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Influence of Some Animal Ecological Factors on the Microbial Quality of the Raw Milk Production from Livestock Farm, Taif, KSA

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Abstract: *The aim of this paper was for fellow-up the influence of some animal ecological factors (AEFs) on the microbial quality (MQ) of the raw milk (RM) production from livestock farm, Taif, KSA. The specimens were collected from a milking cow farms, were differentiated into four groups (Soil, milker, udder and milk). The mean and incidence of micro-organisms (MOs) growth rates and types from understudy specimens, the mean growth rate from control group (CG) in soil, milker, udder and milk were (25, 25, 25 and 12.5%) and from test group (TG) were (65, 50, 40 and 32.5%) respectively. MOs types from CG were (2, 1.5, 0.5 and 0.5) and from TG were (3.6, 3, 2.3 and 1.4) respectively. The mean and the incidence of MOs Spp. were isolated from understudy specimens were resulted from CG (25, 00, 12.5, 25, 00, 00, 00, 00, 00 and 00%) and from TG (66, 41, 25, 72, 22, 19, 25, 13, 6 and 28%) for (Staph., Strept., Entero. Spp., E. coli, Shigella., Proteus., Actino., Clost., Bact. Spp. and fungi) respectively. MOs were isolated related to soil and gastro-intestinal flora also included pathogenic MOs, that were regarded the main sources of contamination and were considered as AEFs. As well as AEFs are not only going to contaminate the milk but also can transmit pathogenic MOs and cause disease for milking animal and also as a media of infection to human. The results were concluded that, some AEFs still affect the MQ of RM production, that indicated the valuable role of AEFs in the rotting of milk and less the economic income in the dairy production.*

Keywords: *Animal Ecological Factors (AEFs), Microbial Quality (MQ), Raw Milk (RM), Livestock Farm, Micro-organisms (MOs), Control Group (CG), Test Group (TG), Species (Spp.), Staph., Strept., Entero., E. coli, Shigella, Proteus, Actino., Clost., Bact., fungi, Rotting.*

1. INTRODUCTION

The RM is sterile in the udder of a healthy cow but may it become contaminated with bacteria mainly during and/or after milking through some of AEFs like udders, milkers, soil, etc.,[1]. The causes of MOs occurred along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment's and water supplies used in sanitary activities. There was tertiary MOs contamination which occurs mainly due to re-contamination of RM after being processed due to un-hygienic conditions and/or poor or improper handling and storage of RM during consumption[2]. In general quality of RM may be lowered when it was contaminated by a number of AEFs such as adulteration, contamination during and after milking[3]. Sources of contamination included pathogenic MOs from animal skin and faecal soiling of the udder, contaminated milking and storage equipment's and water used for cleanliness. Other MOs sources were from air, milkers, handlers, drugs or chemicals used during treatment of animal and from water used for adulteration by unscrupulous and unfaithful workers/sellers which may be contaminated and may cause additional health problems[4]. Unfortunately, these laws and regulations were not often adhered in developing countries making milk-borne diseases a higher health risk to public. This was exemplified by over 75% of RM marketed in many developing countries was sold RM unpasteurized through informal channels[5]. When RM was secreted from a healthy udder, it

contained bacteria (0.5-1X10^3 Colony Forming Unites (CFUs)/ml). After milking environmental contamination occurs, which increased to (5X10^4 CFUs/ml) or reached several millions bacteria/ml. That count level indicated a very poor hygienic standard of RM during milking and handling RM of a diseased animal. The presence of coliform bacteria particularly *E. coli* in RM was an indicator of faecal contamination which implied poor hygienic conditions and un-sanitized environment since these bacteria were of faecal origin. In developing countries, most of the RM was produced by small-holder farmers dominated by local herds of cattle. Their milking units were widely distributed throughout in rural areas with a poor infra-structure, while most of the markets and customers were in urban areas. Therefore, the need for good hygienic practices and a streamlined collection, handling and transport system was important but had been always a challenge. Therefore, proper milking, cleaning and sanitizing procedures of equipment and environments were essential tool to ensure quality of RM. Many countries had implemented laws and regulations concerning the composition and hygienic quality of RM and milk products to protect both the consumers and the public health[6]. Several factors were observed to predispose RM to MOs contamination. Generally, 85.7% of RM samples were higher total bacterial counts (TBCs) than the recommended level of (2X10^6 CFUs/ml). Isolated bacteria included *Listeria Spp., Staph. aureus, E. coli, Salmonella Spp., Pseudomonas aeruginosa* and *Proteus Spp.* The quality of cow RM was poor; unhygienic practices and poor animal husbandry at farm level predispose farmers, consumers and the public to risk of contracting milk-borne infections. It was recommended that, veterinarians (VETs), extension officers and all stakeholders should played their roles in order to ensure safe quality milk delivery to consumers[7]. Prevention and control of MQ of RM was through elimination of MOs from human carriers by general improvements in water supplies, public health education, personal and environmental hygiene. Pathogenic MOs from the environments and equipment can be prevented by adhering to general hygienic practices and environmental cleanliness. Generally, MOs contamination in RM can be minimized through adherence to effective good hygienic practices at farm level, and in order to protect the public against RM-borne infections it was important to screen RM which was informally taken to the market. The lack of awareness of RM-borne infections in many developing countries and consumption of RM milk predisposed small-scale livestock keepers, consumers and the general public at risk of contracting these infections[8].

