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ORIGINAL ARTICLE

Detection of food-borne pathogens with polymerase chain reaction and introduction of food safety supervision system in China

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Abstract

Introduction Disease-causing pathogens in food are transmitted far and wide through current interconnected global food-chains and the morbidity of food-borne diseases is increasing dramatically. It is quite important for every county to share information and experience with each other in order to optimize monitoring system of food safety. *Objectives* To help people from other countries understand more about Chinese food safety information by introducing the application of polymerase chain reaction technique in the detection of food-borne pathogens and the food safety supervision system. *Methods* Official information and articles in Chinese Journal Full-Text Database and other academic databases have been searched and analyzed. *Results* The types of food-borne pathogens detected by polymerase chain reaction have increased dramatically and more applications of quantitative polymerase chain reaction in recent years have been observed. The implementation of a new food safety law has been strengthened and the supervision system of food safety optimized. *Conclusion* Techniques for improving the monitoring of food safety and the implementation of the new food safety law will improve the food safety situation in China significantly. This paper will be very helpful for people to understand more about the current Chinese food safety system.

Introduction

Food-borne illnesses are defined as diseases, usually either infectious or toxic in nature; caused by agents that enter the body through the ingestion of food [World Health Organization, 2007]. Their influence on human beings has increased with the growing trends of globalization, such as the impacts of *Bovine spogiform encephalopathy*, which spread all over Europe in 2001 and the large-scale infection of *Salmonella* in the United States during this year. A similar situation had also occurred in China. For example in 2001, with the *O157:H7* infection which most likely was transmitted via food and had caused about 20 000 reported cases of illness and 177 deaths (He, 2002). In 2004, the swine *Streptococcus*'s infection via infected pork meat had caused 239 cases hospitalized and 39 cases of death [Ministry of Health (MOH), 2005]. The incidence of milk and infant formula adulterated with melamine last year, had caused > 294 000 babies to become ill, with nearly 51 900 hospitalizations and six infant deaths by the end of November 27, 2008 (MOH, 2008a). How to deal with such challenges, especially with diseases caused by food-borne pathogens? Supervision of food safety is the most effective way to reduce such episodes and to protect public health. Effective supervision of food safety relies on a reliable system of supervision, rapid and accurate techniques of detection and valid laws and regulations. Among the rapid and accurate techniques of detection, polymerase chain reaction (PCR) has been considered as the best candidate. How has this analytical technique been applied in China? No systematic information is available so far. This paper is the first one to demonstrate the applications of PCR in the detection of food-borne pathogens in China by searching the Chinese Journal Full-text Database and analyzing the data in four aspects: including the number of related publications, the types of food-borne pathogens detected, the kinds of PCR methods applied and its applications involved in the standard methods. Also the infrastructure of the food safety supervision system and the critical changes to the new food

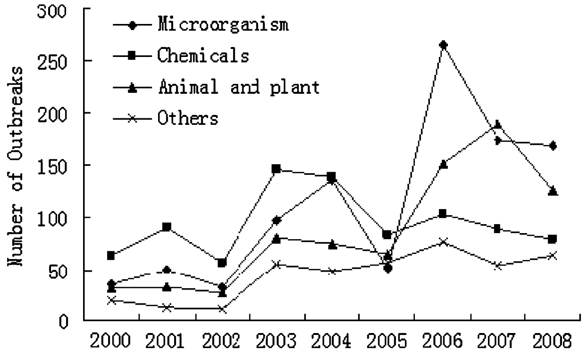

Figure 1 Annual food-borne disease outbreaks by etiology in China.

