Is water at farms a source of *Campylobacter* spp. contamination in live chickens in Khon Kaen Province of Thailand?

Bongkot Noppon1*, Pitak Noimay2, Chaiyaporn Soikum1, Apiradee Sopa1, Chariya Chomvarin3, Takuo Sawada4, and Tetsuo Asai5

1 Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand.

2 Department of Biology, Faculty of Science and Technology, Loei Rajabhat University, Mueang, Loei, 42000 Thailand.

3 Department of Microbiology, Faculty of Medicine, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand.

4 Laboratory for Veterinary Microbiology, Nippon Veterinary and Life Science University, Musashino, 180-8620, Tokyo, Japan.

5 Department of Applied Veterinary Science, Faculty of Applied Veterinary Science, Gifu University, Gifu, Japan.

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Abstract

This study is the first to quantify the *Campylobacter* contamination in chicken farm waters. Water samples from 10 broiler chicken farms in Khon Kaen, Thailand, were collected during winter, summer, and rainy season. Four types of water i.e. 3 drinking water, 1 main tank water, 1 evaporative pond water, and 1 environmental water sample were collected at each farm twice during 1-15, and 16-35 days of rearing. In total, 360 water samples were sampled. The overall occurrence rate of contamination was 0.3% (1/360). Only 1 environmental water sample (1.7%, 1/60) was found contaminated with *Campylobacter* species. Water collected during the later stage of rearing (16-35 days) was positive for *Campylobacter* spp. contamination. The extent of contamination was greater than 230 MPN/100 ml. However, drinking and main tank water were free of *Campylobacter* spp. contamination.

Keywords: *Campylobacter* spp., chicken farm, contamination, MPN, water

1. Introduction

Detection, quantification, and identification of microbial pathogens are prerequisites for water and environmental quality and safety control. The presence of campylobacters in environmental samples is a sign of recent fecal contamination, because not only are campylobacters unable to multiply outside warm-blooded host animals, but also they survive for a shorter time than the usual indicators, fecal coliforms and fecal streptococci (Bolton *et al.*, 1987; Jones, 2001). Thermophilic campylobacters are widespread in the environment, where they are a sign of recent contamination with animal and avian feces, agricultural run-off and sewage effluent (Jones, 2001). The 13-years Norwegian poultry surveillance report indicated that water is one of the routes of transmission to broilers (Hofshagen, 2013). However, limited study has
been conducted to assess the water safety in Thailand. Waterborne outbreaks associated with contaminated drinking water by C. jejuni are rather common in the Nordic countries i.e. Sweden, Norway, and Finland, where in sparsely populated districts groundwater is commonly used without disinfection (Hanninen et al., 2003). The presence of thermophilic campylobacters in streams varies with location, season and agricultural practice (Jones, 2001). Studies of streams in north-west England have shown that campylobacters are absent from streams running through upland moors but present in the same streams running through lowland, grazed pasture (Jones et al., 1990; Jones and Hobbs, 1996). The composition of the Campylobacter population is dependent on the path of the stream (Obiri-Danso and Jones, 1999). Streams running through pasture contain mainly C. jejuni with some C. coli, shed by grazing cattle and sheep (Jones et al., 1999), whereas those draining duck ponds contained a mixture of C. jejuni, C. lari, C. coli and urease-positive thermophilic campylobacters (UPTCs), which are typical of avian sources. A further study showed that campylobacters occurred intermittently in streams, with their density correlating with upstream agricultural locations, such as farmyards, small-holdings and a slaughterhouse, and agricultural events, such as emptying of slurry tanks and the spraying of farm slurry onto land (Jones et al.; Jones and Hobbs, 1996). Thermophilic campylobacters are ubiquitous in rivers, especially those exposed to agricultural run-off and effluent from water treatment plants (Bolton et al., 1987; Jones et al., 1990; Stelzer et al., 1991; Jones and Hobbs, 1996; Popowski et al., 1997; Obiri-Danso and Jones, 1999). The three waterborne outbreaks in Finland caused by C. jejuni were studied. The authors used water sample volumes of 4,000 to 20,000 ml for the analysis of campylobacters depending on the sampling site. Multiple samples obtained from possible sources (water distribution systems and environmental water sources) and the use of large sample volumes (several liters) increased the chance of detecting the pathogen C. jejuni in water (Hanninen et al., 2003).

Limited studies on microbiological quality of water have been conducted in Thailand. Therefore, the present study aimed at detecting and quantifying the amount of Campylobacter spp. contamination on chicken farm waters i.e. drinking water, water from main tank, evaporative pond water, and environmental water. Also, in northeastern Thailand limited studies have been done on the quantitative analysis of Campylobacter load on chicken farm water. This prompted the interest in Campylobacter quantification to assess the Campylobacter load at farm level.

2. Materials and Methods

2.1 Water samples collections:

4 types of water samples i.e. 30 drinking waters (far left, middle, and far right of the drinking lines), 10 main tank waters, 10 evaporative pond waters, and 10 environmental waters were collected during the rainy season, winter and summer month in northeastern Thailand. The amount of 2.5 liter of each water was collected in a sterile plastic bag and transported on ice to the laboratory. The samples were examined within the day of collection. A sterile plastic bag containing 1.0% (w/v) sterile sodium thiosulphate (Amresco, USA) was used merely in case of drinking and main tank waters to neutralize chlorine (Bolton et al., 1982; St-Pierre et al., 2009).

