Isolation of Non-Typhoid *Salmonella* from Humans and Camels with Reference to its Survival in Abattoir Effluents

[1]M.E.M. Mohamed and [2]I.I.A. Suelam

[1]Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Egypt
[2]Diagnostic Laboratory, Veterinary Hospital, Zagazig University, Egypt

Abstract: Non-typhoid *Salmonella* (NTS) are cosmopolitan bacterial zoonotic agents. The present study was undertaken to determine the occurrence of NTS in human and camels and test the survival of *Salmonella enteritidis* in abattoir effluents using volume germ carriers technique. A total of 50 human stool samples including 30 diarrheic and 20 non-diarrheic were collected. Moreover, 108 camel samples were collected from 36 slaughtered camels including feces, mesenteric lymph nodes and liver, 36 each. All samples were cultured by the standard methods for NTS and serotyped. Four (8%) *Salmonella* isolates were obtained from human stool and 6(5.6%) from camel samples. The positive camel samples included 5 feces and one mesentric lymph nodes with the frequencies of 13.9% and 2.8%, respectively. Nevertheless, the liver samples were *Salmonella* free (N=36). Three replicates of volume germ carriers were inoculated with *Salmonella enteritidis* at 7 log CFU/ml in final mix. The contents of germ carriers were examined on days 7, 14, 21 and 28 for the detection of *Salmonella enteritidis* by the most probable number technique. The mean counts of the 3 replicates were 5, 3 and 0.7 log CFU/ml on days 7, 14 and 21, respectively. The mean number of *Salmonella* was decreased by 2.2 log week^{-1}. On the day 28, all the 3 replicates count were found to be under detection limit (< 0 log CFU/ml). The results indicate that aerobic storage of abattoir effluents could minimize the risk of salmonellosis in the environment under the research condition. It can be concluded that camels could be a potential reservoir for NTS to humans and animals.

Key words: Germ carrier · Zoonotic bacteria · Non-typhoid Salmonella · Survival

INTRODUCTION

Non typhoid *Salmonella* (NTS) are zoonotic agents and a wide variety of animals have been identified as a reservoir [1-3]. There are more than 2,500 *Salmonella* serovars in the world with new serovars emerging yearly [4]. Individuals infected with NTS exhibit mild gastrointestinal illness involving diarrhea, chills, abdominal cramps, fever, head and body aches, nausea and vomiting [5]. The prevalence of *Salmonella* species in food production animal herds constitutes a challenge for safe food production [6, 7].

A variety of foods have been implicated as vehicles transmitting salmonellosis to humans, including poultry, beef, pork, eggs, milk, cheese, fish, shellfish, fresh fruit and juice and vegetables [8-10]. There is a lack of available literature about the role of camel as a potential reservoir for NTS in Egypt. Salmonellosis was previously reported in calf camels with enteritis [11]. Moreover,

apparently healthy slaughtered camels could be carriers of *Salmonella* species [12].

Determining the risks associated with spreading of the circulating *Salmonella* serotypes in slaughtered camels could be done by monitoring its survival in abattoir effluents. The survival of *Salmonella* in abattoir effluents may pose a potential public health hazard and contribute to the re-inoculation of the pathogen in the herd environment [13]. The volume germ carrier [14] has been used for microbial tenacity studies. This technique avoids the contamination of investigated substrate because the pores (0.2μ) of the used membrane are permeable to the fluid material to pass and contact the bacteria. Nevertheless, the microorganisms cannot pass. So, the germ carrier enables the tested bacteria to be exposed to liquid substrate resembling the natural matrix. Germ carrier techniques were used previously to study the tenacity of microorganisms in slurry [15] and biogas units [16].

Corresponding Author: Mohamed, M. Elsayed, Department of Zoonoses, Faculty of Veterinary Medicine,
Zagazig University, Egypt, E-mail: bishet68@yahoo.de.