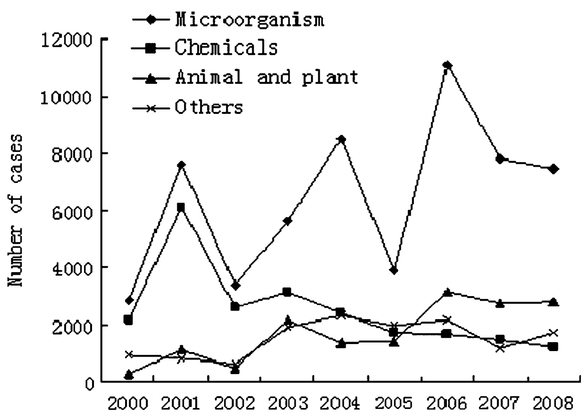

Figure 2 Annual food-borne disease cases by etiology in China.

safety law of China have been addressed, as well as the perspective for food safety supervision in China.

Status quo of food-borne disease in China

The food industry has always been one of the main pillars of the Chinese economy. In 1999, the value of the industrial output of food in China was only 780 billion yuan (US$113.7 billion). In the last 10 years, the annual value of industrial output of food has seen an annual increase of 20% and reached 4.2 trillion yuan (US$617.6 billion) in 2008 (Chinesewine, 2009). However, with the high-speed development of the food industry, China has also been facing severe issues of food safety, especially food-borne diseases. According to reports from Wang (2007) and MOH (2008b, 2009a), there was a total of 3034 outbreaks of food-borne diseases from 2000 to 2008, with the largest percentage of outbreaks (33.26%) and cases (52.82%) pertaining to microorganism; with chemical agents making up 27.98% of the outbreaks and 20.53% of the cases. Animal and plant evidence ranked third (Figures 1 and 2). Bacterial pathogens are still the major pathogens of food-borne disease, including *Vibrio parahemolyticus*, *Salmonella*,

Table 1 The rank of major microbial pathogens for food-borne disease in selected years[1]

Year	First	Second	Third
1992–2001	*V. parahemolyticus*	*Salmonella*	*Proteus*
2003	*V. parahemolyticus*	*Proteus*	*Staphylococcus aureus*
2004	*V. parahemolyticus*	*Bacillus cereus*	*Salmonella*
2005	*V. parahemolyticus*	*Salmonella*	*Proteus*
2008	*V. parahemolyticus*	*Salmonella*	*Proteus*

[1]Liu *et al.* (2004, 2006, 2008), Chen *et al.* (2008), and Xinhua (2009).

Proteus, *Bacillus cereus*, *Escherichia coli*, etc. (Table 1). Clearly, enhancement of detection and surveillance of microbial pathogens is the main key for promoting food safety.

Detection of food-borne pathogens with PCR in China

As bacterial pathogens are still the major cause of food-borne diseases in China, how should they be monitored effectively? Classical microbiological analysis before PCR, carried out regularly, has played an important role in detection of food-borne pathogens. However, in normal screening, especially during public health emergencies, a traditional culture and differentiation assay is laborious and time consuming, and rapid and efficient detection methods need to be introduced. Among the rapid detection methods, PCR is the best option, because of its high specificity, sensitivity and time-efficient characteristics. In this paper the application of PCR technique in China, related to food-borne pathogen detection, will be presented in terms of ① the related Chinese research publications, both in number of publication number and types of detected bacteria; ② the kinds of standard method which including the PCR techniques.

Research publications in Chinese with PCR technique to detect food-borne pathogens

The first research publication in Chinese with a PCR technique to detect food-borne pathogen was in 1990, an article for detection of *HAV* gene (Xi, 1990). Since then, the application of PCR in this field has increased rapidly. The data below were obtained by searching the Chinese Journal Full-Text Database, which is the biggest continuous dynamic updating Chinese journal full-text database in the world and has included over 8200 important kinds of domestic journals that accounts for over 99% of the total in China. From the time of the first publication and to the end of 2008, 1111 research publications on the detection of important food-borne pathogens with PCR were found. The number of

Table 2 Important kinds of food-borne pathogens detected by PCR and the number of corresponding articles

