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A STUDY ON *ESCHERICHIA COLI* AND *CLOSIRIDIUM PERFRINGENS* IN BUFFALOE'S MILK

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SUMMARY

E. coli was recovered from 44.17% of randum samples collected from buffaloe's milk, while, *C. perfringens* could be isolated from 16.67% of examined samples. Serological typing of *C. perfringens* pointed out that type A was present in 45% of positive samples followed by type D (10%). both types of *C. perfringens* A & D were recovered together from 35% of positive samples.

At the surface of solid medium, mixed cultures from both organisms showed that growth of *E. coli* was able to mask the growth of toxigenic isolates of *C. perfringens* with a range from 56% to 84%. This result was confirmed experimentally by determination of mean total bacterial cont (MTBC)/ml of *E.coli* and *C. perfringens* in incubated buffaloe's milk containing nearly equal known number of these bacteria in a separate or mixed condition.

The economic and public health importance of these bacteria as well as suggested measures for improving the quality of buffaloe's milk have been discussed.

INTRODUCTION

Human infections may be caused by the ingestion of milk contaminated with microorganisms. *E. coli* and *C.perfringens* are considered to be the most important causes of such infections. They had been isolated from the intestinal tract of both man and animal (Chordash and Insalata, 1978; and Collee et al., 1989). So, their presence in milk is commonly accepted as an index of faecal contamination (Thatcher and Clark, 1968 and Gudkov and Dolidze, 1975).

In contrast to botulism and staphylococcal food poisoning, *E. coli* and *C. perfringens* food poisoning is not due to ingestion of pre-formed toxin, so that, their bacteriological diagnosis is accomplished by isolation of the organisms from the food. Therefore, this investigation was conducted to determine the incidence of *E. coli* and *C. perfringens* in buffaloe's milk. Also, to determine the relation between the growth of *E. coli* and *C.perfringens* especially in mixed conditions.

MATERIAL AND METHODS

One hundred and twenty random samples of buffaloe's milk were collected aseptically in sterile bottles from different farms in Kafr El-Sheikh Governorate. They were examined for detection and identification of existing *E. coli* and *C. perfringens.*

Handling and preparation of samples were done according to A.P.H.A. (1978).

Isolation technique for *E. coli* was done according to Collee et al. (1989), while that for *C. perfringens* was adopted after I.C.M.S.F. (1978).

Suspected *E. coli* isolates were identified biochemically according to Koneman et al. (1988), while suspected *C. perfringens* isolates

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were identified biochemically according to Collee et al. (1989).

Typing of *C. perfringens* isolates was performed by dermonecrotic test in guinea pigs (Bullen, 1952; Oakley and Warrack, 1953 and Stern and Batty, 1975).

Growth of the standard isolates of *E.coli* was studied against the growth of all recovered toxigenic isolates of *C. perfrinjens.* Each *E.coli* isolate was inoculated in straight line at the center of blood agar plate without neomycin. Across the line of *E. coli,* separate different streaks from the studied isolates of *C. perfreingens* were made. Thus, one half of the plate contained toxigenic *C. perfringens* isolates and the other half contained several different mixtures from isolates of *E.coli* and *C. perfringens.* The inoculated plates were incubated anaerobically at 37°C for 24 hours.

Twenty five flasks, each containing 90 ml of pasteurized buffaloe's milk were prepared and divided into 5 groups (each of 5 flasks). They were inoculated with the studied bacteria to get 2.5×10^6 CFU/ml as follows:

1- The first group was inoculated with five isolates of *E. coli.* Each flask contained one isolate.

2- The second group was inoculated with five isolates of *C. perfringens* type A. Each flask contained one isolate.

3- The third gorup was inoculated with five isolates of *C. perfringens* type D. Each flask contained one isolate.

4- The fourth group was inoculated with *E. coli* and *C. perfringens* type A isolates. Each flask contanined one isolate from each of *E. coli* and *C.perfringens* type A.

5- The fifth group was inoculated with *E. coli* and *C.perfringens* type D isolates. Each flask contained one isolate from each of *E.coli* and *C.perfringens* type D.