The aim: It was further determination of the MOs sources and transmission to the RM. The public will be enlightened on the importance of knowing the MQ of the RM and the effect of some exogenous sources of MOs as AEFs which still affected the MQ of which had hazard effect on the RM quality for farm health and economically on the farm owners.

2. MATERIALS AND METHODS

Study Area: Taif region was the chosen area because the location of our Taif University and livestock farms are available as well as Milking Cows Farm. The agreement was taken from Farms Owner to collect research data and specimens without any memorization of any special information. Data were collected about farms, milkers, cows, and the ways of milking for healthy cows, it was on hand milking methods.

Specimens collection: The work was started by a randomly selection of healthy milking cows for this study. All specimens were collected under control of aseptic condition. Specimens detail were collected from different sources for microbial pattern. A total of 40 specimen were collected, 30 specimen were as 10 of each from (soil, cow udder and milk), and 2 from milker hand, this were considered as TG were collected without any wash or clean, 8 specimen were as 2 of each from (soil, milker hand, cow udder and milk), were collected after wash by antiseptic solution, this specimens considered as CG. All specimens were labeled and located in an ice-packed container immediately after collection and were transported to the Micro. Lab. for isolation and identification, as well were carried out within (1-2hrs.) of collection.

Source of specimen	***No of specimen collected**	
	***CG**	***TG**
Soil	2	10
Milker hand	2	2
Cow udder	2	10
Cow milk	2	10
Total	8	32

**No: Number, *CG: Control Group, *TG: Test Group*

Microbial pattern: Specimens were prepared in ten folded as serial dilution using sterile distilled water, 1ml of each serially diluted sample was aseptically transferred into a sterile 2 petri dishes for each specimen using sterile pipettes. Liquid (bacterial and fungal) medium cooled to about 45^0C was then added to the petri dishes containing the specimen and mixed. The plates were allowed to solidify and then incubated. Bacterial cultures were incubated for 18-24hrs at 37^0C, while fungal cultures were incubated at 35^0C for at least 48-72hrs to 1week. Identification were done by API and confirmed by Micro-scan. The growth rate percentage were calculated according to the law ([Specimen colony number/300] X 100) [9].

Data Analysis: The data and results were recorded during the study period were entered into Microsoft Excel Sheet and were resulted in the tables and graphs [10].