	Year					
Types of pathogens	1990–1992	1993–1995	1996–1998	1999–2001	2002–2004	2005–2008
V. parahemolyticus	0	2	3	3	7	26
Salmonella	0	10	9	16	26	97
Staphylococcus aureus	0	4	7	27	43	80
Escherichia coli	0	4	13	12	34	75
C. botulinum	0	0	2	1	1	5
Bacillus cereus	0	0	0	0	1	7
Proteus	0	0	0	0	1	5
Listeria	0	0	3	3	20	57
Calicivirus	0	0	0	2	2	2
Rotavirus	0	4	11	6	11	31
Campylobacter jejuni	0	0	0	2	6	18
Adenovirus	0	1	10	10	33	29
Avian influenza virus	0	0	4	8	29	69
Streptococcus suis	0	0	0	7	7	52
Astrovirus	0	0	0	1	0	4
Shigella	0	3	7	10	12	39
HAV	1	3	8	6	2	5
HEV	0	8	9	5	3	11
Norovirus	0	1	0	0	3	9
Sapovirus	0	0	0	0	0	3
Total	1	40	86	119	241	624

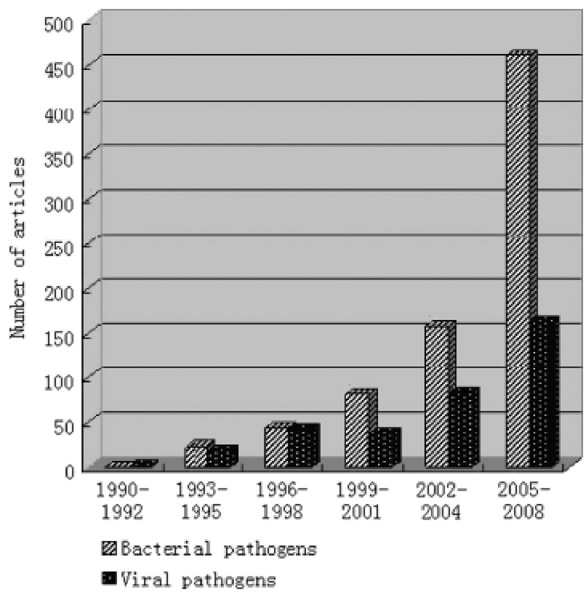

Figure 3 Viral and bacterial pathogens detected by polymerase chain reaction and the number of corresponding articles.

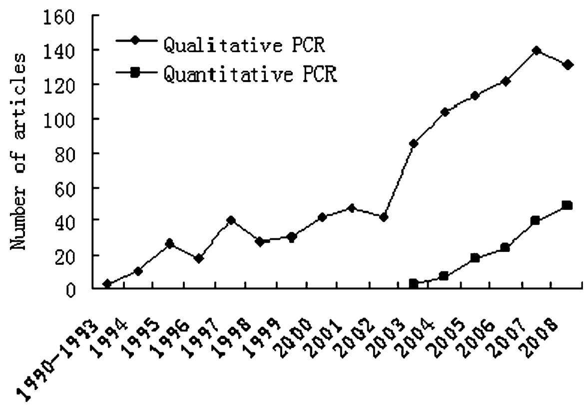

Figure 4 Annual article number of food-borne pathogens detected by qualitative and quantitative PCR from 1990 to 2008.

publications, including for different kinds of pathogens, were analyzed in 3-year intervals (Table 2). For the first 3 years, only one publication was found, which was for the HAV detection. From 1993 to 1995, 40 publications were retrieved, with more than half of the publications being on the detection of food-borne pathogenic bacteria, and 10 of the 40 were for the detection of *Salmonella* spp. After 2001, the publication rate increased dramatically. From 2002 to 2004, the number reached to 241, and in the last 3 years, the number of publications was 624, which was more than double in comparison with those in 2002–2004. At the same time, detection with PCR for food-borne viral pathogens had also increased, but not as much as that for bacteria pathogens (Figure 3). Not only the number and the kinds of publications were analyzed, we have also presented the number of publications for qualitative and quantitative PCR applications yearly (Figure 4). The first publication for quantitative PCR application in food-borne pathogen detection was in 2003 for Avian influenza virus H5N1 (Zhu *et al*., 2003). With the development of PCR and the higher requirement of detection of food-borne pathogens, the detection of food-borne pathogens with PCR has completed its transformation from qualitative to quantitative gradually.

