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A survey of campylobacter in animals

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SUMMARY

A survey of *Campylobacter* species in the faeces or rectal contents of domestic animals was carried out using direct and enrichment culture methods. Campylobacters were isolated from 259 (31 %) of 846 faecal specimens. The highest isolation rate was found in pigs (66 %); lower rates were found in cattle (24 %) and sheep (22 %). In pigs all the isolates were *C. coli*, in sheep and cattle about 75 % were *C. jejuni*. Only five isolations of *C. fetus* subsp. *fetus* were made, all from cattle. More pigs with diarrhoea had *C. coli* in their faeces than healthy pigs (77 % vs 47 %), but such a clear difference in isolation rate between sick and healthy animals was not seen in cattle or sheep.

The enrichment method increased the total isolation rate of *C. jejuni* and *C. coli* by 33 %, but for cattle specimens it increased it by 69 % (from 6·5 % to 21 %). However, the enrichment method failed to detect 16 % of positive specimens (mainly *C. coli*), so direct and enrichment methods should be used for the culture of campylobacters from animal faeces. The results show that cattle, sheep and pigs constitute a large potential source of campylobacter infection for man.

INTRODUCTION

Campylobacters have long been recognized as pathogens in animals under their earlier name of *Vibrio*. It was not until the 1970s that improved isolation techniques developed by Dekeyser *et al.* (1972) and Skirrow (1977) led to the realization that campylobacter enteritis was a common human disease. Infection in man is due almost entirely to *C. jejuni* and *C. coli*, of which the former is the more important.

Although there is much evidence that campylobacter enteritis is a zoonosis and that farm animals are probably the principal sources of infection (Skirrow, 1982), there is little published information on the carriage rates of *C. jejuni* and *C. coli* in these animals, particularly cattle. Nor is it clear whether this group of organisms is important as a cause of enteritis in animals (Prescott, 1981).

The purpose of this investigation was twofold. First, to ascertain the carriage rate of campylobacters in the faeces or rectal contents of animals using both direct and enrichment culture techniques. Secondly, to determine whether these bacteria are present more often in animals with enteric disease, a finding that would suggest a causative role.

MATERIALS AND METHODS

Samples

During 1 year, faecal samples submitted to the Wye Veterinary Investigation Centre and rectal contents obtained from animals at post-mortem examination were cultured for campylobacters.

Most of the faecal samples were sent through the post at ambient temperature; no transport medium was used. They were usually cultured within 48 h of the sample being taken. Post-mortem examination and culture were normally carried out within 24 h of an animal's death.

The faecal samples and animals submitted for post-mortem examination originated from many farms representing a wide range of husbandry systems. Samples from cattle, sheep and pigs were allocated to one of two groups: those from animals with enteritis and/or diarrhoea (the diseased group), and those from animals not having symptoms of enteric illness (the healthy group). The allocation was based on clinical history, specimen appearance and, where applicable, post-mortem findings. The age distribution (0–3 months, 4–6 months, 7 months and over) of the animals represented in each group was roughly similar. Too few samples from other species were submitted for analysis to be of value.

Cultural techniques

Faeces samples were cultured by two methods, as follows.

(i) *Direct culture.* A single 4 mm loopful of faeces was inoculated on to each of the selective agars of Skirrow (1977) and Butzler (Butzler & Skirrow, 1979). Cycloheximide (100 μg/ml) was added to Skirrow's agar to control fungal growth. The Butzler's agar was modified by the inclusion of 0·025% each of ferrous sulphate, sodium metabisulphite and sodium pyruvate (FPB agar supplement of George *et al.* 1978) and nor-epinephrine (0·2 mmol/l) as reported by Hoffman *et al.* (1979). The inoculated plates were incubated at 37 °C for 48 h in microaerobic conditions obtained by twice drawing a partial vacuum of 450 mmHg in anaerobic jars (without a catalyst) and replacing with a gas mixture of 6% oxygen, 10% carbon dioxide and 84% nitrogen (British Oxygen Company Mixed Gases).

(ii) *Enrichment culture.* A single 4 mm loopful of faeces was inoculated into the top 5 mm of a 5 ml volume of semi-solid anaerobic broth (FAB-LabM), in a screw-top bottle, containing FBP supplement, nor-epinephrine and the antibiotic mixture as used in the Butzler formula described above. Tops were screwed down and the bottles incubated at 37 °C for 24 h, after which 2–3 loopfuls (4 mm diam.) were taken from the top 5 mm of broth (the zone of optimum growth) and cultured on the two selective agars as described above.

