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Short Paper

Prevalence of bacterial mastitis in cattle from the farms around Tehran

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Summary

To determine the prevalence of bacterial mastitis in cattle, milk samples positive for California mastitis test (CMT) were cultured during a period of almost 4 years. The bacterial species isolated from 2904 milk samples studied were coagulase negative *Staphylococcus* sp. in 879 (30.27%) samples, *Streptococcus agalactiae* in 642 (22.11%), *S. dysgalactiae* in 332 (11.43%), *E. coli* in 295 (10.16%), *Staphylococcus aureus* in 84 (2.89%), *Bacillus cereus* in 51 (1.76%), *Arcanobacterium pyogenes* in 31 (1.07%), *Pseudomonas aeruginosa* in 6 (0.21%), *Klebsiella pneumoniae* in 4 (0.14%), *Pasteurella multocida* in 1 (0.03%) and *Mycoplasma* sp. in another (0.03%) sample. No growth was found in 578 samples (19.90%). Thirty-one (37%) of 84 animals which were infected with *S. aureus*, had acute infection. We found that contamination of milk with coagulase negative *staphylococci* are the most frequent bacterial infection in dairy cattle around Tehran; it mostly causes subacute form of the disease. *S. agalactiae*, *S. dysgalactiae* and *E. coli* are the second, third and the fourth causative agents.

Key words: Dairy cattle, Mastitis, Pathogenic bacteria, Acute infection, Subacute infection

Introduction

Mastitis is one of the most important economical diseases of dairy cattle. The disease is produced by a variety of Gram-positive and negative bacterial species. Clinical diagnosis of acute mastitis does not usually present a problem to the practicing veterinarian. Diagnosis of subclinical forms may be more difficult but is an important part of any herd survey to establish the disease incidence. In addition to bacterial culture of milk, several indirect tests are employed to ensure the presence of inflammatory exudates and cells in infected milk, such as California mastitis test (CMT) (Carter and Cole, 1990; Hirsh and Zee, 1999) and Somatic cell count (SCC) (Bellamy, 1999) of the bulk milk. Regarding some accepted criteria, milk in a good condition, should not have more than 400,000–500,000 somatic cells per ml. High incidence of SCC indicates the presence of infection or increase genetic susceptibility to mastitis (Bellamy, 1999). Bovine mastitis is mostly caused by *Streptococcus* species like *S. agalactiae*, *S. dysgalactiae* and *S. uberis.* The leading organism is *Staphylococcus aureus*, producing acute suppurative, gangrenous, or chronic mastitis, depending on the infecting strains. In gangrenous mastitis the affected quarter becomes cold and blue-black and eventually sloughs. Tissue necrosis is attributed to the alpha toxin which causes contraction and necrosis of smooth muscle in blood vessel walls, impeding blood flow in the affected quarter. In addition, this toxin causes release of lysosomal enzymes from leukocytes (Quinn *et al*., 2002). Shimi (1997) reported *S. aureus* as one of the most infectious agents which produces mastitis in cattle. The bacteria live around the nipples and penetrate them under inappropriate

conditions of husbandry and milking. Gangrenous mastitis was also seen in a dairy cow with acute infection caused by *Pseudomonas aeruginosa*, which had a similar course of disease to gangrenous mastitis by *S. aureus* (Vodjgani *et al*., 1997).

Tabatabaii and Firouzi (2001) reported that mastitis due to *S. aureus* is not a major problem during the recent years, but contamination of milk and dairy products is still a big problem. Coagulase negative strains such as *Staphylococcus epidermidis* and *Staphylococcus hyicus* subsp. chromogenes are sometimes the causative agents of bovine mastitis, (Carter and Cole, 1990). The main objective of this study was to investigate the prevalence of the bacteria producing cattle mastitis in farms around Tehran.