3. RESULTS AND DISCUSSION

Table1. *Incidence of *MOs growth rates and types from understudy specimens*

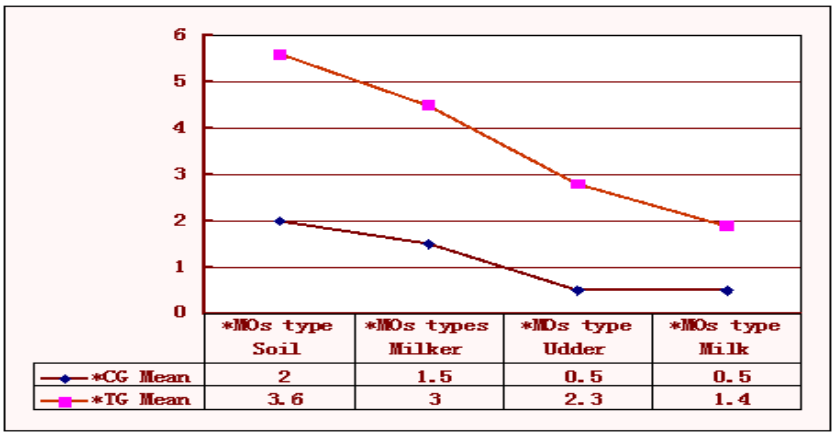
Items	Specimen *No.	Soil		Milker		Udder		Milk	
		Growth rate	*MOs type	Growth rate	*MOs types	Growth rate	*MOs type	Growth rate	MOs * type
***CG.**	1	25%	2	25%	1	25%	1	25%	1
	2	25%	2	25%	2	25%	1		1
Mean		25%	2	25%	1.5	25%	0.5	12.5%	0.5
***TG**	1	75%	4	50%	3	50%	3	50%	2
	2	50%	3	50%	3	25%	3	25%	1
	3	75%	4			25%	2	25%	1
	4	50%	3			50%	2	25%	2
	5	75%	4			50%	3	25%	1
	6	75%	4			25%	2	25%	1
	7	75%	4			50%	2	50%	2
	8	50%	3			50%	2	50%	2
	9	50%	3			25%	2	25%	1
	10	75%	4			50%	2	25%	1
Mean		65%	3.6	50%	3	40%	2.3	32.5%	1.4

**MOs: Micro-organisms, *No.: Number, *CG: Control Group, *TG: Test Group*

Table 2 and Graph (1 & 2): *The mean incidence of *MOs growth rates and types from understudy specimens*

Items	Soil		Milker		Udder		Milk	
	Growth rate	*MOs type	Growth rate	*MOs types	Growth rate	*MOs type	Growth rate	*MOs type
***CG Mean**	25%	2	25%	1.5	25%	0.5	12.5%	0.5
***TG Mean**	65%	3.6	50%	3	40%	2.3	32.5%	1.4

