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Prevalence of *Salmonella enterica* subspecies *enterica* Serovars in Khartoum State, Sudan

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Abstract: This study was undertaken to determine the prevalence of *Salmonella* sp. In human, animals and food items within Khartoum State, Sudan. Samples for analysis were collected from raw and cooked food items, fish, chlorinated drinking water, domestic livestock meat and poultry meat, livestock feces and human fecal samples for restaurant workers. *Salmonella* isolation and identification was performed according to standard previously described methods and the recovered isolates were serotyped and phagetyped at the Public Health Agency of Canada, Office International des Epizooties (OIE') Reference Laboratory for Salmonellosis. A total of 92 *Salmonella enterica* subspecies *enterica* isolates, representing 30 different serovars were recovered from 9.2% of the samples examined. Ninteen of the recovered *Salmonella* serovars were reported for the first time in Sudan. The latest study on Salmonellosis in Sudan dates to 1962 when *Salmonella* sp. were investigated only in animals. We examined various sources for the presence of *Salmonella* sp. therefore, this study provides a baseline study for comparison with any future *Salmonella* surveillance and epidemiological studies in Khartoum State, Sudan.

Key words: *Salmonella* surveillance, serotyping, sudan, epidemiology, salmonellosis

INTRODUCTION

Infectious microbial diseases constitute a major cause of death in many parts of the world, particularly in developing countries. *Salmonella* has been identified as an important food and water-borne pathogen that can infect humans and animals, resulting in significant morbidity and mortality (Laconcha *et al*., 1998; Akkina *et al*., 1999; Poppe *et al*., 1998).

Consumption of food products contaminated with animal feces is a significant risk factor for salmonellosis in humans (Food and Drug Administration, 2007; Kennedy *et al*., 2004; Wells and Butterfield, 1999).

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The worldwide incidence of human nontyphoidal salmonellosis is estimated at 1.3 billion cases, with three million deaths annually (Tassios *et al*., 1997). Frequencies of isolation of *Salmonella* serovars from different clinical sources vary from time to time and from place to place (Nagal *et al*., 2006) and new serovars are isolated throughout the world every year. Currently 2579 *Salmonella* serovars are recognized, with the majority belonging to *Salmonella enterica* subspecies *enterica* (Grimont and Weill, 2007).

In Sudan isolation of *Salmonella* was reported by different investigators. For example, Mamoun *et al*. (1992) isolated 21 *Salmonella* strains from several poultry farms in three different States in the Sudan, all were found to be *S. eneritidis*. Yagoub *et al*. (2005) isolated *Salmonella* sp. from 1.43% (1/70) of raw milk samples collected from Butana dairy factory, farms and milk collection points located in Hillat kuku, Khartoum North. Yagoub *et al*. (2006) isolated *S. paratyphi* A and *S. paratyphi* B from 6% of the white cheese samples collected from retailer shops and restaurants in Khartoum and Omdurman cities during the period from February to November 2001. Yagoub (2009) reported the isolation of *Salmonella* from 6.2% (28/565) of the fish samples (gills, intestine, skin and muscles) collected from fish markets in Khartoum State. Hag Elsafi *et al*. (2009) reported the isolation of four *Salmonella* isolates from 119 fecal samples (3.4%) collected in Khartoum State. Two isolates were serotyped as *S. droganna* and the other two as *S. umbadah*.

Concerted efforts are needed to reduce the risk of *Salmonella* contamination throughout the food supply chain. On-going laboratory-based *Salmonella* surveillance is an essential step to support these efforts. The purpose of this investigation was to determine the prevalence of *Salmonella* from humans, animals, water and different food items in Khartoum State, Sudan.

MATERIALS AND METHODS

Collection of Samples

This study on *Salmonella* was performed in Khartoum State, Sudan during Sep. 2007 to June 2008. A total of 996 samples from five groups of sources were collected: 370 raw and cooked food samples from various vendors within Khartoum State; 97 chlorinated drinking water samples; 309 domestic livestock meat and fecal samples (cattle, sheep, goats, camels) from farms, Khartoum and Omdurman abattoirs and butcher shops and fresh and dried fish from Khartoum fish markets; 193 chicken samples (egg, skin, liver, meat, feces) from poultry farms and markets and 27 human fecal samples from restaurant workers tested for renewal of work permits.

