association with lower rates of clinical *Staphylococcus aureus* mastitis when the milking machine is serviced. This indicates that

a positive effect of maintenance of the milking machine exists. Teats milked without pulsation are more easily penetrated by pathogens and *Streptococcus agalactiae* bacteria have higher survival rates (O'Brien, 1989; Bramley and Schulze, 1991). Although Lacy-Hulbert and Hillerton (1995) found that *Streptococcus agalactiae* mastitis increased with increasing peak flow rates, predominantly contagious pathogens appear to take advantage of the affected teat condition.

Therefore, specific pathogens differ in their opportunistic use of orifice dysfunction to multiply in, or enter the teat canal. This insight may, in part, explain the different findings in IMI rate and associated factors in the literature.

III. MACHINE MILKING AND TEAT CONDITION

TEC is not only influenced by factors as discussed in section II but also by machine milking. Besides, machine milking induces teat swelling and has a large influence on the regeneration of keratin. The two components of milking, vacuum and compressive load (also known as overpressure), should assure milk flow from the teat without damage to the teat tissue. Ideally, teats should only slightly change because of milking. Milking vacuum aids in opening of the teat canal and the flow of milk, but also induces teat swelling (Bramley et al., 1992; Isaksson and Lind, 1992). The compressive load is a mechanical force on the teat-end caused by the collapsing liner, which contributes to the teat canal closure and transport of blood and lymph back to the udder

Ten years ago, Hamann et al. (1994) gave a review of machine-induced teat tissue changes. The major conclusions were: 1) the liner design should be optimised, 2) the average teat-end vacuum should be in a range of 36-40 kPa and 3) the duration of liner closure (liner more than half closed) should be approximately $^1/_3$ second (corresponding to a d-phase of approximately 9 %). These conclusions remain valid.

The teatcup liner is the only part of the milking machine that has direct contact with the cow's teat. The liner design is the most difficult part when optimising milking performance, teat condition and risk of transfer of pathogens (Hamann et al, 1994). The optimum length and diameter of the liner is dependent on the length and diameter of the teat.

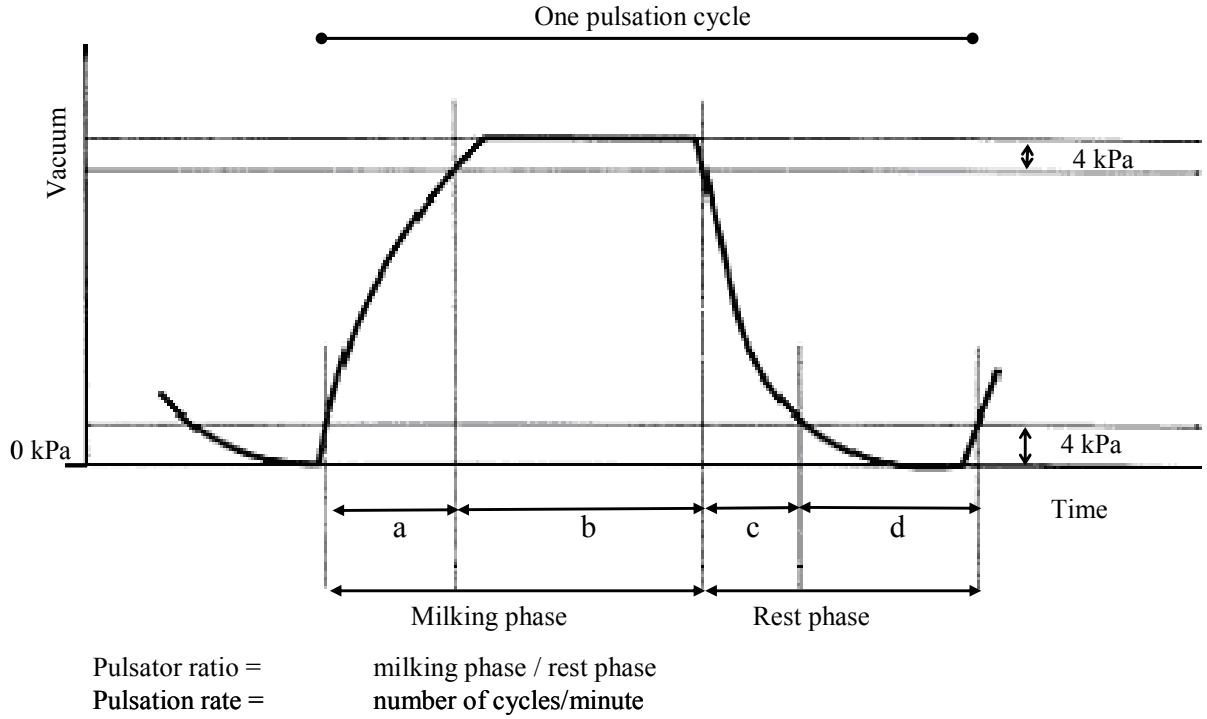


Figure 4: Pulsation chamber vacuum record.

As stated in the General Introduction of this thesis, the compressive load can be estimated from the shape of the curved liner (measured as the r:R ratio) and the pressure difference across the liner just underneath the teat (Chapter 1: Figure 2; Figure 5). The compressive load applied by the liner on the teat should be 8 to 12 kPa, similar to the bovine arterial pressure of 10 kPa (Mein et al., 1987). For successful commercial liners a ratio of r:R =1:5, with an average milking vacuum, the pressure on the teat will be approximately 10 kPa. Very soft, thin walled liners (r:R = 1:15) will result in a much lower compressive load of 3-4 kPa. Thick walled liners with a high tension (ratio r:R = 1:2) will have a high compressive load: 25 kPa.

The compressive load will induce TEC as a normal physiological response to pressure and friction (Hamann, 1987; Mein et al., 2003). An increase in compressive load by positive pressure pulsation, will increase the level of keratin and TEC (Hamann, 1987; Capuco et al., 2000; Figure 5). In recognition of the importance of the compressive load, a measurement of the 'Touch Point Pressure' is proposed as a part of the renewed ISO standards which will be presented in 2005 (ISO 3918, concept 2004).This pressure difference across the liner barrel at which the opposing walls of the liner, when it is mounted in its shell, touch each other, may estimate the compressive load. Different

brands of liners induce different compressive loads (Mein et al., 2003). In Chapter 6, a large proportion of the variance in percentage of teats with rough TEC on a farm could be explained by the brand of the liner.

