

DETECTION OF SALMONELLAE IN HEN EGGS IN THAILAND

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Abstract. Two thousand four hundred and ninety eggs were collected from retail markets in 6 provinces and from laying hen farms in 3 provinces. Eggs were pooled in groups of 3 to obtain 830 samples for testing. Isolation of salmonellae was made from both egg shell and egg contents. Eggs from retail markets were contaminated with salmonellae on egg shells (13.2%) and in egg contents (3.9%). Three (0.4%) samples yield positive both on egg shells and in egg contents. Of the 86 samples from laying hen farms, salmonellae were found on egg shells and in egg contents, 3.5% and 1.2%, respectively. From the 134 strains tested, twenty-four serotypes were confirmed. *Salmonella cerro*, *S. amsterdam* and *S. typhimurium* were predominantly encountered, 4.8%, 4.3% and 1.4%, respectively. Only two samples were contaminated with *S. enteritidis*, one each from open market and laying hen farm, one on egg shells and the other in egg content respectively.

INTRODUCTION

Human salmonellosis is a public health problem worldwide and food animals including avian species are considered as the major source of these infections. Recently, there have been many reports in criminating hen's eggs as the vehicle of the infection. (Chapman *et al.*, 1988; Sharp, 1988; Anon, 1988; 1992; Cowden *et al.*, 1989a,b; Perales and Audicana, 1989; Rodrigue *et al.*, 1990; Humphrey, 1990). The prevalence of salmonellae in egg products, such as frozen unpasteurized eggs has ranged from 32% (Wilder and MacCready, 1966) to 54% (Garibaldi *et al.*, 1969). However, the contamination of whole eggs either in retail markets or on farms was relatively low. Baker *et al.* (1980) found that 3 (0.2%) eggs from 1,400 tested samples were positive. Generally, the occurrence of *Salmonella* contamination rarely exceeded 1% (Perales and Audicana, 1989; Humphrey *et al.*, 1991). The highest rate of reported occurrence of salmonellae in eggs was in Egypt, where 10% of table eggs were positive (WHO, 1985). In Thailand, no confirmed egg-associated outbreaks of human salmonellosis have been reported although sporadic cases may well escape detection. In order to prevent and control the transmission of this foodborne pathogen via eggs to man, we should know the prevalence of the organism. The purpose of the present work was to assess the incidence of salmonellae in eggs collected from retail markets as well as laying hen farms.

MATERIALS AND METHODS

Tested eggs

Two thousand four hundred and 90 eggs were collected from 14 open markets and 9 supermarkets and 7 laying hen farms from October 1991 through June 1992. Of the 14 open markets, 9 located in Bangkok and one each in Chon Buri, Chachoengsao, Lop Buri, Nakhon Ratchasima and Ang Thong Provinces. All supermarkets were in Bangkok. The seven laying hen farms were in Chon Buri (2 farms), Chachoengsao (2), Nakhon Pathom (1) and Saraburi (2). The numbers of eggs from open markets, supermarkets and farms were 1,701,531 and 258, respectively. The eggs from open markets and farms were frequently visibly contaminated with feces, while those from supermarkets were always clean. The eggs were not refrigerated.

Salmonella isolation and identification

Egg samples for salmonellae isolation were grouped in pools of 3. of a total 2,490 eggs, 830 samples were obtained for analysis. Eggs were placed in sterile bags and then 100 ml of buffered peptone water (BPW) was added. They were left in room temperature for 30 minutes and subsequently gently rubbed through the bag for 1 - 2 minutes, in order to release bacteria attached on the shell. Eggs were then removed from the bag

and placed in 95% ethyl alcohol for 1 minute and flamed to disinfect the shell. Eggs were then cracked aseptically and placed into another sterile bag. The egg contents were diluted with 300 ml BPW and homogenized for 1 minute in a Stomacher 400 (Seward Medical, England). The bags containing BPW after washing the egg shells and the emulsion of egg contents were considered as the samples for isolation of salmonellae from the egg shells and contents, respectively. The samples were incubated for 18 hours at 37°C, after which 0.1 ml was inoculated into modified semisolid Rappaport-Vassiliadis medium (MSRV) (De Smedt and Bolderdijk, 1987). The inoculated MSRV plates were incubated for 18 hours at 42°C and 3 motile colonies were stabbed and streaked on triple sugar iron agar and lysine iron agar. Typical colonies were purified and further confirmation was made as described in the previous report (Jerngklinchan and Saitanu, 1993).

RESULTS

Table 1 shows the prevalence of salmonellae in eggs. Salmonellae were frequently found on the egg shells, from which the recovery rates were 12.2, 16.4 and 3.5% of the samples collected from open markets, supermarkets, and farms, respectively. The egg contents were found to be positive in 4.1%, 3.4% and 1.2% of the samples from open markets, supermarkets and farms, respectively. Only 3(0.5%) samples from open markets showed that salmonellae could be isolated from both egg shells and contents.

