

## Bovine Mastitis in Cuba. Review article

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### ABSTRACT

Mastitis is the main disease affecting dairy cattle. Reducing it is one of the most important tasks Cuban cattle raising has today. Hence, it is essential to know the national background, and related research contribution. The literature shows that the frequency of mastitis caused by well-known pathogens has declined; whereas mastitis caused by minor pathogens is more frequent. Research on the incidence of mastitis should be done in the eastern part of the country. The economic losses associated to the disease, antimicrobial sensitivity of the microorganisms involved, and the differences between mechanical and manual milking must be further researched as well. Somatic cell count must also be extended to the rest of the country. Milking routine and hygiene should be improved, increased production of medication to control mastitis, and implementation of research on natural products and alternative treatments must be encouraged (homeopathy and Eucalyptus). A program to control bovine mastitis would be useful, based on recent research done in Cuba, including several products made in the country. Updated international methods to fight the disease are based on state of the art methodologies.

**Key words:** *bovine mastitis, prevalence, etiology*

### INTRODUCTION

Milk is one of the most accomplishing foods. Therefore, many countries consider milk production and delivery a national priority. The best milk production period in Cuba was in the 1980s (900.106 L/year, and 7.2 L/cow). In the 1990s these values decreased (3-4 l/cow) as a consequence from the special period that brought a lot of hardships in terms of inputs, management and efficiency of production (some of which are still present) (Funes-Monzote, Monzote, Lantinga, Keulen *et al.*, 2009). Most Cuban farmers who own small herds in reduced spaces carry out family or subsistence production; they have access to inputs at reasonable prices. However, they do not have much technical and organizational support (Rodríguez, 2010). Today, milk not only represents food with high added value, or the raw material to make by-products, but it is also an important source of income (Ponce, Ribot, Capdevila, Viloch, 2008).

Mastitis is the swelling of the mammary gland, with or without infection. Bacteria, mycoplasma, yeast, viruses, and algae may cause the disease, which is classified as clinical or subclinical. Mastitis is characterized by physical and chemical changes of milk; and patho-physiological changes of the mammary tissue, with possible systemic symptoms (EFSA, 2009). It is a complex disease, caused by the interaction of several factors: the

animal, the environment, microorganisms, and man, who plays a critical role (Valdivieso, 2008). It has a significant incidence due to the high costs associated with the disease, the costliest worldwide (Nielsen, 2009).

Milk production has different features depending on the country or region (Kirk, 2012). Bovine mastitis has become the main cause of discarded milk in Cuba. However, the main negative effect of the disease remains unrecognized: unproduced milk. Accordingly, reducing mastitis is one of the most important tasks of Cuban cattle raising (Ponce, 2009). The aim of this paper is to know the antecedents of the disease in Cuba.

### DEVELOPMENT

#### *Early studies and economic significance*

Plommet (1973) made one of the first contributions to mastitis in Cuba. Then, there were 500 000 dairy cows, and prophylaxis was applied to 124 000 herds of 200-400 cows each, according to two different programs: intensive (California Test (CMT), twice a month to each cow, and tank CMT in every milking session); and extensive (no test was made). Several measures that are not frequently applied today were mentioned, such as, recurrent drying, disinfection of nipples, weekly dismantling and cleansing of the milking equipment, and elimination of chronic animals. The prevalent microorganisms observed in the herds were, *Streptococcus agalactiae* (53%), *Staphylo-*

*coccus aureus* (84%), and *Streptococcus* sp. (9%). They corroborated that mastitis was contagious mainly.

Studies made in Cuba before the 1990s showed losses of 164 pesos/cow/lactation, and reduction of 113% milk production (Fuses et al., 1985a). Novoa *et al.* (2004) calculated the economic losses, and reported high prevalence of subclinical infections as the main cause of production. The total estimate was 456.44 Cuban Pesos, and 40.68 Cuban Convertible Pesos per day. For two herds (averaging 82 milking animals). Sosa, Suárez, Pestano and Purón (2005b) estimated that \$15 333.21 CUP were lost in a quarter, due to poor milk production and medical treatments. Other researchers (Alfonso Pérez and Silveira, 2008) found production losses of 33.2 L daily in four herds, with a total of 192 animals and a mean production of 3.5 L. Relova, Armenteros and Capdevila (2008) calculated annual losses of \$11 059.5 CUP for a twenty-cow herd, using Somatic Cell Counts (SCC) by optic microscopy, and based on unproduced milk, according to Philpot and Nickerson (2000).