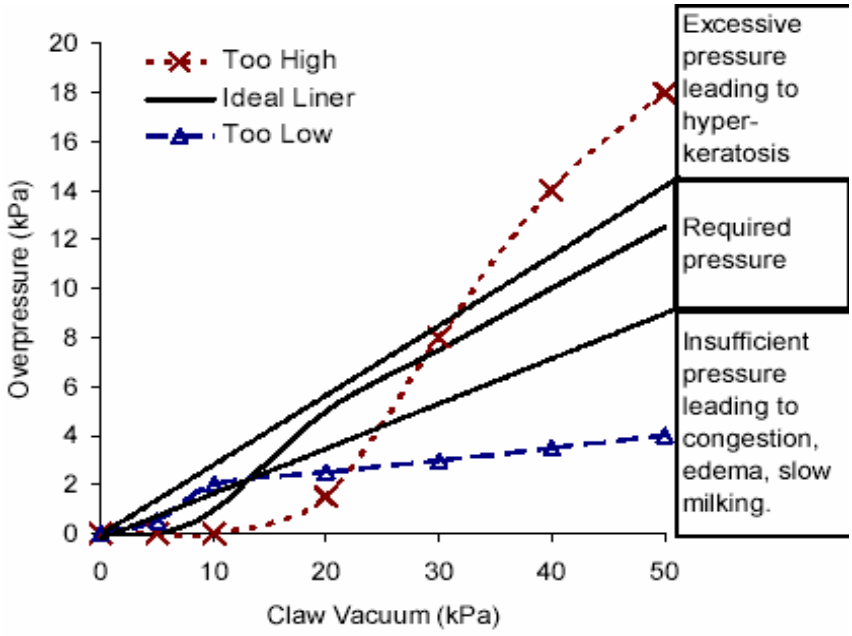


Figure 5. Hypothetical relationship between compressive load (overpressure) applied by different liners and the pressure required to relieve congestion and edema with increasing milking vacuum level (from Mein et al., 2003).

The characteristics of a pulsation cycle and the terminology used when discussing pulsations have been given in Figure 4. Pulsator settings like the length of the phases or the pulsation rate have great impact on machine-induced swelling of teats (Hamann and Mein, 1996; Zecconi et al., 2000). The teat is influenced by compressive load particularly during the d-phase. Therefore, the time span of the compressive load on the teat depends on pulsation characteristics. The ISO standard recommends a minimum length of the d-phase of 150 milliseconds or 15% of the pulsation cycle (ISO 5707, 1996). Shorter d-phases were found to increase IMI (Reitsma et al., 1981; Mahle et al., 1982; Reitsma and Gupta; 1987), and, depending on the liner type, increase teat thickness (Hamann and Mein, 1996). On the other hand, Østeras et al. (1990) found more teat lesions with increasing pulsator ratio (milking phase > 70%). The same authors (Østeras et al., 1995) found the best udder health in herds with a d phase > 330 ms. Increasing the length of compression time (decreasing pulsator ratio or increasing the length of the d-phase) or the degree of compressive load applied by

the liner, increases the peak milk flow during the next pulsation cycle by reducing the degree of congestion in the tissues of the teat surrounding the teat canal (Williams et al., 1981). However, changing the pulsator ratio from 70:30 to 50:50 caused a reduced peak flow rate and prolonged high milk flow period, whereas the main flow rate did not change in more recent research (Pfeilsticker et al., 1995). The effect of the length of the pulsator phases or the pulsator ratio, did not account for a significant proportion of the variation of %ROUGH teats in a herd in our study (Chapter 6). The absence of such a relationship may be due to the fact that, on the farms studied, the lengths of the b- and d-phases were within the recommended ISO standards (ISO 5707, 1996).

Milking with liners mounted under high tension, will increase the compressive load, as discussed earlier, and stimulates keratin production and callosity (Capuco et al., 2000). It is likely that excessive overmilking results in removal of too much keratin and more teat canal injury (Gleeson et al., 2003), and results in teat-ends with thicker and rough callosity rings. Non-conventional milking without pulsation may improve teat condition but will result in an increase in IMI (Woolford and Phillips, 1978; Whittlestone et al., 1980; Capuco et al., 1994; Lacy-Hulbert et al., 1996). The same is true for conventional milking using very soft liners with very light clusters (Paulrud, 2003). The rate of IMI probably increases due to insufficient removal of keratin. Figure 3 represents the hypothesis on keratin regeneration and its relation with TEC and the incidence risk of clinical mastitis.

Some pulsators can rapidly change the pressure in the pulsation chamber during the pulsation cycle of the teatcup, thereby causing short transition phases (a- and c-phase) in the pulsation curve. With short transition phases, the teatcup liner may open and close more rapidly. Although a greater compressive load is exerted on the teat, more teat swelling was found which might lead to increase of TEC (Neijenhuis, 1993; Neijenhuis et al., 1999). However, the effect of the length of the transition phases of the pulsator, did not account for significant variation of percentage of rough TEC in a herd in our study (Chapter 6). The compressive load exerted on the teat will greatly depend on how different liners respond to these short transition phases in the pulsation chamber.

Detachment of the teat cup liners at a milk flow of 400 compared to 200 gram/min/udder, increases average milk flow rate, but not peak flow rate, and lowers the machine-on time, resulting in better teat condition (Rasmussen, 1993). Detachment levels up to 700 gram/min/udder when milking three times a day will decrease machine-on time without leaving too much milk in the udder (Mein, 1998). Quarter take-off decreases milking on empty front teats and decreases roughness of TEC on

front teats (Neijenhuis et al., 2004). Overmilking will lead to more teat lesions (Østeras et al., 1990).

High milking vacuum, combined with wide pulsator ratio, increases machine-induced teat swelling (Hamann et al., 1993). Rasmussen and Madsen (2000) found only a slight improvement in machine-induced teat thickness with lower milking vacuum. The milking vacuum may be a force that induces TEC (Michel et al., 1974; Figure 5), but this was not found in our research (Chapter 6). From the vacuum measurement, the vacuum difference at the teat-end was the only factor that explained a significant part of the variance in percentage of teats with rough TEC between farms.