**MOs: Micro-organisms, *No.: Number, *CG: Control Group, *TG: Test Group*



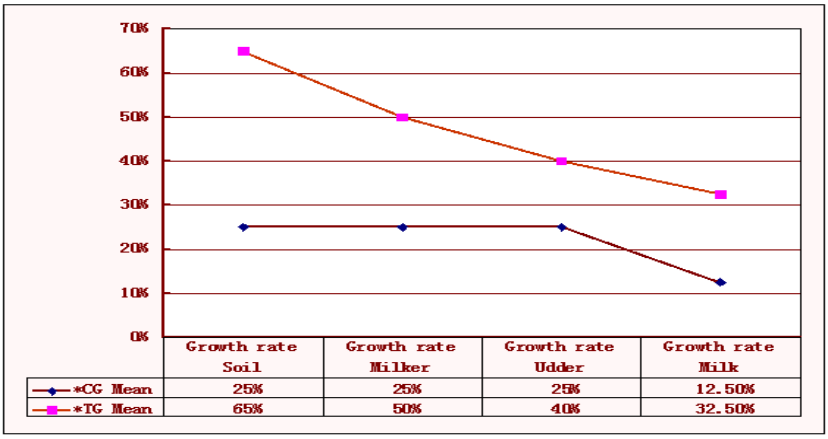


Table (1 & 2) and graph (1 & 2) showed the mean and incidence of MOs growth rates and types from understudy specimens, the mean growth rate from CG were in (soil, milker, udder and milk) were (25, 25, 25 and 12.5%) and from TG were (65, 50, 40 and 32.5%) respectively. Growth rates of TG were compared to CG, which showed of soil as more than double, milker were double, udder less than double and from milk near to triple. MOs were decreased in the value from soil till milk which indicated the sources of MOs contaminated the cows udder and milk so affect the quality of milk. All this items played a role in the fermentation of RM. MOs types from CG were (2, 1.5, 0.5 and 0.5) and from TG were (3.6, 3, 2.3 and 1.4) respectively. MOs types were near to double of TG than CG, that were an indication of the hygienic measures were very important in milk farm because AEFs can transmitted to the animal and contaminated the animal and the milk which affected MQ of RM. The causes of MOs contamination were included contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment's and water supplies used in sanitary activities [2]. Quality of RM may be lowered when it was contaminated by a number of AEFs such as adulteration, contamination during and after milking[3]. The sources of contamination included animal skin and faecal soiling of the udder. Other MOs sources were from air, milkers, handlers, and water used for adulteration [4]. When RM was secreted from a healthy udder, it contained bacteria ($0.5\text{-}1X10^3$ CFUs/ml). After milking environmental contamination occurs, increased to ($5X10^4$ CFUs/ml) or reached several millions bacteria/ml. That count level indicated a very poor hygienic standard of milk during milking and handling. Therefore, the needed for good hygienic practices and a streamlined collection, handling and transport system was important but has been always a challenge. Therefore, proper milking, cleaning and sanitizing procedures of equipment and environments were essential tool to ensure quality of RM. Many countries had implemented laws and regulations concerning the composition and hygienic quality of RM and milk products to protect both the consumers and the public health [6]. Several factors were observed to predispose RM to MOs contamination. Generally, 85.7% of RM samples higher TBCs than the recommended level of ($2X10^6$ CFUs/ml). The quality of RM was poor, unhygienic practices and poor animal husbandry at farm level predispose farmers, consumers and the public to risk of contracting RM-borne infections[7].

Table3. *Incidence of *MOs *Spp. were isolated from understudy specimens*

Items		***MOs *Spp.***									
		***G-positive**			***G-negative**				**Anaerobes**		**Fungi**
Specimen *No.	**Specimen type**	**Staph. *Spp.*	**Strept. *Spp.*	**Entero. *Spp.*	**E. coli*	*Shigella *Spp.*	*Proteus *Spp.*	**Actino. *Spp.*	**Clost. *Spp.*	**Bact. *Spp.*	
*CG											
1	Soil	+		+	+						
	Milker										
	Udder										
	Milk										
2	Soil	+			+						
	Milker										
	Udder										
	Milk										
Total	8	2/8= 25%		1/8= 12.5%	2/8= 25%						
*TG											
1	Soil	+	+		+	+			+		+
	Milker	+	+		+	+					+

	Udder	+	+		+						+
	Milk	+	+								
2	Soil	+	+	+	+						
	Milker	+	+	+	+						
	Udder	+	+		+						
	Milk				+						
3	Soil	+			+		+	+		+	
	Udder	+						+			
	Milk						+				
4	Soil	+			+		+				+
	Udder				+		+				+
	Milk				+		+				
5	Soil	+	+		+					+	
	Udder	+	+		+						
	Milk										
6	Soil	+	+		+	+					
	Udder	+	+			+					
	Milk					+					
7	Soil	+	+	+	+		+				+
	Udder	+		+	+						+
	Milk	+			+						+
8	Soil	+			+			+			
	Udder				+			+			
	Milk				+			+			
9	Soil	+		+	+	+		+			
	Udder				+	+		+			
	Milk							+			
10	Soil	+	+	+	+				+		+
	Udder	+		+					+		
	Milk			+					+		
Total	32	21/32 66%	13/32 41%	8/32 25%	23/32 72%	7/32 22%	6/32 19%	8/32 25%	4/32 13%	2/32 6%	9/32 28%

**MO: Micro-organism, *Spp.: Species, *Staph.: Staphylococcus, *Strept.: Streptococcus, *Entero.: Enterococcus, *E. coli: Escherichia coli, *Actino.: Actinomyces, *Clost.: Clostridium, *Bact.: Bacteriodes,*CG: Control Group, *TG: Test Group*

Table 4 and Graph 3: *The mean incidence of *MOs *Spp. were isolated from understudy specimens*

Items	***MOs *Spp.***									
Specimen	***G-positive**			***G-negative**				**Anaerobes**		**Fungi**
	**Staph. *Spp.*	**Strept. *Spp.*	**Entero. spp.*	**E. coli*	*Shigella *Spp.*	*Proteus *Spp.*	**Actino. *Spp.*	**Clost. *Spp.*	**Bact. *Spp.*	
*CG Mean	25%		12.5%	25%						
*TG Mean	66%	41%	25%	72%	22%	19%	25%	13%	6%	28%

**MO: Microorganism, *Spp.: Species, *Staph.: Staphylococcus,*Strept: Streptococcus, *Entero.: Enterococcus, *E. coli: Escherichia coli, *Actino.: Actinomyces, *Clost.: Clostridium, *Bact.: Bacteriodes,*CG: Control Group, *TG: Test Group*

