A comparison between bulk tank milk samples and individual milk samples from dairy herds in Uruguay

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Abstract

Uruguay is situated between Argentina and Brazil in eastern South America and is the main exporting country of milk and milk products in the region. 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 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 compare results obtained from bulk tank milk (BTM) analysis and individual samples taken from each udder-quarter in the herd, to evaluate whether BTM can be a useful tool in mastitis control.

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 bacteriological analysis. Samples were also taken from the tank on five consecutive days and analysed partly for SCC by the Fossomatic method, and partly for bacteriological analysis. Considered bacteria were common mastitides such as Stafylococci, Streptococci and Coliformes.

The mean value for the total number of bacteria in the bulk tank was 24,227 cfu/ml. The bacteria isolated from the individual cows were not always seen in the tank, and the bacteria found in the tank were not always found in the quarters. The conclusion is therefore that BTM should only be used as a complement to individual sampling.

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Preface

The project behind this degree thesis is a Minor Field Study. Minor Field Studies are carried out within the framework of the Minor Field Studies (MFS) Scholarship Programme, which is funded by the Swedish International Development Cooperation Agency (Sida). The MFS Scholarship Programme offers Swedish university students an opportunity to undertake two months' field work in a developing country to be analysed, compiled and published as an in-depth study or graduation thesis work. The studies are primarily made on subjects of importance from a development perspective and in a country supported by Swedish development assistance.

The main purposes of the MFS programme are to increase interest in developing countries and to enhance Swedish university students' knowledge and understanding of these countries and their problems and opportunities. An MFS should provide the student with initial experience of conditions in such a country. A further purpose is to widen the Swedish human resource base for international development cooperation.

Introduction

Although mastitis occurs sporadically in all species, it assumes major economic importance only in dairy cattle. In terms of economic loss it is undoubtedly the most important disease with which the dairy industry has to contend, partly because milk yield is reduced (Bramley & Kinnon, 1990; Persson Waller & Persson, 2002; Radostits et al., 2002). Mastitis is also the major reason for treating lactating cows with antibiotics (Guterbock et al., 1993; Hallén Sandgren, 1997).

Uruguay is a major milk producer and exporter of dairy products in South America, with approximately 400,000 cows annually yielding 1,400 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 (Gianneechini, 2001).

This study aims to evaluate the difference between the results given by individual samples, taken from each cow in the herd, and bulk tank milk (BTM) samples. From these results it will be evaluated if BTM analysis can be a tool for mastitis control in Uruguay. This study had also another part with the purpose to determine the prevalence of sub-clinical mastitis in the same area of Uruguay. This part of the study will be presented in a degree project written by Sara Ahlner: Prevalence of sub-clinical mastitis in Uruguay.



Fig. 1. A view over the DILAVE-laboratory outside Paysandú.

Definition of mastitis

The term mastitis refers to inflammation of the mammary gland regardless of the cause. It is characterized by physical, chemical and usually bacteriological changes in the milk and by pathological changes in the glandular tissue. The most important changes in the milk include discoloration, the presence of clots and the presence of large number of leucocytes. Mastitis is said to be sub-clinical when there is evidence of inflammation, e.g. a high somatic cell count (SCC) in the milk without any visible abnormality of the milk or udder (Radostits et al., 2002). Often it is more prevalent than the clinical form, it usually precedes the clinical form, it reduces milk production, and it adversely affects milk quality (Gianneechini, 2001). Sub-clinical mastitis can only be diagnosed by the examination of milk samples for the presence of pathogenic bacteria, an increased SCC, or a variety of biochemical changes which are signs of udder disease (Bramley & Kinnon, 1990).

Infection of each mammary gland occurs via the teat canal, the infection originating from two main sources, the infected udder and the environment. In dairy cattle the important infections are those which persist readily in the udder. There are a few bacteria which are common causes of mastitis, these are presented below. The contamination of milkers' hands, wash cloths and milking machine cups by milk from infected quarters may quickly lead to the spread of infection to the teats of other animals (Radostits et al., 2002). No treatment of sub clinical mastitis during lactation should be performed without taking other measures to prevent spreading of infection (Hallén-Sandgren & Ekman, 2002).

