Occurrence of Clinical and Sub-Clinical Mastitis in Dairy Herds in the West Littoral Region in Uruguay

By *R. Gianneechini[1-3], C. Concha[2], R. Rivero[3], I. Delucci[4]* and *J. Moreno López[1]*

[1]Department of Microbiology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, [2]Department of Mastitis and Diagnostical Products, National Veterinary Institute, Uppsala, Sweden, [3]Laboratory Veterinary Direction "Miguel C. Rubino", Paysandú, and [4]National Research Agriculture Institute (INIA), Colonia, Uruguay.

Gianneechini R, Concha C, Rivero R, Delucci I, Moreno López J: Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. Acta vet. scand. 2002, 43, 221-230. – Twenty-nine dairy farms were selected to determine the incidence of clinical mastitis, prevalence of sub-clinical mastitis and bacterial aetiology in the West Littoral Region of Uruguay. In samples taken by the owner and frozen at -20ºC during a week the incidence rate of clinical mastitis was determined as 1.2 cases per 100 cow-months at risk. *Staphylococcus aureus* was the most common isolated pathogen in 37.5% of 40 milk samples from clinical cases obtained in 1 month. No bacteria grew in the 32.5% of the total samples.

A sub-sample including 1077 dairy cows from randomly selected farms was used to determine the prevalence of sub-clinical mastitis. These samples were taken on one visit to each farm. The prevalence was 52.4% on a cow basis and 26.7% on an udder quarter basis. In 55.1% of the quarters of the selected animals with more than 300 000 cells/ml there was no growth. The isolated pathogens from sub-clinical cases and their relative frequencies were: *Staphylococcus aureus* 62.8%, *Streptococcus agalactiae* 11.3%, *Enterococcus sp.* 8%, coagulase-negative staphylococci 7.4%, *Streptococus uberis* 6.4%, *Streptococcus dysgalactiae* 1.8%, *Escherichia coli* 1.5% and *Staphylococcus hyicus* coagulase-positive 0.6%.

mastitis; Uruguay; cows; incidence; prevalence.

Introduction

Mastitis is an inflammation of the mammary gland which, together with physical, chemical and microbiological changes, is characterised by an increase in the number of somatic cells in the milk and by pathological changes in the mammary tissue (*International Dairy Federation,* 1987). The consequences due to reductions in milk yield, changes in milk composition, discarded milk and loss of genetic potential are serious economic losses for the farmers and the dairy industry (*Godkin et al.* 1990). Clinical mastitis, in which abnormal milk is readily detected, and sub-clinical mastitis, in which no change in the milk is apparent,

may both reduce milk production. The reduction in milk production attributed to sub-clinical mastitis may account for 70%-80% of the total losses (*Philpot & Nickerson* 1991).

Uruguay's milk production is among the largest in South America. A total of 410 000 dairy cows (90% Holstein-Friesian) are milked with an annual milk yield of 1462 million litres (*OPYPA* 2000). Uruguay is the main exporting country of milk and milk products in the region but despite this, little research on mastitis has been done. In 1976, *Del Baglivi et al.* found a sub-clinical mastitis prevalence of 51.2% among sampled cows. In 1981, *Laborde et al.*

reported a prevalence of 49.1% in hand-milked and 57.4% in machine-milked cows. These results were obtained 20 years ago with less milk production in the country, representing a screening of selected farms by convenience from the Southern dairy region, without evaluation of clinical mastitis. Furthermore, we considered that the pathogens frequently isolated in those surveys, mainly *Staphylococcus aureus* and *Streptococcus agalactiae* might have changed after this period of time.

Recently, the annual geometric average for somatic cell counts (SCCs) in bulk tank milk samples measured in 80% of the dairy farms in Uruguay was reported to be around 450 000-500 000 cells/ml (*Gonzalez* 1999). These values indicate that at least 51% of the cows are affected by sub-clinical mastitis, considering the ranges estimated by *Philpot & Nickerson* (1991).

