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Research on 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 research contribution. The literature shows that the frequency of mastitis caused by contagious pathogens has decreased; instead, minor pathogens are frequently the main cause. Research on the incidence of mastitis is recommended 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 practices and hygiene should be improved. Increased production of medication to control mastitis, and the implementation of research on natural products and alternative treatments must be encouraged. A program to control bovine mastitis would be useful, based on recent research done in Cuba, including several products achieved in the country, using updated international methods to fight the disease.

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

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 (900.10^6 L/year, and 7.2 L/cow). In the 1990s these values decreased (3-4 l/cow) as a result of the special period that brought a lot of hardships with inputs, management and efficiency of production (some of which are still present) (Funes-Monzote, Monzote, Lantinga, Keulen *et al*., 2009). Most Cuban farmers own small herds in reduced spaces, with family or subsistence production, and 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, Villoch, 2008).

Mastitis is the swelling of the mammary gland, with or without infection. Bacteria, mycoplasmas, yeasts, 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 integration of several factors: the animal, the environment and the microorganisms, and man, who plays a critical role (Valdivieso, 2008). It is significant 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 is not recognized: unproduced milk. Hence, reducing mastitis is one of the most important tasks for 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. There were then 500 000 dairy cows, and prophylaxis was applied to 120 000 herds of 200-400 cows each, according to two different programs: intensively (California Mastitis Test (CMT), twice a month to each cow, and in every milking session); and extensively (no test was made). He mentioned several measures not frequently applied today; such as, recurrent drying, disinfection of nipples, dismantling and cleaning of the milking machine, and elimination of chronic animals. The results of prevalence of microorganisms in herds were, Streptococcus agalactiae (53%), and Staphylococcus aureus (84%), Streptococcus sp. (9%). They showed that mastitis was mainly contagious.

Studies made in Cuba before the 1990s showed losses of $164 pesos/cow/lactation, and reduction of 113% of milk production (Fustes *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 (average of 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 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 thirty-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

In 1980, more systematic studies on the prevalence and etiology of bovine mastitis in Cuban herds were initiated, particularly by the National Center for Animal Health (CENSA). In the 90s, few studies were published on mastitis, due to the harsh economic situation of the country. Since the 2000s, CENSA has directed several studies on the situation of bovine mastitis in important areas of economic significance for the country. Table 1 shows a summary of the studies with mastitis prevalence and etiology, including the prevalence of intrammamary infections (IMI), since 1980.

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 in the farms since the late 1980s; thus, 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 (Ruiz, Peña, González *et al*., 2012).

In the eastern part of the country there are no significant data (Table 1), and most studies were conducted on herds using mechanical milking. Today, a great deal of all the milk produced in Cuba is manually produced 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 latter reported infection of 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 diagnostic, as the cause of granulomatous mastitis (Muñoz, Durand, Quintana, Martínez, 1995).

Although since 2009 CENSA performs electronic SCC, previous research was done with optic microscopy. In order to determine the relation between the California Mastitis Test (CMT), SCC, and the bacteriological exam, Martínez, Fustes and Diallo (1981) found a mean of $180\,10^3$; $720\,10^3$; $1\,300\,10^3$; $5\,300\,10^3$ and $740\,10^3$ cells/mL, for negative CMT, traces, 1; 2; and 3 crosses in the same order. Relova, Armenteros and Capdevila (2008) found $1\,800\,10^3$ cells/mL in a container with milk from 120 primiparous cows. The geometric mean of SCC for 10 herds in Cienfuegos, Cuba, was $906\,10^3$cells/mL (Novoa, Armenteros, Abeledo, Casanovas, Valera *et al*., 2004). Another comparison study reported initial values of $1\,387\,10^3$, and $1\,353\,10^3$ cells/mL of two groups of animals in 238 quarters (Valera, Caballero, Linares, Novoa, and Casanovas, 2005a). Recently, average SCC values were published in Cuba, according to the microbiological diagnostic, mean SCC of negative quarters was $167.4\,10^3$ cells/mL, NSC $623.8\,10^3$ cells/mL, Corynebacterium bovis, $592.2\,10^3$ cells/mL, Staphylococcus aureus $748.3\,10^3$ cells/mL, and Streptococcus agalactiae $1\,303.8\,10^3$ cells/mL (Ruiz *et al*., 2012). International studies, including Latin America, show the need to carry out widespread SCC in the country, 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 payment results derived from the California Mastitis Test.

