

Natural presence of the bacterium *Salmonella* sp. in hen eggs consumed in Costa Rica

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Resumen: Se analizó la presencia natural de *Salmonella* sp. en la cáscara y el contenido de huevos frescos de gallina fértiles, infértiles y rajados vendidos en Costa Rica durante los meses de julio de 1993 a marzo de 1994. Se estudiaron diez lotes de cinco muestras cada uno utilizando la metodología descrita en el Bacteriological Analytical Manual (BAM). La bacteria fue aislada de la cáscara de 38 huevos (25.3%) y del contenido de 28 (18.7%). Hay una mayor incidencia de *Salmonella* sp. en los huevos rajados, así como hay un mayor porcentaje de aislamiento a partir de huevos infértiles que de fértiles. El método usado demostró una mayor efectividad al usar caldo tetracionato incubado por 24 horas como medio de enriquecimiento selectivo, así como al usar agar xilosa-lisina-desoxicolato como medio de cultivo selectivo.

Key words: *Salmonella* sp., fresh eggs, foodborne infection.

Foodborne salmonellosis has been epidemiologically linked to egg and egg products (Watt 1945, McCroan *et al.* 1963, Coyle *et al.* 1988, St. Louis *et al.* 1988) and in recent years, the percent of *Salmonella enteritidis* outbreaks occurring in the world has increased (Anonymous 1992). The analysis of the outbreaks has revealed a significant association with the consumption of scrambled eggs, lightly cooked eggs and shop bought egg sandwiches (Humphrey *et al.* 1989). This implies that some naturally contaminated eggs must have contained large numbers of *Salmonella* cells before cooking (Humphrey *et al.* 1991).

The sources of introduction of *Salmonella* into poultry houses have been widely discussed (Eckroade *et al.* 1992), suggesting the consumption of contaminated feed, vertical transmission and horizontal spread of the bacteria, infected mice introducing it to poultry house environments (El-Assaad *et al.* 1990, Opitz 1992) or insufficient cleaning, but there is no convincing evidence for any of these explanations.

The present study was undertaken to determine the presence of *Salmonella* sp. in

the eggshell and contents of either fertile, infertile and infertile cracked hen eggs consumed in Costa Rica, to establish the potential public health hazard they represent. Infertile cracked eggs were analyzed because they are widely used at present, either by food processing plants or by the general population, which purchases them cheaper than unbroken eggs; 50 hen eggs of each group: fertile, infertile and cracked, all unwashed and fresh were used in this study. Ten lots consisting of five samples of each class of the three categories were purchased from local groceries and farmers from July 1993 to March 1994, and analyzed within a 24 hour period.

Eggshell and egg contents (albumen and yolk) were separately analyzed for the presence of *Salmonella* sp. according to the methodology of the Bacteriological Analytical Manual (Anonymous 1978).

Each complete hen egg was placed in a sterile plastic bag containing 90 ml of lactose broth (preenrichment media, Difco). It was gently hand rubbed through the bag for about

30 seconds and removed for washing with soap (Alconox) and immersed in alcohol 95° for 30 minutes, then cracked and albumen and egg yolk were also placed in 90 ml of lactose broth and homogenized using sterile glass pearls. All preenrichment cultures were incubated for 18-20 hours at 37°C. 1 ml portions of these media were transferred to tubes with 10 ml tetrathionate broth (Difco) and tubes with 10 ml selenite cysteine broth (Difco) for selective enrichment. Incubation of tetrathionate broth was carried out at 43°C, and of selenite cysteine broth at 37°C for 24 and 48 hours respectively.

Each selective enrichment media was streaked into xylose-lysine-desoxycholate agar (XLD) (Difco) and Hecktoen agar (Difco) and incubated at 37°C. After 24 hours, *Salmonella* like colonies were purified and confirmed by biochemical and serological testing. Biochemical reactions included triple sugar iron agar (TSI) (Difco), lysine decarboxylase broth (LDB) (Difco), urease reaction, citrate utilization, Voges-Proskauer reaction and phenylalanine reaction. Serological identification was carried out using the Salmonella Poly A-I + Vi antiserum from SA Scientific Corporation.

