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Short Communication

A comparative study on the use of real time polymerase chain reaction (RT-PCR) and standard isolation techniques for the detection of *Salmonellae* in broiler chicks

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Abstract This study was carried out to compare between conventional cultural isolation methods and real time polymerase chain reaction (RT-PCR) technique for the detection of *Salmonella* in broiler chicks. About 120 livers and intestinal contents samples were collected from 1800 day-old imported and local broiler chicks. The incidence of *Salmonellae* among imported chicks was 11.67% compared to 21.67% among local chicks using conventional cultural isolation methods. *Salmonella newport* (*S. newport*) showed the highest incidence rate in imported chicks, while *Salmonella enteritidis* and *Salmonella typhimurium* were frequently detected in local chicks. The RT-PCR results for detection of *invA* gene of *Salmonella* spp. were 58.33% and 66.67% positive samples in imported and local chicks, respectively. Results have confirmed that RT-PCR technique is rapid, robust, effective and reliable method for detection of Salmonella spp. in broiler chicken when compared to conventional cultural methods. However, RT-PCR should be performed parallel with conventional methods for more accurate detection results of different *Salmonellae* serovars.

1. Introduction

Infection with *Salmonella* is the most frequent food-borne gastrointestinal disease transmitted from animals to humans mainly through water, meat, eggs and poultry [1]. *Salmonella* infection is world-wide food-borne zoonosis and poultry products and byproducts are the common source of infection. Poultry associated *Salmonellae* are the most frequently reported human zoonoses in the European Union which can cause

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relatively vast economic damage due to chronic effects of the infections [2]. Moreover, food borne Salmonella out-breaks can lead to severe economic losses to poultry producers as a result of regulatory actions, market restrictions or reduced consumption of poultry products [3].

Traditional microbiological methods offered standardized procedures for microbial detection. However, they are time consuming (take approximately 4–7 days) and not always compatible with short-time-to-result demand. Therefore, food Microbiology aims for supplementation of classical methods with molecular techniques based on detection of the microbial nucleic acids, which shorten the analysis time and lower the limit of detection [4].

Since *Salmonella* was closely related to both public and animal health, more rapid and sensitive methods for the identification of this bacterium are required. Real Time-Polymerase Chain Reaction (RT-PCR) technology offers several advantages compared with classical bacteriology in terms of speed, detection limit, potential for automation and cost [5]. The use of RT-PCR greatly reduces the time and manpower required when compared with the conventional culture methods; however there are challenges associated with the use of PCR, such as the detection of low levels of contaminating pathogens, the presence of dead cells and the occurrence of inhibitory components. These challenges could be overcome by using the culture enrichment broths [6].

Therefore, this work was designed to compare between conventional cultural isolation methods and RT-PCR technique for detection of *Salmonella* infection in broiler chicks.

2. Materials and methods

2.1. Samples

A total of 1800 day-old broiler chicks representing 900 imported birds and 900 local ones were examined during the period from 2012 to 2013. The local chicks were collected from different Egyptian governorates, while the imported ones were examined in the Reference Laboratory for Veterinary Quality Control on Poultry Production-Animal Health Research Institute, Egypt as a routine work. Under complete aseptic conditions, liver samples and intestinal contents were collected for standard isolation and PCR detection of *Salmonellae*.

2.2. Traditional cultural methods

2.2.1. Isolation of Salmonella spp.

A total of 1 ml of the pooled samples was transferred to 9 ml Buffered Peptone Water (BPW) and incubated aerobically for 16–18 h at 37 °C. Then, 0.1 ml of the incubated BPW was inoculated into Rappaport–Vassiliadis medium (Lab M, Lancashire, UK), with soya broth (RVS) and incubated at 41.5 °C for 24 h. An amount of 1.0 ml of the incubated BPW was inoculated in Muller-Kauffmann Tetra-thionate-novobiocin broth (MKTT-Lab M, Lancashire, UK), and incubated at 37 °C for 24 h. Then plating out on Xylose Lysine Deoxycholate agar (XLD) and Hektoen Enteric agar (HEA-Lab M, Lancashire, UK), was carried out and aerobically incubated at 37 °C for 24 h [7].

2.2.2. Identification of Salmonella isolates

2.2.2.1. Microscopical examination. Suspected colonies were Gram stained according to Quinn et al. [8] and examined microscopically.

2.2.2.2. Biochemical identification. Salmonella isolates were examined using different biochemical reactions [urea hydrolysis on Christensen's urea agar, hydrogen sulfide production, fermentation of sugar including glucose, lactose and sucrose, gas production on Triple Sugar Iron (TSI) agar and lysine decarboxylation on Lysine Iron (Li) agar [9].