Teats need time for closure of the teat canal after milking. Machine-induced teat swelling reflects the penetrability of the teat canal (Chapter 4; O'Brien, 1989; Zecconi et al., 1992), and therefore an increase of IMI may be expected with high values of machine-induced teat swelling (Zecconi et al., 1996). Even when universally accepted milking machine settings are used, some congestion and edema of the teat-end occurs at the end of peak milk flow (Bramley et al., 1992). During the low flow period, hyperaemia in the teat wall will increase teat thickness (Isaksson and Lind. 1992) and can restrict milk flow from the udder to the teat (Williams et al., 1981). Machine-induced teat swelling or teat thickness increases when cows are milked without pulsation, with a high vacuum, or when long low-flow periods occur (O'Brien, 1989; Ziesack et al., 1989; Hamann and Mein, 1990; Bramley et al., 1992; Zecconi et al., 1992, Hamann et al., 1993; Rasmussen, 1993; Hamann and Mein, 1996). Gleeson et al. (2002) found that teat recovery, in terms of altered teat proportions, after milking takes more than 5 hours. This concurs with the results of our research (Chapter 4). Teat canal penetrability is an important parameter for management aspects. It is advised that cows remain standing for 45 minutes after they have been milked. Teat recovery takes a longer time than the expected 45 minutes, thus caution is necessary when increasing the milking frequency. This point is emphasized through research on machine-induced teat swelling with different milking intervals (Neijenhuis and Hillerton, 2003). Short milking intervals can lead to an accumulation of teat damage. Moreover, intervals of 3 hours increase IMI (Smolders et al., 2001).

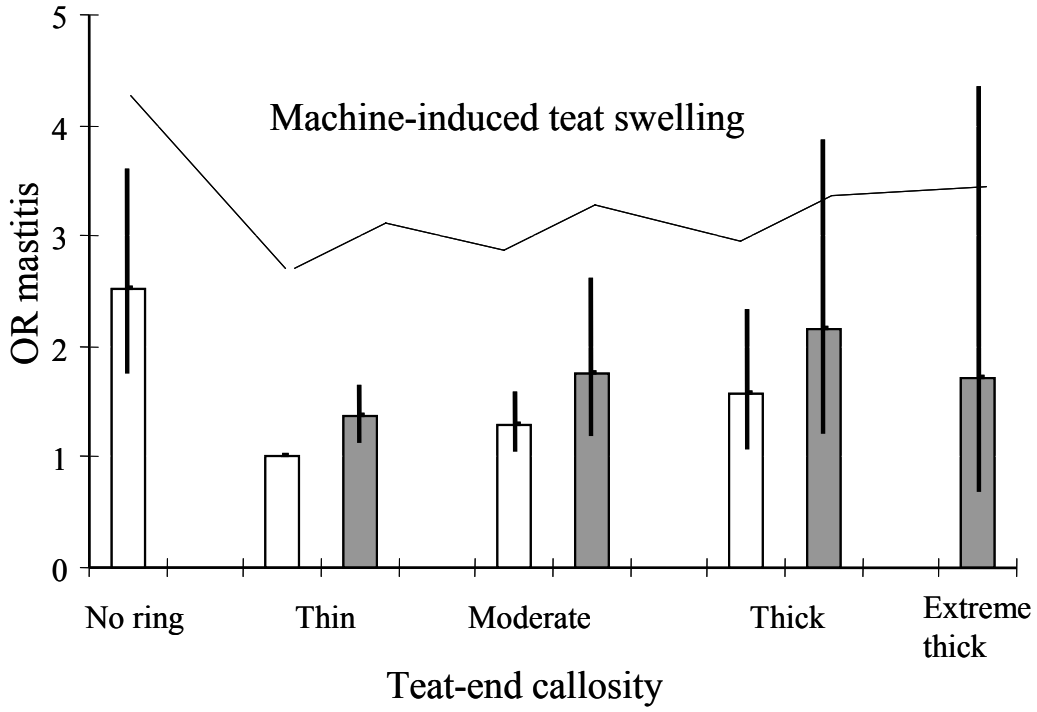


Figure 6. The Odds Ratio (OR) of the incidence risk of clinical mastitis during the month following the TEC score per TEC category (Chapter 5), where the open bars represent the category no ring and smooth ring and the filled bars represent the rough callosity rings(with 95% CI). The line represents the hypothesis on the direction of machine-induced teat swelling.

A hypothesis at the start of this thesis was that machine-induced teat swelling after a single milking has a relationship with the extent of TEC during lactation (Figure 6). Hamann (1987) stated that machine-induced teat stiffness may be even more important than TEC in explaining new IMI. If machine-induced teat swelling does have a relationship with TEC then the measurement of swelling could be used in short term experiments. Machine-induced teat swelling has been shown to increase with increasing pulsator ratio (Hamann and Mein, 1996). Therefore, different pulsator settings (55:45, 65:35 versus 75:25) were tested for the effect on TEC and the relationship with machine-induced teat swelling. Data from this study showed fewer differences than expected, probably because due to the fact that the cows exhibited low levels of TEC during this 9-month experiment (unpublished data). The only significant effect of the widened pulsator ratio from 65:35 to 75:25 was shown in an increased TEC roughness on front teats. Furthermore, there are indications that cows with larger teat cistern changes due to milking, most probably the higher producing cows,

show more TEC. Furthermore, only an indication of a positive correlation between roughness of TEC and machine-induced thickening of the teat-end was found.

The applied vacuum will, not only thicken teats but they will also be elongated during milking. Teat lengthening can be measured with ultrasound in the teat tip as teat canal length (Chapter 4; Gleeson, 2002). The closed teat canal is folded in a spiral fashion (Giesecke et al., 1972). During milking, the spiral state may be unfolded, lengthening the teat canal. This may ease penetration of pathogens into the udder. More discussion on the relationship between teat canal length and IMI can be found in section II.

Thus, machine milking influences the teat condition through the duration and level of both the applied compressive load and vacuum. A strong relationship between machine-induced teat swelling and TEC was not found.

IV SCORING OF MACHINE-INDUCED TEAT CONDITION

As stated in the General Introduction of this thesis, the role of machine milking in mastitis occurrence may be quantified by retrospective analyses of the clinical mastitis cases. Part of the IMI can be explained by a deterioration of the teat condition and thereby a decline of the first line of defence. By quantifying teat condition we can assess the frequency of IMI and thus evaluate the milking process. Insight into teat condition can be useful, both for research purposes as well as for monitoring in the field. In this section, the value of measuring teat swelling and scoring teats will be discussed.

