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Detection of *Salmonella* in Egg Shell and Egg Content from Different Housing Systems for Laying Hens

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Abstract**—**Polymerase chain reaction (PCR) assay and conventional microbiological methods were used to detect bacterial contamination of egg shells and egg content in different commercial housing systems, open house system and evaporative cooling system. A PCR assay was developed for direct detection using a set of primers specific for the invasion by A gene (*invA*) of *Salmonella* spp. PCR detected the presence of *Salmonella* in 2 samples of shell egg from the evaporative cooling system, while conventional cultural methods detected no *Salmonella* from the same samples.

Keywords**—**egg content, egg shell, *invA* gene, PCR, *Salmonella* spp.

I. Introduction

Salmonella spp. is a major food-borne bacterial pathogen, with poultry and poultry products being a primary source of infection to humans [1]. It has most often been associated with consumption of contaminated foods of animal origin, such as poultry, swine, dairy products and eggs [2][3]. Poultry are considered an important source of foodborne disease and the illnesses were associated with the consumption of contaminated eggs. *Salmonella enteritidis* and *S. typhimurium* as well as other serotypes have been isolated from egg shells and egg content [4][5][6][7]. The most commonly used technique for *Salmonella* detection is the conventional culture technique. Conventional selective enrichment and serological tests for *Salmonella* spp. from eggs take 5–7 days and are labor intensive. Thus, rapid and sensitive methods for detecting *Salmonella* are in great demand in order to assure produce safety. PCR technology represents a rapid procedure with high sensitivity and high specificity to detect *Salmonella* in a wide variety of food. Several PCR assays have been developed by targeting various *Salmonella* genes, such as 16S rRNA [8], *agfA* [9], and *viaB* [10], and virulence-associated plasmids [11]. In addition, *invA* gene is one of the most often used to detect *Salmonella* spp. in a variety of food [12][13][14].

The aim of this study was to compare egg contamination in commercial production from different housing systems, determining the prevalence of *Salmonella* spp. on egg shell and egg content by using conventional microbiology detection compared to that detected using *invA* gene of *Salmonella* by PCR technique.

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II. Materials and Methods

A. Egg samples

Fresh chicken eggs were received from different housing systems for laying hens (from Animal research farm, Kasetsart University, Bangkok). In the whole experiment, 20 eggs were received from the open house system and 19 eggs were received from the evaporative cooling system. Unwashed eggs were collected in sterile bags and transported to the laboratory. Aseptic procedures were strictly adopted during collection of samples. Sterile cotton swabs dipped in sterile peptone broth were used to swab the entire surface area of the eggshell then added to the peptone broth, and subsequently incubated for 16-18 h at 37°C.

In order to collect the egg contents, eggs were surface sterilized by immersion in 75% alcohol for 2 min, air dried in a sterile chamber for 10 min then cracked with a sterile knife. Each egg's content was mixed thoroughly and 1 ml of the mixed egg content was inoculated into 9 ml of peptone broth and incubated at 37 °C for 16-18 h.

B. Conventional microbiology detection

After pre-enrichment, 1 ml of enriched cultures of all sample types were transferred to 9 ml of RVS and incubated at 42°C for 18-24 h. At the end of selective enrichment, the broths were plated onto XLD agar and incubated at 37°C for 24 h in order to isolate the suspected colonies. *S. typhimurium* was used as positive control.

C. DNA preparation and PCR assay

Template DNA was prepared from the naturally contaminated egg product enriched by incubation for 16-18 h in peptone broth. After incubation, 1 ml of the pre-enrichment media was centrifuged for 2 min at 13,000 *g*. The bacterial cells were dissolved in 50 µl of H_2O and heated for 10 min at

100°C. The bacterial cells were centrifuged for 2 min at 13,000 *g*. Salmonella specific *invA* gene sequences of Salmonella 139-GTGAAATTATCGCCACGTTCGGGCAA and 141-TCATCGCACCGTCAAAGGAACC were used as primers in this study. PCR was performed in a final volume of 25 µl containing 25 mM $MgCl_2$ 10 mM of dNTPs, 1.5 U of Taq DNA polymerase , 10 pmol of each primer, 1 µl of DNA template and. The mixture was subjected to 30 cycles of amplification in a thermal cycler. The first cycle was preceded by denaturation for 2 min at 95°C. Each cycle consisted of denaturation for 30 s at 95°C, annealing for 30 s at 64°C, and elongation for 30 s at 72°C. The last cycle was followed by a final elongation for 5 min at 72°C. The PCR products were analysed on a 1.2% (w/v) agarose gel electrophoresis.

III. Results

A total of 39 egg samples from animal research farm were tested by conventional methods and by PCR methods for detection of *Salmonella* spp. The results showed that none of the conventional methods detected any positive samples, while analysis of the PCR products from direct boiling of the enriched cultures showed that 2 cultures were found positive of *Salmonella* spp. as shown in Table 1 and Fig. 1. Comparing the different housing systems, we found the contamination only in egg-shell from the evaporative cooling system.

TABLE I DETECTION OF *SALMONELLA* STRAIN BY PCR SYSTEM

Sample	No.of samples	Conventional method	PCR positive results by PCR
Conventional barn housing system			
Egg-shell	20	-	-
Egg content	20	-	-
Evaporative cooling system			
Egg-shell	19	-	2
Egg content	19	-	-

IV. DISCUSSION

The PCR assay and the conventional microbiological method showed a different level of sensitivity for detection for *Salmonella* spp. In this study, part of the *InvA* of Salmonella was amplified and detected *Salmonella* spp. in shell egg from the evaporative cooling system, while no sample was detected by the microbiological method. PCR is a sensitive method with a superior ability to detect *Salmonella* spp. in the presence of other competing bacteria [15][16][17][18][19].

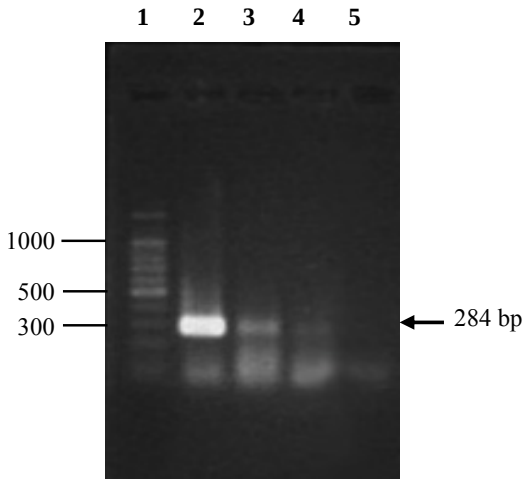

Fig. 1 Representative of PCR amplification of *InvA* gene on 1.2% agarose gel electrophoresis. The expected size for this gene is 284 bp. Lane 1: 100 bp marker. Lane 2: Positive control *S. typhimurium*. Lane 3-4: Salmonella sp. isolated from egg-shell from the evaporative cooling system. Lane 5: Negative control.

The method was also much quicker than conventional techniques taking less than 24 h to obtain a result as opposed to 4-5 d. Thus, the PCR assay targeting the *invA* gene can potentially be used to detect *Salmonella* in egg samples. In addition, when comparing the initial egg shell and egg content contamination between two housing systems for laying hens, only in the samples from the evaporative cooling system was *Salmonella* spp. detected. The pad where the water evaporates may present a risk for bacterial contamination in the evaporative cooling system.

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