#### *Prevalence, CCS, etiology and antimicrobial sensitivity*

More systematic studies were initiated in 1980 on the prevalence and etiology of bovine mastitis in Cuban herds, especially at the National Center for Animal Health (CENSA). In the 90s, studies on mastitis were barely published, due to the harsh economic situation of the country. Since the beginning of the 2000s, CENSA has directed several studies on the situation of bovine mastitis in important areas of economic significance for the country. The table shows a summary of the studies with mastitis prevalence and/or etiology, including the prevalence of intramammary infections (IMI), since 1980 to now.

Overall, the reduction of *Streptococcus agalactiae* occurrence, a pathogen causing bovine mastitis, is remarkable. Values near 10% have been observed in the last ten years, along with the appearance of Negative *Staphylococcus coagulasa* (NSC). The prevalence of *Streptococcus agalactiae* is still high in Cuban herds, which shows poor implementation of basic measures to prevent bovine mastitis. No disease control program has been applied on the farms since the late 1980s; accordingly, *Streptococcus agalactiae*, an udder dependent pathogen, has easily proliferated and

developed (Alfonso et al., 2008). The situation is similar to other countries (Pyörälä and Tamponen, 2009; Schunken, González, Tikofsky et al., 2009), the SCN microorganisms are not emerging pathogens for bovine mastitis in Cuba, today (Ruiz, Peña, González et al., 2012).

In the eastern part of the country there are no significant data (see table), and most studies were made to herds under mechanical milking. Today, a great deal of all the milk produced in Cuba is manual, by the private sector (O.N.E., 2013).

Two studies, at least, have reported the presence of *Arcanobacterium pyogenes* as a pathogen of bovine mastitis (Armenteros, Ponce, Capdevila, Zaldívar, Hernández, 2006; García, Hernández and Silva, 2012). The latest report of infection included 90 calves between 70 and 120 days of age. Both studies were sensitive to the antimicrobials used. *Mycobacterium fortuitum* was reported in Cuba after anatomopathological diagnostics, as the cause of granulomatous mastitis (Muñóz, Durand, Quintana, Martínez, 1995).

Although since 2009 CENSA performs electronic SCC, all previous research was done through optic microscopy. In order to determine the relation between the California test (CMT), SCC, and the bacteriological exam, Martínez, Fustes and Diallo (1981) found a mean of 180 103; 720 103; 1 300 103; 5 300 103; and 14 740 103 cells/mL, for negative CMT, traces, 1; 2; and 3 crosses in the same order. Relova, Armenteros and Capdevila (2008) found 1 800 103 cells/mL in a container with milk from 20 primiparous cows. The geometric mean of SCC for 10 herds in Cienfuegos, Cuba, was 906 103 cells/mL (Novoa, Armenteros, Abeledo, Casanovas, Valera *et al.* 2004). Another comparison study reported initial values of 1 387 103, and 1 353 103 cells/mL of two groups of animals in 238 quarters (Valera, Caballero, Linares, Novoa, and Casanovas, 2005a). Recently, the average SCC values were published in Cuba, according to the microbiological diagnostic, the mean SCC of negative quarters was 167.4 103 cells/mL, NSC 623.8 103 cells/mL, *Corynebacterium bovis*, 592.2 103 cells/mL, *Staphylococcus aureus* 748.3 103 cells/mL, and *Streptococcus agalactiae* 1 303.8 103 cells/mL (Ruiz *et al.*, 2012). International studies, including Latin America, show the need to perform widespread SCC in Cuba, in order to know the average values for the country,

region, herd, and even, animal (Reyes and Bedolla, 2008). Cuba needs SCC urgently to develop dairies and avoid subjective results in the California test, for more fair pay to farmers.

Aguilera (1987b) found penicillin sensitivity in 79.4 and 72.2% of *Streptococcus agalactiae* and *Streptococcus aureus* isolates, respectively. Armenteros, Ponce, Capdevila, Zaldívar and Hernández (2006), in one specialized herd found sensitivity to ciprofloxacin, enrofloxacin, meticillin (except for *Pseudomonas aeruginosa* and *E. coli*), oxytetracycline (except for *Staphylococcus aureus* and *Pseudomonas aeruginosa*), cloxacillin (except for *E. coli* and *Pseudomonas aeruginosa*), cefalexin and gentamicin (except for *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*), and elevated resistance to tetracycline, trimethoprim, bacitracin, penicillin G and neomycin.