Teat condition problems associated with machine milking have been documented since the mid 20th century (Espe and Canon, 1942; Udall, 1947; Pier et al., 1956; Jackson, 1970). Known research on TEC was carried out with a classification system that did not make a distinction between smooth and rough callosity rings (Johansson, 1957; Michel et al., 1974; Bakken, 1981; Graf and Gedek, 1983; Ebendorff and Ziesack, 1991; Shearn and Hillerton, 1996) or included acute lesions (Farnsworth et al., 1978; Sieber, 1980; Thompson and Sieber, 1980). An updated CAB literature search on milking machines and mastitis showed that, in recognition that teat canal defences play a major role in the risk of udder infections, considerable international effort was

launched after 1995 to develop and standardize methods for evaluating the condition of teat tissues after milking (Reinemann et al., 2003).

Teat scoring systems are available for field workers and farmers. Visual assessment of teats, comparison with photographs, and scoring schemes in nominal categories are the most common methods. In addition, many veterinarians and advisers have devised their own unique classification systems. The first publication presenting the TEC classification system described in this thesis (Chapter 2) and the possible relation between TEC and IMI was in 1996 (Neijenhuis and Schuiling, 1996). Since that time, the use of a TEC classification system to quantify changes in teat condition has been adopted and its use is recommended by several authors (Schukken and Kremer, 1996; Van der Haven et al., 1996; Levesque, 1998; Mein et al., 2000; Hulsen, 2003; Levesque, 2004). The TEC classification system is used in several Dutch research projects (Smolders and Bruin, 2000, 2001; Smolders, 2001; Thomassen et al., 2003; Galama et al., 2004). The scoring system is published by the Research station for Cattle, Sheep and Horse Husbandry (currently part of the Animal Sciences Group, Wageningen UR) on a single sheet for practical use in the milking parlour.

Teat condition research was initiated again in various places around the world since the mid 1990's. Several researchers took the initiative to join forces and as a result the Teat Club International[1] was established. The Teat Club International is an informal international group of researchers and extension workers interested in teat condition and machine milking. To provide an internationally acceptable, simple yet reliable method to assess teat condition in the field, the Teat Club International proposed a set of guidelines for observing teat condition and interpreting the findings (Mein et al., 2000; Ohnstad et al., 2003). For routine field evaluation of TEC, particularly for extension workers, veterinary surgeons and farm managers, a simplified version of the detailed research TEC classification system was proposed. Teat-ends fall into four classes (Figure 7):

1. No ring where the teat-end is smooth with a small even orifice (**Score N**; corresponds with score N in the large system).

2. Smooth or slightly rough ring where a raised ring encircles the orifice. The surface is smooth or very slightly rough. No keratin fronds evident (**Score S;** corresponds with score 1A &1B).

[1] The Teat Club International members are currently: J.R. Baines, J.S. Britt, N. Cook, R Farnsworth, T. Hemling, J.E. Hillerton, G. Mein, W.F. Morgan, F. Neijenhuis, I. Ohnstad, M.D. Rasmussen, D. Reinemann, and L. Timms.

3. Rough ring where a raised, roughened ring has fronds of keratin extending 1 – 3mm from the orifice (**Score R;** corresponds with score 1C, 2A &2B).

4. Very rough ring where the keratin extends more than 4mm from the orifice. The rim of the ring is often cracked (**Score VR;** corresponds with score 2C & 2D).

This system was used in Chapter 6 to examine the relationships between severe TEC and machine milking and management.

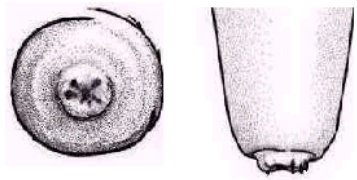
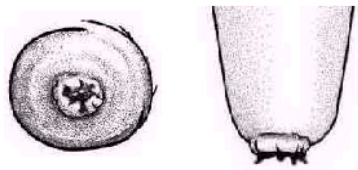
Score	Description	Illustration
N	**No ring** The teat-end is smooth with a small, even orifice. This is a typical status for many teats soon after the start of lactation	
S	**Smooth or Slightly rough ring** A raised ring encircles the orifice. The surface of the ring is smooth or it may feel slightly rough but no fronds of old keratin are evident.	
R	**Rough ring** A raised, roughened ring with isolated fronds or mounds of old keratin extending 1 - 3 mm from the orifice.	
VR	**Very Rough ring** A raised ring with rough fronds or mounds of old keratin extending 4 mm or more from the orifice. The rim of the ring is rough and cracked, often giving the teat-end a "flowered" appearance.	

Figure 7. Teat-end callosity classification system for field work (Mein et al, 2000).

Based on this simplified TEC scoring system, an investigation of the possible causes of TEC is recommended when more than 20% of the teats or cows score R or VR, or more than 10% of the teats score VR (Ohnstad et al., 2003).

Machine-induced swelling can be measured, and thus quantified, with ultrasound pictures or with the cutimeter (Chapter 4). Ultrasound measurements of teat parameters have shown to be a useful tool to study changes in teat properties caused by milking. It is however, a tool that can only be

used for research purposes because it is time consuming and use of the equipment requires a sufficient skill level. Severely swollen teats can be scored by sight. The Teat Club International states that if 20% or more of the teats in a herd exhibit marked swelling or palpable rings at or near the top of the teat or swelling and hardness at or near the teat-end can be classified as firm, hard or swollen, further investigation is required (Mein et al., 2000).

The Teat Club International stated that ideally teat condition should be evaluated on a monthly basis since teat condition may change rapidly. Teat scoring should be carried out immediately after cluster removal. The cows scored should be chosen randomly in order to properly represent the herd. Although scoring is carried out on individual teats, observations are generalised at the cow level. When a cow has one ore more teats with a problem then that cow is considered to have a problem thus assuming that the teat condition scoring of TEC and swollen teats are correlated within a cow (Reinemann et al., 2001). The result of examining teats provides the number of cows with one or more teats affected. When the teat condition problem does not appear to be correlated within cows, a statistical analysis should be carried out on teat level. Agreement between observers on teat condition problem has shown to be good (Morgan, 1999; Houe et al., 2002; Chapter 2). Since the outcome of the teat scoring is made to be a yes/no response, the statistical test is based on a binomial distribution. The Teat Club International recommends scoring all teats of all cows in the herd if time and herd size allow, or at least 80 randomly selected cows or 20% of the herd (whichever is greater) (Reinemann et al., 2001).

V. CONCLUSIONS

A healthy teat of a dairy cow has a good balance between the physiological reaction to machine milking and maintaining its first line of defence mechanism against invading mastitis pathogens. Increasing rates of IMI are related to a high degree of machine-induced swelling, and/or a high level of TECT and/or TECR, and/or the absence of TEC. Pathways through which these machine-induced changes lower the resistance of the teat to bacterial invasion are the openness of the teat canal, harbouring of pathogens in TEC, and significantly increased or decreased levels of keratin regeneration rate.

