

THE ISOLATION AND NATURE OF CAMPYLOBACTERS (MICROAEROPHILIC VIBRIOS) FROM LABORATORY AND WILD RODENTS

D. S. FERNIE* AND R. W. A. PARK

Department of Microbiology, University of Reading, Reading, RG1 5AQ

PLATE XVIII

THE vibrio-like microaerophilic bacteria of the genus *Campylobacter* (Sebald and Véron, 1963) cause infectious infertility in cattle, abortion in cattle and sheep, are associated with dysentery in pigs, and are recognised pathogens of man (Bokkenheuser, 1970). Campylobacters are commensals of the intestinal tract of animals, and have been isolated from faeces voided by healthy cattle (Florent, 1959), sheep (Smibert, 1965) and pigs (Deas, 1960). The occurrence of campylobacters in the intestinal tract of rodents has not been assessed, possibly because campylobacters require for their isolation gaseous conditions that are not normally employed in routine surveys of the intestinal flora. However, campylobacters have been isolated from rats killed on premises where there was an outbreak of dysentery in pigs (Pejtschev, 1969).

This present study of the occurrence and characterisation of campylobacters in rodents was undertaken for two reasons. First, the recognition that campylobacters cause disease in domestic animals and man means that laboratory rodents are used for pathogenicity studies with these organisms. For example, guinea-pigs and hamsters have been used as models for abortion caused by *Campylobacter fetus* (Ristic *et al.*, 1954; Hrabak, Baetz and Bryner, 1976). It is therefore necessary to know if campylobacters are normally present in the intestinal flora of such animals. Second, the epidemiology of diseases involving campylobacters has not been adequately determined and the role of rodents as a source of infection has not been assessed.

MATERIALS AND METHODS

Collection of samples. Freshly voided faecal pellets were collected on three occasions from laboratory rabbits, mice, hamsters, guinea-pigs and rats maintained at Reading University. Wild bank voles (*Clethrionomys glareolus*), field voles (*Microtus agrestis*) and field mice (*Apodemus sylvaticus*) were trapped in areas of woodland and grassland in Berkshire and Hampshire at intervals of 6 weeks over a period of 3 months (Ferne and Healing, 1976) and a faecal pellet was collected from each animal.

Isolation. A faecal pellet was suspended in 10 ml quarter-strength Ringer's solution in a 15-ml McCartney bottle, and the suspension filtered as described by Morris and Park (1971). Three drops of the filtrate were spread over each of two pre-dried plates of blood agar (Blood Agar Base No. 2 (Oxoid) supplemented with 10% (v/v) defibrinated sheep blood) and the

Received 22 Dec. 1976; accepted 14 Jan. 1977.

* Present address: The Wellcome Research Laboratories, Beckenham, Kent.

plates incubated at 37°C in an atmosphere of 30% (v/v) CO₂ in air. After incubation for 4 days plates were scrutinised and colonies of Gram-negative vibrio-like organisms subcultured. If colonies of presumptive campylobacters were not detected the plates were examined after incubation for a further 10 days. Gram-negative cocci present on isolation plates were regarded as campylobacters as rod to sphere morphogenesis is common in old cultures of *Campylobacter* spp.

Characterisation of isolates. Tests for catalase production, gelatin liquefaction, nitrate reduction, glucose fermentation, H₂S production and glycine tolerance were performed as by Morris and Park (1973). Ability to grow in the presence of glycine was also assessed on blood agar with glycine (1% w/v) incubated in 30% (v/v) CO₂ in air. The production of oxidase by campylobacters grown for 3 days on Albimi Brucella Broth (ABB: Pfizer Ltd, Sandwich, Kent) with added Agar No. 3 (Oxoid, 0.1% w/v) was tested by standard procedures (Cruickshank *et al.*, 1975). Tests for tolerance towards and reduction of sodium selenite were performed as described by Bryans and Smith (1960). Ability to grow at various temperatures was assessed in semisolid ABB incubated at either 25°, 37° or 42°C; all other tests were incubated at 37°C. Examination was after 6 days.