Samples were transported refrigerated to the laboratory within 24 h and a 25 g aliquot from each sample was cultured and analyzed for the presence of *Salmonella*.

Isolation and Identification of *Salmonella*

Salmonella were isolated and identified according to the techniques recommended by the International Organization for Standardization (ISO 6579, 1998) and Quinn and colleagues (2004) as described by Molla *et al*. (2004).

For presumptive identification, suspect *Salmonella* colonies were subjected to biochemical tests and tested by slide agglutination with *Salmonella* polyvalent O (Denkafeiken, Japan) and H antisera (Mast Diagnostic, UK).

Salmonella Serotyping and Phagetyping

Salmonella isolates were shipped to the Public Health Agency of Canada, Office International des Epizooties (OIÉ) Reference Laboratory for Salmonellosis for confirmation by serotyping and phage typing as previously described by Molla *et al*. (2006).

RESULTS

A total of 92 out of 996 (9.2%) samples examined were positive for *Salmonella* (Table 1). These were as follows: 9 of the 370 (2.43%) food samples; 22 of the 309 (7.12%) animal meat and fecal samples; 35 of the 193 (18.13%) chicken (egg, skin, liver, meat and fecal) samples; 7 of the 97 (7.23%) water samples and 19 of the 27 (70.34%) human fecal samples. Of the 30 *Salmonella* serovars reported here, seven (23.33%) belonged to serogroup O:7 (C1), whereas six serovars (20.00%) to O:4 (B), four (13.33%) to group O:8 (C2, C3), three (10.00%) to group O:3,10 (E1) and ten of the serovars (33.33%) fit in other five serogroups as revealed in Table 2. The predominant serovars were: Stanleyville, Molade and Kentucky,

Table 1: *Salmonella* isolates from food, animals, chicken, human and water samples

	No. of samples		
Source	Examined	Positive	(%)
Food	370	9	2.43
Animal	309	22	7.12
Chicken	193	35	18.13
Water	97	7	7.23
Human	27	19	70.34
Total	996	92	9.2

Table 2: Serotypes and phagetypes of the recovered *Salmonella* isolates

Serogroup	Antigenic formula	*Salmonella* serotype	No. of isolates	(%)	*Salmonella* phagetype
O:4 (B)	4:fgs:-	Agona	6	6.5	
	4:d:7	Schwarzengrund	2	2.1	
	4:z4,z23:-	Stanleyville	10	10.8	
	4,5:i: 2	Typhimurium	4	4.3	2
	4:eh:-	I:4,12:eh:-	1	1.0	
	4:r,-:z15	I:4:12:r,-:enz15	1	1.0	
O:7 (C1)	6,7:r:5	Infantis	1	1.0	
	Var.14+ 6,7,14:d:l,w	Livingstone	6	6.5	
	6,7:z10:z15	Mbandaka	1	1.0	
	6,7:gms:-	Montevideo	1	1.0	
	6,7:fg:-	Rissen	1	1.0	
	6,7:r:2	Virchow	4	4.3	
	6,7:-:enx	I:6,7:-:enx	1	1.0	
O:8 (C2)	6,8:k:5	Blockley	6	6.5	
O:8 (C3)	6,8:z10:x	Hadar	4	4.3	
	8:20:i:z6	Kentucky	8	8.6	
	8,20:z10:z6	Molade	9	9.7	
O:9(D1)	9:12:gm:-	Enteritidis	3	3.2	21a, Atypical
O:3,10(E1)					
	10:eh:1,w	Meleagridis	1	1.0	
	10:eh:5	Muenster	2	2.1	
	10:1,z13:5	Uganda	2	2.1	
O:1,3,19(E4)	19:gst:-	Senftenberg	6	6.5	
	19:d:1,2	Umbadah	1	1.0	
O:13(G)	23:fg:-	Havana	1	1.0	
	22:z:6	Poona	1	1.0	
O:35(O)	35:fg:-	Adelaide	4	4.3	
	35:z4,z23:-	Alachua	2	2.1	
O:40(R)	40:b:x	Johannesburg	1	1.0	
Other	-:I,z13:5	I:rough form-O:I,z13:1,5	1	1.0	
	-:z29:-	I:rough form-O:z29:-	1	1.0	

