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Campylobacter jejuni and salmonella in raw red meats

A Public Health Laboratory Service Survey*

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SUMMARY

Thirty-one laboratories examined a total of 6169 meat samples, 1236 from abattoirs and 4933 from retail and other outlets. *Campylobacter jejuni* was isolated from 98 (1·6%). A higher isolation rate of 49/1236 (4·0%) was found among abattoir than among retail and other samples (49/4933–1·0%). Twenty-two of the laboratories looked for salmonella; although 94/4002 (2·3%) were positive, in only one sample of minced beef were campylobacter and salmonella found together. Isolation rates for salmonellae were 75/3576 (2·1%) from retail and 19/426 (4·5%) from abattoir samples.

Analysis of the results revealed that (1) the contamination rate of raw red meat by *C. jejuni* is, in general, very low; (2) when contaminated, numbers of organisms are generally also very low; (3) enrichment procedures were of some value; 41/98 (42%) isolates were detected by enrichment only, but, on the other hand 8 (8%) were direct plate positive/enrichment negative; (4) practice at looking for the organism and increased seasonal temperatures over the survey period did not result in a noticeable increase in isolations; (5) there was no apparent correlation between campylobacter and salmonella isolations.

INTRODUCTION

Although the extent of human infection with campylobacter has only become fully recognized in about the past five years, Skirrow (1977) has reviewed literature

* The following Public Health Laboratory Service laboratories kindly participated:
Bath, Brighton, Bristol, Cardiff, Central Middlesex, Chelmsford, Conwy, Dorchester, Exeter, Food Hygiene, Gloucester, Hereford, Hull, Ipswich, Leeds, Lincoln, Liverpool, Luton, Maidstone, Manchester, Middlesbrough, Nottingham, Plymouth, Preston, Shrewsbury, Truro and Whipps Cross.

Also participating were the British Food Manufacturing Industries Research Association, Leatherhead; the Meat Research Institute, Bristol; the Microbiology Department, Guy's Hospital, and the Veterinary Investigation Centre, Newcastle-upon-Tyne.

Confirmation of identification and biotyping was carried out by the Microbiology Department, Worcester Royal Infirmary.

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which shows that it had been established some years earlier that members of this genus could infect man.

It was considered for some time that poultry might be the primary source of human infection since organisms of this type were alleged to cause avian vibrionic hepatitis (King, 1962; Peckham, 1972). Later surveys have indeed shown that, in a manner somewhat analagous to salmonella, high contamination rates may be associated with poultry (Bruce, Zochowski & Ferguson, 1977 – 62% to 68%; Ribeiro, 1978 – 91%; Simmons & Gibbs, 1979 – 48% to > 80%) and that *C. jejuni** could survive commercial processing (Simmons & Gibbs, 1979). Chickens have been directly implicated in only a few human campylobacter infections (King, 1962; Skirrow, 1977; Anon, 1978; Brouwer *et al.* 1979; Schaeffer *et al.* 1979) but the number of such recorded incidents is remarkably small when the high contamination rate of processed chickens is considered. Possibly one of the reasons for this is the apparent inability of the organism to grow in or on chicken meat under the conditions that favour growth of food poisoning bacteria such as salmonella, *Staphylococcus aureus* and *Clostridium perfringens* (Ghosh and Turnbull, unpublished results; Miss R. Blood, British Food Manufacturing Industries Research Association, Leatherhead, personal communication).

While there remained much to be learnt about *C. jejuni* contamination of poultry and its relationship with human disease, virtually nothing at all seemed to be known about contamination rates in red meats, although campylobacters of this group are commonly found in sheep, cattle, and pigs (Butzler & Skirrow, 1979). The Public Health Laboratory Service (PHLS) raw meat survey was set up to determine *C. jejuni* contamination rates of red meats (i.e. other than poultry) as prepared and sold for human consumption.