Evaluation of two strains of *Staphylococcus aureus*, one as reference (ATCC 29740), and another isolated from clinical mastitis by Velázquez and Barreto (2011) found resistance to penicillin G, gentamicin, and to a lesser extent, streptomycin in the natural strain. Although this result is limited by the size of the sample, contrasts with findings by Ruiz *et al.* (2012) where Gentamicin, tetracycline and ciprofloxacin showed the highest sensitivity per cents 69.9, 68.4, and 66.7%, respectively). The *Staphylococcus aureus* isolates were highly sensitive to the microbials used, above 80%, in contrast to the low sensitivity observed for NSC, and contrary to the response of *in vivo* pathogens. They tend to be more resistant than *Staphylococcus aureus* *in vitro*, and to develop multiresistance easily (Sawant, Gillespie and Oliver, 2009).

Further information is needed about antimicrobial sensitivity of pathogens that cause mastitis in Cuba. Although the use of veterinarian medication has been limited over the last decades today, 97% of all vaccines and medication necessary for livestock management is in stock. They have been supplied by the LABIOFAM Group, in charge of developing new products to treat mastitis. Information about the sensitivity of microorganisms to medication is critical for new productions.

#### *Risk factors*

Several papers have identified numerous risk factors leading to bovine mastitis, both clinical and subclinical, in Cuban dairies. Soca, Suárez,

Rivero, Fuentes and Purón (2005) and Alfonso *et al.* (2008) mentioned some risk factors, such as, faulty pre-milking, wet udders, unaligned milking equipment, alterations in the order of activities, poor nipple disinfection after milking, etc. Additionally, the cows with clinical mastitis do not pass when milking is over, there are problems with the milking equipment, and the liners are defective (cracked, dilated and holey). The authors also added the presence of pathogens, like *Staphylococcus aureus* and *E. coli* (90 and 70%, respectively) during swaps made to milkers' hands and liners.

In terms of milking schedules, Armenteros, Peña, Pulido and Linares (2002) observed significant differences in the prevalence values achieved. Equal behavior was observed during the analysis performed between properly working dairies and other farms with defective equipment, especially related to pulsing. Pérez, Guzmán and Vargas (1982) concluded that using defective milking equipment may have a negative effect on the prevalence of mastitis. Cepero, Salado, Aguiar and González (2005b) noted that the most commonly observed problems include the lack of vacuum meters to measure vacuum pressure; pulsators are out of range; the equipment has no maintenance; the absence of cleansing products.

The effects of lactation days on subclinical prevalence of the disease must be taken into account; significant differences were observed between the group of units with more than 180 days of lactation and the group that averaged 180 days (Armenteros *et al.*, 2002b). Even when it was not the purpose of the study (Armenteros *et al.*, 2006), other risk factors observed were the existence of polluted environments, deficiencies in treating clinical cases, and the lack of drying therapy.

One of the most comprehensive studies on risk factors of bovine mastitis in Cuba, Novoa *et al.* (2005) found that placement of liners on clinical cases, and the inclusion of cows with this disease type in the milking sessions were risk factors strongly associated with the prevalence of clinical and subclinical mastitis. Overall, the determinants related to improper routine milking procedures, and wrong handling practices followed by long lactation periods and repetitions, were the most significant. Liner tilting and faulty udder drain-

age, were the risk factors that associated most to the occurrence of the two forms of the disease.

A sui generis study (Oses, Alfonso, Cepero, Saura and Pedraza, 2010), made in the eastern part of the country to assess the impact of climatic variables on the prevalence of bovine subclinical mastitis showed that the highest significant correlations were associated with cloud formation, followed by minimum relative humidity. For other variables, like temperature, no significant relationship to prevalence of subclinical mastitis (SCM) was observed. Increased cloudy skies to 1/8, decreased prevalence of SCM in 8.96 %. January was the month with the highest prevalence of SCM; and December was the lowest.

