Dairy Cows as a Possible Reservoir of Enteropathogenic and Enterohemorrhagic *Escherichia Coli*

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ABSTRACT

Enteropathogenic (EP) and enterohemorrhagic (EH) *Escherichia coli* (EC) were studied in raw milk and fecal shedding of apparently healthy dairy cattle. A total of 120 samples from raw milk and fecal samples (60 samples of each) were collected. The bacteriological examination of the collected samples revealed that EPEC was isolated from 5 raw milk samples (8.3%) and 12 fecal samples (20%). While EHEC was identified in 3 (5%) and 7 (11.6%) in raw milk and fecal samples respectively. Serological investigation revealed that EPEC isolated from raw milk and fecal samples were serotyped as O_{26}, O_{55}, O_{111}, O_{119}, O_{127} and O_{128}. While 7 (70%) EHEC isolated from raw milk and fecal samples were belonged to O_{157}: H_7 serotype. Presence of the same serotypes of EPEC and EHEC in raw milk and fecal samples is an indicative for contamination of raw milk with bovine feces. The public health importance of those microorganisms and suggested control measures to prevent contamination were discussed.

INTRODUCTION

Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhea in infants and clinically is characterized by fever, malaise, vomiting and diarrhea with prominent amounts of mucus but without gross blood *(1)*. EPEC strains O_{111} : H_2 and O_{55} : H_6 are the major classic serotypes in Brazil and are the most important serotypes world wide *(2)*.

Enterohemorrhagic *E. coli* (EHEC) is the cause of hemorrhagic colitis and hemolytic uremic syndrome and associated with clinical syndromes, including mild to severe bloody diarrhea and hemolytic uremic syndrome, and it can result in death *(3)*.

Approximately half of EHEC strains are serotype O_{157} : H_7 *(4)*. Serotype O_{157} : H_7 was responsible for the food borne outbreak that occurred in Japan *(5)*. Outbreaks are associated with the consumption of unpasteurized milk or undercooked ground beef *(6)*.

Cattle are considered to be the major reservoir of the EHEC O_{157}: H_7 and contaminated foods of bovine origin are important vehicle of human infection *(7)*. Milk and milk products contaminated with animal manure have been identified as sources of human infection with the organism *(8)*.

This study was aimed to spotlight on the possible role of dairy cows in transmission of EPEC and EHEC from the zoonotic point of view.

MATERIAL AND METHODS

Sampling

In the present study samples were randomly collected from cows of dairy farms in different localities in Kaliobia governorate. A total of 120 samples of raw milk and fecal samples (60 samples of each) from apparently healthy dairy cows were collected under aseptic condition. The collected samples were labeled and kept in containers with ice packs and transferred to the laboratory without delay. Sampling technique was performed as previously described *(9)*.

Isolation and Identification of *E. coli*

Isolation and Identification of EPEC and EHEC was performed as recorded previously *(10)*. Sample were dipped into test tubes containing 10ml of sterile phosphate buffer saline as a pre-enrichment broth and then incubated for 6 hours at 37°C. Two enteric enrichment broth were inoculated with 1ml of

pre-enrichment broth one for isolation of EPEC and other for EHEC.

Isolation and identification of enteropathogenic *E. coli*

Aliquot of 1ml of pre-enrichment broth were inoculated into MacConkey broth tube contained inverted Durham's tube and incubated at 37°C for 48 hours. Tubes showing acid and gas were considered positive. A loopful from each positive MacConkey broth tube was surface plated on Eosin-Methylene blue (EMB) agar medium and incubated at 37°C for 48hours. Colonies of typical growth, greenish metallic with dark purple center, were picked and streaked onto nutrient agar slants for further biochemical identification ***(11)***.

Isolation and identification of enterohemorrhagic *E. coli*

About 1ml of pre- enrichment broth was dipped into EHEC enrichment broth with 0.02gm/ml novobiocin (Oxoid) and incubated overnight with gentle agitation at 37°C. A loopful of each enriched broth culture was streaked onto Sorbitol- MacConkey (SMAC) agar with 0.05mg/ml cefixime (Oxoid) and incubated at 37°C overnight. Red or pink coloured colonies on SMAC plates were regarded as sorbitol fermenting and colorless colonies as sorbitol non-fermenting. Suspected colonies were picked up and confirmed by biochemical tests ***(12)***.

Serotyping of isolates

Isolates were submitted to slide agglutination tests ***(13)*** using polyvalent and monovalent sera against EPEC serogroup O_{26}, O_{55}, O_{86a}, O_{111}, O_{119}, O_{127} and O_{128}. All sorbitol non-fermenting growth colonies were tested with antisera against EHEC serogroup O_{157} by slide agglutination while H antigen of motile isolates was determined by tube agglutination test ***(14)*** using antisera H_7 commercially available from Denka Seiken Co, LTD, Japan.

