A Comparison Using Gel Electrophoresis of Cell Proteins of Campylobacters (Vibrios) Associated with Infertility, Abortion and Swine Dysentery

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SUMMARY

Ninety-six campylobacters were isolated from cattle, sheep, pigs and fowls, of which 40 were studied in detail. Characterization by routine biochemical tests indicated that all isolates were similar and that differences did not correspond with habitat or pathogenicity. The electrophoresis patterns of acid plus phenol-soluble proteins from the 40 campylobacters allowed the isolates to be placed in three groups which correlated with habitat. Group I comprised all the campylobacters from the bovine genital tract (these isolates were associated with infertility). Group II comprised all the strains isolated from cattle faeces and all the strains associated with sporadic abortions in cattle and sheep. Group III comprised all the campylobacters from healthy pigs and from pigs with swine dysentery. Isolates could not be differentiated by electrophoresis of total soluble proteins. Isocitrate dehydrogenase and peroxidase electrophoresis patterns each allowed the pig campylobacters to be differentiated from cattle and sheep campylobacters, but did not allow finer differentiation. Malate dehydrogenase patterns, which were produced only by the pig campylobacters, provided three groups but these did not correlate with isolation source. It is concluded that there is no qualitative difference in the campylobacter population of the gut of healthy and dysenteric pigs.

INTRODUCTION

Campylobacters (microaerophilic vibrios; Sebald & Véron, 1963) cause infectious infertility and abortion in cattle and abortion in sheep. The organisms are associated with swine dysentery, though their role in the disease is not clear, and have been isolated from sources such as the vagina of women at fertility clinics (Moore, 1954), goats (Lawson, 1959), the domestic fowl (e.g. Whenham, Carlson & Askel, 1961), wild birds (Watson, Hunter & Bellhouse, 1967) and rats (Pejetschev, 1969). In most differential tests employed for bacteria campylobacters appear to be relatively inert; they do not attack the usual range of carbohydrates, hydrolyse gelatin, starch, urea or lipid, haemolyse blood nor produce indole, but nitrite is produced from nitrate.

Florent (1953, 1957, 1959) subdivided campylobacters from cattle, sheep and pigs on the basis of catalase production, growth in the presence of 1.0% glycine, production of H₂S from a medium containing cystine and their isolation site and pathogenicity (see Table 1). Park, Munro, Melrose & Stewart (1962) studied campylobacters, which corresponded biochemically to Florent's *Campylobacter fetus* var. *intestinalis*, from the genital tract of heifers with histories of infertility and showed that they could proliferate in the genital tract

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Table 1. *Characteristics proposed by Florent to differentiate Campylobacter fetus varieties*

Characteristic	<i>C. fetus</i> var. <i>intestinalis</i>	<i>C. fetus</i> var. <i>venerealis</i>
H ₂ S production from medium containing 0.02 % cystine	+	—
Growth in presence of 1.0 % glycine	+	—
Causes infectious infertility in cattle	—	+
Causes sporadic abortion in cattle and abortion storms in sheep, but not infertility in cattle	+	—
Ability to proliferate in genital tract of non-pregnant heifer	—	+
Normal habitat intestinal tract of cattle and other animals	+	—

of non-pregnant heifers. These findings, later confirmed by Florent (1963), showed that because some strains behaved *in vivo* like Florent's *C. fetus* var. *venerealis*, but gave the biochemical reactions of *C. fetus* var. *intestinalis*, separation on the basis of the biochemical tests shown in Table 1 was not always satisfactory. Morris (1971) found that all the campylobacter isolates obtained from both healthy pigs and pigs suffering from swine dysentery biochemically resembled *C. fetus* var. *intestinalis*, differing only in producing more H₂S. But Söderlind (1965) found that many isolates from pigs showed no serological cross-reaction with *C. fetus*.

The biochemical tests currently used to classify campylobacters are not wholly satisfactory and so we have looked for other means of differentiation. This communication describes electrophoretic studies of various protein components of campylobacter, undertaken in the hope of detecting differences between strains, and discusses the results in the light of differences in habitat and association with disease. *Campylobacter bubulus* was not examined because it is not usually associated with disease and because lack of catalase differentiates it from other campylobacters (Thouvenot & Florent, 1954). A preliminary report of this work has been published (Morris & Park, 1970).

