Broilers do not play a dominant role in the Campylobacter 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 (Takkinen 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, Campylobacter 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). Campylobacter 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 caecae were collected. Faecal, intestinal and caecal samples were diluted 1/10 in 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
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 $3 \times 10^6$, $5 \times 10^6$ and $2 \times 10^7$ bacteria ml$^{-1}$ for strains 2005/0154, 2005/102H and 2005/016H, respectively. The titre of the mixture inoculated in the second trial was $9 \cdot 6 \times 10^7$ 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. Additionally, several frequently used media (e.g. Karmali, CCDA and Butzler No. 2) contain ceftoperoxone 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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REFERENCES


