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Prevalence of *Salmonella enterica* Contamination of Camel Milk in Iran

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Abstract: This study was undertaken to conduct a baseline risk analysis of raw camel milk with special emphasis on *Salmonella enterica* serovars. Cross-sectional studies were designed to investigate the prevalence of *S. enterica* serovars in a major camel milk production zone of Iran. A total of 196 samples were assessed for possible presence of *S. enterica*. The samples included composite milk from the individual camel udders, bulk milk from collection and market centres, faeces, soil and water samples. Of the 196 samples tested, 43% (84/196) were found to contain Salmonella species. Out of the 84, only 31% (26/84) was positively identified as *S. enterica*. *S. enterica* was found in all the sample categories that represented the camel milk production environment. The results suggest that raw camel milk contamination by *S. enterica* was influenced by post-harvest handling of the product rather than camel infection by the pathogen. It was concluded that a need exists to formulate better regulation strategies for the safe handling of camel milk on rural Kenyan farms.

Key words: Camel milk, *Salmonella enterica*, milk safety, Kenya, Iran

INTRODUCTION

Camel milk constitutes 12% of 3 billion L of the milk produced nationally in Iran. The average camel milk production per annum is 0.366 million litres and 25% of the national population (17 m), especially those living in the low lands depend on camel milk.

Since, commercial exploitation of camel milk in Iran has grown tremendously, there is a growing public health concern on its safety as it is informally marketed.

The handling of informally marketed milk has been reported to affect the safety and quality of milk with reports of food poisoning due to consumption of camel milk also being reported (El-Nawawi *et al*., 1982).

Salmonella infection in camels has been reported in various countries including Sudan, Palestine (Olitzki and Ellenbogen, 1943), French North Africa, USA (Moran, 1949) and more recently from Somalia (Cheyne *et al*., 1977), Ethiopia (Pegram and Tareke, 1981), Egypt (Refai *et al*., 1984; Yassiem, 1985; Osman, 1995) and UAE (Wernery, 1992). Faye (1997) reported that *S. enteric* typhimurium and *S. enterica* enteritidis are more prevalent in camels. Healthy camels can be carriers of Salmonella and organisms have been isolated from faeces and lymphnodes on slaughter of camels (Zaki, 1956; Hamada *et al*., 1983; El-Nawawi *et al*., 1982; Refai *et al*., 1984; Yassiem, 1985; Selim, 1990).

Camels that are chronic carriers of Salmonella may present a human health hazard through consumption of camel products like milk. This study was undertaken to conduct a baseline risk analysis of raw camel milk with special emphasis on *S. enterica* serovars. The study covered the rural zones in Iran.

MATERIALS AND METHODS

Cross-sectional studies were designed to investigate the prevalence of *S. enterica* serovars. The samples for *S. enterica* analysis included the composite milk from the individual camel udders, bulk milk from collection and market centres, faecal, soil and water samples. For the composite and bulk milk, the containers were shaken to mix the samples well. A cup was used to take the sample after shaking the container. About 25 mL of the milk sample from the cup was poured into a sterile screw cap universal bottle and then capped.

This was then put in a cool box maintained at 4°C. Faecal samples were taken using sterile cotton swabs wrapped on splint wood sticks.

The cotton swab stick was pushed into the rectum in a screw manner of the lactating female camel whose milk sample had been taken. The swab was immediately transferred into sterile Stuart Transport Medium (Oxoid) in a screw cap Bijou bottle. The handle stick was broken and the swab remained in the transport medium.

The bottle was capped and put in the cool box. Water samples were also taken from the boreholes or wells that were being used as sources of drinking water for the camels. Borehole samples were taken by pumping the water out for 5 min and then taking the sample by slanting the mouth of a sterile glass bottle towards the nozzle of the water pipe. Samples (500 mL) were taken and the bottles capped. Well water was taken by lowering a bucket on a rope into the well when the bucket reached the water level, it was swirled to stir the water and then lowered to scoop the water.