In the Nordic countries equivalent SCC values were 198 000, 143 000, 276 000 and 133 000 cells/ml for Sweden, Norway, Denmark and Finland, respectively, indicating a 25%-30% prevalence of sub-clinical mastitis in the region. The incidence of clinical mastitis in the Nordic countries was 21, 30, 56, and 32 cases per 100 cow-years at risk, respectively (*Plym-Forshell et al.* 1995). There is no information available on the incidence of clinical mastitis and its etiological agents in Uruguay.

The establishment of an efficient mastitis control programme requires an effective diagnostic and monitoring system for all dairy herds in a country. Consequently, the purpose of this survey was to determine the actual prevalence of sub-clinical mastitis and the incidence of clinical cases, and to study the bacteriological aetiology in dairy herds from the West Littoral Region of Uruguay. This region, the second most important for milk production in the country, will be the beginning of a National Survey.

Materials and methods
Sample selection
A computerised list of 345 dairy farms from 3 dairy plants in the districts of Paysandú and Río Negro (West littoral region) that represent 80% of the total dairy farms in the county, was used to select the sample. A two-stage sampling design scheme (*Farver* 1987) was used to determine the prevalence of sub-clinical mastitis. The assumptions used to calculate the sample size needed were: 95 per cent confidence, a 3 per cent maximum allowable error in the estimate of prevalence, and an expected prevalence of sub-clinical mastitis of 50 per cent. On the basis of these assumptions, a sample size of 29 dairy farms and a sub-sample of 1077 milking cows was calculated. According to this scheme, for example 80%, 50% and 29% of the cows of herds with 10, 100, and 300 milking cows, were sampled. The purpose of the sampling scheme was to ensure that the ability to detect sub-clinically affected quarters would be approximately the same for herds of all size. The same 29 randomly selected dairy farms were used to determine the incidence rate of clinical mastitis and the prevalence of subclinical mastitis.

Incidence of clinical mastitis
The sampling was performed during one month. Before starting the project the farm owners were trained in sampling and in the identification of clinical cases. They were requested to freeze the samples at -20ºC. Mastitis was identified on the basis of clinical signs, including abnormal milk and/or a hard or swollen udder. Information about cow identity, lactation number and stage of lactation was recorded. As clinical mastitis was considered when a cow had at least one affected quarter during a period of 14 days (*Bartlett et al.* 2001), this reduces the possibility of taking samples twice from the same clinical case. The incidence rate of clinical mastitis was expressed as the number of

clinical cases per 100 cows-month at risk. This was calculated as the number of cases during the time period divided by the number of cow-days at risk during the same time period x 100 (*Kelton et al.* 1998). The SCC was not determined in the clinical cases, considering the effect of freezing at -20°C.

Prevalence of sub-clinical mastitis
In order to determine the prevalence of sub-clinical mastitis, each selected farm was visited once between September and December 1998 and individual quarter milk samples from selected lactating cows were collected for microbiological culture and SCC determinations. A quarter was considered to be sub-clinically affected when clinical signs were not present and the SCC level was greater than the threshold value of 300 000 cells/ml with or without positive isolation of udder pathogens (*Klastrup* 1975). The calving date from each cow was recorded. The prevalence was determined as the proportion of animals or quarters sub-clinically affected (*National Mastitis Council* 1996).

Bacteriology
Before milking, milk samples were collected aseptically for microbiological culture, according to the procedures of the National Mastitis Council, 1999. The samples from clinical cases were frozen at –20°C and sent to the Northwest Regional Laboratory "Miguel C. Rubino", of Paysandú. These samples were thawed after 1 week and analysed.
The samples for determining the prevalence of sub-clinical mastitis were transported immediately to the laboratory in a special box with ice at 4°C and streaked within 24 hours. Milk samples from both clinical and sub-clinical cases (10 μl) were streaked on a bovine blood agar plate, incubated under aerobic conditions at 37°C and analysed at 24 and 48 hours. The isolated micro-organisms were analysed by colony morphology, haemolysis, Gram stain, catalase and potassium hydroxide (KOH 3%) tests and by colony number categories: 1 = <10 colonies, 2 = 10-50 colonies and 3 = >50 colonies, respectively (*National Veterinary Institute, SVA,* 1998). The isolated bacterial strains were stored at -20°C in tryptic soy broth containing 10% of glycerol. The strains were transported on tryptose agar at 4°C to the Mastitis Laboratory SVA, Uppsala, Sweden and were refrozen and stored under the same conditions as in Uruguay until their final identification.

