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Short Communication

A Cross-Sectional Study on the Prevalence of *Salmonella* in Raw Milk in Tandojam and Surrounding Areas, Pakistan

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Abstract

The study on the isolation of *Salmonella* in raw milk marketed in Tandojam town was carried out and raw milk samples were collected from village Saleh Makrani, Tandojam city and Moosa Khatiyani. Eleven milk samples from Saleh Makrani, thirty from Tandojam city and eleven from Moosa Khatiyani were collected which totaled 52 samples. Results indicated that of 2 milk samples collected from Village Saleh Makrani, 2 from Moosa Khatiyani and 4 from Tandojam city were found positive for *Salmonella*. Of the total 52 milk samples, *Salmonella* species was confirmed in eight samples. The overall prevalence of *Salmonella* was in the minimum-maximum range of 13.33-18.18 percent, showing average *Salmonella* prevalence of 15.38 percent. Hence the prevalence of *Salmonella* in milk was relatively higher in the samples collected from village of Haji Muhammad Saleh and Moosa Khatiyani.

Keywords: Raw milk, *salmonella*, prevalence, typhoid, cross-sectional study.

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Introduction

Salmonellae are Gram negative, short plump shaped rods, non-spore forming, noncapsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae, (OIE Manual, 2006). It is one of the commonest causes of food poisoning worldwide. *Salmonella* infections are zoonotic; they can be transmitted by humans to animals and vice versa. There are over 2,000 different types of *salmonella*, they cause illnesses in humans and many animals, such as typhoid fever, paratyphoid fever and foodborne illness salmonellosis (Ryan and Ray, 2004). According to the World Health Organization (WHO), over 16 million people worldwide are infected with typhoid fever each year, with 500,000 to 600,000 of these cases proving to be fatal.

A *salmonella* infection is a foodborne illness caused by *salmonella* bacteria carried by some animals, which can be transmitted from kitchen surfaces and can be in water, soil, animal feces, raw milk, raw meats, and eggs. Infective *Salmonella* must then survive peristalsis, the epithelial surface and the host immune response (Brodsky *et al.*, 2002). *Salmonella* infections can be prevented by paying due attention to cleanliness and to make sure that all food is thoroughly cooked.

Milk is an opaque white liquid produced by the mammary glands of mammals that provides primary source of nutrition for young mammals. Exact components of raw milk vary by species, but it contains significant amounts of saturated fat, protein and calcium as well as vitamin C. Cow's milk has a pH ranging from 6.4 to 6.8, making it slightly acidic (William, 2005).

Raw milk is illegal in many countries as it can be contaminated with potentially harmful microbes. There is an ongoing debate about the benefits and risks of drinking unpasteurised milk. Some people believe health benefits resulting from extra nutrient content of raw milk outweigh the risk of ingesting potentially dangerous microbes, such as *Mycobacterium bovis*, which can cause tuberculosis, and *Salmonella* species. Contamination of milk and other liquids used for human consumption with *Salmonella* is a major

concern worldwide, and rapid, sensitive and reliable methods are needed for detection of these organisms. Since *Salmonella* can contaminate foods and other types of samples, it is imperative to enumerate this harmful organism and to generalize preventive methods. The study therefore was carried out to enumerate *Salmonella* from samples of raw market milk at Tandojam Town and Moosa Khatiyani, District Hyderabad.

Materials and Methods

Present study was carried out for the detection of *salmonella* in raw milk in Tandojam town, district Hyderabad. Experiment was conducted as per protocol of Tecra International Pty Ltd, Australia. A total number of 15 raw milk samples were collected from different vicinities of Tandojam for the detection of *Salmonella*.

Sterilization of Laboratory Equipments

Sterilization of laboratory equipments was carried out in hot air oven at temperature of 160°C for two hours.

Preparation of Broth

Modified Buffered Peptone Water (MBPW) was used in experiment. 25 grams of MBPW were mixed with sterilized water in conical flask up to 250 ml. Then the water was thoroughly shaken with the help of hot plate.

Sterilization of Broth

Sterilization of broth was carried out to ensure the absence of any contamination. The sterilization was done in autoclave, having temperature of 121°C for 15 minutes. Then the broth was transferred to refrigerator for use.

Collection of Raw Milk Samples

A total number of 52 raw milk samples were collected from three different places of Tandojam and surrounding, eleven (11) samples were collected from village of Saleh Makrani, eleven (11) from Moosa Khatiyani and thirty (30) samples from Tandojam city. These samples were collected in sterilized milk sample collection bottles, and were further analyzed.

Inoculation and Incubation

A 10 ml of broth was transferred to each of 15 sterilized test tubes with the help of automated pipette. Then 01 ml of raw milk was transferred to each test tube containing the broth. The tubes were then placed in incubator for 24 hours.

Results

The study was carried out on the isolation of *Salmonella* in milk marketed in Tandojam town and

surrounding areas, district Hyderabad. Three representative areas of Tandojam town were selected for this study, which included village of Haji Muhammad Saleh Makrani Baloch, Moosa Khatiyan and Tandojam city. Total 52 milk samples were collected, out of which 11 milk samples from different Buffalo Farms in village Saleh Makrani, 11 milk samples from Moosa Khatiyan and 30 milk samples from the Tandojam city (Table 1). Each collected milk sample was subjected to isolation for *Salmonella*.