METHODS

Strains

Campylobacters, whose histories are given in Table 3, were isolated as described earlier (Morris & Park, 1971). *Escherichia coli* was strain 16A from our departmental collection and the aerobic vibrio was a member of Gardner & Venkatraman 'O' subgroup 6 (NCTC 4716).

Characterization of isolates

With the exception of nutrient gelatin, which was prepared according to Cruickshank (1965), each test medium was prepared by adding the appropriate solid test compound (Table 3) to freshly reconstituted Albimi brucella broth (ABB: Micro-Bio Labs, 46 Pembridge Road, London, W. 11) made semisolid by the addition of Agar no. 3 (Oxoid; Southwark Bridge Road, London, S.E. 1) (0.1 %, w/v). Hydrogen sulphide and glycine tolerance tests were described by Park *et al.* (1962). Catalase production was demonstrated by effervescence on the addition of 0.5 ml of 10 vol. H₂O₂ to growth in semisolid ABB. Gelatin liquefaction and reduction of nitrate (in semisolid ABB + KNO₃, 0.1 %, w/v) were tested by standard procedures (Cruickshank, 1965). Glucose fermentation was tested by measuring the pH value of cultures in semisolid ABB + D(+)-glucose (0.1 %, w/v) using narrow-range pH

paper. All incubations were at 37 °C and, with the exception of the H₂S tests, which were examined daily (Park *et al.* 1962), the cultures were examined after incubation for 7 days.

Electrophoretic studies

Bulk growth and harvesting of bacteria. Campylobacters were grown in 250 ml conical flasks containing 100 ml ABB. Each flask was inoculated with 10 ml of an exponential phase culture in semisolid ABB, filled with an atmosphere of 30 % (v/v) CO₂ in air, and the plug tightly covered with aluminium foil to reduce gaseous exchange. The aerobic vibrio and *Escherichia coli* were grown aerobically in nutrient broth. Incubation was at 37 °C on a Gallenkamp orbital shaker operating at 150 rev./min. Bacteria from 1500 ml of late exponential-phase culture were harvested by centrifugation at 18000 g for 10 min at 4 °C and washed twice in quarter strength Ringer's solution.

Preparation of protein fractions. Two protein fractions were studied: (a) total soluble proteins, and (b) acid + phenol soluble proteins, both usually being prepared from the same batch of cells. Packed cells, resuspended to 250 mg wet wt/ml in phosphate-buffered saline (100 mM, pH 7.0), were broken by ultrasonic treatment in 40 ml amounts in a Rosett (1965) cell surrounded by a slurry of melting ice. The 100 W ultrasonic disintegrator (MSE, Manor Royal, Crawley, Sussex) was fitted with a 0.75 in. titanium probe and operated at 20 kHz giving a wavelength of about 6.2 µm peak to peak. Treatment was for 4 × 1 min, the probe being switched off during every alternate minute to aid cooling. After centrifugation at 40000 g for 30 min at 4 °C the supernatant fluid, hereafter called the 'total soluble protein sample', was decanted and the protein content determined using Kennedy & Fewson's (1968) modification of the Lowry method. The pellet was washed twice in Ringer's solution and resuspended to about 2 to 3 ml. The amount of protein was determined and the suspension adjusted to give 10 mg protein/ml Ringer's solution. To 1 vol. of this suspension was added 2 vol. phenol + acetic acid + water (4:2:1 parts by weight) and the mixture left at room temperature overnight. The digest was centrifuged in an identical manner to the sonicated suspension and the resulting supernatant fluid, hereafter called the 'acid + phenol soluble (APS) protein sample', was retained. The pellet was discarded. Both the total soluble protein samples and the APS protein samples were frozen at -20 °C if they were not required immediately.