Standard methods including the PCR techniques

The application of PCR techniques in the detection of food-borne pathogens was not only demonstrated in the increased research publications, but it is also involved in the standard methods. There have been 19 standards of examination of food-borne pathogens with PCR in China, including 12 national standards and seven professional standards. Among them, real-time PCR takes up nine standards, in which there are six national standards. Surely, the implementation of these standards makes the normal work of detection organizations faster and more effective. The detailed information was displayed in Table 3.

Infrastructure of supervision system for food safety in China

To improve the food safety situation, better application of the monitoring techniques is important, but the establishment and optimization of sound infrastructure are also a prerequisite for food safety supervision.

Supervision system before the implementation of the new food safety law

Based on the two Acts of 'The Decision of the State Council about Strengthening Food Safety Further' (Central People's Government of the PRC, 2005) and 'The Announcement of the Central People's Government about Identifying Responsibility of Departments in Supervision System for Food Safety Further' (China Organization, 2007), the primary pattern of the supervision system was fragmented, which meant special departments would take charge of special links in the food chain before the implementation of the Food Safety Law of the People's Republic China. The Ministry of Agriculture (MOA) is responsible for regulating the link to primary agricultural products, Administration of Quality Supervision, Inspection and Quarantine of PRC (AQSIQ) for manufacturing and processing, Administration of Industry and Commerce for circulation, MOH for food consumption and State Administration for Food and Drug for general supervising, harmonizing, investigating and prosecuting serious food safety accident. Except for these special departments, some other departments also take part in the supervision system, for example, the Ministry of Commerce works on the control of food circulation; pollutant inspection and control of the food industry are mainly carried out by the Ministry of Environmental Protection, etc. Although this primary infrastructure generally involves the different links from 'farm to table' and plays an important role in supervision of food safety, some serious food safety accidents still reveal the shortcomings of this infrastructure including the duty overlaps of different departments and the lack of a concrete coordination mechanism. A typical example of the shortcomings is the Melamine Crisis. The supervision of milk stations is located at the edge of the cut-off point of MOA and AQSIQ, and the supervision attribution was uncertain. If milk stations were subordinated to dairy enterprises, the AQSIQ was responsible for their quality supervision. If not, just as the service for milking, it belonged to the supervision duty of MOA. Moreover, there was not a harmonization of food standards in China, both the Food Hygiene Standard and Food Quality Standard could work. Obviously, these shortcomings provided good opportunities for milk adulteration with melamine.

The significant alternations of infrastructure of supervision system

Focussing on these disadvantages, there are some significant alternations to the enforcement of the Food safety law on June 1, 2009 (Legislative Affairs Office of the State Council, PR China, 2009). Among them, the most important is that the State Council will firstly establish a Food Safety Committee whose staff comprises the vice-premier of the State Council and senior officials of related departments for food safety. The function of this committee is the macrocontrol of food safety including the analysis of food safety status, arrangements and guidance for food safety, raising the important problems of food safety, etc. The MOH is responsible for the normal duties of this Committee. These concrete duties of the MOH for food safety include comprehensive coordination, risk assessment, standard formulation, information reporting for food safety; formulating the accreditation requirements and detection standards for food detection organizations, investigating and prosecuting serious food safety accidents. In addition the new law re-identifies the duties of departments in order to avoid the overlaps and lack of supervision from farm to table. The outline of new infrastructure of supervision system for food safety was illustrated in Figure 5.

