Prevalence of some public health important pathogens in raw milk and kareish cheese

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Abstract

A total of 100 random samples comprising of 50 samples each of raw milk and raw-milk cheese (Kareish cheese) were collected from different dairy shops, supermarkets and street vendors in Giza and Kalubia governorates. All samples were screened for the presence of *Salmonella spp.*, *Listeria monocytogen*es and *Entrohaemorrhagic Escherchia coli* serotype O157: H7 .*Salmonella spp*. were detected in 1 (2%), 2 (4%) of raw milk and Kareish cheese, respectively. While, *Listeria monocytogen*es could be isolated in 4 (8%), 3 (6%) of raw milk and Kareish cheese, respectively. Whereas, *Entrohaemorrhagic Escherchia coli* serotype O157: H7 could be recovered from 3 (6%) of raw milk samples, but could not be isolated from any of examined Kareish cheese samples. PCR assay was used for identification of isolated *Entrohaemorrhagic Escherchia coli* serotype O157: H7. The public health implications of the isolated strains, as well as suggested control measures were discussed.

Key words: *Salmonella spp. –Listeria monocytogenes- Entrohaemorrhagic Escherchia coli serotype O157: H7:raw milk-kareish cheese –Giza and Kalubia Governorates.*

Introduction

Milk and dairy products are important components of the human nutritious and healthy diet .They provide excellent dietary source of protein, vitamins and other minerals **(Huth *et al.,* 2006).** In addition, inclusion of these products in the diet aids in the prevention of certain diseases such as obesity ,hypertension and diabetes. They are also a good source of Calcium-important for growing teeth and bones ,helping prevention of osteoporosis **(McCarron and Heany, 2004).**

However, raw milk and raw milk dairy products can also pose a potential serious public health risks due to possible contamination with many food borne pathogens **(LeJeune and Rajala, 2009 , Oliver *et al.,* 2009, Brooks *et al.,* 2012 and FDA,2013)**.These pathogenic bacteria can originate directly from dairy animals farm, environment particularly contaminated water sources, utensils used for collection, storage and transportation of milk. Whereas in cheese contaminated via raw milk and workers during handling ,processing as well **(Murinda *et al.,* 2004; Oliver *et al.,* 2005; Kousta *et al.,* 2010, D'Amico and Donnelly, 2010)**. Moreover, raw milk and raw milk cheese has been recognized as vehicles for the transmission of numerous bacterial pathogens of both human and animal origin **(Lind *et al.,* 2007; Baylis, 2009 and Torres-Vitela *et al.,* 2012).** Among these pathogens *Salmonellosis*, *Listeriosis* and *Entrohaemorrhagic Escherchia coli* serotype O157: H7,become recently recognized as a significant causes of human morbidity and mortality. Since 2005, Several outbreaks of these pathogens have been reported (**Dominquez *et al.,* 2009; CDC, 2010; Fretz *et al.,* 2010; CDC, 2011; Gaulin *et al.,* 2012).**

Salmonella spp. has been known to cause illness for over 100 years. They remain the most important food borne pathogens and reported

causes of food poisoning outbreaks in both developing and developed countries. Infection of humans can be acquired through the consumption of foods contaminated with animal feces (**Kennedy *et al.,* 2004; Karns *et al.,* 2005 and Alexander *et al.,* 2009).** Several surveys have detected Salmonella in raw milk and raw milk cheese **(Van-kessel *et al.,* 2004 ; Jayarao *et al.,* 2006; Kinde *et al.,* 2007; Helba *et al.,* 2011 and Van-kessel *et al.,* 2011).** Salmonella spp. are a leading cause of acute gastroenteritis in humans and Salmonellosis remains an important public health problem worldwide.. Additionally, raw milk and raw milk cheese have been implicated in a recent massive outbreaks of human salmonellosis (**CDC, 2003, CDC, 2008, Pastore *et al.,* 2008 and Silva *et al.,* 2012).**

Listeria monocytogenes has been recognized for many years as a potentially lethal food borne pathogen that causes a sever and often fatal illness in human called Listeriosis **(CDC, 2013 and Scallan *et al.,* 2011)**. Gastrointestinal symptoms may precede more serious forms of Listeriosis or may be the only symptoms expressed **(FDA, 2012 and Cartwright *et al.,* 2013)**. *Listeria monocytogenes* is a food borne pathogen, consumption of food contaminated with *L.monocytogenes* is the primary route of transmission in human **(Rossi *et al.,* 2008 and Todar, 2009).** Dairy products currently have a strong association with Listeriosis than other types of food**.(Kasalica *et al.,* 2011)**. Listeriosis outbreak have mostly been linked to consumption of raw milk or soft cheese made of raw milk **(MacDonald *et al.,* 2005; Bille *et al.,* 2006; Yilmaz *et al.,* 2009 and CDC, 2 012).**