2.2.2.3. Serological identification. Suspected *Salmonella* isolates were serologically identified according to Popoff [10]. Briefly, serology was performed using slide agglutination tests with known polyvalent somatic and flagellar antisera (SIFIN, Berlin, Germany) according to Kauffmann-White serotyping scheme.

2.3. Real Time-PCR technique for Salmonella spp. detection

2.3.1. Primers and probes

Oligonucleotide primers and probe sequences used in RT-PCR were published previously and shown in Table 1. Amplification of *Salmonella invA* genes was done and detected using [11–13].

2.3.2. Extraction of Salmonella DNA

DNA was extracted from 1 ml of the pre-enrichment broths using QIAamp DNA Extraction Mini Kit (Qiagen, Germany) following the manufacturers' instructions.

Amplification and detection of specific products were performed using the following cycle profile: a primary denaturation and activation of Taq DNA polymerase at 94 °C for 15 min for one cycle followed by secondary denaturation at 94 °C for 10 s and primer annealing and extension at 60 °C for 20 s for 40 cycles according to Oliveiria et al. [14].

3. Results and discussion

Globally, Salmonella spp. are the most important bacterial pathogens of poultry, where infection causes significant economic losses in poultry rearing and food industries. Losses are also including high mortalities in addition to growth retardation [15]. In addition, human gastroenteritis as a result of infection with poultry-associated *Salmonellae* is a well-known food-borne zoonosis and of health burden [16]. Previous researchers have shown that conventional methods should be carried out along with molecular techniques using RT-PCR [17]. Accordingly, this study has been done to compare conventional cultural isolation methods and RT-PCR technique for detection of *Salmonellae* in broiler chicks.

Obtained results revealed that all *Salmonella* isolates showed pink colonies with black center on XLD media and green to blue colonies with black center on HEA media. Gram stained results revealed the presence of Gram negative straight rods [8]. This study highlights the prevalence of *Salmonellae* in local as well as imported birds. The incidence of *Salmonellae*

Table 1 Fluorogenic primers and probe used for amplification of a 102-bp region of the *invA* gene of *Salmonella* spp.

Primer/probe	Sequence (5′–3′)	Amplified product (bp)
Sal-F	GCGTTCTGAACCTTTGGTAATAA	102 bp
Sal-R	CGTTCGGGCAATTCGTTA	
Sal-TM (probe)	FAM-TGGCGGTGGGTTTTGTTGTCTTCT-TAMRA	

Table 2 Prevalence of different *Salmonella* serovars isolated from different types of samples.

Source of samples	Total number of examined samples	Incidence of *Salmonellae* isolation		Types of isolated serovars		
		Number of positive samples	%*	Serovars	Number of serovars	%**
Imported chicks	900	105	11.67	*S. Newport*	2	11.10
				S. Heistopdenberg	1	5.56
				S. Bochum	1	5.56
				S. Indiana	1	5.56
				S. Bargny	1	5.56
				S. Neftenbach	1	5.56
Local chicks	900	195	21.67	*S. Enteritidis*	6	33.32
				S. Essen	1	5.56
				S. Infantis	1	5.56
				S. Indiana	1	5.56
				S. Typhimurium	2	11.10
Total number	1800	300	16.67		18	100

* The percentage was calculated according to the total number of the examined samples.
** The percentage was calculated according to the total number of positive samples of *Salmonellae*.

among imported chicks reached (11.67%), whereas it was (21.67%) among the local chicks by conventional cultural methods. The rate of isolation from all examined samples reached (16.67%). The increased microbial load in local chicks could be attributed to improper management or biosecurity measures. These results are so far in agreement with others investigators [18–21]. Snow et al. [22] isolated *Salmonella* in a rate of (10.7%) in the United Kingdom, while Ibrahim et al. [23] reported that the incidence of *Salmonella* in broiler was (16.66%) in Beni-Suef governorate, Egypt.

