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Evaluation of microbial quality and mycotoxin (M1) residue in milk of roaming goat flocks

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Abstract
This study was undertaken to evaluate the prevalence of some foodborne microorganisms in raw goat milk and were screened also for the presence of Aflatoxin M1 (AFM1) residues. Thirty samples of raw goat milk were collected randomly from roaming goat flocks and transferred to laboratory under hygienic conditions and examined microbiologically for the prevalence of *Staphylococcus aureus, Escherichia coli*, *Salmonella* and mould and also analyzed for detection of aflatoxin M1 (AFM1). The obtained results revealed that both *Staph. aureus* and *E. coli* were detected in) 43.3% and 33.3%) of the examined samples, respectively while *Salmonella* not detected .Mycological examination showed that moulds were recovered from (60%) of examined samples and the isolated-moulds were belong to species of genera *Cladosporium*, *Penicillium, Aspergillus* and *Fusarium* spp. On the other hand AFM1 was detected in (50%) of analyzed samples with different concentrations. In conclusion, the results reflect the neglected sanitary conditions under which raw goat milk is produced resulting in the possibility of potential public health threat with the pathogenic microorganisms in addition to the harmful cumulative effect of AFM1 residues.

Key words: *Goat milk, E. coli, Staph. aureus , Salmonella, Mould and AFM1*

Introduction

Consumption of food of animal origin occupies an important place in the human diet. Therefore, special attention should be taken to the type and source of food for notification, correction and /or the removal of the risk factors for obtaining high quality products.

Goats' milk is nutritionally closest to cows' milk than other alternatives, it is highly nutritious, contains essential vitamins and minerals and higher amounts of potassium, calcium, iron and vitamin A than cows' milk as well as a good source of high quality protein **(Tomotake, 2006).**

Breeding of goats in small groups by small holders and their management as milking by hands increasing the probable risk of milk contamination with different microorganisms which originate from different sources and negatively impact on milk quality, shelf life and safety **(Lendenbach and Marshal, 2009 and FDA, 2013).**

Although in developing countries goat is very valuable there were few attempts to determine the microbial loads in its milk. The little available published information indicated variety of pathogens could be isolated from raw goat's milk similar to cow's milk including *Escherichia coli, Salmonella spp*. and *Staphylococcus aureus*. Furthermore, storage of milk at ambient temperature for a long time increase the count of microbial load to 100 fold or more if compared with the fresh on **(Migeemanathan *et al*., 2011 and Ombarak and Elbagory, 2017).**

Different types of fungi also are recognized as an important cause of spoilage of various dairy

products leading to high economic losses and the main problem is the ability of some species to produce health hazard mycotoxin which is a major public health concern especially in tropical and subtropical regions **(Sengum *et al.*, 2008; Marwa *et al.*, 2013and Pal and Jadhav, 2013 and Guchi, 2015).**

Mycotoxins are secondary metabolites of various different fungal species and they differ in chemical structure, biosynthetic origins, and biological effects. Aflatoxin, for example, is a hepatotoxic, mutagenic, carcinogenic mycotoxin **(Bennett and Klick, 2003)** produced mainly by fungi of the genus *Aspergillus* particularly *Asp. flavus* and *Asp. Parasiticus* and resulting in a significant economic losses that estimated as 25% or more of the world's food crops to be destroyed annually **(Mostrom, 2016 and WHO, 2018).**

AflatoxinM1(AFM1) is secondary metabolites resulted from biotransformation of aflatoxin B1 and can be present in milk and milk products via feeding of lactating animal aflatoxin contaminated feed **(Dohnal *et al.*, 2014)**. It was estimated that about 1%– 6% of AFB1 in feed is present in milk within a few hours to two days after feeding the diet **(Gürbay *et al.*, 2006 and Mary and Carmen, 2011).**

Therefore, the aim of this study was to detect the prevalance of the most important foodborne pathogens such as, *Staph. aureus; E. coli* and *Salmonella* as well as mould that may be present in raw goat milk and measure the level of AFM1 that may be existed in milk samples.

Materials and Methods

1- Samples collection

A total of thirty goat milk samples were collected from different roaming goat flocks in sterile containers then transported to the lab directly in ice box under aseptic conditions. Each sample was divided into two parts; one part was stored (at 4°C)for microbiological examination and the another part was stored at -20 for chemical analysis.

2- Microbiological examination.

2-1. Preparation of samples (ISO6887-5:2010)

Ten ml of each sample were transferred into 90 ml of sterile peptone water 1% and tenth fold serial dilutions of milk were prepared in peptone water for the following analysis to be performed.

