International Journal of Food Science and Biotechnology
2022; 7(1): 12-15
http://www.sciencepublishinggroup.com/j/ijfsb
doi: 10.11648/j.ijfsb.20220701.14
ISSN: 2578-9635 (Print); ISSN: 2578-9643 (Online)

Quantitative Detection and Result Analysis of Salmonella in Raw Pork

Huang Xin[1], Zhang Weiyu[1], Gong Yunwei[2], Yao Jiatong[1], Zhang Lifu[1], Liu Guihua[1]

[1]Institute of Microbiology Department, Jilin Provincial Center for Disease Control and Prevention (Public Health Research Institute of Jilin Province), Changchun, China

[2]Institute of Microbiology Department, Changchun City Center for Disease Control and Prevention, Changchun, China

Email address:

46169267@qq.com (Huang Xin)

To cite this article:

Huang Xin, Zhang Weiyu, Gong Yunwei, Yao Jiatong, Zhang Lifu, Liu Guihua. Quantitative Detection and Result Analysis of Salmonella in Raw Pork. *International Journal of Food Science and Biotechnology.* Vol. 7, No. 1, 2022, pp. 12-15. doi: 10.11648/j.ijfsb.20220701.14

Received: January 4, 2022; **Accepted**: February 8, 2022; **Published**: February 16, 2022

Abstract: Objective: To quantitatively detect Salmonella in raw pork, obtain the data of Salmonella contamination in raw pork, conduct risk assessment and formulate reasonable risk management measures. Methods: 302 samples of fresh pork were collected from 10 of Jilin province, mainly from agricultural markets and supermarkets. A 12-tube MPN method was used for quantitative detection of Salmonella, and biochemical identification of API20E and serological identification of Salmonella. Sixty-nine strains of Salmonella were detected. Results: The total detection rate of Salmonella was 22.8%. Sixty-nine strains of Salmonella were divided into 20 serotypes, mainly S.Typhimurium, accounting for 33.3%, followed by S. London (13.0%) and S.Rissen (8.7%). The positive samples were mainly concentrated in the 3rd quarter, followed by the 2nd and 4th quarter, and the quantitative results of Salmonella were between 0.015MPN/g and 600 MPN/g, focus on 0.215MPN/g. Conclusion: The detection rate and contamination in raw pork were serious. Serotypes include both common and rare serotypes. The contamination level of samples collected from farmers' markets was higher than those from supermarkets and online shopping. The detection level in the third, second and fourth quarters was higher than that in the first quarter, it means that high temperature is beneficial to breeding bacteria. The detection rate of samples collected from urban and rural areas showed that the contamination of raw pork Salmonella was not affected by the environmental hygiene conditions, mainly from the samples themselves.

Keywords: Raw Pork, Salmonella, Result Analysis

1. Introduction

Salmonella is a pathogen that can infect both humans and animals. When we eat meat that is not clean, we are easily infected by Salmonella and can develop symptoms of food poisoning [1-4]. Salmonella can be found in many meat products. So when process the meat, we should pay attention to cleaning and thorough heating them. Otherwise, it will make the food container surviving bacteria and cause food poisoning [5-8]. At the same time, we can also get Salmonella infections when we store food and process it, such as knives and chopping boards used to cut raw meat, and containers contaminted. If we do not clean them up and use them to store other foods, it can also cause contamination [9, 10]. In our daily life, we should pay special attention to healthy diet, especially when processing meat and pay attention to cross-contamination of raw and cooked food. Fresh pork Samples were collected from 10 regions of Jilin province, mainly from farmers markets and supermarkets to quantitatively detect Salmonella in raw pork. Through this monitoring, we know the condition of Salmonella contamination in fresh pork in our province, obtain the data of Salmonella contamination in raw pork, conduct risk assessment and formulate reasonable risk management measures.

2. Materials and Methods

2.1. Source of Experimental Material

2.1.1. Sample Source

Samples were collected from 10 areas of the province, mainly from farmers markets and supermarkets, individual

samples for online shopping. The ratio of sample size between urban and rural areas (including the urban-rural interface) is about 1:1. The ratio of pork to mince is 1:1. 1 portion of pork and 1 portion of minced meat per stall. The quantity of samples of the same type and the same site shall not exceed 2 copies. No duplicate sampling shall be collected at the same sampling site every quarter. Selected the four corners and the middle of the cut as the experimental sample, and no less than 5 individual or representative parts equally for asepsis operation and put into the sterilizing container. The experiment is conducted quarterly.