Salmonella serotyping is very important to the epidemiology studies [4]. Epidemiologically, it is very important to study the current status of NTS in human and camel sources to fulfill the prevention and control strategies for NTS infections. The present study was carried out to determine the occurrence of *Salmonella* species in humans and camels as well as its survival in abattoir effluents to monitor the potential risk of the circulating *Salmonella* species strains in the examined ecosystem.

MATERIALS AND METHODS

Collection of Samples: Fifty human stool samples from the out-patient clinic of a Public Hospital (Zagazig) and 108 camel samples from Zagazig abattoir, (Egypt) were collected during July and August 2010. From each slaughtered camel; about 100 g individual sample, each from feces (direct from the rectum), mesenteric lymph nodes and liver were obtained. All the examined camels were male and aged from 3 to 5 years. Samples were transported in ice box to the Diagnostic Laboratory, Veterinary Hospital, Zagazig University for analysis.

Isolation and Identification of *Salmonella* Species: Isolation and identification of *Salmonella* species was carried out according to the International Organization for Standardization [17] with some modifications. Briefly, about 25 g of each tissue sample was cut with a sterile scalpel. and the pieces were put in sterile mortar and homogenized with 225 ml of buffered peptone water (BPW). The pre-enriched samples were incubated for 16-20 h at 37°C.

One ml and 0.1 ml of the inoculated pre-enriched broth were transferred aseptically into 10 ml of selenite cyctine (SC) (Difco, USA) and 10 ml of Rappaport-Vassiliadis (RV) (Merck, Germany) and incubated for 24 h at 37°C and 42°C, respectively. Subcultures from SC and RV broth were inoculated onto brilliant green-phenol red-lactose-sucrose agar (BPLS) (Merck) and XLD agar (Oxoid) and incubated at 37°C for 24-48 h. The plate cultures were examined for suspected colonies and confirmed by API-20E strips (Biomereux, France). Serotyping was done in The Animal Health Research Institute, Giza, Egypt.

Preparation of Bacterial Suspension and Volume Germ Carriers: *S. enteritidis* was streaked onto nutrient agar plate. A colony loopful was inoculated in 50 ml nutrient broth and incubated at 37°C for 24 h. Bacterial count of the prepared bacterial suspension was done by the Most Probable Number (MPN) [18]. The volume germ carriers were kindly provided by Professor R. Boehm (Hohenheim University, Animal Hygiene Institute, Stuttgart, Germany). The germ carriers are designed in Technical Centre of Hohenheim University. The germ carrier consists of a hollow synthetic cylinder covered on both sides with semi permeable polycarbonate membrane (pore size 0.2 μ) which are fixed with two tight screwable rings [14]. Twelve grams of autoclaved abattoir effluents were inoculated into each germ carrier filled with 1.2 ml bacterial suspension with concentration of 7 log CFU/ml [16]. The germ carrier was closed by membrane filter (Sartorius, D-37070, Goettingen, Art. No. 23007-25-N) with rubber ring (Viton, Firma Karl Spaech, D-72516). The concentration of the tested bacteria after exposure is determined weekly by counting according to the MPN method as colony forming units/ml (CFU/ml) [18]. The pH of the germ carrier content was measured by pH meter. The detection period was defined as the number of days from preparing germ carriers in abattoir effluents until bacterial counts had fallen below the detection limit of *Salmonella* species (1 CFUg^{-1}) [19]. The decimation time (t_{90}), as defined by Schlundt [20], is the time taken for viable counts of a bacterial population to decrease by one logarithmic unit (log10), which is equivalent to 90% reduction.

Statistical Analysis: Statistical analysis has been done using SPSS version 11.0., Pearson correlation coefficient.

RESULTS

Table 1. Illustrates that 4 out of the 50 examined samples (8%), were found to contain *Salmonella* species. All the isolates (No=4) were serotyped as *S. enteritidis* and were recovered from diarrheic cases. The examined non-diarrheic cases were *Salmonella* free.