2-2. Isolation and identification of *E.coli* (APHA,1992)

From each prepared dilution 1.0 ml was spread over 3 plates of Eosin Methylene Blue (EMB) agar then incubated at 35°C for 24hrs. Suspected *E. coli* colonies were identified microscopically and biochemically then serological identification was done at **Serology department in (Animal Health Research Institute) using *Escherichia coli* Antisera (DENKA SEIKEN CO., LTD.)**

2-3. Isolation and identification of *staph. aureus* (FDA, 2001).

From each prepared dilution 1.0 ml was spread over3 plates of Baired parker agar then incubated at 37°C for 24 - 48hrs then suspected isolated colonies were purified for identification **(McFaddin, 2000).**

2-4. Isolation of *Salmonella* (ISO, 2017)

-Salmonella pre-enrichment

Milk sample (25 ml) were added to 225ml. of sterilized buffered peptone water (1%) and incubated at 37°C for 24 hrs.

-Selective enrichment

From each pre-enrichment 0.1 ml was added to 10 ml of Rappaport Vasiliadis broth and incubated at 41°C for 24 hrs as well as 1ml of pre-enrichment into 10 ml Muller- Kauffmann Tetrathionate – Novobiocin broth was incubated at 37°C for 24hrs.

-Isolation on selective media

0.1 ml from each broth was spread over Xylose Lysine Deoxycholate agar (XLD) agar plates and incubated at 37°C for 24 hrs. Colonial morphology was used as the first step for the bacterial isolates identification then identified microscopically, biochemically and serologically.

2-5. Isolation and identification of moulds (Narange, 2004)

From each prepareddilution1.0 was cultured on Dichloran Rose Bengal Chloramphenicol agar

medium (containing antibiotic 0.05 mg of chloramphenicol / ml) by pouring method then the plates were incubated aerobically at 25°C± 1°C for 5 days. Isolated mould colonies were counted, selected, purified, identified individually by macroscopic (based on colony morphology such as pigmentation, shape and coloration on the dorsal side) and microscopic characteristics under oil immersion **(Pitt and Hocking 2009).**

3- AFM1 determination.

The quantitative analysis of AFM1 in examined samples was performed by competitive ELISA test kit (RIDASCREEN IMMUNOLAB AFM1, Art No. R1111- R- BiopharmGmb H, and Darmstadt, Germany) at **Food Hygiene Departmentin (Animal Health Research Institute)** as described by R- biopharmGmb H **(Anonymous, 1999)**

3-1. Preparation of samples for AFM1 analysis.

The samples should be stored in a cool place, protected against light then centrifuge milk samples for degreasing (3500 rpm at 10°C (50°F) /10 min.). After centrifugation, remove upper cream layer completely by aspirating through a Pasteur pipette and use the skimmed milk directly in the test (100 ul per well).

3-2. Evaluation of AFM1 by ELISA test (ISO 14675 / 2003

The absorbance values obtained for the standards and the samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100 (percentage maximum absorbance). The zero standards are equal to 100%, and the absorbance values were recorded in percentages. The values calculated for the standards were entered in a system of coordinates on graph paper against the AFM1 concentration in ppt.

3-3. Statistical Analysis: (Zar, 1984)

Data were analyzed and results reported as mean ± SE. The calibration curve and trend line equation prepared using available softwareware, Percentage, minimum, maximum and mean ± SE were carried out. The calibration curve and line equation were prepared, data were analyzed and results recorded.

Results and discussion

Table (1). Prevalence and the mean count (log CFU/ml.) of the isolated pathogenic bacteria and mould in examined goat milk samples (30 samples).

Bacterial species	Positive samples		Max. (Log cfu/ml)	Min. (Log cfu/ml)	Mean± SE (Log cfu/ml)
	Number	%			
S. aureus	13	43.3 %	5.84	3.69	5.02 ± 0. 3
E. coli	10	33.3%	5.84	4.11	5.33 ± 0.83
Salmonella	ND	ND	ND	ND	ND
Mould	18	60%	4.84	2.3	2.77± 0.16

ND: Not Detected

Table (2). Serological identification of *E. Coli* isolated from goat milk samples.

No. of isolates	*E. coli* Serotypes											
	O44		O86		O29		O26		O55		O125	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
10	2	20%	1	10%	3	30%	2	20%	1	10%	1	10%

Table (3). Incidence of the identified genera of isolated mould species from examined goat milk samples (n=30).

Positive Samples	*Cladospori-umcladosporidea*	*Asp. Niger*	*Fusarium* Spp.	*Pencillium* Spp.		
18 (60%)	12 (63.1 %)	5 (26.3%)	5 (26.3%)	7 (38.8%)		
				P. Camemberti 1 (14.2%)	*P. Chrysogenum* 2 (28.5%)	*P. Solitum* 4 (57.1%)

N.B. some samples contained more than one type of mould spp.