2.1.2. Culture Medium and Reagent Sources

TTB, XLT4 and swarm agar were purchased from Beijing Land Bridge Biotechnology Co. Ltd.; BPW, Columbia blood plate was purchased from Guangzhou Huankai Biotechnology Co. Ltd.; API20E was purchased from French Merrier Co. Ltd.; Salmonella serum was purchased from Thailand, all media and reagents are used within the period of validity.

2.2. Experimental Method

2.2.1. Sample Processing

Fresh pork samples were collected at the supermarket or farmer's market around the laboratory on the day of the experiment. 300g of the sample was weighed into a sterile homogenized bag containing 150mL BPW (the concentration was 500mL/kg). Minced meat used a homogenized bag with a filter). The rinsing solution of BPW need to be homogenized with a homogenizer for 1-2 minutes.

2.2.2. Dilution the Sample

1 mL of the sample rinse solution is taken with a 1 mL sterile pipette or micropipette and slowly filled along the tube in a sterile test tube containing 9 mL of the dilution. Noted that the tip of the pipette or suction tip not touch the level of the dilution. Shaked the test tube or changed 1 mL aseptic pipette to blow repeatedly to make the mixture well mixed, made it 1:10. Prepared 10-fold series diluted sample homogenate in turn. Replaced 1 mL sterile pipette with each dilution.

2.2.3. Inoculation and Culture

4 suitable continuous dilution were selected, according to the estimation of sample contamination, each dilution was inoculated with 1 mL of sample homogenate to 9 mL BPW pre-enrichment tube, and each dilution was inoculated with 3 tubes. The inoculum was cultured at 36°C±1°C for 18 h ~ 24 h. After pre-cultured tubes were taken out and well mixed, take 0.5 mL± 0.05 mL of bacteria solution from each tube to 10 mL TTB selective enrichment broth, culture them at 42°C±1°C for 18 h ~ 24 h.

2.2.4. Isolation

Took out the tubes and mixed well, then inoculated the cultures on the XLT4 plate with 3mm diameter sterile rings, culture them 36°C±1°C for 18 h ~ 24 h.

2.2.5. Identification

The suspected colonies of trisaccharide iron (TSI) were inoculated on Columbia blood plate and cultured at 36°C±1°C for 18 h ~ 24 h. The culture was inoculated with API20E and its biochemical code was looked up to determine whether it was Salmonella.

2.2.6. Serum Identification

Select the cultures from Columbia blood plate for the agglutination test of multi-valency and factor serum, if factor H agglutination was not good, induced colonies on swarm agar plate, after culturing, serum agglutination test determine the serum type [11, 12].

2.2.7. Results and Report

Calculate the number of test tubes confirmed positive, according to the different distribution of positive tubes, choosed 3 continuous concentration gradient which can get more accurate contamination degree, checked MPN table, reported the most likely result of Salmonella, expressed as MPN/g. This quantitative method needed the concentration (500 mL per kg) of BPW rinse. Each 10 mL rinse solution for 20 g sample. Select 3 consecutive dilutions and look up the MPN table, the MPN value divided by 2 was the actual MPN value.

3. Results

3.1. Result of Salmonella in Different Sampling Sites

There was no significant difference in the detection of fresh meat collected from urban and rural areas, but the contamination rate in the farmers' markets was significantly higher than that in supermarkets and online stores. See Table 1.

Table 1. *Types of sampling sites and corresponding Salmonella strains.*

Type of sampling site	Urban	Rural	Total
Supermarkets	12	11	23
Farmers'markets	22	22	44
Online stores	2	0	2
Total	36	33	69

3.2. The Results of the Serotypes

The total detection rate of Salmonella was 22.8% (69/302). Sixty nine strains of Salmonella were divided into 20 serotypes, mainly S. Typhimurium, accounting for 33.3% (23/69), followed by S. London, accounting for 13.0% (9/69), and S.Rissen, accounting for 8.7% (6/69). See table 2.

