

Prevalence of sub-clinical mastitis in Uruguay

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Abstract

Uruguay is a major milk producer and exporter of dairy products in South America with 1,423.5 million litres of milk being produced annually. Thus, the dairy industry is of great economic importance to Uruguay. Mastitis is an inflammatory reaction of the mammary gland, which has significant health and economic implications. It is the major health problem among dairy cows in Uruguay. Sub-clinical mastitis is a condition in which there is no detectable inflammatory change in the udder and no observable abnormalities in the milk. However, it reduces milk production and adversely affects milk quality. It can also be seen as a reservoir for bacteria that can later cause clinical mastitis, which has an even greater effect on health and economy. It has been shown that sub-clinical mastitis is responsible for 70% of the losses in milk production in Uruguay.

The aim of this study was to determine the prevalence of sub-clinical mastitis in an area of Uruguay. By identifying these cases and the bacteria causing them, udder health can be markedly improved. Seven farms in the Paysandú area were selected. On each farm, samples were collected from each quarter and analysed; partly for somatic cell count (SCC) by the California mastitis test (CMT), and partly for bacteriology. Samples were also taken from the tank for five consecutive days and analysed partly for SCC by the Fossomatic method, and partly for bacteriology. Identification of Streptococci was made by the CAMP-test and SVA-strept. The catalase test was used to differentiate Streptococci from *Corynebacterium bovis*. Staphylococci was identified by the coagulase test.

The prevalence of sub-clinical mastitis was found to be high. On a cow-basis it was 42.2% and on a quarter-basis 21.8%. A number of various factors could be the reason for this and several measures could be taken by the farmers themselves to reduce these infections. The mean value for the total number of bacteria in the bulk tank was 24,227 cfu/ml. These results, however, are accounted for in the degree project: "Comparison between bulk tank milk samples and individual milk samples from dairy herds in Uruguay" written by Anneli Axelsson.

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Introduction

This degree thesis is based on a Minor Field Study (MFS), carried out in the summer of 2002. The project is a case report, describing findings out in the field and should not be seen as a statistical analysis.

Uruguay is a major milk producer and exporter of dairy products in South America, with approximately 400,000 cows annually yielding 1400 litres of milk. Milk production is of great importance to Uruguay's economy and about 70% of its total losses are due to sub-clinical mastitis (Giannechini, 2001). This study aims to evaluate the prevalence of sub-clinical mastitis and which bacteria are most prevalent. With access to this information together with knowledge about risk factors and how different pathogens are spread, udder health can be markedly improved.

A comparison between results obtained from bulk tank milk (BTM) analysis and individual samples taken from each udder-quarter in the herd was also performed to evaluate whether BTM can be a useful tool in mastitis control. This part of the study is presented in a separate degree project written by Anneli Axelsson. For that reason, the results of the BTM analysis are merely presented as numbers and tables in this paper without a following analysis.

Mastitis

Mastitis is the body's defence against an injury caused to the udder tissue (Emanuelson & Philipsson, 1984; Kaneene & Hurd, H. S. 1990, Miller & Dorn, 1990; Hallén-Sandgren et al, 1997; Heringstad et al, 1997). This injury can be caused either by mechanical damage or by an alien substance, e.g. bacteria. The injury activates an inflammatory response in the udder. The inflammation consists of three stages:

1. The acute transient phase, during which local capillary circulation and the permeability of the capillaries increase as the endothelial cells contract. Inter-endothelial gaps are formed through which plasma and its proteins leak in the interstitium, causing oedema. Blood leukocytes begin to adhere to the endothelium (Sandholm et al, 1995b). This leads to a change in the composition of the milk, e.g. the content of lactosis and casein is reduced while the content of saline is increased (Hallén-Sandgren et al, 1997).
2. The subacute phase, during which cells migrate from the circulation to the infection site (Sandholm et al, 1995b). These cells are leukocytes (lymphocytes, macrophages, neutrophils, eosinophils), but epithelial cells from the udder can also be found in the milk (Kehrli & Shuster, 1994). When a bacterial infection occurs, the amount of cells in the milk increases rapidly. The proportions are also changed from mainly lymphocytes and macrophages in a healthy udder, to over 95% neutrophils in an infected udder (Kehrli & Shuster, 1994; Detilleux, Leroy & Volckaert, 1997).

3. The chronic proliferative phase, which is characterised by tissue degeneration, regeneration and formation of fibrotic tissue (Sandholm et al, 1995b).

Mastitis always causes a certain destruction of milk producing tissue which leads to a decrease in milk production. The decrease is affected by a number of factors, e.g. the cow's age and the potency of the inflammation (Hallén-Sandgren et al, 1997).

Sometimes the inflammation fails to eliminate the causal microbe and a degree of unreactivity "tolerance" is formed. This is what is called sub-clinical mastitis (Sandholm et al, 1995b). We distinguish between this and clinical mastitis, in which there is detectable changes in either milk or udder.

Sub-clinical mastitis

Sub-clinical mastitis is a condition in which there is no detectable inflammatory change in the udder and no observable abnormalities in the milk. Often it is more prevalent than the clinical form, it usually precedes the clinical form, it reduces milk production, and adversely affects milk quality (Gianneechini, 2001).

