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Acidification of drinking water inhibits indirect transmission, but not direct transmission of *Campylobacter* between broilers

B.A.D. van Bunnik[a,c,*], W.E.A. Katsma[a], J.A. Wagenaar[a,b,d], W.F. Jacobs-Reitsma[a,1], M.C.M. de Jong[c]

[a] *Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands*
[b] *Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*
[c] *Quantitative Veterinary Epidemiology, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands*
[d] *WHO Collaborating Center for Campylobacter/OIE Reference Laboratory for Campylobacteriosis, The Netherlands*

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ABSTRACT

In this study the effect of acidification of the drinking water of broiler chickens on both direct and indirect transmission of *Campylobacter* was evaluated. In the direct transmission experiment both susceptible and inoculated animals were housed together. In the indirect transmission experiment the susceptible animals were spatially separated from the inoculated animals and no direct animal to animal contact was possible. The transmission parameter β was estimated for the groups supplied with acidified drinking water and for the control groups. The results showed that acidification of the drinking water had no effect on direct transmission ($\beta = 3.7\ \text{day}^{-1}$ for both control and treatment). Indirect transmission however was influenced by acidification of the drinking water. A significant decrease in transmission was observed ($p < 0.05$), with control vs. treatment point estimates being $\beta = 0.075\ \text{day}^{-1}$ vs. $\beta = 0.011\ \text{day}^{-1}$.

Apart from providing quantitative estimations of both direct and indirect transmission of *Campylobacter* in broilers, this study also demonstrates the use of an experimental setup for indirect transmission of *Campylobacter* between broilers to assess the efficacy of candidate measures to reduce transmission.

1. Introduction

Campylobacter causes a substantial number of cases of human gastroenteritis worldwide (Allos, 2001; Tauxe, 2002). The handling and consumption of contaminated poultry products are major risk factors for campylobacteriosis (Saleha et al., 1998). Implementation of measures to control *Campylobacter* in the poultry production chain may reduce the exposure of humans to *Campylobacter*. Such measures can be applied either at the slaughterhouse level, i.e. improving the slaughterhouse hygiene, or they can be applied at primary production level, i.e. on farm hygiene and biosecurity measures, to reduce the incidence of *Campylobacter* colonized flocks. A reduction in the number of colonized poultry flocks will decrease the risk for consumers considerably (EFSA, 2010). One way of reducing the number of colonized poultry flocks is by altering the susceptibility of the host; i.e. the chance of successful colonization after exposure (Byrd et al., 2001). In broiler chickens, fermented liquid feed (FLF) has been shown to reduce the susceptibility to *Campylobacter* and *Salmonella* (Heres et al., 2003a,b; Savvidou et al., 2009). In FLF, lactobacilli are present whose main metabolic products are lactic acid and acetic acid (Giraffa et al., 2010). The effects of FLF are attributed to the high level of organic acids and

* Corresponding author at: Central Veterinary Institute of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands. Tel.: +31 320 238525; fax: +31 320 238668.
E-mail address: bram.vanbunnik@wur.nl (B.A.D. van Bunnik).
[1] Present address: RIVM, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

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the low pH of this feed. Following this line of reasoning, acidified drinking water may be expected to have a similar effect on the susceptibility of broilers to *Campylobacter* as FLF.

The aims of this study were (1) to investigate the effect of acidification of the drinking water on both the direct and indirect transmission of *Campylobacter* between broilers and (2) to explore the use of an experimental system of indirect transmission of *Campylobacter* between broilers for assessing the effect of candidate measures against transmission in a controlled setting. With indirect transmission we mean transmission that occurs in a situation where there is no possibility for contact between susceptible and infectious animals, i.e. they are spatially separated.