Enterohaemorrhagic E.coli (EHEC) serotype O157:H7 is most frequently implicated in human disease and most commonly associated with large outbreaks**.(Rangle *et al.,* 2005; FDA, 2012).** *Enterohaemorrhagic E.coli (EHEC)* produces large quantities of one or more potent toxins (Shiga-like toxin) closely related or identical to the toxins produced by Shigella dysenteriae type 1.Shiga toxins, the main virulence factor contributing to pathogen city consists of two major types Stx1 and Stx2**. (Scheutz and Strockbin, 2005)**.

Most human infections caused by the consumption of contaminated food especially those of bovine origin such as raw cow's milk and raw –milk cheese**.(Bindus Kiranmayi *et al.,* 2010**).Infection with *Enterohaemorrhagic E.coliO157:H7* can cause hemorrhagic colitis (HC) with bloody diarrhea which may develop to a serious and life-threatening complication know as hemolytic uremic syndrome(HUS) which may result in hemolytic anemia and renal failure **(Karch *et al.,* 2005; WHO, 2011).**

Food borne diseases are important and growing public health and economic problems. The risk of food borne diseases has increased over the last 20 years. These diseases impose a substantial burden on health care systems and markedly reduce economic productivity. Therefore, the present study attempts to determine the prevalence of *Salmonella spp*.,*Listeria monocytogenes* and *Enterohaemorrhagic E.coli O157:H7* in raw milk and raw milk cheese (kareish cheese) to estimate the risk for public health from the consumption of these food products and how to ensure dairy products safety.

Material and Methods

Sampling procedure

A total of 100 random samples comprising of 50 samples each of raw milk and raw milk cheese (Kareish cheese) were collected in sterile plastic bags from different dairy shops, supermarkets and street vendors in Giza and Kalubia governorates. Random sampling was done to ensure representative sampling. Collected samples were packed into an ice box and transported immediately to the laboratory under refrigeration and kept at 4ºC for not more than 24 hour before examination. Samples were analyzed for the prevalence of selected bacterial pathogens; *Salmonella* spp., *Listeria monocytogenes* and *Enterohaemorrhagic E.coli O157:H7*.

Microbiological analysis:

Isolation and identification of *Salmonella* spp. was carried out according to the **International Standard Organization "ISO" (2002).**

Isolation and identification of *Listeria monocytogenes and Enterohaemorrhagic E.coli O157:H7* were performed as suggested by **Food and Drug Administration "FDA/ BAM" (2011).**

Detection of *Enterohaemorrhagic E.coli O157:H7* and virulence gene by PCR:

Samples preparation for PCR assay as described by **Jothikumar and Griffiths (2002).** Primer design and PCR assay were applied as mentioned by **Paton and Paton (1988).**

Results

Table (1). Prevalence of *Salmonella* spp. from raw milk and Kareish cheese

Samples	No. of examined samples	Positive samples	
		No.	%
Raw milk	50	1	2
Kareish cheese	50	2	4

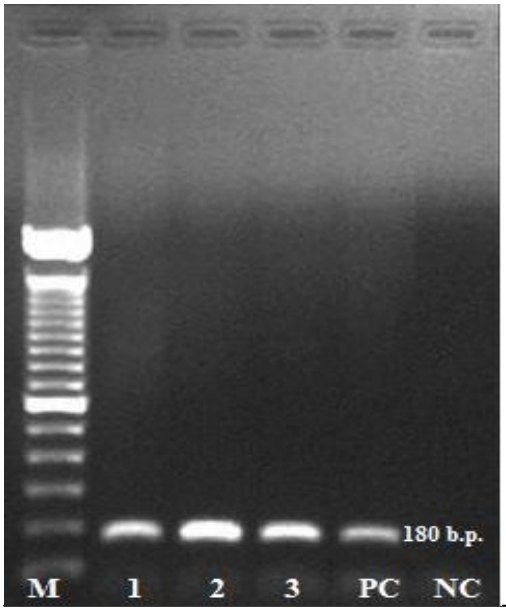
Table (2). Prevalence of *Listeria monocytogenes* from raw milk and Kareish cheese

