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OCCURRENCE OF SOME PATHOGENIC MICROORGANISMS IN SHEEP'S AND GOAT'S MILK WITH SPECIAL REFERENCE TO LIPOLYTIC AND PROTEOLYTIC ENZYMES OF THE ISOLATED *BACILLUS CEREUS*

(With 3 Tables)

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تواجد بعض الميكروبات الممرضة فى ألبان الأغنام والماعز مع إشارة خاصة إلى الأنزيمات المحللة للبروتين والدهون للباسيلس سيريس المعزولة

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لدراسة تواجد بعض الميكروبات الممرضة والمسببة للتسمم الغذائى فى ألبان الأغنام والماعز تم جمع ستين عينة عشوائية من منازل الفلاحين بمحافظة أسيوط بواقع ٣٠ عينة من كل نوع وذلك لمعرفة مدى تواجد ميكروبات الباسيلس سيريس ، المكور العنقودى الذهبى والكلوستريديم بيرفرنجنيز. وقد أوضحت النتائج أن : ميكروب الباسيلس سيريس قد تواجد بنسبة ٥٦,٦٧ ، ٧٣,٣٣% بمتوسطات قدرها ٦,١٣ × ١٠ ^٢ ، ٦,٤ × ١٠ ^٢ لكل مل بينما ميكروب الكلوستريديم بيرفرنجنز قد تواجد بنسبة ٢٠ و ١٠% بمتوسطات قدرها ٣,٥ × ١٠ و ٢,٧ × ١٠ لكل ملى فى ألبان الأغنام والماعز على التوالى . أما بالنسبة لميكروب المكور العنقودى الذهبى فلم يتم عده لكل ملى فى ألبان الأغنام والماعز على التوالى. وعند دراسة نشاط الباسيلس سيريس المعزول لمقدرته على إفراز الانزيمات المحللة للدهون والبروتين دلت النتائج على أن ميكروب الباسيلس سيريس له القدرة على افراز الأنزيم المحلل للدهون بنسبة ٥٢,٩٤ و ٧٧,٢٧% وكذلك المحلل للبروتين بنسبة ١٧,٦٤ و ٤,٥٤% فى ألبان الأغنام والماعز على التوالى . هذا وقد نوقشت الأهمية الصحية لهذه الميكروبات واتخاذ الإجراءات اللازمة لحماية المستهلك والحفاظ على القيمة الغذائية للألبان .

SUMMARY

A total of 60 random samples of sheep's and goat's milk (30 of each) were collected from different farmer's houses in Assiut Governorate and

examined for the occurrence of *B. cereus, S. aureus* and *C. perfringens*, as well as, the lipolytic and proteolytic activities of the isolated *B. cereus*. Results indicated that *B. cereus* were existed in 56.67 and 73.33% of the examined samples with averages $6.13x10^2$ and $6.4x10^2$/ml, while, *C. perfringers* revealed from 20, 10% with 3.5x10 and 2.7x10/ml as averages in sheep's and goat's milk samples, respectively. However, *S. aureus* counts could not be detected in the examined samples of sheep's and goat's milk, respectively. The isolated *B. cereus* from sheep's and goat's milk proved that 52.94 and 77.27% of the isolated organisms were positive for lipolytic activity and 17.64 and 4.54% had a proteolytic activity, respectively. The public health importance of the pathogenic microorganisms was discussed.

Key words: *Sheep's milk, goat's milk, B. cereus, S. aureus, C. perfringens, lipolytic and proteolytic enzymes.*

INTRODUCTION

Sheep and goats are important animals for different areas in the world including Egypt. Milk of sheep and goats comprises fourth and third in terms of global milk production. The microbiological standards for the production and distribution of sheep's and goat's milk are more relaxed than those of cow's milk which has stringent hygiene and quality regulations. Since, the pathogenic microorganisms can gain access to sheep's and goat's milk either by fecal contamination or by direct excretion from the udder into the milk. So dairy industries for milk of sheep and goats become increasily important elsewhere (Maxy, 1993). Several characterizations belong to sheep's and goat's milk. It can be consumed fresh without thermal treatment if it is produced under aseptic condition. It has a high nutritional value with the same concentrations of cow's milk. In addition, to the growing demand for milk of sheep and goats by consumers specially a number of children suffering from intolerance to cow's milk as well as alternative for mal nourished children (Razafindrakoto *et al.*, 1993).