Identification

Campylobacter colonies were initially identified by microscopy and then by the biotyping scheme of Skirrow & Benjamin (1980). The following strains were included as controls: *C. jejuni* biotype 1 (NCTC 11168), *C. jejuni* biotype 2 (NCTC 11392), *C. fetus* ssp. *fetus* (NCTC 5850), *C. coli* (NCTC 11353) and *C. laridis* (NCTC 11352).

RESULTS

Isolation rates

Campylobacters were isolated from 259 (31%) of the 846 faecal specimens examined (Table 1). Two-thirds of the pigs and almost a quarter of the cattle and sheep were positive, whereas only 9% of the other species had campylobacters present.

Table 2 shows that the carriage rates of the cattle and sheep in the diarrhoea groups were little different from those of the healthy ones, whereas 77% of pigs with enteric disease carried campylobacters compared with 47% of healthy pigs (χ^2, $P < 0{\cdot}001$).

Distribution of Campylobacter *species*

Of all the campylobacters isolated, 95% belonged to the thermophilic group. The commonest species in cattle and sheep was *C. jejuni* biotype 1 (Table 3), which accounted for 73% of cattle isolates and 77% of sheep isolates. In pigs all the isolates were *C. coli*. Only five isolations of *C. fetus* subsp. *fetus* were made, all from cattle. Eight cultures died before they could be identified.

Effect of enrichment culture

Campylobacters were isolated by direct culture from 175 (68%) of the 259 positive faecal samples. The remaining 84 (32%) were obtained only by the enrichment technique. However, 41 (16%) of isolates were obtained only on direct culture, i.e. they failed to grow in enrichment cultures. A breakdown of the

Table 1. *The isolation of* Campylobacter spp. *from the faeces or rectal contents of domestic animals*

Animal species	No. tested	No. positive for campylobacter (%)
Cattle	309	74 (24)
Sheep	281	61 (22)
Pigs	178	117 (66)
Goats	26	0 (0)
Horses	14	1 (7)
Antelopes	11	2 (18)
Dogs	8	1 (12·5)
Others	19	3 (16)
Total	846	259 (31)

Table 2. *The isolation of campylobacter from the faeces or rectal contents of animals with symptoms of enteric disease and those without (healthy)*

Animal species	Enteric disease. No. tested	Enteric disease. No. positive for campylobacter (%)	Healthy. No. tested	Healthy. No. positive for campylobacter (%)	Significance (P)
Cattle	198	52 (26)	111	22 (20)	$> 0{\cdot}05$
Sheep	143	35 (25)	138	26 (19)	$> 0{\cdot}05$
Pigs	112	86 (77)	66	31 (47)	$< 0{\cdot}001$

Table 3. *The isolation of* Campylobacter *spp. and biotypes from the faeces or rectal contents of domestic animals*

Animal species	Faeces/ rectal contents	*Campylobacter* species and biotypes. No. (%)					Not identified
		C. jejuni 1	*C. jejuni 2*	*C. coli*	*C.f.f.**	*C. laridis*	
Cattle	74	54 (73)	0 (0)	10 (13)	5 (7)	0 (0)	5 (7)
Sheep	61	47 (77)	3 (5)	8 (13)	0 (0)	1 (2)	2 (3)
Pigs	117	0 (0)	0 (0)	117 (100)	0 (0)	0 (0)	0 (0)
Others	7	5 (72)	0 (0)	1 (14)	0 (0)	0 (0)	1 (14)
Total	259	106 (41)	3 (1·1)	136 (52·5)	5 (2)	1 (0·4)	8 (3)

* *C. fetus* ssp. *fetus*.