Salmonella sp. was isolated from the shell of 38 eggs (25.3%) and from the contents of 28 (18.7%). The rate of isolation of the bacteria from cracked eggs was significantly higher than from non-cracked eggs. Also, there is a greater percent of isolation from infertile eggs than from fertile eggs (Fig. 1).

The greater number of isolations was obtained from the incubation of the selective enrichment media for 24 h (57%) than for 48 h (43%). Also, a greater percent of isolation was obtained from the tetrathionate broth (57%) than from the selenite-cysteine broth (43%). More isolates were obtained using XLD (55%) than Hecktoen agar (45%) as selective plating agars.

Since 1980, intact shell eggs have been identified as an important vehicle for human infections with *Salmonella* sp. (Anonymous 1986). Countries as the US (Anonymous 1992), Czech. Republic (Sramova *et al.* 1993), Germany (Zastrow and Schoneber 1993), Italy (Binkin *et al.* 1993), Argentina (Eiguer *et al.* 1990), confirm that the rise in salmonellosis in recent years is associated with the consumption of contaminated eggs or egg derivatives. Costa Rica lacks real information as to its incidence in its population, since most cases occur spo-

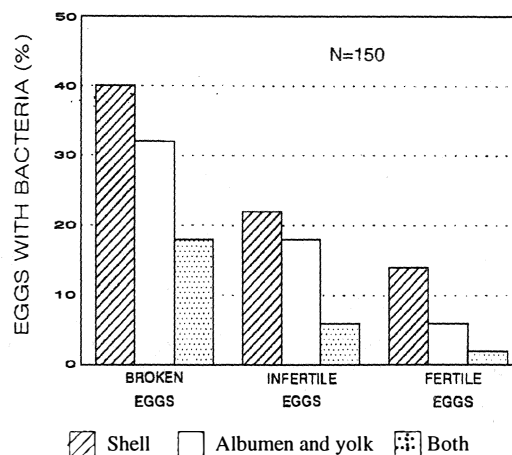


Fig. 1. Percentage of eggs with *Salmonella* sp. in Costa Rica.

* $p \leq 0.005$ between cracked and uncracked eggs.

radically or as limited family outbreaks that are not reported for statistical reference, but the percent of *Salmonella* sp. obtained in egg and eggshell in this work confirms the serious hazard to public health that this bacteria poses.

There was a greater percentage of *Salmonella* sp. in infertile (18%) than in fertile (6%) eggs, this suggests the presence of local flocks infected with the bacteria. Also, the growing use of cracked eggs due to their low price, and the high contamination they present, (32%) increase the probability of the occurrence of alimentary infection outbreaks.

Because of the percentage of *Salmonella* sp. found in egg and eggshells in this work, food containing raw or undercooked eggs poses a risk of infection by this bacteria. Since most serious illnesses or deaths associated with these infections occur among infants, the elderly and immunocompromised persons, special attention should be paid to the diets of these persons.

The US Food and Drug Administration recommends the use of selenite and tetrathionate broths as selective enrichment media (Anonymous 1975, Anonymous 1978, Anonymous 1981). Also, the incubation of the selective preenrichment media at elevated temperatures for at least two days has been reported to enhance the recovery of *Salmonella* from high moisture foods (Silliker *et al.* 1964, Yde and Ghysels 1984). The largest number of isolations in this work was obtained by the combination of tetrathionate broth incubated for 24 h at 43°C

and XLD agar. These methodology differences may be due to the high level of contamination these eggs present.

The use of a combination of a highly selective plating media (Hecktoen) with a less selective one (XLD), also has been recommended for effective isolation (Anonymous 1978). In this study, XLD performed better than Hecktoen agar, but the use of both media allowed for better recovery.

In conclusion, to avoid further spreading of *Salmonella* sp. and to improve the protection of the consumer, certain measures must be taken in the production, processing and handling of hen eggs in commercial and private kitchen. Also, efficient, standardized and responsive methodologies must be developed in order to obtain more reliable results.

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