Major mastitides

Streptococci

Streptococcus agalactiae (Str. agalactiae) and, in 50% of the cases, Streptococcus dysgalactiae (Str. dysgalactiae), are contagious bacteria and the most important reservoirs of these bacteria are infected udders. Contagious micro organisms 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 (Bramley & Kinnon, 1990; Jayarao, 2000). Sores on teats are the commonest sites outside the udder for persistence of the organism. In any large cattle population where Str. agalactiae 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%. Since the advent of antibiotic treatment, Str. agalactiae has been supplanted by Staphylococcus aureus as the major cause of bovine mastitis. Particularly in herds with a high bulk milk cell count, the probability is that Str. agalactiae infection will be common. The infection may persist for up to three weeks on hair and skin and on inanimate materials such as dung and bricks. Only the teat canal is important as a portal of entry, although there is doubt as how the invasion occurs through the sphincter. Suction into the teat during milking or immediately afterwards does occur, but growth of the bacteria into the canal between milkings also appears to be an important method of entry (Radostits et al., 2002).

The CAMP test, which utilizes the lytic phenomenon shown by Lancefield's group B streptococci in the presence of staphylococcal beta-toxin, is sufficiently accurate for the routine presumptive identification of *Str. agalactiae* in large-scale eradication schemes. Breakdowns are usually due to the introduction of infected animals, even heifers which have not yet calved, or the employment of milkers who carry infection with them (Radostits et al., 2002). Economically and ecologically it is not recommended to treat sub-clinical mastitis during the present lactation, with one exception for *Str. agalactiae* when treatment with antibiotics during lactation period could be motivated (Hallén-Sandgren & Ekman, 2002).

Environmental Streptococci are Streptococcus uberis (Str. uberis) and sometimes also Str. dysgalactiae. Both bacteria are common causes of infection and there are relationships between infection and teat injuries, bad milking technique and housing. Str. uberis 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 (Bramley & Kinnon, 1990; Radostits et al., 2002). Some cows become permanently colonized with Str. uberis and may pass very large numbers of the organism in the feces. It is common with findings 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. Str. uberis is the most common cause of clinical mastitis occurring during the dry period (Radostits et al., 2002). Results in the control of Str. uberis are poor probably because infection occurs at times other than during milking. Once the infection has become established in e herd, sporadic cases are likely to occur in spite of good hygienic precautions. Because of the failure of the general control program to restrain the spread of the infection it is more than usually necessary to treat the infected quarters vigorously (Radostits et al., 2002). Str. dysgalactiae is readily controlled by teat dipping and dry cow treatment, suggesting that transmission often is from cow to cow (Jayarao, 2000).

Staphylococci

Staphylococcus aureus (S. aureus) is a contagious mastitis pathogen that colonizes the teats when there is damage to the skin surface. It produces enzymes that allow it to penetrate deep into the mammary tissue. The infection is usually chronic (with signs of clinical mastitis) or sub-clinical, occasionally showing mild clinical signs. 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 (Jayarao, 2000; Persson Waller & Persson, 2002). The infecting bacteria are excreted in milk and, consequently, the milking clusters, the milkers' hands, udder cloths, etc., become contaminated and may act as fomites transferring disease among the herd (Bramley & Kinnon, 1990; Persson Waller & Persson, 2002). At sub-clinic infections with S. aureus, the count of bacteria in the milk can be very low, which could result in the infection not being discovered unless repeated sampling is done with bacteriological analyses (Persson Waller & Persson, 2002).

Haemolytic, coagulase-positive *S. aureus* is the usual cause, although it may be difficult to demonstrate the presence of the organism in per acute cases especially when necrotic tissue is invaded by *Escherichia coli* and *Clostridium spp*. (Radostits et al., 2002).