#### *Prevention, treatment, and control*

The chemical tool to diagnose mastitis (CENMAST) was developed and registered nationally, after research done to find products to handle bovine mastitis. It had sensitivity, specificity, and efficacy of 97.7, 97.5, and 97.6%, respectively. The correlation between the commercial CMT reagent and CENMAST was 0.995; and it was 0.926 in comparison to SCC ( $P < 0.0001$ ) (Escobar and Ponce, 2001). The product has reduced imports of CMT, four times more expensive (Betancourt, Ramírez, Navarro, González, López, Linares, 2010). Despite the availability of these products, the absence of California reagent in the Cuban market in the latest years has caused a decline in the diagnostic of subclinical mastitis. Therefore, the search for other reagents that can replace California and provide the same efficiency, may have an important impact on dairy cattle and quality. Accordingly, several studies have been made to evaluate other products, like Dodecilo, with positive results (Ferrer and Valdéz, 2009).

Recently, molecular research of bovine mastitis pathogens has been started, beginning with *Staphylococcus aureus* (Peña and Uffo, 2010). The genetic variability of isolates of the pathogen in the milk has been demonstrated. Research using 98 strains from 17 genotypes demonstrated that the Cuban strains (98.0%) of *Staphylococcus aureus* are high biofilm producers. Also, because the t605 genotype has a strong capacity to form biofilm, it has a significant pathogenic potential in terms of bovine mastitis that may confer high resistance and persistence capacities to infect the mammary glands (Peña, Uffo, 2013).

In the 1980s several complementary studies were developed in Cuba to identify efficient disinfectants. Initially, the disinfectants with different concentrations were identified, skin-friendly pH and emolient (Fustes, Martínez, Tablada and Suárez, 1895b). The best bactericidal effects were evaluated in vitro (Fustes, Martínez, Tablada, Suárez and de la Vega, 1985c). Finally, efficacy was evaluated in two dairies, using the two solutions with proven bactericidal activity in the lab; a third dairy with no disinfection, was used as control. The control herd underwent 65 intramammary infections, while the instances where the benzalconio chloride at 0.75% with emolient and tamed iodine were applied (1% free iodine), 32 and 33 infections (50.8 and 49.2% reduction, respectively) were produced (Fustes, Martínez, Tablada, Suárez, Pérez, 1985d).

Perhaps the Cuban product with the highest impact potential on udder health is Udertan, considered the first natural internationally recognized post-milking mammary disinfectant (Ponce et al., 2007). Additionally, the safety advantages offered by Udertan have been corroborated; no risks have been observed to industry and consumer's health (Armenteros et al., 2002a). IIM reduction in 73.3 and 44.4% have been reported in *S. aureus* and *S. agalactiae*, respectively, as well as 71.7% for the clinical cases (Armenteros et al., 1998). Unfortunately, raw material shortages (*Rhizophora mangle* L bark), due to environmental issues that caused a halt in the production of Udertan. Today, disinfection and post-milking sealing of the udder is one of the frequent problems of milking practice (Relova, Armenteros and Capdevila, 2008).

A new version of Diralec®, Diralec-02 has been under development for a few years. The device will include subclinical mastitis detection through electricity, with an accuracy of  $\pm 0.01$  units (Ramírez et al., 2007). This will allow the dairy industry and laboratories to achieve different milk quality parameters faster, based on the logistic and economic advantages provided by a domestic device.

In the 1980s evaluation of clinical mastitis was made by penicillin injection through the abdominal aorta, though the long-term highest concentration was found in the most affected quarters. Elimination was reported in the apparently healthy quarters (Aguilera et al., 1980). Further comparison research including the intramammary

and intraorta routes to inject streptopenicillin showed greater efficacy of intramammary treatment of subclinical mastitis (Aguilera, and Martínez, 1981b), and intraorta treatment for clinical mastitis (Aguilera, 1983). Aguilera (1987b) evaluated drying and found a reduction in the number of quarters with *Streptococcus agalactiae*, and *Staphylococcus aureus*, of 78.4 and 52.8%, respectively. In the control group, the reduction of infected quarters was 24.2 and 20.0%. The new mastitis pathogen isolates accounted for 14.8% in the treatment group, and 34.2% in the control group. Ramírez *et al.* (2000) made a separate experiment in few quarters (76), in the province of Granma, using acriflavina as drying treatment for cows. Even when the results were positive, the treatment protocol was too complex in comparison to the antimicrobials used for that purpose. Currently, LABIOFAM produces gentamycin (Pérez, Guevara, Rodríguez, Ortíz, 2010), the only available choice for intramammary treatment in the country.

