

RISK OF *SALMONELLA* IN A SUBURBAN REGION OF VIENTIANE, LAO PEOPLE'S DEMOCRATIC REPUBLIC

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Abstract. The study examined non-typhoid *Salmonella* infection incidence in a suburban region of Vientiane, Lao People's Democratic Republic (PDR), an area that has undergone rapid economic development in recent years. The research was conducted in two rural villages located in the suburb of Vientiane during the period 2005 - 2013. Two new methods of non-typhoid *Salmonella* detection, namely, MY Phenomenon/MIDO Ring and enhanced visibility of color change in media, were used to monitor the changes in non-typhoid *Salmonella*-positivity rate over the 9-year period. Both methods were effective in detecting non-typhoid *Salmonella*. Non-typhoid *Salmonella* infection rate in one village decreased during the study period. However, further research regarding non-typhoid *Salmonella* in Lao PDR is necessary from an economical point of view.

Keywords: *Salmonella*, MY Phenomenon/MIDO Ring test, Lao PDR

INTRODUCTION

Southeast Asia is experiencing a period of rapid economic growth. Countries in this region are promoting sustainable development and the Lao People's Democratic Republic (PDR) is no exception (World Bank, 2009). For example, the gross domestic product (GDP) of Lao PDR has doubled in the past decade and the quality of life has changed significantly as infrastructure systems have improved (World

Bank 2009). Knowledge of disease transmission and effective public health interventions have controlled many infectious diseases, including food-borne diseases; however, infectious diseases are still the most common threat to public health in Lao PDR (Midorikawa *et al*, 2016).

Lao PDR began gradually ending its national isolation policy in 1991 and, since then, the government has made improvements in public health system aimed at decreasing risk of contracting infectious diseases in the country (Midorikawa *et al* 2010). Infectious diseases are also known as transmissible diseases or communicable diseases.

Research conducted in 1994 found that a majority of participants tested for *Salmonella* are infected with *Salmonella*

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(Midorikaw *et al*, 1996). While *Salmonella typhi* and *S. paratyphi* have been a major public health problem in Lao PDR, non-typhoid *Salmonella* cases were not noted. Given that the last survey was conducted in 1994, further *Salmonella* research among Laotians is required. Therefore, a baseline research examining the risk of non-typhoid *Salmonella* in Lao PDR was started in 2005 and is ongoing. In this study non-typhoid *Salmonella* infection rates in villages in Lao PDR were investigated, using two new detection methods (Midorikawa *et al*, 2005).

MATERIALS AND METHODS

Study sites

Two study sites were chosen. Phai Lom Village (BPL) is located in Saitani District in the suburbs of Vientiane, Lao PDR. Endemic cholera was present in 1995 in this region. The infection rate of food poisoning diseases, such as *Salmonella*, was investigated from 2005 to 2013. Koksaa Village (BKS) is located in a suburb of Vientiane near BPL and in Saitani District. Research in this village was conducted between 2008 and 2013.

The research study obtained ethical clearance from National Ethics Committee for Health Research, Lao PDR Ministry of Health (No.102/NECHR).

Detection of non-typhoid *Salmonella*

Fecal samples from people living in the three villages were collected and transported in agar [Seed swab gamma (γ) 2: Eiken, Kagaku, Tokyo, Japan] to laboratory in Suzuka University Medical Science, Mie, Japan. Two new detection methods previously developed (Midorikawa *et al*, 2009a) were used to identify the presence of non-typhoid *Salmonella*. Both methods are based on H₂S production by

Salmonella, which is detected by reaction with citric acid and by enhanced visibility of color change in triple sugar iron (TSI) agar supplemented with an increased concentration of NaCl (from 0.5% to 3%) that suppresses H₂S production (Midorikawa *et al*, 2014). The monitoring steps were as follows (Fig 1):

1) *Salmonella* culture samples were inoculated in a selective agar enrichment Rappaport-Vassiliadis soya peptone (RVS) broth (Oxoid, Lenexa, KS) and incubated for 24 or 48 hours at 37°C.

2) Samples were then inoculated on deoxycholate hydrogen sulfide lactose (DHL) agar (Eiken Kagaku, Tokyo, Japan), a selective agars for *Salmonella*, and incubated for 24 hours at 37°C.

3) Putative *Salmonella* colonies were inoculated on DHL agar. A paper disk (10 mm diameter) containing citric acid was placed on the center of the agar medium. The agar medium then was incubated for 24 hours at 35-37°C.

4) Putative *Salmonella* colonies were inoculated on TSI medium containing 0.5% NaCl (the standard method) and incubated for 24 hours at 37°C.

5) Putative *Salmonella* colonies were inoculated on TSI medium supplemented with 3% NaCl (the modified method) and incubated for 24 hours at 37°C.

6) Putative *Salmonella* colonies were inoculated on lysine indole motility (LIM) agar (Eiken Kagaku, Tokyo, Japan) and incubated for 24 hours at 37°C.

