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RISK FACTORS OF WATER POLLUTION ON MASTITIS AND MILK SOURING IN DAIRY FARMS

(With 3 Tables)

By

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العوامل المنذرة لتلوث المياه في حدوث إلتهاب الضرع وفساد الحليب في مزارع الألبان

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أجريت الدراسة في مزرعة تشكو من زيادة عدد حالات إلتهاب الضرع رغم إتخاذها كافة الإجراءات الوقائية ولإستبيان سبب المشكلة تم أخذ عينات من الأرباع المصابة بإلتهاب الضرع (٣٩) لعمل عزل بكتيري وإختبار حساسية ومسحات من أقماع المحلب وعينات لبن من خلال خزان الحليب ومياة من مواسير تغذية المياه للمحلب وخزان المياة لعمل عد بكتيري كلي وكولي فورم وعزل بكتيري. دلت النتائج على أن الميكروبات المعزولة من حالات إلتهاب الضرع هي:

Enterobacter aerogenes, E.coli, Enterobacter hafnia, Klebsiella pneumoniae, Staph, aureus., Staph epidermidis, Strept. ubris.

ودلت نتائج إختبار الحساسية على فعالية مركب الريفامبسين والإنروفلوكساسين على معظم هذه الميكروبات. ان الميكروبات معظمها من مجموعة *Enterobacteriaceae* . العد البكتيري والكولي فورم مرتفع في لبن خزان الحليب وأقماع المحلب ومياة مواسير المحلب وخزان المياة .

تم عزل الميكروبات *Enterobacter aerogenes, E.coli* من حالات إلتهاب الضرع وأقماع المحلب وعينات لبن خزان الحليب ومياه مواسير المحلب ومياة خزان المياه. إتضح أن المياه بما تحمله من ميكروبات تتحمل النصيب الأعظم في تلك المشكلة بتلويثها المحلب مرة أخرى في دورة الغسيل بعد الحلب وقبل الحلب في الوجبة التالية. وعليه يوصى بالفحص البكتيريولوجي الدوري لكل من حالات إلتهاب الضرع والحلب وخزان الحليب والمياه ومصادرها بالمزارع للوقوف على مصدار التلوث أولا بأول وتجنبا للخسائر الإقتصادية الناتجة عنها.

SUMMARY

A commercial dairy farm had a serious mastitis problem in spite of its control hygienic measures. For detecting the cause of this problem samples were taken from a- clinical mastitis quarter milk, b-bulk milk tank, C-milking machine, d-water source. The samples were examined for total bacterial count, coliform count,isolation and identification of the isolated microorganisms. Antimicrobial susceptibility testing was performed for isolates of clinical mastitis quarter milk. The total bacterial count recorded an average number of 5.3xl 06 , 7.8x I 06 33.45xl 06 and 38.9x I 06 Cfu/ml for bulk milk, milk clusters, water pipes and water tank respectively while the coliform count was 4.2x *105* 44xl 05 2.35xl 06 and 21.7xl 06 respectively. The drug ofchoice based on antimicrobial sensitivity testing was rifamycin and enrofloxacin. Members of the family *Enterobacteriaceae* were the main pathogens isolated from clinical mastitis,bulk milk milking machine water pipes and water tank. From the results obtained it could be concluded that water pollution with members of the family *Enterobacteriaceae is* considered as the main source of pollution. The result of this work emphasizes that periodical bacteriological investigation of farm water, bulk milk I affected quarters and milking machine is an essential measure for minimizing losses of dairy industry.

Key words: *Water pollution, mastitis, milk, dairy farms.*

INTRODUCTION

Bovine intramammary infection or mastitis is a complex infectious disease, which results in major economic losses for dairy agriculture. Mastitis control involves detection and treatment of existing infections and prevention of new infections (Morris et al., 1978).
Bacteriological culture of milk is the gold standard method for determining the cause of clinical mastitis in dairy cows. Although substantial progress has been made world wide in reducing subclinical mastitis caused by contagious pathogens, clinical mastitis remains a frequent and costly disease of dairy cows, even in well managed herds (Erskine et al., 1988). The microorganisms responsible for most episodes of clinical mastitis in well managed herds are Gram negative (primarily coliform) and Gram positive (primarily Streptococcus

spp.other than *Streptococcus agalactiae and* Staphylococcus spp. other than *Staphylococcus aureus)* where each group of microorganisms is responsible for approximately one third of the cases (Hogan et al., 1989,Barkema et al., 1998,). These bacteria are ubiquitous on dairy cows and farms.