Table 3: Distribution of *Salmonella* serovars by source

	Source and number of serovars					
Serovar	Food	Animal	Chicken	Human feces	Water	Total
Agona	1	0	0	5	0	6
Schwarzengrund	0	0	0	2	0	2
Stanleyville	0	4	6	0	0	10
Typhimurium	1	1	1	1	0	4
I:4,12:eh:-	0	1	0	0	0	1
I:4:12:r,-:enz15	0	1	0	0	0	1
Infantis	0	0	0	1	0	1
Livingstone	1	2	0	1	2	6
Mbandaka	0	0	1	0	0	1
Montevideo	0	0	0	1	0	1
Rissen	0	0	1	0	0	1
Virchow	0	0	4	0	0	4
I:6,7:-:enx	0	1	0	0	0	1
Blockley	1	3	2	0	0	6
Hadar	0	0	4	0	0	4
Kentucky	0	0	6	2	0	8
Molade	2	0	2	0	5	9
Enteritidis	2	0	0	1	0	3
Meleagridis	0	0	0	1	0	1
Muenster	0	0	2	0	0	2
Uganda	0	2	0	0	0	2
Senftenberg	0	4	0	2	0	6
Umbadah	0	1	0	0	0	1
Havana	0	0	1	0	0	1
Poona	0	0	1	0	0	1
Adelaide	0	2	2	0	0	4
Alachua	0	0	2	0	0	2
Johannesburg	0	0	0	1	0	1
I:rough-O:I,z13:1,5	1	0	0	0	0	1
I:rough-O:z29:-	0	0	0	1	0	1
Total	9	22	35	19	7	92

followed by Agona, Blockley, Senftenberg and Livingstone. The four *S. typhimurium* isolates were phagetype 2. Two of the three *S. enteritidis* isolates were phagetype 21a and one was an atypical phagetype.

Nineteen of the *Salmonella* serovars reported here were recovered for the first time in Sudan. These included: Umbadah, Stanleyville, Agona, Blockley, Hadar, Alachua, Molade, Kentucky, Schwarzengrund, Infantis, Virchow, Mbandaka, Rissen and Meleagridis. This in addition to other five unnamed serovars including two rough isolates.

Table 3 shows that serovars Typhimurium and Livingstone were isolated from four of five sources examined, whereas Molade and Blockley were isolated from three of the sources. The remaining serovars were either isolated from only one or two sources.

S. molade (71%) was the most common serovar among the water isolates. The most common serovar isolated from human feces was Agona (26%). The most commonly isolated serovars from food were Molade (23%) and Enteritidis (22%). In animal feces, serovars Stanleyville and Senftenberg (each 18%) were dominant and in chicken feces, Stanleyville and Kentucky (each 17%) were the dominant serovars.

DISCUSSION

In this study, *Salmonella* prevalence was highest in human feces (70.43%) followed by chicken feces (18.13%), water (7.23%), animal feces (7.12%) and various food items (2.43%). *S. agona* was the most predominant serovar recovered from human feces but it was not the

most prevalent serovar recovered from food items. Serovars Schwarzengrund, Infantis, Montevideo, Meleagridis and Johannesburg were recovered only from human feces. *S. stanleyville* was detected in both animal and chicken faeces but not from the other sources. Serovars Mbandaka, Rissen, Virchow, Hadar, Muenster, Havana, Poona and Alachua were detected only in chicken feces with varying degrees of frequency. Serovars I: 6, 7:-: enx, *S. uganda* and *S. umbadah* were only recovered from animal feces. One rough *Salmonella* serovar was recovered from a food item only and another rough serovar was isolated from one human fecal sample. The remaining serovars were isolated in low frequency from several sources. Remarkably, the human-associated *Salmonella* serovars *S. typhi* and *S. paratyphi* were not recovered from human subjects in this study, although they had been isolated in a few of the previous studies performed in Sudan (Unpublished work).