ORGANIZATION AND PROCEDURES

Twenty-seven PHLS and four other laboratories volunteered to participate in the survey which ran from February to August 1979, encompassing cold and warm months. As each laboratory had to fit this investigation into an existing work programme and as campylobacter isolation methods were largely empirical at the time, no rigid criteria were laid down for the methods to be used for the collection and preparation of samples.

Samples were collected either by members of the local Environmental Health Department or by the laboratories themselves.

In general the samples were transferred from abattoirs or retail premises to the laboratory in cold boxes. In one instance (laboratory no. 5 in Table 6) in which there was a field laboratory attached to an abattoir, sampling of carcasses was done with swabs. In this case, two swabs were used; the first was moistened with ISO maintenance broth (0·8% NaCl + 0·1% peptone) and the second, a dry swab was used to mop up excess fluid.

Samples from two bacon factories were for the purposes of this survey classed as 'retail'. In all, 6169 samples were collected; the main categories and the numbers

* We use the term *C. jejuni* to include organisms conforming to both *C. jejuni* and *C. coli* (Véron & Chatelain, 1973) but where a distinction between the two has been made this is indicated in the text.

Table 1. *Samples examined* (31 *Laboratories*)

Minced beef	Minced pork	Sausage/sausage meat			Other meats	Source		Total
		Beef	N/S	Pork		Abattoir	Other	
2046	376	101	1114	254	2278	1236	4933	6169

Table 2. *Examples of 'other meats' listed in Table* 1

Bacon
Beefburger – various forms
Black pudding
Bovine brisket/clod/forequarter/ shin/flank/heart/liver/ muscle/neck/tripe/skirt
Kidney – cow/rabbit
Kebab – Doner – raw
Lamb diaphragm/heart/kidney/ liver/breast/rolled stuffed breast
Mutton – minced
Ox tail
Pork – belly/boneless steak/chop/ diaphragm/diced/heart/ liver/muscle/pie meat/sausage with Protena/spare ribs/ streaky/stuffed shoulder/ tripe
Pork/beef – minced
Rabbit – fresh
Steak – blade/chuck/diced/flash and quick fry/frying/rump/Vienna
Tomato sausage meat

examined within each category are given in Table 1. Examples of 'other meats' in Table 1 are listed in Table 2.

Except for one laboratory (no. 19 in Table 6) which preferred impression plates, all laboratories commenced examination by adding quarter-strength Ringer's solution to the meat sample prior to stomaching (Colworth Stomacher, A. J. Seward, Bury St. Edmonds) or blending to homogeneity. Most laboratories appeared to be satisfied with simple w/v sample/diluent combinations of 25, 50 or 100 g to 25, 50 or 100 ml (not necessarily respectively). Occasionally higher or lower weights or volumes than these were used or less simple ratios. Different types of samples called for different amounts of diluent; for example, sausage meat absorbed relatively large volumes before it acquired a moist homogeneous consistency.

The homogenized sample (usually 0·1 ml) – or swabs in the case of laboratory no. 5 – was spread onto lysed horse blood agar supplemented with vancomycin, polymyxin and trimethoprim and incubated at 43 °C under microaerobic conditions as recommended by Butzler & Skirrow (1979); the plates were examined at 24 and 48 h. One laboratory (no. 22 in Table 6) inoculated 0·1 ml of the homogenized sample into 3 ml peptone water, incubated this for 2 h at 37 °C, filtered it through 0·65 μm filters and inoculated two drops of filtrate onto blood agar plates without antibiotics; the plates were incubated at 37 °C in 10 % CO_2. In all laboratories when presumptive colonies were observed, the number was recorded.