Table 3 Standards for detection of food-borne pathogens with PCR[1]

Food-borne pathogens	Level of standard	Number of standard	Type of PCR	Time of implementation (yyyy-mm-dd)
Avian influenza virus subtype H7	National standard	GB/T 19438.3-2004	Real-time PCR	2004-02-15
Avian influenza virus subtype H9	National standard	GB/T 19438.4-2004	Real-time PCR	2004-02-15
Avian influenza virus	National standard	GB/T 19438.1-2004	Real-time PCR	2004-02-15
Avian influenza virus	Professional standard of Ministry of Agriculture	NY/T 772-2004	Conventional RT-PCR	2004-02-17
Virulence factor of *Streptococcus suis* type 2	National standard	GB/T 19915.8-2005	Real-time PCR	2005-11-01
Streptococcus suis type 2	National standard	GB/T 19915.5-2005	Multi-PCR	2005-11-01
Hemolysin gene for *Streptococcus suis* type 2	National standard	GB/T 19915.9-2005	PCR	2005-11-01
Streptococcus suis type 2	National standard	GB/T 19915.4-2005	triple-PCR	2005-11-01
Streptococcus suis type 2	National standard	GB/T 19915.7-2005	Real-time PCR	2005-11-01
Streptococcus suis type 2	National standard	GB/T 19915.3-2005	PCR	2005-11-01
Enterobacter sakazakii	Professional standard of import-export inspection and quarantine	SN/T 1632.3-2005	Real-time PCR	2006-02-01
Norovirus	Professional standard of import-export inspection and quarantine	SN/T 1635-2005	Conventional RT-PCR and Real-time PCR	2006-02-01
Listeria monocytogenes	Professional standard of import-export inspection and quarantine	SN/T 0184.2-2006	Multi-PCR	2007-03-01
Pathogens in food, including *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Campylobacter jejuni*, *O157:H7*, *V. parahemolyticus, V. cholerae*, *V. vulnificus*, *V. alginolyticus*	Professional standard of import-export inspection and quarantine	SN/T 1870-2007	Real-time PCR	2007-10-16
Pathogens in food, including *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Campylobacter jejuni*, *O157:H7*, *V. parahemolyticus*, *V. cholerae*, *V. vulnificus*, *Enterobacter sakazakii*	Professional standard of import-export inspection and quarantine	SN/T 1869-2007	PCR or BAX automatic detection system of pathogens with PCR	2007-10-16
Brucella	Professional standard of import-export inspection and quarantine	SN/T 1942.2-2007	PCR	2008-03-01
Hepatitis A virus	National standard	GB/T 22287-2008	Conventional RT-PCR and Real-time PCR	2008-12-01
Listeria monocytogenes	National standard	GB/T 4789.30-2008	BAX automatic detection system of pathogens with PCR	2009-03-01
O157:H7	National standard	GB/T 4789.36-2008	BAX automatic detection system of pathogens with PCR	2009-03-01

[1]Ministry of Agriculture of the PRC (MOA) (2004), Standardization Administration of the PRC (SAC) (2004a–c, 2005a–f, 2008, 2009a, b), Administration of Quality Supervision, Inspection and Quarantine of the PRC(AQSIQ) (2006a, b, 2007a–c, 2008).