Preparation of gels. Gels to separate total soluble proteins were prepared by a modification of the method of Davis (1964). One vol. solution A (tris, 36.3 g; 1 M-HCl, 48.0 ml; *N,N,N',N'*-tetramethylethylene-diamine, 1.0 ml; H₂O to 100 ml) was mixed with 2 vol. solution B (acrylamide, 30.0 g; *N,N'*-methylenebisacrylamide, 0.8 g; potassium ferricyanide, 0.015 g; H₂O to 100 ml), and 4 vol. solution C (ammonium persulphate, 0.3 g; H₂O to 100 ml) and 1 vol. H₂O, and was left for 1 h at room temperature to polymerize. Gels to separate APS proteins were prepared by a modification of the method of Razin & Rottem (1967). Twelve millilitres of solution D (urea, 30.0 g; acrylamide, 7.5 g; *N,N'*-methylenebisacrylamide, 0.2 g; 35 % (v/v) aqueous acetic acid to 100 ml) was added to 10 mg ammonium persulphate in a Buchner flask, which was then evacuated to 160 Torr and shaken occasionally during 5 min. The vacuum was released and 0.04 ml of *N,N,N',N'*-tetramethylethylene-diamine was added and distributed by gently rotating the flask. Polymerization was at 37 °C for 90 min. Both types of gel were cast by pipetting into glass running tubes (75 mm × 5 mm) to a height of 62 mm and layering a few drops of water (for soluble protein) or 75 % (v/v) aqueous acetic acid (for APS protein) on the surface of the gels. Tubes were positioned vertically in an Analytical Polyacrylamide Electrophoresis Apparatus (Shandon, Pound

Table 2. *Mixtures for the detection of enzymes*

Enzyme to be detected	Reagents	Quantity (ml)
Malate dehydrogenase	Clark-Lubs buffer (150 mM), pH 10.0	5.0
	MTT tetrazolium* (2.4 mM)	2.0
	N-methylphenazonium methosulphate, PMS (1 mM)	1.0
	NAD (14 mM)	1.0
	Sodium malate (1.5 M)	1.0
Isocitrate dehydrogenase	Tris-HCl buffer (100 mM), pH 8.5	4.2
	MTT tetrazolium (2.4 mM)	2.0
	PMS (1 mM)	1.0
	NADP (13 mM)	0.8
	Magnesium chloride (100 mM)	1.0
	Sodium isocitrate (13.5 mM)	1.0
Peroxidase	Tris-HCl buffer (100 mM), pH 7.1	6.0
	Pyrogallol (20 mM)	3.0
	Hydrogen peroxide (0.74 mM)	1.0

* 3-(4,5-dimethyl thiazolyl-2)-2,5 diphenyl tetrazolium bromide.

Lane, London, N.W. 10) and the reservoir fluid added (for soluble protein: glycine, 28.8 g; tris, 6.0 g; H₂O to 1 l; for APS protein: 10 % (v/v) aqueous acetic acid).

Application of sample. Samples containing 250 to 300 µg protein were applied through the reservoir fluid using 1.0 ml disposable syringes fitted in a home-made calibrated screw device. Soluble protein samples contained (per 0.1 ml) 0.02 ml aqueous bromophenol blue (0.1 %, w/v) and 0.18 ml sucrose-Sephadex (Broome, 1963); APS protein samples were applied without these additions. Following Clarke (1964) no spacer gel was used.

Electrophoretic separation of sample. For soluble protein fractions the upper electrode was made the cathode and a constant current equivalent to 5 mA/gel supplied from a Vokam type 2541 Power Pack (Shandon). Electrophoresis was usually terminated when the bromophenol blue, assumed to move with the chloride-glycinate interface, had migrated about 45 to 55 mm into the gel. For APS protein fractions the upper electrode was made the anode and a constant current equivalent to 3 mA/gel was delivered for 30 min, followed by 5 mA/gel for 40 min. To allow comparison of proteins run on different occasions one of the gels in each run was always used for protein from a reference strain (H22).

Staining of APS proteins and soluble proteins was by 1.0 % Amido Black prepared in 7.0 % (v/v) aqueous acetic acid, unbound stain being removed after 60 min by washing in 3.0 % (v/v) aqueous acetic acid contained in a bath fitted with a stirring device.

Detection of enzymes in soluble protein preparations. At the completion of electrophoresis unstained gels containing soluble proteins were expelled from the running tubes, quickly dried with filter paper and put into specimen tubes. Equal volumes of the enzyme reaction mixture and 2.0 % (w/v) aqueous molten Agar no. 1 (Oxoid) were mixed at 50 °C and poured into the specimen tubes to cover the gel (Table 2). Gels were incubated at 37 °C in the dark until the colour developed, and were photographed within 10 min.

