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Isolation of *Campylobacter fetus* from recent cases of human vibriosis

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SUMMARY

Campylobacter fetus was isolated from five recent cases of human vibriosis, of which two were adults and three were children. One adult presented with pericarditis and the other with recurrent pyrexia. *Campylobacter fetus* subsp. *intestinalis* which resembled cattle strains serologically, was isolated under CO_2 or anaerobic conditions from blood cultures of these patients. Two of the three children had kwashiorkor and the third was only 8 days old. Isolates identified as *Campylobacter fetus* subsp. *jejuni* were cultured from blood of these patients, two of whom had diarrhoea. Three patients succumbed, despite adequate antibiotic therapy. The epidemiology of the disease is discussed and it is suggested that infection may have been from the patients' own flora.

INTRODUCTION

Microaerophilic vibrios infect several animal species including Man, and isolates from cattle have been from all parts of the world (Food and Agriculture Organization, 1964; Neitz, 1965). In animals the organism has been cultured from various sites and infections may cause endometritis and placentitis, resulting in infertility, delayed conception and abortion (Gilman, 1960). Since the first authenticated report of a human infection due to *Vibrio fetus* in France (Vinzent, Dumas & Picard, 1947), cases have been reported from several other countries (Darrell, Farrell & Mulligan, 1967; White, 1967; Raahave, 1969; Bokkenheuser, 1970; Cornere, Robinson & Paykel, 1971; Dolev, Altmann & Padeh, 1971; Park, McDonald, Twohig & Cook, 1973). Clinical features have been: abortion, fever, gastroenteritis, thrombophlebitis, septic arthitis, local abscesses, pericarditis (Killam, Crowder, White & Edmonds, 1966), meningitis and septicaemia (Bryner, 1975). The taxonomic position of these organisms has recently been reviewed (Véron & Chatelain, 1973; Smibert, 1974), and most authors now place them in

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the genus *Campylobacter*. Because of the rarity of these isolations from humans and the uncertainty of the epidemiology of the disease we report herein our experience with a series of five cases.

METHODS

Blood for culture was collected by venipuncture after sterilization of the skin with 70% alcohol, and 5 ml was put into each of 100 ml glucose broth and 100 ml thioglycolate broth. Subcultures were done in 5% CO_2 and anaerobically 24 and 48 h later and then after 1 week and 2 weeks, on blood and MacConkey agar plates. Organisms were isolated from blood cultures of four patients and from pericardial fluid of one patient. Growth was established initially on outdated human blood agar under increased CO_2 atmosphere or under anaerobic conditions. Identification was established by morphology, biochemical reactions and serology.

The identifications were confirmed by the Center for Disease Control (C.D.C.), Atlanta, Georgia and Onderstepoort Veterinary Research Institute, Pretoria.

RESULTS

Clinical presentation

One patient presented with a localized infection, and four had septicaemia (Table 1).

Localized infection

The organism was isolated from a pericardial aspirate of a patient in cardiac failure. The case history is as follows:

Case no. 1. A 38-year-old Zulu female was admitted to hospital complaining of increasing dyspnoea for the previous fortnight, bilateral lower chest pain, dry cough and nausea. There was no previous history of illness. On examination she was pale, dyspnoeic, sweating and both legs were oedematous. She had a four finger hepatomegaly and jugular venous pressure was 10 cm above the sternal angle. No splenomegaly or deep vein thrombosis was found. An electrocardiogram was compatible with pericarditis. X-ray of the chest revealed an enlarged cardiac shadow and bilateral pleural effusions.

She was treated for her cardiac failure and 2 days later 1·2 l of blood-stained fluid was aspirated from the pericardium, but no organisms were isolated on media incubated anaerobically and aerobically. Anti-tuberculosis therapy (streptomycin, I.N.H. and P.A.S.) was started. Over the next 5 weeks the signs of cardiac failure improved slightly but she developed a maculo-papular rash. Pericardial aspiration was repeated on three occasions. The first specimen was found to contain Gram negative bacilli on microscopic examination, but organisms were not isolated on culture. The second specimen was negative on direct microscopy and culture. From the third aspirate, obtained 6 weeks after admission, *C. fetus* was isolated. Rifampicin and cephalothin were then added to the previous drug therapy.