The little research done in Cuba on treatments and products to control mastitis have focused on natural products. *In vitro* studies demonstrated that Hook *Eucalyptus citriodora* tinctures (20 and 80%) were more effective ( $P < 0.05$ ) than *Eucalyptus saligna* tinctures, and both showed better antimicrobial activity than gentamycin, penicillin and streptomycin against *Staphylococcus aureus* (Velázquez and Barreto, 2011). Barreto, Velázquez, Rodríguez and Rodríguez (2007) evaluated hydroalcoholic extracts (20%) of *Eucalyptus citriodora* Hook (54 g/L) to treat subclinical mastitis. They used 44 quarters and found that the tincture had a curative action after 72h. It was easy to make, and less costly than other treatments. Homeopathy is another treatment variant for bovine mastitis, which has been studied in Cuba, mainly in the province of Cienfuegos. It is an easy and inexpensive therapeutic method which does not interfere with the quality of milk by-products, because it is not accumulated or excreted in the milk (Valera *et al.*, 2002). Studies of 238 quarters divided in two groups (one receiving homeopathic treatment and one negative control), have reported a decline in prevalence, from 69.7% to 33.6% in the treated group. The control group had an increase of up to 86.6%, beginning in 68.0%. The herds that were not treated had 8.24 fold higher risk of subclinical mastitis; the negative incidence

of the treated herd was 36.1% (Valera *et al.*, 2005b). Variability of SCC depended on the treatment applied (Valera *et al.*, 2005a).

Cnemast and Udertan, and Diralect are part of the Integrated Program to Enhance Milk Production and Quality (PROCAL) (Ceballos *et al.*, 2009). The program includes scientific and technological advances in products, technologies, services, training and supervision, from primary to industrialized production, for the last 15 years in Cuba, and the international results in the area. One of the 10 stages of a recent version of PROCAL has the sole purpose of eradicating mastitis. PROCAL was first applied in 1996, and after 10 years, it had been applied in 200 dairies, about 1.109 L (Ponce, 2007). For some years now, the intention has been to include PROCAL in the Good Dairy Production Practices (GDPP), so a guideline was designed for its application, in which mastitis is a vital issue from the beginning (Villoch and Ponce, 2010). However, the implementation of GDPP has not been started in Cuba. The reduction of mastitis is too big an assignment to implement through just one stage in PROCAL or GDPP. The present conditions, and the important strategic priority milk production has for the country's development, require a national program for prevention and control of bovine mastitis. Considering that the ability of manufacturers to control mastitis depends on access to information, diagnostics and animal health products (Zadoks and Fitzpatrick, 2009).

A Cuban standard (CEN, 1987) establishes the procedures to control bovine mastitis. It classifies the units according to the existing control (low intensive control, non-intensive control, supervision); it also provides epidemiological classification (unaffected units, controlled focus, and uncontrolled focus), using detailed parameters for each category, though they are not applicable now. In addition to the standards, there are detailed measures to follow in each category: final nipple disinfection, dry treatment, elimination of cows with more than two quarters lost, or repeated episodes of clinical mastitis during lactation, cleansing, and systematic maintenance of milking equipment, clinical examination of dry cows, clinical cow milking at the end, and immediate treatment of clinical cows. Many of them are absent in most Cuban herds.

The recent news stories about milk production and mastitis (Hernández, 2011, Delgado, 2012, Castro and Delgado, 2012) proved the importance of the sector. Mastitis represents a problem due to the losses it causes (Nielsen, 2009, Carrier, 2009), but farmers do not recognize it that way, and only worry when their milk is not sold at \$0.35 CUP/L.

Armenteros (2002) noted that their results stress the need to reactivate the program for bovine mastitis control, and Alfonso et al. (2008) concluded that failure of milking practices, poor conditions of the milking equipment, and advanced lactation period, are factors that effect on the elevated prevalence values described. The disease is considered a sanitary disaster; hence, the need to reestablish the Program for Mastitis Prevention and Control is an imperative.