RESULTS

Isolation and biochemical characterization

Ninety-six campylobacters were isolated from cattle, sheep, pigs and fowls, and 40 of these isolates were kept for detailed study. All produced catalase and reduced nitrate to nitrite, but did not produce acid from glucose, grow in nutrient gelatin or haemolyse blood. Other biochemical characteristics and the history of each isolate are given in Table 3. Three

Table 3. *Source, history and biochemical reactions of campylobacters*

No. of isolates	Isolation material	Animal	Geographical location	Disease associated with isolate	H ₂ S production from ABB*	H ₂ S production from ABB+ 0.02 % cystine	Growth in presence of 1 % glycine	Growth in presence of 1.5 % glycine	Biochemical type
4	Vaginal mucus	3 heifers	Reading	Infertility	—	—	—	—	Type A
2	Vaginal mucus	2 heifers	Reading	Infertility	—	+	+	—	Type B
1	Prepuce	1 bull	Langford	Infertility	—	+	+	—	
1	Aborted foetus	1 cow	Weybridge	Sporadic abortion	—	+	+	—	
2	Faeces	2 heifers	Reading	None	—	+	+	+	
5	Foetus	5 sheep	Weybridge	Abortion storm	—	+	+	+	Type C
4	Bile	4 chickens	Reading	Vibronic hepatitis	—	+	+	—	
24	Rectal swabs	10 pigs	Reading	None	+	++	+	+	
8	Rectal swabs	6 pigs	Reading	None	+	++	+	—	
3	Rectal swabs	2 pigs	Reading	Swine dysentery	+	++	+	+	Type C
14	Faeces	8 pigs	Cambridge, Langford, Newbury	Swine dysentery	+	++	+	+	
7	Colon	5 pigs	Langford, Reading	Swine dysentery	+	++	+	+	
3	Colon	2 pigs	Langford, Reading	Swine dysentery	+	++	+	—	
2	Caecum	2 pigs	Langford, Reading	Swine dysentery	+	++	+	+	Type C
6	Caecum	4 pigs	Langford, Reading	Swine dysentery	+	++	+	—	
5	Ileum	3 pigs	Langford	Swine dysentery	+	++	+	—	
5	Duodenum	3 pigs	Langford	Swine dysentery	+	++	+	—	

* ABB, Brucella Broth (Albimi) + 0.1 % (w/v) Agar no. 3 (Oxid).

Table 4. *Correlation between isolation sites, biochemical characteristics and electrophoresis patterns of cell proteins of campylobacters*

Isolate		Biochemical reactions	MDH pattern	Isolation site	APS pattern
Cattle	1847	Type A	ND	{ Vaginal mucus Vaginal mucus Vaginal mucus }	Group I
	1854				
	271A				
	1848	Type B	ND	{ Vaginal mucus Vaginal mucus Prepuce }	
	1855				
	32-2				
Sheep	1873	Type B		{ Faeces Faeces }	Group II
	1874				
	w11			{ Aborted foetus Aborted foetus Aborted foetus Aborted foetus }	
	102				
	s2				
	661				
Pig	H6		Group A	{ Rectal swab (H) Rectal swab (H) Caecum (SD) Ileum (SD) }	Group III
	H21				
	10				
	C3/1				
	H1	Type C	Group B	{ Rectal swab (H) Rectal swab (H) Rectal swab (H) Rectal swab (H) Rectal swab (H) Rectal swab (H) Rectal swab (H) Rectal swab (H) Rectal swab (H) Faeces (SD) Faeces (SD) Rectal swab (SD) Rectal swab (SD) Rectal swab (SD) Caecum (SD) Colon (SD) }	
	H4				
	H5				
	H7				
	H10				
	H11				
	H22				
	H23				
	N1				
	N2				
	1				
	2				
	3				
	16				
	A5/1				
	H2	Group C		{ Rectal swab (H) Rectal swab (H) Colon (SD) Caecum (SD) Duodenum (SD) Caecum (SD) Colon (SD) Colon (SD) Rectal swab (SD) }	
	H3				
	6				
	9				
	A2/1				
	B4/B				
	B5/B				
	C5/1				
	B6/1				

ND, not detectable; H, healthy animal; SD, swine dysentery; MDH, malate dehydrogenase; APS, acid + phenol soluble proteins.