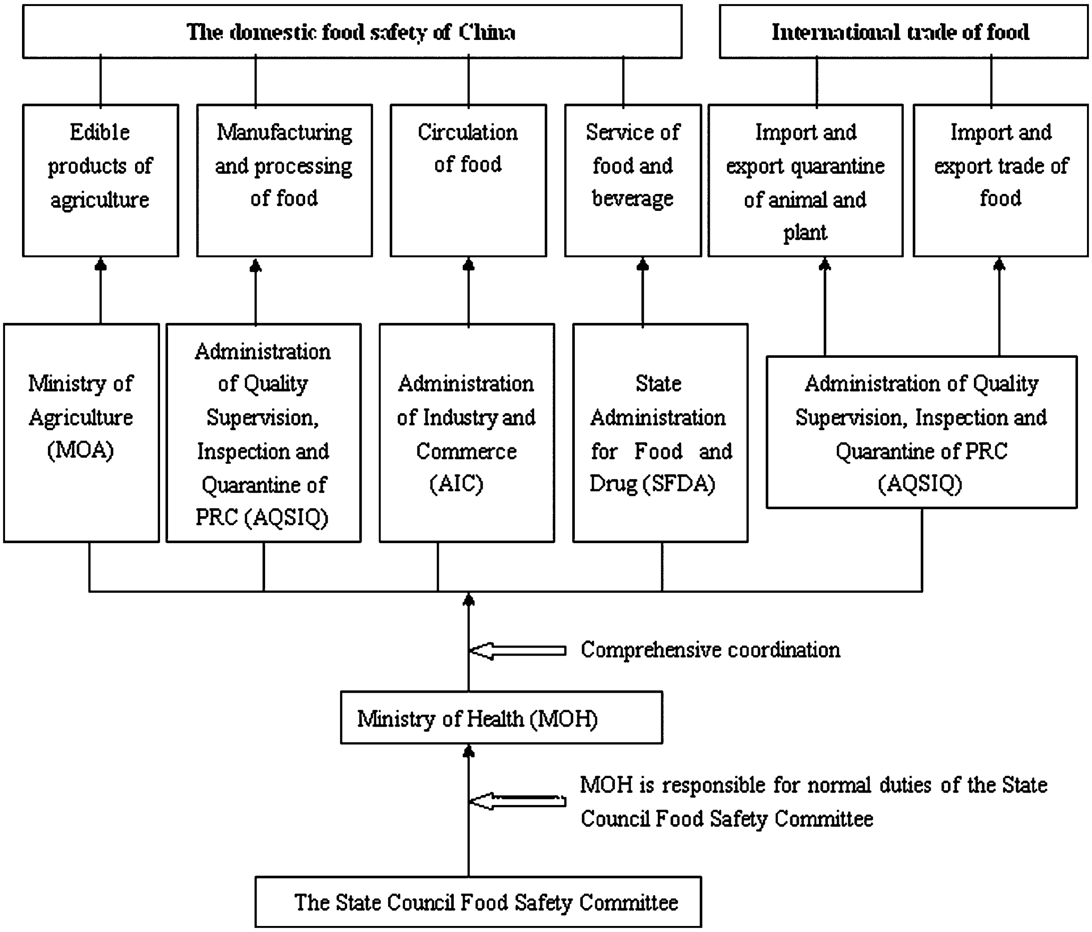

Figure 5 Responsibilities of departments in supervision system for food safety.

The promulgation and implementation of new food safety law of China

As demonstrated above, the alternation of the infrastructure for the supervision system for food safety is a quite substantial characteristic of the new Food Safety Law, though it is not the only characteristic for optimizing the supervision system for food safety in China. To some extent, the supervision of food safety in China was insufficient and needed to be improved continuously. Therefore, the promulgation and implementation of new Food Safety Law undoubtedly are widely concerned and of great significance for supervision of food safety in China.

The background of promulgation of the food safety law

The first law related to food safety was the Food Hygiene Law which was implemented on October 1, 1995 (Legislative Affairs Office of the State Council, PR China, 1995). Although the Food Hygiene Law has made a great and positive impact on the hygienic quality of food, it generally formed in a supervision mode of dependent response, which could no longer adapt to the needs of modern food industry in the rapid development of China's economy. The important food safety incidents in 2004 made the government and experts realize that there were some conflict and intersection between the Food Hygiene Law and Product Quality Law, so it was imperative to enact a single law of supervision for food safety by combing the two previous ones and to gradually complete the transformation from reactive to proactive. Through a 5-year effort, especially in reaction to milk and infant formula adulterated with melamine, this new Food Safety Law was adopted at the 7th Session of the Standing Committee of the 11th National People's Congress on February 28, 2009, and was implemented on June 28, 2009, which demonstrates that supervision of Chinese food safety heads into a 'new era.'