Formation of a MIDO Ring on DHL agar (MY Phenomenon) indicates presence of non-typhoid *Salmonella* (Fig 2). A color change in TSI agar (black) resulting from FeS formation from H₂S production indicates non-typhoid *Salmonella* (Fig 3). Positive *Salmonella* isolates as shown on

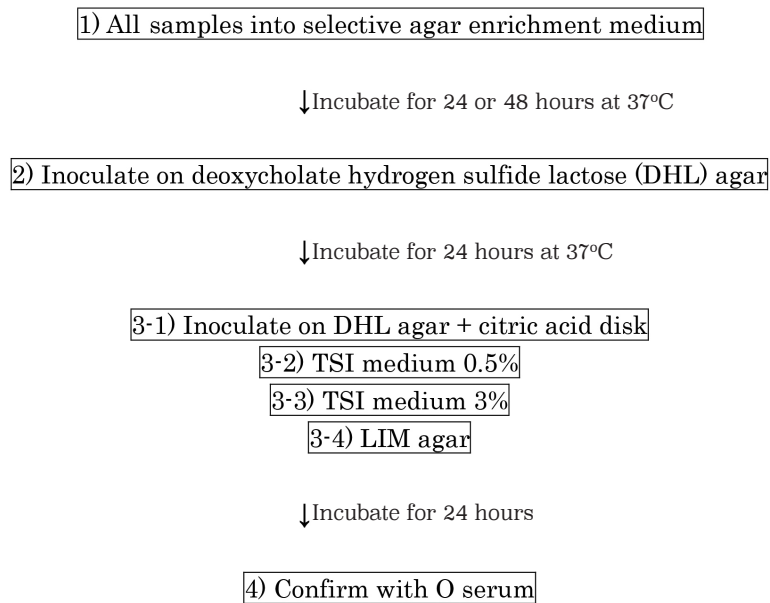


Fig 1—Steps involved in detecting non-typhoid *Salmonella*.

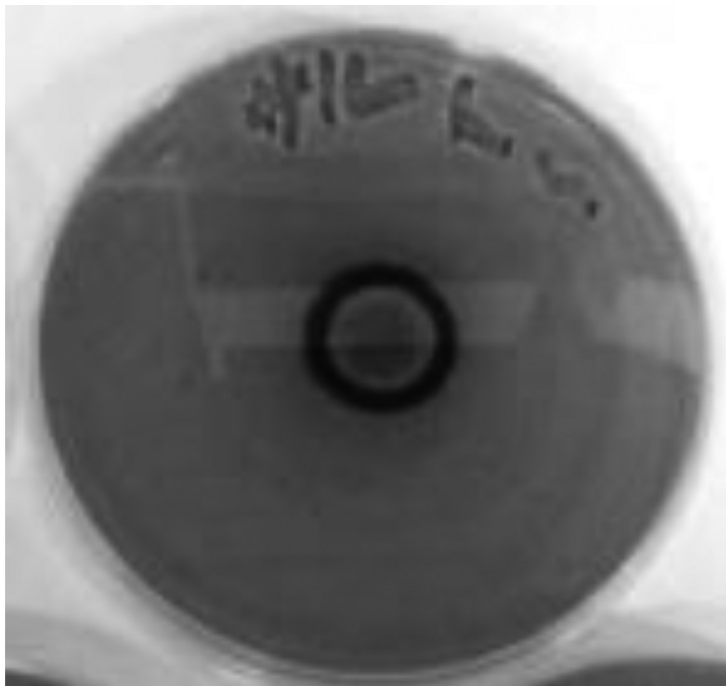


Fig 2—Formation of MIDO Ring in MY Phenomenon test of non-typhoid *Salmonella*.

TSI, LIM and DHL agars were confirmed by O serum test (Denka Seiken, Tokyo, Japan).

Statistical analysis

Chi-square test was used to analyze change in *Salmonella* positivity rate. A $p < 0.01$ is considered significant.

RESULTS

Detection of non-typhoid *Salmonella*

For detecting non-typhoid *Salmonella*, both the MY Phenomenon and the medium supplemented with higher (3%) NaCl concentration tests were effective. Citric acid enhanced H₂S production, while the higher NaCl concentration decreased H₂S production to enhance visibility of the color change in TSI agar. Both methods facilitated the detection of non-typhoid *Salmonella*. A MIDO Ring was apparent in all non-typhoid *Salmonella* isolates detected in this study, while a color change indicating glucose fermentation was seen when the NaCl concentration of TSI agar was 3% (Fig 3). *Salmonella* identified by the two methods belonged to Derby and Anatum type as determined by the O serum assay (data not shown).