Table 2. *Detection of different serotypes of Salmonella.*

Latin name	Strains	Percentage
S. Typhimurium (Group B)	23	33.3
S. London (Group E1)	9	13.0
S.Rissen (Group C1)	6	8.7
S.Saintpaul (Group B)	5	7.2
S. Sinstorf (Group E1)	4	5.6
S. Choleraesuis (Group C1)	3	4.3
S. Bovismorbificans (Group C2)	2	2.9
S. Essen (Group B)	2	2.9
S. Derby (Group B)	2	2.9
S. Okefoko (Group E1)	2	2.9
S. Nchanga (Group E1)	2	2.9
S. Stanleyville (Group B)	1	1.4
S. Riggil (Group C1)	1	1.4

Latin name	Strains	Percentage
S. Montevideo (Group C1)	1	1.4
S. Kentucky (Group C3)	1	1.4
S. Meleagridis (Group E1)	1	1.4
S. Saugus	1	1.4
S. Aarhus	1	1.4
S. Montevideo II	1	1.4
S. Freiburg	1	1.4
Total	69	22.8

3.3. Results of Quantitative Detection of Salmonella in Four Seasons

The positive samples were mainly concentrated in the third quarter, followed by the second and the fourth quarter, and the quantitative results of Salmonella were between 0.015 MPN/g and 600 MPN/g. It was concentrated at 0.215 MPN/g, followed by 0.018 MPN/G and 0.0465 MPN/g. See table 3.

Table 3. *Quantitative detection of Salmonella in different quarters.*

Result MPN/g	First quarter (strains)	Second quarter (strains)	Third quarter (strains)	Fourth quarter (strains)	Total (strains)
0.015	1		3	1	5
0.018		2	2	4	8
0.037			1	1	2
0.046		3	2	3	8
0.075			1		1
0.1			1		1
0.115		3	1	2	6
0.175		1			1
0.18		1			1
0.215		4	4	2	10
0.37				2	2
0.465			4	2	6
1.0			1		1
1.05		1			1
1.2			3		3
1.9			1		1
3.1		1			1
3.4	2				2
5.5			2		2
7.5			1		1
11.5				1	1
12			2		2
55		2			2
600			1		1
Total	3	18	30	18	69

4. Discussion

4.1. The Contamination of Salomonella in Fresh Food and the Significance of Detection

Salmonella is a group of Gram-negative bacteria that live in the intestines of humans and animal, and the biochemical reaction is related to the antigen structure. Now Salmonella has more than 2500 serotypes. A few serotypes, such as Salmonella Typhi, Salmonella Paratyphi A, Salmonella Paratyphi B and Salmonella Paratyphi C, have direct pathogenic effects on humans, cause enteric fever but not on non-human hosts. The vast majority of serotype hosts range from livestock, poultry, wild vertebrates, cold-blooded animals, mollusctiles, annular animals, arthropods (including flies), etc. Some salmonella are pathogens of anthropozoic diseases, which can cause human food poisoning or septicemia. As a result of Salmonella contamination, such as inadequate heating or tool contamination and Cross-contamination of raw and cooked food leading to Salmonella food poisoning. Therefore, the objective of this surveillance is to obtain the data of Salmonella contamination in raw pork. It is significance to establish reasonable risk management, measures for the risk assessment of Salmonella in fresh pork [11, 12].

4.2. Distribution of Salmonella in Raw Pork

4.2.1. Distribution of Salmonella in Different Sampling Sites

There was no significant difference in the detection of raw meat collected from urban and rural areas, indicating that Salmonella in raw pork was mainly due to the contamination of pig intestinal faeces during slaughtering. At present, there is no significant difference between urban and rural sanitary condition. But in the circulation link, the market pollutionis obviously higher than the supermarket and the net purchase, which shows that the sanitary condition and storage condition of the supermarket and the net store are obviously better than the market.

4.2.2. Distribution of Salmonella in Different Seasons

Positive samples were mainly concentrated in the third quarter, followed by the second and fourth quarters. The quantitative results of Salmonella were between 0.015 MPN/g and 600 MPN/g, concentrated at 0.215 MPN/g. Followed by 0.018 MPN/G and 0.0465 MPN/g. This indicates that Salmonella grows quickly with the increase of temperature, so the positive rate and pollution amount also increase.

4.2.3. Distribution of Salmonella Serotypes

Sixty-nine strains of Salmonella were divided into 20

serotypes, mainly S. Typhimurium accounting for 33.3%; followed by S. London (13.0%) and S.Rieson (8.7%). This result is similar to Salmonella food poisoning, S. Typhimurium is the dominant strain in our province.

Through this monitoring, we know the condition of Salmonella contamination in fresh pork in our province. Surveillance results showed that the contamination was very serious, mainly S. Typhimurium. The detection rate of samples collected from farmers' markets was higher than that from supermarkets and online shopping. There is little difference between urban and rural areas. Serotypes include both common and rare serotypes. The pollution level of samples collected from farmers' markets was higher than those from supermarkets and online shopping. The positive samples were mainly concentrated in the third quarter, followed by the second and the fourth quarter, indicating that high temperatures were more conducive to the breeding bacteria. The detection rate of samples collected from urban and rural areas showed that the contamination of raw pork was not affected by the environmental hygiene conditions, mainly from the samples themselves. In the supervision of fresh pork, on the one hand, attention should be paid to the pollution from pig intestinal faeces in the slaughter process, on the other hand, it is necessary to ensure the sanitation of the environment and the storage temperature in the storage process. It is also necessary to ensure the thorough heating and to prevent the cross-contamination of tools and containers during processing [13-15].