Samples	No. of examined samples	Positive samples	
		No.	%
Raw milk	50	4	8
Kareish cheese	50	3	6

Table (3). Prevalence of *Enterohaemorrhagic E.coliO157:H7* from raw milk and Kareish cheese

Samples	No. of examined samples	Positive samples	
		No.	%
Raw milk	50	3	6
Kareish cheese	50	N.D	0

N.D: Not detected



M: Marker
Lane 1-3: Positive raw milk samples
Lane PC: Positive Control
Lane NC: Negative Control.
Photo (1): Electrophoretic pattern of *Enterohaemorrhagic E.coliO157:H7*

Discussion

In the present study, a total of 100 random samples comprising of 50 samples each of raw milk and raw-milk cheese(Kareish cheese) were analyzed for the prevalence of selected bacterial pathogens; *Salmonella* spp., *Listeria monocytogenes* and *Enterohaemorrhagic E.coliO157:H7*

Results in table (1) revealed that, *Sallmonella spp.* could be isolated from 1 (2%) and 2 (4%) of analyzed raw milk and kareish cheese samples, respectively.

Previous studies have shown a wide range of estimates for the prevalence of *Salmonella spp*. in raw milk and raw-milk cheese (Kariesh cheese). Regarding *Salmonella spp*. in raw milk, nearly similar results were reported by **Van-kessel *et al.*, (2004) and Karns *et al.*, (2005)** found that the prevalence of *Salmonella spp*. was 2.6 and 2.6% of analyzed raw milk samples, respectively**.** Moreover, our present results were in agreement with that stated by **Ebtesam *et al.*, (2008)** and **Chao *et al.*, (2007)** ***Salmonella spp.*** could be isolated from 2.5 and 1.9% of examined raw milk samples, respectively. However, lower prevalence of *Salmonella* spp. in raw milk samples was detected by **Chye *et al.*, (2004)** and (**Moshtagi and Mohamadpour, 2007)** showed that an incidence of 1.4 and 1.6% of *Salmonella spp*. recovered from tested raw milk samples. In the contrary to this result, **Jayarao *et al.*, (2006)** and **Tajbakhsh *et al.*, (2012)** recorded higher incidence 6 and 4% , respectively of *Salmonella* spp in raw milk samples. On the other hand **D' Amico and Donnely (2010); Hill *et al.*, (2012**) failed to detect *any Salmonella spp*. in examined raw milk samples.

The likely source of *Salmonella spp.* in the raw milk is fecal contamination .Dairy cattle are natural reservoir of this organism and often shed in the feces of animals . Additionally , the environment of the dairy farm is conductive to contamination particularly contaminated water sources , utensils used for collection , storage and transportation of milk (**Fossler *et al.*, 2004 and Oliver *et al.*, 2005**).

Kareish Cheese is one of the most ancient Egyptian soft, white pickled cheese,, which manufactured by traditional methods. It is made from cow or buffalo skimmed milk or from mixtures of these milks using 5-7% salt. It is typically manufactured in small dairy farms under unsatisfactory hygienic conditions .In recent years, Kareish cheese has gained more popularity among Egyptian consumers due to its low fat content **El-Sayed *et al.*, (2011**).

The obtained results of *Salmonella spp*. in Kareish cheese were in accordance with that reported by **Abd El-Atty and Meshref (2007)** detected *Salmonella spp***.** in 4% of Kareish cheese**.** Also these results were in good harmony with that obtained by **El-Kosi (2001); Ebtesam *et al.,(*2008) and Mahmoodi(2010)** they found 3.3% of analyzed raw-milk cheese positive to *Salmonella spp*. Our results is higher than those mentioned by **Colak *et al.*,(2007) and Kahraman *et al.*, (2010)** isolated *Salmonella spp.* in an incidence of 2.4 and 1.9% from tested raw-milk cheese , respectively.. In contrast to our recorded results, significantly higher prevalence of *Salmonella spp*. was detected in raw- milk kareish cheese by **Kinde *et al.*, (2007)** recorded an incidence of 13%. However, **El-sayed *et al.*, (2011) and Brooks *et al.*, (2012)** reported that *Salmonella spp*. Was not recovered from any of the examined raw-milk kareish cheese samples.