The most common way to detect sub-clinical mastitis is by measuring the somatic cell-count (SCC) in the milk. Through this, the degree of inflammatory changes can be seen, as it is more likely that the mastitis has been in progress for a longer time and is more widely spread if the SCC is high than if it is low. A higher SCC can also indicate that the inflammation is caused by more aggressive bacteria or that other quarters of the udder are also infected (Hallén-Sandgren & Ekman, 1994a; Hallén-Sandgren et al, 1997). The SCC is also affected by lactation number and lactation stage (Kennedy et al, 1982; Emanuelson & Persson, 1984). SCC increase with the number of lactations. This could be explained by the fact that the risk of infection increases with age (perhaps because the immune system in an older cow is not as efficient) or that the udder has been subjected to many attacks during earlier lactations, which makes it easier for bacteria to gain access to the udder (Dettelleux, Leroy & Volckaert, 1997). Regarding lactation stage SCC is considerably decreased during the first two months of lactation and then gradually increases until dry-off (Kennedy et al, 1982). This SCC raise, however, affects all quarters, which is a very unusual state in mastitis (Radostits, 2000). A high milk production and genetics are also factors involved in high SCC (SHS, 1999).

Other indirect tests are the chloride content and electrical conductivity, and the test for bovine serum albumin. These tests are more accurately diagnostic of damage to mammary epithelium and will not be further discussed here (Radostits, 2000).

The prevalence of sub-clinical mastitis in Sweden is calculated as the percentage of cows with an udder health class of 6-9 at least once during the year. Udder health class is defined as an average determination based on three consecutive SCC:s. The class 6-9 indicates a 60-90% probability that one or more udder quarters would have a sub-clinical infection. Class 6 has 300, 000 to 400, 000 cells/ml range of SCC, while with class 9 the SCC level is above 600, 000 cells/ml.

Eleven measures of SCCs are performed and recorded annually for each cow (Giannechini, 2001).



Picture 1. Milk sampling on farm 5.

Mastitis bacteria

Streptococci

Streptococcus agalactiae

Str. agalactiae is a contagious bacteria and the most important reservoir of this bacteria is the infected udder. Contagious microorganisms are well adapted to survival in the udder and usually establish mild clinical infections of long duration (chronic infections). These bacteria are shed in milk from infected quarters, and transmission to uninfected quarters and cows occurs mainly at milking time. Important objects that may transmit these bacteria are contaminated milking machines, udder wash cloths, and the hands of machine operators.

Although the main source of infection is the udder of infected cows, contamination of the environment may provide a ready source of infection when hygiene is poor. The teats and skin of cattle, milkers' hands, floors, utensils and clothes are often heavily contaminated. Sores on teats are the commonest sites outside the udder for persistence of the organism. The infection may persist for up to three weeks on hair and skin and on inanimate materials such as dung and bricks. *Str. agalactiae* is also known to cause higher a SCC than other types of infection and in herds with a high bulk milk cell count it is likely that *Str. agalactiae* is

common. The infection tends to reach a peak in the younger age groups (Radostits, 2000).

In any large cattle population where the disease is not controlled in any way, most herds will be found to be infected and the average morbidity rate among the cows will be about 25%. Where good hygienic measures and efficient treatments are in general use, the morbidity rate in the cattle population will be considerably below this. Since the advent of antibiotic treatment, *Str. agalactiae* has been supplanted by *Staph. aureus* as the major cause of bovine mastitis

Streptococcus dysgalactiae

Str. dysgalactiae is in 50% of the cases regarded a contagious bacteria, but is also an environmental bacteria. In healthy cows it can be found in the tonsils. Otherwise, it is mainly found in lesions on the skin, teats and teat canal. It is readily controlled by dry cow treatment, suggesting that transmission often is from cow to cow (Jayarao, 2000).

Streptococcus uberis

Str. uberis is, as *Str. dysgalactiae*, both environmental and contagious. It is a common inhabitant of the skin, lips and tonsils of cows in infected herds, the skin of the belly often carrying the largest population. Some cows become permanently colonized with *Str. uberis* and may pass very large numbers of the organism in the feces. This observation is also linked with the finding of large numbers of the organism in straw bedding on farms where this form of mastitis persists. Infection of the mammary gland appears to be secondary to infection of the skin and both appear to be more prevalent during the cool months of the year. It is a bacteria increasingly associated with a raised SCC (Hughes, 2001).

Str. uberis has a long survival period in the udder and once the infection has been established in a herd, sporadic cases are likely to occur in spite of good hygienic precautions. Results in the control of *Str. uberis* are poor, probably because infection occurs at times other than during milking. Because of the failure of the general control program to restrain the spread of the infection it is more than usually necessary to treat infected quarters vigorously. Treatment of all dry cows also seems necessary (Radostits, 2000).

Staphylococci

Staphylococcus aureus

S. aureus is a contagious mastitis pathogen that colonises the teats when there is damage to the skin surface. It produces enzymes that allow it to penetrate deep into the mammary tissue and causes a significant reduction in milk yield and rate of milking. Transmission occurs mainly through contaminated milking machines, udder wash cloths, and the hands of machine operators. It can survive outside the cow for a shorter period of time (Svensk mjölk & Alfa Laval Agri Scandinavia AB, 2003). The infection is usually chronic (with signs of clinical mastitis) or sub-clinical, occasionally showing mild clinical signs. The bacterial count in the milk is usually low in a sub-clinical infection with *S. aureus* and is therefore rarely detected

without repeated sampling (Persson Waller, 2002). Staphylococcal antibodies are found in the blood of infected cows but they appear to afford little protection against mastitis due to these bacteria. This may be due to the low titer of the antibodies in the milk (Radostits, 2000).

Because most newly infected animals do not show clinical signs and because the abscesses that form are difficult to treat; *S. aureus* is a challenging organism to control in a dairy herd and eradication is not possible (Jayarao, 2000). Chronically infected animals will never be free of the bacteria and should be culled in connection with drying-off (non pregnant cows). Fighting *S. aureus* in a herd requires a systematic plan which can be summed up in four key words: sampling, culling, grouping and dry cow therapy (Svensk mjölk, 2003).

