Camel as a transboundary vector for emerging exotic *Salmonella* serovars

Nahed H. Ghoneim, Khaled A. Abdel-Moein and Hala Zaher

Faculty of Veterinary Medicine, Department of Zoonoses, Cairo University, Cairo, Egypt

ABSTRACT

The current study was conducted to shed light on the role of imported camels as a transboundary vector for emerging exotic *Salmonella* serovars. Fecal samples were collected from 206 camels directly after slaughtering including 25 local camels and 181 imported ones as well as stool specimens were obtained from 50 slaughterhouse workers at the same abattoir. The obtained samples were cultured while *Salmonella* serovars were identified through Gram's stain films, biochemical tests and serotyping with antisera kit. Moreover, the obtained *Salmonella* serovars were examined by PCR for the presence of *invA* and *stn* genes. The overall prevalence of *Salmonella* serovars among the examined camels was 8.3%. *Stn* gene was detected in the vast majority of exotic strains (11/14) 78.6% including emerging serovars such as *Salmonella* Saintpaul, *S. Chester*, *S. Typhimurium* whereas only one isolate from local camels carried *stn* gene (1/3) 33.3%. On the other hand, none of the examined humans yielded positive result. Our findings highlight the potential role of imported camels as a transboundary vector for exotic emerging *Salmonella* serovars.

KEYWORDS

Camels; exotic *Salmonella* serovars; vector

1. Introduction

Camels are multipurpose animals, humans reared camels for their meat, milk, hide and other purposes. Currently, it is important to note the upsurge in infectious diseases while camels were known to be infected with several pathogens, including major zoonoses such as brucellosis, tuberculosis, anthrax and salmonellosis [1]. Camels able to move very long distance and even cross country borders thereby they have incriminated in the introduction of exotic zoonotic diseases likewise what happened in Egypt as importation of camels from Sudan to Egypt was assumed to stand behind the introduction of rift valley fever virus into Egypt resulting in large outbreak of rift valley fever in 1977 [2,3]. Because of importation of camels is mainly for meat consumption, so the risk of introduction of emerging food-borne pathogens including new *Salmonella* serovars cannot be ruled out. Salmonellosis constitutes an important food-borne disease in both industrialized and developing countries resulting in severe illness among humans [4,5]. The virulence of *Salmonella* strains is represented by carrying virulence genes some of them are known to be involved in adhesion and invasion process such as *invA* gene but others are associated with the actual clinical manifestations of the pathogen such as *stn* gene [6]. However, over 2500 *Salmonella* serovars in the world are known, new serovars are still emerging each year [7]. *Salmonella* serovars were distributed throughout the world with predominance of some serovars in certain geographical

areas and emergence of others from time to time [8]. Moreover, movement of people, food animals and manufactured food products across boundaries facilitated the pan distribution of *Salmonella* strains among countries [9,10]. In Egypt, Salmonellosis was reported in camels and it was illustrated that apparently healthy slaughtered camels could be carriers of *Salmonella* species but little is known about the possible role of imported camels in the entrance of new emerging *Salmonella* serovars into Egypt [11]. Therefore, the current study was carried out to investigate the potential role of imported camels as a transboundary vector for exotic *Salmonella* serovars into Egypt which confer a serious public health implication.

2. Materials and methods

2.1. Collection of samples

Fecal samples were obtained from the rectum of 206 apparently healthy slaughtered camels (*Camelus dromedarius*) (25 local camels and 181 imported camels which came mostly from Sudan). Samples were collected directly from rectum just after slaughtering and placed in sterile cups. The age range of the examined camels was (3–5) years. Moreover, stool specimens were collected from fifty slaughterhouse workers (six were suffered from diarrhea and forty-four were apparently healthy), all humans participated in this study gave their consents. Both animal and human samples were collected from Bassitn abattoir, Cairo, Egypt. Samples were transported

in icebox to the laboratory of Zoonoses Department, Faculty of Veterinary Medicine, Cairo University for bacteriological examination.

2.2. Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella* was carried out according to the International Organization for Standardization ISO (6579) [12]. Briefly, fecal samples were pre-enriched in sterile buffered peptone water (BPW) (Oxoid, UK) in a ratio of 1 g of the sample to 9 ml of BPW. The pre-enriched samples were incubated for 16 to 18 hours at 37 °C. Then, one ml of the pre-enrichment broth was transferred aseptically into 10 ml of enrichment broth Rappaport Vassiliadis (RV) (Oxoid, UK) and incubated for 24 hours at 42 °C. After enrichment step, a loopful of enriched RV broth was plated on Xylose Lysine Desoxycholate agar (XLD) (Oxoid, UK) and incubated at 37 °C for 24–48 hours.