The milking machine must be adjusted to the cow in order to achieve an optimal, square milk flow profile. The pulsation characteristics of the liner, and the applied milking vacuum, should be within

the physiological borders to make milk flow possible but also give a suitable compressive load to avoid congestion and edema of the teat tissue. Development of a more suitable method of milking than just one method of pulsation and vacuum during a whole milking may be controlled milking on basis of actual quarter milk flow. Too high peak flow rates should be avoided because this may increase the erosion of keratin too much, through shear forces of the milk in the teat canal.

The liner should have correct dimensions in terms of length and diameter relative to the average herd teat dimensions and give the teat good support and pulsation during milking. The compressive load of the liner on the teat should approximate the bovine arterial pressure of 10 kPa. Of course variation between cows within a herd should be as small as possible, since there will only be one milking machine, and especially one kind of liner, that must be as near feasible to 'one size fits all'.

A strong relationship between machine-induced teat swelling and TEC was not found. Machine-induced teat swelling may decrease milk flow which could lead to milking on empty teats, longer machine-on time and higher levels of TEC.

Specific pathogens differ in their opportunistic use of orifice dysfunction in order to multiply in or enter the teat canal. Environmental pathogens seem to benefit from teat swelling because the teat canal cannot close quickly enough or completely. Contagious pathogens may penetrate the teat canal more easily when a high level of TEC is present in which they can harbour. This insight may, in part, explain the different findings in IMI rate and associated factors in the literature.

Classification of teat condition is an essential tool in milking machine research and a useful monitoring tool of the quality of milking in the field. Protocols for systematic evaluation of teat condition are available. The original eight-category TEC classification system should be used in milking machine research. The simplified four-category TEC classification system can be used in field evaluation, comparable to other monitoring instruments (such as body condition score, rumen fill score, locomotion score and hygiene score). Ultrasound pictures facilitate quantification of machine-induced teat swelling during research and scoring of visibly swollen teats provides a good method for assessing teat condition changes caused by congestion or edema in commercial herds. A teat with a smooth thin callosity and with low machine-induced swelling is a healthy teat. Deterioration of teat condition will increase the risk of new IMI.

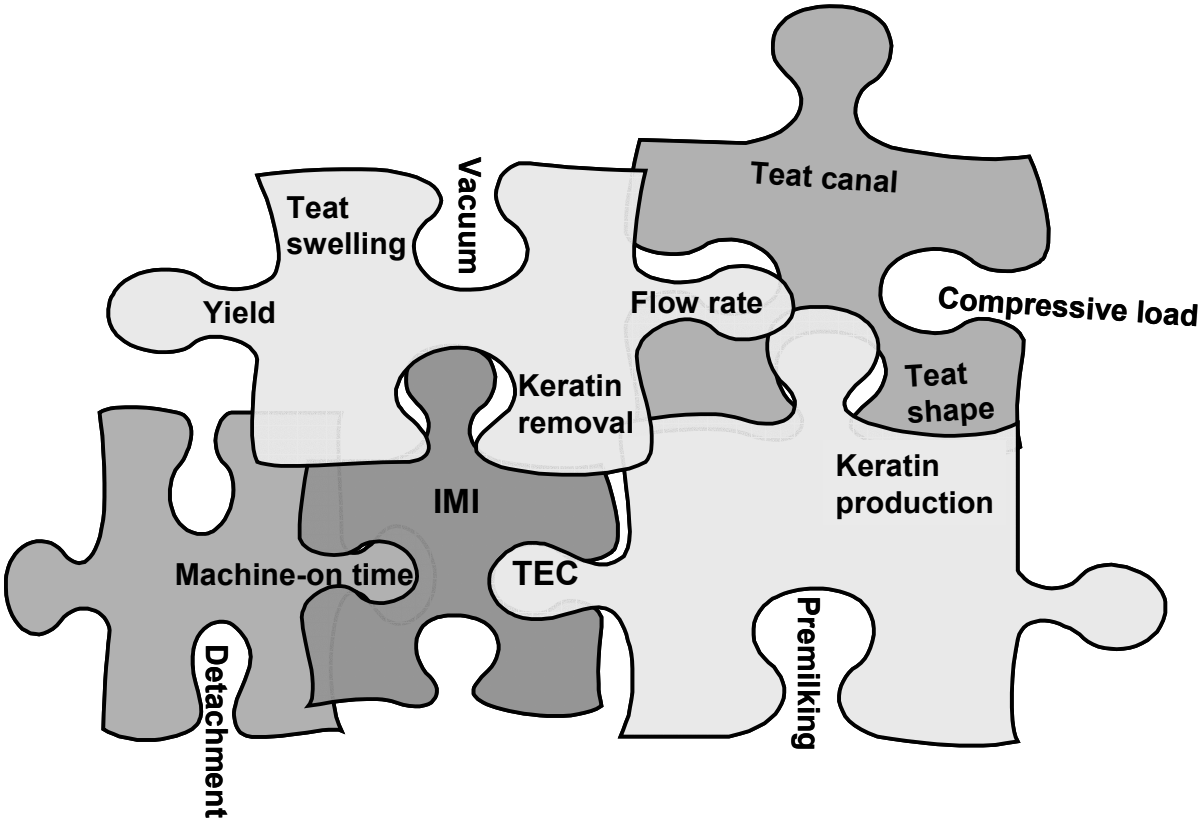


Figure 8. Mechanisms by which machine milking increases the rate of new intramammary infection (IMI) by changing the resistance of the teat to bacterial invasion.

Although the jigsaw puzzle of the mechanisms underlying the change of resistance of the teat to bacterial invasion, factors contributing to it and IMI is not completely solved, this thesis provides us with better insights into the relationship between teat condition and IMI (Figure 8).

VI. FURTHER RESEARCH

In this thesis we found cow- and milking machine factors that influence the rate of IMI through changing the teat's resistance to penetration of pathogens. The relationship between cow- and milking factors is like a jigsaw puzzle with some relationships as yet unclear (Figure 8). Interesting elements of this puzzle include the relationships between machine-induced teat swelling and TEC, and the role of milk yield and milk flow curve and peak milk flow as well as the fit of teat in the liner and compressive load. Further research should be conducted to establish the differences in teat

swelling of cows with high and low yields, high and low peak milk flow rates and high and low levels of TEC.

Teats without any callosity ring, and thus without a beneficial, physiological reaction to milking, should be examined more closely. These quarters may deviate in milk flow pattern or teat canal characteristics. Different machine settings may be necessary in order to avoid an increase in IMI.

