Journal of Animal and Veterinary Advances 11 (6): 746-749, 2012
ISSN: 1680-5593

Situation of Salmonella Contamination in Food in Hebei Province of China from 2009~2010

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Abstract: 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 the market in Hebei province of China in 2009-2010. These foods were examined for the presence of Salmonellae by cultural methods and biochemical tests. The isolates were characterized using serotyping testing. The 9 (6.9%) of 139 samples were positive for Salmonella, the most common serovar being *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. *Salmonella paratyphi* (1) and *Salmonella choleraesuis* (2) were also isolated. All other products were negative. 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 and potential hazards from antimicrobial resistance in Salmonella.

Key words: Salmonella, surveillance, serotype, antimicrobial, resistance, China

INTRODUCTION

Salmonella sp. 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; Haque *et al*., 2007; Taddele *et al*., 2011). About >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-E. Most human infection is caused by the limited number of serovars such as *S. choleraesuis*, *S. typhimurium*, *S. enteritidis* and *S. gallinarum*. With the rapid development of economy and the improvement of people's living conditions, people pay more attention to food security in recent years (Osman *et al*., 2010; Callaway *et al*., 2008; Bassullu and Tolunay, 2010).

However, deadly case caused by food contaminant occurs occasionally. In a variety of animal products for human consumption, food poisoning caused by Salmonella is the most common and ranks front row (Simpore *et al*., 2009; Moniruzzaman *et al*., 2011). In Sweden, one most serious case was the pork contaminated with *S. typhimurium* in 1953 in which 7717 cases were poisoned and approximately 90 patients died from it (Glynn and Bradley, 1992). Salmonella is one of the zoonotic pathogens which is of great importance on public health science. Whether people can be infected 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 (USFDA, 2004; Coburn *et al*., 2007; Crump *et al*., 2002). 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. The purpose of this study was to detect the prevalence of *Salmonella* sp. in a variety of food animal species and foods available at the market in Hebei province of China in 2009-2010. Monitoring the occurrence and distribution of Salmonella from food is important to detect possible outbreaks, to identify possible sources of infection and to target prevention and control measures.

MATERIALS AND METHODS

A total of 139 samples were collected including pork (29), chicken (21), beef (19), mutton (17), eggs (35), fish

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

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

[a]Biochemical identification results consistent with S1-S3 model is suspected Salmonella

(20), shrimp (3), jellyfish (2) from markets in Hebei province and were kept at -20°C (Eggs at 4°C) in the laboratory.

Isolating culture: At the market, 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°C for 4 h, 10 mL of each was transferred to 100 mL selenite-cystine enrichment media and incubated at 37°C 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 18~24 h at 37°C and streak plating onto CAS color plates to isolate Salmonella. After 24 h of incubation at 37°C, presumptive Salmonella colonies were used to inoculate triple sugar iron agar, lysine decarboxylase media, D-Mannitol media and ONPG media (Youxian, 2005) which were then incubated for 24 h at 37°C. 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. The single bacterial colony was made into liquid of 0.5 M. It was used to the sevovar agglutination after boiling. A clean glass slide was prepared for the somatic antigen (O). Choose two areas with 1×2 cm on the slide. 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 were compared as control. Those in the saline positive strain for the rough-shaped and can not type. The strains which were positive 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. At last, the subset was identified. For the flagellar antigen (H), the method was carried out as described previously and identified H antigen according to Table 2. At last, all the Salmonella researchers have identified were further identified and confirmed in the professional laboratory of Salmonella identification, Chengdu Institute of Biological Products.