Flasks of the first group which containing *E. coli* were incubated aerobically at 37°C for 24 hours. Meanwhile, flasks of other groups were incubated anaerobically at 37°C for 24 hours.

Sterile ten fold serial dilutions from each flask were prepared in quarter-strength ringer's solutions (Collee et al., 1989). Pipette 0.1 ml from each dilution onto three macConkey's agar plates for *E. coli* and/or three neomycin sheep blood agar plates for *C. perfringens* (Kamel, 1973).

Plates inoculated with *E. coli* were incubated aerobically and those inoculated with *C. perfringens* were incubated anaerobically at 37°C for 24 hours.

In each group, the plates of suitable dilution of each flask were counted and mean of five flasks was calculated. Thus, mean total bacterial count (MTBC)/ml of *E. coli* as well as of *C. perfringens* type A and of *C. perfringens* type D in a separate or a mixed condition was determined.

RESULTS AND DISCUSSION

As shown in Table (1), *E. coli* was recovered from 44.17% of examined buffaloe's milk samples. Nearly similar results were obtained by Saudi (1978). Higher incidence percent (89%) was recorded by Bogdanowicz and Nockiewicz (1973). While lower incidence percent (3.7% & 10%) was recorded by Gupta (1986) and Riad (1988) respectively.

Presence of *E. coli* in milk indicates faecal contamination and reflects the unhygienic conditions of production and handling especially that produced under village conditions (Singh and Ranganthan, 1978), Moreover, it may indicates the presence of clinical or subclinical cases of mastitis in donor animals (Gedek, 1984).

E. coli had been considered to be a potential pathogene for man and domestic animals where it needs time to replicate in food or water (Mehlman et al., 1976; and Gangarosa, 1978). It was recorded in many cases of food poisoning (Matsievskii et al., 1971 and Tullock et al., 1973). In milk, it is implicated as a cause of diarrhoeal

outbreaks around the world which may result from ingestion of large numbers (10^6 - 10^9) of the organism (Mehlman et al., 1967; and Gangarosa, 1978). Also, it is responsible for the death of young children, the discomfort of many vacationers, severe cholera-like syndrome and shigella like illness; (Kornacki and Marth, 1982). Because of the greater likelihood of poor sanitation, these diseases are more common in the developing nations of the world than in more in more developed coumtries (Kornacki and Marth, 1992).

Results presented in Table (1) revealed also that *C. perfringens* could be isolated from 16.67% of the examined samples. Nearly simlar incidence was reported by Moustafa et al. (1975). While, higher findings were obtained by et al. (1975). While, higher findings were obtained by El-Bassiony (1980).

Serological typing of recovered isolates of *C. perfringens* showed that 18 out of 20 (90%) positive samples were contained two toxigenic types of *C. perfringens* (A &D). Type A was present in 45% of positive samples followed by type D (10%). Both types of *C. perfringens* A&D were recovered together from 35% of positive sample (Table 2).

From public health point of view, *C. perfringens* type A is the most important one of clostridia causing food poisoning (willis, 1977). While, type D had been implicated in cases of gastroenteritis (Kohn and Warrack, 1955).

Tables (3 & 4) demonstrated the results of testing of growth of *E. coli* against the toxigenic types of *C. perfringens.* Totally, *E. coli* was able to mask or overcome the growth of studied types of *C. perfringens* from 56-84% on solid media. This result was confirmed experimentally by determination of mean total bacterial count (MTBC)/ml of *E. coli* as well as of *C. perfringens* types A&D in incubated buffaloe's milk inoculated with these bacteria either separate or mixed together. MTBC/ml of *E.coli* in a separate inoculum was 3×10^9 and in mixed inocula ranged from 3×10^9 to 3.1×10^9. MTBC/ml of *C. perfringens* type A in a separate inoculum was 4×10^6 and in mixed inoculum was 2.9×10^6. MTBC. of *C. perfringens* type D in a separate inoculum was 3.8×10^6 and in a mixed inoculum was 2.8×10^6.