To gain more insight into the pathways underlying the coherence of TEC, machine-induced teat swelling an IMI, further research is needed. This research should be focussed on the relationship between keratin production and keratin loss in relation to milking machine and factors such as teat dimensions, vacuum, compressive load and peak flow rate.

Peak flow rate appears to be an important factor in the resistance of the cows' teat to IMI. Research should be conducted on controlled milking based on actual quarter milk flow which should establish square quarter milk flow profiles with non excessive peak flow rates. In the literature, a maximum peak flow rate of 1.6 kg/min/quarter has been reported (Grindal and Hillerton, 1991). This should be evaluated.

Important steps have been made in qualification of liners in terms of quantifying the compressive load applied to the teat. However, knowledge on the combination of the liner, pulsator ratio and milking vacuum with cows' teat requires further attention when installing a milking machine on a farm. Furthermore a method is needed for non-invasive real-time measurement of the compressive loads on teats for liner design, and during milking in the field.

Cows are dynamic in teat dimensions, milk yield, and milk flow patterns during milking. Due to this variability, vacuum close to the teat (milking and pulsation vacuum), and the movement of the liner, will vary during milking. These variations make it difficult to analyse the impact of small changes in components or settings of the milking machine on the teat. The development of a dynamic model can standardise these influential cow-characteristics during the milking process, thereby enabling a more complete estimation of the factors that vary at the borderline of teat/liner. The impact of small changes for example vacuum patterns or liner movement on the teat may become much clearer.

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SUMMARY

The dairy cow's teat is the first line of defence against mastitis pathogens. The milking process may affect the teat's condition, increasing the risk of mastitis. It is well-proven that teat-ends with severe erosions or broken skin will have an increased risk of mastitis. However, more common changes in teat condition because of milking have not been related to udder health problems. The focus of this thesis was on the relationship between teat-end condition, machine milking and occurrence of mastitis. In this thesis, two types of changes of teat-end condition were distinguished: callosity rings around the orifice and machine-induced teat swelling.

First a classification system of the callosity rings around the orifice was defined: the teat-end callosity (TEC) classification system. In this system, a distinction is made between roughness of the callosity ring (TECR) and thickness of the callosity ring (TECT). Teats can be scored in 8 categories. In an experiment, it was demonstrated that TECT and TECR increased rapidly during the first 8 weeks after calving. Cow factors such as days in milk, parity, machine-on time, and teat-end shape were associated with TEC.

In order to measure machine-induced teat swelling, a methodology, using ultrasound, has been developed. Using this method, the changes of teat tissue in relation to machine milking and the recovery time of teat tissue after milking were evaluated. Machine milking had a large effect on the length of the teat-canal, the width of the teat-cistern, the width of the teat-end, and the thickness of the teat-wall. 8 h after milking, the teat-end width and the teat-canal length still differed from before milking. The teat-wall thickness and the teat-cistern width were recovered after 6 and 8 h.

The developed TEC classification system was used in a 1½ year longitudinal field study on 15 farms to examine the relationship between TEC and the incidence of clinical mastitis. From that study, it was clear that clinical mastitis does have a relationship with TEC. The relationship between TEC and mastitis was different for different mastitis pathogens. *E. coli* mastitis occurred more in quarters where teats had less TEC. Specific pathogens differ in their opportunistic use of orifice dysfunction to multiply, or enter the teat canal. Pointed teat-ends had higher TECT and TECR than flat or inverted teat-ends and TECT increased with a higher milk yield at peak production.

The same longitudinal dataset was used to focus on TEC as a risk condition for mastitis. Teats with a thin and smooth TEC ring showed the lowest incidence risk of clinical mastitis. They can be regarded as physiologically normal teats. An increase in the risk of clinical mastitis was observed

when thickness and/or roughness of TEC increased. This is caused by less tightly closed teat canals after milking, a too high turnover of keratin, or the harbouring of pathogens in TEC. An increase in TECT and TECR can be used as an early warning signal for enhanced risk of clinical mastitis. Quarters without any callosity ring also showed an increased incidence risk of clinical mastitis during the next month. The higher risk of IMI in teats without a callosity ring may be caused by a decreased rate of keratin regeneration in the teat canal.

To evaluate TEC in the field, a simplified 4-category scoring system is suggested and used in an observational study on 200 dairy farms. Cows that have an increased risk of clinical mastitis due to more TEC build up because of milking, were the unit of interest in this study. Therefore, categories of TEC that gave an increased risk on mastitis were combined into one group: %ROUGH. Variation in %ROUGH between farms is explained by cow factors such as teat-end shape and machine-on time and milking machine factors such as the liner and the vacuum.

The overall conclusion of this thesis is that a healthy teat of a dairy cow has a good balance between the physiological reaction to machine milking and maintaining its first line of defence mechanism against invading mastitis pathogens. Increasing rates of IMI were related to one or more of the following: a high degree of machine-induced swelling, a high level of TECT, a high level of TECR and the absence of TEC. Pathways through which these machine-induced changes lowered the resistance of the teat to bacterial invasion are the openness of the teat canal, harbouring of pathogens in TEC, and significantly increased or decreased level of keratin regeneration rate. Part of the impaired reaction of the teat to machine milking may lay in the peak milkflow rate. Suggestions are made to adjust the characteristics of machine milking to the milk flow profile of an individual cow. This can minimise machine-induced teat condition problems.

Teat condition changes can be used as an early warning signal for enhanced risk of clinical mastitis. Classification of teat condition is an essential tool in milking machine research and a useful monitoring tool of the quality of milking in the field. Protocols for systematic evaluation of teat condition are available.

SAMENVATTING

De spenen van melkkoeien zijn de eerstelijns afweer tegen mastitis veroorzakende bacteriën. Het melken kan de speenconditie aantasten. Het is bewezen dat ernstige speenpunt beschadigingen lijden tot meer mastitis. Maar de meer algemeen voorkomende veranderingen in de conditie van de speen door het melken zijn niet gerelateerd aan uiergezondheid problemen. Dit proefschrift was gericht op het verband tussen speenconditie, melken en het optreden van mastitis. In dit werk zijn twee typen van speenconditie veranderingen bekeken: speenpuntvereelting en zwelling van spenen door het melken.