Coagulase Negative Staphylococci

Coagulase Negative Staphylococci (CNS) are bacteria that are normally present on the cow's skin, but can cause mastitis in the teat canal (Jayarao, 2000). Although staphylococci can multiply on the surface of the skin and provide a source of infection for the udder, the cutaneous lesions are usually infected originally from the udder.

It does appear that although these bacteria are capable of causing microscopic lesions, and in some cases increased leukocyte counts in the milk, they are not nearly as pathogenic as hemolytic staphylococci. The infection is generally mild and transient and the tissue reaction is usually so mild that the CMT is negative. If the infection is capable of causing loss of productivity, current standards for the diagnosis of mastitis will need to be reassessed. CNS appear to have the advantage that they resist colonisation of the teat duct and teat skin by coagulase-positive staphylococci (Radostits, 2000).

Coliforms and other Gram-negative bacteria

Coliforms (*E. coli*, *Klebsiella spp.* and *Enterobacter aerogenes*) are environmental organisms and are frequently isolated from bulk tank milk. They can survive for long periods in the udder (Hughes, 2001). The coliform bacteria are considered as opportunists, and contamination of the skin of the udder and teats probably occurs primarily between milkings when the cow is in contact with contaminated bedding, rather than at the time of milking (Jayarao, 2000).

Faeces, which commonly provide the source of *E. coli*, can contaminate the perineum and the udder directly or indirectly through bedding, calving stalls, dry lot grounds, udder wash water, udder wash sponges and cloth rags, teat cups and milkers' hands. Cows with chronic coliform mastitis also provide an important source of the organism and direct transmission probably occurs through the milking machine. Coliforms can rapidly build-up in moist, milky residues in milking equipment, which then becomes the major source of contamination of the milk produced (Bramley & McKinnon, 1990). Inadequate drying of the base of the udder and the teats after washing them prior to milking can lead to a drainage of coliform-contaminated water down into the teat cups and subsequent infection.

However, it is now well recognised that the presence of coliforms in raw milk is not evidence of direct faecal contamination, and can not be relied on to detect inadequate udder cleaning before milking.

Sawdust and shavings used as bedding, which are contaminated and harbouring *E. coli* are considered to play a major role in the epidemiology of coliform mastitis. Wet bedding, particularly sawdust and shavings, promote the growth of coliform bacteria, especially *Klebsiella spp.* (Radostits, 2000).

A high proportion of new infections occur around two weeks before and two weeks after drying-off. During lactation, susceptibility is highest at calving and decreases considerably as the lactation progresses. (Bramley & McKinnon, 1990). Coliform mastitis has been reported world-wide and is most common in dairy cattle which are housed during the winter months or kept in total confinement in a dry lot. The disease is uncommon in dairy cattle which are continuously in pasture. Gram-negative non-coliform organisms can cause severe mastitis including outbreaks of clinical mastitis (Jayarao, 2000).

Corynebacterium bovis

Corynebacterium bovis is a highly contagious organism and weakly pathogenic in nature. Intramammary infections, when detected, are usually sub-clinical and rarely have been shown to cause clinical mastitis. Reports suggest that cows with *C. bovis* infection generally tend to have lower milk production. If there are many cows with *C. bovis* in a herd, this could result in an elevated bulk tank SCC (Jayarao, 2000).

It is commonly held that the presence of *C. bovis* in a quarter reduces the chances of infection by pathogens, but the evidence for this view is inconclusive and the effect may be different for different pathogens. The level of infection is low in herds where teat dipping and dry period treatment are practised (Radostits, 2000).



Picture 2. The Giordano farm (farm 1).

Factors affecting the occurrence of mastitis

The udder has three important defence mechanisms. First, an uninjured, closed teat canal efficiently prevents pathogens from entering the udder. To keep the teat canal in good condition, it is important to reduce the risk of teat tramp and to use a proper milking technique. Secondly, an efficient immune response is very important. The immune response is weakened by e.g. stress, malnutrition and feed and water of poor quality (Hallén-Sandgren et al, 1997). Most cases of clinical mastitis occur during the period around calving (Schepers, Smolders & Hanekamp, 1993; Kehrlí & Shuster, 1994). This can be partly explained by the fact that the immune system is suppressed during that period, which has been proved in several studies (Kehrlí & Shuster, 1994). Finally, the risk of mastitis is decreased through washing out of pathogens at milking (Hallén-Sandgren et al, 1997).

The prevalence of infectious mastitis is determined by the frequency and duration of new infections. The basic strategy in a successful programme against mastitis is to bring down the amount of new infections to a minimum. The duration of the infection is determined by the pathogen, but also by the cow's ability to fight the infection. A number of factors have a negative effect on this ability (the immune system): virus infections, stress, general health situation, unsuitable climate, selenium- and E/A-vitamin deficiency, feed damaged by mould etc. (Hallén Sandgren & Ekman, 1996).

To minimise the cow's exposure to pathogens it is important to have a correct milking order in which cows with mastitis are milked last, good milking technique, regular control and tending of the milking equipment, good hygienic quality of feed and water, clean cows and a clean environment (Hallén-Sandgren et al, 1997).

Health problems in milk production

The most common health problem among dairy cows is probably mastitis followed by metabolic and hoof problems (Giannechini, 2001). Mastitis causes losses in the form of costs for veterinarians and treatment, discarded milk due to treatment with antibiotics, decrease in milk production, premature culling, replacement of animals, more work, poor milk quality and increased risk of infection in the future (Eriksson, 1991; Schepers, Smolders & Hanekamp, 1993; de Jong & Lansbergen, 1996; Heringstad, Klemetsdal & Ruane, 1996 Hallén-Sandgren et al, 1997).