Response to treatment is comparatively poor but although *S. aureus* is still preeminent as a cause of sub clinical mastitis its prevalence has been significantly curbed by modern control programs based on test dipping, dry period treatment, well planned slaughter and correct order during milking. Cows that are known carriers of the infection should be slaughtered continuously. It is of great importance to make clear to the farmer that a cow with chronic *S. aureus* infection is not going to get well (Radostits et al., 2002; Svensk Mjölk, 2003b).

Coagulase-negative staphylococci (CNS) have in general been disregarded as mammary pathogens. 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 haemolytic staphylococci. The tissue reaction is usually so mild that the CMT is negative. 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 (Radostits et al., 2002).



Fig. 2. S. aureus with double hemolysis on a blood agar plate.

Coliforms and other Gram-negative bacteria

The term coliform mastitis includes the mastitides in cattle caused by *Escherichia coli* (*E. coli*), *Klebsiella spp.* and *Enterobacter aerogenes* (Radostits et al., 2002). Coliforms are environmental organisms and are frequently isolated from bulk tank milk. 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. Gram-negative non-coliform organisms can cause severe mastitis including outbreaks of clinical mastitis (Jayarao, 2000). Coliform mastitis has been reported worldwide 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 (Radostits et al., 2002).

Bulk tank milk on the farm can become contaminated with gram-negative bacteria present on teats, the teat ends, teat canal, udder surfaces, mastitic udders, and contaminated water used to clean the milking systems and those that are resident in the milking system (Jayarao, 1999).

Corynebacterium bovis

Corynebacterium bovis (*C. bovis*) is a highly contagious organism and weakly pathogenic in nature. *C. bovis* is commonly found as constant inhabitants of mammary glands. 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. Experimentally induced infection of quarters with *C. bovis* causes a mild but significant rise in the milk cell count and a persistent infection of the teat duct epithelium but there is neither clinical abnormality nor change in milk composition (Radostits et al., 2002). If there are many cows with *C. bovis* in a herd, this could result in an elevated bulk tank SCC (Jayarao, 2000; Radostits et al., 2002).

Although *C. bovis* is rarely a cause of udder disease it is frequently found in random milk samples. Because it is highly infectious and is susceptible to teat disinfection it has been suggested that its prevalence could be used as an indicator of teat-dipping efficiency in a herd, either of the intensity of the dipping or of the efficacy of the dip (Bramley & Kinnon, 1990; Radostits et al., 2002).

The method of bulk tank milk sampling

The culture of bulk tank milk for diagnosis of mastitis causing bacteria was begun on dairy farms in the early 1970s. Workers in California and Minnesota were among the first to use this technique. Today, numerous systems are in use due to the further development of BTM culture techniques, however, there is no standardized procedure used (Farnsworth, 1992; Jayarao, 2000). Studies conducted over the last decade have shown that examination of BTM is of practical value in terms of time and cost of analysis for diagnosing multiple problems (current and potential) that might exist in a dairy herd related to milk quality and mastitis pathogens (Jayarao, 2000). Over the last years, using BTM sampling as a tool to ascertain BTM quality and troubleshoot herds with mastitis has received a lot of attention, especially from veterinarians and dairy health consultants who view milk quality and mastitis as an important aspect of their consultancy services for their clients. Progressive milk cooperatives have also become aware of the benefits of monitoring BTM for milk quality and mastitis pathogens. They often use it to reward their dairy producers who excel at producing quality milk and have low incidence of mastitis in their herd. In addition, the milk producers and cooperatives look at BTM analysis as an important part of their quality assurance program (Jayarao, 2000).