The results presented in **Table (1)** showed the frequency of bacterial isolation that may be present in goat milk samples. Among the bacteria predominantly *Staph. aureus* is one of the most common agents in bacterial food poisoning outbreaks and the contaminated food are often the cause of food intoxication as a result of its enterotoxin production. **Necidova *et al.* (2012)** and **Janstova *et al.* (2014)** found that *Staph. aureus* count in the range from 10^3 - 10^5cfu/ g is sufficient to produce amount of heat resistant enterotoxin toxin enough for consumer illness that produced at temperatures range of 10°C to 46°C **(Tortora *et al*., 2005).**

The current results revealed that, Staphylococciare the most frequent bacteria isolated from goat milk samples. *Staph. aureus* was detected in (43.3%) of the examined samples with a mean count value of 5.02 ± 0.3 log cfu/ml. Nearly similar results (40%) recorded by both **Zakary *et al.*** (**2011**) and **Aboshamaa (2014),** while lower results (12%, 31.43%, 2.15% and 10.7%) were recorded by **Heba *et al.* (2017), Ombarak and Elbagory (2017), Acosta *et al.* (2018)** and **Mahlangu *et al.* (2018),** respectively.

The contamination of milk could be transmitted from the skin of human beings to mammary glands or to milk itself during the hand milking and unhygienic practices in the milking process **(Fadel and Jehan, 2009 and Abeer *et al*., 2010)**.

With respect to the environmental factors, **Hagi *et al.* (2010)** observed that the microbial load of milk could affected by the feeding behavior of animals either indoors or outdoors feeding with an increase in *Staphylococcus* Spp. during outdoor feeding as in goat behavior.

Jan and Jaskowski (2014) recorded that although the sampled animals may be clinically normal at the time of sample collection, subclinical mastitis may also share with milk contamination with staphylococci as it was the most prevalent pathogens of the mammary gland in small ruminants with subclinical mastitis (60- 80.7% of examined goats) and *Staph. aureus* represented 37% of the isolates causing subclinical mastitis in goats and responsible for 35% of the economic loss in the dairy industry **(Le Loir *et al.*, 2003, Da Silva *et al*., 2004 and Maria *et al.*, 2010).**

Also *E. coli* is recognized as a serious foodborne pathogen and has been associated with several outbreaks worldwide and dairy products have been reported as a main source of those outbreaks (**scotter *et al*., 2000 and Espie *et al.*, 2006).**

The microbial load of the examined milk samples **(Table 1)** revealed that *E. coli* was isolated from (33.3%) of the examined samples which relatively higher than that (23% and

14.3%) reported by **Heba *et al*. (2017) & Ombarak and Elbagory (2017),** respectively. On the other side, there were other studies showed slightly higher results (44%) that was recorded by **Abd El-Aal and Awad (2008)**.

Contamination by *E. coli* can be attributed directly to the surrounding unhygienic environment as faecal contamination as previous studies has been confirmed that ruminants were one of the important reservoirs for *E. coil* and they shed the bacteria into the environment throughout their faeces without suffering from clinical signs **(Osman *et al*., 2013 and AL-Zogibi *et al*., 2015)**.

Furthermore, other factors help in milk contamination with various microorganisms such as hand milking with long milking time, dirty teats, as goats are nearer to the soil, small ruminants are very hairy, times and the conditions under which the herds or flocks are raised **(Kalantzopoulos *et al*., 2002; Portolano *et al*.; 2007 and Millogo *et al*., 2010)** .

The serological identification of isolated *E. coli* revealed that the isolated strains belonged to six *E. coli* serotypes O44, O86, O29, O26, O55 and O125 **(Table 2).**

However, *Salmonella* spp. couldn't be isolate from the examined samples. The absence of this dangerous microorganism in raw ovine milk has also been pointed out by **Abd El-Aal and Awad (2008) & Ombarak and Elbagory (2017).**

Moulds can also be important microbial populations within raw milk as a rich nutrient source and they are influenced by different factors and environmental conditions as the physiological state of animal as well as the weather, type of feeding and season **(Bullerman *et al*., 1984 and Callon *et al*., 2007).**

Results in the present study revealed that moulds were recovered from 18 (60%) of examined milk samples **(Table1)** and the isolates belonged to 4 genera of moulds were *cladosporiumcladosporidea* (63.1%), *Asp. niger* (26.3%), *Fusarium Spp*. (26.3 %) and *penicillium Spp.* (38.8%) which including *P. Camemberti* (14.2%), *P. Chrysogenum* (28.5%) and *P. Solitum* (57.1%) from the isolated samples **(Table 3).**

These results agreed with the results recorded by **Bourabah *et al*. (2013)** who reported that *Asperagillusniger* was isolated from 23.4% of goat milk samples.

