African Journal of Microbiology Research Vol. 6(2), pp. 365-370, 16 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1050
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Situation of *Salmonella* contamination in food in Hebei Province of China in 2009 - 2010

Qiumei Shi[1,2], QiuYue Wang[2], Yanying Zhang[2], Cuizhen Chen[2], Hai Fang[2]*, Zengqiang Yuan[1] and Liliang Xia[1]

[1]Key Laboratory of Preventive Veterinary Medicine of Hebei Province, Hebei Normal University of Science and Technology. Qinhuangdao, 066600, China.
[2]Institute of Biophysics, Chinese Academy of Sciences, State Key Laboratory of Brain and Cognitive Science, Chaoyang District, Beijing, 100101, China.

Accepted 22 November, 2011

A total of 139 samples including 29 pork, 21 chicken, 19 beef, 17 mutton, 18 chicken's egg, 10 duck's egg, 20 fish and 5 seafood samples were collected from market in Hebei province of China in 2009-2010. These samples were examined for the presence of *Salmonellae* by cultural methods and biochemical tests. The isolates were characterized using serotyping testing. As a result, nine (6.9%) of them were positive for *Salmonella*, in which the most common serovar were *Salmonella typhimurium* (6/9). The positive rates of eggs, fish, chicken, pork and beef were 11.1% (2), 10% (2), 9.5% (2), 6.9% (1) and 5.3% (1), respectively. In addition, *Salmonella paratyphi* (1) and *Salmonella choleraesuis* (2) were also isolated. Potential hazard of *Salmonellosis* did not be underestimated in free market. In order to reduce *Salmonellosis*, it is important to strengthen food sanitation management especially to animal products. Results from these studies could form a basis for risk assessment and future interventions, which intended to reduce the incidence of *Salmonella* in Hebei province of China.

Key words: *Salmonella,* surveillance, serotype.

INTRODUCTION

Salmonella spp. is gram-negative and facultative anaerobe bacteria consisting of non-spore forming bacillia. *Salmonella* are relatively widespread in the environment and within food animals (Rodriguez et al., 2006; Humphrey, 2000). More than 2500 serovars of *Salmonella* have been identified in different parts of the world (Jones et al., 2008). According to the antigenic structural, *Salmonella* are classified to 34 strains, such as A, B, C, D, E etc. Most human infection is caused by the limited number of serovars, such as *S. choleraesuis*, *S. typhimurium, S. enteritidis*, *S. gallinarum*.

With the rapid development of economy and improvement of people's living conditions, people pay more attention to food security in recent years in China (Osman et al., 2010; Callaway et al., 2008).

However, deadly case caused by food contaminant occurs occasionally. *Salmonella enterica* bacteria are estimated to cause 1.3 million cases of gastroenteritis each year in the United States (Mead et al., 1999). In a variety of animal products for human consumption, food poisoning caused by *Salmonella* is the most common and ranks front row. In the United States *Salmonella* was estimated to represent the leading cause of foodborne illnesses due to bacterial pathogens in 2006 (Centers for Disease Control Prevention, 2007a). In China, 70 - 80% cases of bacterial food poisoning were caused by *Salmonella* and approximately 90% of livestock products which were contaminated by *Salmonella* were meat, egg and milk (Zhang et al., 2001). *Salmonella* is one of the zoonotic pathogens, which is of great importance on public health science. Whether people can be infected

*Corresponding author. E-mail: Fanghaihb@163.com. Tel: 0335-8387028 or 13803352036. Fax: 0335-2039084.

Table 1. The selected standards in the five biochemical tests[a].

	Hydrogen sulfide	Lysine	Motility	D-Mannitol	ONPG
S1	+	+	+	+	-
S2	+	+	+	+	+
S3	-	+	+	+	-

[a]Biochemical identification results consistent withS1-S3 model is suspected *Salmonella.*

depends on the serovars of *Salmonella* and their physical conditions. Many serovars of *Salmonella*, (e.g. *S. typhimurium* and *S. enteritidis*) can lead to the food-borne diseases such as nausea, intestinal cramps, diarrhea, vomiting and possible arthritic symptoms, and can be an intracellular pathogen (Centers for Disease Control Prevention, 2007a; Zhang et al., 2001; Glynn and Bradley, 1992). Therefore, it is essential to test *Salmonella* in food in order to dispose contaminated food in time and maintain a strong defense against them entering into the consumer market.