5. Conclusion

The detection rate and contamination in raw pork were serious. Serotypes include both common and rare serotypes. The contamination level of samples collected from farmers' markets was higher than those from supermarkets and online shopping. The detection level in the third, second and fourth quarters was higher than that in the first quarter, it means that high temperature is beneficial to breeding bacteria. The detection rate of samples collected from urban and rural areas showed that the contamination of raw pork Salmonella was not affected by the environmental hygiene conditions, mainly from the samples themselves.

Fund Project

Health Technology Innovation Project of Jilin Province (2016J034).

References

[1] Zhang Zhenkai, Huang Yunneng, Guo Bingcai, etc. Surveillance of drug resistance and plasmid of Salmonella Paratyphi [J]. Chinese Journal of Antibiotics, 2004, 29 (10): 610-913.

[2] Ran Lu. Surveillance trends of foodborne pathogens and foodborne diseases [J]. Food Safety, 2001. 13 (4): 42-45.

[3] Rao Qiuhua, Chen Jianhui, Luo Tuyan, Yao Qinghua. Detction and analysis of Salmonella in aquatic feed [J]. China Agricultural Science and Technology, 2010. 12: 79-81.

[4] Wang Dongmei, Dong Kaizhong, etc. Detection and analysis of Salmonella in frozen pork [J]. Journal of Northwest University for Nationalities: Natural Science Edition, 2009. 30 (74): 63-65.

[5] Xue Chengyu, Yu Xiaojie, Xie Pinghui, Dong Rui, Zheng Xiaohuan, An Hong, Yan Jun. Analysis of Salmonella contamination in food and application of VITEK microbial identification system [J]. Primary health care, 2011, 25 (5): 75-76.

[6] Li Hongxing, Wang Xiaomei, Ma Li. Analysis of Salmonella in animals [J]. Chinese Journal of Health Inspection, 2005, 15 (5): 581-582.

[7] Jiang Lin, Zhong Deqiao, Jiang Tao, Zheng Huaying. Report on detection of Salmonella in self-cooked Meat Products [J] Chinese Journal of Medical Laboratory Science, 2010, 11 (3): 148-149.

[8] Li Yueting, Gong Yunwei, Liu Guihua. Drug Resistance of food-borne Salmonella and pathogenic E.coli [J]. Chinese Journal of Health Inspection, 2016, 26 (22): 3335-3336.

[9] Li Jiandong, Chen Yizhong, Chu Xinwei, etc. Antimicrobial resistance and plasmid characterization of Salmonella isolated from restaurant workers [J]. Chinese Journal of Public Health. 2005, 21 (2): 205-206.

[10] Pan Zhiming, Jiao Xinan, Liu Wenbo, etc. Surveillance of drug resistance of Salmonella Pullorum [J]. Journal of Animal Husbandry and veterinary medicine, 2002, 33 (4): 377-383.

[11] He Xiaoqing. Examination of bacteria in sanitary and epidemic prevention [M]. Nanchang: Xinhua Publishing House, 1989.

[12] Rapid detection of multiple pathogens in food-PCR method-part 1: Rapid Detection Method for Salmonella in food [S). Industry Standard of entry-exit inspection and Quarantine of PRC.

[13] Liu Guihua, Huang Xin, Zhang Weiyu, Wang Yanqiu. Detection and analysis of antimicrobial resistance of Salmonella in faeces of foodborne disease patients [J]. Science Journal of Public Health, 2020, 8 (3): 77-82.

[14] Liu Guihua, Huang Xin, Wang Yanqiu, Zhang Weiyu, Gong Yunwei, Huang Biao. Detection and analysis of Yersinia contamination in meat products in Changchun [J]. Asia-Pacific Journal of Food Science and Technology, 2020, 2 (1): 1-5.

[15] Liu Guihua, Zhang Weiyu, Huang Xin, Gong Yunwei. Surveillance and analysis of antibiotic resistance of Yersinia in fresh meat food. Science Discovery, 2021, 9 (2) 32-36.

Biography

Huang Xin 1981, female, A native of Changchun City, Jilin Province, master degree of hygienic microbiology, deputy chief technician.