Public health problems associated with consumption of raw sheep's and goat's milk and their milk products have been well documented (Barrett,1986; Keene *et al.*, 1997; De Valk *et al.*, 2000; Kalman *et al.*, 2000; De Buyser *et al.*, 2001 and Harrington *et al.*, 2002). Various bacterial illnesses due to *B. cereus, S. aureus* and *C. perfringens* have been linked to consumption of sheep's and goat's milk (Al-Graibawi and Youssef, 1985 and Bautista *et al.*, 1988).

Occurrence of *B. cereus* in milk has been reported since 1916. Several cases of food poisoning outbreaks due to *B. cereus* have been reported to the National Center of Disease Control (CDC) (Galbraith *et al.*, 1982; Cohen *et al.*, 1984 and Van Netten *et al.*, 1990). *B. cereus* could be isolated in variable percentages from milk and other dairy products (Chopra *et al.*, 1980; El-Nawawy *et al.*, 1982; Ahmed *et al.*, 1983; Saad,1985 and Abdel-Hakiem, 1992).

B. cereus is capable of causing both lipolytic and proteolytic activities due to heat-stable extracellular lipases and proteases which contribute to milk spoilage (Sorhaug and Stepaniak, 1997) and reduce the shelf life of processed milk. Free fatty acids are produced by hydrolysis of milk fat by lipase enzyme, while bitter flavour is due to digestion of casein by proteases which can survive UHT (Harper *et al.*, 1980) causing deterioration of dairy foods during storage and after processing (Chopra and Mathur, 1983).

Among the microbial pathogens, *Staphylococcus aureus* occupies a unique position with a minimum number of 500,000/g, variable coagulase positive supports evidence of staph food poisoning (Allison, 1949). The organism has been reported as agents of foodborne illness for the first time in Phillipines (Barber, 1914). Comprehensive reviews on staphylococcal food poisoning and on its occurrence in milk have been published (Bryan, 1988). General symptoms of staphylococcal intoxication in human are typical gastrointestinal disturbances occur within 3-8 hs after consumption of toxin, including salivation, diarrhea with vomiting. Complications may accompany severe attacks but the illness is seldom fatal (Bergdoll, 1970 and 1972). Moreover, *S. aureus* has been reported to cause staphylococcal diseases (pyoderma, otities externa, urinary tract and wound infections and septicemia (Johnson, 1981).

Also, the organism was responsible for high incidence of subclinical mastitis in dairy animals (Olsvik *et al.*, 1981). Raw milk is subjected to contamination by *S. aureus* either directly or indirectly from different sources including the producing animal, milkers, handlers, infected water supplies, rodents, flies and those who are throat and nasal carriers as they give chance for postpasteurization contamination. More attention and extensive work have been conducted by different investigators in various countries to throughout light on *S. aureus*. Several cases of *S. aureus* food poisoning outbreaks due to consumption of sheep's and goat's milk or their milk products have been recorded by

several investigators (Farina, 1955; Buyser *et al.*, 1985; Bautista *et al.*, 1988, Gross *et al.*, 1988 and Muehlherr *et al.*, 2003).

C. perfringens has been recognized as one of the principal causes of human food poisoning due to ingestion of food containing large numbers of vegetative cells of the organism (10^6/ml or g). the organism is more widely distributed than any other pathogenic bacterium, occurring in soil, dust and dirt, and its spores can survive for long periods. Also the organism is considered as a normal inhabitant of the intestinal tract of warm blooded animals (Smith and Holdeman, 1981) and about 25% of the human populations excrete the bacterium in their faeces. So, its presence in milk and milk products is accepted as an index of faecal or soil contamination. Therefore, there is a complete relationship between hygienic production of milk and the organism (Gudkov and Dolidze, 1975). Consequently, the organism can reach raw foods and food ingredients and because of its ability to produce resistant spores may persist in low numbers in some processed food products.