Monitoring the occurrence and distribution of *Salmonella* in food is important to detect possible outbreaks, to identify possible sources of infection and to target prevention and control measures. The aim of this study is to detect the prevalence of *Salmonella* spp. in a variety of food animal species and foods available at the market in Hebei province of China between January 2009 and March 2010.

MATERIALS AND METHODS

A total of 139 samples were collected including pork (29), chicken (21), beef (19), mutton (17), eggs (35), fish (20), shrimp (3), jellyfish (2) from 8 cities about (?) markets which are located in the utility area and retail fresh meats in Hebei Province between January 2009 and March 2010. Following the standards of the collecting samples, all the samples were collected. Each market was sampled only once and were kept at -20℃ (eggs at 4℃) in the laboratory (Appendix).

Isolating culture

At the markets where the samples were obtained, approximately 25 g of pork, chicken, beef and mutton (freezing) were purchased and cut into several pieces. All samples were placed directly into 225 ml Buffered Peptone Water (BPW) as pre-enrichment media. After the cultures were incubated at 37℃ for 4 h, 10 ml of each was transferred to 100 ml selenite-cystine enrichment media, and incubated at 37℃ for 18 - 24 h. If the samples were fresh (such as eggs and marine product), pre-enrichment media is unnecessary. Samples were placed directly into 25 ml sterile physiological saline and homogenated. The selenite-cystine enrichment media were incubated for 18-24h at 37℃ and streak plating onto CHROMagar color plates which are the choose plates to isolate *Salmonella*. After 24 h of incubation at 37℃, presumptive *Salmonella* colonies were used to inoculate triple sugar iron agar, lysine decarboxylase media, D-Mannitol media and dulcitol ß-galactosidase (ONPG) media (FDA-CFSAN, 2006), which were then incubated for 24 h at 37℃. According to the Table 1, colonies were roughly selected. The identities of *Salmonella* isolates were confirmed by biochemical tests using the semi-automatic Microbe analyzer K-3401.

Serotyping

Confirmed isolates were further serotyped for agglutination with *Salmonella* O and H antigens (Lanzhou Institute of Biological Products). The single bacterial colony was added into the physiological saline and made into liquid of 0.5M (about 1.5×10^8 CFU/ml). It was used to the sevovar agglutination after boiling. A clean glass slide was prepared for the somatic antigen (O). Two areas with 1 cm × 2 cm on the slide were chosen. Each area was put 1/2 loop-ring of the bacterium. A region in which the left part add 1 drop of polyvalent somatic antigen (O) antiserum, the right part of anther region with 1 drop of saline as control. The colonies were admixture into emulsion by the inoculation loop for 1 min, and then observed with dark background. Any degree of agglutinate phenomenon is positive reaction.

Slide agglutination was done with the A - F polyvalent somatic antigen (O) serum in order to identify the O antigen. At the same time, physiological saline was compared as control. Those, in the saline positive strains for the rough-shaped, cannot be typed. The positive strains in the A - F blood serum forming serum agglutination were continue to aggregate with the factor serum O4, O3, O10, O7, O8, O9, O2 and O11. Then O groups were determined. If the strains were positive in the O3 and O10, continued to do with the O10, O15, O34 and O19. Then, the subsets were identified. For the flagellar antigen (H), the method was carried out as described previously and identified H antigen according to Table 2. All the *Salmonella* were further identified and confirmed in the professional laboratory of *Salmonella* identification in Chengdu Institute of Biological Products of China.

RESULTS

A total of 139 samples were collected in Hebei province from January 2009 to March2010 and the situation of *Salmonella* contamination in food was detected in this study. Nine (6.9%) of 139 samples were positive for *Salmonella.* The result was showed in Table 3. In different samples, the prevalence of *Salmonella* in samples of chicken's egg and fish were 11.1 and 10.0%, while the positive rates of chicken, pork and beef were 9.5, 6.9, and 5.3%, respectively. *Salmonella* in mutton, duck's egg and other marine product were not found (Table 3). In different areas, the food samples from Baoding, Hengshui, Xingtai, Qinhuangdao and Tangshan were contaminated by *Salmonella* (Table 4). Positive of *Salmonella* isolates were further serotyped for agglutination with *Salmonella* O and H antigens. The results were showed in the Table 5. The most common serovar were *S. typhimurium* (6/9). Two strains of *S. paratyphi* and one strain *S. choleraesuis* were detected, respectively.

Table 2. Flagellar (H) antigen in common *Sallmonella* (Types A – F).

