Assiut Vet. Med. J. Vol. 59 No. 137 April 2013

OCCURRENCE AND VIRULENCE OF *YERSINIA ENTEROCOLITICA* IN RAW AND PASTEURIZED MILK

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ABSTRACT

Received at: 3/3/2013

Accepted: 3/4/2013

Seventy random samples of raw and pasteurized milk (35 of each) were collected from different localities in Assiut City, Egypt, and examined bacteriologically for the presence of *Yersinia enterocolitica*. The samples were collected in summer months, 2012. The results revealed that *Yersinia enterocolitica* was detected in 20.0 and 5.7 % of the examined marketable raw and pasteurized milk samples, respectively. The virulence of the recovered isolates of *Yersinia enterocolitica* was tested by using virulence test (Guinea pig conjunctivitis) and revealed that non of the isolated strains harbouring virulence determinants (Avirulent strain).

***Key words:** Occurrence, Virulence, Yersinia Enterocolitica, milk.*

INTRODUCTION

Yersinia enterocolitica is Gram negative, motile, coccobacilli and the colonies are dark and red centered colonies "Bulls eye like" with transparent border on CIN agar. These micorganisms are psychrotrophic milk-borne enteric pathogen; widely spread in the environment and indigenous to the gastrointestinal tract of warm blooded animals including dairy cattle (Marshall, 1992).

Yersinia enterocolitica can enter the milk from faeces, bedding and imporoperly cleaned teats and milk handling equipments contaminated with soil or water (Robinson, 1990). Both environmental strains and serotypes associated with human diseases were found. The infections may manifest itself in a variety of forms depending upon the organism itself (serotypes and biotypes); the dose of infection; genetic factors; age and physical condition of the host (Bottone, 1977 and Larson, 1979).

Contact with infected animals, persons to person contact within an infected family or consumption of contaminated food are possible modes of *Yersinia enterocolitica* transmission (Sonnenwirth and Weaver, 1970 and Gutman *et al.*, 1973). The types of infection reported include gastroenteritis, terminal ileitis, mesenteric lymphadenitis in childhood or adolescence, polyarthritis among adults, erythema nodosum and eye infection in older people (Winblad, 1973 and Bottone, 1977). However, the most common manifestation of clinical yersinosis is acute gastroenteritis which is characterized by diarrhea, abdominal pain, fever, vomiting and pseudoappendicitis (Black *et al.*, 1978 and Vidon & Delmas, 1981).

Several outbreaks of food poisoning caused by *Yersinia enterocolitica* were associated with consumption of milk and its products (Eley, 1996). The first definitive and well documented food-associated outbreak of yersinosis occurred in 1979 in Oneida country, New York, due to consumption of chocolate milk where over 220 individuals, primary school children were stricken with an acute intestinal illness (Black *et al.*, 1978). The bacterium was recovered from raw milk in many countries including Australia; Canada; Czechoslovakia; and USA (Aldova *et al.*, 1973). Furthermore, several outbreaks of *Yersinia enterocolitica* have been associated with consumption of pasteurized milk (Varnam & Evans, 1991 and Ackers *et al.*, 2000).

Pathogenic strains of *Yersinia enterocolitica* has the capability to produce heat stable enterotoxin (St) that gives a positive test with infant mouse assay, and other experimental virulence tests. Moreover, the authors observed that, this capacity was characterized for all human isolates of Yersinia enterocolitica serotype O:3 (Pai and Mers, 1978), and the heat stable enterotoxin (St) of *Yersinia enterocolitica* was similar to that produced by *E. coli*.

Therefore, this study was planned to throw the light on the occurrence of *Yersinia enterocolitica* in marketable raw and pasteurized milk in Assiut City, Egypt.

MATERIALS and METHODS

Seventy random samples of marketable raw and pasteurized milk (35 of each) were collected from different supermarkets, dairy shops and street vendors in Assiut City, Egypt, during the period of summer

months of 2012. The raw milk samples (250 ml of each) were collected in sterile plastic bags and the collected samples were transferred to the laboratory in an insulated ice box with a minimum of delay to be examined bacteriologically for the presence of *Yersinia enterocolitica*. Pasteurized milk packages which were manufactured by different milk companies were collected from different supermarkets and dairy shops.

I-Isolation and identification of *Yersinia enterocolitica*

The apparently normal raw milk samples were mixed thoroughly and tested for heat treatment by Storch test according to A.P.H.A. (1985) before being subjected to examination.

Pasteurized milk packages were thoroughly mixed and sterilized by cotton wetted by alcohol at the sit of package opening then by automatic pipette 10 ml of sample was taken off.

A- Enrichment procedure.

A tube of phosphate buffer saline was inoculated by 1 ml of each prepared raw or pasteurized milk sample. The inoculated enrichment broths were incubated in refrigerator at 4°C for 14 to 21 days (Greenwood and Hoeper, 1989).