biochemical types were found. Type A did not produce H_2S from semisolid ABB or semisolid ABB + cystine within 7 days, nor grow in the presence of 1.0 % or 1.5 % glycine. This type biochemically resembled *Campylobacter fetus* var. *venerealis* (Florent, 1959). Type B produced H_2S from semisolid ABB + cystine within 3 days, but not from semisolid ABB even after 7 days, and grew in the presence of 1.0 % glycine. The three Type B isolates from the bovine genital tract were unable to tolerate 1.5 % glycine, but all the other ovine and

Fig. 1. Electrophoresis of total soluble protein from (a) *Campylobacter* H22 from a rectal swab of a healthy pig, (b) *Campylobacter* B4/B from the caecum of a pig with swine dysentery, and (c) *Escherichia coli*.

bovine Type B campylobacters did tolerate this. Type B isolates biochemically resembled *C. fetus* var. *intestinalis* (Florent, 1959). Type C produced copious amounts of H_2S from semisolid ABB+cystine, and some H_2S from ABB without added cystine, within 3 days, and grew in the presence of 1.0 % glycine. The majority of Type C isolates grew in the presence of 1.5 % glycine.

Type A strains were from bovine genitalia; Type B strains were from bovine genitalia, bovine and ovine aborted fetuses, bovine faeces and avian bile; and Type C strains were from the porcine gut. The name *Vibrio coli* was first applied by Doyle (1948) to campylobacters from the porcine gut but he stated that *V. coli* did not reduce nitrate. The species has not been formally transferred to the new genus *Campylobacter* and so its status is unclear.

Electrophoresis

Rigid standardization of procedure and protein concentration, and inclusion of a reference bacterial extract in each run, were essential for interpretation of electrophoresis patterns. At least three sets of samples were examined for each isolate. Photographs were the best permanent record, although not all the bands were visible on the prints; electronic plots of optical density of the gels were unsatisfactory.

Total soluble proteins. Strains gave identical patterns of total soluble proteins; even a strain of *Escherichia coli* gave a pattern which had few detectable differences from that of the campylobacters (Fig. 1).

APS proteins. Using electrophoresis of APS protein campylobacters could be placed into three groups (Table 4). Group I comprised all the cattle genital campylobacters, regardless of biochemical characteristics. Group II comprised all the sheep campylobacters and all

Fig. 2. Electrophoresis of APS protein of a Group I campylobacter from vaginal mucus of a heifer (*Campylobacter* 1848) and a Group II strain from faeces of a heifer (*Campylobacter* 1874).

Fig. 3. Electrophoresis of APS protein of a Group II campylobacter from an aborted sheep foetus (*Campylobacter* w11) and a Group III strain from a rectal swab of a healthy pig (*Campylobacter* H22).

Fig. 4. Electrophoresis of APS protein from Group III campylobacters and *Vibrio*. (a) From a rectal swab of a healthy pig (*Campylobacter* H22), (b) from the caecum of a pig with swine dysentery (*Campylobacter* B4/B), (c) aerobic vibrio (NCTC 4716).

the cattle faecal strains. Group I and Group II were very similar. Group III comprised all the pig campylobacters, regardless of glycine tolerance and anatomical site of isolation. Group III patterns were very different from Group I and Group II patterns. Fig. 2, 3 and 4 show the APS protein patterns of the three Groups. Fig. 4 also shows the APS protein pattern of an aerobic vibrio grown in nutrient broth.

Enzymes. Electrophoresis patterns of campylobacters from pigs differed from those from cattle and sheep in gels stained for malate dehydrogenase, isocitrate dehydrogenase and peroxidase. Faecal and genital campylobacters or cattle and sheep campylobacters could not be differentiated by patterns of enzyme activity.

All strains produced two isozymes of isocitrate dehydrogenase (ISD). The mobilities of pig campylobacter ISD were sufficiently greater than those of cattle and sheep strains (Fig. 5) to allow differentiation. One band of peroxidase activity was usually produced by pig campylobacters and two bands, both with greater mobility, by cattle and sheep strains (Fig. 6). Some preparations (W11, 1855, H3, H6, H22, 6) after storage or repeated freezing and thawing gave additional bands of activity (Fig. 7). Three malate dehydrogenase (MDH) patterns (referred to as MDH Group A, MDH Group B and MDH Group C) were found in pig campylobacters. All contained one major band, which was in an identical position in all groups, but the number and position of minor bands differed (Fig. 8). As with peroxidase preparations, additional bands of activity were observed in old MDH preparations. There was no correlation between MDH groups and anatomical or geographical isolation sources or the state of health of the pig (Table 4). Protein samples from cattle and sheep campylobacters, prepared in an identical manner to the porcine campylobacter extracts, had too

Fig. 5. Isocitrate dehydrogenase electrophoresis patterns of campylobacters, (a) from a pig (*Campylobacter* H22), (b) from a heifer (*Campylobacter* 1848).