The water from the bucket was then poured into a sterile 500 mL glass bottle, capped and then placed in the cool box. For soil samples, 200 g was scooped in the middle of the boma and wrapped in clean polythene papers then transferred to the cool box. All samples were transported to the laboratory at KARI-Marsabit in a cool box within 12 h of sampling. A total of 196 samples were taken and analysed for the isolation of *S. enterica*.

Enumeration of *S. enterica* from the various sample categories: About 25 mL of milk and water and 25 g of faecal and soil samples were pipetted and weighed, respectively and inoculated into 225 mL of Buffered Peptone Water (BPW) (Oxoid) as a preenrichment.

The suspensions were incubated at 37°C for 24 h. After the incubation, the mixtures were shaken gently and using a sterile pipette, 1 mL was pipetted and transferred into 10 mL Rappaport Vassiliadis medium (Oxoid). After incubation in a water bath at 42°C for 24 h, a loopful of growth was streaked onto both Xylose Lysine Desoxycolate (XLD) (Oxoid) agar and Brilliant Green agar (BGA) (Oxoid). The agar plates were incubated at 37°C for 24-48 h. Colonies that appeared dark on XLD and those that appeared pink on BGA were taken to be non-lactose fermenters and were purified on MacConkey agar (Oxoid). The purified colonies on Mac-Conkey agar were inoculated into the Triple Sugar Iron (TSI) (Oxoid) agar slants by stabbing the butt and streaking the slant. The colonies that appeared shiny, yellow and mucoid were taken to be lactose fermenters and were also purified on MacConkey agar. Pure isolates (3-4 colonies) that were lactose negative on culture and purified on MacConkey agar were inoculated into 10 mL preparations of fermentable sugars (glucose, lactose, manitol, sorbitol, citrate, Triple Sugar Iron (TSI) and urea) with phenol red as the indicator and incubated at 37°C for 24 h.

Serotyping of *S. enterica* isolates: For serological identification, a slide agglutination test using O grouping polyvalent sera and Vi serum was used. All the reagents were left to reach room temperature. On a clean microscope slide, a drop of antiserum was placed at one end and a drop of sterile normal saline (0.85% NaCl) was placed at the opposite end of the same slide. Three to four colonies from the non-selective media were suspended in 0.3 mL sterile saline and a dense cell suspendsion was made. One loopful of the cell suspension was put onto each of the drops of serum and normal saline and mixed well. The cell suspension and normal saline served as controls. The slide was gently shaken for 1 min. Agglutination within 1 min was regarded as positive for polyvalent O-group. Whenever any isolate agglutinated with one of the polyvalent O-sera, it was again tested against the corresponding monovalent antisera using the same procedure as earlier.

The reason for testing with monovalent O was to avoid cross-reaction with non-salmonella genera like Escherichia, Shigella, Citrobacter and Proteus. The name of the serum that produced agglutination was considered as the name of the O-antigen possessed by the tested *Salmonella* sp.

When there was no agglutination after 1 min from the above test, the same above procedure was carried out using Vi serum instead of the polyvalent sera. When a positive reaction was found, a dense suspension of the organism in sterile saline was made and autoclaved at 121°C for 15 min. After cooling to room temperature, the agglutination test was repeated with polyvalent serum and Vi serum using the heated cells. Live cells that were negative with polyvalent serum and positive with Vi serum before heating and positive with polyvalent serum and negative with Vi serum after heating were taken to be *S. enterica* Typhi.

For H-antigen, a tube agglutination test was used. H polyvalent and monovalent sera were left to reach room temperature. Pure cultures of suspected *Salmonella* sp. (grown for 8 h at 37°C in non-selective broth were diluted with an equal volume of saline containing 1% formalin. An aliquot (0.4-0.5 mL) of the antigen suspension was added to 0.05 mL of each specific H serum in small test tubes. A control was prepared that only contained the antigen suspension. The tubes were shaken well for 2 min, allowed to stand in a water bath at 50-52°C for 1 h and then observed for agglutination.

The name of the serum that produced agglutination corresponds to the name of the H-antigen possessed by the test organism.