A decrease in bulk milk SCC (somatic cell count) has been observed in virtually all countries, and based on that, an associated decrease in prevalence of major pathogens may be expected. However, clinical mastitis incidence has not decreased, in some cases increased or resulted in a higher proportion of systemic sick cows when bulk milk SCC decreased. (Hogan et al., 1989. Miltenburg et al., 1996.; Barkema et al., 1998.; Elbers et al., 1998).

The farm of the present study although it has a decreased number of SCC in bulk milk, its complain was an increased number of clinical mastitis with low quality of bulk milk. The present work aimed to establish the sources of bacterial contamination of bulk milk and the cause of increasing mastitis through bacteriological investigations of clinical mastitis cases,bulk milk tank,milking machine and water supply.

The therapeuic treatment of the reported mastitis cases was suggested according to the antimicrobial susceptibility testing.

MATERIALS and METHODS

The subjects of this study were four hundred and fifty dairy cows in a commercial dairy farm (Alex.Desert road) that were milked thrice daily in Alfa Laval milking parlor with automatic removal of milking units. Preparation of udder for milking included udder washing with runing water, drying with individual paper towel, examination of foremilk and mammary gland for abnormalities. Mastitis control farm program was based on postmilking teat dipping in 1% iodine and antibiotic therapy of clinical cases and nonlactating cows The farm had been low SCC, but clinical mastitis remained a serious problem.. Farm system for milking machine followed the forgoing steps for cleaning and disinfecting:

a- cleaning with cold water "open cycle".
b- Cleaning with caustic soda ((500 gm/501iter of hot water at 70'C) in closed cycle for 5 minutes.
c- using the cold water"open cycle"

d- Washing with quaternary ammonium compounds in closed system for 15 minutes.
e- using nitric acid (50%) in closed cycle twice weekly.
f- Washing with cold water "open cycle".
g- Rinsing with cold water before milking.

Animals:

Fourty seven Friesian dairy cows suffering from mastitis were enrolled in the study. Samples for bacteriology:

A-Quarter milk samples(Clinical mastitis):

Cases of mastitis were characterized by shedding of abnormal milk (flakes, clots, or discoloured milk) or presense of swollen or indurated mammary quarters at milking time All cows included in the study had clinical mastitis of only one mammary quarter at the time of sample collection.

Quarter milk samples from cows with clinical mastitis were taken as recommended: After cleaning the teats of the affected quarters (N. =39) with 70% ethanol and discarding the fore discharges of these quarters, milk samples were collected in a duplicate sterile maccartney bottles from each affected quarter before treatment was administered (day o).

B-Bulk milk tank:

In a sterile 250 ml bottle bulk milk sample was taken from the two milk tanks (n. =2).

C-Milking machine:

Samples From the teat cups were taken using a sterile cotton swab, which was moistened with sterile quarter strength Ringer'solution, the swab was immersed in a tube containing 10 ml quarter strength Ringer's solution

D-Water samples:

In a sterile 500 ml bottles, under restricted hygienic conditions, water samples (n--2) were taken from:
a- water pipes in milking parlor
b- main water farm tank (N. =2).

The samples were kept in an icebox and transported to the laboratory for total bacterial count according to (A.P.H.A.,1985) and coliform count according to Thatcher and Clark (1978).

Isolation and identification of the microorganisms:

Samples (Milk or water) were centrifuged at 3000 RPM for 15 min. From the sediment, a sterile loopful was inoculated into a nutrient

broth (Difco), brain heart infusion broth (Oxoid), then incubated aerobically overnight at 37C for enrichment and enhancement of bacterial growth. Subcultures were streaked on nutrient agar, 5% sheep blood agar and macconkey bile salt media. After incubation, suspected colonies were described for their appearance, haemolytic activity and morphological characters. Smears from the colonies were stained with Gram's method and examined microscopically then divided generically according to staining reaction, shape and cell arrangement. The isolates were identified biochemically according to Bailly and Scott (1978), Finegold and Martin (1983) and Cruickshank et al., (1984).

Antimicrobial susceptibility testing:

This test was done for the isolated microorganisms from the affected quarters according to Quinn et al., (1994).

