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Prevalence of Mastitis Resulted from Bacteria of Enterobacteriaceae Group (Coli Forms) and Determining Their Antibiotic Sensitivity in Traditional Farms of Sarab City, Iran

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Abstract: Mastitis in livestock means transformation and pathogenesis in udder tissue which may affect one or all quarter of cattle and lead to non-usable of the milk. Although, mastitis may be seen in all livestock, it is of high economical importance in dairy cattles. The studies reveal that notwithstanding disease agent, mastitis is more prevalent in most countries. Coli form bacteria are opportunist. All environmental resource which are in contact with udder are considered as important for pathogenicity of organism. When udder gland is infected, *Escherichia coli* bacterium propagates at high numbers and produces a strong endotoxin resulting in edema and mastitis. About 1000 dairy cattles of traditional farms of Sarab and its surrounding villages were considered as understudy statistical population out of which 752 were lactating cattles. Preparing milk samples of these cattles according to the following stages, the samples were studied) measuring individual cell count of milk samples of understudycattles) conducting CMT (California Mastitis Test) for cows' milk whose cell count in over than 150000 in mL) sterile sampling from milk of quarter whose CMT result were positive and transferring the samples to bacteriology laboratory) centrifuging milk and culture sample from deposit and cream resulted in the culture media) conducting of differential tests for colonies related to Enterobacteriaceae of positive suspected lactose in order to determine species and genus of the isolated bacteria) conducting of antibiogram test.

Key words: Enterobacteriaceae (coli forms), antibiotic, mastitis, milk, species, colonies

INTRODUCTION

Mastitis in livestock means transformation and pathogenesis in udder tissue which may affect one or all quarter of cattle and lead to non-usability of the milk (Bradley and Green, 2001). Mastitis refers to inflammation of livestock's udder tissue ignoring its reason. It is characterized by physical, chemical and usually bacteriological changes in milk and also changes resulted in the udder due to the disease (Dopfer *et al*., 1999). The most important changes observed in the milk include change of its color, clotted milk, increasing number of leukocyte in milk and also inflammation, high temperature, pain and hardening of the udder tissue (Kaipainen *et al*., 2002). In some cases, the infected udders can not be easily or through palpation or superficial testing of milk recognized and indirect tests such as counting leukocytes found in milk are practically required. Although, mastitis may be shown in all livestock, it is of high economical importance in dairy cattle. From economical and public health viewpoint, it is regarded as the most important disease confronted by animal husbandry and milk production industry. The studies made it clear that notwithstanding disease agents, mastitis is more prevalent in most countries. Etiologically, the disease is divided into two groups: contagious mastitis and environmental mastitis (Anderson *et al*., 1982).

Now a days, efforts aimed at decreasing the disease as an essential and important indicator in animal breeding and foodstuff hygiene and its side effects considering significant progression and advancement in public health and animal husbandry industry. Mastitis imposes significant losses through reduction of milk production, discarding milk high expenses of treatment, replacing infected cows with healthy ones and sometimes, perishing the livestock (Hill *et al*., 1979). Contaminated milk creates problems in production of dairy products and endangers public health. Importance of the subject and reputation of Sarab as an important bestial pole in northwest of Iran persuaded us to specify contamination status through studying mastitis prevalence in dairy cattles of the region and necessary actions taken to enhance knowledge of region's veterinarians and cattlemen about this disease

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and improve hygienic conditions of the region farms through identifying effective antibiotics by use of antibiogram test (Hogan *et al*., 1989).

Studies manifest that environmental mastitis is common in all dairy cattles. More control of contagious mastitis in cattle more relative importance of environmental mastitis. Unlike contagious mastitis, environmental mastitis relates more to clinical cases rather than under-clinical infection. Conducted researches reveal that all infections resulted from environmental pathogenesis appears during dry period of cows. Clinically, they appear during the first 75 days of dairy period. This supports the idea that infections of dry period are contributed in appearing of clinical cases at the initial periods of cows lactation (Powers *et al*., 1986). Environmental pathogenesis agents are important factors of intra-udder infection and clinical mastitis in heifers and at the time of parturition. Also, it has been observed that 5-10% of infected quarter in heifers have been contaminated by environmental pathogenesis agents during parturition (Nikerson, 1990). During every lactation and dry period, environmental pathogenesis agents equally affect cows that are kept in a limited and closed place. Cows demonstrate maximum of new cases of clinical mastitis at the beginning of lactation. Mastitis increases in every dry and lactation period as a result of number of parturition (Nickerson, 1990; Hill *et al*., 1979).