RESULTS AND DISCUSSION

A total of 120 samples from raw milk and fecal samples of apparently healthy dairy cows were screened bacteriologically to detect EPEC and EHEC (Table 1). The results proved that EPEC was identified in 5 samples of raw milk (8.3%) and 12 fecal samples (20%). Our results are lower than that isolated from raw milk ***(15)***.

Sorbitol non-fermenting EHEC was isolated from 3 raw milk samples (5%) and 7 fecal samples (11.6%). Our findings were lower than those identified in raw milk ***(16)*** and fecal samples ***(17)*** respectively. While were higher than those ***(8,18)*** in raw milk and fecal samples respectively.

Table 2 pointed out that serotyping of EPEC isolated from raw milk and fecal samples were belonged to O_{26} , O_{55} , O_{111} , O_{119} , O_{127} and O_{128} serotypes. A finding was similar to that recorded in Kenya ***(19)***.

Table 3 declared that 7 (70%) sorbitol non-fermenting EHEC isolated from raw milk and fecal samples were $O_{157} : H_7$ serotype.

Similarly it has been reported that $O_{157} : H_7$ the most common serogroup, was isolated from healthy cattle ***(20)***. Despite the disease causing by $O_{157} : H_7$ in human, the organism does not appear to be the cause of the disease in cattle. Therefore, healthy cattle harboring *E. coli* $O_{157} : H_7$ may enter the food chain ***(21)***. Therefore, to ensure the safety of milk and milk products, Hazard Analysis and Critical Control Point (HACCP) programs must be instituted in the dairy processing plants. These programs involve identification of critical point for contamination or transmission of pathogens and the establishment of control programs of these critical point to decrease risk of pathogens entering human food chain.

In conclusion the present study showed that the same serotypes of EPEC and EHEC could be recovered from raw milk and fecal samples of apparently healthy dairy cows that might be incriminated as possible reservoir of human *E. coli* infection.

Table 1. Occurrence of EPEC and EHEC isolated from dairy cows

Samples	Number	EPEC		EHEC	
		Number	%	Number	%
Raw milk	60	5	8.3	3	5
Fecal samples	60	12	20	7	11.6
Total	120	17	14.1	10	8.3

Table 2. Occurrence of EPEC serotypes isolated from dairy cattle

EPEC serotypes	Raw milk samples		Fecal samples	
	Number	%	Number	%
O_{26}	-	-	1	8.3
O_{86a}	-	-	-	-
O_{55}	-	-	1	8.3
O_{111}	1	20	2	16.6
O_{119}	2	40	3	25
O_{127}	1	20	2	16.6
O_{128}	1	20	3	25
Total	5	100	12	100

Table 3. Occurrence of EHEC O_{157} : H_7 serotype isolated from dairy cows

Samples	Examined isolates	EHEC O_{157} : H_7 serotype	
		Number	%
Raw milk	3	2	66.6
Fecal samples	7	5	71.4
Total	10	7	70

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الملخص العربي

الماشية الحلابة كمستودع ممكن للأيشريشيا كولاى الباثولوجية والمنزفة للدم المعوي

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أجريت هذه الدراسة للكشف عن ميكروب الايشريشيا كولاى الباثولوجية والمنزفة للدم المعوى في عينات اللبن وبراز الماشية الحلابة السليمة ظاهريا . وقد تم جمع عدد ١٢٠ عينة من اللبن الخام وبراز الماشية وأجريت التجارب المعملية اللازمة لعزل الميكروب وتصنيفه وكانت نسبة عزل الإيشريشيا كولاى الباثولوجية ٥ (٨,٣%) ، ١٢ (٢٠%) في كل من عينات اللبن الخام وبراز الماشية . بينما أسفرت النتائج عن عزل الإيشريشيا كولاى المنزفة للدم في عينات اللبن الخام وبراز الماشية بنسبة ٣(٥%) ، ٧(١١,٦%) علي التوالى. النتائج الإيجابية للفحص السيرولوجي للعترات المعزولة من كل من اللبن الخام وبراز الماشية أسفرت عن تصنيف الإيشريشيا كولاى الباثولوجية إلي عترات O_{26} و O_{55} و O_{111} و O_{119}و O_{127}. بينما ٧(٧٠%) من الإيشريشيا كولاى المنزفة للدم تم تصنيفها إلى عترة H_7 : O_{157} . هذا ويغير وجود نفس عترات ميكروب الإيشريشيا كولاى الباثولوجية والمنزفة للدم في عينات اللبن الخام وبراز الماشية مؤشر لتلوث هذا اللبن ببراز الماشية وتم مناقشة الأهمية الصحية للميكروب والطرق المقترحة لتفادي التلوث به .