Als eerste is een systeem ontwikkeld om de vereelting van speenpunten te classificeren: het speenpuntvereelting (TEC) classificatiesysteem. In dit systeem wordt een onderscheid gemaakt tussen de dikte (TECT) en de rafeligheid (TECR) van de eeltring. Het systeem heeft 8 klassen. In een experiment werd vastgesteld dat de mate van TECT en TECR snel oplopen gedurende de eerste 8 weken na afkalven. Koefactoren zoals lactatiestadium, pariteit, machine melktijd, en speenpuntvorm lieten een associatie zien met TEC.

Om speenzwelling te kunnen meten werd een methode ontwikkeld, waarbij echografie werden gebruikt. Met deze methode werden de veranderingen in de dimensie van de speen door het melken vastgesteld. Ook de tijd dat deze veranderingen aanhielden werden gemeten. Machinaal melken had een groot effect op de lengte van het tepelkanaal, de diameter van de tepelcisterne, de breedte van de speenpunt en de dikte van de speenwand. De breedte van de speenpunt en de lengte van het tepelkanaal waren zelfs 8 uur na het melken nog niet op het niveau van voor het melken. De dikte van de speenwand en de diameter van de tepelcisterne waren gelijk aan de waarde voor het melken na 6 en 8 uur.

Om de relatie tussen TEC en de incidentie van mastitis vast te stellen is het speenpuntvereelting classificatiesysteem gebruikt in een longitudinaal onderzoek van 1½ jaar op 15 bedrijven. Hieruit bleek dat klinische mastitis een relatie heeft met TEC. De relatie verschilde voor de te onderscheiden mastitis veroorzakende pathogenen. Bij kwartieren waarvan de spenen minder vereelting vertoonden, kwam *E. coli* mastitis meer voor dan mastitis veroorzaakt door andere pathogenen. Specifieke pathogenen verschillen in hun opportunistisch gebruik van afwijkingen van het slotgat om zich te vermenigvuldigen of het tepelkanaal binnen te dringen. Puntige spenen vertoonden meer TECT en TECR dan platte of ingestulpte speenpunten en TECT nam toe bij hogere melkgiften gedurende de piek van lactatie.

Dezelfde longitudinale data werden gebruikt om te kijken of TEC risico verhogend werkt op het voorkomen van mastitis. Spenen met een dunne en gladde eeltring hadden de laagste incidentie van klinische mastitis en kunnen gezien worden als fysiologisch normale spenen. Een toename van klinische mastitis werd gevonden als de vereelting dikker of rafelig was. Dit wordt veroorzaakt door een minder goed afgesloten tepelkanaal na het melken, een te hoge regeneratie van keratine of doordat mastitis veroorzakende pathogenen zich beter in de eeltring kunnen nestelen. Maar ook spenen zonder een eeltring hadden een hogere kans op klinische mastitis in de volgende lactatiemaand. De reden hiervoor zou kunnen liggen in een te lage regeneratie van keratine in spenen zonder eeltring. Een toename in TECT en TECR kan worden gebruikt als een waarschuwingssignaal voor de verhoogde kans op mastitis.

Een vereenvoudigd vereelting score systeem met 4-klassen is gebruikt om het voorkomen van TEC te evalueren in de praktijk en toegepast op 200 bedrijven. Koeien met een verhoogde kans op mastitis door toegenomen eeltvorming door het melken, was hierbij de variabele die gebruikt werd. Om die reden werden de categorieën van vereelting die een verhoogde kans op mastitis gaven, in één groep samengevoegd: %RAFELIG. Variatie in de grootte van de groep %RAFELIG werd beïnvloed door koefactoren zoals speenpuntvorm en melktijd. Ook factoren die gerelateerd zijn aan het melken zoals de gebruikte tepelvoering en het melkvacuüm beïnvloedden %RAFELIG.

De conclusie van dit proefschrift is dat gezonde spenen van melkkoeien een goede balans hebben tussen de fysiologische reactie op het melken en het behoud van het eerstelijns afweer mechanisme tegen binnendringende mastitispathogenen. Een toename van de kans op mastitis wordt veroorzaakt door één of meer afwijkingen in de speenconditie: een ernstige zwelling van de spenen door het melken, toename van de dikte van de eeltring, het rafelig worden van de eeltring of juist geen reactie op het melken in de vorm van eelt. Een verlaagde eerstelijns afweer, waardoor mastitispathogenen makkelijker binnendringen, kan veroorzaakt worden door: het onvoldoende sluiten van het tepelkanaal, het zich beter kunnen nestelen van bacteriën als TEC is toegenomen en een te hoge of juist te lage regeneratie van keratine. Een achterliggende oorzaak zou een hoge piek melkssnelheid kunnen zijn. Een aanbeveling wordt gedaan om de instellingen van de melkmachine aan te passen aan de melkafgifte curve van de individuele koe. Op deze wijze kunnen negatieve gevolgen van het melken op de speenconditie te minimaliseren.

Veranderingen in de speenconditie zijn een signaal voor een verhoogde kans op mastitis. Classificeren van de speenconditie is een essentieel middel voor het doen van onderzoek naar de

werking van de melkmachine en een nuttig instrument in de praktijk om de kwaliteit van het melken te beoordelen. Protocollen voor systematische beoordeling van de speenconditie zijn beschikbaar.