Staphylococci. The coagulase test was performed following the methodology used by the SVA. The samples were checked for positive coagulase reaction after 2, 4, 10, and 24 h. The differentiation of coagulase-positive staphylococci (CPS) was carried out according to *Capurro et al*. (1999). The inoculation of Peptone agar (P agar) supplemented with 7 mg of acriflavine per ml was conducted according to *Wallace et al.* (1998). Coagulase-negative staphylococci (CNS) were identified according to *Thorberg & Brändström* (2000) with 2 modifications: (1) a commercially available substrate tablet (Rosco, Taastrup, Denmark) was used to test *ß*-galactosidase activity; and (2) the acetone test was performed as described by *Roberson et al.* (1992).

Streptococci. Streptococcal and enterococcal bacteria were identified according to procedures used at the SVA. The *ß*-CAMP synergistic haemolysis test was performed with a *S. aureus ß*-haemolysin strain on a bovine blood agar plate. The Streptex ZL50 kit (Murex Biotech Ltd, Central Road, Dartford, Kent, UK), was used to identify the Lancefield group. Twelve different biochemical reactions were performed using the microplate system for biochemical identification of streptococci (SVA-strept). Unidentified esculin-positive strains

were inoculated on Slanetz-Bartley (SlaBa) agar (Oxoid Limited, Basingstoke Hants, UK) (*Slanetz & Bartley* 1957). To differentiate enterococcus species, each suspicious strain was streaked on a SlaBa agar plate and incubated at 44°C for 2 days. *Enterococcus faecalis* ATCC 29212 and *Str. dysgalactiae* CCUG 39323 were used as positive and negative controls, respectively.

Coliforms. The coliforms were differentiated according to the tests performed at the SVA. The PI test (PGUA + Indol, SVA 1998) determines whether the bacterial strain produces the enzyme *ß*-D-glucuronidase (p-nitrophenyl-*ß*-D-glucopyranosiduronic acid – PGUA) and tryptophanase (amino acid tryptophan-indol test). The Bactidrop™ Oxidase test (Remel, Lenexa, KS, USA) was used to detect the presence of cytochrome oxidase. The strains were included in a miniaturised identification system for enterobacteria and other Gram-negative bacteria such as Api 20E for oxidase-negative and Api 20NE for oxidase-positive strains (api Bio Merieux S.A., 69280 Marcy-l'Etoile, France).

Somatic cell counts
The SCCs in sub-clinical mastitis were performed at the Milk Quality Laboratory of the National Agriculture Research Institute (Instituto Nacional de Investigaciones Agropecuarias, INIA) experimental station, "La Estanzuela", in Colonia, Uruguay. All samples were collected and transported in 10 ml plastic tubes with a tablet of bronopol (Broad Spectrum Microtabs II, D & F Control Systems Inc., Chaska, MN, USA) and analysed within 48 h. The content of somatic cells was determined by the fluoro-opto-electronic cell counting method (Somacount 300, Bentley, Instrument Inc., Chaska, MN, USA).

Table 1. Frequency of micro-organisms isolated from milk samples of clinical mastitis cases.

Microorganism	Number of isolates	Percentage
Staphylococcus aureus	15	37.5
Escherichia coli	5	12.5
Coagulase Negative Staphylococci	3	7.5
Staphylococcus hyicus	(2)	
Staphylococcus chromogenes	(1)	
Streptococcus agalactiae	2	5
Streptococcus uberis	1	2.5
Enterococcus sp.	1	2.5
Negative	13	32.5
Total	40	100

Results
Incidence of clinical mastitis
Milk samples from 40 clinical cases of mastitis were collected from a population of 3351 cows at risk. The incidence of clinical mastitis cases was determined as 1.2 cases per 100 cow-months. The cases were obtained from 29 dairy farms, with cows between first and third lactations, and they were from one to twelve weeks after calving.