Materials and Methods

During a period of almost four years, 2904 milk samples were collected by standard milk sampling technique (Carter and Cole, 1990; Quinn *et al*., 1994). The samples were submitted to our laboratory (Bacteriology Lab., Faculty of Veterinary Medicine, University of Tehran) in sterile screw-capped tubes. Bacterial culture was performed based on method described by Carter and Cole (1990). Nutrient agar, 5% sheep blood agar and MacConkey agar were used for primary cultivation of all milk samples. Culture plates were incubated at 37^{o}C for 24–48 hrs. Mycoplasma agar and broth were utilized for cultivation of *Mycoplasma* spp. in milk as described by Carter and Cole (1990). The plates were examined for bacterial colonies. Gram stain was performed to distinguish Gram-positive and negative organisms and to reveal the bacterial shapes. The type of bacterial haemolysis (alpha, beta and none) was determined on blood agar plates. Some primary and specific biochemical tests were subjected for bacterial diagnosis as follows. Catalase test was performed to distinguish *streptococci* and *staphylococci.* Leuffler's serum test was used for detection of *Arcanobacterium pyogenes*. Oxidase test used to distinguish the Enterobacteriaceae spp. from Gram-negative non-Enterobacteriaceae organisms. Coagulase test was used for detection of coagulase positive *Staphylococci*. CAMP test was used to recognize *S. agalactiae*. It was necessary to complete the differentiation of some Gram-positive and Gram-negative coccobacilli using the carbohydrate fermentation reaction tests. Finally, antimicrobial sensitivity tests were performed as standard plate procedure described by Carter and Cole (1990), to help the treatment of some kind of mastitis. Statistical tests (Chi-square and Fisher's exact tests) were employed to compare the categorical variables.

Results

Bacterial cultures were performed on all milk samples positive for CMT. This test had been done in the farm before submitting the samples to the laboratory. The relative frequency of bacterial species was determined during four-year period of this investigation. The results of bacterial cultures are summarized in Table 1.

Discussion

We found that coagulase negative *staphylococci* is the most frequent organism causing contamination of milk in industrial dairy cattle around Tehran; in most cases, it also causes subacute mastitis. The statistical analysis also showed that the frequency of these bacteria and their proportion are significant during four years ($P<0.01$, $P<0.02$ and $P<0.0005$). The most frequent occasions of contamination were encountered in the first and second years of investigation. In this study, *S. epidermidis*, a coagulase negative strain, was isolated from the majority of samples. This strain is occasionally haemolytic on sheep blood agar. Shimi (1997) suggested that *S. epidermidis* can produce subacute mastitis in cattle which is sensitive to antibiotics. Presence of this organism around the nipples induces the aggregation of leukocytes in that area which in turn prevents invasion of serious mastitis-producing bacteria. *S. hyicus* was also separated from two cases. *S. hyicus* subsp. chromogenes is believed to be a coagulase negative, non-haemolytic

Table 1: Frequency and proportion of isolated bacteria from milk samples collected from cows with mastitis during consecutive years

Species of bacteria	First year	%	Second year	%	Third year	%	Forth year	%	Total	% of total	P-value
S. coagulase negative	209	36.16	238	24.79	303	35.65	129	25	879	30.27	0.0005
S. agalactiae	90	15.57	338	35.21	78	9.18	136	26.36	642	22.11	0.0005
S. dysgalactiae	96	16.61	101	10.52	61	7.18	74	14.34	332	11.43	0.0005
E. coli	41	7.09	56	5.83	147	17.29	51	9.88	295	10.16	0.0005
S. aureus	17	2.94	24	2.5	18	2.12	25	4.84	84	2.89	0.02
B. cereus	10	1.73	17	1.76	21	2.47	3	0.58	51	1.76	NS
A. pyogenes	16	2.77	1	0.11	2	0.23	12	2.33	31	1.07	0.0005
P. aeruginosa	-	0.0	3	0.31	2	0.23	1	0.19	6	0.21	NS
K. pneumoniae	1	0.17	-	0.0	-	0.0	3	0.58	4	0.14	0.01
P. multocida	-	0.0	-	0.0	1	0.12	-	0.0	1	0.03	NS
Mycoplasma spp.	1	0.17	-	0.0	-	0.0	-	0.0	1	0.03	NS
No. growth	97	16.78	182	18.96	217	25.53	82	15.89	578	19.90	0.0005
Total	578		960		850		516		2904	100	