Contagious pathogeneses: *Streptococcus agalactiae*, *Staphylococcus aureus*, *Mycoplasma bovis*.

Environmental pathogeneses: Species of environmental streptococcus including *Streptococcus uberis* and *Streptococcus dysgalactiae* as the most common ones and environmental coli forms including negative gram *Escherichia coli* bacterium, species of *Cytrobacter*, species of *Entrobacter*, *Entrococcus faecalis*, *Entrococcus faecium* and other negative gram bacteria such as *Serratia*, *Pseudomonas* and *Proteous* (Barrow and Hill, 1989).

More other infectious factors may result to sever mastitis. Usually, they are sporadic and affect one or a few number of cows. These factors include *Nocardia asteroides*, *Haemophilus somnus*, *Pasteurella moltocida*, *Pasteurella haemolytica*, *Mycobacterium bovis* and *Bacillus cereus*. Mycotic factors such as *Trichosporon*, *Aspergillus fumigatus* and some viruses may create mastitis in cattle but are not so important (Hill *et al*., 1979).

Some of different serotypes of *Escherichia coli*, famous kinds of capsulated Klebsiella and Enterobacter aerogenes are responsible for appearing of coli form mastitis in cattles. Environment of dairy cattles is the initial source of coli form infections. This is in contrary with contagious pathogeneses because infectious udder glands are their source. Exposing of non-infectious quarter to environmental pathogeneses may occur every time during cows life including milking, dry period and before first parturition in heifers (Pankey, 1989).

Escherichia coli isolated from milk of cows suffering from mastitis is known as opportunistic bacterium with endotoxin and lipopolysaccharide which make part of external layer of cellular wall of all negative gram bacteria. Sensitivity of coli form bacteria isolated from milk or cows' environment varies concerning bactericidal activity of cow serum. In return, all isolated cases lead to sever mastitis resisting against serum. All environmental resource which are in contact with udder are regarded as an important source for pathogenesis organism. Coli form bacteria are opportunistic and udder skin and teat are often infected at milking intervals and when the cow contacts with contaminated bed (Barrow and Hill, 1989). Livestock dung are generally source of *Escherichia coli* and can directly and indirectly infect the udder through bed, place of parturition, barnyard, water used for washing udder, towels used for drying the udder, milking sets and milkers' hands. Also, cows suffer from chronic coli form mastitis constitute an important infection source (Dopfer *et al*., 1999). When udder gland is infected, *Escherichia coli* bacterium propagates at high numbers and produces a strong endotoxin resulting in changing of permeability of vessels of udder tissue, edema, acute mastitis and increase of Neutrophiles in the milk. Super acute mastitis in cows is a sever disease identified with sudden dryness and toxemia (Erskine *et al*., 1995).

Anorexia, sever weakness, trembles, fever over than 40°C are common. Cow may be more toxemic or fevered. It has diarrhea before appearing of evident changes in udder gland or milk. Lactation varies from watery viscosity to a diluted serous yellow liquid with very small pieces. Super acute coli form mastitis appears quickly. Some cows may perish within 6-8 h. This period may last for 24-48 h, too (Hogan *et al*., 1989).