Presumptive *Salmonella* colonies were selected to make pure cultures. Isolates were identified as *Salmonella* sp. after Gram's stain films and full biochemical identification using commercially available identification test RapID ONE (Remel, USA). Afterwards, the obtained isolates were serologically confirmed and serotyped to recognize *Salmonella* serovars in the Central Laboratory of Egyptian Ministry of Health based on somatic (O) and flagellar (H) antigens with commercially available anti-sera kit (SIFIN, UK).

2.3. Molecular investigation

DNA was extracted from *Salmonella* isolates by PrepMan®Ultra Sample Preparation Reagent (Applied Biosystems) according to manufacturer's instructions. The molecular detection of *invA* gene of *Salmonella* and *stn* gene (*Salmonella* enterotoxin gene) were performed by conventional PCR assay using the primers listed in Table 1.

The PCR amplification of *invA* gene was carried out using HotStarTaq® Master Mix (Qiagen, Germany) and the reaction was performed with a thermal profile of 95 °C for 15 min followed by 35 cycles of denaturation (94 °C for 30 s), annealing (64 °C for 30 s) and extension (72 °C for 45 s) followed by final extension at 72 °C for 10 min while specific band was detected after electrophoresis step at 284 bp [13]. Furthermore, the molecular detection of *stn* gene was performed using HotStarTaq® Master Mix (Qiagen, Germany) at the following conditions: initial denaturation at 95 °C for 15 min then 25 amplification cycles of denaturation, annealing and extension at 94, 59

and 72 °C for 1 min in each step respectively followed by final extension at 72 °C for 10 min. Then, amplicons were entered electrophoresis step to detect specific bands at 617 bp (Figure 1) [6].

2.4. *Stn* gene sequencing and phylogenetic analysis

Amplicons of *stn* gene from *S. Wingrove* (from local camel); *S. Typhimurium* (exotic strain); *S. Saintpaul* (exotic strain); *S. Chester* (exotic strain); *S. enterica sub sp. salama* (exotic strain) were purified using Qiaquick kit (Qiagen, Germany) and sequencing step was conducted. The sequences were blasted in GenBank to identify the most similar sequences from which *S. Typhi*, *S. Paratyphi A* and three strains of *S. Typhimurium* were selected to make sequences identity matrix using BioEdit software version (7.0.9). Moreover, *stn* gene sequence of *S. Wingrove* (local strain) was aligned against the sequenced exotic strains and the retrieved ones from the GenBank as previously mentioned then phylogenetic tree was done through neighbor-joining method and 500 replicates of bootstrap using MEGA 6 software version(6.06) (Figure 2).

2.5. Accession numbers

Nucleotide sequences of *stn* gene from *S. Typhimurium*; *S. Chester* and *S. Saintpaul* obtained in the present study were deposited in the GenBank under the following accession numbers: KU981177; KU981178; KU981179 respectively.

3. Results

Of the 206 camels examined, 17 (8.3%) yielded *Salmonella* serovars in their feces. 3 (12%) from local camels and 14

Figure 1. Occurrence of *stn* gene among isolated *Salmonella* serovars from examined camels.

Note: Lanes M: DNA Ladder (100 bp); Lane 1: Negative control (sterile nuclease free water); Lane 2: Negative sample (*S. Kottbus*); Lanes 3–6: positive samples show specific bands at 617 bp.

Table 1. List of primers used for detection of *InvA* and *stn* genes.

Target gene	Primer	Primer sequence	PCR product (bp)	Reference
<i>InvA</i>	<i>Sal-f</i>	5'-GTGAAATTATCGCCACGTTCCGGGCAA-3'	284	[13]
	<i>Sal-r</i>	5'-TCATCGCACCGTCAAAGGAACC-3'		
<i>stn</i>	<i>stn-f</i>	5'-TTG TGT CGC TAT CAC TGG CAA CC -3'	617	[6]
	<i>stn-r</i>	5'-ATT CGT AAC CCG CTC TCG TCC -3'

Figure 2. Phylogenetic tree was constructed by neighbor-joining method to show the evolutionary history of the analyzed sequences. The analysis involved 10 sequences of *stn* gene of 5 sequenced isolates from the current study as well as 5 sequences from GenBank. The analysis was done using MEGA6 software.