Bulk tank sampling is a simple, essential, and economical method of monitoring a dairy herd. Culturing can supply two important types of information: 1) presence or absence of a bacterial group, and 2) identification of predominant bacterial groups in BTM (Laboratory handbook, 1999; Robertson and Bailey, 1999). The method has both benefits and limitations. The benefits are that it saves time, time needed to collect individual quarter milk samples, and the time needed to conduct laboratory tests, and it is less expensive as compared to a whole herd culture analysis. It can also be an important part of a quality assurance program. The limitations are that it does not provide information on an individual cow basis either for milk quality or mastitis. Also information about herd management practices related to milking and milk hygiene is needed to interpret the BTM analysis reports (Jayarao, 2000).

The more often BTM is sampled, the more useful the information. Samples taken over consecutive days or weeks are most useful (Laboratory handbook, 1999; Robertson and Bailey, 1999). Current evidence suggests multiple-day sampling adds accuracy because it helps to overcome variation in shedding of organisms and, in the case of environmental bacteria, variation in milkers (Farnsworth, 1992; Robertson and Bailey, 1999).

Correct interpretation of bulk tank cultures relies heavily on correct and aseptic sampling techniques. Before collecting the sample the milk should be agitated in the bulk tank for 10 minutes prior to collection (Laboratory handbook, 1999; Robertson and Bailey, 1999). The sample should always be obtained, using a clean sanitized dipper, through the milk tank lid and not through the milk valve because bacteria from residual milk in the valve can cause inaccurate results. Refrigerate the sample until processing, which should occur within 36 hours for SCC evaluation. Freezing has little effect on culture results but causes inaccurate SCCs because of cell lysis (Robertson and Bailey, 1999).

The first question to ask when interpreting BTM cultures is whether or not the samples are positive for *Str. agalactiae*, *S. aureus* or *Mycoplasma spp*. Presence of these pathogens in BTM almost always indicates the presence of infected quarters in the herd. The number of these organisms found on culture is determined by the number of infected cows and herd mates, and the severity of infection (Godkin & Leslie, 1993). However, negative culture results do not necessarily mean that the herd is negative for infections caused by these pathogens (Farnsworth, 1992; Laboratory handbook, 1999; Robertson and Bailey, 1999). Coliforms and environmental streptococci may originate from intramammary infections, but more

common sources of elevated counts caused by these bacteria are milking wet udders, milking hygiene in general, organic soil in milk lines, cracked inflations, inadequately heated wash water, and inadequate cooling of milk (Farnsworth, 1992; Godkin & Leslie, 1993; Laboratory handbook, 1999).



Fig. 3. Sterilizing of the dipper before taking milk sample from the bulk tank.

Description of the study area

Uruguay has 3.2 million inhabitants and is situated at latitude $30-35^{\circ}$ S, longitude $53-58^{\circ}$ 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.

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 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 (Gianneechini, 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 is produced per cow per day and 3,500 litres of milk are produced per lactation. The principal breed is the Holstein Friesian (90%) (Gianneechini, 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 cooperative 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 cooperatives (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/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 are above the maximum levels (Persson Waller, 2002).

Bulk tank 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).



Fig. 4. A typical small dairy farm in Uruguay.

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).

Health problems in milk production

The most common health problem among dairy cows is probably mastitis followed by metabolic and hoof problems. The clinical mastitis incidence rate was 1.2 cases per 100 cow months at risk. The sub-clinical mastitis prevalence was 55%. Mastitis is a major cause of losses in milk production in all European countries and probably also in Uruguay (Gianneechini, 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 (Gianneechini, Concha & Franklin, 2002; Persson Waller, 2002).



Fig. 5. Sterile tubes for individual milk samples.

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. The samples for bacteriology were collected in test tubes and placed on ice, for transport to the laboratory, and then cultured the same day. 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.

Somatic cell count

Although there is swelling, heat, pain and indurations in the mammary gland in many cases, a large proportion of mastitic glands are not readily detectable by manual palpation or by visual examination of the milk using a strip cup. Because of the very large numbers of such sub clinical cases the diagnosis of mastitis has come to depend largely on indirect tests which depend, in turn, on the leukocyte content of the milk (Radostits et al., 2002).