In Sweden, culling and loss of production composes the main part of the costs. In an average herd, 20% of the cows are treated yearly for mastitis and the culling is 10% (Hallén- Sandgren & Ekman, 1994b). Cell-counts are made two or three times a month, depending on which dairy the farmer delivers to. The limit is 400, 000 cells/ml (according to an EU decree) during a period of three months. Sweden has a well organised system of health registration, including mastitis, which is used e.g. in breeding programmes (Eriksson, 1991). This, in combination with the economic consequences of delivering milk with too high cell-counts, has led to a considerable reduction of cells in the milk delivered to the dairies (Hallén-Sandgren et al, 1997).

In Uruguay, the sub-clinical mastitis prevalence was 55% in 2001 according to a study made in the west Littoral region. The limit for delivering milk is 800, 000 cells/ml (Giannechini, 2001). In some farms, treatment routines in cases of mastitis have been defined by the farm veterinarian together with the farmer. However, this is probably not the case on most farms. In general, the costs for the veterinarian are considered to be high and therefore they are not consulted.

Antibiotics can be bought without prescription at special stores. The decision on what type of antibiotics to use for clinical cases or at drying-off is often based on the price of the product. Clinical cases of mastitis are mostly treated with intramammary preparations. On most farms, blanket dry cow therapy is used, i.e. 100% of the cows are treated with long-acting intramammary antibiotics at drying-off (Persson Waller, 2002).

Description of the study area

Uruguay has 3.2 million inhabitants and is situated at latitude 30-35°S, longitude 53-58°W, between Argentina and Brazil in the eastern part of South America. The country has a total area of 178,000 km². Most of the people live in the cities, mainly in Montevideo, and only 9% in the rural areas. The climate is temperate and the average temperature is 11°C in winter and 27°C in summer.

Livestock are of great importance to Uruguay's economy, with 10, 295, 000 cattle and 16, 493, 000 sheep. Approximately 94% of the total land area is productive soil and around 80% is used for livestock production (Giannechini, 2001).

The dairy sector in Uruguay

The dairy production represents a very important sub-sector, 1,060,000 hectares are used for milk production, with a total number of 5,500 dairy farms producing 1,423.5 million litres of milk annually and 4,500 dairy farms delivering 1,134.8 million of litres to the dairies each year. Uruguay is the main exporting country of milk and milk products in the region. Together with Argentina, Brazil and Paraguay it is a member state of the South Common Market (MERCOSUR). The main products for export are powder milk, cheese, ultra-high temperature (UHT) milk, and butter (Giannechini, 2001). Approximately 700 million litres are used to produce dairy products for export (Persson Waller, 2002).

A total of 702,000 animals compose the Uruguayan dairy herd, of which 400,000 are milking cows. An average of 13.3 litres of milk are produced per cow per day and 3,500 litres of milk are produced per lactation. The principal breed is the Holstein Friesian (90%) (Giannechini, 2001). Most of the production is situated in the southern and south-western parts of the country. The production has increased consistently from 1977 to 1999, especially during the last decade. The number of cows per herd varies between 10 and 1000 cows. Most milk is produced during September to December (Persson Waller, 2002).

The biggest dairy plant is the co-operative Conaprole with several dairy plants in the country. Conaprole has >70% of the market. There are also a number of small dairy plants, which are run privately or as co-operatives (Persson Waller, 2002).

In 1996, the government established new regulations for the hygienic quality of milk. The maximum somatic cell count (SCC) was set at 800,000 cells/ml and the maximum total bacterial count (TBC) was set at 200,000 cfu/ml, based on a geometric mean for three months. Some dairy plants give extra premium payment for milk of hygienic quality better than the maximum limits, but the limits used differ between the companies. There are no deductions made for high SCC or TBC. The dairy plants are of the opinion that they have to accept all milk even if the SCC and/or TBC is above the maximum levels (Persson Waller, 2002).

Bulk milk samples are regularly analysed for SCC, TBC, fat, protein, freezing point and antibiotic residues. Most dairy farms are privately-owned family farms of varying size. The educational level of the farmers and their personnel is very variable (probably rather low on average) (Persson Waller, 2002).

Milk production is based on all-year grazing and low levels of supplementary feeding, mainly during winter. If supplementary feed is given, it consists mainly of home-produced silage and concentrates. The cows and the calves are not housed at any time. Artificial insemination is becoming increasingly popular (Persson Waller, 2002).

The milking is performed in a milking house and most farmers have cooled milk tanks. In general, cows are machine-milked twice daily, and the udder is cleaned before milking using water from a hose. Forestripping is probably common, but paper towels are not used. Teat dipping after milking is probably performed on most farms. It is not common to use a milking order based on udder health (Persson Waller, 2002).

The possibility to take samples from individual cows to measure milk production and milk quality has existed for years. However, only a small proportion (<30%) of the milking cows are included in such cow control programmes (cow recording) at this point, mainly the so-called pedigree cows. On some farms, individual cows are tested using CMT, sometimes by a veterinarian (Persson Waller, 2002).



Picture 3. The laboratory outside Paysandú.

Materials and methods

Seven dairy farms in the Paysandú area were selected. The farms had between 13 and 43 cows each. One California Mastitis Test (CMT) and one sample for bacteriology were taken from each quarter of the udder of each cow. There is a great deal of variation in the cell counts at various times during milking and the most significant sample is the one taken just before the evening milking. We therefore collected our samples at this time of day (Radostits, 2000). The first few streams was rejected because their cell count and bacterial count are likely to be a reflection of the disease situation within the teat, rather than the udder as a whole. (Radostits et al, 2000).