Salmonella was isolated from different sources with an overall prevalence of 9.2%, which is comparable to the isolation frequency (11.99%) reported by Roy *et al.* (2002) in USA. The prevalence percentage reported demonstrates the widespread occurrence and distribution of *Salmonella* in Khartoum State, Sudan. This prevalence was clearly higher than the range of 3.34-4.0%, reported in the limited studies previously conducted in Sudan (Soliman and Khan, 1959; Khan, 1962, 1970; Yagoub and Mohamed, 1987; El Tom *et al*., 1999; Yagoub *et al*., 2006; Elsafi *et al*., 2009). Several studies in other developing countries have reported a higher overall prevalence of *Salmonella* (human, food and animal) such as 68.2% in Ethiopia, 51.2% in Argentina, 25.9% in Korea and 72% in Thailand (Cardinale *et al*., 2003). It is important to recognize that the prevalence and distribution of *Salmonella* serovars varies from region to region (Dominguez *et al*., 2002; Uyttendaele *et al*., 1998) and isolation rates depend upon the country where the study was carried out, the sampling plan and the detection limit of the methodology (Roberts, 1982; Uyttendaele *et al*., 1998). Consequently, it is difficult to make comparisons between *Salmonella* surveillance surveys conducted in different countries. However, the serovars isolated from the various sample types in our survey in Sudan were comparable to the results reported by various investigators in other countries. For example, Baudart *et al*. (2000) reported the prevalence of *S. agona, S. enteritidis, S. infantis, S. mbandaka, S. muenster, S. rissen, S. typhimurium, S. montevideo* and *S. virchow* in different aquatic environment. The same serovars plus *S. senftenberg* were isolated by Saha *et al*. (2001) from faecal samples from hospitalized diarrhoeal children in India. As the case in this study, Liebana *et al*. (2001, 2002), Tamada *et al*. (2001) managed to isolate *S. mbandaka, S. montevideo* and *S. livingstone* from animal sources. The findings of Delicato *et al*. (2004) and of Fernandez *et al*. (2003) were also comparable to our results. They reported the isolation of *S. enteritidis*, *S. infantis* and *S. typhimurium* from human faeces. *S. typhimurium* and *S. enteritidis* in particular, are regarded worldwide as significant pathogenic serovars, with certain phagetypes being associated with serious illness in both humans, chickens and animals (Dominguez *et al*., 2002; Jorgensen *et al*., 2002; Roy *et al*., 2002; Mohammad *et al*., 2006). There were only a few *S. typhimurium* and three *S. enteritidis* strains isolated in this initial study, so it is not possible to comment on the predominant phagetypes of these two serovars in Sudan. Further surveillance would be needed to determine any trend in phagetypes among the different sources.

Many animal species harbor *Salmonella* and can act as potential reservoirs for human infections. *Salmonella* may enter the food chain through carcass contamination with animal feces at slaughter and during processing, or through food or food handlers. However, human infection may also occur through contaminated water, pets and exotic animals. Measures taken to control these routes of transmission are an effective way of preventing

salmonellosis. The collection of prevalence data of *Salmonella* serovars is an important component of a successful epidemiological surveillance for public health management in any country.

The high isolation rate of *Salmonella* from the human subjects indicates that more strict measures should be implemented in the food industry to curtail the spread of salmonellosis in Sudan. For example, monitoring of restaurant workers should be performed at more frequent intervals rather than the current mandatory annual check-up for renewal of work permits. This should be accompanied by organized training programs involving not just restaurant employees from the shop floor but suppliers of food items and corporate administrators. More involvement by public health authorities in surveillance programs is needed to ensure that public safety regulations are properly implemented.

This initial survey has provided useful information about the status of salmonellosis in Khartoum, Sudan. We have demonstrated that *Salmonella* was isolated mostly from humans followed by chickens. The *S.* Kentucky serovar was common to both groups, as well as food, suggesting that chicken and chicken-food products could be a potential source of salmonellosis in the food chain. Furthermore, *S.* Kentucky serovars are known for their resistance to ciprofloxacin, the antibiotic of choice for the treatment of typhoid in Sudan, (Roy *et al*., 2002). Thus this phenotype may spread to other serovars which might have greater potential for infection.

CONCLUSIONS

We have isolated 30 different *Salmonella* serovars, 19 of which were reported for the first time in Sudan, including the new serovar *S. umbadah.* This is of significance for both the animal and public health sectors in Sudan. Results of this study will provide a baseline for future human and animal *Salmonella* surveillance programs. The high frequency of *Salmonella* isolation in human feces underlines the necessity for continuous coordinated and collaborative Salmonellosis monitoring programs. We recommend the establishment of a national authority for administering such programs to identify the most prevalent serovars, their patterns of antimicrobial sensitivities, the genes conferring resistance and setting standards for poultry industry in Sudan.

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