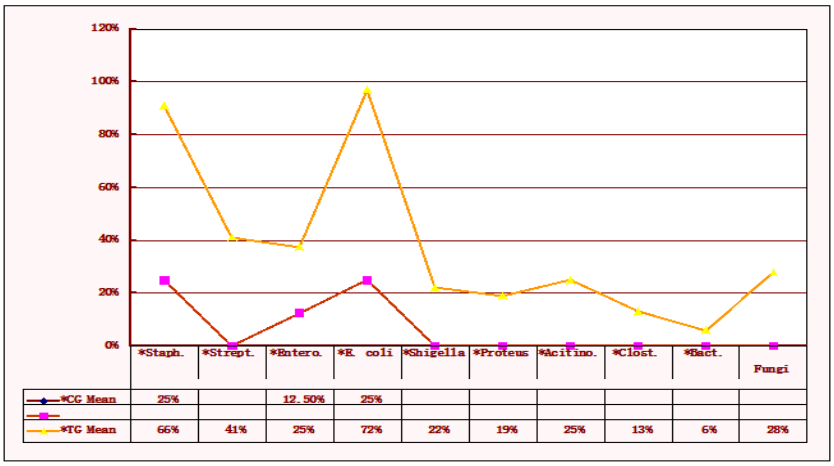


Table (3 & 4) and graph 3 showed the mean and the incidence of MOs *Spp*. were isolated from understudy specimens were resulted in the present of G-positive, G-negative, anaerobes and fungi were in decline percentage respectively. MOs were from CG as (25, 00, 12.5, 25, 00, 00, 00, 00, 00 and 00%) and were from TG as (66, 41, 25, 72, 22, 19, 25, 13, 6 and 28%) for (*Staph.*, *Strept.*, *Entero.*

Spp., E. coli, Shigella, Proteus, Actino., Clost., Bact. Spp. and fungi) respectively. MOs were isolated which related to soil, gastro-intestinal flora (coliform bacteria) and included pathogenic MOs, that were regarded the main sources of contamination and were considered as AEFs. As well this factors not only contaminate the RM but also can transmit pathogenic MOs cause disease for milking animal and also as a media to infect the human. The results were concluded in the some AEFs still affect the MQ of RM production, that indicated the valuable of AEFs in rotting the RM and less economic income in dairy production. The presence of coliform bacteria particularly *E. coli* in RM was an indicator of faecal contamination which implies poor hygienic conditions and un-sanitized environment. In developing countries, most of the RM was produced by small-holder farmers dominated by local herds of cattle. Therefore, the need for good hygienic practices and a streamlined collection, handling and transport system was important but had been always a challenge. Therefore, proper milking, cleaning and sanitizing procedures of equipment and environments were essential tool to ensure quality of RM. Many countries had implemented laws and regulations concerning the composition and hygienic quality of RM and milk products to protect both the consumers and the public health[6]. Several factors were observed to predispose RM to MOs contamination. Generally, 85.7% of RM samples were higher TBCs than the recommended level of ($2X10^6$ CFUs/ml). Isolated bacteria included (*Staph. aureus, E. coli,* and *Proteus Spp.*). The quality of RM was poor, unhygienic practices and poor animal husbandry at farm level predispose farmers, consumers and the public to risk of contracting RM-borne infections. It was recommended that VETs, extension officers and all stakeholders should played their roles in order to ensure safe quality RM delivery to consumers[7]. Prevention and control of MQ of RM were through elimination of MOs by general improvements in water supplies, public health education, personal and environmental hygiene. Pathogenic MOs from the lactating animals can be controlled through improvements in animal husbandry and maintenance of good animal practices, and those from the environments and equipment can be prevented by adhering to general hygienic practices and environmental cleanliness. Generally, MOs contamination the RM can be minimized through adherence to effective good hygienic practices at farm level, and in order to protect the public against RM-borne infections it was important to screen milk which is informally taken to the market[8].

4. CONCLUSIONS

The AEFs had a greater influence on the MQ of RM and contributed to zoonotic pathogens. Strictly hygienic measures should be applied during milking and RM handling practices, achievable by educating on good animal husbandry practices. VETs, have to make periodic surveillance visit to small-scale livestock farms and create awareness, advice or conduct training on good animal health and hygienic measures management systems for reducing AEFs effects to contaminate the RM.

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