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Hygienic status of raw milk adulterated with added water in El-Beheira governorate

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A B S T R A C T

Milk is known as a full diet because it includes the essential elements needed for growth, development and body maintenance, such as proteins, fat, sugar, minerals and vitamins. However, it has been noted that milk also could be considered as a vehicle for pathogenic microorganisms. Milk likely to be intentionally/unintentionally polluted at different production, manufacturing and marketing stages. Therefore, the following study was conducted to ascertain the prevalence of zoonotic Y. enterocolitica in raw milk and to evaluate the risk effects of milk adulteration with addition of water with an emphasis on the quality of this water that might be used in the adulteration process. Raw cow milk samples were gathered randomly from different households at El-Beheira province, Egypt and subjected to some chemical and microbiological investigations. One hundred and thirty-two raw milk samples and 53 water samples were randomly collected from the same source. The results showed that the isolation rate of Y. enterocolitica from the milk samples was 8.3%. The percentage of milk fraud by water addition was 52.8%. Our findings suggested that milk contaminated and adulterated by water addition could be a potential source for many zoonoses. Also revealed high adulteration percentage of milk with addition of low hygienic quality water that represents a great health hazard in addition to poor nutritional quality.

Keywords: Prevalence, Y. enterocolitica, Milk, Adulteration, Water.

1. Introduction

Y. enterocolitica, Yersinia pestis and Yersinia pseudotuberculosis are considered from the most common milk-borne human pathogens (Rahimi et al. 2014). Yersinia are transmitted to human through contaminated food or water (Revell and Miller 2001). Y. enterocolitica is a dangerous foodborne pathogen responsible for foodborne gastroenteritis (EFSA, 2018). Other clinical syndromes associated with Y. enterocolitica are enterocolitis, acute mesenteric lymphadenitis, mimicking appendicitis, arthritis and systemic infections (Neubauer et al., 2001). Milk is nutrient-dense, containing high-quality proteins, carbohydrates, fats, minerals, and vitamins (Neumann et al., 2002). Milk proteins are important for human health and supply the body with amino acids that are essential for proper infant and adult growth (Espinosa et al., 1992). Milk compositional quality is determined by its physico-chemical properties including butter fat, solid non-fat (SNF), protein, added water, freezing point and specific gravity (Connor, 1995). Milk adulteration was firstly recognized as adding water to increase volume and then after addition of various substances with different intended purposes (Cassoli et al., 2011). These adulterants include urea, starch and preservatives such as different antibiotics (Tipu et al., 2007). Water addition to milk interferes with the hygienic, nutritional, compositional and processing qualities of milk (Donkor et al., 2007). Sellers usually use milk adulteration with water, which is cheap, easily to be homogenized and cannot be detected by the consumers when compared with other substances used to adulterate milk (Adam, 2009).

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This study aimed to demonstrate the incidence rate of *Y. enterocolitica* in the examined raw milk samples. The risk effects of adulteration of milk with added water. In addition, the quality of water that could be used in milk adulteration is being investigated.

2. Materials and Method

Samples collection and preparation

One hundred and thirty-two randomly collected raw cow milk samples were purchased from different farmers' houses at El-Beheira governorate, Egypt. The collected samples (about 500 ml. each) were transferred as directly and rapidly as possible in an ice box to Food and Feed Safety laboratory, Damanhour, El-Beheira, Egypt. Each sample was divided into two equal parts after proper mixing. The first part was used for chemical examination and the second was used for Yersinia spp. isolation.

Isolation of Yersinia spp. (Wauters, 1973; Collee et al, 1989)

a. Pre-enrichment:

One ml of each sample of milk was firstly enriched in 9 ml of phosphate buffered saline with incubation at 25˚C for 2 days.

b. Selective enrichment:

One ml of pre-enrichment culture was added to 90 ml of Yersinia enrichment broth then incubated at 25˚C for 5 days.

c. Plating procedure:

A loopful of each enriched culture was streaked on the surfaces of Yersinia-selective agar (Cefsulodin–Irgasan–Novobiocin (CIN) followed by incubation at 30˚C for 48hrs. The suspected small colonies with a deep red center and a rather sharp border surrounded by a clear colorless zone were picked and streaked onto slopes of tryptone soya agar medium supplemented by yeast extract (TSAYE). The slopes were incubated at 30˚C for 24hrs.

d-Biochemical testing

Isolated colonies were biochemically confirmed by catalase, utilisation of citrate, triple sugar oxidase, urease and Kligler's iron test (FDA, 2001).

Detection of percentage of added water in milk samples

The MilkoTester (LTD Europe) equipment used to analyze percentage of added water in raw milk samples.

Examination of the hygienic quality of water samples

Fifty-three water Samples (50 ml each) were randomly collected from the same sources of milk samples. Water Samples were assayed for total ATP using an Aquasnap Total Sampling device and a Hygiena ATP bioluminometer (EnSURE Hygiena, UK). ATP measurement was conducted according to the manufacturer's instructions. The amount of light detected (Relative Light Units or RLU) is linearly correlated to the quantity of ATP collected equates to 1 fmol of ATP) per millilitre (RLU /ml). For the purposes of initial ATP testing, the pass/fail guidelines were kept at the default values that the unit came with, which were Pass: <10 RLU, Caution: 10-30 RLU, and Fail: >30 RLU.

Statistical analysis

Data were analyzed by statistical package for social science (SPSS) version 10 software package. Quantitative variable was expressed as mean and standard deviation.2.2. Organic acids used:

3. Results

Table (1) Prevalence of Yersinia Species and Y. enterocolitica in examined milk samples (n=132).