B- Isolation of *Yersinia enterocolitica* (Schiemann, 1979):

loopfuls from the incubated enrichment broths were directly inoculated onto Yersinia selective agar plates (Cefsulodin Irgasan Novobiocin) as described by Schiemann, 1979 and incubated at 35°C for 24h till the appearance of dark red colonies (1.5 mm diameter) surrounded by a transparent border (bulls eye like). These colonies were subcultured on nutrient agar slants and incubated at 37°C for 24h and then maintained in a refrigerator for further confirmation and identification.

C- Identification of presumptive colonies.

I- Microscopic examination.

Gram's stain was performed for each isolate and examined under oil immersion lens for presence of Gram negative coccobacilli with rounded edges or short rods.

II- Biochemical reactions: according to (Schiemann and Devenish, 1982):

a-Kligler iron agar: Each isolate was stabbed and streaked into KIA slants and incubated at 35°C for 5 days. The positive result of *Yersinia enterocolitica* was characterized by a pink colored butt and a red slant with no gas or H_2S production.

b-Urea hydrolysis: Slants of Christensen's urea agar were inoculated with the suspected organisms and incubated at 37°C for 24h and examined daily up to 5 days. Hydrolysis of urea was indicated by red colour development.

c- Sugar fermentation: Pure cultures of the isolated organisms were inoculated into sterile test tubes containing 10 ml of 1% peptone water with Durham's tube. The medium was containing 1% of the required sugar (salicin and sucrose) with a bromocresol purple as indicator. The inoculated tubes were incubated for 48h at 35°C for sucrose and salicin. Positive results for *Yersinia enterocolitica* were indicated by fermentation of sucrose with gas production and fermentation of salicin without gas production.

III- Detection of virulence of *Yersinia enterocolitica* recovered from raw and pasteurized milk samples.

The virulence of *Yersinia enterocolitica* has been associated with several properties including the ability to autoagglutination. The virulence of all isolates confirmed as *Yersinia enterocolitica* were examined according to techniques recommended by (Schiemann, 1981) as following:

Guinea pig conjunctivitis (Schiemann, 1981):

Each isolate of *Yersinia enterocolitica* was grown on trypticase soy agar with yeast extract (0.6%) plates and incubated at 22 °C for 48h. The growth was washed off and the bacterial concentration of 10^9 cells/ml was used. A 10 ul amount of the prepared bacterial suspension was inoculated into the right eye of a Guinea pig (the left eye was used as control). The animals were examined daily for symptoms of conjunctivitis. A positive result was recorded where there was evidence of swelling of the conjunctiva, eye ball depression and accumulation of fluid or mucous with recovery of the organisms that were introduced at 2 days.

RESULTS

Table 1: Prevalence of *Yersinia enterocolitica* in the examined marketable milk samples.

Samples	Number of examined samples	Positive samples	
		No.	%
Raw milk	35	7	20
Pasteurized milk	35	2	5.7

Table 2: Virulence of *Yersinia enterocolitica* strains isolated from examined marketable milk samples.

Number of tested isolates	Virulence strains		Avirulent strains	
	No.	%	No.	%
9	0	0	9	100

DISCUSSION

Table 1 showed that, *Yersinia enterocolitica* strains could be isolated from 7 (20%) of the examined raw milk samples, this finding was higher than that reported for Schiemann and Toma (1978); Saad and Moustafa (1989); Moustafa (1990); Ahmed (1997) and Bahout and Moustafa (2004). Nearly similar incidences were reported by Pugina *et al.* (1984); Quaglio *et al.* (1988); Rindi *et al.* (1989); Desmasure *et al.* (1997) and Ebrahim (1998). While, lower incidences were detected by Hamama *et al.* (1992); Khalil *et al.* (1993); Jamshidian and Babakhani (1999) and Uraz and Yucel (1999). On contrast, El-Leboudy (1989); El-Kholy (1990); Abdel-Hady (1993) and Basha *et al.* (2008) failed to detect Yersinia organisms in Egyptian raw milk samples. Higher incidences rates (till 50%) were reported by Norberg (1981); Ray (1983); Hafez (1988) and Abdel-Khalek (1998).

Concerning pasteurized milk samples, Table 1 revealed that, *Yersinia enterocolitica* could be isolated from 2 (5.7%) of the examined samples. Higher incidence was reported by Walker and Gilmour (1986), while lower incidence was reported by Schiemann (1978). In contrast to this finding, Moustafa *et al.* (1983); Tassinari *et al.* (1994); Bruce *et al.* (1995); Jamshidian and BabaKhani (1999); Padilha (2001) and Romia (2001) failed to isolate *Yersinia spp.* from pasteurized milk samples, while Abdel-El All and Atta (2009) could isolate *Yersinia spp.* from pasteurized milk samples.

The presence of *Yersinia enterocolitica* in raw milk samples is an indicator for poor hygiene or cross contamination (Roberts *et al.*, 1995) and its presence in pasteurized milk samples could be explained either by post pasteurization contamination or by presence of a heat resistant strain but insufficiently cleaned milk equipment was the most frequently incriminated source of pasteurized milk contamination with Yersinia microorganism.

High incidence of *Yersinia enterocolitica* in milk was significantly related to high bacterial counts (Cotton and White, 1992) and implies that these products are a likely sources of contamination with Yersinia (Adriana *et al.*, 1994).