The samples for bacteriology were collected in test tubes and placed on ice, for transport to the laboratory, and then cultured the same day. The microflora of the milk when it leaves the farm is determined by the temperature to which it has been

cooled, the storage temperature, the time elapsing before collection and the initial microflora. Where milk is cooled to and stored at $\leq 4^{\circ}\text{C}$, the low temperature will normally prevent bacterial multiplication for at least 24h, and the microflora is, therefore, similar to that present initially (Bramley, 1990).

Two bulk tank milk samples from each farm were also taken for five consecutive days. The samples were taken immediately after milking time with a sterile ladle from the top of the tank. One sample was sent to a laboratory for Fossomatic cell counting and the other sample was cultured the same day on five different media for bacteriology.



Picture 4. Making agar plates.

Somatic cell count

A high level of cells in the milk indicates an inflammatory process in the udder and is therefore an important tool in the diagnosis of mastitis. The CMT was used for somatic cell count (SCC) of the individual quarters and the Fossomatic method for the bulk tank samples.

California Mastitis Test

CMT is a cow-side test for detecting mastitis in milk. It reflects accurately the total leukocyte and the polymorph count of the milk. It has the advantage that it can be used on the bulk milk from the cow, from individual cans and from a herd bulk tank as well as on individual quarters. Cows in the first week after calving or in the last stages of lactation always give a strong positive reaction (Radostits, 2000).

The CMT test estimates the DNA content of the milk. It is based on an anionic detergent, Na-lauryl sulphate (SDS), which dissolves cell membranes and nuclei.

Consequently DNA is released and it forms a transient gel with the detergent. The more DNA there is in the sample, the higher the viscosity of the gel.

Equal volumes of milk and the 3% Na-lauryl sulphate (2-3 ml of both) are mixed on the test plate having wells reserved for each of the four quarters. The formation of a viscous gel is evaluated as the plate is rotated gently. The result is evaluated after five seconds. A few seconds later, the gel begins to deteriorate. As most of the DNA in milk originates from somatic cells, CMT reflects the somatic cell content of milk. The result is interpreted visually. Inter-quarter comparison is recommended. A pH-indicator (bromocresol purple) is also included in the reagent. Mastitis milk is slightly more alkaline than normal milk, and the gel turns purple in the presence of bromocresol purple. Therefore, one has actually two indicator principles within the same test, DNA-quantification and pH-analysis. (Sandholm et al, 1995a).

This, together with the reading of the blood agar plates for each quarter, is a good indication for mastitis. The appearance of the milk after the adding of the reagent was classified with a number from one to five, corresponding to a certain cell count range (table 1).

Only colonies from quarters with CMT three or higher were considered, except for Coagulase Negative Staphylococci (CNS), which is a minor pathogen and only considered in quarters with CMT four or higher. *C. bovis* is also a minor pathogen and was not considered in the results concerning the number of infected quarters and sub-clinical mastitis.

Table 1. *CMT results (4)*

| CMT | Cell count range (cells/ml) |
|-----|-----------------------------|
| 1 | < 200,000 |
| 2 | 150,000-500,000 |
| 3 | 400,000-1,500,000 |
| 4 | 800,000-5,000,000 |
| 5 | > 5,000,000 |

The Fossomatic method

Bulk tank milk samples for Fossomatic cell counting were collected in small containers containing Bronopol, a substance that prevents cell lysis. The samples were sent to a laboratory to be evaluated by the Fossomatic method. This method involves dying of cell nuclei by addition of a DNA-specific fluorescent dye to the milk. The fluorescent nuclei of the cells are then counted automatically, using the principle of patch count fluorescence microscopy (Sandholm et al, 1995a).



Picture 5. Our advisor, Carlos, sterilising the ladle for the bulk tank sample.

Bacterial culturing of quarter samples

One μl of each quarter sample was cultured on blood agar and incubated at 37°C for 24 hours.



Picture 6. One test tube for each udder quarter and, in the background, the reagent used for the CMT.

Streptococci

Small colonies were tested for catalase production. Catalase-negative colonies were identified as Streptococci and further differentiated. One colony from each of the quarters that had growth of Streptococci was clean-streaked on blood agar and incubated at 37°C for 24 hours. Differentiation was done by CAMP-test and SVA-strept. The CAMP-test was performed by making a streak with a control strain of *S. aureus* in the middle of a blood agar plate. Material from each Streptococcus-infected quarter was streaked out perpendicular to the *S. aureus* strain and the plate was incubated at 37°C for 24 hours. A hemolysis zone between the Streptococci and the *S. aureus* strain is a positive result. A positive result is seen for *Str. agalactiae* in almost 100% of the cases and in about 50% for *Str. uberis*. *Str. dysgalactiae* is negative for CAMP (Sandholm et al, 1995b).

SVA-strept. is a microplate system for biochemical identification of mastitis Streptococci. The plate has twelve different media evaporated in wells and eight strains of Streptococci can be tested on each plate. Material from each Streptococcus strain was mixed in three millilitres of sterile distilled water and four drops of the solution were used to inoculate each of the twelve wells in one row. The wells were covered with a plastic film and incubated at 37°C for 24 hours. Before the reading, two drops of ninhydrin reagent was added for the Sodium Hippurate test. The plate was then read and the Streptococci differentiated according to a schedule.



Picture 7. The ninhydrin reagent being added to the SVA strept.