Mould contamination of milk samples may be attributed to improper hygienic practice at environment such as container, air, equipments, water etc….as an external sources of contamination (**Vacheyrou *et al*., 2011).**

Also, fungi were detected as an important causative agents of mycotic mastitis in ruminants either clinical or subclinical mastitis, and even in the apparently healthy goat which may be the internal source of fungal contamination of milk **(Quinn *et al*., 2011; Bourabah *et al*., 2013 and IIhan *et al* ., 2016).**

In addition, seasonal trend reflects the fact that warm climate and inadequate refrigeration are the principal causes of fungal contamination of milk and concentrated feed stuffs that may be stored under inadequate conditions increasing the risk of raw milk contamination by mould and /or its toxin **(Bilandžića *et al*., 2014).**

Table (4). Incidence and concentration of AFM1 (ppt) in the examined goat milk samples.

No. of samples	Positive samples.		Maximum concentration	Minimum concentration	Mean ± SE
	No.	%			
30	15	50 %	39.65	5	11.9± 1.9

Detection limit: 5 ppt. The permissible limit is 50ppt and for children is 25ppt (EC, 2006)

Therefore, feeding of animal on mould contaminated food with a count more than10^6 /g and kept under humid conditions cause a potential of mycotoxin production resulting intoxication to both animals and consumers, so aflatoxins contaminated agricultural crops is a worldwide problem **(Yin *et al.*, 2008 and Marwa *et al*., 2013).**

Aflatoxin M1 is the most important heat stable mycotoxins found in milk produced through hydroxylation of aflatoxin B1 **(Sadeghi *et al*., 2009)** and due to its high hepatocarcinogenic potential, its level permitted in milk and dairy products is strictly regulated as the limit of AFM1 in milk and dairy products is 50 ppt according to European Union (EU) and 500 ppt in the United States (US) and (25 ppt) for milk intended for consumption by nursing infants and children **(EC, 2006)**.

AFM1 concentration in the examined raw goat milk samples were determined and compared to (EU) limit for milk. AFM1 levels were detected in 15(50 %) of the examined samples with quantities don't exceed the legal limits of EU 0.05 µ/l (50 ppt) with a mean value 11.9 ± 1.9 **(Table 4),** but there was one sample its toxin concentration (39.65 ppt) exceeds the legal limit for children 0.25 µ/l (25ppt) and other samples contain this toxin in concentrations (22.51 and 16 ppt) that are relatively near to the legal limit of children and even with low concentration. It should put in consideration the cumulative effect of this toxin with the prolonged ingestion of contaminated milk.

Lower incidences of M1 (20% and 40%) were recorded by **Hussain *et al.* (2010) and Fardos *et al.* (2017),** respectively. On the contrary, higher incidences 48.5% and 63.6% were recorded by **Ozdemir (2007) and Ghanem and Orfi (2009),** respectively.

Also, **Ruangwises *et al.* (2013)** recorded that the incidence of M1 containing goat milk samples was 54.4% and only 7 samples (14.3%) were contaminated with concentrations exceed the EU legal limit.

Feeding patterns of goats in Egypt which allowed goats to graze on pasture in the morning and are brought back into the enclosed areas for concentrate feedstuffs play an important rule in the contamination of milk with AFM1 if compared with pattern of cow which are generally kept in enclosed areas and fed with a large proportion of AFB1-contaminated feedstuffs; corn, cotton seed, and concentrated feed **(Motawee *et al*., 2009 and Hussain *et al*., 2010).**

In this connection, **Virdis *et al.* (2008)** concluded that the levels of AFM 1 concentration in goats milk produced from goats fed on grass and naturally growing bushes was lower than that measured in those were mainly fed on concentrates.

Conclusion and Recommendations

Results obtained in this study highlighted the poor microbiological and sanitary condition of milk produced from roaming goat flocks and presence of AFM1 in milk samples indicated that they are fed on AFB1 contaminated food therefore:

1. Awareness of consumers about that fresh goat milk has high nutritive values but it shouldn't be consumed in its raw state or used for production of milk products and should be boiled well before its use to eliminate most microorganisms that may be transmitted for

consumer.

2. More efforts needed to increase the awareness of small holders about the probable risk of transmission of different contaminants during lactation and handling.

3. As milk is a source of nutrients of particular importance for infants, it is essential to adopt measures to minimize feed contamination by mycotoxins. Thus, increasing the awareness of small holders about special care should be taken with the quality of lactating goat's feedstuff and its storage conditions.

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