Foodborne illness caused by *C. perfringens* occurs within 8-15 h after ingestion of the contaminated food with large numbers of living bacteria which subsequently release toxin in the alimentary canal. The symptoms include abdominal cramps, gas and diarrhea (nusea and vomiting are rare). Also, *C. perfringens* type A is accepted as an etiological agent of gas gangarene in man and animals (Hobbs, 1965), in addition, to acute matitis (Johnston, 1986). Milk and dairy products have been implicated as a cause of *C. perfringens* food poisoning (Weinberg and Prevot, 1937; Woodward *et al.*, 1970; Anon, 1982).

Owing to the implication of sheep's and goat's milk in several cases of foodborne illnesses, the present work was planned to:

1- Study the occurrence of *B. cereus, S. aureus* and *C. perfringens* in the examined sheep's and goat's milk.
2- Detection of the lipolytic and proteolytic activities of the isolated *B. cereus*.

MATERIAL and METHODS

Collection of samples:

60 samples of sheep's and goat's milk (30 of each type) were collected randomly from different farmers' houses in Assiut Governorate. The samples brought to the laboratory with a minimum of delay in clean, dry and sterile containers in an ice box and kept in refrigerator.

Preparation of samples:

Each sample was thoroughly mixed and subjected to Storch's test (Lampert, 1975) to exclude heat treated samples.

Microbiological examination:

1- Enumeration of *Bacillus cereus*: by using the direct plating technique on Manitol egg yolk agar supplemented with polymyxin B sulphate according to (Lancette and Harmon, 1980). Isolates were identified morphologically and biochemically according to the methods adopted by MacFaddian (1976).

2- Enumeration of *Staphylococcus aureus*: using Baird-Parker agar (Baird-Paker, 1962) and identification according to Finegold and Martin (1982).

3- Enumeration of *Clostridium perfringens*:

Applying the direct planting technique using sulphate polymyxin sulfadiazine (SPS) agar as recommended by (Angelotti *et al.*, 1962). Suspected colonies were picked up for further confirmation according to Mead *et al.* (1981).

1- Detection of lipolytic activity: (Oxoid, 1998)

Ready poured plates of tributyrin agar were inoculated with pure culture of *B. cereus* and incubated at 30°C for 3 days. Positive plates regarded as a zone of clear medium surrounds lipolytic colonies.

2. Detection of proteolytic activity (Harrigan and Maccance, 1976).

The isolated and identified strains of *B. cereus* were inoculated onto prepoured plates of standard plate count agar with 10% sterile skim milk (Stanley *et al.*, 1986) and then incubated at 25°C for 48 hs. Positive results were recorded as a clear zone around the colonies.

RESULTS

The obtained results were tabulated in Tables 1-3.

Table 1: Statistical analytical results of some pathogenic microorganisms in the examined sheep's milk samples.

Microorganisms	Positive samples		Count		
	No/30	%	Min.	Max.	Average
Bacillus cereus	17	56.67	$1x10^2$	$4.5x10^3$	$6.13x10^2$
S. aureus	-	-	-	-	-
Clostridium perfringens	6	20	1x10	$3.4x10^2$	3.5x10

Table 2: Statistical analytical results of some pathogenic microorganisms in the examined goat's milk samples.

Microorganisms	Positive samples		Count		
	No/30	%	Min.	Max.	Average
Bacillus cereus	22	73.33	$1x10^2$	$8.0x10^3$	$6.4x10^2$
S. aureus	-	-	-	-	-
Clostridium perfringens	3	10	1x10	5x10	2.7x10

Table 3: Lipolytic and proteolytic activities of *Bacillus cereus* isolated from sheep's and goat's milk.