Seventeen of the laboratories attempted enrichment culture on all their samples; a further two used enrichment for some of their samples. The enrichment broth used by most of the laboratories was prepared according to the formula given in a PHLS Communicable Disease Report (1978, no. 47). This had been used with success in the isolation of campylobacter from milk socks during the investigation of a milkborne outbreak, and consisted of nutrient broth 375 ml, horse blood 25 ml, vancomycin 10 mg/l, polymyxin B sulphate 2500 i.u./l and trimethoprim lactate

5 mg/l. Three laboratories reported trying out other enrichment broths. Procedures for inoculation of the enrichment broth varied widely and ranged from 0·1 ml of meat homogenate in 20 ml enrichment broth to the addition of an equal volume of enrichment broth to a homogenate of 100 g of sample in 100 ml Ringer's solution. The enrichment broth was incubated 24 h at 43 °C microaerobically and was then subcultured to blood agar supplemented with antibiotics as for the direct plate procedure.

Smears from suspect colonies checked for positive oxidase and catalase reactions, were identified as *C. jejuni* by their characteristic morphology on gram staining, dark ground, or phase contrast microscopy. Some strains were sent in thioglycollate or cooked meat broth to the Worcester Royal Infirmary laboratory for biotyping.

Examination for the presence of salmonellae in samples was carried out using generally accepted techniques. Five laboratories included total viable, coliform, and *Escherichia* (faecal) *coli* counts on their samples.

RESULTS

The overall *C. jejuni* and salmonella isolation rates resulting from the survey are given in Table 3. Of 6169 samples examined for *C. jejuni* just 98 (1·6 %) were found to be positive; this compared with the finding of salmonella in 94 of 4002 (2·3 %) samples. However, only on a single occasion were the two organisms found in the same sample (minced beef).

Table 3. *Overall isolation rates*

C. jejuni		Salmonella	
No. samples examined	No. positive	No. samples examined	No. positive
6169	98 (1·6 %)	4002	94 (2·3 %)

Only in one sample (minced beef) were both salmonella and campylobacter found together.

Table 4. *Comparison of* C. jejuni *isolation rates in retail and abattoir samples*

Abattoir samples		Retail and *other samples	
No. samples examined	No. positive	No. samples examined	No. positive
1236	49 (4·0 %)	4933	49 (1·0 %)

* e.g. bacon factory.

When samples were considered in terms of those collected from abattoirs and the remainder (Table 4), a higher rate of isolation of *C. jejuni* (49/1236 – 4·0 %) was found among abattoir than among non-abattoir samples (49/4933 – 1·0 %). Similarly the salmonella isolation rate among abattoir samples (19/426 – 4·5 %) was greater than that in non-abattoir ones (75/3576 – 2·1 %).

Retail beef and minced beef and abattoir pork accounted for most (72) of the 98 isolates of *C. jejuni* (Table 5). The same types of sample also accounted for a substantial proportion (39) of the salmonella isolates; however, in this case, sausage meat also yielded a relatively high number (28) of isolates.

Table 5. *Foods* vs *Campylobacter and Salmonella isolations*

Food	Retail/ abattoir	Number positive for campylobacter (%)	Number positive for salmonella (%)
Minced beef	R	21/2015 (1·0)	21/1492 (1·4)
Minced pork	R	1/342 (0·3)	8/243 (3·3)
Sausage/sausage meat	R	2/1448 (0·1)	28/962 (2·9)
Other			
Beef	R 9	74/2278 (3·2)	6
Beef	A 6		1
Beefburger	R 1		2
Pork (including parts)	R 9		7
Pork (including parts)	A 32		12
Lamb (including parts)	R 6		3
Lamb (including parts)	A 10		6
Rabbit kidney	R 1		0
			37/879 (4·2)