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 California Mastitis Test was used for somatic cell count (SCC) of the individual quarters and the Fossomatic method for the bulk tank samples.

California Mastitis Test

The California mastitis test (CMT) is a cow-side test for detecting mastitis in milk. It is the most commonly used test and has proved to be highly efficient, especially in the hands of a skilled operator (Jayarao, 2000; Radostits et al., 2002). It reflects accurately the total leukocyte and the polymorph count of the milk. A healthy cow should have less than 100,000- 150,000 cells per millilitre milk (Svensk Mjölk, 2003a). A single fourth of the udder can have two million cells per millilitre without any clinical signs of mastitis (Hallén Sandgren & Ekman, 1994b). Counts of less than 250,000/ml are considered to be below the limit indicative of inflammation although most normal quarters show less than 100,000/ml (Radostits et al., 2002).

A little milk from each teat was extracted and a CMT reagent added to the milk. The formation of a gel indicates a high level of DNA and a dark purple colour indicates a high pH (Sandholm et al., 1995a). 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). This, together with the reading of the blood agar plates for each quarter, is a good indication for mastitis. Cows in the first week after calving or in the last stages of lactation always give a strong positive reaction,

not necessarily indicating mastitis, this fact was also considered when interpreting the results (Radostits et al., 2002).

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.

СМТ	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

Table 1: CMT results (Sandholm et al., 1995a)

The Fossomatic method

The level of cells in the bulk tank is affected by how many of the cows in the herd that have mastitis and how severe the inflammation is. It is often a small part of the animals that make the level of cells increase (Hallén Sandgren & Ekman, 1994a). Persistently elevated, herd somatic cell counts indicate a high prevalence of sub clinical infection within the herd (Godkin & Leslie, 1993).

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 dyeing 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).

Bacterial culturing of quarter samples

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

Streptococci

Small colonies were tested for catalase production. The catalase test detects the enzyme catalase that converts hydrogen peroxide to water and gaseous oxygen. The reagent is 3% hydrogen peroxide. A loopful of the bacteria was taken from the top of the colonies and placed on a microscope slide and a drop of 3% hydrogen peroxide was added. An effervescence of oxygen gas, within a few seconds, indicates a positive reaction. 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 haemolysis 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.

Staphylococci

S. aureus was identified directly on the blood agar plate by recognition of yellow colonies with α - and β -haemolysis. 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*.



Fig. 6. Ruben with the cooling bag for milk samples.

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, Edwards, McConkey and Acriflavin supplemented agar, respectively.

Streptococci

Edwards's 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 Acriflavin supplemented agar (P-agar) is selective for *S. aureus*. The difference between the total number of colonies on the two media was used to estimate the number of CNS.



Fig. 7. Collecting individual milk samples early in the morning.

Coliforms and other Gram-negative bacteria

McConkey agar was used as a selective medium for Coliforms and other Gramnegative 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.

Legal limits for coliform counts, unlike for pasteurized milk, have not been established for bulk tank milk. However, it is generally accepted that counts >1000 colony forming units (cfu)/ml of raw milk indicate milk produced under unhygienic conditions (Jayarao, 1999).

Total number of bacteria

All colonies on blood agar were counted to determine the total number of bacteria. This is also called Standard Plate Count (SPC) and gives an indication of the total aerobic bacteria present in milk. Bulk tank milk SPC of <1000 cfu/ml is an indication that milk from clean and healthy cows has been collected under hygienic conditions. Under current conditions it is extremely difficult to totally prevent contamination of milk, but SPC counts of less than 5,000 cfu/ml can be achieved. SPC of <10,000 can be achieved by most farms. High SPC in raw milk can be due to improper cleaning of the milking system or presence of *Str. agalactiae* mastitis infection in a herd. Milking cows with soiled udders and teats and mastitis, unclean or sanitized milking equipment, and the inability to cool milk rapidly to less than 4, 4 °C can increase the SPC of raw milk (Jayarao, 2000).



