SHORT COMMUNICATION

Prevalence of Pathogens Causing Subclinical Mastitis in Argentinean Dairy Herds

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ABSTRACT

The objective of this study was to investigate the prevalence of different mastitis pathogens in Argentinean dairy farms. Composite milk samples were collected of 2296 cows from 51 randomly selected herds in Córdoba, Argentina. Somatic cell count was determined in all samples and bacterial examination of the milk samples with a SCC exceeding 200,000 cells/mL was performed. Of all the cows, 54% were diagnosed with subclinical mastitis (SCC ≥200,000/mL). Bacteria were isolated in 83.1% of milk samples subjected to bacteriological analysis. The most frequently isolated pathogen was coagulase-negative staphylococci (CNS) (52.1%), followed by *Staphylococcus aureus* (21.3%), *Corynebacterium* spp. (5.2%), *Streptococcus agalactiae* (4.4%) and *Streptococcus dysgalactiae* (4.4%). This study demonstrates that among the major pathogens isolated, the contagious bacteria caused most of the subclinical infections of dairy cows in Cordoba, Argentina. Moreover, CNS was the most relevant group of minor pathogens causing subclinical mastitis.

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INTRODUCTION

Over one hundred different microorganisms have been isolated from bovine mastitis, but the most frequently isolated microorganisms are staphylococci, streptococci, and gram-negative bacteria (Oliver *et al.*, 2004; Hussain *et al*., 2012; 2013). The importance of the main mastitis pathogens has changed over time, probably because of marked changes in the dairy industry, and it varies among not only countries but also among mastitis forms. Subclinical mastitis is more prevalent than clinical mastitis. Although it is not manifested as visible changes in either the mammary glands or in the milk it does increase the somatic cells in milk. Therefore, it is not easily recognised by farmers and it can lead to large production losses (Hogeveen *et al*., 2011). Moreover, cows with subclinical infections should be considered as a source of new infections within herds. The subclinical mastitis is still a major problem in dairy herds in Argentina. However, no updated data of large numbers of random selected dairy farms in our country are available (Calvinho and Tirante, 2005).The objective of this study was to investigate the prevalence of pathogens associated with subclinical mastitis in composite milk samples collected from Argentinean dairy herds.

MATERIALS AND METHODS

The farms involved in the cross-sectional study constituted a sample of 51 dairies located in Córdoba province, Argentina, randomly selected from the roster provided by the producers' association of the region, with a herd size between 100 and 250 cows. The studied herds represented a sampling fraction of 15.1%. The herds were visited once between March and September 2007. The assumptions used to calculate the sample size of cows were: 95% confidence, a 10% maximum allowable error in the estimate of prevalence, and an expected prevalence of sub-clinical mastitis of 20%. On the basis of these assumptions, on average 45 lactating cows (SD 3) in each herd were sampled by the same investigator. In total, 2296 composite milk samples were collected (Oliver *et al.*, 2004). Before sampling, the first streams of milk were discarded, and teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry. The milk samples were transported on ice to the laboratory immediately. All samples were frozen and then thawed before they were bacteriologically processed, which occurred within 20 d post-sampling. The SCC values of all composite milk samples were measured using the

Soma count 300 (Bentley, USA 1997) within the collection day. Bacterial examination of the milk samples with a SCC ≥200000 cells/mL was conducted. A volume of 0.01 mL of milk was streaked on trypticase soy agar plates (Sigma-Aldrich, USA) containing 5% sheep blood and incubated at 37°C, and plates examined for growth at 24 and 48 h. A sample was considered bacteriologically positive when growth of more than 3 cfu of a particular organism and <3 colony types on the plate was detected. For *Staphylococcus*, a minimum of 1 cfu was required. A mammary gland was considered infected by CNS when growth of ≥500 cfu/mL of a particular organism and only one colony type on the plate were isolated. Samples yielding three or more colony types were considered to be contaminated. No significant growth was defined as plates that were not contaminated and no organism had more than 3 cfu. For Gram-positive cocci, catalase tests were performed to distinguish catalase-negative *Streptococcus* spp. from catalase-positive *Staphylococcus* spp. Coagulase-positive staphylococci were further identified with biochemical methods. Coagulase-negative staphylococci were differentiated from *Micrococcus* by furazolidone sensibility test, and were classified as CNS. Streptococci and Gram-negative bacilli were classified according to Oliver *et al.* (2004). *Corynebacterium* spp. and *Arcanobacterium* spp. were confirmed by colony morphology, Gram staining and catalase reaction. Coagulase negative staphylococci and *Corynebacterium* spp. were considered minor pathogens.

RESULTS AND DISCUSSION

Composite milk samples were analysed from a total of 2228 cows of the Holando-Argentino (Holstein) breed. Cases of clinical mastitis were not included in the current study. The percentage of cows (1201/2228) having subclinical mastitis (SCC ≥200,000/mL) was 53.9% (95% CI=51.8-56.0).This result is similar to those reported in Korea, namely 54.3% on a cow basis (Nam *et al.*, 2010). In different countries, studies have determined the prevalence of subclinical mastitis (Haltia *et al.*, 2006; Kalmus *et al.*, 2011; Abera *et al.*, 2012; Saidi *et al.*, 2013) but milk sample selection (quarter or composite), SCC determination method and SCC threshold have varied among studies, making comparisons difficult.