Electrophoresis of acid plus phenol soluble (APS) protein. For electrophoretic studies campylobacters were grown on blood agar in Petri dishes. Each plate was inoculated by spreading 0.1 ml of an exponential phase culture in ABB over the surface and incubated in an atmosphere of 30% (v/v) CO₂ in air. After incubation for 3 days at 37°C growth was washed off the surface of 70 plates with 350 ml quarter-strength Ringer's solution and the bacteria were harvested by centrifugation at 18 000 *g* for 15 min. at 4°C and washed twice in quarter-strength Ringer's solution. Subsequent procedures were as described by Morris and Park (1973) except that (i) the suspensions of disrupted bacteria were adjusted to give 30 mg protein per ml Ringer's solution before digestion with phenol+acetic acid+water (4 : 2 : 1 parts by weight), and (ii) proteins were stained by immersion of the gels in Coomassie Blue (0.25%) in methanol+acetic acid+water (5 : 1 : 5 parts by volume) for 3 h at 22°C; unbound stain was removed by immersing the gels in methanol+acetic acid+water at 37°C for 1 h, followed by washing in 7% (v/v) aqueous acetic acid. Three sets of samples were examined for each isolate, and a reference extract was included in each run.

Bacteria. The following organisms were compared with isolates from rodents in electrophoretic studies: *Campylobacter coli* (Véron and Chatelain, 1973) strains V6, V8 and A2 isolated from the faeces of pigs with swine dysentery; *Campylobacter fetus* subsp. *fetus* (Véron and Chatelain, 1973) strain W2 isolated from an aborted sheep foetus.

RESULTS

Isolation of campylobacters from rodents

Attempts were made to isolate campylobacters from a sample of faeces from each of 121 rodents (table). Colonies of Gram-negative organisms morphologically resembling campylobacters were detected after 4 days on plates inoculated with faecal material from three of 16 female rats, two of five female bank voles and all of eight male bank voles. All isolation plates were incubated for 14 days and all colonies examined by staining and microscopy but campylobacters were not detected from any of the other samples.

Characterisation of isolates

All of 13 strains isolated from bank voles and rats were motile slender curved rods; coccid forms predominated in cultures incubated for more than 5 days. On blood agar incubated for 3 days at 37°C colonies were non-haemolytic, irregular and tended to spread. Each colony had an entire edge, was light grey and 2–4 mm in diameter. Isolates were oxidase and catalase