The most prevalent isolated pathogens in clinical cases were *S. aureus* (37.5%) and *Escherichia coli (E. coli)* (12.5%), while 32.5% samples were negative. The results of bacteriological findings in clinical cases are summarised in Table 1.

Prevalence of sub-clinical mastitis
Testing for prevalence of sub-clinical mastitis was carried out on 4308 foremilk quarter samples collected from 1077 cows between the first and sixth lactations with less 270 days. In total, 564 (52.4%) cows and 1138 (26.4%) quarters were diagnosed with sub-clinical mastitis.

The 45% of quarters with more than 300 000 cells/ml showed positive bacteriological findings, while 55% of the quarter samples over this

Table 2. Classification of quarters according to cell counts and bacteriological findings on sub-clinical cases

Cell count (Fossomatic method)	Sampled quarters	
	Negative bacteriological findings	Positive bacteriological findings
<300 000 cell/ml [1]	3158 (99.6%) healthy	12 (0.4 %) latent infection
>300 000 cell/ml [1]	627 (55%) non infectious mastitis	511 (45%) infectious mastitis

[1] Threshold value

threshold value did not present bacterial growing. Only twelve quarters with positive bacteriological findings below the threshold value (300 000 cells/ml) were obtained in our survey (Table 2).

The most frequently isolated pathogen was *S. aureus,* followed by *Str. agalactiae, Enterococcus sp.,* CNS, *Str. uberis, Str. dysgalactiae, E. coli* and *S. hyicus* (coagulase-positive). The numbers of isolated pathogens from sub-clinical cases are described in Table 3.

In the present work, CNS was diagnosed at species level for the first time in the country. In this way the presence of *S. hyicus* and *S. chromogenes* was determined in the clinical cases (Table 1) and in the sub-clinical cases *S. hyicus, S. chromogenes, S. epidermidis, S. simulans, S. warneri, S. haemolyticus* and CNS novobiocin resistant strains (Table 3).

Discussion

In this survey, the incidence rate of clinical mastitis was 1.2 cases per 100 cow-months at risk, which as annual incidence can be estimated as 14.4 cases per 100 cow-years at risk. Thus, an estimation of the annual incidence rate of clinical cases was based on the monthly screening. In Sweden, the incidence of 21 cases per 100 cow-years reported by *Plym-Forshell et al.* (1995), and 18 cases per 100 cow-years, reported by *Hallén-Sandgren* (2000), is higher

than in Uruguay. The incidence rates reported from other Nordic countries are also considerably higher. *Plym-Forshell et al.* (1995) report 30, 56, and 32 cases of clinical mastitis per 100 cow-years at risk in Norway, Denmark and Finland, respectively. However, according to

Table 3. Relative frequencies of micro-organisms isolated from milk samples of subclinical mastitis cases.

Microorganism	Number of isolates	Percentage
Staphylococcus aureus	321	62.8
Streptococcus agalactiae	58	11.3
Enterococcus sp.	42	8.2
Coagulase Negative Staphylococci	37	7.4
Staphylococcus hyicus	(13)	
Staphylococcus chromogenes	(11)	
Staphylococcus epidermidis	(3)	
Staphylococcus simulans	(2)	
Staphylococcus warneri	(1)	
Staphylococcus haemolyticus	(1)	
CNS novobiocin resistant strains	(6)	
Streptococcus uberis	32	6.4
Streptococcus dysgalactiae	9	1.8
Escherichia coli	8	1.5
Staphylococcus hyicus (coagulase-positive)	3	0.6
Total	511	100

* In brackets, number of strains to respective staphylococci.

Bartlett et al. (1992) the incidence rates observed in studies on herds from different geographical locations should be compared with caution, since the differences in the incidence rate of clinical mastitis in dairy herds are associated with factors such as climate, breed, level of production, and management. In our case it should be pertinent to carry out a new survey during one year, including all the milk production regions of the country in order to avoid seasonal and ecological influences on the incidence rate of the disease.