RESULTS AND DISCUSSION

The prevalence of *S. enterica* in the sampled regions and the market outlets is shown in Table 1. Of the 196 samples tested, 84 were found to contain *Salmonella* species. Out of the 84, 31% (26/84) were positively identified as *S. enterica*.

Table 1: Prevalence of *Salmonella enterica* in sampled regions and market

Region	N	Udders	Bulk milk	Faeces	Water	Soil
AK	36	10	5	4	1	1
BL	35	11	5	2	1	0
CN	20	4	1	0	3	0
DG	30	5	0	0	3	1
Enh	19	2	2	0	1	0
FM	16	3	2	2	3	0
GI	14	4	4	2	2	0
HNm*	26	0	0	0	0	0
Total	196	39	19	10	14	2
Incidence (%)		20	10	5	7	1

*Market isolates, A-G are isolates from different production areas and Districts of Northern Kenya. AK-Kalacha, BL-Logologo, CN-Ngumit, DG-Gudas, Enh-North horr, FM-Moyale, GI-Isiolo, HNm-Nairobi market

Salmonella incidence was more prevalent at the production level than market level. At market level, there was no isolation of Salmonella.

Milk was the main source of Salmonella (individual camel milk and pooled milk). However, it is not quite clear whether the origin of Salmonella was endogenous that is from the camel itself or exogenous that is from the camel environment. At the udder (milking) level, the incidence of *S. enterica* was twice as high (incidence of 20%) than at the collecting centres (incidence 10%) (Table 1). Based on serology, Table 2 shows the two serovars that were identified based on poly O and Factor O agglutination tests. *S. enterica* was found in all the sample categories that represented the camel milk production environment, these being the milk, faeces, water and soil (Table 3). The serovar typhi was found in all four sample categories accounting for 46% (12/26) of the positively identified *S. enterica* while serovar Paratyphi C was found in three categories accounting for 54% (14/26) but missing in soil category (Table 3).

Individual camel udders and collecting centers (bulk) had a higher incidence (57%) of *S. enterica* contamination as compared to other categories. The serovars typhi and Paratyphi C had almost the same prevalence. Water and faeces accounted for 19% of *S. enterica* contamination (Table 3).

S. enterica was found in all the sample categories that represented the camel milk production environment. Milk at the udder harvesting level and the bulk milk at collection points had the highest incidence (>20%) of the pathogen (Table 3). The environment in which any food is produced is a key factor contributing to its quality.

The environment in which camel milk is produced has been proven in this study to be a contributory factor to the contamination of the camel milk by *S. enterica*. The fact that Salmonella were found in a wide spectrum of the categories sampled including water, faeces, soil and milk is an indication that faecal contamination of camel milk production and market chain is common.

Table 2: Serological identification of *Salmonella enterica* isolates

Serovar isolate	n	Poly O-Ag	Factor O-Ag	Subgroup	Serotype
Paratyphi	15	A-G	C-factor 6, 7	C1	Paratyphi C
Typhi	11	A-G	D-factor 9 (Vi)	D1	Typhi

Table 3: *Salmonella enterica* serovars present in 196 samples taken from camel milk production environment

Sample category	No. of positive isolates	Incidence (%)	*S. enterica* serovar (n)
Milk(udder/bulk)	15	57	Paratyphi C (7), Typhi (8)
Water	5	19	Paratyphi C (3), Typhi (2)
Faeces	5	19	Paratyphi C (4), Typhi (1)
Soil	1	4	Paratyphi C (0), Typhi (1)
Total	26		Paratyphi C (14),Typhi (12)

Two serovars of *S. enterica* were isolated from camel milk in this study; *S. enterica* Paratyphi C and *S. enteric* Typhi. The latter is a strict human-associated serovar that causes septicaemia. This host-adapted serovar is transmissible through faecal contamination of food (Todar, 2005). Camel milk is produced in the same environment where human faecal waste is deposited as pastoralists normally do not construct pit latrines. Whenever rains come, the faecal waste could be carried as surface runoff to the nearest water body, commonly streams that are seasonal, dams, boreholes and shallow wells. These are in turn used as sources of drinking water for camels and humans. The milkers and milk-handlers of camel milk could be carriers of *S. enterica* Typhi.

