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The relationship between intestinal *Campylobacter* species isolated from animals and humans as determined by BRENDA

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SUMMARY

Intestinal thermophilic *Campylobacter* species produce stable patterns when subjected to bacterial restriction endonuclease DNA analysis (BRENDA); this technique is therefore of considerable value in epidemiological studies. BRENDA was used to examine thermophilic *Campylobacter* species from humans, wild and domestic animals. One hundred and ninety-four (61%) of 316 isolates of *Campylobacter jejuni* from humans had BRENDA patterns which could be matched to those of animal isolates. Poultry appear to be a major source of infection for *C. jejuni* in humans with nearly half (49·7%) of the human isolates giving patterns which were indistinguishable from those isolated from poultry. A total of 60 BRENDA types were identified from 316 human isolates and 11 of these had the same pattern as those isolated from poultry. One of the three *Campylobacter coli* BRENDA types recovered from poultry was indistinguishable from a human isolate type. Pigs appear to be only a minor source of *C. coli* infection for humans in New Zealand. Rats were found to be infected with strains of *C. jejuni* with BRENDA patterns indistinguishable from those infecting humans, poultry and a horse. None of the 102 isolates of *Campylobacter* species from wild birds gave BRENDA patterns similar to those of isolates from humans.

INTRODUCTION

Until recently the epidemiological relationship between the intestinal thermophilic *Campylobacter* species recovered from different species of animals and humans has been uncertain. The major reason for this has been the lack of a sensitive and reliable system for differentiating strains within the same species of *Campylobacter*.

The biotyping scheme of Skirrow & Benjamin (1980) has received international recognition and provides a method for differentiation between *C. jejuni*, *C. coli* and *C. laridis*. It also allows for the subdivision of *C. jejuni* into biotypes 1 and 2 but is unable to differentiate many of the strains. Blaser (1980) has discussed the inadequacies of the methods of classification of intestinal thermophilic *Campylobacter* species in relation to epidemiological investigations. Skirrow (1982)

Table 1. *The number and source of thermophilic* Campylobacter *species examined*

Source of isolate	*C. jejuni*	*C. coli*	*C. laridis*	Total
Human	316	25	0	341
Chicken	98	30	0	128
Pig	0	147	0	147
Duck	7	2	0	9
Gull	34	11	43	88
Horse	1	0	0	1
Rat	18	0	0	18

pointed out that it is unlikely that there will be any major advances in the epidemiology of campylobacter until a comprehensive and practical strain identification scheme is developed. Attempts have been made to resolve the problem of strain differentiation of *C. jejuni* and *C. coli* by serological methods by a number of people including Penner & Hennessy (1980), Lior *et al.* (1982) and Itoh *et al.* (1982). Serogrouping by one method does not always give good discrimination and the use of two methods together (heat-stable and heat-labile antigens) is cumbersome.

Bacterial restriction endonuclease DNA analysis (BRENDA) has already been shown to be a useful technique for the identification of a broad spectrum of different organisms, including *Leptospira interrogans* (Marshall, Wilton & Robinson, 1981) *Vibrio cholerae* (Kaper *et al.* 1982), *Rickettsia prowazekii* (Regnenery *et al.* 1983), *Moraxella bovis* (Marshall *et al.* 1985) and *Campylobacter coli* (Kakoyiannis, Winter & Marshall, 1984).

The aim of this study was to use the BRENDA technique to investigate the epidemiology of intestinal thermophilic *Campylobacter* infections in animals and humans.

MATERIALS AND METHODS

A total of 731 isolates of thermophilic *Campylobacter* (473 *C. jejuni*, 215 *C. coli* and 43 *C. laridis*) was subjected to BRENDA analysis. Table 1 shows the source and the numbers of all the isolates examined.

The BRENDA technique has been described previously by Marshall, Wilton & Robinson (1981) and Kakoyiannis, Winter & Marshall (1984). This method involves the extraction and purification of the DNA from a pure culture of campylobacter, digestion of this DNA using a suitable restriction enzyme, followed by electrophoresis of this digested DNA. Ethidium bromide is incorporated in the agarose gel and buffer used for electrophoresis so that the bands of DNA of different molecular weight can be visualized using a UV transilluminator. A photograph of the DNA electrophoretic pattern is taken as a permanent record. The photographic negatives of the DNA patterns were compared by eye when illuminated by diffuse back lighting.