2.2 Culture medium and incubation:

Bolton broth (Oxoid, UK) supplemented with 5.0% (v/v) defibrinated sheep blood, Campylobacter Antibiotic Selective Supplement, SR 0117E (Oxoid, UK) and Campylobacter Growth Supplement, SR 0232E (Oxoid, UK) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA, Oxoid, UK) with the earlier mentioned supplements were employed for the MPN enumeration, and detection of Campylobacter spp. in water samples. MPN tubes were incubated in the microaerophilic atmosphere generated from Anaerocult C gas pack (Merck, Germany) at 42°C for 48 hours.

2.3 MPN method for Campylobacter count:

The enumeration method was employed followed that of Bolton et al. (1982), Savill et al. (2001), and St-Pierre (2009) with minor modification. Briefly, the 3 tubes Bolton broth (Oxoid) with selective antibiotic supplement (SR0117E, Oxoid), Campylobacter Growth Supplement (SR 0232E, Oxoid) with 5.0% defibrinated sheep blood, and microaerophilic atmosphere (Anaerocult C, Merck) were used at 42°C for 48 hours. This condition is well acknowledged as selective for Campylobacter species by previous authors. After incubation, one loopful from tubes that showed bacterial growth was streaked onto mCCDA Agar plates (Oxoid), then examined to genus level by typical colony characteristics (creamy white with swarming), cell shapes (s-shape), and specific biochemical tests in this case oxidase (+) and catalase (+) tests, respectively.

3. Results and Discussion

3.1 Water sample collection

Among the 360 water samples collected from 10 broiler chicken farms in Wang Noi District, Khon Kaen Province, northeastern Thailand, the prevalence of contamination with Campylobacter spp. was 0.3% (1/360) (Table 1).

3.2 MPN method for Campylobacter count

Results showed that one of the water samples collected during the later period of rearing (16-35 days) was Campylobacter spp. positive, while all water samples
collected during the early stage of rearing (1-15 days) were negative for Campylobacter species.

The present study indicated that drinking water samples and water samples from main tank were free of Campylobacter spp. contamination. For environmental waters, Campylobacter spp. was detected in 1.7% (1/60) of the samples (Table 1). The Campylobacter load by MPN enumeration revealed that the extent of contamination exceeded 230 MPN/100 ml of environmental water (Table 2).

In summary, in the present study, Campylobacter spp. was not detected in any of the drinking and main tank water samples using the conventional plating procedure after the MPN enumeration method. Among environmental waters, Campylobacter spp. was detected in 1.7% (1/60) of the samples. The extent of contamination exceeded 230 MPN/100 ml of water. The contaminated water sample was collected during the later period of rearing (16-35 days) whereas all water samples collected at the early stage of rearing (1-15 days) were free of Campylobacter. The present findings were similar to that of Chaveerach et al. (2004) in The Netherlands. Their results showed that the drinking water was free of Campylobacter spp. throughout the study at the chicken age of 1-15 days to 16-35 days. Nevertheless, in the present study one environmental water sample was positive for Campylobacter species. Chaveerach et al. (2004) stated that water is not a prominent vehicle for Campylobacter spread throughout a chicken flock. The present study found that the overall Campylobacter positive rate in environmental water sample was 1.7% (1/60). The overall prevalence of Campylobacter contamination was 0.3% (1/360) for all types of water samples. A study in northern Thailand revealed that the prevalence of Campylobacter on farms was lower in environmental samples than in samples collected from live animals in the northern Thailand study (Padungtod and Kaneene, 2005). It can be drawn from the present study that drinking waters at the chicken farm are free from Campylobacter spp. contamination especially at the early stage of rearing. Therefore, we conclude that water is not the likely source of Campylobacter spp. contamination in live birds, and measures to monitor the safety are still warranted at farm level.

Acknowledgments

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References


Table 1. Campylobacter positive sample of the chicken farm water in northeastern Thailand

<table>
<thead>
<tr>
<th>Age of chicken</th>
<th>Main tank</th>
<th>Drinking</th>
<th>Environmental</th>
<th>Evaporative pond</th>
<th>Total no. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15 days</td>
<td>0.0 (0/30)</td>
<td>0.0 (0/90)</td>
<td>0.0 (0/30)</td>
<td>0.0 (0/30)</td>
<td>0.0 (0/180)</td>
</tr>
<tr>
<td>16-35 days</td>
<td>0.0 (0/30)</td>
<td>0.0 (0/90)</td>
<td>3.3 (1/30)</td>
<td>0.0 (0/30)</td>
<td>0.6 (1/180)</td>
</tr>
<tr>
<td>Total</td>
<td>0.0 (0/60)</td>
<td>0.0 (0/180)</td>
<td>1.7 (1/60)</td>
<td>0.0 (0/60)</td>
<td>0.3 (1/360)</td>
</tr>
</tbody>
</table>

Table 2. Campylobacter count of the chicken farm water in northeastern Thailand by MPN enumeration method

<table>
<thead>
<tr>
<th>Age of chicken</th>
<th>Main tank</th>
<th>Drinking</th>
<th>Environmental</th>
<th>Evaporative pond</th>
<th>Total no. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-15 days</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16-35 days</td>
<td>0</td>
<td>0</td>
<td>&gt;230</td>
<td>0</td>
<td>&gt;230</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>&gt;230</td>
<td>0</td>
<td>&gt;230</td>
</tr>
</tbody>
</table>