Surgical drainage of a left pleural empyema and intrapericardial abscess was undertaken. Histological examination showed oedematous granulation tissue but no evidence of tuberculosis. She then became jaundiced but this diminished on

Table 1. *Summary of clinical symptoms of patients with vibriosis*

Case no.	Age	Sex	Site of isolation	Race	Symptoms	Treatment	Outcome
1	38 yrs	F	Pericardial fluid	Black	Pericarditis	Streptomycin, I.N.H., P.A.S., rifampicin, cephalothin, penicillin, gentamicin	Died
2	23 yrs	M	Blood	White	Fever, vomiting	None	Survived
3	21 mths	F	Blood	Black	Diarrhoea, vomiting, kwashiorkor,	Penicillin, Streptomycin, ampicillin, kanamycin	Survived
4	21 mths	F	Blood	Black	Fever, bronchopneumonia, malnourished	Penicillin, kanamycin	Died
5	8 days	F	Blood	Black	Cough, diarrhoea, jaundiced	Penicillin, kanamycin	Died

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Table 2. *Antibiotic disk sensitivities of* Campylobacter fetus *isolates*

	Conc. (μg)	Case no. 1	2	3
Ampicillin	25	S	R	PR
Carbenicillin	100	S	*	S
Cephalothin	30	S	R	R
Chloramphenicol	25	S	*	S
Cloxacillin	5	R	R	R
Erythromycin	5	*	S	*
Gentamicin	10	S	*	S
Kanamycin	30	S	*	S
Nalidixic acid	30	R	*	*
Neomycin	30	S	*	*
Nitrofurantoin	200	S	*	*
Penicillin G	1	R	R	R
Streptomycin	10	R	S	S
Sulphafurazole	200	S	S	S
Tetracycline	25	S	S	S
Trimethoprim/sulphamethoxazole	25	S	S	R

PR, Partially resistant; S, sensitive; R, resistant; * = not done.

cessation of rifampicin therapy. Further surgical draining of the pleural empyema and penicillin and gentamicin administration were of no avail and the patient succumbed. Autopsy was not performed.

Bacteriology. The organism was isolated on a human blood agar plate, incubated anaerobically for 48 h. No growth was obtained aerobically, but on subculture growth was established under CO_2. Tentative identification as *C. fetus* was made on the following characteristics: Gram-negative comma-shaped bacillus with spiral forms. On primary isolation only anaerobic culture was successful. The following tests were negative: glucose, lactose, sucrose, dulcitol and mannitol fermentation, urea hydrolysis and gelatin liquefaction.

Subcultures were sent to the C.D.C. Laboratories, Altanta, Georgia, and the Veterinary Research Institute, Onderstepoort, where the identification was confirmed. The Onderstepoort laboratory classified it as *Vibrio fetus intestinalis* (Serotype 01), which is now known as *Campylobacter fetus* subsp. *intestinalis* (Smibert, 1974). This serotype is frequently isolated from cattle in South Africa, where it causes abortions and sterility.

A serum specimen obtained from the patient 53 days after admission, was tested against 2 animal strains of *C. fetus* antigens (65 and 661) at Onderstepoort. These showed a mild degree of aggultination to a dilution of 1/1250 with antigen 65 and 1/32 with antigen 661, but the reactions were reported to be weaker than those experienced with animal sera.

Antibiotic sensitivity tests to a wide range of antibodies by the disk method showed resistance to cloxacillin, nalidixic acid, penicillin and streptomycin, but susceptibility to the other antibiotics tested (Table 2). The minimal inhibitory concentration (MIC) of cephalothin was 10 μg/ml and resistance to streptomycin was demonstrated to 100 μg/ml by the tube dilution method.