The characteristics of the food safety law for strengthening supervision system

It is obvious that hygiene do not means safety. Addressing the weak links of supervision, the Food Safety Law has made some targeted innovations. In this new law, there are 104 rules in 10 chapters which illustrate the purpose and significance, scope of jurisdiction, organizations of enforcement, responsibility for socio-group, monitoring and the risk assessment for food safety, management of food company, detection and inspection standard, management of the import and export of food, dealing with a food safety accident, supervision and administration of food production, the terminology of food safety, etc. as well as detailed aspects to ensure the food safety like 'from farm to table.' By comparison with the Hygiene Law, the most important changes in the new law are characterized as follows:

1. There will be only one food safety standard in China in place of two standards, Food Hygiene Standard and Food Quality Standard and there will not be any other mandatory standard in the food field.
2. The established Food Safety Committee will be the general coordinator for food safety in China.
3. The food producers and operators are the persons of primary responsibility for product quality and food safety and the local government's responsibility for local food safety supervision will be established first.
4. The systems of food withdrawal for disqualified food in the market, and monitoring and assessment of food risks will be added to reduce possible food hazards to the minimum and enhance the ability to cope with food risks.
5. The supervision of food additives will be emphasized and strengthened so as to regulate food producers to use food additives legally.

These changes will increase the efficacy of the Infrastructure of Supervision System for Food Safety in China.

Perspective

More rapid detection methods for food-borne pathogens will be developed and applied. With the higher requirements of food safety monitoring and the promotion of the national '10th five years' key project of the Ministry of Science and Technology (Gao & Zhou, 2008), the detection techniques for food-borne pathogens are being rapidly developing in China, especially in the application of PCR techniques, as well as with methods of harmonization with other countries. For instance, the MOH has set the strategy for PCR applications in city level CDC and above, and about 2000 CDC staff national wide has been trained in our school (West China School of Public Health) to conduct PCR analysis. The PCR technique will be involved in more standard methods for food borne pathogen detection. Because quantitative PCR assay has been proved to be rapid, quantitative, sensitive and with fewer contamination problems, real-time PCR will more significantly applied in the detection of food-borne pathogens compared with other styles of PCR. However, we still have to do more work. Actually, there are many other methods, which also possess rapid speed, sensitivity and accuracy, just as outlined in some professional standards, local standards and industry standards. In order to find the most suitable method as the monitoring tool for food-borne diseases in China, we need to introduce ourselves to other countries and learn from their advanced experience to form harmonized detection methods and corresponding system of accreditation. Only in this way can our detection data be recognized by other countries, and it may also be helpful for the global surveillance of food-borne diseases and for eliminating the technical barriers.

Advanced detection techniques have provided strong support for the development of food safety supervision in China. The food safety supervision system has also been optimized through the implementation of the new food safety law. However, China still faces many challenges in the whole monitoring processes from 'farm to table.' Firstly, active surveillance and a source tracing monitoring network should be established in order to improve the level of the early warning mechanism for food hazards. Secondly, popularization of hazard analysis and critical control can help to ensure the safety of agricultural product and food. Thirdly, the personnel involved in food safety should be trained through the establishment of education organizations for food safety. Finally, more cooperation with international organizations and other countries could harmonize global supervision of food quality and safety.

In conclusion, the Chinese food safety supervision system has been improved both at the micro and macrolevels as demonstrated by the application of PCR and a food safety supervision infrastructure described in this paper. It will be very helpful for the people from other countries to know China better, as well as to contribute to the harmonization of global food quality and safety monitoring.

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