Table 2 demonstrates the serotypes of salmonellae. Twenty-four serotypes were confirmed from 134 tested strains. Generally, *S. cerro*, *S. amsterdam* and *S. typhimurium* were commonly found on egg shells and in egg contents no matter what the origins of the samples.

DISCUSSION

It would appear from the present study that the prevalence of salmonellae on egg shells and in egg contents is quite high. The rate of contamination of egg shells from the markets varied from 3.5 - 16.4% while the contamination in egg contents was ranged from 1.2 - 4.1%. The contamination of eggs may occur through transovarian passage. *S. pullorum* and *S. gallinarum* commonly infect the ovaries of laying hens and the organisms can be transmitted in the yolk of the eggs (Bryan, 1968; Pomeroy, 1984; Snoeyenbos, 1984). With other salmonellae there is some controversy as to how often this occurs. Shivaprasad *et al* (1990) and Forsythe *et al* (1967) could detect *S. enteritidis* from the shells but not from contents of eggs delivered from experimentally infected hens. However, Cox *et al* (1973), Timoney *et al* (1989) and Humphrey *et al* (1989a) detected salmonellae in the egg contents in their experiments which showed that salmonellae other than *S. pullorum* and *S. gallinarum* could undergo vertical transmission. Infected hens excreted salmonellae in feces and this may contaminate eggs (Forsythe *et al*, 1967; Cox *et al*, 1973). Salmonellae contaminating the shells can multiply and penetrate into the chorioallantoic membranes and yolk sacs (Wil-

Table 1

Prevalence of salmonellae from hen eggs collected from retail markets and layer farms.

| Samples location | Open markets (n = 567) | Supermarkets (n = 177) | Laying hen farm (n = 86) | Total (n = 830) |
|-------------------|---------------------------|---------------------------|-----------------------------|--------------------|
| Shell | 69(12.2)* | 29(16.4) | 3(3.5) | 101(12.2) |
| Content | 23(4.1) | 6(3.4) | 1(1.2) | 30(3.6) |
| Shell and content | 3(0.5) | 0 | 0 | 3(0.4) |
| Total | 95(16.8) | 35(14.8) | 4(4.7) | 134(16.1) |

* Number of positive sample (%)

Table 2

Salmonellae serotypes from hen eggs collected from retail markets and layer farm.

| Serotype | Open markets | | | Supermarket | | Farm | | Total |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------|
| | Shell(S) | Content(C) | S and C | S | C | S | C | |
| <i>S. cerro</i> | 26* | 6 | - | 8 | - | - | - | 40(4.8)** |
| <i>S. amsterdam</i> | 15 | 12 | 1 | 8 | - | - | - | 36(4.3) |
| <i>S. typhimurium</i> | 2 | 3 | 1 | - | 6 | - | - | 12(1.4) |
| <i>S. tennessee</i> | 5 | 1 | - | - | - | - | - | 6(0.7) |
| <i>S. mbanbaka</i> | 3 | - | - | 3 | - | - | - | 6(0.7) |
| <i>S. singapore</i> | 2 | - | - | 3 | - | - | - | 5(0.6) |
| <i>S. emek</i> | 3 | - | - | 1 | - | - | - | 4(0.5) |
| <i>S. montevideo</i> | 3 | - | - | - | - | - | - | 3(0.4) |
| I.6, 7 : 1, V :- | - | - | - | - | 3 | - | - | 3(0.4) |
| I.6, 7 : Z ₁₀ :- | 2 | - | - | - | - | - | - | 2(0.2) |
| Other serotypes | 4 ^a | 2 ^b | 1 ^c | 4 ^d | 2 ^e | 3 ^f | 1 ^g | 17(2) |
| Total | 65 | 24 | 3 | 27 | 11 | 3 | 1 | 134(16.1) |

* = Number of samples positive

** = Number of samples positive (percentage)

a = *S. alachua*, *S. enteritidis*, *S. infantis* and I.6, 7 : d :- (1 each)b = *S. alachua* and *S. schwarzengrund* (1 each)c = *S. potsdam*d = *S. abany*, *S. lexington*, *S. oslo* and I.6, 7 :- (1 each)e = *S. agona* and *S. albania* (1 each)f = *S. blockey*, *S. enteritidis* and *S. thompson* (1 each)g = *S. london*

liams and Dillard, 1968; Williams *et al*, 1968; Padron, 1990). Salmonellae remained viable and multiplied at 25°C but not at 4°C or 10°C (Clays and Board, 1991; Lock and Board, 1992). Once the organism survived and grew in the eggs contents, they were able to survive in partially cooked eggs (Humphrey *et al*, 1989b). Eggs in the open markets were unwashed and not refrigerated. These conditions are favorable for the growth of bacteria and penetration into the egg contents. Because of the high rate of contamination, hen eggs may be a vehicle of human salmonellosis in this country. We recommend that eggs should be washed, followed with the application of sanitizing chemicals and kept at refrigeration temperature. 4°C, for prevention the multiplication of salmonellae. Consumers should also be encouraged to cook well.

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