CAMPYLOBACTERS FROM RODENTS

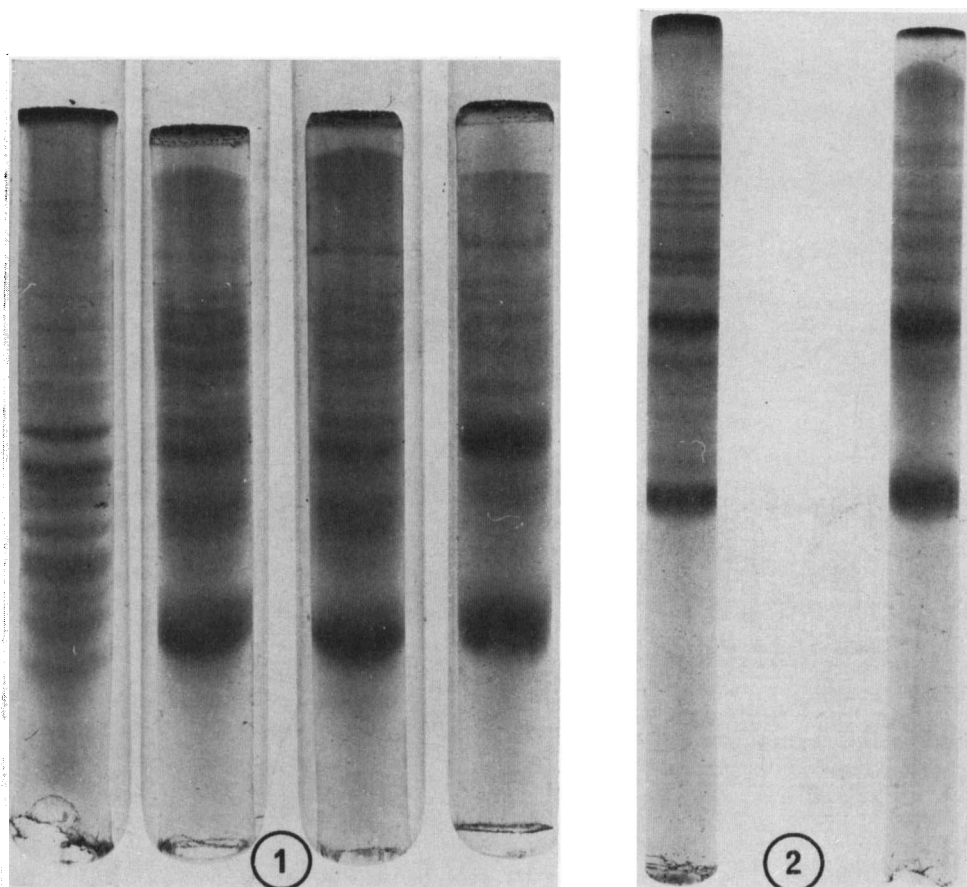


FIG. 1.—Electrophoretograms of acid plus phenol soluble (APS) proteins of campylobacters from (left to right) an aborted sheep foetus (*Campylobacter fetus* strain W2), a bank vole (strain Cg6), a bank vole (strain Cg12) and a pig with dysentery (*Campylobacter coli* strain V6).

FIG. 2.—Electrophoretograms of APS proteins of (left to right) campylobacters from a rat and of *C. coli* strain V6 from a pig with dysentery.

TABLE
Isolation of campylobacters from faeces of rodents

Species	Campylobacters isolated from	
	males	females
Rabbit (laboratory, Californian)	0/8	0/7
Mouse (laboratory, LACA)	0/11	0/10
Guinea-pig (laboratory, Hartley)	0/4	0/0
Rat (laboratory, Wistar)	0/13	3/16
Hamster (laboratory, Syrian)	0/5	0/5
Bank vole (wild)	8/8	2/5
Field vole (wild)	0/11	0/6
Field mouse (wild)	0/9	0/3

* Numerator = number of animals from which campylobacters were isolated; denominator = number of animals examined.

positive, reduced nitrates to nitrites, and did not grow on blood agar incubated either in air or anaerobically. There was no liquefaction of gelatin, and acid was not produced in media containing glucose. By these criteria our isolates resembled the catalase positive members of *Campylobacter* from cattle, pigs, sheep and man (Véron and Chatelain, 1973).

All strains produced H₂S when grown in ABB supplemented with 0.02% cystine, but only the strains isolated from rats produced H₂S in ABB alone. Isolates from bank voles were inhibited by 0.1% sodium selenite in brucella agar, whereas strains from rats were tolerant of and reduced sodium selenite. All strains grew abundantly at 37° and 42°C, but not at 25°C or in or on media containing 1% glycine. Isolates from bank voles resembled *C. fetus* subsp. *venerealis* biotype *intermedius* (Véron and Chatelain, 1973), a cause of infectious infertility in cattle (Berg, Jutila and Firehammer, 1971) in their reactions but differed by growing at 42° but not at 25°C (Firehammer and Berg, 1965). Except for their inability to grow in media containing 1% glycine, strains from rats resembled *C. coli* (Morris and Park, 1973), an organism that may be involved with a spirochaete in the aetiology of swine dysentery (Ferne, Griffin and Park, 1975).