Uruguay has a high level of SCCs (458.000 cells/ml) in bulk tank milk (*Gonzalez* 1999) and a low incidence of clinical mastitis compared with the Nordic countries. This agrees with the results obtained by *Erskine et al.* (1988), who reported an incidence of 4.2 cases of clinical mastitis per 100 cow-months at farms with low SCCs ($\leq$150 000 cells/ml) and 2.9 cases per 100 cow-months on farms with high SCCs ($\geq$700 000 cells/ml). *Schukken et al.* (1989) demonstrated also that farms with a very low SCC level showed an increase of clinical mastitis, with high prevalence of *E. coli* infections. Recently, *Suriyasathaporn et al.* (2000) and *Beadeau et al.* (2002) have remarked that herd situations with high proportion of cows with low SCCs appeared to be at increased risk of subsequent clinical mastitis.

In the present study, the prevalence of sub-clinical mastitis was 52.4% as measured on a cow basis, and 26.7% as measured on a quarters basis. These results are higher than those reported in Sweden, namely 30% on a cow basis (*Swedish Dairy Association* 2000) and Finland, namely 37% on a cow basis (*Myllys et al.* 1998). These differences can be attributed to the lack of an udder health programme in Uruguay.

According to *Brolund* (1985), the diagnosis of sub-clinical mastitis is based on a quarter foremilk sample for SCCs, together with bacte-

riological findings. In our survey both parameters were included. The threshold value used to perform the diagnosis in each quarter foremilk sample was 300 000 cells/ml according to the standard applied in Nordic countries (*Klastrup* 1975). We have considered that this old parameter is a realistic one keeping in mind the actual levels of udder health in Uruguay and Southern America. Furthermore, working with cows of the same breed, *Giraudo et al.* (1995) in Argentina have determined an arithmetic mean of 494 000 cell/ml in bacteriologically negative healthy quarters.

The bacterial strains isolated from cases of clinical mastitis were principally *S. aureus* (37.5%) and *E. coli* (12.5%). The percentages of other pathogens isolated were: CNS 7.5%, *Str. agalactiae* 5% and *Str. uberis* 2.5% and *Enterococcus* sp. 2.5%, with 32.5% of cultures being negative (Table 1). These results were substantially different with respect to bacteriological findings in Sweden (*Hallén-Sandgren* 2000) where *S. aureus* (25%) was the principal pathogen in clinical cases and the most prevalent environmental pathogens were: coliforms (23%), *Str. uberis* (18%), *Str. dysgalactiae* (16%) and *Archanobacterium pyogenes (A. pyogenes)* 11%. Slightly less than 1% of *Str. agalactiae* and 4% CNS were isolated.

It is known that control measures for mastitis such as teat dipping and dry cow therapy are adequate to control contagious pathogens (*S. aureus* and *Str. agalactiae*), but are not effective against coliforms. However, dry cow therapy may be of some value in controlling environmental streptococci. This should serve as a reason for explaining the difference of prevalence among contagious and environmental udder pathogens in clinical cases. In Uruguay, these measures have been discontinued, while in Sweden and other Nordic countries they are included in control programmes. Animal management systems may be another reason, *Gold-*

berg et al. (1992) reported a lower incidence of environmental pathogens on teat ends in pastured cattle than in confined cattle. This indicate an increased risk of exposure to environmental pathogens in confined herds while in grazing systems bacterial contamination of teats is minimised. However, muddy conditions in pastures or areas where cows congregate, for example in the installations around the parlour at milking time, may contribute significantly to environmental mastitis in a dairy herd during the rainy season (*Smith & Hogan* 1995). In Uruguay the cows are on grazing during all year.

As mentioned, in our survey 32.5% of the bacteriological cultures were negative for clinical cases. This result was not remarkably different from the 38% and 27% of negative samples obtained by *Giovannini et al.* (2000) and *Miltenburg et al.* (1996), respectively, but is higher than the 18% negative samples reported by *Bartlett et al.* (1992). *Zorah et al.* (1993) stated that in their study between 18% and 38% of milk samples from clinical mastitis yielded no pathogens on culture. The same authors, in reviewing the failure to isolate pathogens, suggested the following reasons: (1) a spontaneous bacteriological cure, (2) the presence of too few viable bacteria, (3) inhibition of bacteria by antibiotics, and (4) the bacteria have continued to be killed after removal of milk samples prior to culture. Analysing the same problem, *Sears et al.* (1990) found that *S. aureus* was shed in a cyclical manner from mammary glands and the sensitivity of culturing a single quarter milk sample to determine the infectious status of a quarter at any one point during the infections was 75%. Glands exhibiting a low shedding cycle are at higher risk of a false negative result when a single-quarter sample is used to detect infection status.