Table 2. Prevalence of *Salmonella* species in the examined camels and humans.

Sample origin	No. examined	Positive samples for <i>Salmonella</i> species	
		No.	(%)
Local camels	25	3	12
Imported camels	181	14	7.7
Total camels	206	17	8.3
Humans	50	0	0

Table 3. Serotyping of *Salmonella* isolates and the occurrence of (*stn*) gene among them

Isolate origin	Serotypes (number)	Positive for <i>stn</i> gene	
		No.	(%)
Exotic	<i>S. Saintpaul</i> (2)	2	100
	<i>S. Cerro</i> (2)	1	50
	<i>S. Papuana</i> (1)	1	100
	<i>S. Reading</i> (2)	1	50
	<i>S. Butantan</i> (1)	0	0
	<i>S. Anatum</i> (1)	1	100
	<i>S. Chester</i> (2)	2	100
	<i>S. Typhimurium</i> (1)	1	100
	<i>S. enterica sub sp. salamae</i> (1)	1	100
	Rough strain (1)	1	100
Local	Total (14)	11	78.6
	<i>S. Typhimurium</i> (1)	0	0
	<i>S. Wingrove</i> (1)	1	100
	<i>S. Kottbus</i> (1)	0	0
	Total (3)	1	33.3

(7.7%) from imported ones. On the other hand, all human stool specimens were negative (Table 2). *Salmonella* isolates were serotyped as shown in (Table 3). All *Salmonella* isolates pose *invA* gene whereas 12 of them carried *stn* gene. *Stn* gene was more prevalent among exotic isolates

(11/14) while only one isolate from local camels carried *stn* gene (Table 3). Furthermore, *stn* gene sequencing and phylogenetic analysis revealed that exotic strains were separated from the local one in two different clusters whereas both local and exotic strains show high genetic relatedness to *stn* gene sequences of virulent strains (*S. Typhi*, *S. Paratyphi* and *S. Typhimurium*) (Table 4 and Figure 2).

4. Discussion

The results of the current study revealed that 8.3% of the examined camels were *Salmonella* positive. Such prevalence was in line with that obtained by Munch et al. [14] in the United Arab Emirates and Raufu et al. [15] in Nigeria who detected *Salmonella* in 8 and 6% of the examined camel fecal samples respectively. Higher results were obtained by Molla et al. [11] in Ethiopia (15.1%) and Mohamed and suelam (2010) [16] 13.9% in Egypt. Also, This finding was strongly supported by Wernery and Kaaden [1] who documented that camels could be chronic carriers of *Salmonella* species and intermittently shed the bacteria in their feces, especially at times of stress such as during parturition, concurrent diseases, starvation, overcrowding and transport; these carriers may constitute a human health threat through contact with infected animals or consumption of contaminated camel products.

The distribution of positive results among local camels and imported ones revealed prevalence rates (12%) and (7.7%) respectively. Importantly, imported camels yielded a wide array of exotic serovars that comprise

Table 4. *Stn* gene sequence identity matrix of *S. Wingrove* (from local camel), sequenced exotic *Salmonella* serovars and some *Salmonella* strains retrieved from GenBank.

	<i>S. Typhimurium</i> L16014	<i>S. Typhimurium</i> KF032246	<i>S. Typhi</i> JX293347	<i>S. Typhimurium</i> JX293346	<i>S. Paratyphi A</i> JX293348	<i>S. enterica sub sp.</i> <i>salamae</i> **	<i>S. Saintpaul</i> **	<i>S. Chester</i> **	<i>S. Typhimurium</i> **	<i>S. Wingrove</i> *
<i>S. Wingrove</i> *	ID	0.967	0.97	0.977	0.96	0.973	0.975	0.977	0.972	0.979
<i>S. Typhimurium</i> **	0.967	ID	0.953	0.96	0.967	0.961	0.963	0.963	0.958	0.958
<i>S. Chester</i> **	0.97	0.953	ID	0.987	0.949	0.966	0.968	0.965	0.967	0.97
<i>S. Saintpaul</i> **	0.977	0.96	0.987	ID	0.954	0.977	0.979	0.975	0.973	0.977
<i>Salmonella enterica</i> <i>sub sp. salamae</i> **	0.96	0.967	0.949	0.954	ID	0.953	0.953	0.953	0.942	0.949

