
SHORT REPORT

National surveillance of *Salmonella* Enteritidis in commercial eggs in Japan

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SUMMARY

A total of 105 033 eggs were collected across Japan from June 2010 to January 2011 and tested for *Salmonella* Enteritidis to provide data for the risk profiling of *S. Enteritidis* in eggs by the Food Safety Commission of Japan. *S. Enteritidis* isolates were recovered from three samples (20 eggs/sample) and these samples were different in regard to sampling period, grading and packaging centre and farm. The prevalence of *S. Enteritidis* in commercial eggs in Japan is estimated at ~0.003% which was a tenfold decrease in prevalence compared to similar surveillance in the mid 1990s. The decrease in the contamination in commercial eggs is considered a contributory factor in the decrease of foodborne diseases associated with *S. Enteritidis* in this period.

Key words: Egg, *Salmonella* Enteritidis, surveillance.

Human salmonellosis is one of the most significant foodborne illnesses worldwide. In Japan, *Salmonella*-related foodborne diseases have decreased during 2000–2010; however, it still ranks as one of the top three bacterial agents causing foodborne disease. *Salmonella* Enteritidis has been the most frequently isolated serotype over the past 10 years and the foodborne disease outbreaks caused by *S. Enteritidis* in Japan have been mainly attributed to the consumption of foods associated with contaminated eggs [1].

In 2006, the Food Safety Commission of Japan started to organize the risk profile of *S. Enteritidis* contamination in eggs in order to conduct a risk assessment. In this process, it was pointed out that a nationwide estimate of *S. Enteritidis* contamination in eggs was necessary for the risk assessment. In the

present study, commercial eggs were collected across Japan and tested for *S. Enteritidis*.

Commercial eggs were collected at 15 of 47 prefectures (Hokkaido, Aomori, Miyagi, Gunma, Ibaraki, Tokyo, Kanagawa, Mie, Kyoto, Osaka, Hiroshima, Ehime, Oita, Kumamoto, Kagoshima) across Japan. A total of 600 eggs of 15 different types of package labels (40 eggs/package label) were purchased at retail stores in each area and 9000 eggs were collected in 15 areas at every sampling time. In total, 108 000 eggs were collected during 12 samplings from June 2010 to January 2011. Eggs were collected twice monthly from September to December. These eggs were transported to our laboratory at room temperature. Tests for *S. Enteritidis* were started within 3 days of receiving the eggs. When egg shell cracks were detected, those eggs were discarded, leaving 105 033 of 108 000 collected eggs for testing. These shell cracks were mainly due to accidents during the transport of the eggs.

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Tests of the egg contents were performed according to methods described by the *Salmonella enteritidis* Pilot Project [2]. Contents of 20 eggs were pooled and homogenized in a sterile plastic bag and incubated for 3 days at room temperature (26 °C). After the incubation, one loop (10 µl) of each culture was inoculated onto CHROMagar Salmonella agar (Becton Dickinson, USA) and desoxycholate-hydrogen sulphate-lactose agar (Eiken Co. Ltd, Japan) supplemented with novobiocin (20 mg/l), then incubated at 37 °C for 24 h. Suspected *Salmonella* colonies were isolated and identified biochemically with API20E (Sysmex bioMérieux Co. Ltd, Japan) and *Salmonella* isolates were then tested for agglutination with O antisera and tube agglutination with H antisera. *S. Enteritidis* was confirmed according to the Kauffman–White scheme [3]. Phage typing of *S. Enteritidis* was performed at the National Institute for Infectious Diseases, which is the reference laboratory for phage typing of *Salmonella* in Japan.

The Clopper–Pearson exact test was used to calculate 95% confidence intervals (CI) for the prevalence of *S. Enteritidis* in liquid eggs [4].

