



Prevalence and risk factors for *Campylobacter* spp. in chicken broiler flocks in Reunion Island (Indian Ocean)

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ABSTRACT

Our objectives were to determine *Campylobacter* prevalence in broiler chicken flocks in Reunion Island and to define specific practices associated with the presence of *Campylobacter* spp. Infection in Reunionese broiler flocks. Fifty broiler flocks were studied in Reunion Island from May 2007 to February 2009. A questionnaire was submitted to the farmers and samples of fresh droppings were collected to assess the flock's *Campylobacter* status. Fifty four percent of the flocks were infected by *Campylobacter* spp.: 30% (95% CI: 28.71–31.29) were infected with *Campylobacter coli* and 17% (95% CI: 15.95–18.05) with *Campylobacter jejuni*; only 7% (95% CI: 6.28–7.72) were infected by both species at the same time.

Several poultry houses in the farm (OR = 11.2; [1.05–92]) and cleaning without any detergent (OR = 13.1; [2.1–78.3]) increased the risk of *Campylobacter* infection. A distance higher than 500 m between broiler farms (OR = 0.27; [0.1–0.8]) and use of disinfectant during the rearing period decreased this risk of infection (OR = 0.15; [0.1–0.75]).

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1. Introduction

Thermophilic *Campylobacter* species are recognized as being the most common bacterial cause of gastroenteritis in the world. It is predominantly *Campylobacter jejuni* and *Campylobacter coli* which cause acute bacterial food-borne gastroenteritis (World Health Organisation: <http://www.who.int/mediacentre/factsheets>; Moore et al., 2005). As an example, *Campylobacter* remains a leading cause of acute bacterial enteritis in the USA, with an estimated 2.4 million cases annually (Mead et al., 1999). *Campylobacter* is an emerging public health problem for both developed and developing countries (Prasad et al., 2001). The most important post infectious manifesta-

tion is Guillain–Barré syndrome (Smith, 1995). Generally, the most severe cases are induced by *C. jejuni* and Guillain–Barré syndrome occurs in 1 per 3000 *C. jejuni* infections (Leonard et al., 2004) but the risk of severe infection developing may be 40 times higher for subjects who are HIV positive (Sorvillo et al., 1991). In 2007 a total of 200,507 confirmed cases of *Campylobacteriosis* were reported from 24 European countries corresponding to an incidence of 45.2 cases per 100,000 inhabitants. *Campylobacteriosis* also has a significant economic impact when taking into account loss of working hours and health care costs (Roberts et al., 2003).

Poultry is regarded as one of the most important reservoirs for *Campylobacter* and represents a very important vehicle for the transmission of *Campylobacter* to humans by contaminated food, particularly through the consumption of raw or undercooked products (Humphrey et al., 2007). As various studies have shown, *Campylobacter* has infected broiler chickens in poultry farms (Stern et al., 2001) and

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in retail stores (Zhao et al., 2001); but this bacteria has also been isolated from domestic animals, including cattle, swine and sheep (Diker et al., 2000).

Poultry become contaminated by *Campylobacter* spp. particularly through faeces, foodstuffs, litter and also aerosol (Jacobs-Reitsma, 1997). Intestinal colonization in broiler chicks is rarely detected before the age of 7 days. Once colonized, chicks remain asymptomatic carriers until they reach slaughter age. Nevertheless, the epidemiology of *Campylobacter* in broiler production is still, not fully understood (Humphrey et al., 2007) and the importance of vertical transmission from parents to their offspring is unclear (Callicott et al., 2006). Horizontal transmission is, at this time, believed to be responsible for flock colonization (Bull et al., 2006).

Reunion Island has been producing industrial poultry for 60 years and is one of the main industries on the island. Poultry status is particularly important because it is the most eaten meat in the island and in the Indian Ocean and because 100% chicken sausages (meat and skin) are often prepared.

Thus, our objectives were to determine *Campylobacter* prevalence in broiler chicken flocks in Reunion Island and to define specific practices associated with *Campylobacter* spp. infection in Reunionese broiler flocks.