N.B. *Strain from local camel current study; **exotic strains current study.

some dangerous ones such as *S. Typhimurium*, *S. Saintpaul* and *S. Chester*. These serovars have a great public health importance as *Salmonella* Typhimurium is the most commonly isolated serotype from humans with nontyphoid salmonellosis and accounts for death of nearly 50% of salmonellosis patients [17] because it has multiple virulence factors responsible for efficient colonization and invasion of gastrointestinal tract [18]. In similar vein, *S. Saintpaul* is usually incriminated in large food-borne outbreaks likewise those occurred in USA in 2008 and in 2013 [19,20]. Similarly, *S. Chester* was responsible for multistate food-borne outbreak in USA as well as another one in Canada [21,22].

All *Salmonella* isolates in the current study pose *invA* gene which encodes protein that responsible for epithelial cell invasion [23]. On the other hand, *stn* gene which encodes *Salmonella* enterotoxin that plays an important role in acute gastroenteritis [6,24,25] was more prevalent among exotic isolates (78.6%) than those obtained from local camels (33.3%) mentioning that *stn* gene was present in all exotic *S. Saintpaul*, *S. Typhimurium*, *S. Chester* serovars regarding these serovars of great public health importance as aforementioned above.

Interestingly, the results of *stn* gene sequencing of *S. Wingrove* (from local camel) and the selected exotic serovars as well as the retrieved *stn* sequences of *S. Typhi*, *S. Paratyphi A*, *S. Typhimurium* revealed high level of identity between the exotic serovars and *S. Typhi* (isolated from patient's blood) and *S. Paratyphi A* (isolated from patient's blood) especially *S. Saintpaul* (more than 0.975) and *S. Chester* (more than 0.966). Curiously, the phylogenetic tree constructed here demonstrates two clusters, one of them comprises both exotic *S. Saintpaul* and *S. Chester* as well as *S. Typhi* and *S. Paratyphi* while the other one represented only by *S. Wingrove* but exotic strains of *S. Typhimurium* and *S. enterica sub sp. salamae* are considered out-group. Therefore, the phylogenetic analysis does not only point out the difference between the local strain and the exotic ones but also highlights the public health importance of the recovered exotic strains *S. Chester* and *S. Saintpaul* which were grouped in the same cluster with highly virulent *Salmonella* serovars (*S. Typhi* and *S. Paratyphi*).

On the other side, none of fifty examined slaughterhouse workers yielded positive result nevertheless, the risk to contract *Salmonella* from infected animals cannot be excluded.

In conclusion, the occurrence of such enterotoxigenic exotic *Salmonella* serovars particularly *S. Saintpaul*, *S. Typhimurium*, *S. Chester* among the imported camels may contaminate camel carcasses and subsequently meat during slaughtering process and thereby these strains find its way toward human gut. Therefore, such findings alarm both veterinary and public health authorities about the potential role of imported camels as a vector for exotic emerging *Salmonella* serovars which