Aguilera (1987b) found penicillin sensitivity in 79.4 and 72.2% of Streptococcus agalactiae and Streptococcus aureus, respectively. Armenteros, Ponce, Capdevila, Zaldívar and Hernández (2006), found sensitivity to ciprofloxacin, enrofloxacin, meticillin (except for Pseudomona aeruginosa and E. coli), oxytetracycline (except for Staphylococcus aureus and Pseudomona aeruginosa), cloxacillin (except for E. coli and Pseudomona aeruginosa), cephalexin and gentamicin (except for Staphylococcus aureus, E. coli and Pseudomona 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) in which 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 antimicrobials used, above 80%, in contrast to the low sensitivity observed for NSC, and to the response of in vivo pathogens. They tend to be more resistant than Staphylococcus aureus in vitro, and they show multiresistance more 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 available. They have been supplied by the LABIOFAM Group (ECURED, 2013), 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 (2005a), and Alfonso *et al*. (2008) mentioned some risk factors, such as, faulty bluntness, wet udders, unaligned milking equipment, changes in the order of activities, and poor nipple disinfection after milking. Additionally, the cows with clinical mastitis do not pass when milking is over, there are problems with the milking equipment, and the cups are defective (cracked, dilated and holey). The authors also added the presence of pathogens, like Staphylococcus aureus and E. coli (90 and 70%, respectively) after hand swaps made to dairy cows and cups.

Armenteros, Peña, Pulido and Linares (2002 b) observed significant differences in the prevalence values achieved, in terms of milking schedules. Equal behavior was observed during the analysis performed between properly working dairies and other farms with defective equipment, especially in terms of 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; out of range pulsing; lack of equipment maintenance; and 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), additional risk factors also include the existence of polluted environments, treatment deficiencies of clinical cases, and no drying therapy.

One of the most comprehensive studies on risk factors of bovine mastitis in Cuba, Novoa *et al*. (2005) found that placement of cups in 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 cases. Cup tilting and faulty udder drainage were the risk factors that most associated with 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, aimed at evaluating 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. As to other variables, like temperature, no significant relationship to prevalence of subclinical mastitis (SCM) was observed. January was the month with the highest prevalence of SCM; and December was the lowest.

Prevention, treatment, and control

A 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 0.926 compared to SCC ($P < 0.0001$) (Escobar and Ponce, 2001). The product has helped reduce imports of CMT, four times more expensive (Betancourt, Ramírez, Navarro, González, López, Linares, 2010). Despite the availability of these products, in the latest years, the absence of California reagent in the Cuban market has caused a decline in subclinical mastitis diagnosis. Hence, 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 Dodecyl, with positive results (Ferrer and Valdéz, 2009).

Recently, molecular research of pathogens that cause bovine mastitis has been started, beginning with Staphylococcus aureus (Peña and Uffo, 2010). In it, 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 desinfectants were identified at different concentrations, skin-friendly pH and emollient features (Fustes, Martínez, Tablada and Suárez, 1895b). The 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 was used as control. The control herd underwent 65 intramammary infections, while the instances where the benzalkonium chloride at 0.75% with emollient and iodophor 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 post-milking mammary disinfectant, internationally recognized (Ponce *et al*., 2007). Additionally, the advantages offered by UDERTAN in terms of safety have been corroborated; no risks have been observed to industry and the consumer’s health (Armenteros *et al*., 2002a). IIM reduction in 73.3 and 44.4% have been reported in S. aureus and S. agalatiae, respectively; *as well as 71.7% for the clinical cases (Armenteros et al., 1998).* Unfortunately, raw material shortage (Rhyzophora mangle L bark), due to environmental issues, caused a halt in the production of UDERTAN. Today, disinfection and post-milking sealing of the udder is one frequent problems of milking practice (Relova, Armenteros and Capdevila, 2008).

A new version of Diralec®, Diralec-02 has been developed for a few years. The device will include subclinical mastitis detection through electricity, with an accuracy of ± 0.01 units (Ramírez *et al*., 2007). This will allow the dairy industry and laboratories to achieve faster different milk quality parameters, relying 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 highest concentration for a long time 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 had greater efficacy of the intramamary 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, reduction of infected quarters was 24.2 and 24.0%, respectively. 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 a 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. Currently, LABIOFAM produces gentamicin (Pérez, Guevara, Rodríguez, Ortíz, 2010) as 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 alternatives. In vitro studies demonstrated that Hook Eucalyptus citriodora tinctures (20 and 80%) were more effective ($P < 0.05$) than Eucalyptus saligna Sm tinctures, and both showed better antimicrobial activity than gentamicin, penicillin and streptomycin against Staphylococcus aureus (Velázquez and Barreto, 2011). Barreto, Velázquez, Rodríguez and Rodríguez (2007) evaluated hydro alcoholic 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 it was less costly than other treatments. Homeopathy is another treatment variant for bovine mastitis, which has been studied in Cuba, in the province of Cienfuegos. It is an easy and cost-effective therapeutic method; it does not interfere with the quality of milk by-products, because is not cumulated or excreted in the milk (Valera *et al*., 2002). Studies of 238 quarters divided in two groups (one homeopathic and one negative control), have reported a decline in prevalence, from 69% 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 36.2% (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 is completely aimed at eradicating mastitis. PROCAL was first applied in 1996, and after 10 years, it has been used in 200 dairies, about $1 \cdot 10^9$ 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 a 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 (Zadoks and Fitzpatrick, 2009).