Type	Flagellar(H) antigen	
	Phase 1	**Phase 2**
A	a	none
B	g, f, s	none
B	i, b, d	2
C1	k, v, r, c	5, Z15
C2	b, d, r	2,5
D(produce gas)	d	none
D(no gas)	g, m, p, q	none
E1	h, v	6, w, x
E4	g, s, t	none
E4	i	--

Table 3. Positive rates of *Salmonella.*

Species	Sample	Positive	Positive rates (%)
Total	139	9	6.5
Pork	29	2	6.9
Chicken	21	2	9.5
Beef	19	1	5.3
Mutton	17	0	0
Chicken's egg	18	2	11.1
Duck's egg	10	0	0
Fish	20	2	10.0
Shrimp	3	0	0
Jellyfish	2	0	0

Table 4. Results of Salmonella in different foods.

Species	Area	Serovar
Chicken	Baoding	*S. typhimurium*
	Baoding	*S. paratyphi A*
Chicken's egg	Qinghuangdao	*S. typhimurium*
Pork	Tangshan	*S. paratyphi A*
	Hengshui	*S. typhimurium*
Beef	Xingtai	*S. typhimurium*
	Hengshui	*S. typhimurium*
Tilapia	Qinghuangdao	*S. choleraesuis*
Croaker	Qinghuangdao	*S. typhimurium*

DISCUSSION

This study was detected *Salmonella* in meat, beef, eggs in Hebei province, and makes count to the pollution condition poisoned by *Salmonella* in partly areas in Hebei in order to provide data on food security in the markets. The result demonstrated that food samples from supermarkets in Hebei of China were contaminated with

Table 5. The antigens of the *Salmonella.*

Type	Serovar	Somatic(O) antigen	Flagellar(H) antigen	
			Phase 1	Phase 2
A	*S. paratyphi A*	1, 2	a	None
B	*S. typhimurium*	1, 4, 5	i	
C1	*S. choleraesuis*	6, 7, (vi)	c	5

Salmonella spp. (6.5%). The result for *Salmonella* contamination in pork samples (6.9%), chicken samples (9.5%) and beef samples (5.3%), were consistent with previous study by Luyi (2007), in which 7.5, 7.5, 2.4% of pork, chicken and beef samples were contaminated with *Salmonella* spp. in Shanghai province, respectively. Five of eighty-six meat samples (5.8%) were positive for *Salmonella* whereas rate of *Salmonella* in meats samples from Fujian province was 12.1% in 2002 (Zheng, 2005). Pork, chicken product samples and beef were contaminated with *Salmonella* spp. In Cangzhou city, it was reported that fresh and cooked meat were contaminated with *Salmonella* spp. by Wang et al in 2008 (Crump et al., 2002). *Salmonella* contamination in chicken in Hebei province was higher (9.5%) than other kinds of meat in this study. The relatively high rate of may be attributed to the not rigorous hygienic supervision on the slaughter, transportation and processing, which aggravates the contamination of disease-causing bacteria. In recent years the contamination with *Salmonella* in domestic meat foods has not solved and there might be health hazards in meat products. Government should take effective measures to detect and supervise in order to improve the security of meat food,

Two *Salmonella* strains were detected in the chicken samples only from Baoding, but not from other areas. Such regional concentration of *Salmonella* contamination may be related to means of collective slaughter and the hygienic conditions in produce markets. The prevalence of *Salmonella* in chicken's eggs is highest. These data suggested that *Salmonella* as well as other contaminating bacteria may pose a threat to food safety. In Qinhuangdao 2 aquatic products with *Salmonella* were detected in the test. In Zaoqiang County and Hengshui City, *Salmonella* were found in pork and beef.

As shown in Table 4, contaminated food in Hebei Province is chiefly of *S. typhimurium* (6/9) and *S. paratyphi-A* (2/9). In the survey on *Salmonella* contamination of meat conducted by Hu Huiling and Chen Weiwei in Fuzhou, Quanzhou, Longyan and Youxi, Fujian Province, the top 4 serotypes among the separated 10 are *Salmonalla derby* (25.8%), *S. typhimurium* (22.6%), *Salmonella agona* (16.1%) and *Salmonella wetevreden* (12.9%).The results of this test was similar to the survey mentioned above, implying food contamination by *S. typhimurium* is severe in some areas of our country. The main symptom is diarrhea for a person infected with *S. typhimurium*, which can provide guidance for timely treatment and prevention of *S. typhimurium*-related diseases in some regions. Most *Salmonella* and their serotypes detected in our survey have been described about the relevant diseases, which demonstrate that no new serotype *Salmonella* was found.