may initiate food-borne outbreaks. Thus, strict quarantine should be applied on imported camels with bacteriological examination to detect and eliminate *Salmonella* carriers as well as adequate hygienic measures should be implemented at abattoirs to avoid contamination of animal carcass during slaughtering process in order to get safe camel meat.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- [1] Wernery U, Kaaden OR. Infectious disease of camelids. Berlin: Blackwell Wissenschafts Verlag; 1995.
- [2] Ghoneim NH, Woods GT. Rift valley fever and its epidemiology in Egypt: a review. *J Med*. 1983;14:55–79.
- [3] Kamal SA. Observations on rift valley fever virus and vaccines in Egypt. *Virology*. 2011;8:532. doi:10.1186/1743-422X-8-532
- [4] Acha PN, Szyfres B. In: Zoonoses and communicable diseases common to man and animals, volume I: bacterioses and mycoses. 3rd ed. Pan American Health Organization; Washington, D.C. 2003; USA; 2001.
- [5] Eguale T, Engidawork E, Gebreyes WA, et al. Fecal prevalence, serotype distribution and antimicrobial resistance of *Salmonellae* in dairy cattle in central Ethiopia. *BMC Microbiol*. 2016;16:20. doi:10.1186/s12866-016-0638-2
- [6] Murugkar HV, Rahman H, Dutta PK. Distribution of virulence genes in *Salmonella* serovars isolated from man & animals. *Indian J Med Res*. 2003;117:66–70.
- [7] Kim S. *Salmonella* serovars from food borne and waterborne disease in Korea, 1998–2007: total isolates decreasing versus rare serovars emerging. *J Korean Med Sci*. 2010;25:1693–1699.
- [8] Molla B, Alemayehu D, Salah W. Sources and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997–2002. *Ethip J Health Dev*. 2003;17:63–70.
- [9] D'Aoust JY. *Salmonella* and the international trade. *Int J Food Microbiol*. 1994;24:11–31.
- [10] Plummer RAS, Blissett ST, Dodd CRR. *Salmonella* contamination of retail chicken products sold in the UK. *J Food Prot*. 1995;58:843–846.
- [11] Molla B, Mohammed A, Salah W. *Salmonella* prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedaries*) in Eastern Ethiopia. *Trop Anim Health Prod*. 2004; 36: 451–458.
- [12] International Organization for Standardization 6579. Microbiology of food and animal feeding stuff-Horizontal method for the detection of *Salmonella*. Geneva: ISO; 1998.
- [13] Rahn K, De Grandis SA, Clarke RC, et al. Amplification of an *invA* gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. *Mol Cell Probes*. 1992;6:271–279.
- [14] Münch S, Braun P, Wernery U, et al. Prevalence, serovars, phage types, and antibiotic susceptibilities of *Salmonella* strains isolated from animals in the United Arab Emirates from 1996 to 2009. *Trop Anim Health Prod*. 2012;44:1725–1738.
- [15] Raufu I, Odetokun IA, Oladunni F, et al. Serotypes, antimicrobial profiles and public health significance of *Salmonella* from camels slaughtered in Maiduguri central abattoir, Nigeria. *Vet World*. 2015;8:1068–1072.
- [16] Mohamed ME, Suelam I. Isolation of non-typhoid *Salmonella* from humans and camels with reference to its survival in abattoir effluents. *Glob Vet*. 2010;5:356–361.
- [17] Kennedy M, Villar R, Vugia DJ, et al. Hospitalizations and deaths due to *Salmonella* infections, Food Net, 1996–1999. *Clin Infect Dis*. 2004;38:S142–S148.
- [18] Foley SL, Lynne AM. Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. *J Anim Sci*. 2008; 86: E173–E187.
- [19] Mody RK, Greene SA, Gaul L, et al. National outbreak of *Salmonella* serotype Saintpaul infections: importance of texas restaurant investigations in implicating Jalapeno Peppers. *PLoS One*. 2011;6:e16579. doi: 10.1371/journal.pone.0016579
- [20] Centers for Disease Control and prevention (CDC). Multistate outbreak of *Salmonella* Saintpaul infections linked to imported cucumbers. Atlanta (GA): CDC; 2013. Available from: <http://www.cdc.gov/salmonella/saintpaul-04-13/index.html>
- [21] Centers for Disease Control and prevention (CDC). Multistate Outbreak of Human *Salmonella* Chester Infections. Atlanta (GA): CDC; 2010. Available from: <http://www.cdc.gov/salmonella/2010/cheesy-chicken-9-9-10.html>
- [22] Taylor J, Galanis E, Wilcott L, et al. An outbreak of *Salmonella* Chester infection in Canada: rare serotype, uncommon exposure, and unusual population demographic facilitate rapid identification of food vehicle. *J Food Prot*. 2012;75:738–742.
- [23] Osman KM, Marouf SH, Alatefeh N. Antimicrobial resistance and virulence-associated genes of *Salmonella enterica* subsp. *enterica* serotypes Muenster, Florian, Omuna and Noya strains isolated from clinically diarrheic humans in Egypt. *Microb Drug Resist*. 2013;19:370–377.
- [24] Chopra AK, Huang JH, Xu XJ, et al. Role of *Salmonella* enterotoxin in overall virulence of the organism. *Microb Pathog*. 1999;27:155–171.
- [25] Majtan T, Majtanova L, Timko J, et al. Oligonucleotide microarray for molecular characterization and genotyping of *Salmonella* spp. strains. *J Antimicrob Chemother*. 2007;60:937–946.