Table 6. *Laboratory isolation rates*

Lab. no.	Total Campylobacter isolations: No. isolations	Total Campylobacter isolations: No. samples examined	Abattoir samples: No. isolations	Abattoir samples: No. samples examined	Retail samples: No. isolations	Retail samples: No. samples examined	No. of human isolations same period
1	22	262	1	6	21	256	8
2	20	616	20	614	0	2	9
3	15	319	14	142	1	177	95
4	9	423	0	0	9	423	n.a.
5	7	160	6	100	1	60	n.a.
6	4	100	0	0	4	100	n.a.
7	4	250	4	30	0	220	75
8	3	294	0	0	3	294	36
9	3	137	0	0	3	137	151
10	2	130	0	75	2	55	7
11	2	49	0	10	2	39	18
12	1	92	0	0	1	92	59
13	1	208	1	12	0	196	410
14	1	254	0	0	1	254	13
15	1	116	0	0	1	116	86
16	1	61	0	3	1	58	n.a.
17	1	105	0	0	1	105	3
18	1	97	0	0	1	97	12
19	0	711	0	0	0	711	n.a.
20	0	273	0	0	0	273	67
21	0	227	0	129	0	98	54
22	0	226	0	76	0	150	n.a.
23	0	213	0	0	0	213	30
24	0	210	0	0	0	210	16
25	0	129	0	0	0	129	3 p. wk
26	0	119	0	10	0	109	21
27	0	108	0	0	0	108	n.a.
28	0	99	0	0	0	0	79
29	0	79	0	0	0	0	13
30	0	72	0	0	0	0	21
31	0	22	0	0	0	0	20

n.a.: not applicable or not available.

Table 7. *Isolation by direct plate (DP) and enrichment (En.)*

Total isolates	DP+/En. ND	DP+/En. Neg.	Both +	DP−/En.+	Not known
98	33	8	14	41	2

ND, not done.

Table 8. *Total viable counts (TVC)* vs *isolation of Campylobacter and Salmonella (five laboratories)*

TVC	Number of samples	Number from which Campylobacter isolated (%)	Number from which Salmonella isolated (%)
$\geqslant 10^7$	108	4 (3·7)	2 (1·9)
$\geqslant 10^6$	232	10 (4·3)	4 (1·7)
$\geqslant 10^5$	293	5 (1·7)	5 (1·7)
$\geqslant 10^4$	129	1 (0·8)	2 (1·6)
$< 10^4$	68	2 (2·9)	1 (1·5)

Table 9. *Hippurate and* H_2S *biotyping of 54 of the survey Campylobacter isolates*

		C. jejuni 1	*C. jejuni* 2	*C. coli*	Total
Pork	Abattoir	0	0	19	19
	Retail	3	1	3	7
Beef	Abattoir	4	1	0	5
	Retail	12	2	1	15
Lamb	Abattoir	5	0	0	5
	Retail	3	0	0	3
					54

Table 6 shows that *C. jejuni* was isolated from meat samples by 18 of the 31 participating laboratories, with 57 (58 %) of the isolations being made in only three of the laboratories. Also apparent from Table 6 is that (1) the number of isolations made by the various laboratories bore little relation to the number of samples they examined; (2) in those laboratories where it was applicable, the patterns of isolations from meat samples were not reflected in the patterns of human *C. jejuni* isolations made over the same period.

Enrichment procedures carried out by 19 of the laboratories clearly enhanced the isolation rate (Table 7). Of the 98 isolations, 41 (42 %) would not have been detected without enrichment. On the other hand eight isolates were direct plate positive/enrichment negative. Fifty-five of the 98 (56·1 %) isolates were detected by direct plating and with one exception, the *C. jejuni* colony counts recorded ranged from 1 to 20 per 0·1 ml meat slurry. The single exception was a sample of pig spleen from a bacon factory which contained $1{\cdot}2 \times 10^4$ c.f.u./ml.

With the 22 *C. jejuni* positive samples on which total viable counts (TVC) were carried out, there appeared to be an association between high percentages of *C. jejuni* isolations and high TVCs (Table 8). However, the numbers of samples examined were too low to assess the significance of this observation. No such association between salmonella isolations and TVC was seen in this analysis; the

percentage of salmonella isolated was approximately the same from each of the five groups of samples derived from classification according to TVC (Table 8). Fifty-four of the isolates were biotyped at Worcester Royal Infirmary by the method of Skirrow & Benjamin (1980*a*). The differential distribution of these strains into *C. jejuni* biotype 1, *C. jejuni* biotype 2 and *C. coli* is shown in Table 9.

