j.Egypt.vet.med.Assoc 78, no 4. 505 – 511 (2018)

BACTERIOLOGICAL SURVEY ON *SALMONELLA* SEROVARS FROM SLAUGHTERED CATTLE

By

Maher, A., Atia[1], I, Rayan, I.A.1, Abdelmordy, W. [1], Saad A., Atia, S.A[2]. and Orabi,A.*[3]

[1] Veterinary Services Department, Egyptian Armed Forces., Egypt.

[2] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt

[3*] Corresponding author: Email: drorabi2012@yahoo.com , Tel : 01124666847

ABSTRACT

Although more than 2400 *Salmonella* serovars have been identified worldwide, only about a dozen serovars account for more than 75% of the isolates reported from human beings and animals. Bacterial pathogens are especially of most serious concern regarding the issues of meat safety to consumers. In the present study standard ISO 6579 method was used to investigate the presence of *salmonella*e in slaughtered cattle. *Salmonellae* were detected in 28 % gall bladder samples, 24 % duodenum samples, 10 % hide swabs,10 % liver samples, 8 % Pre-femoral (pre- corral) lymph node swabs, 6 % Pre-scapular lymph node swabs and 0 % raw muscle meat samples. *Salmonella* isolates were identified as *S. Muenster* (n=17), *S. Typhimurium* (n=15), *S.* Kentucky (n=4), *S. Anatum* (n=3), *S. Nyborg* (n=3) and *S.* Livingstone (n=1).

Key words:

Slaughtered cattle*, salmonellae***,**Incidence.

INTRODUCTION

Cattle may be reservoir of several bacterial pathogens that are present in their gastrointestinal tract without any clinical signs in animals shedded microorganisms in the feces may infect other animals as well as contaminate hides in abattoirs. Furthermore, the bacteria can be also transferred to the carcasses during the slaughter and dressing processes **(Bell,1997).**

The European Union countries are the second most common food group where meat products are contributing to human salmonellosis in 2005. Contamination of beef during slaughter and processing is the major risk of subsequent food-borne infection of the consumers **(Norrung and Buncic 2008).** Foodborne diseases represent an important public health problem, significantly affecting the health of the population with major economic consequences

(FAO, 2002). Bacterial pathogens are especially of most serious concern regarding the issues of meat safety to consumers **(Sofos, 2008).** A wide array of pre-harvest, harvest, and post-harvest processes cause human pathogen contamination of raw meat products **(Li and Mustapha, 2005)**. The main reservoir of zoonotic *Salmonella* is food animals and beef represents one well-recognized source of human infection **(USDA, 2007);** and the main sources of infections in industrialized countries are animal-derived products, notably fresh meat products and eggs. In developing countries, contaminated vegetables, water, and human-to-human transmission are believed to contribute to comparatively larger proportion of human cases than those in industrialized countries **(Acha and Szyfres, 2001)**. *Salmonella* is an infectious agent causing typhoid, paratyphoid and enteritis (food poisoning) in man and many other diseases in domestic and wild animals as well as birds. The genus *Salmonella* is a member of the bacterial family *Enterobacteriaceae*. It is a Gram negative, straight, non-spore forming rods peritrichous flagellated, facultative anaerobic and can grow well under both aerobic and anaerobic conditions **(Wagner, 2010).** More than 2400 serovars of *Salmonella enterica* are recognized now and are capable of infecting a variety of animal species, poultry as well as human.*Salmonella enterica* serovars,Typhimurium, Enteritidis, Agona, Virchow, Montevideo, Hadar.....etc. are the serotypes most commonly associated with human salmonellosis **(Rajashekara, *et al*., 2000 and Cormican *et al*., 2002).**So the current study aimed to survey on *Salmonella* recovered from meat with special reference to biochemical and serological identification of recovered isolates from cattle samples.