Even though we did not find *Salmonella* in the other products that we examined, it must be emphasized that the survey was very limited, and no conclusion as to the potential danger can be drawn from these preliminary results. These data are representative of only the ingredient loads sampled. Nevertheless, these data illustrate the suggestion that *Salmonella* contaminating bacteria can be found in food in the market. To diminish *Salmonella* contamination rates in food especially meats, some strategies should be taken. These strategies include reducing pathogen carriage on-farm practices, increasing hygiene at both slaughter and meat processing, increasing consumer education efforts and avoiding the cross-contamination of undercooked meat products during food handling and preparation.

ACKNOWLEDGEMENTS

This work was supported by Science Technology department Project of Hebei Province (NO.08220401D, NO.10960408D) and forty seven of China Postdoctorate Surface Science Fund (NO.20100470565).

REFERENCES

Callaway TR, Edrington TS, Anderson RC, Byrd JA, Nisbet DJ (2008). Gastrointestinal microbial ecology and the safety of our food supply as related to Salmonella. J. Anim. Sci., 86(14 Suppl): 163-172.

Centers for Disease Control Prevention (2007a). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 States, Morb. Mortal Wkly Rep., 56:336–339.

Crump JA, Griffin PM, Angulo FJ (2002). Bacterial contamination of animal feed and its relationship to human foodborne illness. Clin. Infect. Dis., 35(7):859-865.

FDA-CFSAN (2006). Foodborne pathogenic microorganisms and natural toxins handbook..

Glynn JR, Bradley DJ (1992). The relationship between infecting dose and severity of disease in reported outbreaks of Salmonella infections. Epidemiol. Infect., 109(3):371-388.

Hu Huiling, Chen Weiwei (2002). Investigation on Salmonella Contamination Status in Raw Meat. Strait. Chinese J. Prev. Med., 8(6): 52-53.

Humphrey TJ (2000). Public-health aspects of Salmonella infections. In: Wray C, Wray A, editors. Salmonella in domestic animals. Wallingford: CABI Publishing.

Jones TF, Ingram LA, Cieslak PR, Vugia DJ, Tobin-D'Angelo M, Hurd S, Medus C, Cronquist A, Angulo FJ (2008). Salmonellosis outcomes differ substantially by serotype. J. Infect. Dis., 198(1):109-114.

Luyi (2004). Food contamination analysis of food-borne pathogens in Shanghai in 2003. Shanghai Chinese J. Prev. Med., 16(9): 499-451.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999). Food-related illness and death in the United States. Emerg. Infect. Dis., 5:607-625.

Osman KM, Yousef AM, Aly MM, Radwan MI (2010). *Salmonella* spp. infection in imported 1-day-old chicks, ducklings, and turkey poults: a public health risk. Foodborne Path. Dis., 7(4):383-390.

Rodriguez A, Pangloli P, Richards HA, Mount JR, Draughon FA (2006). Prevalence of Salmonella in diverse environmental farm samples. J. Food Prot., 69(11): 2576-2580.

Wang Chundong, Lujun, Li Shunli, Li Gengsheng, Li Xiuzhi, Dai Guojun (2009). Survey of foodborne pathogens in Cangzhou City in 2008. Chinese Hebei Medical J., 31(8): 5-9.

Zhang YH, Wu YG, Du YZ (2001). Development in the Rapid detection of *Salmonella*.Prog. Vet. Med., 22(2): 39- 41

Zheng Youxian (2005). Detection of *Salmonella* in Foodborne pathogens. Chinese Henan J. Prev. Med., 16(2): 80.

Appendix. Numbers of samples collected from the different areas.

Places	Pork	Chicken	Beef	Cutton	Chicken's egg	Duck's egg	Fish	Shrimp	Jellyfish
City of Tangshan	6	2	2	2	2	2	2	0	0
City of Shijiazhuang	5	4	3	2	2	2	4	0	0
City of Cangzhou	3	2	2	2	2	2	2	0	0
City of Zunhua	2	2	2	2	2	0	2	0	0
City of Baoding	2	2	2	2	3	2	2	0	0
City of Xingtai	3	2	2	2	3	0	0	0	0
City of Qinhuangdao	5	5	2	3	3	2	6	3	2
City of Hengshui	3	3	4	2	2	0	2	0	0
Total	29	21	19	17	18	10	20	3	2