P-value at $p<0.01$, $p<0.02$ ($P<0.05$) and $p<0.0005$ ($P<0.001$) are significant; NS: non-significant

bacteria that may produce pigment. It is rarely the causative agent of mastitis in cattle (Carter and Cole, 1990). *S. agalactiae, S. dysgalactiae* and *E. coli* are the next important causative organisms of cattle mastitis regarding disease related symptoms. Tabatabaii and Firouzi (2001) reported that *E. coli* is a serious causative agent of mastitis during the cold seasons of year. The relative frequencies of these bacteria were also significantly high during four years, especially for *S. agalactiae* in the second and fourth years, *S. dysgalactiae* in the first and fourth years, and *E. coli* in the third year of investigation.

Workinen *et al*., (2002) reported that *Staphylococcus* species constituted 57% of the isolates, of which the predominant cause of bovine mastitis (40.5%) was *S. aureus.* In our study, although the prevalence of *Staphylococcus* spp. was predominant, *S. aureus* was not the most important cause of dairy cattle mastitis. Our findings are similar to that of Nessru *et al*., (1997), Edwards *et al*., (1982) and Workinen *et al*., (2002) who reported *Streptococcus* sp. (*S. agalactiae and S. dysgalactiae*) as the second and the most common cause of bovine mastitis.

Hemmatzadeh and Aghili (2000), in a one-year investigation, showed that 55.8% of the isolated bacteria were contagious. *E. coli* was a major cause of bovine mastitis followed by *S. agalactiae*, *S. aureus* and *S. dysgalactiae*, 44.2% had environmental agents.

Douglas *et al*., (2000) reported that there were different strains of *S. uberis* in New Zealand dairy cattle, so that prevention and treatment of this type of mastitis is somewhat complicated. In our investigation, no *S. uberis* could be isolated from milk samples. Tabatabaii and Firouzi, (2001) suggested that *S. uberis* causes mastitis in cattle, during the first period of lactation, which produces acute inflammation of mammles.

As it is shown in Table 1, the frequency was significant for *A. pyogenes* ($P<0.0005$), *S. aureus* ($P<0.02$) and *Klebsiella pneumoniae* ($P<0.01$), but not significant for *Bacillus cereus*, *P. aeruginosa*, *Pasteurella multocida* and *Mycoplasma* sp. during our investigation.

Heringstad (1999) pointed out that genetic specification plays an important role in producing clinical mastitis in the Norwegian cattle. On the other hand, it is suggested that clinical mastitis occurs in a period of only six weeks around calving. Therefore, the infection would occur 15 days before and 30 days after the first calving. Recently, Bellamy (1999) suggested that SCC are of important concern to dairy farmers. High quality milk must have fewer than 400,000 cells per ml to be suitable for human consumption. High SCC indicates increasing genetic susceptibility to mastitis. Breeding to achieve a low SCC is a long-term strategy to reduce both herd cell counts and increase resistance to mastitis. Recent observation by Heringstad (1999) revealed that the bulls genetic properties, regarding SCC, are correlated with its immunological response to the important mastitis pathogen, such as *S. aureus*, suggesting bulls can be pre-

selected for increased resistance to *S. aureus* in their offsprings. In this regard, bacterial species, their requirement for growth, genetic background, etc. are the factors responsible for susceptibility of cattle to mastitis. Gharagozloo *et al*., (2001) during an investigation, reported that 257 (56.2%) of 475 dairy cows were suffered from mastitis with *Streptococcus* sp., coagulase negative *Staphylococcus* and *E. coli*. They mentioned that the increased SCC is due to subacute mastitis, which reduce lactose, fat and protein in milk. Production of milk reduces by increasing of each 100,000 cells per ml to the maximum permitted number of cells per ml.

No attempts were made to investigate other organisms such as *Chlamydia* spp. and fungi, etc. that may be responsible for infection of the 578 (19.90%) milk samples that had no growth results.

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