MATERIAL AND METHODS

Samples:

Three hundred fifty swabs were tested for *Salmonella* spp. from January 2016 to February 2017 from Ismailia Governorate, Egypt. The collected samples included 50 raw muscle meats,50 livers,50 gall bladder,50 hide,50 pre-scapular lymph nodes, 50 pre femoral (pre-crural) lymph nodes and 50 duodenum swabs (Table 1). All samples were appropriately labeled and held at 4°C, transported to the laboratory within 1-4 hrs. and analyzed for *Salmonella* within 24 hrs.All samples were subjected immediately to bacteriological examination for *Salmonella* microorganism.

Isolation and identification of *Salmonellae* (Quinn *et al*., 2011).

Each swab was inoculated onto 10 ml peptone water broth and incubated aerobically at 37°C for 24 hours. Then a loopful from each inoculated and incubated enrichment fluid medium was subculture onto the Rapapport-Vassiliadis broth tube. The tubes were incubated at 42°C for 16-18 hours. Then a loopful from each inoculated and incubated enrichment fluid medium was subculture onto the surface of a plate of XLD agar. The plates were incubated at 42°C for 24 hours. Smears from suspected colonies were prepared and stained with Gram's stain to be examined microscopically before being transferred into semisolid agar for further biochemical identification. Serological identification of *Salmonella* isolates was done at the Central Public Health Laboratories, Ministry of Public Health, and Cairo, Egypt. Typing of *Salmonella* organisms included the detection of the major components of the "O" antigen and both phases of "H" antigen. Isolates that were preliminary identified biochemically as *Salmonella* were subjected to serological identification according to Kauffman and White Scheme **(Kauffman, 2001)**.

RESULTS

Bacteriological findings of examined cases from cattle carcass revealed that 14 out of 50 (28 %) gall bladder samples were positive for *Salmonella*, 12 out of 50 (24 %) duodenum samples were positive for *Salmonella*, 5 out of 50 (10 %) hide swabs was positive for *Salmonella*, 5 out of 50 (10 %) liver samples was positive for *Salmonella*, 4 out of 50 (8 %) A pre-femoral (pre-crural) lymph node swab was positive for *Salmonella,* 3 out of 50 (6 %) Pre-scapular lymph node swabs was positive for *Salmonella* and zero out of 50 (0 %) raw muscle meat samples was positive for *Salmonella* as shown in (Table 2). The results of serotyping of *Salmonella* isolated from cattle carcass in (Table 3) showed that 43 isolates of *Salmonellae* were identified into 6 serovars, namely; *Salmonella* Muenster (n=17), *S.* Typhimurium (n=15), *S.* Kentucky (n=4), *S.* Anatum (n=3), *S.* Nyborg (n=3) and *S.* Livingstone (n=1) with an incidence of (39.5 %), (34.9 %), (9.3 %), (6.9%), (6.9 %), (2.3 %), respectively .

Table (2): Incidence of *Salmonella* in cattle carcass according to different sites of sampling.

Samples	n=sample	Positive for *Salmonella*	(%)
Gall bladder	50	14	28
duodenum	50	12	24
hide swabs	50	5	10
Liver	50	5	10
Pre-femoral lymph node	50	4	8
Pre-scapular lymph node	50	3	6
Raw muscle meat	50	0	0
Total	350	43	12.28

Table (3): Serotyping of *Salmonella* isolated from collected samples.

Salmonella serovars	Antigenic Formula			No.	*Salmonella** %	Samples** %
	O antigen	H antigen				
		Phase 1	Phase 2			
S. Muenster	3,(10) (15) (15,34)	eh	1,5	17	39.5	4.85
S. Typhimurium	1,4,(5),12	i	1,2	15	34.9	4.28
S. Kentucky	8,20	i	z_6	4	9.3	1.14
S. Anatum	3,(10) (15) (15,34)	eh	1,6	3	6.9	0.85
S. Nyborg	3,(10),(15)	eh	[1,7]	3	6.9	0.85
S. Livingstone	6,7,14	d	Lw	1	2.3	0.28