Staphylococci

S. aureus was identified directly on the blood agar plate by recognition of yellow colonies with α - and β - hemolysis. Other Staphylococci colonies were tested for coagulase production. The coagulase test was performed by adding bacteria to 0.5 millilitres of plasma, diluted 1:3, and incubating it at 37°C. Reading was done at 2, 4 and 24 hours when the tested colonies were compared with a positive control containing a control strain of *S. aureus*. Clotting of the plasma is a positive reaction and indicates *S. aureus*. Negative colonies are CNS.

Corynebacterium bovis

Small colonies from the quarter samples were tested for catalase production. Catalase-positive colonies were identified as *C. bovis*.

Bacterial culturing from bulk tank milk samples

For each of the five consecutive days, ten μ l of milk was streaked out on blood, mannitol, acriflavin supplemented (P), Edwards and McConkey agar, respectively.

Streptococci

Edwards agar was used as a selective medium for Streptococci. Selected colonies from these plates were clean-streaked on blood agar and then differentiated by CAMP-test and SVA-strept. as for the quarter samples.

Staphylococci

Mannitol agar was used as a selective medium for Staphylococci; and the total number of Staphylococci was counted on these plates. The P-agar is selective for *S. aureus*.

Coliforms and other Gram-negative bacteria

McConkey agar was used as a selective medium for Coliforms and other Gram-negative bacteria. Coliforms grow as purple colonies and other Gram-negatives as yellow. These colonies were counted separately and together. Purple colonies were clean-streaked on blood agar and incubated at 37°C for 24 hours. A few of these colonies were mixed in 0.2 millilitres of sterile distilled water each. One P-disc (PGUA) and one I-disc was added to each tube and incubated at 37°C for one to four hours. A yellow colour indicates a positive PGUA-reaction. Two drops of Ehrlich indicator were then added to each tube and a pink colour indicates a positive reaction for Indole production. A positive result for both tests indicates that the strain is *E. coli*. Negative results were classified as Gram-negative bacteria.

Total number of bacteria

All colonies on blood agar were counted to determine the total number of bacteria.

Results

California Mastitis Test

The results of the CMT were used as a tool in determining which quarters had sub-clinical mastitis. A CMT of 3 or higher together with bacterial growth in an udder quarter (with the exception of CNS for which a CMT of 4 or higher was required; and *C. bovis* which was not considered at all as a mastitis pathogen) was regarded as sub-clinical mastitis. For CMT results, see Table 2.

Table 2. *CMT results, % of quarters*

| CMT | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 | Farm 6 | Farm 7 | Mean value |
|-----|--------|--------|--------|--------|--------|--------|--------|------------|
| 1 | 33.8 | 56.1 | 43.4 | 37.9 | 40.5 | 27.7 | 42.7 | 40.3 |
| 2 | 30.9 | 22.3 | 32.9 | 40.8 | 36.5 | 22.3 | 29.8 | 30.8 |
| 3 | 33.8 | 14.4 | 17.1 | 9.7 | 19.2 | 27.7 | 20.5 | 20.3 |
| 4 | 1.5 | 4.3 | 6.6 | 11.6 | 1.9 | 17.9 | 6.4 | 7.2 |
| 5 | 0 | 2.9 | 0 | 0 | 1.9 | 4.4 | 0.6 | 1.4 |

Streptococci

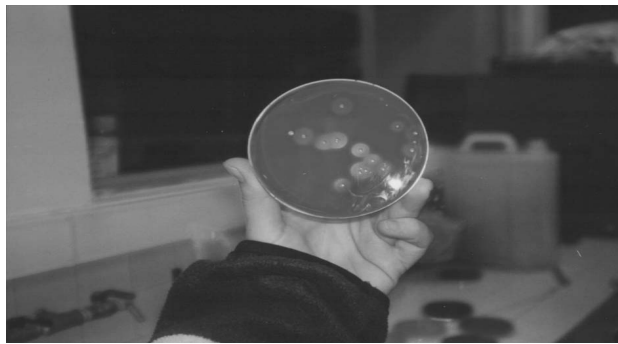
Individual samples have shown that out of 181 cows, *Str. agalactiae* was found in one cow (0.6%), *Str. dysgalactiae* in eight cows (4.4%) and *Str. uberis* in seven cows (3.9%). In two cows, a Streptococcus was found but could not be typed (in table 3 called *Str. spp.*). For results of Streptococci in the individual samples, see Table 3.

For *Str. agalactiae*, a count over 6000 CFU/ml in the bulk tank milk is considered high (Jayarao, 2000). For results of bulk tank milk, see Table 5.

Staphylococci

Results from individual samples showed that out of 181 cows, 62 were infected with *S. aureus* (34.3%) and 10 out of 181 cows were infected with CNS (5.5%). For results of Staphylococci in the individual samples, see Table 3.

Results from individual samples have shown that For *S. aureus*, a count in excess of 500 CFU/ml in bulk tank milk is considered high (Jayarao, 2000). For results of Staphylococci in the bulk tank, see Table 5.



Picture 8. *Staphylococcus aureus* on blood agar.

Coliforms and other Gram-negative bacteria

These bacteria were only analysed from bulk tank milk and not from quarter samples. For Coliforms, a count in excess of 50 CFU/ml in bulk tank milk is considered high (Jayarao, 2000). The results of bulk tank milk analysis for Gram-negative bacteria and Coliforms are presented in Table 5. No *E. coli* were identified and are therefore not presented in Table 5.

Corynebacterium bovis

C. bovis was cultured only from the individual samples and not from the bulk tank. The results have shown that out of 181 cows, 21 were infected with *C. bovis* (11.6%). *C. bovis* is, however, a minor pathogen and is therefore not considered in the total number of infected cows or quarters. Nor is it considered in the prevalence

of sub-clinical mastitis. For results of *C. bovis* in the individual samples, see Table 3.