Presence of *Salmonella spp*. in kareish cheese could be attributed to contaminated raw milk used in the manufacture of cheese. Moreover, contamination of cheese by *Salmonella spp*. may occur during handling and processing at any stage and might be the workers as well (**Kousta *et al.*, 2010**). **Egyptian standard (ES), (2005)** set zero tolerance for *Salmonella spp.* in both raw milk and soft cheese.

Most human infections of Listeriosis are foodborne primarily associated with the consumption of contaminated raw milk and raw-milk dairy products **(Kells and Gilmour, (2004).** Dairy products are associated with almost half of the cases of listerosis reported in Europe, mainly with consumption of raw milk or products made from raw milk **Jemmi and Steph-**

an, (2006).

Results achieved in table (2) showed that, the prevalence of *Listeria monocytogenes* in raw milk 4(8%) were nearly in agreement with those found by **(Abbas and Jaber, 2012)** ; **Van-Kessel *et al.,* (2011).** They recorded isolation of *Listeria monoctogenes* in an incidence of 7.3 and7.1% from the analyzed raw milk samples. Also, nearly similar results reported by **Jayarao and Henning (2001)** recovered *Listeria monocytogenes* from 6.1%of tested raw milk samples

While lower prevalence of *Listeria monocytogenes* in raw milk were reported by **Jayorao *et al.,* (2006)** who mentioned isolation of *Listeria monoctogenes* from raw milk in an incidence of (2.8%). Also, **Navratilova *et al.,* (2004)** could isolate 15 samples of raw milk contaminated with *Listeria monocytogenes* in an incidence (2.1%) and **Mahmoodi (2010)** who reported that the prevalence of *Listeria monocytogenes* in raw milk samples was 3.3% .

In comparison, **Luljeta *et al.,* (2009)** reported significantly higher incidence rate of *Listeria monocytogenes* contamination in raw milk samples 22%. Whereas, **Vanegas *et al.,* (2008)** could isolate *Listeria monocytogenes* from raw milk samples in an incidence of 16.1%.

However, *Listeria monoctogenes* could not be detected from the examined raw milk samples by **D' Amico and Donnely (2010); Sobrinho *et al.,* (2012).**

Concerning *Listeria monoctogenes* in Kareish cheese (table 2) demonstrated that *Listeria monoctogenes* could be isolated fom 3(6%) of analyzed cheese samples. **Torres-Vitela *et al.,* (2012)** recorded same results , isolated *Listeria monoctogenes* from kareish cheese in an incidence of 6%. Moreover, (**Rudolf and scherer., 2001) and Mahmoodi *et al.,* (2010)** reported nearly similar results, Listeria monocytogenes was recovered from 6.4 and 6.7% of examined cheese samples.

On the other hand, lower prevalence were detected by **Kinde *et al.,* (2007) and Mena *et al.,* (2004)** found that incidence of *Listeria monoctogenes* was 2 and 4% of tested cheese samples. In the contrary, our results were significantly lower than those stated by **El-Sayed *et al.,* (2011) and Baracelli *et al.,* (2011)** isolated *Listeria monoctogenes* from 14.2 and 13.3%of analyzed raw-milk cheese samples .Additionally, *Listeria monoctogenes* was not detected in analyzed cheese samples by **Kongo *et al.,* (2006) and Moraes *et al.,* (2009).**

Dairy farms have been identified as a reservoir of *Listeria monocytogenes* , **Borucki *et al.,* (2004) and Mohammed *et al.,* (2009)** *Listeria monocytogenes* have been shown to be particularly persistent in production plants, primarily due to an ability to form biofilms in milking equipments and processing plants, **Nightingale *et al.,* (2004) and Latorre *et al.,* (2010).** This ability to form biofilms could represent a continuous source of bacteria in the raw milk and raw-milk cheese, **Beresford *et al.,* (2001).**

In addition, during storage of raw milk on the farm *Listeria monocytogenes* can grow and multiply even at refrigerated condition. The ability to persist in dairy farm environments, dairy processing plants and multiply under refrigeration temperatures makes *Listeria monocytogenes* a significant threat to public health, **Jemmi** and **Stephan, (2006)** and **Yilmaz *et al.,* (2009).**