The above mentioned results are summarized in that *E. coli* was able to mask the growth of toxigenic types of *C. perfringens.* This is because of the growth rate of *E. coli* was higher than that of *C. perfringens* as recorded from exaerimentally inoculated milk. A short lag phase and rapid logarihmic phase of *E. coli* in contrast to *C. perfringens* are the most accepted explains of this result. Also, we must put in mind the role of motility on spreading of bacteria whereas. *E. coli* is a motile organism and *C. perfringens* is a non-motile (Koneman et al., 1988 and Collee et al., 1989).

E. coli was able to decrease the growth of *C. perfringens* in milk containing both organisms. This result may be due to the production of colicins by *E. coli* which have a role on the

Table (1): Incidence of *E.coli* and *C. perfringens* from buffaloe's milk.

Isolated bacteria	Total number of examined samples	Positive samples	
		Number	Precentage
E. coli	120	53	44.17%
C. perfringens	120	20	16.67%

Table (2):Typing of *C. perfringens* isolates.

Recovered types of *C. perfringens*	Positive samples	
	Number	Precentage
a) Toxigenic types:		
Type A	9	45%
Type D	2	10%
Both types (AgD)	7	35%
Total	18	90%
b) Non toxigenic types:	2	10%
Overall total	20	100%

Table (3) : Effect of *E. coli* on masking the growth of *C. perfringens* types A& D.

Isolates of *E. Coli*	Types of *C. perfringens*				Total ** (25)	
	Type A. (16)		Type D** (9)			
	N_1	P_1	N_1	P_1	N_2	P_2
Isolate No. 1	13	81.25%	8	88.89%	21	84%
Isolate No. 2	12	75.00%	7	77.78%	19	76%
Isolate No. 3	11	68.75%	6	66.67%	17	68%
Isolate No. 4	11	58.75%	5	55.56%	16	64%
Isolate No. 5	9	65.25%	5	55.65%	14	56%

* : Number of studied *C. perfringens* type A isolates was 16.
** : Number of studied *C. perfringens* type D isolates was 9
***: Total number of studied *C. perfringens* isoaltes was 25
N_1: Express the number of masked isolates from each type of *C. perfringens.*

N_2: Express the total number of masked isoaltes *C. perfringens.*
P_1: Express that the precent was calculated according to the number of studied isolates from each type of *C. perfringens.*

P_2: Express that the percent was Calculated according to the total number of studies isolates from each type of *C. perfringens.*

No: Number

Table (4): Mean total bacterial count of *E. coli* and *C. perfringens* inoculated either separate or in a mixed condition in pasteurized buffaloe's milk 24 hours post-incubation.

Bacterial count	*E. Coli*	Separate inocula		Mixed inocula			
		C. perfrin gens Type A.	*C. perfrin gens* Type D.	*E. Coli. & C.perfringens* Type A.		*E. Coli. & C.Perfringens.* Type D.	
				E. Coli.	*C. Perfrin gens.* Type A.	*E. Coli.*	*Perfrin gens.* Type D.
MTBC/ml.	$3x10^9$	$4x10^6$	$3.8x10^6$	$3x10^9$	$2.9x10^6$	$3.1x10^9$	$2.8x10^6$

MTBC/ml. Mean total bacterial count per ml. of inoculated milk.

inhibition of growth of other bacteria. Production of colicins from *E. coli* was recorded by Mokhamed and Kozarov (1985), Djqnne (1986), Ayhan and Aydin (1989), and Cong et al. (1992).

In this paper, our results include the following conclusions;

1- Improperly handled raw milk provides a good medium for transmission of pathogenic organisms to consumer and may at times induces food poisoning. To safegurad consumers, strict hygienic measures and suitable regulations should be imposed for production, handling and distribution of raw buffaloe's milk.

2- The usual media used for isolation of *C. perfringens* from milk should be developed. This development include the addition of inhibitor substances to other bacteria and activators to *C. perfringens* on both enriched liquid media and solid media to become sepecific for isolation of *C. perfringens*.

3- Further studies should be made on the use of colicins as milk additives to inhibit bacterial growth and their public health significance.

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