## CONCLUSIONS

There is a need to carry out further studies of Bovine mastitis in Cuba. The accuracy and current circumstances (new price of milk) of related economic losses are unknown. A reduction in the frequency of mastitis caused by contagious pathogens, like *Staphylococcus aureus* and *Streptococcus agalactiae*, has been observed; with the emergence of new minor pathogens. It is important to study bovine mastitis in the eastern part of Cuba, and also to further study the disease in hand-milked herds. SCC must be extended throughout the country, to access data that make possible greater and faster development of the Cuban milk industry. Gentamycin, tetracycline, and ciprofloxacin are the most sensitive antimicrobials among mastitis pathogens. Further studies must be developed on this topic. Mechanical milking has a lot of risk factors for the occurrence of the disease, due to equipment deterioration (hand milking is also included), and the milking practices must be improved. Although there are a number of successful products for diagnostic and control of bovine mastitis, production and recovery of others, like UDERTAN is required. Research on natural products, like homeopathic treatments and Eucalyptus have not contributed with specific products to control the disease. A program for bovine mastitis control must be implemented, from recent results of research in Cuba, including some products developed over the years, in concert with state of the art technology in the world.

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**Table. Studies of prevalence and/or etiology of bovine mastitis results in Cuba**

Reference	Location	Milking ty	#	Prevalence (%)				
				Herds (quarters)	Atroph	Clinical mastitis	Subclinical mastitis	IIM
(Aguilera and-1981a)	-	-	1 (304)	10.2	47.4	29.6	-	<i>Streptococcus agalactiae</i> <i>Corynebacterium</i> sp. <i>Streptococcus</i> spp. 5,1
(Aguilera and-1981b)	-	-	(124)	-	-	70.2	81.7	<i>Staphylococcus</i> sp. 34 <i>Streptococcus</i> sp. 22,6
(Martínez <i>et al.</i>	-	-	6 (527)	-	-	44.2	59.2	<i>S. agalactiae</i> 19,5 <i>Corynebacterium</i> sp. <i>Staphylococcus aureus</i>
(Guzmán <i>et al.</i>	Mecánico	-	4 (3 000)	-	-	51.0	-	-
(Aguilera, 1981)	-	-	(503)	-	-	-	-	<i>S. agalactiae</i> 53,6 <i>S. aureus</i> 22,2
(Fustes <i>et al.</i> , Occidente	-	-	25 (11 488)	1.7	1.1	25.2	-	-
(Ronda <i>et al.</i> , LHA	-	-	19 (6 188)	-	6.8	45.1	-	-
(Fustes and M-	-	-	-	2.8	1.7	29.1	-	-
(Aguilera, 1981)	-	-	4 (828)	5.7	14.5	45.5	51.5	<i>S. agalactiae</i> 25,7 <i>S. aureus</i> 9,9
(I.M.V., 1989-	-	-	-	-	2.1	38.6	-	-
(Escobar and -	-	-	4 (1 000)	-	-	63.4	-	-
(Novoa <i>et al.</i> , CFG	Mech.	-	5 (1 016)	4.3	1.1	67.0	-	<i>S. agalactiae</i> 55,0 <i>S. aureus</i> 20,0
(Armenteros <i>et al.</i> , PRI LHA MTZ VCL	Mech.	-	56 (12 274)	3.7	3.0	76.5	45.1	<i>S. aureus</i> 30,5 <i>Corynebacterium</i> bov. <i>S. agalactiae</i> 8,3
(Novoa <i>et al.</i> , CFG	Mech.	-	10 (2 024)	-	5.0	62.2	45.6	<i>S. aureus</i> 29,3 <i>C. bovis</i> 8,8 <i>S. agalactiae</i> 6,8
(Cepero <i>et al.</i> , VCL	Mech.	-	5 (480)	-	-	18.1	-	-
(Soca <i>et al.</i> , 2MAY	Mech.	-	2 (328)	4.8	3.9	67.0	-	-
(Cepero <i>et al.</i> , VCL	Manual	-	3 (568)	-	-	25.9	-	-
(Armenteros <i>et al.</i> , ART	Mech.	-	1 (568)	-	12.1	22.9	42.8	<i>S. aureus</i> 19,2 <i>Streptococcus</i> spp. 8,3

(Relova <i>et al.</i> , MAY	Mech.	1 (80)	2.5	26.3	76.9	53.7	SCN 5,7 <i>Corynebacterium sp.</i> 4 <i>C. bovis</i> 27,5 <i>S. agalactiae</i> 12,5 <i>S. aureus</i> 1,2
(Ruiz <i>et al.</i> , 2PRI ART MAY SSP CMG	Both	35 (1 484)	3.0	0.9	38.2	63.7	SCN 24,2 <i>C. bovis</i> 18,9 <i>S. aureus</i> 11,8 <i>S. agalactiae</i> 10,0

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