The prevalence of different *Salmonella* serovars isolated from different types of samples is illustrated in Table 2. Out of the 900 imported day-old broiler chicks, 105 samples (11.67%) were positive for *Salmonellae*. Also, *S. newport* had

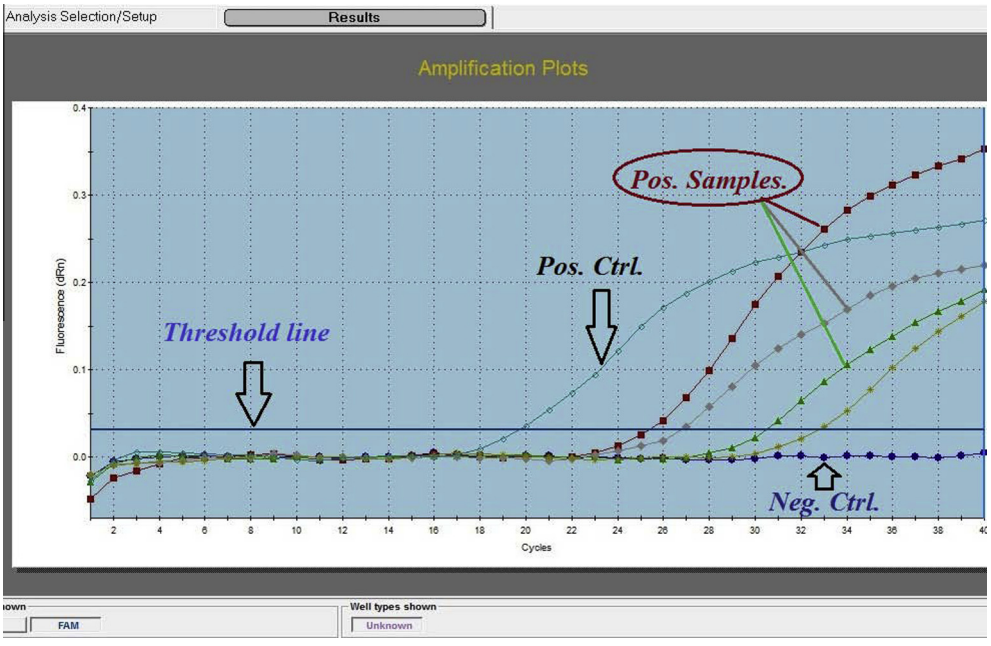

Figure 1 RT-PCR results of some positive *Salmonella* detected in samples of examined broiler chicks.

Table 3 Comparison between the results of detection of *Salmonella* spp. by conventional cultural methods and RT-PCR technique.

Source of samples	Number of examined samples	conventional cultural methods		RT-PCR technique	
		Positive	%*	Positive	%
Imported chicks	900	105	11.67	525	58.33
Local chicks	900	195	21.67	600	66.67
Total	1800	300	16.67	1125	62.50

* The percentages was calculated according to the total number of the examined samples.

the highest incidence (11.10%). However, other serovars; *S. heistopdenberg*, *S. bochum*, *S. indiana*, *S. bargny* and *S. neftenbach* were lower in their incidences (5.56%, all respectively). For the local chicks, out of the 900, 195 samples (21.67%) were positive for isolation of *Salmonella* spp. Where, *S. enteritidis* and *S. typhimurium* gave the highest incidence (33.32% and 11.10% respectively), while the other serovars including *S. essen*, *S. infantis* and *S. indiana* were the lowest in their incidences (5.56%, all respectively).

Regarding the incidence of *Salmonella* serovars that isolated from local broiler chicks in the present study, 6 isolates of *S. enteritidis* (33.32%) were isolated. Similarly, *S. enteritidis* was considered to be diagnosed more frequently as recorded by Abd-Allah [24] who detected 10 serotypes (40%) of *S. enteritidis* out of 25 isolated *Salmonella* strains. Heriksstad et al. [25] considered *S. enteritidis* as the most common species of *Salmonella* spp. isolated worldwide. Abd El-Ghany et al. [26] reported on *Salmonella* serotypes circulating in broiler chicken farms in Kalubia governorate, Egypt including *S. enteritidis*, *S. infantis*, *S. chiredzi*, *S. kentucky*, *S. typhimurium* and *S. tsevie* where *S. enteritidis* and *S. typhimurium* are the most prevalent ones.

The RT-PCR technique for detection of *invA* gene of *Salmonella* spp. was carried out after non selective enrichment in BPW after 18 h in 37 °C to improve sensitivity and dilute PCR-inhibitory substances and time management [27,28]. The results of RT-PCR amplification of some positive samples are shown in Fig. 1. The incidence of *Salmonella* among imported chicks was determined as 58.33% compared to 66.67% among the local ones. The rate of isolation from all examined samples was 62.50%. The comparison between detection results of *Salmonella* spp. by conventional cultural methods and RT-PCR technique is described in Table 3.

In conclusion, this study has addressed an interesting subject since *Salmonella* is a common bacterial disease of poultry and of zoonotic concern. It has shown that RT-PCR technique is rapid, accurate, and more sensitive. Moreover, such molecular technique greatly reduces the time and manpower required if compared to conventional cultural methods. However, it should not be used solely and be done in combination with the conventional cultural methods for more accurate detection results of different serovars.

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