Raw milk and inadequately pasteurized milk have been implicated in transmission of *Yersinia enterocolitia* infection to humans (Black *et al.*, 1978).

Contamination of milk with *Yersinia species* was explained by Meadows and Sudden (1982), they found that, *Yersinia species* might gain entry to milk during collection, contaminated water, soil and milker's hands.

Pathogenic strains of *Yersinia enterocolitica* are capable of causing illness in human with a wide range of symptoms. In children and adolescents, symptoms of gastroenteritis, mesenteric lymphadenitis and psudoappendicitis are predominating, where in adults, symptoms of acute abdominal disorders and arthritis (Larsen, 1980; Roberts *et al.*, 1995 and Marth & Steel, 2001). *Yersinia enterocolitica* represented by six biovars (1A, 1B, 2-5). The biovar 1A strains are generally regarded as avirulent as they lack of plasmid for yersinia virulence (PYV) and major chromosomal virulence genes. Despite this, some biovar 1A strains produce disease symptoms undistinguishable from that produced by known pathogenic biovars (1B & 2-5) (Bahgat and Virdi, 2011).

Well-defined virulence determinants that allow the bacteria to become established in their hosts and overcome host defences. A number of strains obtained from patients with diarrhea, however, lack these genes. Accordingly, the mechanisms by which they cause disease are uncertain. Most of these isolates belong to biotype 1A. Strains of this biotype are also frequently isolated from a variety of non clinical sources such as, food, soil, water and healthy animals and there is evidence that some of these strains are avirulent (Grant *et al.*, 1998).

Avirulent Yersinia strains (*Y .entirocolitica biogroup* 1A, *Y. intermedia, Y. kristensenii* and *Y. frederiksenii*) lack the virulence plasmid. They are widely distributed in the environment and can frequently be isolated from clinical samples (Hoffmann *et al.*, 2002).

Table 2 revealed the virulence of isolated *Y. enterocolitica* strains, fortunately all the 9 strains were avirulent strains due to lacking of virulence determinants, these strains may be of *Y. enterocolitica* biogrup 1A. These results agreed with Pritchard *et al.* (1995), while disagreed by many investigators, Abdel-Hady (1993), El-Prince and Sabreen (1998), Bahout and Moustafa (2004), who demonstrate the virulence of isolated strains of Y. enterocolitica.

The lack of pathogenecity in *Y. enterocolitia* strains in the examined raw and pasteurized milk samples in this study may attributed to the source of infection which may be the environment which harbouring mostly avirulent strains.

In conclusion this investigation emphasized that, raw and pasteurized milk were contaminated with *Y. enterocolitica* and this may reflect the lack of hygienic supervision, poorly cleaned & sterilized dairy farm and processing plant equipments. Therefore, to improve quality of raw and pasteurized milk bacteriology and prevent consumers from being infected by this and other organisms strict hygienic measures should be adopted during milk collection and distribution to avoid infection with the pathogens.

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مدي تواجد وضراوة ميكروب اليرسينيا انتيروكوليتيكا في اللبن الخام والمبستر

محمود فرغلي حسين ، منال محمد امين ، انسي أديب صادق ، ايمن منير ابراهيم كريم

يعتبر اللبن ومنتجاته من اكثر الأغذية استهلاكاً نظراً لقيمتة الغذائية العالية إلا انه قد يكون مسبباً لكثير من الأمراض عن طريق التلوث بالميكروبات أثناء الإنتاج والنقل والتداول والاستهلاك. ومن هذه الميكروبات اليرسينيا انتيروكوليتيكا والتي تعتبر احد الميكروبات المحبة للبرودة والتي تلعب دوراً خطيراً في التأثير علي الصحة العامة للإنسان لما قد يسببه من حالات تسمم غذائي مصحوبة باضطرابات معدية ومعوية حادة ومزمنة. لذا أجريت هذه الدراسة لفحص عدد ٧٠ عينة من اللبن الخام والمبستر (٣٥ لكل منها) والتي تم جمعها من مصادر مختلفة بمحافظة أسيوط في صيف عام ٢٠١٢. وقد أسفرت النتائج عن تواجد ميكروب اليرسينيا انتيروكوليتيكا بنسبه ٢٠% (٧ عترات) في عينات اللبن الخام وبنسبه ٥.٧% (٢عترة) في عينات اللبن المبستر. هذا وقد تم اختبار ضراوة عترات اليرسينيا انتيروكوليتيكا المعزولة من اللبن الخام والمبستر من خلال قدرتها علي إحداث التهاب في العين اليمني لحيوان خنزير غينيا (Guniea pig) وقد اتضح أن كل العترات المعزولة وعددها ٩عترات عديمة الضراوة. وقد تم مناقشة الأهمية الصحية للميكروبات المعزولة والاشتراطات الصحية أثناء إنتاج وتصنيع وتداول الألبان الخام والمبسترة وكذلك الاقتراحات الواجب إتباعها لتحسين جودة اللبن للحفاظ علي صحة المستهلك.