The paratyphi group occurs in almost all domestic animals and is transmissible to man. They are known to cause food poisoning in humans. Serovar Paratyphi C occurs sporadically and its mode of transmission is direct or indirect contact with faeces or contaminated food (PHAC, 2001). As a health hazard, serovar Paratyphi C causes bacterial enteric fever. It has also been reported by Kariuki *et al*. (1999) that Paratyphi C is resistant to most antimicrobials. In this study, raw camel milk contamination by *S. enterica* was more strongly influenced by post-harvest handling of the product rather than *S. enterica* infection of the camel (Table 1). In some studies, the presence of *S. enterica* in camels has been reported in disease assessment, especially camel calf diarrhoea (Salih *et al*., 1998, Shigidi *et al*., 1998; Nation *et al*., 1996; Malik *et al*., 1967; Ambwani and Jaktar, 1973; Wernery, 1992) and in lymph-nodes and intestines of slaughtered dromedaries in Egypt (Refai *et al*., 1984; Yassiem, 1985). The lower prevalence of Salmonella in raw camel milk in this study does not mean food borne illness may not be caused but in fact should be considered a potentially hazardous situation. The presence of the Salmonella in camel milk must be supported by several factors in the environment or chain of production and marketing. For *S. enterica* to contaminate the camel milk, it must have gone through the chain of infection. It must have had a source (host) and a mode of transmission to the milk. The milk has all the factors that support its growth. The sources of the pathogen constitute the risk factors that may be associated with the prevalence or incidence of the same pathogen in the environment. In this case, pastoralists, camels, milkers, milk-handlers, equipment used in milking and handling milk, water, soil, etc. are the likely sources of Salmonella in the environment of camel milk production. The pastoralists and the camels may be healthy carriers and they may persistently shed the pathogen in the environment and through the milk. The pathogen finds its way into other transmissible avenues like water, soil, milk and equipment. This cycle forms a

web of causation of the pathogen in the environment. In the pastoralist environment, there is no human or animal waste disposal system. The same water sources are used for domestic work and animal watering. This web of causation of the pathogen based on the risk factors mentioned before is maintained at production level. This may explain the reason why there is high incidence of *S. enterica* at production level. There was no Salmonella isolated at the market level (Table 1). The time lapse from production to market centres seems to be a factor in the apparent absence of the Salmonella from the milk. The ambient temperatures at the production level (ASAL) are normally high, during the transportation of the milk; there is no temperature control hence microbial growth is not limited. The situation is made worse by the poor infrastructure that characterizes these areas and the long distances to the lucrative markets in urban centres. Under these conditions, the milk undergoes physico-chemical changes due to the unlimited multiplication of the microbial population. This leads to acid development that may selectively inhibit *S. enterica* from multiplying (Foster and Spector, 1995; Abee *et al*., 1995; Juven *et al*., 1991). Intrinsic factors of camel milk such as cationic peptides that have antimicrobial properties may also inhibit *S. enterica* from multiplying.

The survival tactics of *S. enterica* in these conditions include developing resistance to these cationic peptides (Christensen *et al*., 1988; Kagan *et al*., 1990; Cotter and DiRita, 2000), entering Viable But Non-Culturable (VBNC) state (Eriksson de Rezende *et al*., 2001; Anriany *et al*., 2001; Chmielewski and Frank, 1995) and escaping from the extracellular environment of the milk to the intracellular environment using the leukocytes in milk (Galan *et al*., 1992; Rosenshine and Finlay, 1993) hence macrophage survival (Dunlap *et al*., 1992) may explain the apparent absence of the Salmonella from the market milk.

CONCLUSION

This study has shown that camel milk within the studied area is contaminated with *S. enterica* and there is a clear indication of faecal contamination of camel milk. The serovars involved were *S. enterica* serotype Typhi and *S. enterica* serotype Paratyphi C. *S. enterica* serotype Typhi is highly host-adapted to humans. This suggests that there is direct and indirect human faecal contamination of the camel milk and water through the chain of production and marketing.

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