Pathogen	Positive samples	
	No.	%
Yersinia Species	41	31
Y. enterocolitica	11	8.3

Table (2) Incidence of fraud by water addition in examined milk samples (n=53).

Samples	*Positive samples*	
	No.	*%*
Milk samples	*28*	*52.8*

Table (3) Percentage of added water in examined milk samples (n=28).

Samples	Minimum	Maximum	Mean ± SD
Milk samples	0.3	21	4.93 ± 8.54

Table (4) Total ATP level in examined water samples (RLU) (n=53).

Samples	Minimum	Maximum	Mean ± SD
Water samples	0	201	19.70 ± 36.4

Table (5) Quality of examined water samples according to ATP level (n=53).

Samples	<10 RLU	10-30 RLU	>30 RLU
Water samples No.	34	9	10
Water samples %	64	17	19

4. Discussion

Y. enterocolitica is one of the most serious food poisoning pathogens with widespread nature in dairy products, meats, water and the environment (Logue et al., 1996; Mayrhofer et al., 2004). In this study, Yersinia spp. were detected in 31% of raw milk samples (table 1). Similar findings were reported by Hamama et al., (1992), in which 36.6% samples of raw milk were positive for Yersinia spp.. Higher prevalence of Yersinia spp. in raw milk samples (45.2% and 52.2%) was reported by Tassinari et al., (1994) and Askr et al. (2013), respectively. Compared with our results, a lower incidence of Yersinia spp. (11.7, 24%) was observed by Özdemir and Arslan (2015) and Ali and Al-Samarai (2020) studies, respectively. Results in table (1) revealed that Y. enterocolitica was isolated from 8.3% of the examined samples. Contamination of raw milk with Y. enterocolitica has reported in different studies, for instance, 39% (Darwish et al., 2015), 22% (Ahmed et al., 2019), 19.4% (Abd El Aal and Atta, 2009), 7.7% (Ruusunen et al., 2013), 5.8% (Jamali et al., 2015) and 3.1% (Bonardi et al., 2018). In dairy farms, raw milk could be contaminated with Yersinia spp. during milking, post-milking contamination, contamination from human handlers, environment, water, improper cleaning of milking utensils, improper transport and storage conditions (Tassinari et al.,1994; Ray 2004). Yersinia is a psychotropic microorganism which can grow at refrigeration temperatures during storage and transport; therefore, they could offer a potential food safety hazard in cold chain food products like raw milk (Dallal et al.,2004).

Chemical composition of raw milk plays an important role in assessing organoleptic properties, shelf life and flavor of processed milk products. In the milk industry, water is the most used adulterant (Das et al., 2016). Water interferes with processing qualities of milk, lowers the its nutritional value and poses a chemical and microbial health hazard. In this study, 53% of raw milk samples were obviously adulterated with water (Table 2). Findings of this study were higher than those of a study done by Omore et al., 2002 (15%) and Ondieki et al., (2017) (42.7%). In contrary, Lateef et al., (2009), Adam, (2009) and Amin, (2016) reported higher percentage of milk samples adulterated by water, 93.33 and 95, 68%, respectively.

Results in table (3) revealed that the added water percent in the adulterated samples ranged from 0.3 to 21%. Our findings were similar to those reported by Amin, (2016) and Adam, (2009) who found that street vendors' milk samples were adulterated with water, ranging from 5.96 to 23.84 % and 1.6-19 %, respectively. These results are higher than those reported by Kabui (2012) and Kabui (2014) where the percent of added water were, 1.88- 6.79 % and 1.43- 6.79%, respectively. Prevalence of added water in milk samples can be associated with the fact that milk produced by farmers is marketed raw directly to consumers while bulk milk produced in large scales is sold to milk processing factories which are very strict on the quality of milk purchased where poor compositional quality milk is usually rejected by processors resulting in big economic losses to farmers (Muzira et al., 2006; Kabui et al., 2012).

Findings in this study showed adulteration of significant numbers of the examined raw milk samples with added water. So, it was important to check the quality of randomly collected water samples that might be used in the adulteration process. Traditional heterotrophic plate count technique is time-consuming and limited to culturable bacteria. So, Rapid and precise detection methods, such adenosine triphosphate (ATP) measurement have recently emerged to determine microbe activity in drinking water. Adenosine triphosphate - a universal energy molecule present in all bacterial, plant and animal cells were used in this assay. ATP value directly depends on the degree of microbial contamination and organic pollution of water (Belov and Vasilyev, 2020). Thus, the concentration of ATP reflects the value of the total microbial number, which give an idea about the hygienic status of water samples. Values in table (4) showed that the mean value of ATP in the examined water samples was 19.70 ± 36.4 RLU. Our findings were far higher than those reported by Siebel et al., (2008) who found that total ATP concentrations from the drinking water samples varied in the range of 0.005–0.094 nM ATP with an average of 0.023 (±0.017) nM ATP. Results in Table (5), showed that after examination of water samples, we noticed that 64% of the examined water samples were within permissible limits, 17% of them were within the border line, while 19% of the examined samples were hygienically unfit to be used according to Manning et al., (2015). These findings confirm that water addition to milk not only decrease the compositional and nutritional quality, but also carry a great public health risk.

5. Conclusions

Consumption of raw milk could be associated with significant public health threats for consumers as it might be contaminated with Yersinia and other food borne pathogens. Milk adulteration by water addition could be a potential source of different pathogens combined with poor nutritional quality. Therefore, Application of highly recommended hygienic practices and regulations, such as application of HACCP and on-site pasteurization is important to improve the hygienic quality of milk and to safeguard the consumer from being possible health hazard organisms.

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