***Total No. of *Salmonella* isolated from cattle carcass (43).**

****Total No. of samples collected from cattle carcass (350).**

DISCUSSION

The incidence of human salmonellosis is rising in most countries where surveillance networks have been set up. World Health Organization (WHO) recorded that the problem became one of the diseases of public health significance. While multiple drug resistant *S. Typhi* (the causative agent of typhoid fever in man) is responsible for numerous outbreaks possessing a major threat to the affected persons due to failure of treatment of the disease **(Breuil *et al*., 2000 and Wain *et al*., 2003)**. In many global surveillance data indicated that the number of salmonellosis has increased mainly associated with the consumption of raw or undercookedeggs,poultry,meat or dairy products, demonstrating the importance of controlling this pathogen in food production **(Braden, 2006; Kimura *et al.*, 2004; Zhao *et al.*, 2001).** Cattle are a major reservoir for *Salmonella* that is carried in the intestinal tract of healthy animals and excreted in feces **(Chapman *et al,* 1993).**

Due to chemical composition and biological characteristics, meat and meat products are highly perishable foods. Meat an excellent source for growth of many notorious microorganisms such as mesophilic and psychrophilic bacteria those can cause infection in human, spoilage of meat and economic loss **(Kalalou and Ahami, 2004).** In the present study standard ISO 6579 method was used to investigate the presence of *salmonellae* in slaughtered cattle. *Salmonellae* were detected in 28 % gall bladder samples, 24 % duodenum samples, 10 % hide swabs,10 % liver samples, 8 % Pre-femoral (pre-crural) lymph node swabs, 6 % Pre-scapular lymph node swabs and 0 % raw muscle meat samples as shown in (Table 2). *Salmonella* isolates were identified as *S.* Muenster (n=17), *S.* Typhimurium (n=15), *S.* Kentucky (n=4), *S.* Anatum (n=3), *S.* Nyborg (n=3) and *S.* Livingstone (n=1).

Local Slaughterhouse environment is observed conducive for the growth of microorganisms, which can rapidly render the meat unsafe for human consumption. The poor hygiene and sanitation prevailing in the abattoirs as well as the shops encourage microbial contaminations and growth. The higher microbial load in the shops further enhances the chances of early meat spoilage **(Sudhakar *et al,* 2007).**Microbiological contamination of carcasses occurs mainly during processing and handling, such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments **(Abdallah *et al.,* 2009**). The external contamination of meat constitutes a constant problem in most developing countries, in the abattoir itself where there are large numbers of potential sources of contamination by microorganisms **(Davis *et al.,* 2002).** Every treatment done to the food animal carcass from the point of

slaughtering until it ready for consumption,including preparation of the carcass, transportation and handling, will add to the bacterial load of its meat **(Ali, 1992).**

CONCLUSION

Our study concluded that *main Salmonella* serovars recovered from slaughtered cattle *S.* Muenster*, S.* Typhimurium, *S.* Kentucky, *S.* Anatum*, S.* Nyborg*,* and *S.* Livingstone.

REFERENCE

Abdallah, M.A.; Suliman, S.E.; Ahmed, D.E. and Bakhiet, A.O. (2009): Estimation of bacterial contamination of indigenous bovine carcasses in Khartoum (Sudan). Afri. J. Microbio. Res., 3 (12):882-886.

Acha, P. N., and Szyfres, B. (2001): *Salmonella.* In B. Szyfres (Ed.), Zoonoses and communicable diseases common to man and animal. PAHO Science and Technical Publication, No. 5.

Ali, S.A. (1992): Evaluation of some animal and plant protein sources in the diet of the shrimp Penaeus indicus. Asian Fisheries Science 5, 277-289.

Bell RG. (1997): Distribution and sources of microbial contamination on beef carcasses. J Appl Microbiol 82: 292-300.

Braden CR. (2006): *Salmonella* enterica serotypes Enteritidis and eggs: a national epidemic in the United States. Clin Infect Dis 43:512-517.

Breuil, J., Brisabois, A., Casin, I., Armand-Lefèvre, L., Frémy, S., Collatz, E. (2000): Antibiotic resistance in *salmonellae* isolated from humans and animals in France: comparative data from 1994 and 1997. Journal of Antimicrobial Chemotherapy, 46, 965 - 971.