Out of the 108 examined samples, 6 (5.6%) were positive for Salmonella isolation and were recovered from feces and mesenteric lymph node, with the percentages of 13.9 and 2.8, respectively. The examined liver samples were *Salmonella* free. Out of the 6 serotyped *Salmonella* species isolates, both *S. enteritidis* and *S. typhimurium* (No=3 each) were identified (Table 2).

Table 1: Occurrence of non-typhoid *Salmonella* in the examined human stool samples

		Positive samples		
Source	No of examined samples	No	%	Serotype
Diarrheic cases	30	4	13.3	*S. enteritidis*
Non-diarrheic cases	20	0	0.0	-
Total	52	4	8.0	*S. enteritidis*

Table 2: Occurrence of non-typhoid *Salmonella* in the examined apparently healthy slaughtered camels samples

		Positive samples			
Source	No of examined samples	No	%	*S. enteri*tidis	*S. typhim*urium
Feces	36	5	13.9	3	2
Mesenteric lymph nodes	36	1	2.8	0	1
Liver	36	0	0.0	0	0
Total	108	6	5.6	3	3

Table 3: Survival of *S. enteritidis* in abattoir effluent using volume germ carrier technique

Time (day)	pH at sampling (mean of 3 replicates)	CFU/ml (Mean of 3 replicates)
0	7.1	7 log
7th	7.3	5 log
14th	7.4	3 log
21st	7.6	0.7 log
28th	7.5	<0 log

The survival of *S. enteritidis* in abattoir effluents using volume germ carriers is illustrated in Table 3. The mean *Salmonella* counts of recorded 3 replicates for each sampling time (0, 7th, 14th and 21st days) was 7 log, 5 log, 3 log and 0.7 log CFU/ml, respectively. The detection period of *Salmonella enteritidis* in abattoir effluents was < 28 days. Moreover, the decimation time (t_{90}) of *Salmonella* in abattoir effluents was found to be 2.2 log week^{-1}. The correlation between storage time and counting was significant at the 0.01 level (Pearson correlation coefficient).

DISCUSSION

Continuous surveillance studies for NTS in human and animals are important, since new *Salmonella* serovars are emerging yearly and serotyping is very important to the epidemiology study [4]. The present study demonstrated a high incidence rate (13.3%) of *Salmonella* infection in the examined human diarrheic cases. It was found that all serotypes (N=4) isolated from human stool samples were *S. enteritidis*. This lead to the conclusion that the circulating serotype in human at Zagazig Centre is *S. enteritidis*. Most human infections are caused by a limited number of *Salmonella* serovars which may vary from country to country and overtime [21]. High incidence

of *S. enteritidis* was also reported in Korea by [4], who reported that the percentage of *S. enteritidis* from all isolated *Salmonella* species from 1998 to 2007 was 47.5%. Moreover, *S. enteritidis* was the most prevalent serovar among the NTS serovars in the world between 2000-2002 [22]. In Kenya, *S. enteritidis* was isolated from 33.2% of other NTS serotypes and 4.8% of human cases [5].

A total of 6 *Salmonella* species isolates representing 2 different serotypes were isolated from the examined camels. The isolated *Salmonella* serotypes were *S. enteritidis* and *S. typhimurium* (3 isolates each). This lead to the conclusion that both serotypes are the predominant serotypes in camels at Zagazig centre. So, camels and its products could be a potential reservoir for NTS salmonellosis not only for the remaining camels but also to human and other animal species. *S. enteritidis* and *S. typhimurium* are the most common serovars causing human salmonellosis [23].