RESULTS And DISCUSSION

The results showed that both total bacterial and coliform count of bulk milk, milk clusters water pipes and water main tank recorded increasing cfu /ml. The total bacterial count recorded an average number of 5.3xl 06 , 7. gX,06 33.45xl 06 and 3 8.9x 1 06 CfU/Ml for builk milk,milk clusters, water pipes and water tank respectively while the coliform count was 4.2xl *05* 44xl *05* 2.3 5x 1 06 and 21.7xl 06 respectively (Table 1). From this table it is noticed that the bacterial counts, with their total or coliform counts, are much higher in case of milk cluster saaamples than those of bulk milk samples. This remarkable increase is due to the passage of milk through the milking equipment which gets contaminated from the polluted water source since the last step of farm cleaning system is rinsing with cold water in closed system. The water pollution is quite evident on observing the high bacterial counts (total and coliform) of the water samples especially those taken from the water tank. It is worthmentioning that the most predominant,,bacterial isolates were the coliform bacteria *(Enterobacter aerogenes and E.coli*).The presence of these coliforms especially *E.coli* in the milk is a great threat to the consumer in case of inefficient pasteurization since they cause certain cases of gastroenteritis especially in children (Cruickshank et al., 1984). Moreover, Quinn et al. (1994) reported that the members of Enterobacteriacae a such as *Enterobacter aerogenes,* and *Ecoli,* have lipopolysaccharides in the outer membrane of the cell wall that are potent endotoxins, which are released on the death

and lysis of the bacteria. The more pathogenic members of the enterobacteriaceae have other factors such as adhesins for attachment to host cells and capsules that are antiphagocytic.

Coliforms are also responsible for spoilage of milk and its products which includes acid production, sliminess, ropiness, bitter flavour, grassy unclean, feacal oudor as will as rancid and soapy flavour (Stead, 1986). Such spilage will disgrade the value of the milk and consequently leads to economic losses to the milk plant.

As regards the examination of 39 cases of clinical mastitis it is shown that (Table 3) members of the family *Enterobacteriaceae* were the main pathogens isolated n=31 (79.48%) which included *[Enterobacter aerogenes n-17(43.58%), E.coli n--6(15.38%), Enterobacter* and *n--5(12.82%),Klebsiella pneumoniae* n--3(7.69%)].

Staphylococcus spp.were isolated in a percentage of 12.82% represented as: *Staphylococcus aureus* n--2(5.1%) and *Staph.epidermidis* n--3(7.9%) in addition *Streptococcus ubris* was isolated in a percentage of 7,6% (n--3).

A decrease in bulk milk SCC has been observed in virtually all countries, and based on that, an associated decrease in prevalence of major pathogens may be expected. However clinical mastitis incidence has not decreased (Hogan et al., 1989).

Therapy of clinical cases assists the cow's defenses to overcome the infection. The treatment aims to provide antimicrobial concentrations in the udder, which are equal or higher than the minimum concentrations needed for the common mastitis pathogens (Francis, 1989). The drug of choice based on antimicrobial sensitivity testing (Table3) was rifamycin and enrofloxacin . Rifamycin inhibited the growth of *Enterobacter aerogenes (52.94%), Ecoli (50%) Enterobacter hafnia (60%), Staphylococcus aureus (50%), Staphylococcus epidermidis (33.3%) and Streptococcus ubris* (66.6%), while Enrofloxacin inhibited the growth of these organisms with an inhibition percentage of 29.7, 66.6, 60, 50, 33.3. and 66.6 respectively. Effective antimicrobial therapy depends on the susceptibility of the pathogen, pharmakokinetic characteristics of the drug, the amount of the drug given at one time, the route , frequency of administration, the duration of treatment, its half life, concentration and persistence at the site of infection (Quinn et al., 1994).

From the results obtained it could be concluded that water pollution with members of the family *Enterobacteriaceae* would cause a

great risk to the farm represented in contamination of the bulk milk, and maintain a permanent source of recontamination and pollution of milking machine after cleaning, which renders the cleaning system unefficient. Moreover, washing of the milking machine in the last step of the cleaning system with cold water and rinsing it with cold water before milking refresh the presence of the contaminant bacteria mainly *Enterobacter aerogenes&Ecoli in* milking clusters, and milking machine accessories. It should be noted that other factors such as vacuum pressure, dilated teat sphincter, with an individual variance of mammary gland immune status for each cow in presence of contaminated clusters would result in mammary gland infection.

The result of this work emphasizes that periodical bacteriological examination of farm water, bulk milk, affected quarters and milking machine is an essential measure for minimizing losses of dairy industry

REFERENCES

A.P.H.A. (1985): Standard methods for the examination of dairy products. 15th Ed.,American Public Health Association, Washington,D.C.

Bailly, W.R. and Scott, E.G. (19 78): Diagnostic Microbiology 5th Ed. The C.V.Mosby Company. Saint Louis.