Chapman P. A., P. M. Zadik and C. A. Siddons, (1993): J. Med. Microbiol. 39:155-158.

Cormican, M., DeLappe, N., O'Hare, C., Doran, G., Morris, D., Corbett-Feeney, G., Fanning, S., Daly, M. *et al.* (2002): *Salmonella* enterica serotype Bredeney: antimicrobial susceptibility and molecular diversity of isolates from Ireland and Northern Ireland. Appl Env Microbiol 68, 181-186

Davis, M. A. and Conner, D. E. (2002): Factors Affecting Campylobacter Incidence on Various Poultry Meat Products. pp. 446 - 449, IN Conference Proceedings - 7th WPSA Asian Pacific Federation Conference/12th Australian Poultry and Feed Convention.

Kauffmann. F., and W. Das - Kauffmann (2001): Antigenic formulas of the *Salmonella* serovars. WHO co-operating center for reference and research on Salmonella.8th Ed, cited by pop off, M.Y., Paris, France.

FAO.(2002): FAO/WHO global forum of food safety regulators. Marrakech, Morocco. Rajashekara. G., E. Haverly, D.A. Halvorsan, K.E. Ferris, D.C. Lauer, K.V. Nagaroja Multidrug-resistant *Salmonella* typhimurium DT104 in poultry J. Food Prot., 63 (2000), pp. 155-161.

Kalalou I, M. Faid and A. Ahami (2004): Elect. J. of Biotic. 7: 0717-3458.

Kimura AC, Reddy V, Marcus R, Cieslak PR, Mohle-Boetani JC, Kassenborg HD *et al.* (2004): Chicken consumption is a newly identified risk factor for sporadic *Salmonella* enterica serotype Enteritidis infections in the United: a case-control study in Food Net sites. Clin Infect Dis 38 (Suppl 3):244 -252.

Li, Y., and Mustapha, A. (2005): Application of a multiplex PCR for the simultaneous detection of Escherichia coli O157:H7, *Salmonella* and Shigella in raw and ready-to-eat meat products. Meat Science, 71, 402 - 406.

Norrung B and Buncic S.(2008): Microbial safety of meat in the European Union. Meat Sci 78:14-24.

Quinn, P.J., Markey, B.K., Leonard, F.C., FitzPatrick, E.S., Fanning, S.; and Hartigan, P.J. (2011): Veterinary microbiology and microbial disease. Second. ed: Blackwell Science Ltd. ISBN 978 - 1 - 4051-5823 -7.

Sofos, J. N. (2008): Challenges to meat safety in the 21st century. Meat Science, 78, 3e13, Resistance integrons and super-integrons; a review. Clinical Microbiology and Infection, 10, 272-288.

Sudhakar, G. B., A. T. Sherikar, A. M. Paturkar, V. S. Waskar and R. J. Zende (2007): J. Food Cont. 18:854 - 858.

U.S.D.A. (2007): A statistical model for attributing human salmonellosis to meat, poultry, and eggs. Food safety summit, Arlington, Virginia. Attributing human *salmonellosis* to food sources. http:www.fsis.usda.gov/PPT/RBIguo.ppt261,2 Retrieved 28.07.10.

Wagner, A. B., Jr. (2010): Bacterial food poisoning. http://generalhealthtopics.com/ bacterial-food-poisoning-330.html Retrieved 02.08.10.

Wain, J., Nga, D., Kidgell, C., James, K., Fortune, S., Diep, S., Ali, T., Gaora, Parry, C., Parkhil, J. (2003): Molecular analysis of inch II antimicrobial resistance plasmids from *Salmonella* serovar typhi strain associated with typhoid fever. Veterinary World, EISSN: 2231-0916.

Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D.G. White, D. Wagner and J. Meng, (2001): Prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork and beef from the Greater Washington, D.C., Area. Applied and Environmental Microbiology, 67(12): 5431-5436.