Fig. 8. The adding of ninhydrin for the Sodium Hippurate test.

Results

California Mastitis Test

The results of the CMT were used as a tool in determining which quarters had subclinical mastitis. For CMT results, see Table 2.

		,	5 1		
CMT	Farm 1	Farm 2	Farm 3	Farm 4	Fa

 Table 2: CMT results. % of quarters

СМТ	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

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 4.

Streptococci

Individual samples have shown that out of 181 cows, *Str. agalactiae* was found in one cow, *Str. dysgalactiae* in eight cows and *Str. uberis* in seven cows. In two cows, a Streptococcus was found but could not be typed (in the table called *Str. Spp.*).

For *Str. agalactiae*, a count over 6000 cfu/ml in the bulk tank milk is considered high (Jayarao, 2000).

For results of Streptococci in the individual samples, see Table 3. For results of bulk tank milk, see Table 4.

Staphylococci

Results from individual samples showed that out of 181 cows, 62 were infected with *S. aureus*.

Results from individual samples have shown that 10 out of 181 cows were infected with CNS (Table 3).

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 4.

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 4. No *E. coli* were identified and are therefore not presented in Table 4.

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* (see Table 3). *C. bovis* is, however, a minor pathogen and is therefore not considered in the total number of infected cows or quarters.

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

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



Fig. 9. The sterilizer used for sterilization of the milk tubes.

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 4). For the total number of bacteria in bulk tank milk, a count over 10, 000 is considered high (Jayarao, 2000).

Table 4: Summary of bacteriology results, bulk tank samples from 5 days

	Tot.	SCC, average	Tot. No.	<i>S</i> .	CNS	Str.spp
	No. of	5 days (* 10^3 ,	of bacteria	aureus	(cfu/ml)	(cfu/ml)
	cows	cells/ml)	(cfu/ml)	(cfu/ml)		
Farm 1	17	336	30,320	4,400	8,660	212,600
Farm 2	35	330	13,250	560	660	<100
Farm 3	19	297	15,260	720	7,380	760
Farm 4	26	734	8,920	100	3,340	<100
Farm 5	13	857	48,000	6,550	36,575	<100
Farm 6	28	774	8,680	700	5,100	760
Farm 7	43	734	45,160	2,800	22,840	<100

Table 4, cont: Summary of bacteriology results, bulk tank samples from 5 days

	Str.	Str.	Str.	Coliforms	Gram
	dysgalactiae	agalactiae	uberis	(cfu/ml)	negatives
	(cfu/ml)	(cfu/ml)	(cfu/ml)		(cfu/ml)
Farm 1	39,200	<100	<100	2,660	3,220
Farm 2	320	<100	<100	<100	<100
Farm 3	<100	<100	<100	<100	<100
Farm 4	<100	780	<100	220	<100
Farm 5	980	<100	35,300	140	<100
Farm 6	1,760	<100	220	180	<100
Farm 7	24,080	<100	21,620	6,420	4160

Comparison between individual samples and samples from the bulk tank

On three of the seven farms the results from individual cows and from the bulk tank matched perfectly. On two of the farms, *Str. uberis* was found among the individuals and not in the tank. On one of the farms, *Str. dysgalactiae* was found among the individuals but not in the tank, and on one of the farms *Str. agalactiae* was found among the individuals but not in the tank.

On three of the farms the problem was the opposite; a bacteria was found in the tank, but not among the individuals. On one of these farms, *Str. uberis* and *Str. dysgalactiae* were found in the tank, but none of the cows were infected with these bacteria. *Str. dysgalactiae* was also found in the tank on one of the other farms, but not among the individuals. On one of the farms, *Str. agalactiae* was found in the tank but none of the cows were infected with the tank but none of the cows were infected with the bacteria.