Table 1: Number of milk samples collected for the isolation of *Salmonella*.

S. No	No. of samples collected	Address	Vicinity
1	3	Wali Muhammad Baloch, Buffalo farm, Saleh Makrani, Tandojam	Village Saleh Makrani
2	5	Rasool Bux Baloch, Buffalo farm, Saleh Makrani, Tandojam	
3	2	Detha Parra, Saleh Makrani, Tandojam	
4	1	Chandia Parra, Saleh Makrani, Tandojam	
Sub total		11	
1	11	Moosa Khatiyan, near Tandojam	Moosa Khatiyan
Sub total		11	
1	9	Mid-City, Tandojam	Tandojam city
2	5	Railway Crossing, Tandojam	
2	5	Railway Station, Tandojam	
4	3	Nakka, Tandojam	
5	8	Work shop/ Open Air Restaurants	
Sub total		30	
Grand total	52		

Isolation of Salmonella

Table 2 shows that out of 52 milk samples 08 milk samples were positive for *Salmonella*, 02 from the

village of Saleh Makrani, 02 from Moosa Khatiyan and 04 from Tandojam city, while 44 milk samples were found negative for *Salmonella*.

Table 2: Isolation rate of *Salmonella* in raw milk.

S. No	No. of samples collected	No. of positive samples	Isolation Rate %	Vicinity
1	3	1	33.33	Village Saleh Makrani
2	5	1	20	
3	2	0	0	
4	1	0	0	
Subtotal positive samples		02		
1	11	2	18.18	Moosa Khatiyan
Subtotal positive samples		02		
1	9	1	11.11	Tandojam city
2	5	1	20	
3	5	1	20	

4	3	1	33.33
5	8	0	0
Subtotal positive samples			04
Grand	52	08	
Total			

Overall Prevalence of *Salmonella*

Table 3 shows overall prevalence of *Salmonella* in milk samples collected from different areas of Tandojam.

Table 3: Overall prevalence (%) of *Salmonella*.

Species	Vicinity								Overall
	Vill: M. Saleh		M. Khatiyani		T. Jam city		Min	Max	
	+ve	-ve	+ve	-ve	+ve	-ve	(+ve)	(+ve)	
<i>Salmonella</i>	18.18	81.81	18.18	81.81	13.33	86.66	13.33	18.18	15.38

Discussion

Milk is most used liquid after water in all age human beings, and the prevalence of *Salmonella* in fresh milk is one of the major causes of various bacterial infections. *Salmonellae* are closely related to *Escherichia* genus and are found worldwide in warm- and cold-blooded animals, in humans, and in nonliving habitats. They cause illnesses in humans and many animals, such as typhoid fever, paratyphoid fever, and the foodborne illness salmonellosis (Ryan and Ray, 2004). Contamination of milk and other liquids used for human consumption with *Salmonella* is a major concern worldwide and rapid, sensitive, and reliable methods are needed for detection of these organisms. Since *Salmonella* can contaminate foods and other types of samples, it is imperative to isolate these harmful organisms and to generalize preventive methods.

The results of the present study indicated that of total 52 raw milk samples collected from various representative areas of the Tandojam, the prevalence of *Salmonella* was confirmed in eight. In village of Haji Muhammad Saleh 18.18 percent samples were found positive and 81.81 percent negative for *Salmonella*. In Mossa Khatiyani area 18.18 percent milk samples were found positive and 81.81 percent milk negative; in Tandojam area 13.33 percent milk samples were found positive for

Salmonella. The overall prevalence of *Salmonella* was in the minimum-maximum range of 13.33-18.18 percent, showing average *Salmonella* prevalence of 15.38 percent. Hence, prevalence of *Salmonella* in milk was relatively higher in the samples collected from village of Haji Muhammad Saleh and Mossa Khatiyani.

Results are in agreement with those of Hassan *et al.*, (2000) isolated *Salmonella* spp. from 1.5 % milk samples they collected and *Salmonella* spp. were prevalent in milk filters in New York dairy herds. Wedderkopp *et al.*, (2001) tested milk samples using LPS-ELISA and indicated that the risk for a dairy herd to receive *Salmonella* spp. infection increases with the diseases status among the nearest neighbors and with the prevalence of seropositive herds in the geographic area.

A considerable research work has been found published in different research journal in relation to aspects of the present study. Sorensen *et al.*, (2003) analyzed the fifty dairy herds in Alberta were tested for the presence of *Salmonella*. Four (8%) dairy herds had at least 1 cow shedding *Salmonella*. Van Kessel *et al.*, (2004) determined the prevalence of *Salmonella* and fecal coliforms in bulk tank milk in the United States and Twenty-two samples (2.6%) were culture-positive for *Salmonella*. Breurec *et al.*, (2010) studied ninety two of raw milk samples, which failed to meet official standards.

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