Previous reports from various countries have reported salmonellosis in camels in Sudan [24], Egypt [25], UAE [26], Iran [27] and Ethiopia [12]. Moreover, Selim [28] reported that healthy camels can be carriers of *Salmonella* species and NTS have been isolated from feces and lymph nodes of our investigated slaughtered camels. Camels that are chronic carriers of *Salmonella* species may present a human health hazard through consumption of

camel products [29]. The shedding of *Salmonella* species in chronic carrier camels is intermittent, especially at times of stress such as during parturition, concurrent diseases, starvation, overcrowding and transport [30].

In the present study, *Salmonella* species were isolated from camel feces with the percentage of 13.9 as shown in Table 2. The recorded infection rate of *Salmonella* species in camel feces is near to those reported by Molla *et al*.[12] whose result was 15.1% and *Salmonella* species were isolated from camel-calf diarrhea (13%) in eastern Sudan [11]. On the other hand, a higher infection rate with salmonellosis in calf camel (11/15, 73.3%) was recorded by Pegram and Tareke [31]. Fecal shedding of *Salmonella* species from diarrheic calf camel was reported with 13% by Salih *et al*. [11]. The present study recorded incidence of salmonellosis of 5.6% in camels which is higher than that found (4.3%) in UAE by Wernery [26]. The variation of *Salmonella* species incidence could be attributed to the overcrowding and transportation stresses which increase the excretion of *Salmonella* species. Concerning the occurrence of *Salmonella* species in the mesenteric lymph nodes, we recorded lower percentage (2.8) than that reported by Molla *et al*. [12] (15.9%).

In the present study, the survival of *Salmonella enteritidis* in aerobic stored abattoir effluents was determined. As shown in Table 3, the tested bacteria was detectable through days 21. The mean counts of 3 replicates were 5, 3 and 0.7 log CFU/ml on 7th, 14th and 21st days, respectively. The mean number of Salmonellae decreased by 2.2 log week^{-1}. On the 28th day, all the 3 replicates count were found to be under detection limit (< 0 log CFU/ml). A previous study was done by Lung *et al*. [32] who found that *Salmonella enteritidis* (10^7 CFU/g) in cow manure were not detectable after 48 h of composting at 45°C and 4 days at room temperature. Moreover, *Grewal et al*. [33] reported that *Salmonella* species in liquid manure at temperature lower than 32°C require 2-8 weeks to reduce the concentration of *Salmonella cerro* to undetectable levels. The obtained results reveal that *Salmonella enteritidis* was not detectable on 28th day in stored abattoir effluents; this indicates that the tested organisms were either dead or live under detection limit of the used isolation technique. The obtained regression lines (2.2 log week^{-1}) is higher than that found by Paluszak *et al*. [34] who found that *Salmonella senftenberg* decreased by 0.296 log week^{-1} in peat soil amended with slurry at 20°C. *Salmonella senftenberg* was no longer detected after 7 days in swine slurry applied to clay soil [19].

In conclusion, this study has shown that the predominant *Salmonella* species in human at Zagazig city are *Salmonella enteritidis*. Camel meat could be a potential reservoir for zoonotic *Salmonella* species. Further molecular epidemiological studies could be done to study the kinetic of incriminated *Salmonella* species clones in food chain. Under the research conditions, storage of abattoir effluents for 4 weeks or more could reduce the exposure risk to non-typhoid *Salmonella* in the environment.

ACKNOWLEDGMENT

REFERENCES

1. Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin and R.V. Tauxe, 1999. Food-related illness and death in the United States. Emergency Infectious Disease, 5: 607-625.

2. Adak, G.K., S.M. Long and S.J. O'Brien, 2002. Trends in indigenous food borne disease and deaths, England and Wales: 1992 to 2000. Gut, 51: 832-841.

3. Hald, T., D.M. Wong and F.M. Aarestrup, 2007. The attribution of human infections with antimicrobial resistant *Salmonella* bacteria in Denmark to sources of animal origin. Food Borne Pathology Disease, 4: 313-326.

4. Kim, S., 2010. Salmonella serovars from food borne and waterborne disease in Korea, 1998-2007: total isolates decreasing versus rare serovars emerging. J. Korean Medical Sci., 25: 1693-1699.