Table 4. *Comparison of direct and enrichment methods for isolating* C. jejuni *and* C. coli

Animal species	Total no. of isolates	Culture method								
		Direct positive, enrichment negative			Direct negative, enrichment positive			Direct positive, enrichment positive		
		*C. jejuni**	*C. coli*	Total (%)	*C. jejuni**	*C. coli*	Total (%)	*C. jejuni**	*C. coli*	Total (%)
Cattle	64	6	2	8 (12·5)	37	7	44 (68·75)	11	1	12 (18·75)
Sheep	58	3	0	3 (5)	20	2	22 (38)	27	6	33 (57)
Pigs	117	0	26	26 (22)	0	13	13 (11)	0	78	78 (67)
Others	6	2	0	2 (33)	1	0	1 (17)	2	1	3 (50)
Total	245	11	28	39 (16)	58	22	80 (33)	40	86	126 (51)

* *C. jejuni* biotypes 1 and 2.

isolation rates of *C. jejuni* and *C. coli* by both cultural methods for each animal species is shown in Table 4. Although this comparison shows that an additional one-third (33 %) of all isolates were made with the enrichment method, this increase was much greater in cattle. In this species 69 % would have been missed had reliance been placed on direct culture alone. By contrast, in pigs only 11 % would have been missed in this way.

DISCUSSION

This investigation shows that campylobacters are commonly present in the faeces of cattle, sheep and pigs. The carriage rates in cattle (24 %) and sheep (22 %) were similar, but they were only about one-third of that in pigs (66 %). The numbers of other species examined were too small for any significant conclusions to be drawn, although it is notable that no isolation was obtained from the 26 goats tested. Goat's milk is usually consumed unpasteurized and so presents a potential health risk. There is no reason to believe that campylobacters do not occur in this species, as *C. jejuni* has recently been found to be the cause of abortion in a goat in America (Anderson *et al.* 1983). More goats need to be examined in this country to establish carriage rates for this organism.

The high isolation rate found in pigs was similar to that found in several other countries. In Germany Gorgen, Kirpol & Bisping (1983) reported rates of 48–100 %, and in Finland Hänninen & Roevuori (1981) found 55 %. Prescott (1981), however, reported that only 2·5 % of pigs were carriers in his survey in Canada. In our study the carriage rate in pigs with evidence of enteric disease was considerably higher than in those with no obvious symptom of enteritis (77 % vs 47 %). Such a clear difference was not observed in cattle and sheep. Taylor & Olubumni (1981) concluded that *C. coli* may be a cause of enteritis in pigs, and these findings support this view.

About one-third of the campylobacters were isolated only by the enrichment method. The value of this was particularly evident in cattle and sheep where, without enrichment, the isolation rate fell from 24 % to 7·8 % and from 22 % to 13·2 % respectively. The lower figures are comparable to the 2·5 % for cattle and 13·5 % for sheep obtained in Canada by Prescott (1981) and the 6 % for cattle obtained in Finland by Hänninen & Roevuori (1981); in both cases only direct culture methods were used. Yet Svedham & Kaijser (1981) in Sweden, also using a direct culture method, found 19 % of cattle to be carriers. There is no obvious explanation for the higher proportion of cattle isolates obtained by enrichment compared with sheep, but probably fewer organisms are present in cattle. The enrichment technique in pigs contributed less (11 %) to the total number of isolations. This suggests that either more organisms are present in pigs, allowing easier isolation by the direct method, or that the isolation of *C. coli* – the only species found – is not favoured by the enrichment procedure; indeed, most of the campylobacters that failed to grow in the enrichment cultures were *C. coli*. A combination of direct and enrichment methods is therefore necessary to ensure the maximum isolation of campylobacters from animal faeces.

C. jejuni, and to a lesser extent *C. coli*, are the main cause of human campylobacter enteritis. This survey shows that 20 % of the cattle and sheep tested

were excreting these species of campylobacter, and of these 85% were *C. jejuni*. On the other hand, all of the campylobacters isolated from pigs were *C. coli* which, in general, accounts for only about 5% of human infections (Skirrow, 1982). Thus despite high carriage rates, pigs appear to be of less importance as a source of human infection. A possible explanation is that the commonly used method of forced-air cooling of pig carcasses after slaughter causes a profound drop in surface campylobacter counts through drying (Oosterom *et al.* 1983). Nevertheless, in Germany Sticht-Groh (1982) showed that pig offal was commonly contaminated with *C. coli*, and in Yugoslavia Kalenić *et al.* (1983) reported that in the summer and autumn at least three-quarters of the campylobacters isolated from human patients were *C. coli*.

Three conclusions may be drawn from this survey: (1) that cattle, sheep and pigs form a large potential source of human campylobacter infection; (2) that *C. coli* may be a cause of diarrhoea in pigs, as more were found in those with diarrhoea than in those without; (3) that both direct and enrichment culture methods are necessary for the satisfactory culture of campylobacters from the faeces of these animals.

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