Salmonella positivity rates

Salmonella positivity in the two participating villages in Lao PDR were determined between 2005 and 2013. At the beginning of the study in 2005, 28/63 (44%) villagers living in BPL were *Salmonella* positive, and the incidence of non-typhoid *Salmonella* was 29%. In the following year, non-typhoid *Salmonella* was detected only in 7/24 (29%) stool samples, but in 2007 non-typhoid *Salmonella* was detected in 22/60 (37%) stool samples and by 2012 non-typhoid *Salmonella* was detected in 19/151 (13%) stool samples, a significant change when compared to 2007 ($p = 0.0001$). In BKS, located close to BPL, in 2008 *Salmonella* incidence was 14% (8/57 stool samples), and in 2009 this remained unchanged at 15% (4/27 samples), but dropped to 9% (4/41 samples) in 2013, but the change is not significant when compared with 2008.

DISCUSSION

In this study to monitor non-typhoid *Salmonella* in fecal samples two newly developed detection methods were employed, namely, MY Phenomenon/MIDO Ring and enhanced visibility of color change in media (Midorikawa *et al*, 2009a,b). Advantage of the former method is that only non-typhoid *Salmonella* forms Mido ring but not typhi or paratyphi, while the advantage of the latter method is that glucose fermentation in TSI medium only occurs in the presence of 3% NaCl.

In the previous study in 1994, non-ty-

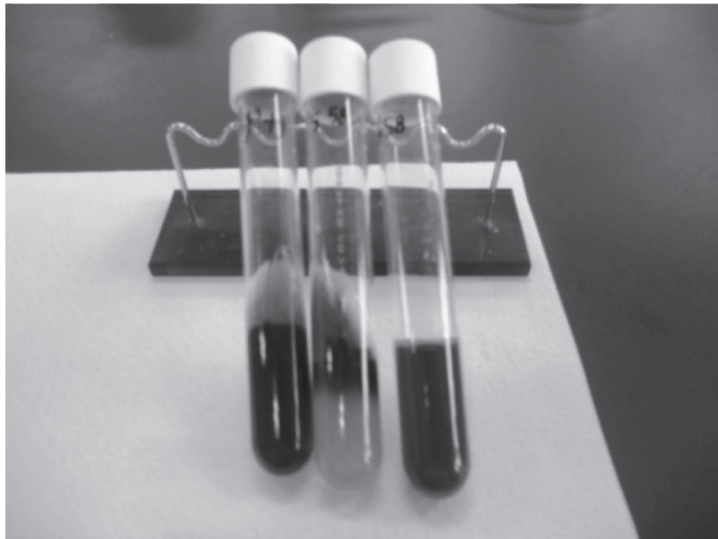


Fig 3—Enhanced visibility test of non-typhoid *Salmonella*. Left: TSI medium with 0.5% NaCl; Center: TSI medium with 3% NaCl; Right: LIM medium.

phoid *Salmonella* positivity is 27% (Midorikawa *et al*, 1996), a value much higher than that (0.01%) reported in Japan (Hara-Kudo and Takatori, 2009). The results from 2006 and 2007 also showed infection rates of non-typhoid *Salmonella* of approximately 30%. Survey of non-typhoid *Salmonella* infection rate in BPL was not conducted between 2008 and 2010, but result from BKS, located near BPL, in 2008 showed a 14% infection rate. In Puxai Village, a rural village located in Attapeu Province in southern Lao PDR, a 13.8% infection rate of non-typhoid *Salmonella* was detected in 2009 (unpublished data). These results suggest that non-typhoid *Salmonella* infection is not limited to the Vientiane area but probably can be found throughout Lao PDR.

As the rates of non-typhoid *Salmonella* infection in BPL between 2005 and 2007 were considerably higher than that found in BKS in 2008, we surmise that BPL had a high endemic rate of non-typhoid

Salmonella during that period. However, compared with the results of 2005 - 2007, the incidence rate of non-typhoid *Salmonella* in BPL decreases significantly in 2012. Although the research sites differed in 2008 and 2009, limiting the generalization of these results, it is possible that the rate of non-typhoid *Salmonella* is decreasing gradually all over the country. This could be due to improvements in the sanitary systems in the food industry and to the environment as a whole. Typhoid *Salmonella* is one of the main causes of gastro enteric disease in Lao PDR, but non-typhoid *Salmonella*, however, has not been as thoroughly researched because it is not as dangerous as typhoid *Salmonella*. However, a person who carries non-typhoid *Salmonella* would not be qualified for jobs in a food factory for Japan (Yamane, 2006). In particular, the holding of the 25th Southeast Asian Games in Vientiane in December 2009 seemed to play an important role in this improvement (VIENTIANE-2009, 2009) when the Ministry of Health made efforts to improve food hygiene before the Games. In addition, GDP of Lao PDR has doubled in the past 10 years and economic growth has made it possible to reduce the risk of contracting infectious diseases (Watanabe *et al*, 2011).

Given the globalization of the food trade, Lao PDR will need to increase efforts to reduce the risk of *Salmonella* presence in the food exporting industry. In addition, *Salmonella* poses a major public health problem and surveillance of *Salmonella* infections should be continued (Gordon and Graharn, 2008; Dhanoa *et al*, 2009; Yen *et al*, 2009).

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with this manuscript.

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