2. Materials and methods

2.1. Study sample

Our study was undertaken from May 2007 to February 2009 and involved 50 broiler chickens flocks in Reunion Island (Indian Ocean). Only one broiler flock was studied on each farm. The location and the day of placing chicks were provided by the poultry company. The aim of the study was explained to the farmers by phone. In our selection, only 2 farmers refused to participate in the study and because of climatic cyclonic conditions, 3 more poultry farms were excluded from the study.

The first industrial chicken farm on Reunion Island was created 60 years ago and the main slaughter house was built in the 1990s. The poultry houses are open sided with artificial or dynamic ventilation. Various kinds of poultry houses exist but most are industrial “Colorado” or “Louisiana” types along with some locally made houses called “pei” built with old tobacco driers. The basic foodstuffs are imported from Argentina, France, and Mauritius. Parents belong to the Hubbard strain and they are imported from continental France. Each year 12 million hatching eggs are produced, around 75,000 breeders are imported and 8.5 million chicks are sold on the island; 98% of chicks are locally produced and the remaining 2% come from hatching eggs or from day old chicks from continental France. The typical prophylactics consist of vaccinations against Newcastle and Gumboro diseases.

2.2. Data collection

Each chicken farm was visited once at the end of the rearing period. The visit was done between 43 and 45 days

of age (before slaughtering) to define the flock’s *Campylobacter* status.

Twenty pooled samples of five fresh droppings were taken to assess the *Campylobacter* status of the flock. The droppings were taken according to a systematic walking pattern in the poultry house (Refregier-Petton et al., 2001). Fresh droppings were placed in a sterile plastic bag using a sterile glove and transported to the laboratory within 4–6 h after sampling.

Data were collected from a questionnaire submitted to each farmer concerning poultry house characteristics, practices and treatment of day-old chicks, management of dead birds, control of rodents and other domestic animals, watering practices, farm staff, cleaning and disinfection. This questionnaire was always submitted by the same person and it was pre-tested in a preliminary study. The final questionnaire consisted of closed-ended questions. Only the manager was interviewed.

2.3. Isolation procedure and identification

In all our analyses, *Campylobacter* culture was carried out in a micro-aerophilic atmosphere (7% O₂, 10% CO₂, 83% N₂) at a temperature of 42 °C (Campygen, Oxoid).

The protocol used is based on Denis et al. (2001). Samples were added to 90 ml of Preston broth (Oxoid, France) with preston antibiotic supplement and incubated for 24 h. Streaking on Virion plates and on Karmali plates was performed after 24 h of enrichment steps. After 48 h of incubation on agar plates, suspected colonies were isolated on blood agar plates for purification (Merck).

2.4. DNA extraction and polymerase chain reaction (PCR)

Identification of every isolate was confirmed by PCR using SYBR Green Jumpstart (Sigma) using specific primers: Col 2 and Col 3 for *C. coli*. For *C. jejuni*, map1 and map2 were used (Denis et al., 2001). Control positive strains used was ATCC 33560 for *C. jejuni* and ATCC 33559 for *C. coli*. Each PCR tube contained SYBR green (ADD MIX) (12.5 µl), primer (1 µl for each) and H₂O (8 µl). DNA amplification was carried out in a thermocycler Biorad using an initial denaturation step at 94 °C for 2 min followed by 35 cycles. Cycling conditions were as follows: denaturation 95 °C for 15 s, annealing 59 °C for 1 min, extension 72 °C for 1 min.

2.5. Statistical analysis

The flock was the unit of observation; it was considered infected by *Campylobacter* when one or more samples taken at the end of the rearing period was positive. Thus our outcome variable was dichotomous (infected versus non infected).

All variables were categorical with a number of categories per variable limited. All frequencies of categories were >10%. Relationships between risk factors were checked (χ^2). To assess the association between explanatory variables and *Campylobacter* status of the flock, two stage procedures was used. The first step consisted of performing an univariable analysis to test the association between *Campylobacter* infection and each independent

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