DYNAMICS OF THERMOPHILIC *Campylobacter* COLONIZATION IN BROILER FLOCKS REARED ON REUSED LITTER

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ABSTRACT

The study of *Campylobacter* epidemiology might help the development of strategies to reduce broilers contamination. To evaluate the dynamics of *Campylobacter* colonization in broilers, a longitudinal study was conducted in an experimental broiler farm in Southern Brazil. Three consecutive flocks were reared on reused litter from three previous flocks. Depopulation occurred at 42-day, and cleaning, disinfection and fermentative litter treatment were performed during 14 days between flocks. Studied flocks consisted of 180 *Campylobacter*-negative chicks obtained from the same hatchery. Cloacal swabs, litter and darkling beetles (*Alphitobius diaperinus*) were sampled once a week from 7 up to 42-day of age. Cloacal swabs from flock 2 became *Campylobacter*-positive sooner than flocks 1 and 3. According to logistic regression analysis, it was estimated that 50% of cloacal swabs from flock 2 were positive at 17.9-day compared to 28.4-day from flocks 1 and 3. At 42-day, between 97.3% and 100% of broilers from all flocks were colonized by *C. jejuni*. *C. jejuni*-positive litter samples were detected at 21 (flock 2 and 3) and 35-day (flock 1), and remained positive throughout the end of the rearing period. *C. jejuni* was also isolated from darkling beetles sampled after 28 and 35-day in flocks 2 and 3, respectively. Cleaning, disinfection and treatment of reused litter between flocks reduce residual *Campylobacter* contamination in broiler farm. However, *Campylobacter* contamination spreads quickly among broilers as soon as they became colonized, emphasizing the role of birds in the amplification of contamination in the production environment.

KEY WORDS: Broilers, *Campylobacter jejuni*, longitudinal study

INTRODUCTION

The intestinal tract of broilers is frequently colonized by thermophilic *Campylobacter* (*C.*), which are found in high numbers in the caecal content. Horizontal transmission of *Campylobacter* is considered the major route for colonization of housed broilers, while the vertical transmission is not considered to be a common route (Ridley *et al*., 2011). Nevertheless, broilers are free of *Campylobacter* at day of hatch, although intensively reared broiler flocks become *Campylobacter*-positive at 2 to 3 weeks of age (Ridley *et al*., 2011). Infection spreads rapidly to most of broilers in a flock. At 36-42 days of age, over 60% of the flocks might be colonized by thermophilic *Campylobacter* (Evans & Sayers, 2000). The implementation of general biosecurity measures effective at broiler farms at preventing *Campylobacter* flock colonization has proven difficulty, indicating that additional interventions will be required (Ridley *et al*., 2011).

On the other hand, the reuse of broiler litter from healthy flocks after depopulation and fermentative litter treatment for up to 6 times is a regular practice in Brazilian broiler farms, but there is little information about *Campylobacter* survival on this recycled broiler

litter. Moreover, the study of *Campylobacter* epidemiology might help the development of effective strategies to reduce broilers contamination. To evaluate the dynamics of *Campylobacter* colonization in intensively reared broilers on reused litter, a longitudinal study was conducted in an experimental broiler farm in Southern Brazil.

MATERIALS AND METHODS

This study was carried out in an experimental broiler farm in Southern Brazil. Three consecutive broiler flocks were intensively reared on reused litter from three previous healthy flocks. Depopulation occurred at 42-day, and cleaning, disinfection and fermentative litter treatment were performed during 14 days between flocks. Studied flocks consisted of 180 *Campylobacter*-negative chicks obtained from the same commercial hatchery. Cloacal swabs, broiler litter and live darkling beetles (*Alphitobius diaperinus*) were individually sampled once a week from 7 up to 42-day of age.

All samples were transported to the laboratory in insulated boxes with ice packs and processed within 2 hours of sampling. Next, samples were individually processed as previously described (Stern *et al*., 2001) and plated onto Preston Agar (PA) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA), which were incubated in a microaerobic atmosphere (5% O_2, 10% CO_2 with the balance N_2) at 41.5°C for 24-48 h. Suspect Gram-negative bacterial colonies exhibiting curved or spiral rods were presumptively identified as *Campylobacter* and were tested for catalase, oxidase, hippurate hydrolysis and indoxyl acetate hydrolysis. *C. jejuni* subsp. *jejuni* (ATCC 33560) was used as positive control. Considering the effects of broilers age and flocks, logistic regression was used to analyze the results. Based on estimates of model parameters, it was estimated the birds age to detect 50% and 95% of *Campylobacter*-positive cloacal swabs (Demetrio, 2001).