Electrophoretograms of APS protein

The similarity of campylobacters from rodents to species of *Campylobacter* involved in diseases of farm animals, and the somewhat trivial differential tests available for *Campylobacter*, prompted us to use gel electrophoresis of APS protein as an aid to differentiation; Morris and Park (1973) found that results with this technique showed good correlation with habitat and pathogenicity of campylobacters from cattle, sheep and pigs. All isolates from bank voles consistently gave the same APS protein pattern, which was different from the pattern consistently given by strains of *C. coli* and that given by *C. fetus* (fig. 1) All isolates from rats exhibited an APS protein pattern similar to, though not identical with that given by strains of *C. coli* (fig. 2).

DISCUSSION

The present study demonstrates that campylobacters are more widespread in rodents than hitherto recognised. Campylobacters were isolated from the majority of apparently healthy wild bank voles examined, which suggests that campylobacters are normal commensals in the gut of this species. In addition, we were able to isolate campylobacters from three of 29 rats that had been bred and reared for use in the laboratory; this confirms the report of Pejtschev (1969) that rats can be a reservoir of campylobacters. Thus some human campylobacter infections of uncertain origin (Darrell, Farrell and Mulligan, 1967; Bokkenheuser, 1970) may have originated from contact with rodents rather than with domestic animals.

This study emphasises that the routine tests for distinguishing between campylobacters are inadequate and confirms the findings of previous workers that there is often little or no correlation between reactions of campylobacters in these tests and association with disease (Park *et al.*, 1962; Berg *et al.*, 1971; Morris and Park, 1973). Our isolates from rats resembled *C. coli*, the organism associated with swine dysentery. Pejtschev (1969) reported similar findings and suggested that rats might be a reservoir for campylobacter infection of pigs. However, electrophoretograms of APS proteins revealed distinct differences between our rat isolates and *C. coli*. Although we did no pathogenicity studies these results suggest that the rat isolates are not *C. coli*. Similarly, our isolates from bank voles were, apart from growth temperatures, identical to a type of *C. fetus* that causes infertility in cattle. The striking differences between electrophoretograms of APS proteins from vole isolates and from *C. fetus* indicate that the similarity in routine biochemical tests is superficial. Morris and Park (1973) reported that all of their strains of *C. fetus* from cattle and sheep, associated with either infertility or abortion, exhibited similar APS protein electrophoretograms. Thus we consider that vole isolates are unlikely to be a source of campylobacter infections in cattle or sheep.

It is apparent that *Campylobacter* cannot be subdivided on the basis of routine biochemical tests in a way that provides reliable diagnostic or epidemiological information. As new strains are found it will be necessary to use more detailed methods for differentiation. Electrophoresis of APS proteins may be useful for this purpose.

SUMMARY

Faeces voided by eight species of laboratory or feral rodents were cultured for campylobacters by means of selective methods. Campylobacters were isolated from bank voles and from rats, but not from rabbits, laboratory mice, hamsters, guinea-pigs, field mice or field voles. In routine biochemical tests isolates from bank voles resembled a type of *Campylobacter fetus* that causes infectious infertility in cattle; isolates from rats resembled *Campylobacter coli* associated with swine dysentery. Electrophoretograms of acid plus phenol soluble proteins revealed striking differences between isolates from rodents, *C. fetus* and *C. coli*. It is concluded that campylobacters are more widespread in rodents than hitherto realised, and that routine methods for differentiating

campylobacters do not allow an adequate correlation with pathogenicity or habitat.

We are grateful to Mr T. D. Healing for helpful discussion and for providing clinical material from feral rodents. We thank Mr J. Gaitens and Mr B. Sandells for providing laboratory-bred rodents, and the Agricultural Research Council for financial support.