2. Materials and methods

2.1. Experimental design

2.1.1. Direct transmission experiment

The direct transmission experiment consisted of one control group and one treatment group and was carried out in duplicate, resulting in four groups in total. Each group consisted of 23 animals. Throughout the experiment the groups were housed in separate stables. From day 0 (day of hatching) until day 12 all animals in a group were housed together. The two control groups received tap water whereas the treatment groups continuously received acidified drinking water. A commercially available acid (Forticoat®, Selko BV) was diluted until a final pH of 4 (approximately 2 ml acid on 1 L water). Active ingredients of the commercially available acid are: sorbic acid, formic acid, acetic acid, lactic acid, propionic acid, ammonium formate, L-ascorbic acid, citric acid, mono- and diglycerides of edible fatty acids and 1,2-propanediol. At day 12, ten animals per group were randomly selected from each group, inoculated with *Campylobacter* by gavage (see Section 2.4) and housed separately. On day 16 the inoculated animals were placed back with the rest of their group. Colonization was monitored by taking cloacal swabs on a daily basis from day 14 onwards. The swabs were processed within 2 h for the analysis of the presence of *Campylobacter*. If an animal was found positive on 5 consecutive days, swabs were taken only once a week. The experiment was ended 20 days post inoculation. At that day all chickens were euthanized and caecal contents were qualitatively analysed for the presence of *Campylobacter*.

2.1.2. Indirect transmission experiment

The indirect transmission experiment consisted of one control group and one treatment group and was carried out in duplicate. Each group consisted of 9 animals. The two control groups received tap water, the treatment groups received acidified drinking water (Forticoat®, Selko BV, pH 4). From day 0 (day of hatching) until day 4 all animals in a group were housed together. On day 4, animals were housed individually according to the housing plan depicted in Fig. 1. This setup was chosen to equalize the infection pressure experienced by each susceptible bird as much as possible. Twelve days after hatching 5 animals from each group were orally inoculated with 1 ml of *Campylobacter*

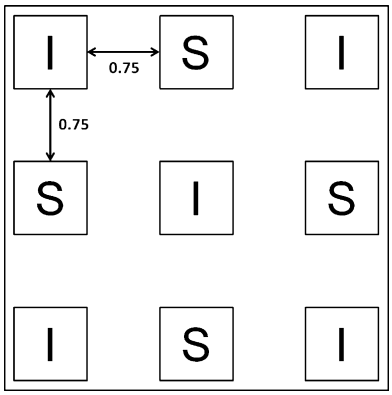

Fig. 1. Schematic overview of the housing of the animals during the indirect transmission experiment. S: susceptible animal; I: infectious animal. Distances are given in metres.

(see Section 2.4). To monitor colonization, from day 12 onwards, swabs were taken on a daily basis from all animals, both inoculated and susceptible. If an animal was found positive for *Campylobacter* on 5 consecutive days, swabs from that animal were taken on a weekly basis. The experiment was ended 21 days post inoculation. All animals were euthanized and the caeca were removed and qualitatively analysed for the presence of *Campylobacter*.

2.2. Housing

Animals were housed in wire cages placed directly on the floor. Wood shavings were provided as bedding material; feed was supplied ad lib.; drinking water was supplied via an open water drinking system. No flow of water was possible between infectious and susceptible animals. Drinking water was refreshed on a regular basis. Before the start of the experiment all stables used in the experiment were cleaned and disinfected and samples were taken from different areas inside the stable, to check for the absence of *Campylobacter*.

2.3. Animals

Eggs from commercial broilers (type Ross 308) were incubated in an in-house facility. Day of hatching is day 0 in the experiment. On day 1 and day 8 cloacal swabs were taken from all animals to check for the absence of *Campylobacter*. These samples were incubated in mCCD (modified cefoperazone charcoal deoxycholate) broth (Nutrient Broth no. 2, Oxoid CM0067 with *Campylobacter* selective supplement (Oxoid SR0204E) and *Campylobacter* growth supplement (Oxoid SR0232E)) for 24 h and plated on mCCDA (modified cefoperazone charcoal deoxycholate agar) and incubated again to check suspected *Campylobacter* colonies after 24 and 48 h (see Section 2.5 for complete procedure). All animals were uniquely tagged so they could be tracked throughout the experiment.

All animal experiments were in compliance with national and institutional regulations and as such approved by the institute's ethical committee.