Barkema,H.W.; Schukken, Y.H. and I.am, T.J.G.M. (1998) Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts.J.Dairy Sci. 81:411-419.

Cruckshank, R.; Duguid, J.P.J.:Marinion,B.P. and Swain,R..H.A.(1984): Medical Microbiology 12th Ed. Vol.11 Churchill Livignstone Edinburgh, London and New York.

Elbers, A.RrW.; Miltenburg, J.D, and Delang, D. (1998): Risk factors for clinical mastitis in a random sample of dairy herds from the southern part of the Netherlands. JDairy Sci.81:420-426.

Erskine,R.J., Eberhart, R.J. and Hutchinson,L.J.(1988): Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. J.Dairy Sci. 72: 1547-1556.

Finegold, S.M., and Martin, W.J. (1983): Diagnostic Microbiology. 6 Ed. The C.V. Mosby Company. St. Louis Toronto, London.

Francis, P.G. (1989): Update on mastitis.111 mastitis therapy.Br.Vet.J. 145: 302-311.

Hogan, J.S., Smith, K.L., Hoblet, K.H. (1989): Field survey of clinical mastitis in low somatic cell count herds. J.Dairy Sci.72: 1547-1556.

Miltenburg, J.D., Delang, D. and Crawels, A.P.P. (1996): Incidence of clinical mastitis in a random sample of dairy herds in southern Netherlands. Vet Rec. 139: 204-207.

Morris,R.S.; Blood,D.C;Williamson, N.B.;Cannon,C.M. and Cannon, R.M.(1978): A health program for commercial dairy herds for changes in mastitis prevalence. Australian Veterinary Journal 54: 247-251.

Quinn, P.J;CarterME; Marker, B. and Carter, G.R.. (1994): Clinical veterinary microbiology. Wolfe Publishing,Year Book Europe Limited.

Stead, D. (1986): Nficrobial lipase. Their characteristic role in food spoilage and industerial uses.J.Dairy Res. 53: 481-505.

Thatcher,ES. and Clark D.S. (1978): Microorganisms in foods,their significance and methods of enumeration, 2nd Ed. ICMSF, Academic Press, New York.

Table (1) Total bacterial count and coliform count of the different samples

Sample	Total bacterial count	Coliform count
Bulk milk tank	$5.31x10^5$	$4.2x10^5$
Milk clusters	$78x10^6$	$44x10^5$
Water from pipes	$33.45x10^6$	$2.35x10^6$
Water from tank	$38.9x10^7$	$21.7x10^6$

Table (2) The isolated microorganisms isolated from the different samples

Sample	*Enterobacter aerogenes*	*E.coli*	*Enterobater hafnia*	*Klebsiella pneumoniae*	*Staph .aureus*	*Staph epidermidis*	*Strept .ubris*
Clinical mastitis	+	+	+	+	+	+	+
Bulk milk	+	+				+	
Milking clusters	+	+		+		+	
Water from pipes	+	+					
Water tank	+	+					

Table(3) The antimicrobial sensitivity results for microorganisms isolated from 39 clinical mastitis cases.

	Mic ug/ml	*Enterobacter aerogenes(17)* S	%	*E.coli(6)* S	%	*Enterobater hafnia(5)* S	%	*Klebsiella pneumoniae(3)* S	%	*Staph .aureus (2)* S	%	*Staph.epidermidis(3)* S	%	*Strept.ubris (3)* S	%
Florfenicol	30 Difco	5	29.4	4	66.6	4	80	1	33.3						
Tetradelta	25 Upjon	2	11.4	3	50	--									
Rifamycin	30 Oxoid	9	52.94	3 50		3	60			2	100	2	66.6	1	33.3
Sulpha &trimethoprim	30 Oxoid	--	--	3	50					1	50	1	33.3	2	66.6
Streptomycin	10 Oxoid	--	--	3 50											
Cephalixin	10 Oxoid	5	29.4	3 50											
Penicillin	10 Oxoid	--	--	--								2	66.6	1	33.3
Gentamycin	10 Oxoid	--	--	--						1	50				
Tetracyclin	30 Oxoid	5	29.4	3 50						1	50			1	33.3
Enrofloxacin	5 Oxoid	5	29.4	4 66.6								1	33.3		
Amoxycillin	10 Oxoid	--	--	4 66.6		3	60	1	33.3	1	50	1	33.3	2	66.6
				3 50						1	50	1	33.3	2	66.6

Abreviation: S= the organismshows sensitivity to the agent

%=the percentage of sensitivity is related to the total number of the species.