5. Kariuki, S., G. Revathi, N. Kariuki, H. Kiiru, J. Mwituria, J. Muyodi, J.W. Githinji, D. Kagendo, A. Munyalo and A. Hart, 2006. Invasive multidrug-resistant non-typhoidal *Salmonella* infections in Africa, zoonotic or anthroponotic transmission? J. Medical Microbiol., 55: 585-591.

6. Davies, R.H and C. Wray, 1996. Persistence of *Salmonella* Enteritidis in poultry units and poultry food. British Poultry Sci., 37: 589-596.

7. Threlfall, J.E., B. Rowe and L.R. Ward, 1993. A comparison of multiple drug resistance in Salmonellae from humans and food animals in England and Wales, 1981 and 1990. Epidemiology Infection, 111: 189-197.

8. Espi, E., H. DeValk, V. Vaillant, N. Quelquejeu, F. LeQuerrec and F.X. Wei, 2005. An outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections linked to the consumption of imported horse meat in France. Epidemiology Infection, 133: 373-376.

9. Mazurek, J., E. Salehi, D. Propes, J. Hoh, T. Bannerman, L.M. Nicholson, M. Bundesen, R. Duffy and R.L. Moolenaar, 2004. A multistate outbreak of *Salmonella enterica* serotype *typhimurium* infection linked to raw milk consumption-Ohio, 2003. J. Food Protection, 67: 2165-2170.

10. Varma, J.K., K.D. Greene, J. Ovitt, T.J. Barrett, F. Medalla and F.J. Angub, 2005. Hospitalization and antimicrobial resistance in *Salmonella* outbreaks. 1984-2002. Emergency Infectious Disease, 11: 943-946.

11. Salih, O., M.T. Shigidi, H.O. Mohamed, P. McDoungh and Y. Chang, 1998. The bacterial causes of Camel-calf (*Camelus dromedarius*) diarrhea in Eastern Sudan. Proceedings of the third Annual Meeting for Animal Production under Arid Conditions, pp: 132-137.

12. Molla, B., A. Mohammed and W. Salah, 2004. *Salmonella* prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedaries*) in Eastern Ethiopia. Tropical Animal Health and Production, 36: 451-458.

13. Baloda, S.B., L. Christensen and S. Trajcevska, 2001. Persistence of a *Salmonella enteric* serovars *typhimurium* DT12 clone in a piggery and in agricultural soil amended with *Salmonella*-contaminated slurry. Applied and Environmental Microbiol., 67: 2859-2862.

14. Rapp, A., 1995. Hygienisch-mikrobiologische Untersuchungen zum verhalten von ausgewaehlten Bacterien und Viren waehrend der laengerfristigen Speicherung von Fluessigmist in Guellegemeinschaftsanlagen. Doctor Thesis, University of Hohenehim, Animal Hygiene Institute, Germany.

15. Winter, D.W., A. Martens, A. Weber and R. Boehm, 1995. Inaktivierungskinetic von ECBO-virus in cerschiedenen Prozesstufen moderner Guelleaufarbeitungsanlagen: Untersuchungen mit der Filtersandwitch-Keimtraegermethode. 2. Symposium on Desinfection, Disinfection, Deratization in Animal Health and Environment Protection. Umag, Croatia Sept. 28-30. ISBN 953-96576-01.

16. Drca, M., 2007. Seuchenhygienisch-mikrobiologische Untersuchungen an einer mesophil betriebenen Biogasanlage zur Verwertung von Speiseresten in Verbindung mit methodischen Untersuchungen zum Nachweis von *Salmonellen* und *Escherichia coli* aus biologischem Material. Doctor Thesis, Faculty of Veterinary Medicine, Leipzig University, Germany.

17. ISO, 1998. Microbiology of food and animal feeding stuff-Horizontal Methods for the detection of *Salmonella*. ISO, 6579, International Organization for Standardization, Geneva.