All DNA preparations were digested with *Hind* III but 12 isolates found to belong to five different BRENDA types using that enzyme (each type included at least two isolates) were subjected to further BRENDA analysis using a second endonuclease enzyme (*Xho* 1).

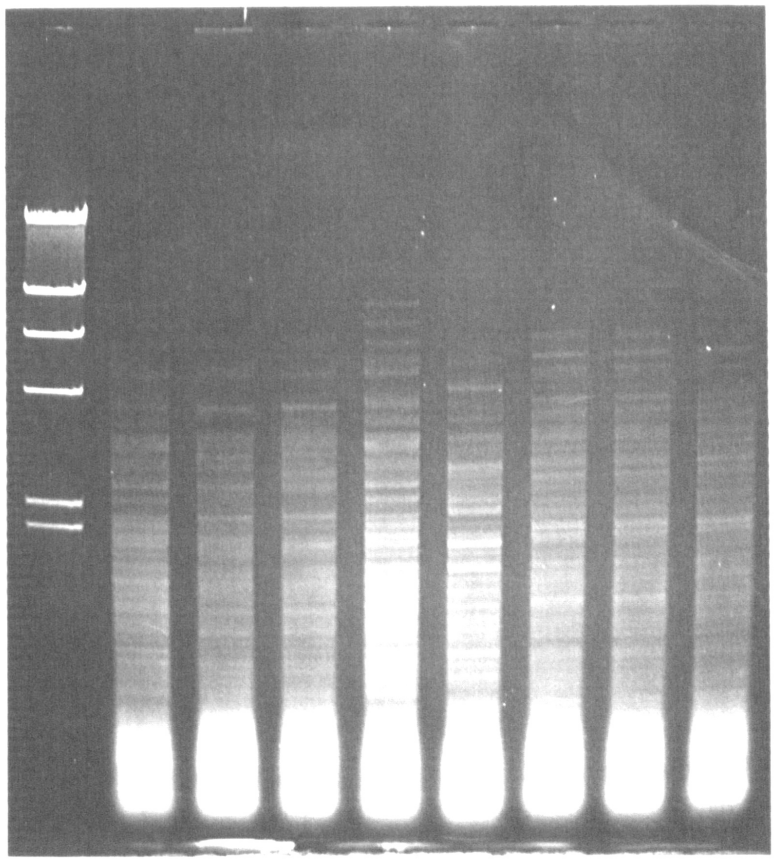

Fig. 1. BRENDA patterns of a selection of human isolates produced by using *Hind* III. Lane 1, λ DNA 23·1 kb, 9·4 kb, 6·5 kb, 4·3 kb, 2·3 kb, 2·0 kb fragments. Lanes 2 and 5 have produced patterns the same as each other but different from the others on this gel. Lanes 3 and 4 have similar patterns but are distinguishable from each other. Lane 3 has a pattern which is indistinguishable from poultry isolates from farm 4 (Fig. 2). Lane 6 is different from all other patterns on this gel. Lanes 7, 8 and 9 have similar patterns to each other, although lane 9 can be distinguished from 7 and 8.

RESULTS

Human isolates. A total of 316 human *C. jejuni* isolates was tested and they produced 60 readily distinguishable BRENDA patterns; 113 (35%) of the isolates were indistinguishable from 11 of 23 of the BRENDA patterns produced by poultry isolates. For examples of these patterns see Fig. 1, lane 3 and Fig. 2, lanes 2–5. A further 50 (15%) were similar although distinguishable in appearance from the BRENDA types found in poultry having up to four band differences. One of the BRENDA types found among both human and poultry *C. jejuni* isolates was also found in an isolate recovered from a rat. One of the patterns given by human *C. jejuni* isolates was indistinguishable from a horse isolate and another indistinguishable from a calf isolate.