A total of 105 033 eggs (5400 pooled samples) were tested and *S. Enteritidis* was isolated from three of the pooled egg samples. These three cases of *S. Enteritidis* contamination seem to be independent as the farm of origin, grading and packaging centre, distributor and place of purchase were all different from each other. The phage types of these *S. Enteritidis* isolates were PT1 (two isolates) and PT47 (one isolate) which were two major phage types of *S. Enteritidis* isolates recovered from foodborne diseases in Japan, 2010.

Assuming that only one egg out of 20 was contaminated with *S. Enteritidis* in each of the three positive samples, 0.0029% (95% CI 0.0025–0.0032) is the prevalence of *S. Enteritidis* of liquid eggs in Japan. This postulation appears reasonable because *Salmonella* egg contamination was not detected during a recent survey conducted by the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan [5] which tested 20 300 eggs, making the rate less than 1/20 300. The prevalence of *S. Enteritidis* obtained from a 1990–1992 survey was 0.03% (7/26 400), and this decreased to about 0.003% (3/90 100) in a 2004 study [6, 7]. These surveys were performed in limited areas of Japan, and the prevalence of 2004 was biased because eggs from known *Salmonella*-contaminated farms were

also tested. In the present study, the eggs were randomly collected across Japan and the rate of *S. Enteritidis* egg isolation would be the nearest to the true national contamination rate in data obtained so far.

The decrease of *Salmonella* contamination from the late 1990s to 2000s might be the result of various efforts for *Salmonella* control in the late 1990s. In 1998, *S. Enteritidis* and *S. Typhimurium* were designated as notifiable infectious diseases by the revision of the Act on Domestic Animal Infectious Disease Control. Moreover, the standards of chicken egg or household egg handling guidelines (e.g. the setting of best-before date) were set and the use of inactivated *Salmonella* vaccine for *S. Enteritidis* or *S. Typhimurium* was approved. In response to these measures, producers increased efforts to produce clean eggs. After these approaches, the detection rate of *S. Enteritidis* in layer farms had decreased by half [8] and foodborne diseases caused by *Salmonella* decreased by 90% (757 cases in 1998 to 73 cases in 2010).

In summary, the rate of *S. Enteritidis* contamination in commercial eggs is estimated at 0.003% following the Japan-wide surveillance. The decreased rate of *S. Enteritidis* egg contamination mirrors decreases in foodborne illness in humans due to *Salmonella* in Japan over this time. These decreases of *Salmonella* contamination could be the results of various approaches for *Salmonella* control from farm to table. To the best of our knowledge, the results of the present study provide the most recent dataset of the prevalence of *S. Enteritidis* in shell eggs at retail shops in Japan.

DECLARATION OF INTEREST

None.

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REFERENCES

1. **Ministry of Health, Labour and Welfare of Japan.** Food poisoning investigation reports (<http://www.mhlw.go.jp/topics/syokuchu/04.html>). Accessed 5 October 2011.
2. **Schlosser WD, et al.** 'Salmonella enteritidis Pilot Project' Progress Report. Washington, DC: United States Government Printing Office, 1995.
3. **Grimont PAD, Weill FX.** *Antigenic Formulas of the Salmonella Serovars*, 9th edn. Paris: Institut Pasteur, 2007.
4. **Clopper CJ, Pearson ES.** The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika* 1934; **26**: 404–413.
5. **Sasaki Y, et al.** *Salmonella* prevalence in commercial raw shell eggs in Japan: a survey. *Epidemiology and Infection* 2010; **139**: 1060–1064.
6. **Nakanishi, H.** Present status of gastroenteritis due to *Salmonella* Enteritidis and its preventive measures [in Japanese]. *Shokuhin Eiseigaku Zasshi* 1993; **34**: 318–322.
7. **Lapuz R, et al.** The role of roof rats (*Rattus rattus*) in the spread of *Salmonella* Enteritidis and *S. Infantis* contamination in layer farms in eastern Japan. *Epidemiology and Infection* 2008; **136**: 1235–1243.
8. **Sato S.** Control of *Salmonella* in western countries and Japan [in Japanese]. *Kakishippigaku Bunkakaihou* 2003; **9**: 2–4.