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# Biotypes and serotypes of thermophilic campylobacters isolated from cattle, sheep and pig offal and other red meats

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## SUMMARY

In this study we examined 730 faecal samples of offal (mainly liver), mince-meat and sausage meat collected from abattoirs and retail butchers' shops for campylobacters. *Campylobacter jejuni* or *C. coli* were isolated from 30·6, 10·5 and 6% of sheep, cattle and pig offal samples respectively. Specimens collected from abattoirs were, in general, more often contaminated than material obtained from retail butchers' shops. Only 1·4% of minced meats and sausage meats contained campylobacters. Most isolates (89·5%) were *C. jejuni* biotype 1 (Skirrow & Benjamin, 1980) of serotypes 1 and 2 (Penner & Hennessy, 1980). This study shows that animal offal is frequently contaminated with *C. jejuni* of biotypes and serotypes commonly isolated from human beings with campylobacter enteritis.

## INTRODUCTION

Campylobacter enteritis is now a well-recognized disease, yet in most sporadic infections the source and mode of transmission is unclear, even though food is thought to be a common vehicle.

Poultry have been incriminated in several outbreaks (Blaser, Taylor & Feldman, 1983) and there have been at least two reports of infection following the consumption of meat (Oosterom *et al.* 1980; Anon, 1982). Contamination with campylobacters during poultry processing has been extensively investigated (Smeltzer, 1981; Oosterom *et al.* 1983*a*) and the contamination of cattle, sheep and pig carcasses at slaughter has also been studied (Stern, 1981; Sticht-Groh, 1982; Oosterom *et al.* 1983*b*).

In a large nationwide survey the incidence of campylobacters in red meats was reported to be 4·0% in abattoir samples but only 1% in retail samples (Turnbull & Rose, 1982). This low isolation rate was confirmed by a failure to detect campylobacters from retail and wholesale butchers' premises (Bolton, Dawkins & Robertson, 1982). In contrast, work carried out in Sweden indicated that minced meats were frequently contaminated with campylobacters (Svedham, Kaijser & Sjogren, 1981).

Since the contamination of poultry giblets and intestines is particularly common (Grant, Richardson & Bokkenheuser, 1980; Christopher, Smith & Vanderzant, 1982; Barot, Mossenthal & Bokkenheuser, 1983), we decided to determine the incidence of campylobacter contamination in offal from cattle, sheep and pigs. We

also included samples of minced meat and sausage meat in the survey so that we could make more valid comparisons with the Swedish results.

## MATERIALS AND METHODS

### *Samples*

Samples of offal, minced meat and sausage meat were collected from abattoirs and retail butchers' in five Lancashire towns. Of 730 samples examined, 153 were of cattle offal, 232 sheep offal, 67 pig offal, 135 minced meats (mostly minced beef or designated minced 'meat') and 143 sausage meats. Most offal samples were liver, but some kidney and a few hearts were included.

### *Isolation studies*

A 30–50 g quantity of each sample was added to 90 ml of buffered peptone water (Oxoid, CM 509) and homogenized in a Colworth stomacher (Seward Laboratory, London); 40 ml of the homogenate were dispensed into each of two screw-topped plastic containers (120 ml vol). About 60 ml of modified Preston broth (Bolton *et al.* 1983) were added to each container, the tops were tightly closed and the broths incubated at 42 °C. All broths were subcultured after 24 hr incubation onto plates of Preston agar (Bolton & Robertson, 1982) which were then incubated microaerobically at 42 °C for up to 42 hours.

Suspect campylobacter colonies were identified by colonial morphology, positive oxidase reaction, typical motility and cell morphology on examination by dark field microscopy.

### *Typing of isolates*

Most isolates were biotyped using the scheme of Skirrow & Benjamin (1980) and serotyped at the Public Health Laboratory, Manchester, England, according to the Penner serotyping scheme (Penner & Hennessy, 1980).

## RESULTS

*C. jejuni* or *C. coli* were isolated from 30·6, 10·5 and 6% of sheep, cattle and pig offal samples respectively, but from only 1·4% of minced meats and sausage meats (Table 1). Of the offal samples collected from abattoirs 25% were positive compared with 16% from retail butchers' shops. The effect of blanching (immersion in boiling water for thirty seconds) was tested in 22 offal samples; campylobacters were isolated from 14 before blanching, but from only 2 after blanching.

The biotypes of 86 of the 94 campylobacter isolates are presented in Table 2. These comprised 89·5% *C. jejuni* biotype 1, 3·5% *C. jejuni* biotype 2 and 7% *C. coli*. *C. laridis* (Benjamin *et al.* 1983) was not isolated from any of the samples in this study. *C. jejuni* biotype 1 was the predominant biotype associated with both cattle and sheep offal. This biotype was also the only one found amongst the isolates from minced meats and sausage meats.