18. DeMan, J.C., 1983. MPN tables, corrected. European J. Applied Microbiol. Biotechnol., 17: 301-305.

19. Boes, J., L. Alban, J. Bagger, V. Mogelmose, D.L. Baggesen and J.E. Olsen, 2005. Survival of *Escherichia coli* and *Salmonella typhimurium* in slurry applied to clay soil on a Danish swine farm. Preventive Veterinary Medicine, 69: 213-228.

20. Schlundt, J., 1984. Survival of pathogenic enteric bacteria in anaerobic digestion and on slurry-treated land. Disease Abstract International, C45, pp: 1025.

21. Fashae, K., F. Ogunsola, F.M. Aarestrup and R.S. Hendriksen, 2010. Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. J. Infection in Developing Countries, 4: 484-494.

22. Galanis, E., D.M. Wang, M.E., Patrick, N. Bensztein, A. Cieslik, T. Chalermchikit, A. Aidara-Kane, A. Ellis, F.J. Angulo and H.C. Wegener, 2006. World Health Organization Global Salm-Surv. Web-based surveillance and global *Salmonella* distribution, 2000-2002. Emergency Infectious Disease, 12: 381-388.

23. Morpeth, S.C., H.O. Ramadhani and J.A. Crump, 2009. Invasive non-Typhi *Salmonella* disease in Africa. Clinical Infectious Disease, 49: 606-611.

24. Curason, G., 1998. Une malade du dromadaire analogue au farcin du boeuf. Bull. Soc. Cent. Medecine Veterinaire, 71: 491-496.

25. Osman, A.R., 1995. Pathological study on intestinal infections in camels. Thesis, Faculty of Veterinary Medicine, Cairo University.

26. Wernery, U., 1992. The prevalence of *Salmonella* infections in camels (*Camelus dromedarius*) in the UAE. British Veterinary J., 148: 445-450.

27. Tadjbakheh, H., G.H. Aba-Ali and M. Haghigt, 1992. Incidence des proeurs de *Salmonella* chez les moutons et les chameaux en Iran. Revue de Medecine Veterinaire, 143: 361-365.

28. Selim, A.M., 1990. Salmonellosis in camels in Egypt. In: M.F. Wardeh, R.T. Wilson and A.A. Saied, Proc. International Conferance On Camel Production And Improved Dec, Tobruk Libya, pp: 10-13.

29. Matofari, J.W., A. Shitandi, P.L. Shalo, N.J. Nanua and M. Younan, 2007. A survey of *Salmonella enterica* contamination of camel milk in Kenya. African J. Microbiol. Res., 1: 46-50.

30. Wernery, U. and O.R. Kaaden, 1995. Infectious diseases of Camelids, (Blackwell Wissenschafts-Verlag, Berlin., pp: 12-49.

31. Pegram, R.G. and F. Tareke, 1981. Observation on the health and Afar livestock. Ethiopian Veterinary J., 5: 11-15.

32. Lung, A.J., C.M. Lin, J.M. Kim, M.R. Marshal, R. Nordstedt, N.P. Thompson and C.I. Wei, 2001. Destruction of *Escherichia coli* O157:H7 and *Salmonella enteritidis* in cow manure composting. J. Food Protection, 64: 1309-1314.

33. Grewal, S., S. Rajeev, S. Sreevatsan and F. Michel, 2006. Persistence of *Mycobacterium avium* subsp. *paratuberculosis* and other zoonotic pathogens during simulated composting, manure packing and liquid storage of dairy manure. Applied and Environmental Microbiol., 72: 565-574.

34. Paluszak, Z., A. Ligocka, B. Breza-Boruta and H. Olrzewska, 2003. The survival of selected fecal bacteria in peat soil amended with slurry. Electronic J. Polish Agricultural Universities, 6: 1-6.