Twenty-five human isolates of *C. coli* were tested by BRENDA and produced

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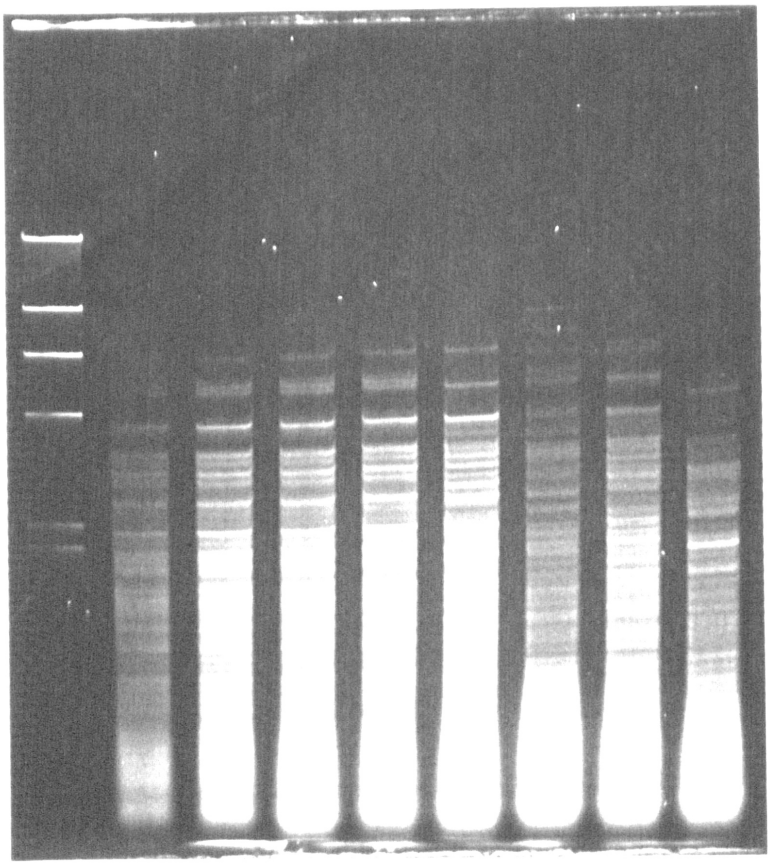

Fig. 2. BRENDA patterns of a selection of poultry isolates produced using *Hind* III. Lane 1, λ DNA 23·1 kb, 9·4 kb, 6·5 kb, 4·3 kb, 2·3 kb, 2·0 kb fragments. Lanes 2–5, poultry isolates obtained from the same farm (Farm 4), which are indistinguishable. Lane 6, a distinguishable but very similar pattern from the same farm. Lane 7 and 8 came from farms 1 and 2 respectively. Lane 9 is a *C. coli* from farm 7.

24 unique patterns. The patterns from two of these were indistinguishable from the patterns given by a poultry isolate. Another isolate gave a pattern identical to that of a pig isolate.

Chicken isolates. A total of 23 different BRENDA types of *C. jejuni* and 3 different BRENDA types of *C. coli* were recorded from 98 isolates of *C. jejuni* and 30 *C. coli*. Most of these isolates originated from 8 farms and 2 lots, each of 10 packets of fresh chicken pieces purchased from a supermarket. Six additional chicken isolates were provided by the National Health Institute (NHI). As can be seen from Table 2, flocks were infected with from 1 to 4 different BRENDA types. Examples of these are shown in Fig. 2. This range of different BRENDA types was even seen in different flocks on the same farm. However, only one BRENDA type of *C. coli* was ever recovered from any one flock or from any one batch of processed chicken pieces.

One of the three BRENDA types of *C. coli* isolated from chickens was found to be indistinguishable from an isolate of *C. coli* from a human patient (Kakoyiannis, Winter & Marshall, 1984).

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Table 2. *Source, rate of infection and number of isolates of* C. jejuni *and* C. coli *from poultry together with the number of BRENDA patterns produced*

Source of isolate	Farm no.	Rate of infection or contamination of samples	No. of isolates analysed by BRENDA		No. of BRENDA patterns produced	
			C. jejuni	*C. coli*	*C. jejuni*	*C. coli*
Poultry farm	1	20/20	10	0	1	—
	2	20/20	10	0	1	—
	3	20/20	10	0	1	—
	4	30/40	10	0	2	—
	5	7/10	5	0	3	—
	6	9/10	27	0	3	—
	7	20/20	10	3	4	1
	8	17/20	0	17	—	1
Supermarket survey (packets fresh chicken pieces)	Batch 1	10/10	10	0	2	—
	Batch 2	10/10	0	10	—	1
National Health Institute			6		6	—
			98	30	23	3

Pig isolates. BRENDA analysis of 147 isolates of *C. coli* from 126 pigs gave a total of 76 different BRENDA types. Twenty-eight were isolates from animals at one piggery and the remaining 48 types comprised 86 isolates from animals at 19 other piggeries. Fourteen of the 28 BRENDA types found at one piggery were recovered from 14 sows and 23 of their piglets. In six cases the DNA patterns of the isolates from the piglets were indistinguishable from those of their sows. The remaining 14 BRENDA types were recovered from older growing pigs in the same piggery. Some pigs were infected with up to three different BRENDA types of *C. coli*. Only one pig isolate had a BRENDA pattern indistinguishable from that of a *C. coli* isolated from a human.

