

DETECTION OF SOME PATHOGENIC BACTERIA IN RAW FARM MILK

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ABSTRACT

The objective of this study was performed to detect the incidence of Escherichia coli, Salmonella, Yersinia enterocolitica in raw farm milk samples. A total of 50 raw milk samples were collected from different dairy farms in Kafrelsheikh Governorate. The isolates were identified biochemically and serologically. The obtained results revealed that E. coli, Salmonella and Yersinia enterocolitica were detected in 32%, 10% and 18%, respectively. Additionally 50% of recovered E. coli were entero-hemorrhagic (O₂₆:H₁₁ and O₁₁₁:H₄), 12.5% were enteropathogenic (O₁₁₄:H₂₁) and 37.5% were entero-toxigenic (O₁₂₅:H₂₁; O₁₂₇:H₆). The results also exhibit species of isolated salmonellae were S. typhimurium (40%), S. enteritidis were 60%. The public health significance of isolated strains as well as suggested control measures were discussed to obtain high quality milk and healthy for human being.

Keywords: Farm milk, *E. coli*, *Salmonella*, *Y. enterocolitica*.

INTRODUCTION

Milk ranks high among other foods and is considered as the most perfect food for human from birth to senility as it is not only has good sensory properties and all nutrients required for the body for rapid growth but also could prevent or reduce risks of many nutritional deficiency diseases (*Kalkwarf et al., 2003 and Marshall et al., 2003*).

Raw milk is still used by large number of farm families and workers and by a growing segment of the general population who believe that the milk is not only safe but also imparts beneficial health effects that are destroyed by pasteurization (*Lejeune and Rajala-Schultz, 2009*).

Milk and products derived from milk can harbor a variety of microorganisms and can be important sources of food borne pathogens. The presence of food borne pathogens in milk may be due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. The bacteria can access to the milk through colonization of the teat canal or an infected udder (clinical and sub clinical mastitis) or gets contaminated from milk utensils and water supply used (*Gruetzmacher and Bradle, 1999 and Hayes et al., 2001*).

The presence of bacteria in milk has many undesirable effects on the quality and safety of milk and its products (*Szteyn et al., 2005*) and the milk becomes unsuitable for further processing (*Nanu et al., 2007*). Raw milk is usually colonized by a variety of pathogens such as Enterohaemorrhagic *E.coli*, *Staph. aureus*, *S. typhimurium* and *Y. enterocolitica*, which considered as a public health hazard (*Marco and Wells-Bennik, 2008*).

E.coli is a normal inhabitant of intestine of animals and humans but its recovery from food may be of public health concern due to the possible presence of entero-pathogenic and/or toxigenic strains which lead to sever gastro-intestinal disturbance (*Soomro et al., 2002*). *E.coli* is among many pathogenic microorganisms which can access to milk and some of dairy products (*Quinn et al., 1994*) which considered a reliable indicator of contamination by manure, soil and contaminated water (*Todar, 2008*).

Milk and milk products have been identified as the vehicle for transmission in approximately 5% of salmonellosis cases (*CDC, 2000*). Contamination of raw milk with *Salmonella* spp is mostly due to infected persons and contamination of the environment, since natural infections of the udder are rare and seldom contribute to human food poisoning (*Mubarak et al., 2010*).

Y. enterocolitica is rapidly worldwide as enteric pathogen and has become major cause of diarrhea. Since the natural reservoir of *Y. enterocolitica* infection was latent infection in the intestinal tract of wild and domestic animals. The main source of contamination of raw milk with *Y. enterocolitica* was not mammary gland but probably feces (*Quinn et al., 1994*).

Therefore this study was carried out to determine the prevalence of some pathogenic bacteria spread by contamination of raw milk. The public health significance of the isolated microorganisms was discussed and suggests control and preventive measures.

MATERIALS AND METHODS

Collection of samples:

A total of 50 raw farm milk samples were collected randomly from different dairy farms in Kafrelsheikh Governorate, Egypt. Milk samples were transferred directly to the laboratory with a minimum of delay, where they prepared for examination.

Isolation of *Escherichia coli* according to (*Atlas et al., 1995*).

Isolation of *Salmonella* as recommended by (*ISO 6579: 2002*).

Isolation of *Yersinia enterocolitica* according to (*Schieman, 1982*) and (*Walker and Glimour, 1986*).