MATERIALS AND METHODS

About 1000 dairy cows of traditional farms of Sarab and its surrounding villages were considered as understudy statistical population out of which 752 were lactating cattles. Milk samples were collected from these cows and somatic cell count and bacteriological tests were conducted on the samples. Milk samples with cell

count over than 150,000 were regarded as milk of non-infected udder. CMT (California Mastitis Test) tests were conducted for those samples with cell count over than 150,000 in a mL. Then, milk samples collected from infected udders were prepared for bacteriological tests to insolate the bacterium through use of media. The used media to isolate the *Escherichia coli* from other bacteria include MacConkey Agar, Blood Agar, Eozine Methylin Blue, T.S.I, S.I.M, Simmons' Citrate Agar, liquid urea and Peptone water.

Antibiotic discs are used to test antibiotic sensitivity. Each disc has been identified and marked with abbreviatory mark of the antibiotic. The disc diffusion was employed per interpretation chart provided by the manufacturer of discs depending on the diameter of zone of inhibition. To determine effects of antibiotics, the test should be done with pure culture (Erskine *et al*., 1992).

RESULTS AND DISCUSSION

Outcomes resulted from culture of milk of cows suspected to suffer from bacterial mastitis during 12 months including summer, fall, winter and spring of the next year Table 1. The results demonstrate that 23% of the samples were infected by bacteria of coli form group out of which 15% was *Escherichia coli*, 4.8% was Klebsiella and 3.2% was Enterobacter. Effective antibiotics on coli forms include Neomycin, Gentamycin, Cannamycin and trimethoprime, respectively. Among them, Neomycin is more effective antibiotic.

Considering environmental bacteria which lead to increase of cell count, the resulted mastitis are often curable but reaction of cows immune system varies in challenge with bacterium species such that *E. coli* lead to massiveness and feverishness of the udder (Dopfer *et al*., 1999). In this case, cell count may even increase up to 10 million within 4 h due to aggressive state of body and finally kill the cow because the bacterium highly propagates in udder and the toxin produced by the bacterium reduces livestock's resistance (Powers *et al*., 1986). At summer and initial periods of lactation, amount of cell count increases due to stress and mismanagement factors. This is while cell count decreases in winter and due to frequently milking. Most of infections of coli form mastitis are created by *Escherichia coli* and then Klebsiella and Enterobacter lead to infection. Mastitis is regarded as the most important cause of economical losses in dairy cattle. Several pathogeneses agents including coli forms are contributed in mastitis. Cattlemen use self-remedy and widely apply antibiotics in livestock's treatment because of lack of required information in this regard. This leads to resistance of bacteria against antibiotics and makes treatment of these livestock more difficult.

Table1: Outline result from culture of cow's milk suspected to suffer from becterials mastitis

Seasons	Cell count and infection	Values
Summer	Number of cell counted cows	198
	Samples with cell count over than 150,000	87
	Samples with positive result of milk bacterial culture	36
	Sum of cattles infected by coli form mastitis	18
	Samples infected by *E. coli*	7
	Samples infected by Klebsiella	8
	Samples infected by Enterobacter	3
Autumn	Number of cell counted cows	228
	Samples with cell count over than 150,000	102
	Samples with positive result of milk bacterial culture	48
	Sum of cows infected by coli form mastitis	33
	Samples infected by *E. coli*	12
	Samples infected by Klebsiella	15
	Samples infected by Enterobacter	6
Winter	Number of cell counted cows	184
	Samples with cell count over than 150,000	97
	Samples with positive result of milk bacterial culture	65
	Sum of cows infected by coli form mastitis	24
	Samples infected by *E. coli*	10
	Samples infected by Klebsiella	9
	Samples infected by Enterobacter	5
Spring	Number of cell counted cows	142
	Samples with cell count over than 150,000	65
	Samples with positive result of their bacterial culture	43
	Sum of cows infected by coli form mastitis	25
	Samples infected by *E. coli*	11
	Samples infected by Klebsiella	8
	Samples infected by Enterobacter	6

CONCLUSION

The resulted outcomes reveal that 23% of the samples were infected by bacteria of coli form group out of which 15, 4.8 and 3.2% were *E. coli*, Klebsiella and Enterobacter, respectively. Effective antibiotics on coli forms include Neomycin, Gentamycin, Kanamycin and trimethoprim. Among them, Neomycin is more effective antibiotic.

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