



Original Article

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

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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

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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

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

% (n/N) positive for <i>Campylobacter</i> spp.					
Water samples					
Age of chicken when collected water	Main tank	Drinking	Environmental	Evaporative pond	Total no. of samples
1-15 days	0.0(0/30)	0.0(0/90)	0.0(0/30)	0.0(0/30)	0.0(0/180)
16-35 days	0.0(0/30)	0.0(0/90)	3.3(1/30)	0.0(0/30)	0.6(1/180)
Total	0.0(0/60)	0.0(0/180)	1.7(1/60)	0.0(0/60)	0.3(1/360)

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

% (n/N) positive for <i>Campylobacter</i> spp.					
Water samples					
Age of chicken when collected water	Main tank	Drinking	Environmental	Evaporative pond	Total no. of samples
1-15 days	0	0	0	0	0
16-35 days	0	0	>230	0	>230
Total	0	0	>230	0	>230

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.

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