Table 3. *Summary of bacteriological results, individual samples. Number of infected cows*

| Bacteria and total | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 | Farm 6 | Farm 7 | Total |
|--------------------------------|--------|--------|--------|--------|--------|--------|--------|-------|
| CNS | 0 | 3 | 1 | 0 | 1 | 4 | 1 | 10 |
| <i>S. aureus</i> | 1 | 6 | 1 | 6 | 12 | 17 | 19 | 62 |
| <i>Str. uberis</i> | 0 | 1 | 0 | 2 | 2 | 2 | 0 | 7 |
| <i>Str. dysgal.</i> | 2 | 1 | 0 | 1 | 4 | 0 | 0 | 8 |
| <i>Str. agal.</i> | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| <i>Str. spp.</i> | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| <i>C. bovis</i> | 0 | 2 | 0 | 1 | 0 | 11 | 7 | 21 |
| Total no. of infected cows | 4 | 10 | 2 | 8 | 12 | 19 | 18 | 73 |
| Total no. of infected quarters | 5 | 11 | 4 | 15 | 27 | 46 | 42 | 150 |

Prevalence of sub-clinical mastitis

Individual samples have shown that out of 181 cows, 73 had sub-clinical mastitis. The prevalence on a cow-basis was 42.2% and on a quarter-basis 21.8%. For details of each farm, see Tables 3 and 4.

Table 4. *Prevalence of sub-clinical mastitis, %*

| | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 | Farm 6 | Farm 7 | Mean value |
|---------------|--------|--------|--------|--------|--------|--------|--------|------------|
| Cow-basis | 23.5 | 28.6 | 10.5 | 30.8 | 92.3 | 67.9 | 41.9 | 42.2 |
| Quarter-basis | 7.4 | 7.9 | 5.3 | 14.6 | 51.9 | 41.1 | 24.6 | 21.8 |

Fossomatic cell count

The farm with the highest cell count had a mean value of 857, 000 cells/ml over five days. The farm with the lowest number had 297, 000 cells/ml. The mean value of all the farms together was 588, 000 cells/ml. For results of the Fossomatic cell count, see Table 5.

Total number of bacteria in the bulk tank

The average total number of bacteria on the seven farms was 24,227 cfu/ml. The highest number was 48,000 cfu/ml and the lowest 8,680 cfu/ml (see Table 5). For the total number of bacteria in bulk tank milk, a count over 10, 000 is considered high (Jayarao, 2000).

Table 5. Summary of bacteriology results, bulk tank samples from 5 days

| | Farm 1 | Farm 2 | Farm 3 | Farm 4 | Farm 5 | Farm 6 | Farm 7 |
|--|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Tot. No. of cows | 17 | 35 | 19 | 26 | 13 | 28 | 43 |
| SCC, average 5 days (* 10 ³ , cells/ml) | 336 | 330 | 297 | 734 | 857 | 774 | 734 |
| Tot. No. of bacteria (cfu/ml) | 30320 | 13250 | 15260 | 8920 | 48000 | 8680 | 45160 |
| <i>S.aureus</i> (cfu/ml) | 4400 | 560 | 720 | 100 | 6550 | 700 | 2800 |
| CNS (cfu/ml) | 8660 | 660 | 7380 | 3340 | 36575 | 5100 | 22840 |
| <i>Str.spp</i> (cfu/ml) | 212600 | <100 | 760 | <100 | <100 | 760 | <100 |
| <i>Str. dysgalactiae</i> (cfu/ml) | 39200 | 320 | <100 | <100 | 980 | 1760 | 24080 |
| <i>Str. agalactiae</i> (cfu/ml) | <100 | <100 | <100 | 780 | <100 | <100 | <100 |
| <i>Str. uberis</i> (cfu/ml) | <100 | <100 | <100 | <100 | 35300 | 220 | 21620 |
| Coliform (cfu/ml) | 2660 | <100 | <100 | 220 | 140 | 180 | 6420 |
| Gram negative (cfu/ml) | 3220 | <100 | <100 | <100 | <100 | <100 | 4160 |

Discussion and conclusions

The purpose of this study was to evaluate the prevalence of sub-clinical mastitis. We found that the prevalence of sub-clinical mastitis was high. The farm with the fewest infected cows had a 10.5% prevalence and the farm with the highest had a 92.3% prevalence. This means that on the farm with the lowest prevalence of sub-clinical mastitis, one in ten cows were infected. There are probably several reasons for this, possible factors being:

1. The costs for treatment and consultation by a veterinarian are high and most of the farmers have limited economic resources. Farmer confidence in veterinarians is also low.
2. The level of education is often low on the smaller farms which leads to greater risks concerning the spread of infections.
3. Teat-dipping is common, but is not performed on all farms.
4. None of the farms in this study used a milking-order based on udder quality and infection. For certain pathogens, this is the main way of spreading.

5. There are no payment reductions for high somatic cell counts or high levels of bacteria in the milk. The dairy plants receive all the milk from the farms, regardless of these parameters.
6. We found the number of bacteria to be generally higher on farms where employees milked the cows, compared with farms where the owners themselves did the milking. This could imply that as an employee you tend to be less thorough than if you own the cows you are milking.
7. The cows in Uruguay graze all the year. During rainy periods, the udders can get very muddy and the washing before milking is not always sufficient.
8. Less than one-third of the cows in Uruguay are included in a health programme.
9. The use of antibiotics is high and uncontrolled. Antibiotics can be purchased by anyone without a prescription and this increases the risk for the development of resistant bacteria.