Septicaemic form

In this group one patient had been suffering from recurrent bouts of fever assumed to be malaria. The case history of this patient was as follows:

Case no. 2. A 23-year-old white student, who had at the age of 10 years suffered from proved malaria in Zambia. Since then he had had recurrent bouts of fever which had been presumed to be due to malaria. The present illness dated back 3 weeks when he experienced a rigor and felt ill, he vomited and sweated profusely. The only positive clinical finding was a pyrexia of 40 °C. Blood cultures were taken and an organism isolated later identified as *C. fetus*. The patient was discharged without treatment before bacteriological isolation had been achieved. He re-attended, but no further positive blood cultures or bone marrow cultures were obtained. Widal, Weil Felix, Paul Bunnell and Brucella agglutination tests were negative. Examination of blood smears revealed no malarial parasites. Brucella Coombs test, on a previous admission, had been positive for *B. abortus* to a titre of 50 and was negative on this admission.

Bacteriology. Curved, Gram-negative rods were isolated from a set of blood cultures taken whilst the patient was experiencing rigors. Blood cultures taken at a later date when the patient was clinically well, failed to grow any organisms.

This strain grew well under increased CO_2 atmosphere and did not grow aerobically or anaerobically. Carbohydrates were not attacked and urea was not hydrolysed. Voges Proskauer, citrate utilization, gelatin liquefaction, methyl red and indole production were negative. The organisms were motile and produced catalase. After overnight incubation growth was scant but colonies reached a diameter of 1–2 mm after 5 days incubation under CO_2 on horse blood agar, with a smooth, entire, glossy, low convex, opaque, butyrous and slightly buff coloured appearance. Antibiotic sensitivity was determined by the disk method and the culture was found to be sensitive to streptomycin, erythromycin, tetracycline and co-trimoxazole whilst being resistant to penicillin, ampicillin, cephalosporin group and cloxacillin (Table 2). Electron microscopy revealed the structure of a vibrio, with a single flagellum at one pole (Plate 1).

The other three patients in this group were African children, two of whom were malnourished. The case history of one is as follows:

Case no. 3. A 21-month-old African female child with severe kwashiorkor and a history of diarrhoea and vomiting was admitted to hospital. She had marked hair changes, severe oedema over the whole body, and a 3 cm hepatomegaly. There was no splenomegaly, she was not jaundiced and was afebrile. A blood culture taken on the day of admission yielded *C. fetus*.

She was given penicillin V 500,000 units intramuscularly 6 hourly and streptomycin 100 mg intramuscularly 12 hourly for 4 days. The streptomycin was then stopped and the penicillin continued for a further 12 days. Five days after admission the patient became febrile (Fig. 1) and developed bilateral bronchopneumonia. Kanamycin 60 mg 12 hourly intramuscularly and ampicillin 125 mg 6 hourly intramuscularly for 24 h, and then orally, was given for 11 days. The patient then became afebrile and was discharged.

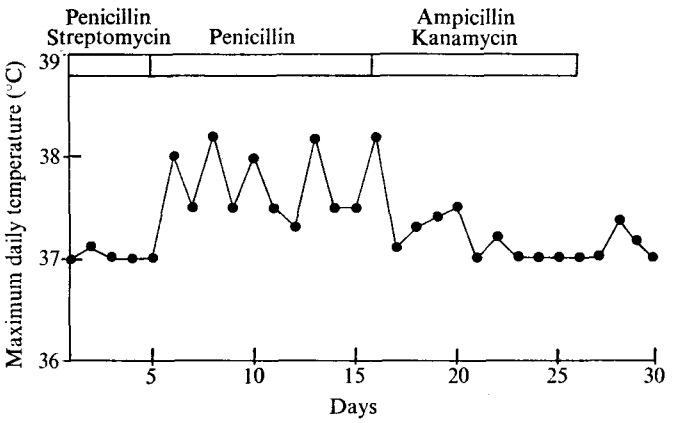

Fig. 1. Temperature chart of patient with *Campylobacter fetus* septicaemia.

Bacteriology. The organisms were isolated from a routine blood culture in thioglycollate broth. After 8 days incubation a sub-culture on human blood agar incubated anaerobically without added CO_2 yielded small curved rods. This culture was identified by the Veterinary Research Institute, Onderstepoort as *Vibrio fetus intestinalis* serotype C, and by the C.D.C. laboratories as a 'related vibrio' (King, 1957). This organism is now known as *Campylobacter fetus* subsp. *jejuni* (Smibert, 1974). Similar strains have been isolated from sheep in South Africa (A. P. Schutte, personal communication) and may be associated with gastro-intestinal symptoms in man (King, 1957). Antibiotic sensitivity tests (Table 1) showed resistance to penicillin and partial resistance to ampicillin, but sensitivity to streptomycin and kanamycin.