Fig. 6. Peroxidase electrophoresis patterns of campylobacters, (a) from a pig (*Campylobacter* H22), (b) from a heifer (*Campylobacter* 1848).

Fig. 7. Effect of freezing and thawing on peroxidase patterns of campylobacters, (a) from a pig (*Campylobacter* H22), (b) from a pig (*Campylobacter* 6), (c) from a heifer (*Campylobacter* 1855).

Fig. 8. Malate dehydrogenase electrophoresis patterns of pig campylobacters of Group A, Group B and Group C. Group A, *Campylobacter* 10. Group B, *Campylobacter* H22. Group C, *Campylobacter* 6.

little malate dehydrogenase for detection on the gel. This was not a reflexion of the situation in the whole cell since respiration experiments with an oxygen electrode indicated that all the campylobacters studied oxidized malate at comparable rates (Morris, 1971).

DISCUSSION

There is no clear classification of the catalase-positive, microaerophilic vibrios despite many studies, including those of Bond (1957) using colony morphology; Mohanty, Plumer & Faber (1962) using tolerance to NaCl, glucose and formate; King (1962) using optimal growth temperatures; Plastringe, Williams & Trowbridge (1964) using antibiotic sensitivity; and Firehammer & Border (1968) using phage typing. In the biochemical tests now most commonly used for these organisms we found no correlation between the reactions and association with disease.

Norris (1964), by studying the isozymal forms of esterase on starch gels, clearly demonstrated that the distribution of different forms of esterase among bacteria could be of value in taxonomic studies. Electrophoresis of enzymes and total proteins has now been used in both the identification and classification of bacteria. Lund (1965) demonstrated differences at the species level by examining by gel electrophoresis water-soluble proteins of some group D streptococci. Razin & Rottem (1967) examined phenol-acetic-acid extracts of *Mycoplasma* membranes and reported that species-specific patterns were produced in polyacrylamide gels. Haas, Davidson & Sacks (1972) found that a similar technique was of little value in differentiating *Mycobacterium*; but Sacks, Haas & Razin (1969) reported that gel electrophoresis of phenol-acetic-acid extracts of Enterobacteriaceae differentiated the family mainly at the generic level. In our studies with campylobacters electrophoresis differentiated at three levels. Electrophoresis of the soluble protein differentiated campylobacters from *Escherichia coli*, but did not differentiate between campylobacters. APS protein differentiated between large groups of campylobacters, and some enzyme electrophoresis patterns differentiated between small groups of strains. The correlation of APS protein patterns with habitat suggests that differentiating campylobacters using electrophoretic methods may be more significant than differentiating by routine biochemical tests. Furthermore, the marked differences revealed by electrophoresis between porcine campylobacters and bovine and ovine campylobacters indicate that pigs are unlikely to serve as a source of infection for cattle and sheep and vice versa.

Campylobacters derived from the genital tract of cattle with infectious infertility all produced the same APS patterns (Group I), irrespective of whether they were biochemical type A or B; campylobacters from aborted cattle or sheep fetuses and from cattle faeces produced patterns similar to Group I but differing in at least one band (Group II). Thus APS patterns of isolates from these animal species may be of use in diagnosis, though differences are slight. Enzyme patterns revealed no differences.

Campylobacters are present in large numbers in the gut of pigs suffering from swine dysentery and have been suggested as the causative organism. As they are also present, though in smaller numbers, in healthy pigs, it is important to know if the isolates from diseased and healthy animals differ. That electrophoresis revealed no correlation with porcine disease can be explained in one of three ways. (i) Campylobacters in diseased pigs are mostly pathogenic types, unlike those in healthy pigs, and, though different from one another, are very similar with regard to electrophoresis as well as biochemical reactions so that differences have not yet been regularly detected. (ii) Only non-pathogenic campylobacters were isolated by us from both healthy and diseased pigs, even when pathogenic types were

present. (iii) There is no qualitative difference in the campylobacter population of healthy and diseased pigs.