Duck and gull isolates. Four BRENDA types were identified from the 7 isolates of *C. jejuni* from ducks and 2 BRENDA types from 2 isolates of *C. coli*. Twenty-two different BRENDA types of *C. jejuni* were identified from amongst the 34 isolates from black-backed gulls (*Larus dominicanus*). Eleven *C. coli* isolates from the gulls consisted of 6 BRENDA types whereas the 43 *C. laridis* were of 27 BRENDA types. None of the duck or gull isolates gave patterns resembling those of the human isolates.

Rat isolate. Four BRENDA types were identified from the 18 *C. jejuni* isolates from rats (*Rattus norvegicus*). Fifteen of these isolates gave the same BRENDA type and the remaining three each gave distinct BRENDA types. Two of these latter types were indistinguishable from those of two human isolates as well as to that of one chicken isolate and that of a horse isolate.

All isolates which were found to produce identical DNA patterns with the first enzyme (*Hind* III), also had identical patterns when digested by the second enzyme (*Xho* 1).

DISCUSSION

Two of the most widely used serotyping systems, those of Lior *et al.* (1983) and Penner, Hennessy & Congi (1983) have identified 91 and 60 serogroups respectively of thermophilic *Campylobacter* species. Approximately 7 and 3% respectively of organisms tested proved to be ungroupable by these two systems and in the Penner system up to 20% of the typable isolates reacted with more than one antiserum (Penner, Hennessy & Congi, 1983). Serological grouping of thermophilic *Campylobacter* species does not necessarily indicate a definite identity among isolates of the same serogroup (Karmali *et al.* 1983). The identification of a large number of BRENDA types among the intestinal thermophilic *Campylobacter* species is not therefore surprising. One of the advantages of BRENDA analysis is that a DNA 'fingerprint' can be obtained from all isolates and therefore it is reasonable to expect that the total number of types will be greater than for a serotyping scheme where a proportion of isolates are not typable. Also, it is unlikely that any serotyping scheme or other routinely used system of subspecies identification, will be as sensitive at recognizing different genomic types as BRENDA typing. For a system such as that using BRENDA, the stability of the patterns produced by isolates of *C. jejuni* and *C. coli* is of paramount importance. In a number of *in vivo* experiments not reported here, it has been shown that the challenge strains maintain stable BRENDA patterns during the entire duration of the experiments, a period of months. Such stability has also been demonstrated *in vitro* where four strains of *C. coli* of different BRENDA types retained their specific restriction endonuclease DNA patterns after 23 passages on agar over a period of 45 days (Kakoyiannis, Winter & Marshall, 1984). Further evidence of the stability of the DNA 'fingerprint' is provided by the recovery of similar BRENDA types of *C. jejuni* and *C. coli* from different chickens of the same flock and the recovery of *C. coli* from the same DNA pattern from both sows and their piglets.

The minor differences seen between some BRENDA patterns give the impression that these isolates are closely related and possibly derived from one another. However, in the experiments testing the stability of patterns, no changes even of a minor nature were ever observed. On the basis of the stability experiments and epidemiological information, the evidence suggests the view that these minor changes may represent a unique BRENDA type and not recent DNA mutations. For outbreak investigations, isolates must have indistinguishable patterns in order to consider them as being derived from a common stock.

The use of the restriction endonuclease enzyme *Hind* III produced sufficient DNA fragments to provide a satisfactory genomic 'fingerprint'. The number of bands of digested DNA produced by *Hind* III ranged from approximately 30 to 60. The usefulness of this enzyme has also been shown in limited studies of isolates of *C. jejuni* by Penner *et al.* (1983) and Bradbury *et al.* (1984). The present work indicates that the use of the second enzyme (*Xho* 1) provides no extra taxonomic data.