Furthermore, the freezing of milk samples has an effect on the ability to isolate specific bacteria. Freezing and increased storage time result in a decreased number of samples containing *E. coli* and increase the number of samples with CNS without an effect on the number of samples testing positive for streptococci or S. aureus (*Schukken et al.* 1989). Our samples were frozen for one week.

In our survey, *S. aureus* was the pathogen most frequently isolated from sub-clinical cases (62.2%), followed by *Str. agalactiae* (11.3%), *Enterococcus sp.* (8.2%), CNS (7.4%), *Str. uberis* (6.4%), *Str. dysgalactiae* (1.8%), *E. coli* (1.5%) and *S. hyicus* coagulase-positive strain (0.6%) (Table 3). According to *Hallén-Sandgren* (2000), in Sweden the most important isolations from sub-clinical cases were *S. aureus* (37%), CNS (31%) and *Str. uberis* (14%), whereas *Myllys et al.* (1998) reported CNS (53.5%) to be the most common in Finland.

The low SCC of 180 000 cells/ml (geometric means) in Sweden and 130 000 cells/ml in Finland (*Hallén-Sandgren* 2000) is associated with a good control of contagious udder pathogens (*Str. agalactiae* and *S. aureus*) using post-milking teat dipping and dry cow therapy. These measures, however, are not efficient in preventing infections caused by environmental and opportunistic bacteria such as CNS (Smith & Hogan 1995). Uruguay has a high geometric mean of SCC 458 000 cell/ml (*Gonzalez* 1999), which is attributed to poor control of mastitis.

Also, in this study 3 *S. hyicus* coagulase-positive strains have been isolated, representing 0.6% of all pathogens isolated from sub-clinical cases (Table 3). The finding of a low number of coagulase-positive strains other than *S. aureus* concurs with the results obtained by *Capurro et al.* (1999) in Sweden. However, the groups of CNS isolated in this survey were similar to those isolated from milk samples in Sweden (*Birgersson et al.* 1992).

Acknowledgements

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Sammanfattning

Förekomsten av klinisk och subklinisk mastit i mjölkbesättningar i Västkustregionen i Uruguay.

Tjugonio mjölkgårdar valdes ut for att bestämma incidensen av klinisk mastit, prevalensen av subklinisk mastit och den bakteriella etiologin i West Littoral Region i Uruguay. Från prover tagna av ägaren, som frusits ned till -20ºC i en vecka, bestämdes incidensen av klinisk mastit till 1,2 fall per 100 komånader. *Staphylococcus aureus* var den vanligaste patogenen som isolerades, den återfanns i 37,5% av 40 mjölkprover från kliniska fall, samlade under en månad. Av det totala antalet prover var 32,5% utan bakterieväxt. Prov tagna från ytterligare 1077 mjölkkor från slumpmassigt utvalda gårdar användes for att bestamma prevalensen av subklinisk mastit. Dessa prover togs vid besok på respektive gård. Prevalensen var 52,4% avseende kor, och 26,7% avseende juverfjärdedelar. 55% av juverfjärdedelarna hos de utvalda djuren, med över 300 000 celler/ml hade ingen vaxt av bakterier. De isolerade patogenerna, från subkliniska fall, och deras relativa frekvenser var: Staphy-

lococcus aureus 62,8%, Streptococcus agalactiae 11,3%, Enterococcus sp. 8,2%, koagulasnegativa Staphylokocker (CNS) 7,4%, Streptococcus uberis 6,4%, Streptococcus dysgalactiae 1,8%, Escherichia coli 1,5% och koagulaspositiv Staphylococcus hyicus 0,6%.