RESULTS

Table (1): Incidence of isolated pathogens from examined raw farm milk samples:

Pathogen	No. of examined samples	Positive samples	
		No.	%
<i>Escherichia coli</i>	50	16	32
<i>Salmonella spp</i>	50	5	10
<i>Yersinia enterocolitica</i>	50	9	18

Table (2): Serological identification of *E.coli* isolates:

Strain characteristics	Positive samples		<i>E.coli</i> strains	No.	% from the +ve samples
	No.	% from the +ve samples			
EHEC	8	50	O ₂₆ :H ₁₁	4	50
			O ₁₁₁ :H ₄	4	50
EPEC	2	12.5	O ₁₁₄ :H ₂₁	2	100
ETEC	6	37.5	O ₁₂₅ :H ₂₁	3	50
			O ₁₂₇ :H ₆	3	50

Table (3): Serological identification of *Salmonellae* isolates:

Type of isolates	Positive samples		Antigenic structure	
	No	% from +ve samples	O	H
<i>S.Typhimurium</i>	2	40	1,4,5,12	i:1,2
<i>S.Enteritidis</i>	3	60	1,9,12	g,m:1,7

DISCUSSION

Raw milk is considered a high risk food as it is highly nutritious and serves as an ideal medium for bacterial growth. Several factors contribute to milk contamination such as poor hygienic milking conditions, contaminated equipment, milking utensils and poor personal hygiene.

Pathogenic bacteria in milk have been a major factor for public health concern since the early days of the dairy industry. Many diseases are transmissible via milk products. Traditionally raw or unpasteurized milk has been a major vehicle for transmission of pathogens (*Vasavada, 1988*). Many microorganisms can get access to milk, among these, *E.coli* which is often used as a marker organism. Recovery and counting of *E.coli* is used a reliable indicator of fecal contamination and possible presence of entero-pathogenic and /or toxigenic microorganisms which constitute a public health hazard. Most of *E.coli* are harmless, but some known as pathogenic bacteria causing severe intestinal and extra intestinal diseases in human (*James et al., 2004*).

The results given in table (1) showed that *E.coli* was detected In 16 (32%) of the examined farm milk samples. Our findings were nearly similar to those obtained by *Abbas et al. (2013)* while lower incidence results were reported by *Mayada and Fatma (2013) and Merwad et al. (2014)*.

Our results disagreed with microbiological standards for milk (*FAO/WHO, codex standard, 2000*) in EU and US who stated that raw milk should be negative for *E.coli*.

Table (2) showed the serological identification of isolated *E.coli* from raw farm milk samples belonged to serovars: O₂₆: H₁₁ and O₁₁₁:H₄ (EHEC) were 4 (50) % for each; O₁₁₄: H₂₁ (EPEC) was 2 (100%) and O₁₂₅:H₂₁ and O₁₂₇:H₆ (ETEC) were 3 (50%) for each.

Serotypes like O₂₆ has been found to be associated with infantile diarrhea among neonates and adult human patients suffering from gastroenteritis as reported by (*Nishikawa et al., 2002*). Thus these serotypes may be of zoonotic importance.

The public health importance of isolated Entero-pathogenic serovars had been attributed to its enterotoxin, which is implicated in causing gastro enteritis, epidemic children diarrhea, and sporadic diarrhea in children as well as food poisoning (*Hassan and Afify, 2007*), the recurrent outbreaks of life threatening human infections were attributed to STEC / EPEC contaminate milk and milk products (*Martin and Beutin, 2011*).

A high percentage of human salmonellosis occurs through the consumption of raw milk or dairy products manufactured with raw milk (*CDC, 2003*).

The results achieved in table (1) revealed that incidence of salmonella in the examined farm raw milk samples was 5 (10%). lower findings were obtained by *Hassan et al. (2000)* and *Tajbakhsh et al. (2012)*. On the other hand *Ekici et al. (2004)*, *Mhone et al. (2012)* and *Zeinhom and Gihan (2014)* failed to detect any salmonella from examined raw milk samples.

Our results were disagreed with Egyptian standard of raw milk (*Es, 154/1/2005*) which stated that raw milk should be free from salmonella spp. and microbiological standards for milk (*FAO/WHO, codex standard, 2000*) in EU and US stated that raw milk should be negative for salmonella spp.

Salmonellosis is one of the most important zoonotic bacterial pathogen of food-borne infection all over the world. The most important serotypes of salmonella are *S. typhimurium* and *S. enteritidis* (*Fashae et al., 2010 and Hendriksen et al., 2011*). Salmonella spp. can cause gastrointestinal disease. The main sources of transmission are water, eggs and raw foods (*Karns et al., 2005*).

The results given in table (3) show the incidence of salmonella serotypes in the examined samples were *S. typhimurium* 2 (40%) and *S. enteritidis* 3 (60%). It is noticed that *S. enteritidis* was the most prevalent isolated salmonella serotypes from examined raw milk samples, followed by *S. typhimurium* and this gradual prevalence reported by (*Niaid, 2000*).