Milk examined	No. of B. cereus isolated	Activities			
		Lipolytic		Proteolytic	
		No.	%	No.	%
Sheep's milk	17	9	52.94	3	17.64
Goat's milk	22	17	77.27	1	4.54

DISCUSSION

Results recorded in Tables 1 and 2 show that B. cereus could be detected in percentages of 56.67 and 73.33% with average counts of $6.13x10^2$ and $6.4x10^2$/ml in sheep's and goat's milk, respectively. These results were somewhat lower than that obtained by Chopra *et al.* (1980) and El-Nawawy *et al.* (1982) who could isolate the organism with 100%. Also, the results obtained were higher than the results obtained by Saad (1985) and Abdel-Hakiem (1992). The wide variety of foods that have been implicated in *B. cereus* food borne illness outbreaks may be due to the wide spread distribution of the organism and the ability of its spores to survive long-term storage in dried products due to their high thermal resistance. As the organism gains entry into milk from many sources during production, handling and storage that makes the microbiological standards for production and distribution of sheep's and goat's milk are more relaxed than those of cow's milk which is subjected to stringent hygiene and quality regulations. Also, the organism is capable of causing mastitis of short duration (Raevuori and Koiranen, 1978) through contamination of the udder with spores. Data illustrated in Tables 1 and 2 pointed out that *C. perfringens* could be detected in 20 and 10% with averages of 3.5x10 and 2.7x10/ml of the tested sheep's and goat's milk, respectively. These results are to less extent in agreement with those

obtained by Abdel-Hakeim (1992). Several investigators could isolate the organism from milk and other milk products El-Bassiony (1980) and Saad (1995). But till now only limited data on the microbiology of sheep's and goat's milk were recorded in other countries (Robert's 1995, Little and Louvois, 1999 and Foschino *et al.*, 2002). The higher incidence and counts of *C. perfringens* in raw sheep's and goat's milk samples are indicative for neglected sanitary measures during, handling, production and distribution, where the organism is considered to be more widely distributed than any other pathogenic bacterium occurring in soil, dust and among the intestinal microflora of warm-blooded animals and consequently, it is considered as one of faecal contaminants (Romagnoli and Brazzi, 1960). The organism has the ability to contaminate raw food and food ingredients due to its heat resistant spores which survive cooking temperature (1 to 5 h at 100°C) or germinate in slow cooling to a number sufficient to induce food poisoning (Hobbs, 1965).

S. aureus organism failed to be detected in sheep's and goat's milk samples (Tables 1 and 2). These results are considered completely in contrast with the results obtained by Jensen and Hughes (1980), Al-Grabawi and Yossef (1985), Bautista *et al.* (1988) and Muehlherr *et al.* (2003). Failure of *S. aureus* to be detected may be due to the high count of contaminating bacteria other than *S. aureus* that have a limited effect on the rate of staphylococcal multiplication (Smith, 1957 and Johns *et al.*, 1975).

Concerning the detection of lipolytic and proteolytic activities of the isolated *B. cereus*, several investigators have reported the rules of extracellular enzymes in virulence Abdel-Rahman and Ahmed (1988), Cox and Macrae (1989), Saad (1994) and Abdel-Hakiem (1992).

Interpretation of results given in Table 3 revealed that *B. cereus* isolated from sheep's and goat's milk achieved 52.94 and 77.27% for lipolytic and 17.64 and 4.54% for proteolytic activities, respectively. These results were somewhat in agreement with Cox and Macrae (1989) who detected lipolytic activity from raw and pasteurized goat's milk. However, Abdel-Rahman and Ahmed (1988) reported that *B. cereus* is a proteolytic mesophile and thermophile.

Since the ewe's and goat's milk is consumed raw or insufficiently heated it can act as a source of human infection specially due to the nature of the sporeforming organisms that have a thermal resistance and can produce a thermostable protease enzyme which lead to spoilage of such contaminated foods (Chopra and Mathur, 1983). So,

there is a need for heat treatment of sheep's and goat's milk for public health reasons and to prolong its shelf-life. Sanitary restrictions and hygienic measures should be adopted to improve the quality of ewe's and goat's milk to safe guard consumers from food poisoning hazard.

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