Although few of the Uruguayan farmers are affiliated to a mastitis program and feel that they can't afford to consult a veterinarian; there are a number of measures the farmers themselves can take to improve udder health in their herd:

- A correct milking order where cows with mastitis or high SCCs are milked last. Mainly important for contagious bacteria such as *Str. agalactiae* and *S. aureus*.
- Culling of cows with *Staph. aureus* and cows with continued high SCCs, since these individuals almost never recover and are a source of infection.
- Teat dipping after each milking. After milking, the teat canal remains open for up to two hours (Sandholm, 1995c). Teat dipping in a disinfectant at this stage is an important measure to prevent microorganisms from gaining access to the teat canal. This is especially true for environmental bacteria (e.g. in the bedding when the cow lies down after milking) such as coliforms and *Str. dysgalactiae*.
- A routine for cleansing of the milking machine. This is to prevent transmission of infection from cow to cow during milking.
- A good hygienic standard can keep environmental bacteria to a minimum.
- Lubricate chapped teats to prevent colonisation of teat sores by e.g. *Str. agalactiae*.
- Dry cow treatment is a valuable method to keep the mastitis situation under control. However, it should be used restrictively and only after a bacterial diagnosis.

Farm 1

This was a small farm with a total of 17 cows. 23.5% of the cows had sub-clinical mastitis and the main problem was *Str. dysgalactiae*. This farm would likely benefit from dry cow treatment and teat dipping.

Farm 2

This farm was twice as big as farm 1, with 35 cows. The prevalence of sub-clinical mastitis was 28.6%. The main problem was *S. aureus*, which is the most common mastitis bacteria and also the most difficult to control. This farm would need to take

measures like dry cow treatment, culling of chronically infected cows and a milking order where infected cows are milked last.

Farm 3

This farm had a total of 19 cows and 10.5% of the cows had sub-clinical mastitis. That means that only two of the cows were infected, which makes this the best farm in our study. The bacteria that was found was Staphylococci. The cow with *S. aureus* should be milked last until drying-off and then culled.

Farm 4

This was, for our study, a medium-sized farm with 26 cows. The prevalence of sub-clinical mastitis was 30.8%. Here, the problem with *S. aureus* was rather big. After diagnosis, these cows should be milked last and chronically infected animals culled at the next drying-off. Dry cow treatment is also a good measure to take when dealing with Staphylococcal mastitis.

Farm 5

This was the smallest farm in our study with only 13 cows. 92.3% had sub-clinical mastitis, which means that 12 out of 13 cows were infected. This was definitely the farm with the most problems in our study. Not only did 12 out of 13 cows have sub-clinical mastitis; but all of the 12 cows were infected with *S. aureus*. Culling of all cows but one is, of course, out of the question. Therefore, dry cow treatment seems to be the best action to take in this herd.

Farm 6

This farm had 28 cows and a sub-clinical mastitis prevalence of 67.9%. The main problem here was *S. aureus*. A milking order where infected cows are milked last and dry cow therapy could improve these numbers. Cows chronically infected with *S. aureus* should be culled at the next drying-off.

Farm 7

This was the biggest farm in our study with a total of 43 cows. The prevalence of sub-clinical mastitis was 41.9%. Here, as in most of the farms in our study, the main problem was *S. aureus*. A milking order, dry cow treatment and culling of chronic cases are advisable measures to be taken.



Picture 9: All test tubes and agar was sterilised in this pressure cooker.

Sammanfattning

Uruguay är en stor mjölkproducent och exportör av mjölkprodukter i Sydamerika och producerar 1 423,5 miljoner liter mjölk årligen. Mjölkindustrin är alltså av stor ekonomisk betydelse för Uruguay. Mastit är en inflammatorisk reaktion i juvret och har stor betydelse vad gäller ekonomi och hälsa. Det är det huvudsakliga hälsoproblemet bland mjölkkor i Uruguay. Subklinisk mastit är ett tillstånd då man inte ser några inflammatoriska förändringar på juvret eller några förändringar i mjölken. Däremot ger det en minskning av mjölkproduktion och påverkar påtagligt mjölkens kvalitet. Det kan också ses som en reservoar för bakterier som senare kan orsaka klinisk mastit vilket har en ännu större effekt på hälsa och ekonomi. Det har visats att subklinisk mastit står för 70% av förlusterna i Uruguays mjölkproduktion.

Målet med den här studien var att utreda prevalensen av subklinisk mastit i en region i Uruguay. Genom att identifiera dessa fall och vilka bakterier som orsakat dem kan juverhälsan förbättras betydligt. Sju gårdar runt Paysandù valdes ut. På

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varje gård togs prover från varje juverfjärdedel och analyserades sedan, delvis för celltal m.h.a. California Mastitis Test (CMT) och delvis för bakteriologi. Prover togs också från tanken fem dagar i rad och analyserades delvis för celltal m.h.a. Fossomatic cell count och delvis för bakteriologi. Prevalensen subklinisk mastit visade sig vara hög. På ko-basis var prevalensen 42,2% och gällande juverfjärdedlar 21,8%. Flera olika faktorer kan vara orsakande och det finns ett antal åtgärder bonden själv kan vidta för att reducera antalet infektioner. Medelvärdet för totalantal bakterier i tankmjölken var 24 227 cfu/ml. Dessa resultat redogörs i examensarbetet: "Jämförelse mellan tankmjölksprover och individuella mjölkprover från mjölkbesättningar i Uruguay", skrivet av Anneli Axelsson.



Picture 9. Our supervisor in Uruguay, Ruben Giannechini.

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