The serotypes of campylobacter strains isolated from each type of sample are listed in Table 3. Serotype 1 was the commonest and accounted for 53·8% of isolates from cattle offal and for 45·8% of isolates from sheep offal. Serotype 2 was found only in sheep offal and was isolated from 22% of these samples. The

Table 1. *Isolation of thermophilic campylobacters from 730 samples of offal, minced meat or sausage meat*

| Type of meat | Source [abattoir (A) or retail (R)] | Number of samples | Number positive for *Campylobacter* spp. (%) |
|---|---|---|---|
| Cattle offal | A | 56 | 9 (16) |
| | R | 97 | 7 (7) |
| Sheep offal | A | 107 | 41 (38) |
| | R | 125 | 30 (24) |
| Pig offal | A | 41 | 1 (2·5) |
| | R | 26 | 3 (11·5) |
| Minced meats | R | 135 | 3 (2·2) |
| Sausage meats | R | 143 | 1 (0·7) |

Table 2. *Biotypes* of 86 campylobacter isolates*

| Type of meat | *C. jejuni* biotype 1 | *C. jejuni* biotype 2 | *C. coli* |
|---|---|---|---|
| Cattle offal | 12 | 0 | 2 |
| Sheep offal | 60 | 2 | 3 |
| Pig offal | 1 | 1 | 1 |
| Minced meats | 3 | 0 | 0 |
| Sausage meats | 1 | 0 | 0 |

* Skirrow & Benjamin (1980).

Table 3. *Distribution of campylobacter serotypes* from offal, minced meat or sausage meat samples*

| Serotype | Cattle offal | Sheep offal | Pig offal | Minced meat/ sausage meat |
|---|---|---|---|---|
| 1† | 7 | 27 | 1 | 1 |
| 2 | — | 13 | — | — |
| 3 | — | 1 | — | 1 |
| 4‡ | 2 | 4 | — | 1 |
| 5 | 1 | — | — | — |
| 6,7 | 1 | — | — | — |
| 8 | — | 6 | — | — |
| 11 | — | 1 | — | — |
| 23 | — | 4 | 1 | 1 |
| 49 | 1 | — | — | — |
| Not typable | 1 | 3 | 1 | 1 |
| Totals | 13 | 59 | 3 | 4 |

* Penner & Hennessy (1980).
† Includes cross-reactions with types 2 and 8.
‡ Includes cross-reacting types 13, 16 and 50.



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remaining isolates were distributed amongst 11 other serotypes; only 6 isolates (7·6%) were non-serotypable.

## DISCUSSION

Although the incidence of salmonella in red meats (including offal) and sausage meat have been the subject of several studies (Dixon & Peacock, 1965; Roberts *et al.* 1975; Abbott & Robertson, 1980; Banks & Board, 1983) there has been only one large scale study of the incidence of campylobacters in these food products (Turnbull & Rose, 1982). These authors indicated that campylobacters could be found in 1·0% of minced beef, 0·3% of minced pork and 0·1% of sausage meats. Although in the present study the number of samples was smaller, our incidence in minced meat was 2·2% and in sausage meat 0·7%. This higher isolation rate is probably due to the use of more sensitive culture methods. However our results confirm the conclusions of Turnbull & Rose (1982) that minced meat and sausage meat produced in the U.K. are infrequently contaminated with campylobacters. In Sweden, Svedham, Kaijser & Sjorgen (1981) isolated *C. jejuni* from all nine samples of minced meat that they tested. As this number of samples is small the true incidence of *C. jejuni* in Scandinavian meat has still to be determined. It may be that the culture methods used by these Swedish workers are much more sensitive than those used in either the Turnbull & Rose survey or in the present study. Alternatively there may be greater contamination of minced meats in Sweden due to different abattoir or processing techniques.

Offal comprised only a small proportion of the total samples examined in the Turnbull & Rose survey and appears to have been ignored as a potential source of *C. jejuni*. In the present study campylobacters were found in 20% of offal samples but the incidence in different types of offal varied. They were most frequently isolated from sheep offal (30·6%), cattle offal (10·5%) and less so from pig offal (6%). These differences possibly reflect the level of intestinal carriage of campylobacters in these animals.

The fact that blanching converted 12 of the 14 offal samples from positive to negative suggests that they are surface contaminated during the evisceration process; this has been observed in poultry production (Barot, Mosenthal & Bokkenheuser, 1983). In general campylobacters were more often isolated from offal samples collected at abattoirs than at retail butchers' shops; a finding which parallels that in other red meats (Turnbull & Rose, 1982). This observation is not unexpected because atmospheric oxygen kills these microaerophilic organisms.

Offal has not been directly linked with human campylobacter infection, yet it is a likely source of infection (Skirrow, 1982). Although offal for human consumption is usually cooked before eating it is common practice to feed raw offal to domestic pets, which can then transmit infection to their human contacts (Blaser, Taylor & Feldman, 1983). Furthermore it is well recognized that inadequate kitchen or personal hygiene can result in human infection. In a recent survey of kitchens which processed poultry we were able to demonstrate that campylobacters present on chicken carcasses could be transferred to work surfaces and the hands of operatives (Dawkins, Bolton & Hutchinson, 1984) and the same could apply to offal. The similarity in biotypes and serotypes found in offal and infected patients adds weight to this argument. *C. jejuni* biotype 1 of Penner serotypes 1 and 2, which

made up 62 % of isolates in our study, are among the most prevalent in man (Jones *et al.* 1984).

We believe that offal is a potentially important source of campylobacters that could be associated with sporadic human infection.

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