The limited number of BRENDA types of *C. coli* in poultry, compared with *C. jejuni* could be a reflection of a lower infectivity of *C. coli* for poultry, and presumably for other species of animals with the exception of pigs. The matching

of BRENDA patterns of a number of *C. jejuni* and *C. coli* isolated from chickens and humans is in keeping with serological findings by others (Lior *et al.* 1981; 1982; Kosunen *et al.* 1982; Munroe, Prescott & Penner, 1983). Although a direct link between infected chickens and human infections has been noted, the frequency with which this occurs is not known. Similarities in BRENDA patterns of isolates from both sources found in this study and the high levels (10^6 c.f.u./carcass) of contamination by campylobacter on processed carcasses (Shanker *et al.* 1982; Bamford, 1982) supports the view that chickens are an important source of infection for humans.

Although many *C. coli* BRENDA types were seen in pigs, only one of these was indistinguishable from an isolate of *C. coli* from humans. This difference between *C. coli* from pigs and those of human origin has also been recorded by Munroe, Prescott & Penner (1983) using the serological typing system of Penner (Penner & Hennessy, 1980). These findings are in contrast with those of Lior *et al.* (1981, 1982) who reported that serotypes of *C. coli* from pigs commonly occur in humans. Hudson & Roberts (1982) observed that the contamination rate of pig carcasses by campylobacter is considerably reduced after chilling the carcasses for 24 h at 0 °C. *Campylobacter* spp. could not be isolated from more than 2 and 26 % of the dry and wet areas of the carcasses respectively when the initial rate of carcass contamination was approximately 60 %. The number of campylobacter recovered were generally less than 1/cm^2 and is in contrast to the high level found in chicken carcasses (Shanker *et al.* 1982; Bamford, 1982). This finding may explain why we have found only one human isolate of *C. coli* with the same BRENDA type as a pig isolate and why Munroe, Prescott & Penner (1983) showed no common serotype between those recovered from humans and those from pigs.

The finding that two of the four BRENDA types seen in rats were indistinguishable from isolates from humans, chickens and a horse, is of interest. Although this finding cannot be taken as direct evidence that the human isolates were of rat origin, it raises the possibility that rats may be carriers of infection for humans and other animals.

Cattle isolates have been shown by DNA restriction endonuclease analysis (Bradbury *et al.* 1984) and by serotyping (Lior *et al.* 1981; 1982; Lauwers, 1982; Munroe, Prescott & Penner, 1983) to have similarities with isolates from humans and indeed several well-confirmed milk-borne outbreaks have also been reported. The only isolate of *C. jejuni* from cattle which was examined in the present work by BRENDA was identical to that of an isolate from a human.

If some BRENDA types of *C. jejuni* and *C. coli* are harboured largely by one species of animal, as seems true for poultry isolates, then BRENDA typing of isolates from human outbreaks of disease will become an essential part of any epidemiological investigation.

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REFERENCES

Bamford, V. W. (1982). *Campylobacter*: experience at state health laboratories. Zoonosis Seminar, 3–4 December, Murdoch University, Western Australia.

Blaser, M. J. (1980). *Campylobacter fetus* subspecies *jejuni*: the need for surveillance. *Journal of Infectious Diseases* **141**, 670–671.

Blaser, M. J., Gravens, J. & Powers, B. W. (1979). *Campylobacter* enteritis associated with unpasturised milk. *American Journal of Medicine* **67**, 715–718.

Bradbury, W. C., Pearson, A. D., Marko, M. A., Congi, R. V. & Penner, J. L. (1984). Investigation of a *Campylobacter jejuni* outbreak by serotyping and chromosomal restriction endonuclease analysis. *Journal of Clinical Microbiology* **19**, 342–346.

Hudson, W. R. & Roberts, T. A. (1982). The occurrence of *Campylobacter* on commercial red-meat carcases from one abattoir. In *Campylobacter: Epidemiology, Pathogenesis, Biochemistry* (ed. D. G. Newell), p. 273, Lancaster: MTP Press.

Itoh, T., Saito, K., Yanagawa, Y., Sakai, S. & Ohashi, M. (1982). *Campylobacter* enteritis in Tokyo. In *Campylobacter: Epidemiology, Pathogenesis, Biochemistry* (ed. D. G. Newell), pp. 5–12, Lancaster: MTP Press.

Kakoyiannis, C. K., Winter, P. J. & Marshall, R. B. (1984). Identification of isolates of *Campylobacter coli* from animals and humans by bacterial restriction endonuclease DNA analysis (BRENDA). *Applied and Environmental Microbiology* **48**, 545–549.