Florent (1957) and Deas (1960) found that some of their campylobacter isolates from pigs did not produce H_2S from their routine growth medium (Florent used semisolid Martin's broth and Deas used tryptose phosphate broth). All the porcine campylobacters examined by Davis (1961), and us, produced H_2S in Albimi brucella broth; Andress & Barnum (1968) reported similar findings. Thus porcine strains that do not produce H_2S may be less widespread than the results of Florent and Deas suggest. In view of this, the intensive examination of isolation plates throughout the present work, and the study of many isolates from this laboratory and elsewhere, we consider it unlikely that we had an unrepresentative collection. We have insufficient evidence to choose between the first and last alternatives but, because the electrophoretic separation of malate dehydrogenase detected differences in the fine structure of that protein, we favour the last explanation; that is there is no qualitative change in the type of campylobacter in the gut of pigs when they succumb to dysentery. Cultural studies by Hurvell & Reiland (1970) and transmission experiments by various workers (reviewed by Harris & Glock, 1971) support this conclusion. Our minds are still open on the question of whether campylobacters play an important role in swine dysentery, since, in common with many workers, we found many more campylobacters in animals with swine dysentery than in healthy animals.

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REFERENCES

- ANDRESS, C. E. & BARNUM, D. A. (1968). Pathogenicity of *Vibrio coli* for swine. II. Experimental infection of conventional pigs with *V. coli*. *Canadian Journal of Comparative Medicine and Veterinary Science* **32**, 529-532.
- BOND, J. M. (1957). Characteristics of colonial forms of *Vibrio fetus*. *American Journal of Veterinary Research* **18**, 449-455.
- BROOME, J. (1963). A rapid method of disk electrophoresis. *Nature, London* **199**, 179-180.
- CLARKE, J. T. (1964). Simplified 'disc' (polyacrylamide gel) electrophoresis. *Annals of the New York Academy of Sciences* **121**, 428-436.
- CRUICKSHANK, R. (1965). *Medical Microbiology*, 11th edn. London: Livingstone.
- DAVIS, B. J. (1964). Disc electrophoresis. II. Method and application to human serum proteins. *Annals of the New York Academy of Sciences* **121**, 404-427.
- DAVIS, J. W. (1961). Studies on swine dysentery. *Journal of the American Veterinary Medical Association* **138**, 471-473.
- DEAS, D. W. (1960). Observations on swine dysentery and associated vibrios. *Veterinary Record* **72**, 65-68.
- DOYLE, L. P. (1948). The etiology of swine dysentery. *American Journal of Veterinary Research* **9**, 50-51.
- FIREHAMMER, B. D. & BORDER, M. (1968). Isolation of temperate bacteriophages from *Vibrio fetus*. *American Journal of Veterinary Research* **29**, 2229-2235.
- FLORENT, A. (1953). Isolement d'un vibriion saprophyte du sperme du taureau et du vagin de la vache (*Vibrio bubulus*). *Comptes rendus des séances de la Société de biologie* **147**, 2066-2069.