Table (3) revealed that the antigenic structure of isolated salmonellae from the examined milk samples were *S. typhimurium* (O strain,1.4.5.12), (H strain,i:1,2); *S. enteritidis* (O strain 1,9,12), (H strain g,m: 1,7).

Y. enterocolitica is the most common agent of genus Yersinia that is associated with a spectrum of clinical syndromes in man, with gastroenteritis as the most frequently encountered manifestation. Most cases are sporadic or occur in small clusters, but large outbreaks have been reported worldwide in families, schools, hospitals, and in association with community gatherings (*Leclercq et al., 2005*).

Table (1) revealed that the incidence of *Y.enterocolitica* in examined raw farm milk samples was 9 (18%). Lower incidence was reported by *Hanifian and Khani (2012)* and *Sarah (2014)* while higher results reported by *Bernardino et al. (2013)*. On contrast *Zeinhom and Gihan (2014)* failed to detect *Y.enterocolitica* in examined milk samples.

The previously our obtained results were disagreed with Egyptian standards of raw cows milk (*ES, 154 /1/2005*) that stated that raw milk should be free from *Y.enterocolitica* and microbiological standards for milk (*FAO/WHO, codex-standard, 2000*) in EU and US that stated that raw milk should be negative for *Y.enterocolitica*.

In other words, in regions in which dairy herds have been bred in small traditional farms rather than industrial ones and been milked by hand rather than by milking machine, the occurrence of *Y.enterocolitica* was higher. Probably milk could get contaminated with animal feces during the milking process in which contamination rate depend on hygienic practices prior and during milking (*Hanifian and Khani, 2012*).

Fredriksson and Korkeala (2003) stated that *Y.enterocolitica* has been isolated most frequently in temperate areas of the world and the majority of cases being reported from cooler regions of Europe and North America .The incidence of *Y.enterocolitica* organisms were much higher during winter than summer seasons (*Toora et al., 1989*).

Conclusion and Recommendations:

The results obtained in our study showed that presence of pathogenic bacteria such as *E.coli*, *Salmonella* spp. and *Y. enterocolitica* in milk, this suggests that raw milk should be considered as a vehicle for transmission of potentially pathogenic bacteria. Since a lot of people still

drink raw milk especially in rural areas, this emphasizes the need for educational efforts to improve dairy farmers' awareness of milk-borne zoonoses, how these pathogens are transmitted to milk, risk factors associated with milk-borne pathogens and how to obtain fresh clean milk.

For achieving good quality milk produced within the dairy farm, some hygienic steps should be taken in consideration such as application of some hygienic practices during milking routine such as cleanliness of animal's udder and teats and diseased animals should be isolated. Cleanliness of milker's hands and also he should wear clean clothes and head cap were very efficient in improving milk quality and control of zoonotic diseases. Also diseased persons must not come in contact with animals.

All equipment that come in contact with milk should be efficiently clean and sterile. The use of refrigeration during processing, storage and handling of the milk and dairy products are also essential.

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الكشف عن بعض مسببات الأمراض البكتيرية فى حليب المزارع الخام

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أجريت هذه الدراسة لتحديد مدى انتشار بعض مسببات الامراض البكتيرية فى حليب المزارع الخام وتشمل كل من ميكروب الايشيريشيا كولاى، السالمونيلا واليارسينيا انتيروكوليتيكا وتم تجميع العينات (50 عينة) من مزارع مختلفة فى محافظة كفرالشيخ وقد أسفرت هذه الدراسة عن تواجد الميكروبات الآتية وهى الايشيريشيا كولاى، السالمونيلا واليارسينيا انتيروكوليتيكا بنسبة 32%، 10% و 18% على التوالي. وباستخدام التصنيف السيرولوجى تم تحديد انواع ميكروب الايشيريشيا كولاى المعزولة كالاتى:

EPEC (O₁₁₄:H₂₁); ETEC (O₁₂₅:H₂₁andO₁₂₇:H₆); EHEC (O₂₆:H₁₁andO₁₁₁:H₄) بنسبة 50%، 12.5% و 37.5% على التوالي. (S. Enteritidis) و 60% (S. Typhimurium) وبالنسبة لميكروب السالمونيلا تم تصنيفها الى 40% .

وتم مناقشة الاهمية الصحية للميكروبات المعزولة حيث انها تمثل خطورة على الصحة العامة وقد اظهرت النتائج الحاجة الى السيطرة على هذه الميكروبات والحد من انتشارها فى الحليب الخام للحفاظ على صحة المستهلك.