Of the 1201 milk samples subjected to bacteriological examination, 141 (11.7%, 95% CI=9.9-13.6) showed no bacterial growth and 62 (5.2%, 95% CI=3.9-6.4) were contaminated. In total, 998 of 1201 (83.1%; 95% CI=81.0-85.2) samples were culture positive, out of which 1117 bacteria were isolated: a single bacterial isolate was identified from 879 (88.1%) samples and two bacterial isolates were cultured from 119 (11.9%) samples.

The results of bacteriological findings are summarized in Table 1. Among the composite milk samples with bacterial growth, CNS was the most commonly isolated bacterial group, followed by *Staphylococcus aureus* and *Corynebacterium spp.* *Streptococcus agalactiae* and *Streptococcus dysgalactiae* were isolated from 49 samples each one. Among Gram-negative bacteria, *Enterobacter* spp. and *Escherichia coli* spp. were the most frequently observed bacteria, followed by *Pseudomonas* spp., *Klebsiella* spp. and *Serratia* spp.

Table 1: Frequency of pathogens causing subclinical mastitis on Argentinean dairy herds

Bacterial isolates	n	% (95% CI)
Coagulase-negative staphylococci	582	52.1 (50.6-53.6)
Staphylococcus aureus	238	21.3 (20.3-22.3)
Corynebacterium spp.	58	5.2 (4.9-5.5)
Streptococcus agalactiae	49	4.4 (4.1-4.6)
Streptococcus dysgalactiae	49	4.4 (4.1-4.6)
Staphylococcus intermedius	43	3.8 (3.6-4.1)
Enterobacter spp.	26	2.3 (2.2-2.5)
Escherichia coli	23	2.1 (1.9-2.2)
Streptococcus acidominimus	21	1.9 (1.8-2.0)
Arcanobacterium spp.	8	0.7 (0.7-0.8)
Enterococcus spp.	7	0.6 (0.6-0.7)
Streptococcus uberis	5	0.4 (0.4-0.5)
Pseudomonas spp.	5	0.4 (0.4-0.5)
Serratia	3	0.3 (0.3-0.3)
Total	1117	

In different countries, studies have been conducted to determine the prevalence of organisms causing mastitis. Sample selection, cultivation techniques and the criteria used when diagnosing a sample as bacteriologically positive vary among studies. Therefore, the results are not strictly comparable with those obtained in this study.

Traditionally, the most common mastitis-causing agents have been classified as minor and major pathogens according to the degree of inflammation they produce in the mammary gland. In the present research, the prevalence of major pathogens was slightly lower than the minor pathogens, 42.7% and 57.3%, respectively (Table 1). Among the major pathogens isolated, *S. aureus* was the predominant agent. Other studies also reported that *S. aureus* was found to be the most prevalent organism isolated from milk samples (Haltia *et al.,* 2006; Kalmus *et al.*, 2011; Abera *et al.*, 2012; Saidi *et al.*, 2013). In Argentina, previous studies mostly conducted during the 1990s obtained similar percentages of *S. aureus* from mastitic milk samples (Calvinho and Tirante, 2005).

In this study, CNS represents the most prevalent bacterial group of minor pathogens found in subclinical infections of dairy cows. Their importance has increased and they have become the most frequently isolated group of species from bovine milk in several countries, and are being currently considered emerging pathogens of bovine mastitis (Nam *et al.,* 2010; Kalmus *et al.*, 2011: Khan *et al.*, 2013). However, conflicting results have been presented in the literature regarding the association between CNS species and the SCC (Thorberg *et al.*, 2009). Accordingly, attention must be paid towards the identification of CNS species and their associations with cow milk SCC in Argentinean dairy herds.

Argentina is characterized by a predominately grass-based production and, therefore, the cows have a reduced exposure to risk factors for environmental mastitis when compared to cows that produce milk while they are housed as it occurs in other countries. In this work, the prevalence of contagious major pathogens such as *S. aureus* and *S. agalactiae* was slightly higher than the environmental major pathogens, accounted for over 60%. *Streptococcus dysgalactiae* was the most frequent environmental major pathogen isolated from the animals.

In spite of the fact that all standard mastitis control practices are still applied among small and medium size dairy farms in Argentina (Vissio *et al.*, 2013), this could be one of the reasons why contagious major pathogens are

predominant in subclinical mastitis on these farms. Our findings are similar to that described in studies conducted in dairy herds with different management practices (Haltia *et al.*, 2006).

Conclusion: This study demonstrates that among the major and minor pathogens isolated, the contagious bacteria caused most of the subclinical infections of dairy cows in Cordoba, Argentina. Moreover, CNS was the most relevant bacterialgroup of minor pathogens causing subclinical mastitis. Knowledge of the bacterial species as bovine subclinical mastitis agent would be helpful for the development of mastitis control programs and, consequently, for improving the health status of dairy herds.

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