The other two patients, a 21 month old undernourished African child and an 8 day old African child were treated with intramuscular penicillin and kanamycin, but failed to respond and both died shortly after admission. An untypable *C. fetus* was isolated from a blood culture of the older child and *C. fetus jejuni* were isolated from a blood culture of the younger child.

DISCUSSION

The epidemiology of *Campylobacter* in man is obscure (King, 1957) but animals, including chickens, may be the source of some human infections (King, 1962; M. B. Skirrow, personal communication). Close contact with cattle, immediately before infection, was established in many of the previously reported cases. An association with animals could not be established with certainty in our patients, but was very likely in our first case as she lived in a rural area where most inhabitants have their own cows. The organism isolated from this patient was identified as *C. fetus intestinalis* biochemically and the patient's serum showed agglutinins to a high titre against antigens from animal strains, suggesting that the strain belonged to a serotype found in cattle. Two other strains (cases 3 and 5) were identified as *C. fetus jejuni*, which has previously been isolated from sheep in the Republic of South Africa.

A previous history of animal contact cannot always be established in cases of human vibriosis. King (1962) suggested that the organisms may be carried by humans in their bodies for long periods, as in animals. If debilitated by other diseases, they become infected by part of their own flora and in cases of abortion the organism may be venereally transmitted (King, 1962). Further evidence that *Campylobacter fetus* is present in the human intestine was obtained by Butzler, Dekeyser, Detrain & Dehaen (1973). By means of selective faecal culture techniques they established that campylobacters of the '*jejuni*' group are a common cause of acute enteritis, mainly in children but also in adults. The depression of cell-mediated immunity in kwashiorkor is well established (Smythe *et al.* 1971) and results in susceptibility to Gram-negative septicaemia (Smythe & Campbell, 1959). Two of the children in this series had kwashiorkor with diarrhoea and the third was only 8 days old. One adult was in cardiac failure. The clinical history in these patients suggests, therefore, that the organisms were derived directly from their own flora, but indirectly from domestic animals, in which similar serotypes have been isolated.

Campylobacter is rarely isolated from humans. This is because several vibrios are only able to grow in an atmosphere of increased CO_2. Under routine isolation procedures, such organisms may be overlooked unless a suitable milieu is provided. Although morphologically similar to the type species of the genus *Vibrio*, *V. cholerae* (Park, 1961), the microaerophilic vibrios have been shown to differ from it in DNA base ratios and in other respects and have consequently been placed in a separate genus, *Campylobacter* (Sebald & Véron, 1963). The best known of these microaerophilic vibrios is *C. fetus* (*Vibrio fetus*) (Smith & Taylor, 1919). The other species included in the genus are *C. fetus*, *C. sputorum* and *C. fecalis* (Smibert, 1974). *C. fetus* and *C. fecalis* are catalase positive, whereas *C. sputorum* is catalase negative. *C. fetus* is differentiated from *C. fecalis* by the inability of the former to produce H_2S in peptone iron agar whereas the latter does. Three subspecies of *C. fetus* are now recognized: *C. fetus fetus*, *C. fetus intestinalis* and *C. fetus jejuni*. *C. fetus fetus* does not produce H_2S in a medium containing cysteine with lead acetate impregnated paper strips as the detection system, and grows at 25 °C but not at 42 °C. *C. fetus intestinalis* and *C. fetus jejuni* are distinguished from *C. fetus fetus* by their ability to produce H_2S as detected by lead acetate impregnated paper strips. *C. fetus intestinalis* grows at 25 °C but not at 42 °C whereas *C. fetus jejuni* grows at 42 °C but not at 25 °C. As the epidemiology of human vibriosis is uncertain, it is important to characterize any vibrio isolate fully, using biochemical and serological techniques. Attempts should be made to develop other methods of characterization, such as bacteriophage typing (Bryner, Ritchie, Foley & Berman, 1970).