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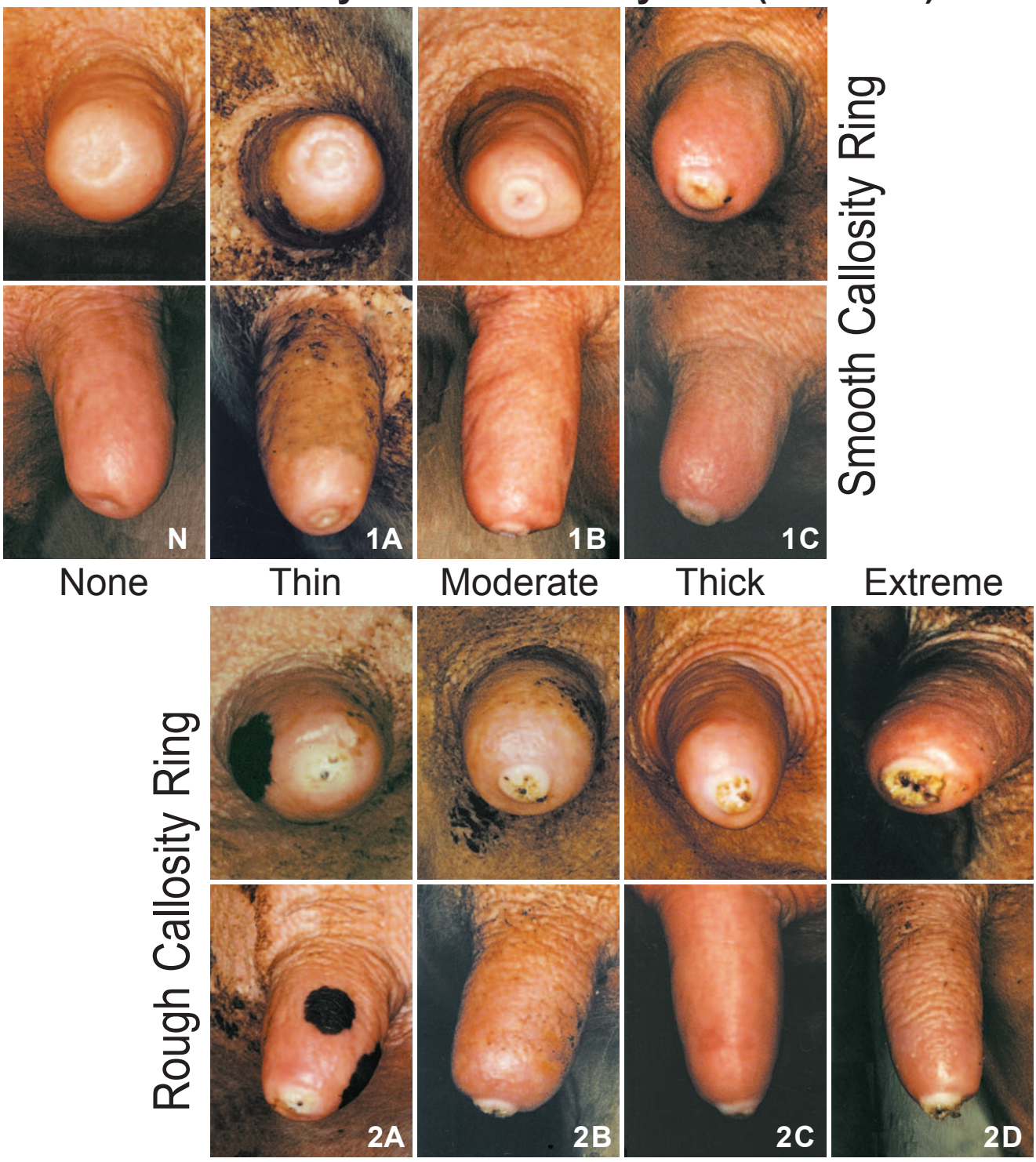
CURRICULUM VITAE

Francesca Neijenhuis werd geboren op 5 mei 1966 in Rijswijk. In 1986 behaalde ze haar VWO-diploma aan de Christelijke Scholengemeenschap Rijswijk. In datzelfde jaar werd begonnen met de studie Zoötechniek aan de Landbouwuniversiteit Wageningen. Zij rondde haar studie af in 1992 waarbij de accenten in de afstudeerfase lagen op bedrijfseconomie, melkwinning en epidemiologie.

Op 1 mei 1992 werd zij aangesteld bij het Proefstation voor de Rundvee- Schapen en Paardenhouderij te Lelystad waar zij zich bezig hield en houdt met onderzoek rondom melkwinning. De werkgever veranderde een aantal malen van naam, waardoor zij thans als wetenschappelijk onderzoeker werkzaam is bij de Animal Sciences Group van Wageningen Universiteit en Research Centrum bij het onderdeel Praktijkonderzoek. De basis van het onderzoek dat in dit proefschrift is beschreven werd uitgevoerd bij het Praktijkonderzoek.

Teat-end callosity classification system (research)



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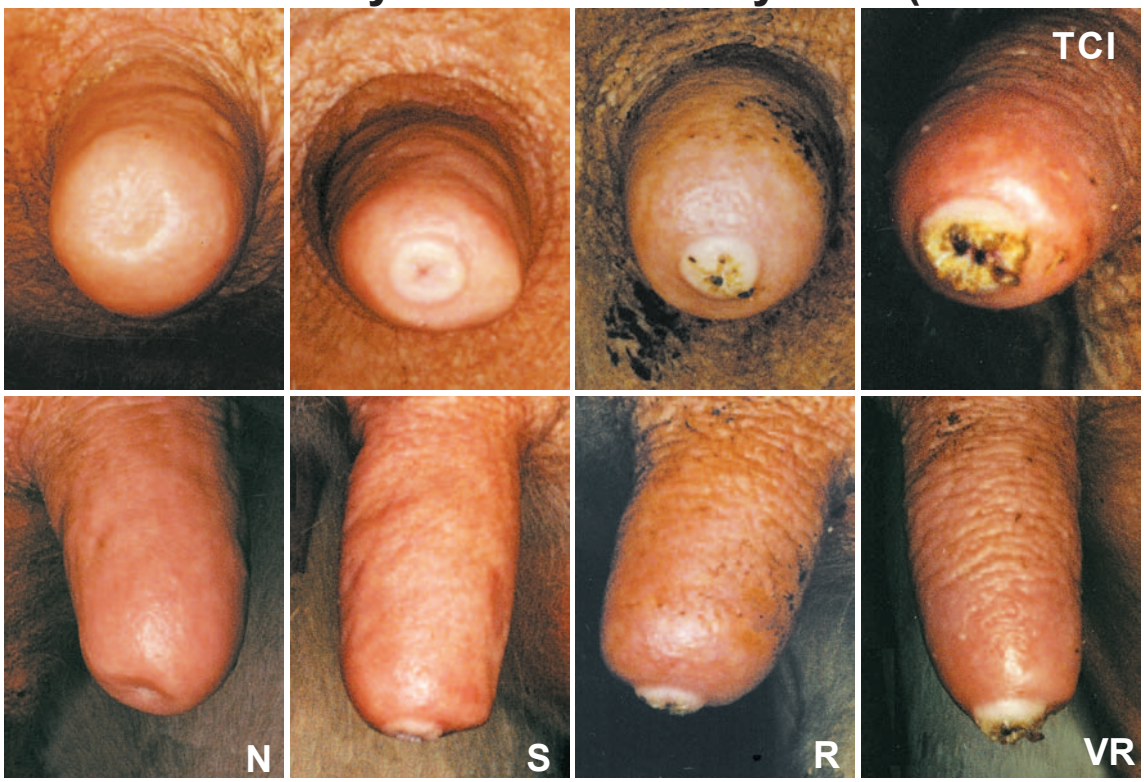
Teat-end callosity classification system (field work)



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