Kaper, J. B., Bradford, H. B., Roberts, N. C. & Falkow, S. (1982). Molecular epidemiology of *Vibrio cholerae* in the U.S. Gulf Coast. *Journal of Clinical Microbiology* **16**, 129–134.

Karmali, M. A., Penner, J. L., Fleming, P. C., Williams, A. & Hennessy, J. N. (1983). The serotype and biotype distribution of clinical isolates of *Campylobacter jejuni* and *Campylobacter coli* over a three-year period. *Journal of Infectious Diseases* **147**, 243–246.

Kosumen, T. V., Pollanen, S., Hanninem, M. L. & Danielsson, D. (1982). Are the *Campylobacter jejuni* strains from different species similar? In *Campylobacter: Epidemiology, Pathogenesis, Biochemistry* (ed. D. G. Newell) pp. 45–49. Lancaster: MTP Press.

Lauwers, S. (1982). Serotyping of *C. jejuni:* a useful tool in the epidemiology of *Campylobacter* diarrhoea. In *Campylobacter: Epidemiology, Pathogenesis, Biochemistry* (ed. D. G. Newell), pp. 96–97. Lancaster: MTP Press.

Lior, H., Woodward, D. L., Edgar, J. A. & Laroche, L. G. (1981). Serotyping by slide agglutination of *Campylobacter jejuni* and epidemiology. *Lancet* ii, 1103–1104.

Lior, H., Woodward, D. L., Edgar, J. A., Laroche, L. J. & Gill, P. (1982). Serotyping of *Campylobacter jejuni* by slide agglutination based on heat labile antigenic factors. *Journal of Clinical Microbiology* **15**, 761–768.

Lior, H., Woodward, D. L., Laroche, L. G., Lacroix, R. & Edgar, J. A. (1983). Serotyping by slide agglutination and biotyping of *Campylobacter jejuni* and *Campylobacter coli*. *Proceedings of the Second International Workshop on Campylobacter infections*, p. 87, Brussels.

Marshall, R. B., Wilton, B. E. & Robinson, A. J. (1981). Identification of leptospira serovars by restriction endonuclease analysis. *Journal of Medical Microbiology* **14**, 163–166.

Marshall, R. B., Winter, P. J., Cooper, B. S. & Robinson, A. J. (1985). Subspecies differentiation of *Moraxella bovis* by restriction endonuclease analysis (BRENDA). *New Zealand Veterinary Journal* **33**, 67–70.

Munroe, D. L., Prescott, J. F. & Penner, J. L. (1983). *Campylobacter jejuni* and *Campylobacter coli* serotypes isolated from chickens, cattle and pigs. *Journal of Clinical Microbiology* **18**, 877–881.

Penner, J. L. & Hennessy, J. N. (1980). Passive haemagglutination technique for serotyping *Campylobacter fetus* subsp. *jejuni* on the basis of soluble heat-stable antigens. *Journal of Clinical Microbiology* **12**, 732–737.

Penner, J. L., Hennessy, J. N. & Congi, R. V. (1983). Serotyping of *Campylobacter jejuni* and *C. coli* on the basis of thermostable antigens. *European Journal of Clinical Microbiology* **2**, 378–383.

Penner, J. L., Hennessy, J. N., Mills, S. D. & Bradbury, W. C. (1983*b*). Application of serotyping and chromosomal restriction endonuclease digest analysis in investigating a laboratory acquired case of *Campylobacter jejuni* enteritis. *Journal of Clinical Microbiology* **18**, 1427–1428.

REGNENERY, R. L., TZIANABOS, T., ESPOSITO, J. J. & MCDADE, J. E. (1983). Strain identification of epidemic typhus rickettsia (*Rickettsia prowazeki*) by DNA restriction endonuclease analysis. *Current Microbiology* **8**, 355–358.

SHANKER, S., ROSENFIELD, J. A., DAVEY, G. R. & SORREL, T. C. (1982). *Campylobacter jejuni* incidence in processed broiler and biotype distribution in human and broiler isolates. *Applied and Environmental Microbiology* **43**, 1219–1220.

SKIRROW, M. B. (1982). *Campylobacter* enteritis – the first five years. *Journal of Hygiene* **89**, 175–184.

SKIRROW, M. B. & BENJAMIN, J. (1980). Differentiation of enteropathogenic *Campylobacter*. *Journal of Clinical Pathology* **33**, 1122.