REFERENCES

- BERG, R. L., JUTILA, J. W. AND FIREHAMMER, B. D. 1971. A revised classification of *Vibrio fetus*. *Am. J. vet. Res.*, **32**, 11.
- BOKKENHEUSER, V. 1970. *Vibrio fetus* infection in man. I. Ten new cases and some epidemiologic observations. *Am. J. Epidem.*, **91**, 400.
- BRYANS, J. T. AND SMITH, A. G. 1960. Physiological properties of pathogenic and non pathogenic *Vibrio* species isolated from cattle, sheep and chickens. *Cornell Vet.*, **50**, 331.
- CRUICKSHANK, R., DUGUID, J. P., MARMION, B. P. AND SWAIN, R. H. A. 1975. Medical microbiology, 12th ed., *London*, vol. 2, p. 180.
- DARRELL, J. H., FARRELL, B. C. AND MULLIGAN, R. A. 1967. Case of human vibriosis. *Br. med. J.*, **2**, 287.
- DEAS, D. W. 1960. Observations on swine dysentery and associated vibrios. *Vet. Rec.*, **72**, 65.
- FERNIE, D. S., GRIFFIN, R. M. AND PARK, R. W. A. 1975. The possibility that *Campylobacter* (*Vibrio*) *coli* and *Treponema hyodysenteriae* are both involved in swine dysentery. *Br. vet. J.*, **131**, 335.
- FERNIE, D. S. AND HEALING, T. D. 1976. Wild bank voles (*Clethrionomys glareolus*) are possibly a natural reservoir of campylobacters (microaerophilic vibrios). *Nature, Lond.*, **263**, 496.
- FIREHAMMER, B. D. AND BERG, R. L. 1965. The use of temperature tolerance in the identification of *Vibrio fetus*. *Am. J. vet. Res.*, **26**, 995.
- FLORENT, A. 1959. Les deux Vibrioses génitales: la Vibriose due a *V. fetus venerialis* et la Vibriose, d'origine intestinale due a *V. fetus intestinalis*. *Meded. VeeartsSch. Rijksuniv. Gent.*, **3**, No. 3.
- HRABAK, R., BAETZ, A. L. AND BRYNER, J. H. 1976. Cystine aminopeptidase activity (oxytocinase) in pregnant guinea pigs: normal and infected with *Campylobacter fetus*. *Am. J. vet. Res.*, **37**, 343.
- MORRIS, J. A. AND PARK, R. W. A. 1971. The isolation of microaerophilic vibrios. In *Isolation of anaerobes*, edited by D. A. Shapton and R. G. Board, *London*, p. 207.
- MORRIS, J. A. AND PARK, R. W. A. 1973. A comparison using gel electrophoresis of cell proteins of campylobacters (vibrios) associated with infertility, abortion and swine dysentery. *J. gen. Microbiol.*, **78**, 165.
- PARK, R. W. A., MUNRO, I. B., MELROSE, D. R. AND STEWART, D. L. 1962. Observations on the ability of two biochemical types of *Vibrio fetus* to proliferate in the genital tract of cattle and their importance with respect to infertility. *Br. vet. J.*, **118**, 411.
- PEJTSHEV, P. 1969. Ein fall von *Vibrio fetus* bei *Rattus norvegicus*. *Zentbl. VetMed. B*, **16**, 480.
- RISTIC, M., MORSE, E. V., WIPF, L. AND MCNUTT, S. H. 1954. Transmission of experimental *Vibrio fetus* infection among guinea pigs and hamsters. *Am. J. vet. Res.*, **15**, 309.
- SEBALD, M. AND VÉRON, M. 1963. Teneur en bases de l'ADN et classification des vibrios. *Annls Inst. Pasteur, Paris*, **105**, 897.
- SMIBERT, R. M. 1965. *Vibrio fetus* var. *intestinalis* isolated from the faecal and intestinal contents of clinically normal sheep: isolation of microaerophilic vibrios. *Am. J. vet. Res.*, **26**, 315.
- VÉRON, M. AND CHATELAIN, R. 1973. Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. *Int. J. syst. Bact.*, **23**, 122.