- FLORENT, A. (1957). Analogies entre certaines souches de *Vibrio coli* et certaines souches de *Vibrio foetus*. Adaption des premières à l'intestin de la bête bovine. *Comptes rendus des séances de la Société de biologie* **151**, 1055-1057.
- FLORENT, A. (1959). Les deux vibrioses genitales de la bête bovine: la vibriose venerienne, due à *V. foetus venerealis*, et la vibriose d'origine intestinale due à *V. foetus intestinalis*. *Proceedings of the XVth International Veterinary Congress, Madrid* **2**, 489-493.
- FLORENT, A. (1963). Apropos des vibrions responsables de la vibriose genitale des bovins et des ovins *Bulletin, Office International des Epizooties* **60**, 1063-1074.
- HAAS, H., DAVIDSON, Y. & SACKS, T. (1972). Taxonomy of *Mycobacteria* studied by polyacrylamide-gel electrophoresis of cell proteins. *Journal of Medical Microbiology* **5**, 31-37.
- HARRIS, D. L. & GLOCK, R. D. (1971). Swine dysentery: a review. *Iowa State University Veterinarian* **33**, 4-11.
- HURVELL, B. & REILAND, S. (1970). *Vibrio coli* hos grisar. *Nordisk veterinärmedicin* **22**, 456-462.
- KENNEDY, S. I. T. & FEWSON, C. A. (1968). Enzymes of the mandelate pathway in bacterium N.C.I.B. 8250. *Biochemical Journal* **107**, 497-506.
- KING, E. O. (1962). The laboratory recognition of *Vibrio fetus* and a closely related *Vibrio* isolated from cases of human vibriosis. *Annals of the New York Academy of Sciences* **98**, 700-711.
- LAWSON, J. R. (1959). Vibriosis. In *Infectious Diseases of Animals. Diseases due to Bacteria*. Edited by A. W. Stableforth and A. Galloway. London: Butterworths.
- LUND, B. M. (1965). A comparison by the use of gel electrophoresis of soluble protein components and esterase enzymes of some group D streptococci. *Journal of General Microbiology* **40**, 413-419.
- MOHANTY, S. B., PLUMER, G. J. & FABER, J. E. (1962). Biochemical and colonial characteristics of some bovine vibrios. *American Journal of Veterinary Research* **23**, 554-557.
- MOORE, B. (1954). Observations on a group of anaerobic vaginal vibrios. *Journal of Pathology and Bacteriology* **67**, 416-423.
- MORRIS, J. A. (1971). *Studies on Campylobacter spp. (microaerophilic vibrios)*. Ph.D. Thesis, University of Reading.
- MORRIS, J. A. & PARK, R. W. A. (1970). The use of polyacrylamide gel electrophoresis of cell proteins to differentiate between microaerophilic vibrios. *Journal of Applied Bacteriology* **33**, iv.
- MORRIS, J. A. & PARK, R. W. A. (1971). The isolation of microaerophilic vibrios. In *Isolation of Anaerobes*, pp. 207-217. Edited by D. A. Shapton and R. G. Board. London: Academic Press.
- NORRIS, J. R. (1964). The classification of *Bacillus thuringiensis*. *Journal of Applied Bacteriology* **27**, 439-447.
- PARK, R. W. A., MUNRO, I. B., MELROSE, D. R. & 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. *British Veterinary Journal* **118**, 411-420.
- PEJTSCHKEV, P. (1969). Ein Fall von *Vibrio fetus* bei *Rattus norvegicus*. *Zentralblatt für Veterinärmedizin, Reihe B* **16**, 480-482.
- PLASTRIDGE, W. N., WILLIAMS, L. F. & TROWBRIDGE, D. G. (1964). Antibiotic sensitivity of physiologic groups of microaerophilic vibrios. *American Journal of Veterinary Research* **25**, 1295-1299.
- RAZIN, S. & ROTTEM, S. (1967). Identification of *Mycoplasma* and other microorganisms by polyacrylamide-gel electrophoresis of cell proteins. *Journal of Bacteriology* **94**, 1807-1810.
- ROSETT, T. (1965). Cooling device for use with a sonic oscillator. *Applied Microbiology* **13**, 254-256.
- SACKS, T. G., HAAS, H. & RAZIN, S. (1969). Polyacrylamide-gel electrophoresis of cell proteins of Enterobacteriaceae. A possible taxonomic aid. *Israel Journal of Medical Sciences* **5**, 49-55.
- SEBALD, M. & VÉRON, M. (1963). Teneur en bases de l'ADN et classification des vibrions. *Annales de l'Institut Pasteur* **105**, 897-910.
- SÖDERLIND, O. (1965). The isolation of *Vibrio coli* from pigs. *Veterinary Record* **77**, 193-196.
- THOUVENOT, H. & FLORENT, A. (1954). Étude d'un anaérobie du sperme du taureau et du vagin de la vache, *Vibrio bubulus* Florent 1953. *Annales de l'Institut Pasteur* **86**, 237-241.
- WATSON, W. A., HUNTER, D. & BELLHOUSE, R. (1967). Studies on vibriotic infection of sheep and carrion crows. *Veterinary Record* **81**, 220-226.
- WHENHAM, G. R., CARLSON, H. C. & ASKEL, A. (1961). Avian vibriotic hepatitis in Alberta. *Canadian Veterinary Journal* **2**, 3-7.