Broilers do not play a dominant role in the *Campylobacter fetus* contamination of humans

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Campylobacter fetus causes severe infections in humans and can be isolated from various mammals and reptiles. However, although poultry are considered to be the main reservoir of *Campylobacter jejuni*, little is known about the presence of *C. fetus* in poultry. Thus, specific pathogen-free chickens were experimentally inoculated with a mixture of either three non-thermotolerant or four thermotolerant human strains of *C. fetus*. Faecal samples were regularly sampled after inoculation and caeca and intestines were collected 21 or 40 days after inoculation. All samples were analysed for the presence of *Campylobacter* using culture techniques. No *Campylobacter* could be re-isolated. This result strongly suggests that broilers do not play an important part in the *C. fetus* contamination of humans.

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INTRODUCTION

Campylobacter species are recognized as a common bacterial cause of human gastroenteritis in developed and developing countries. In Europe, according to *Campylobacter* surveillance systems from 18 countries, more than 130 000 *Campylobacter* infections were reported in 1999, with a mean number of 71 declarations per 100 000 inhabitants in the EU (Takinen *et al.*, 2003). In France, according to the *Campylobacter* National Reference Centre, the most frequently isolated *Campylobacter* species in hospital laboratories are *Campylobacter jejuni* (72.1%), *Campylobacter coli* (13.1%) and *Campylobacter fetus* (12.2%). *Campylobacter fetus* is also more frequently isolated in hospital laboratories than private ones (Gallay *et al.*, 2005). Indeed *C. fetus* seems to be more invasive than *C. jejuni*/*C. coli* and may cause enteritis, abortion, bacteraemia, endocarditis or meningitis in humans (Monno *et al.*, 2004). *C. fetus* causes abortion in sheep and cattle and has been isolated from various mammals and reptiles (Tu *et al.*, 2001). One of the reservoirs of *C. jejuni* is considered to be poultry, but little is known about the presence of *C. fetus* in poultry. In a published study, *C. fetus* has been found in turkey samples (Logue *et al.*, 2003), but identification methods were based on phenotypic tests, which may be inconsistent. To verify the possibility that poultry are a reservoir for *C. fetus*, the aim of the present study was to assess the ability of *C. fetus* to colonize chickens. Because the body temperature of chickens is approximately 42 °C, typical *C. fetus* strains and *C. fetus* strains able to grow at

42 °C were tested (Smibert, 1984). Indeed, such thermotolerant strains have been implicated in bacteraemia in immunocompromised patients (Woo *et al.*, 2002).

METHODS

Three non-thermotolerant *C. fetus* strains (2005/0154, 2005/102H and 2005/106H) and four thermotolerant *C. fetus* strains (2005/418H, 2005/350H, 2005/965 and 2005/1074) were used. All strains were of human origin and had been isolated from blood, except for strain 2005/1074, which was isolated from human faeces. Strains were identified according to phenotypic and PCR methods as described by Menard *et al.* (2005).

In the first trial, the three non-thermotolerant *C. fetus* strains were grown in 10 ml Brucella broth (AES Laboratory) for 18 h at 37 °C under microaerobic conditions (5% O₂, 10% CO₂ and 85% N₂). Tenfold dilutions were prepared and plated onto blood agar (AES Laboratory) for enumeration and the three cultures were pooled. The mixture was used to inoculate 15 specific pathogen-free (SPF) and *Campylobacter*-free chickens, each bird receiving 1 ml of the mixture *per os*. At the time of inoculation, the chickens were 25 days old and were reared in an isolator. Faecal samples were collected before inoculation and on days 2, 3, 4, 7 and 13 post-inoculation (p.i.). On day 21 p.i., birds were humanely killed and their intestines and caeca were collected. Faecal, intestinal and caecal samples were diluted 1/10 in Preston broth and inoculated directly and after 18 h onto Butzler, Skirrow or Karmali media (Oxoid). Plates were incubated at 37 °C under microaerobic conditions and were observed for *Campylobacter*-like colonies after 24–72 h.

In the second trial, an inoculum was prepared as described previously with the four thermotolerant *C. fetus* strains. The titre of the mixture was determined. Fifteen 3-week-old SPF chickens were inoculated with 1 ml of the mixture *per os*. Faecal samples were collected from all

Abbreviations: p.i., post-inoculation; SPF, specific pathogen-free.

animals before inoculation and on days 2, 6, 8, 12, 29 and 35 p.i. Birds were humanely sacrificed on day 40 p.i. and intestinal and caecal samples were taken as described previously. Cultures were performed as in the first trial except that all plates were incubated on Karmali media in duplicate at 37 and 42 °C.

RESULTS AND DISCUSSION

Results showed that the three non-thermotolerant and four thermotolerant *C. fetus* strains could be cultured on Skirrow, Karmali and Butzler media at 37 °C, but only the four thermotolerant strains grew at 42 °C. In the first trial, the titres of the inoculated cultures were 3×10^8 , 5×10^8 and 2.4×10^8 bacteria ml⁻¹ for strains 2005/0154, 2005/102H and 2005/106H, respectively. The titre of the mixture inoculated in the second trial was 9.6×10^7 ml⁻¹.

In both trials, no clinical signs were observed after inoculation and *Campylobacter* was not isolated from chicken faecal samples from day 2 to day 21 p.i. (first trial) or from day 2 to day 40 p.i. (second trial). No *Campylobacter* species could be isolated from intestines or caeca.

Most epidemiological studies concerning the presence of *Campylobacter* in poultry are based on incubation of plates at 42 °C. This incubation temperature is appropriate for *C. jejuni*, *C. coli*, *Campylobacter lari* and *Campylobacter upsaliensis*, but may hamper the isolation of most *C. fetus* strains. Moreover, several frequently used media (e.g. Karmali, CCDA and Butzler No. 2) contain cefoperazone or cefazolin, antibiotics that may inhibit the growth of some *C. fetus* strains (Penner, 1988). For these different reasons, it was important to determine whether *C. fetus* was rarely reported from chickens because most *C. fetus* strains are not able to colonize birds, whose internal temperature is approximately 42 °C, or because of inadequate laboratory methods. According to our results, using appropriate media and incubation conditions, it seems that non-thermotolerant and thermotolerant strains of *C. fetus* of human origin are not able to colonize chickens. This strongly suggests that broilers do not play a dominant role in the *C. fetus* contamination of humans.

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