Table 2: Flagellar (H) antigen in common Sallmonella (Types A~F)

	Flagellar (H) antigen	
Type	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	--

RESULTS AND DISCUSSION

A total of 139 samples were collected in Hebei province from 2009~2010 and the situation of Salmonella contamination in food was detected during this study. These included 29 pork, 21 chicken, 19 beef, 17 mutton, 18 chicken's egg, 10 duck's egg, 20 fish and 5 seafood samples. Identities of Salmonella isolates were detected by five selected biochemical tests and the semi-automatic microbe analyzer K-3401. The 9 (6.9%) of 139 samples were positive for Salmonella. The result was shown in Table 3. In different kinds of foods, 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, Baoding, Hengshui, Xingtai, Qinhuangdao and Tangshan were all contaminated with Salmonella (Table 4). Confirmed isolates were further serotyped for agglutination with Salmonella O and H antigens. The results were shown in the Table 5 clearly. The most common serovar were *Salmonella typhimurium* (6/9). The 2 strains of *S. paratyphi* and 1 strain *S. choleraesuis* were detected. This experiment has detected the 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 market. The present study demonstrated that food samples from

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	0.0
Chicken's egg	18	2	11.1
Duck's egg	10	0	0.0
Fish	20	2	10.0
Shrimp	3	0	0.0
Jellyfish	2	0	0.0

Table 4: Result 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*

Table 5: The antigens of the Salmonella

			Flagellar (H) antigen	
Type	Serovar	Somatic (O) 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

markets and supermarkets in partly areas in Hebei were contaminated with *Salmonella* sp. (6.5%). The result for Salmonella contamination in pork samples (6.9%), chicken samples (9.5%) and beef samples (5.3%) were in close agreement with that of Luyi (2004) who reported that 7.5, 7.5 and 2.4% of the pork, chicken and beef samples, respectively were contaminated with *Salmonella* sp. in Shanghai province. Several studies have also indicated that Salmonella is present in food. It was reported (Chundong *et al*., 2009) that 5 of 86 meat product samples (5.8%) were positive for Salmonella whereas 6.9% pork product samples, 9.5% chicken product samples and 5.3% beef product samples were contaminated. Huiling and Weiwei (2002) reported the rate of Salmonella in some kinds of meats samples from partly areas of Fujian province was 12.1% in 2002. Salmonella contamination in chicken in Hebei province was higher (9.5%) in the study. The relatively high rate 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 of demostic meat food by Salmonella has not solved and there might be health hazards in meat products. In order to improve the security of the meat, the government should take effective measures to detect and supervise.

Table 6: Result of different serovars in the selected biochemical test[a]

Serovar	Hydrogen sulfide	Lysine	Motility	D-Mannitol	ONPG
S. typhimurium (Baoding 1)	-	+	+	-	+
S. paratyphi A (Baoding 2)	+	+	+	-	+
S. typhimurium (Qinhuangdao)	-	+	+	-	+
S. paratyphi A (Tangshan)	+	+	+	-	+
S. typhimurium (Hengshui)	-	+	+	-	+
S. typhimurium (Xingtai)	-	+	+	-	+
S. typhimurium (Hengshui)	-	+	+	-	+
S. choleraesuis (Qinhuangdao)	+	+	+	+	+
S. typhimurium (Qinhuangdao)	-	+	+	-	+

[a]One strain in baoding is identified *S. typhimurium* and two strains of *S. paratyphi* A

Two Salmonella strains were detected in the chicken samples we collected in Baoding. But the samples of chicken in other areas were negative. Such regional concentration of Salmonella contamination is perhaps related to the means of collective slaughter and the hygienic conditons in produce markets. The prevalence of Salmonella in chicken's eggs is highest. These data illustrate the suggestion that Salmonella as well as other contaminating bacteria may pose a threat to food safety. In Qinhuangdao, a coastal city abundant with aquatic products, 2 samples with Salmonella were detected in the test. The Quality Check Department is then advised to strengthen the testing and controlling in fish and other aquatic products so that the people have access to wholesome fish. As to the meat products of Zaoqiang County and Hengshui City, Salmonella were found in pork and beef, so the government is required to be conscientious about the source of food and process of slaughtering.

As shown in Table 6, contaminated food in Hebei province is chiefly of *S. typhimurium* and *S. paratyphi*-A. In the survey on Salmonella contamination of meat conducted by Hu Cuiling 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, implying food contamination by *S. typhimurium* is severe in some areas of the 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 the survey have been described about the relevant diseases which demonstrate that no new serotype Salmonella was found.

CONCLUSION

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.

ACKNOWLEDGEMENT

The Qiumei Shi and Qiu Yue Wang contributed equally to this research.

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