Discussion and conclusions

The purpose of this study was to evaluate whether bulk tank milk analysis can be a tool in controlling sub-clinical mastitis. The evaluation of bulk tank milk analysis as a possible tool for illustrating the situation of sub-clinical mastitis in the herd gave mixed results. On some farms, bacteria that had been identified from the cows could not be found in the tank. In other farms, it was the other way around, bacteria that were found in the bulk tank, were not identified among the individual samples; see Table 5 for a summary. Like the situation in many other countries, the problem with *S. aureus* is considerable in this area.

Farm 1

There is a good relationship between individual samples and samples from the tank. This farm has problems with both environmental and contagious bacteria. They need to consider the order of cows during milking, hygiene during milking and hygiene of the equipment. In this farm you could receive a lot of information only from the bulk tank samples.

Farm 2

The 6 cows infected with *S. aureus* were not fully reflected in the results from the tank. Neither were *S. uberis* or *S. dysgalactiae* found in the bulk tank milk sample. The reason for this could be that there may be such low numbers of contagious pathogens being shed at the time of bulk tank culture that none are grown on culture media. This farm must consider the milking order to prevent spreading of *S. aureus* and they should also gradually slaughter *S. aureus* positive cows.

Farm 3

Here we have a good correlation between individual and bulk tank samples. The only infected cow in the herd has produced a mild reflection in the bulk tank sample. It is though important to identify this cow to prevent spreading of the bacteria.

Farm 4

Also on this farm, the problem is the same as on farm 2, just considering the sample from the bulk tank you could not realize that a major part of the herd is infected with *S. aureus*. Neither was *Str. uberis* found in the tank. The one cow infected with *Str. agalactiae* has caused a mild rise of the bacteria in the tank.

Farm 5

On this farm the correlation is good. They have problems with mastitis in general and *S. aureus* in particular. The total count of bacteria is also far too high and considering the coliforms in the tank there are also problems with the hygiene and maybe the equipment. A lot of improvement needs to be done here and this will best be performed in cooperation with a veterinarian.

	Tot. No. of bacteria in bulk tank milk (cfu/ml)	No. of infected cows	Bacteria found in bulk tank samples	No. of cfu/ml
Farm 1	30,320	S. aureus 1 Str. dysgalactiae 2 Str. spp. 2	S. aureus Str. spp. Str. dysg. Coliforms Gram neg.	4,400 212,600 39,200 2,660 3,220
Farm 2	13,250	S. aureus 6 Str. uberis 1 Str. dysgalactiae 1	S.aureus Str. dysg.	560 320
Farm 3	15,260	S. aureus 1	S. aureus Str. spp.	720 760
Farm 4	8,920	S. aureus 6 Str. uberis 2 Str. dysgalactiae 1	S. aureus Str. agal. Coliforms	100 780 220
Farm 5	48,000	S. aureus 12 Str. uberis 2 Str. dysgalactiae 4	S. aureus Str. dysg. Str. uberis Coliforms	6,550 980 35,300 140
Farm 6	8,680	S. aureus 17 Str. uberis 2 Str. agalactiae 1	S. aureus Str. spp. Str. dysg. Str. uberis Coliforms	700 760 1,760 220 180
Farm 7	45,160	S. aureus 19	S. aureus Str. dysg. Str. uberis Coliforms Gram neg.	2,800 24,080 21,620 6,420 4,160

Table 5: Summary of individual and bulk tank samples, CNS andC. bovis not considered

Farm 6

These results are difficult to explain since a large amount of *Str. dysgalactie* was found in the tank but none of the cows were found infected with this bacterium. In this case, there might have been contamination of the milk from some part of the equipment, environment or when taking the sample. On this farm there were also many cows infected with *S. aureus*. This fact was not satisfactorily reflected in the bulk tank sample. *Str. uberis* in the tank is good correlated with number of infected cows.