RESULTS AND DISCUSSION

All strains isolated from cloacal swabs, broiler litter and darkling beetles sampled (*Alphitobius diaperinus*) were identified as *C. jejuni*. Cloacal swabs from broiler flock 2 became *Campylobacter*-positive sooner than flocks 1 and 3, while there was not statistical difference between broilers flock 1 and 3 (Figure 1). According to logistic regression analysis, it was estimated that 50% of cloacal swabs from flock 2 were positive at 17.9-day compared to 28.4-day from flocks 1 and 3 (Table 1). Microbiology analysis of cloacal swabs at 42-day displayed that between 97.3% and 100% of broilers from all flocks were colonized by *C. jejuni*. In fact, Evans & Sayers (2000) showed that when a broiler flock became colonized, virtually all cloacal swabs were positive within a week, indicating that *Campylobacter* infection spreads very quickly amongst housed broilers.

C. jejuni-positive litter samples were detected at 21 (flock 2 and 3) and 35-day (flock 1), and remained positive throughout the end of the rearing period. According to Stern et al. (2001), broiler flocks are rarely found to be positive during the first 2 weeks of life, but the point at which those destined to become positive begin to shed the organisms is considered important, because it may be possible to delay the event sufficiently to avoid contaminated birds entering the processing plant.

Nevertheless, *C. jejuni* was also isolated from darkling beetles sampled after 28 and 35-day in flocks 2 and 3, respectively. *Alphitobius diaperinus* might be a reservoir of *Campylobacter* in broiler farms. However, the broiler depopulation associated to cleaning, disinfection and fermentative litter treatment performed during 14 days between broiler

flocks longitudinally monitored seems to be able to reduce or eliminate the detection of *Campylobacter*-positive darkling beetles at broiler farm.

CONCLUSION

Cleaning, disinfection and treatment of reused litter between flocks reduce residual *Campylobacter* contamination in broiler farm. However, *Campylobacter* contamination spreads quickly among broilers as soon as they became colonized, emphasizing the role of birds in the amplification of contamination in the production environment.

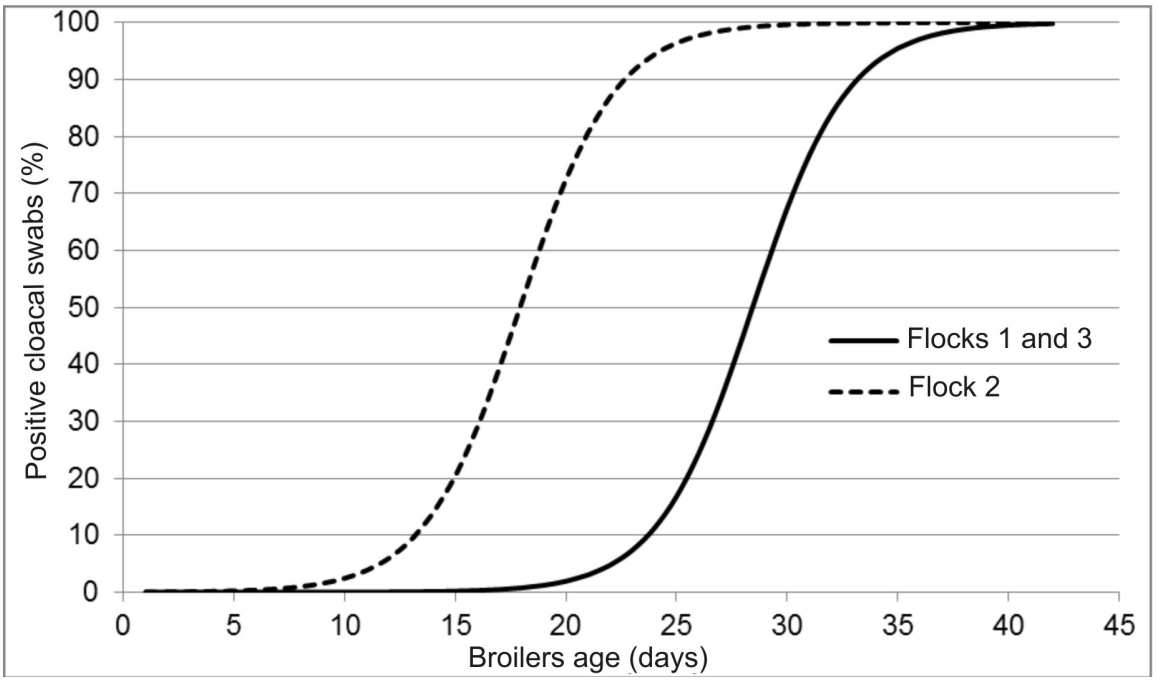

Figure 1. Percentage of *Campylobacter*-positive cloacal swabs according to adjusted logistic model.

Table 1. Birds age (days) to achieve 50% and 95% of *Campylobacter*-positive cloacal swabs depending on the broiler flock.

Broiler flock	Percentage of positive cloacal swabs	
	50%	**95%**
1 and 3	28,4	34,8
2	17,9	24,3

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