Human vibriosis may not respond to treatment even if the organisms are sensitive to the antibiotics *in vitro*. Our first case was on antituberculosis treatment, including streptomycin and rifampicin, for 4 weeks without any obvious improvement. Intravenous cephalothin (MIC 10 μg/ml) was administered also without effect. After 2 days therapy with gentamicin and benzyl penicillin the patient died, apparently of endotoxic shock. Two of the three children from whom *C. fetus*

jejuni were isolated died, despite treatment with kanamycin and penicillin. The antibiotic senstivities of the strains from these two patients were not tested. Kanamycin was effective in treating the third case in which the culture was sensitive *in vitro*, and the second case recovered without treatment. Vibriosis should therefore be considered in cases of pyrexia of unknown origin, because of the potentially lethal nature of the septicaemic form of the disease. Early diagnosis is imperative for effective treatment, and a recently developed serological test (Bokkenheuser, 1972) may be useful for this purpose.

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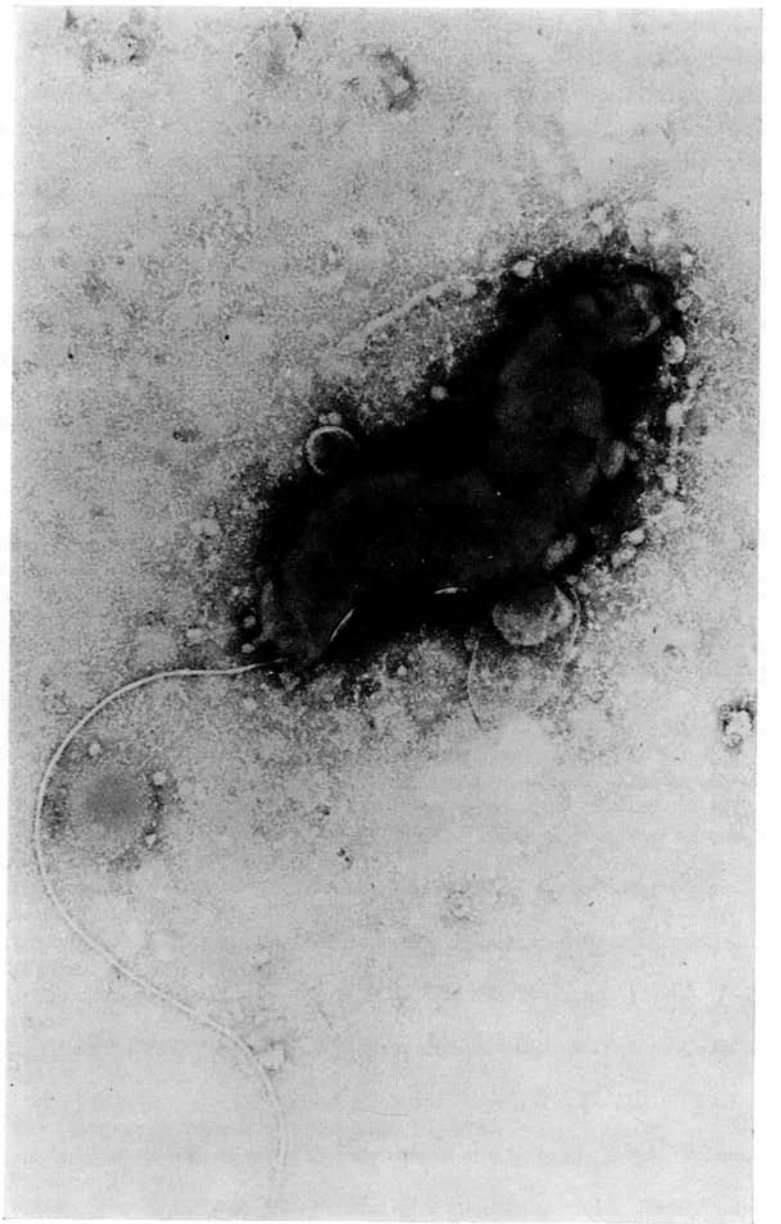

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EXPLANATION OF PLATE

Photomicrograph of *Campylobacter fetus*. × 30,000.

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