Farm 7

Here the problem is the same as on farm 6, Streptococci in the tank and none of the cows infected. *S. aureus* has a satisfying correlation between tank and individuals. There are also some hygienic problems on this farm considering the high total count of bacteria, the Coliformes and the Gram negatives.

The fact that *E. coli* was not found in any of the bulk samples could have a connection with the way cattle are kept in Uruguay. Infections with *E. coli* are mostly a problem when cattle are kept in-doors and the bacteria are often found in bedding materials. Since cattle is kept grazing the whole year in Uruguay, they seem to have less problems with this environmental bacteria. Also, E. coli seldom causes sub-clinical mastitis.

I 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.

The SCC is too high in all of the farms. Less than one-third of the cows in Uruguay are included in a health programme. If a routine bulk tank monitoring was performed in most farms, this could help indicating milking hygiene deficiencies and milk line cleaning problems. Producers who use a bulk tank monitoring system would probably be more successful in decreasing their bulk tank SCC and the prevalence of contagious pathogens than are those who do not use a monitoring system. Using bulk tank milk analysis and taking actions to correct deficiencies help ensure a high-quality product for the consumer and higher producing cows with fewer mastitis problems. Currently, bulk tank milk sample culturing is unfortunately an imprecise procedure. A variety of factors influence both bacterial isolation and interpretation of results. It is advisable to combine the use of bulk tank cultures with other information, such as bulk tank SCC and cultures on individual cows. A veterinarian should also always make a visit to the farm and together with the farmer evaluate milking routines, if teat dipping is performed, the hygiene in general, quality of the food etc. to see if there are any improvements that can be done. A problem here is that 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. 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. Maybe if there were some kind of punishment for having high SCC in the bulk tank, this would stimulate farmers to take part of an udder health program.

Conclusion is that, to be of any value, bulk tank milk analysis should only be used as a complement to individual samples.



Fig.11. We made all agar ourselves.

Sammanfattning

Uruguay ligger mellan Argentina och Brasilien i östra Sydamerika och är det främsta exportlandet av mjölk och mjölkprodukter i regionen. Därför är mjölkproduktionen av stor ekonomisk betydelse i Uruguay. Mastit är en inflammatorisk reaktion i juvret och har betydande effekter på hälsa och ekonomi. Det är det främsta hälsoproblemet hos mjölkkor i Uruguay. Subklinisk mastit är ett tillstånd där det inte finns några synliga inflammatoriska förändringar i juvret och heller inga makroskopiska förändringar i mjölken. Dock kan vid detta tillstånd mjölkproduktionen reduceras och kvaliteten på mjölken försämras. Studier har visat att subklinisk mastit är orsaken till 70% av förlusterna inom mjölkproduktion i Uruguay.

Målet med denna studie var att jämföra resultat från mjölkprover tagna ur mjölktanken, och individuella mjölkprover tagna från varje juverfjärdedel i besättningen. Detta för att utröna om tankmjölksprover kan vara ett användbart redskap för mastitkontroll.

Sju gårdar i området kring Paysandú valdes ut. På varje gård samlades prover in från varje juverfjärdedel och analyserades, dels analyserades cellhalten med hjälp av California Mastitis Test (CMT) och dels odlades proverna för bakteriologisk analys. Prover togs också från tanken i fem på varandra följande dagar och analyserades dels med avseende på cellhalt genom Fossomatic cell count, och dels för bakteriologisk analys. De bakterier som analyserades är vanliga mastitorsakande bakterier: Stafylokocker, Streptokocker och Koliformer.

Medelvärdet för det totala antalet bakterier i tanken var 24,227 cfu/ml. De bakterier som isolerades från de individuella proverna kunde inte alltid isoleras från proverna tagna från tanken, och de bakterier som isolerades från tankmjölksproverna återfanns inte alltid i fjärdedelsproverna. Slutsatsen blir därför att mjölkprover från tankmjölk endast ska användas som ett komplement till individuell provtagning.

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