A Cuban standard (CEN, 1987) establishes the procedures to control bovine mastitis. It classifies the units according to their 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 lost quarters, 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. Most of them are absent in the Cuban herds.

The recent publication of articles 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 producers do not recognize it that way, and only worry when their milk is not sold at $0.35 CUP/L.

Armenteros *et al.* (2002b) noted that their results stress on the need to reactivate the program for bovine mastitis control, and Alfonso *et al*., 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.

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. Gentamicin, 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-based products have not contributed with specific results to control the disease. A program for bovine mastitis control must be implemented, taking into account 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 1. Studies of prevalence and/or etiology of bovine mastitis

Reference	Location	Type of milking	# Herds (quarters)	Prevalencia (%)				
				Atrophied	Clinical mastitis	Subclinical mastitis	IIM	Main microorganisms

(Aguilera and Martínez, 1981a)	-	-	1 (304)	10.2	47.4	29.6	-	*Streptococcus agalactiae* 60.9 *Corynebacterium* sp. 10.9 *Streptococcus* spp. 5.1
(Aguilera and Martínez, 1981b)	-	-	(124)	-	-	70.2	81.7	*Staphylococcus* sp. 34.1 *Streptococcus* sp. 22.0
(Martínez *et al.*, 1981)	-	-	6 (527)	-	-	44.2	59.2	*S. agalactiae* 19.5 *Corynebacterium* sp. 16.7 *Staphylococcus aureus* 8.7
(Guzmán *et al.*, 1982)	-	Mechanical	4 (>3 000)	-	-	51.0	-	-
(Aguilera, 1983)	-	-	(503)	-	-	-	-	*S. agalactiae* 53.6 *S. aureus* 22.2
(Fustes *et al.*, 1985a)	Occidente	-	25 (11 488)	1.7	1.1	25.2	-	-
(Ronda *et al.*, 1985)	LHA	-	19 (6 188)	-	6.8	45.1	-	-
(Fustes and Martínez, 1987)	-	-	-	2.8	1.7	29.1	-	-
(Aguilera, 1987a)	-	-	4 (828)	5.7	14.5	45.5	51.5	*S. agalactiae* 25.7 *S. aureus* 9.9
(I.M.V., 1989)	-	-	-	-	2.1	38.6	-	-
(Escobar and Ponce, 2001)	-	-	4 (>1 000)	-	-	63.4	-	-
(Novoa *et al.*, 2001)	CFG	Mechanical	5 (1 016)	4.3	1.1	67.0	-	*S. agalactiae* 55.0 *S. aureus* 20.0
(Armenteros *et al.*, 2002b)	PRI LHA MTZ VCL	Mechanical	56 (12 274)	3.7	3.0	76.5	45.1	*S. aureus* 30.5 *Corynebacterium bovis* 9.2 *S. agalactiae* 8.3

(Novoa *et al*., 2004)	CFG	Mechanical	10 (2 024)	-	5.0	62.2	45.6	*S. aureus* 29.3 *C. bovis* 8.8 *S. agalactiae* 6.8
(Cepero *et al*., 2005a)	VCL	Mechanical	5 (480)			18.1		
(Soca *et al*., 2005b)	MAY	Mechanical	2 (328)	4.8	3.9	67.0		
(Cepero *et al*., 2006)	VCL	Manual	3 (568)	-	-	25.9	-	-
(Armenteros *et al*., 2006)	ART	Mechanical	1 (568)	-	12.1	22.9	42.8	*S. aureus* 19.2 *Streptococcus* spp. 8.3 SCN 5.7 *Corynebacterium* sp. 4.4
(Relova *et al*., 2008)	MAY	Mechanical	1 (80)	2.5	26.3	76.9	53.7	*C. bovis* 27.5 *S. agalactiae* 12.5 *S. aureus* 1.2
(Ruiz *et al*., 2012)	PRI ART MAY SSP CMG	Both	35 (1 484)	3.0	0.9	38.2	63.7	SCN 24.2 *C. bovis* 18.9 *S. aureus* 11.8 *S. agalactiae* 10.0