One further observation was that the isolations of both *C. jejuni* and salmonellae were made at a steady rate over the period of the survey. Practice gained with time at looking for the organisms and increasing seasonal temperatures as the survey progressed did not result in a detectable increase in isolation rates.

DISCUSSION

Although the overall isolation rate of *C. jejuni* from meat samples was 1·6% (Table 3) there was a strikingly uneven distribution among the laboratories (Table 6). The highest rates recorded were 14/142 (10%) in abattoir (laboratory no. 3 in Table 6) and 21/256 (8%) in retail samples (laboratory no. 1). Some confusion arose in relation to the sources of eight of the latter 21 isolates when confirmation of their identity was being carried out. However, it could be interpreted that, while campylobacter contamination rates in red meat were generally found to be low, the results in these laboratories indicate that, at the upper limit, 5–8% of retail and 10% of abattoir samples may be contaminated. The higher isolation rate among abattoir samples as compared with retail and other samples is compatible with the fact that there is, so far, no evidence that *C. jejuni* multiplies readily in or on meat.

No data could be found with respect to *C. jejuni* in raw red meats with which to compare the results presented here. On the other hand, as reviewed by Bryan *et al.* (1979) and Abbott & Robertson (1980), there are many reports on the prevalence of salmonellae in meats and meat products. A very wide range of isolation rates without any particular pattern can be seen in these reports. As with *C. jejuni*, the distribution of salmonella isolations among the different laboratories was very uneven; isolations made by two of the laboratories accounted for some 50% of all the isolations reported. However, *C. jejuni* and salmonella contamination of the meat samples in this survey showed little in common. The laboratory with the highest campylobacter isolation rate (no. 1 in Table 6) found no salmonellae, whereas a laboratory recording 28 salmonella isolations in 711 samples found no campylobacters. The two organisms were only found together on one occasion, in a sample of minced beef.

The numbers of samples examined by the various enrichment methods were insufficient to allow a useful comparison. Nor were the numbers of positive isolations sufficient to make it possible to recommend any one particular ratio of sample to enrichment broth out of the many that were used. Although enrichment procedures clearly enhanced isolation rates (Table 7) the fact that eight samples were positive on direct plates yet negative by enrichment indicated that some of the enrichment methods were not fully reliable. A recent report by Bolton & Robertson (in the Press) suggests that higher isolation rates would have been attained had their enrichment broth been generally used.

The numbers of campylobacter positive samples on which total viable counts

were made were also too small to assess the significance of the apparent association in Table 8 between high isolation rates and high TVCs. Three of the five laboratories which recorded counts also submitted coliform counts and one included (faecal) *E. coli* counts; these were proportionately lower than the TVCs and supplied no additional data in relation to the isolation of *C. jejuni*.

In those laboratories in which faeces from patients were being examined for the presence of campylobacters, the numbers of specimens examined were not available and therefore the isolation rates from faeces could not be given. However, the isolation figures given (Table 6) serve to show that the laboratories were not, in general, finding *C. jejuni* a difficult organism to isolate. Although the food and faecal examinations would have been carried out at separate benches, this adds a degree of confidence to the meat isolation figures.

The number of campylobacter isolations from the various types of meat sample were again too low to permit a detailed analysis and projection of biotypes that could be expected in meats. All the abattoir pork strains were typed as *C. coli* (Table 9), which suggested they were of autogenous origin (Skirrow & Benjamin, 1980*b*). On the other hand, the four *C. jejuni* (*sensu strictu*) strains from retail pork samples suggested that they may have come from other sources. The predominance of *C. jejuni* biotype 1 in the beef and lamb samples is consistent with their origin in these species.

In summary, the results of this survey have shown that the high contamination rates by *C. jejuni* that have been found in chickens (Bruce *et al.* 1977; Ribeiro, 1978; Simmons & Gibbs, 1979) are not parallelled in red meats.

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