We could concluded that both of examined raw milk and Kareish cheese samples not comply with the requirements of Egyptian Standards both must be free from *Listeria monocytogenes*. According to the microbiological specifications of the **Egyptian standard (ES) (2005)** for white soft cheese, set zero tolerance for *Listeria monocytogenes*. The **FDA** has had a "zero tolerance" policy for *Listeria monocytogenes* since the mid 1980s. Thus, the **FDA** has defined the appropriate level of food safety to be zero tolerance and have set the regulatory trigger at this value. **Chen *et al.,* (2003).**

our study (Table 3) revealed that, *Enterohemorrhagic E. coli 0157:H7* strain was isolated from 3(6%) samples out of 50 samples in raw milk samples. Similar studies on milk reported by **Abbas *et al.,* (2012); Abdul-Raouf *et al.,* (1996) and Allerborger and Dierich**

(1997) reported 5%, 5.8% and 5.5% of tested milk samples to be positive for *Enterohemorrhagic E. coli 0157:H7* and **Klie *et al.,* (1997)** found that (6.3%) of the unpasteurized milk analyzed in Germany was contaminated with this serotype, similar results (5.9%) of cheese samples were reported by **Aksu *et al.,* (1999).**
Lower results were reported by **Oksuz *et al.,* (2004)** they found *an incidence of Enterohemorrhagic E. coli 0157:H7* in 1 out of 100 samples (1%), while **Reuben *et al.,* (2003)**, reported 2% prevalence rate in raw milk samples. Additionally, **Jayarao *et al.,* (2006)** found that the incidence was 2.4% in analyzed raw milk samples.
In the contrary , **Chye *et al.* (2004) and Momtaz *et al.* (2012)**, demonstrated that a significant higher prevalence rate of 33.53%, 13.7% *Enterohemorrhagic E. coli 0157:H7* in raw milk samples, respectively. *Enterohemorrhagic E. coli 0157:H7* was not detected in different studies reported by **Hassan and Elmalt (2003), Abd El-Atty (2007) and Jayarao and Henning (2001)** could not isolated *Enterohemorrhagic E. coli 0157:H7* in raw milk samples**.**
Dairy cattle have long been identified as a major reservoir for *Enterohemorrhagic E. coli 0157:H7*. They are not usually pathogenic for adult cattle. The presence of *Enterohemorrhagic E. coli 0157:H7* on dairy farms and in the feces of dairy cattle has been well documented, **Cho *et al.,* (2006).** Therefore, milk is at risk for contamination with *Enterohemorrhagic E. coli 0157:H7* that is present in dairy cattle feces and dairy farm environment, **Solomakos *et al.,* (2009) and Van-kessel *et al.,* (2011).**
Results in Table (3) showed that, *Enterohemorrhagic E. coli 0157:H7* have not been found in any of the 50 samples kareish cheese examined.
These results were similar with that stated by (**Hassan and El Malt , 2008**; **Chaleshtari and Jazayeri, 2011; Rahimi, *et al.,* 2011**and **Brooks *et al.,* 2012)** in traditional raw milk soft cheese made from raw milk**.** Lower findings in study conducted in Egypt showed that, 2% of Kareish cheese samples were positive for *Enterohemorrhagic E. coli 0157:H7* **(Abd El-Atty and Mesherf, 2007).** Higher results obtained by **Oksuz (2004)** reported higher value (4.0%) and **El-Sayed *et al.,* (2011)** stated an incidence of 19% in kareish cheese samples.
Bacteriologically positive raw milk samples were positive with PCR and the specific PCR product (180-bp fragment) were detected by agarose gel electrophoresis (Photo 1). **Egyptian standard (ES) (2005)**, set zero tolerance for *E. coli* in soft cheese and no limits stated for *E. coli* in raw milk.

Conclusion

Our study revealed that raw milk is contaminated with *Salmonella spp*., *Listeria monocytogenes.* And *Enterohemorrhagic E. coli 0157:H7*, While Kareish cheese is contaminated with *Salmonella spp.* and *Listeria monocytogenes* . Based on the results of this study drinking raw milk or eating raw-milk cheese constitute public health hazards to consumers
Therefore, in order to safeguard the consumer from being infected and to prevent the products from contamination the following suggestions are to be considered.
*They should avoid drinking raw milk and consuming dairy products manufactured from raw milk and consume only heat treated and dairy products manufactured from heat treated milk.
*Heat Treatment is the cornerstone of milk safety. It is the most effective method of enhancing the microbiological safety of milk by destroying pathogenic microorganisms in milk.

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