Septicaemia and meningitis caused by dysgonic fermenter-2 (DF-2)

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SUMMARY A patient developed dysgonic fermenter-2 (DF-2) septicaemia and meningitis after having been bitten by a dog. The pathogenic organism was isolated from the oral cavity of the dog, which, it is believed, is the first time that this has been done. The growth characteristics of the organism and the immunological response of the patient were studied.

The isolation of the infecting agent from the dog confirms that the oropharyngeal flora of dogs is a reservoir of the organism: the fastidiousness and slow growth of this Gram negative bacillus may account for the small number of reported cases of infection caused by this organism.

DF-2 (dysgonic fermenter-2) is a fastidious Gram negative bacillus, which was first isolated from the blood and spinal fluid of a patient after a dog bite.¹ A recent review of the literature on the organism described 28 cases, half of which were associated with dog bites and the remainder of which had a history of dog or animal contact.²⁻⁴ Underlying illness was also a common feature; only three patients were reported to have been in previously good health. Eleven of the 28 had a splenectomy, which seems to be a high risk factor.

The epidemiological evidence associating DF-2 infection with dog contacts strongly implies that the dog is the reservoir of this organism. Indeed, the bacillus has been found in the oral secretions of four out of 50 dogs screened at random⁵ and in one dog whose owner developed DF-2 post-splenectomy sepsis, although he had denied ever being bitten by the dog.⁶ Attempts to isolate the organism from the animal after a dog bite resulting in DF-2 infection have, until now, been unsuccessful.^{1,7}

We report a case of DF-2 meningitis and septicaemia from a previously healthy patient that developed after a dog bite; successful isolation of the organism from the oral cavity of the dog responsible; growth characteristics of the organism and the immunological response of the patient. To our knowledge only two other cases of DF-2 have previously been reported in the United Kingdom.^{8,2}

Case history

A 63 year old man presented with a four day history

of fever, rigors, arthralgia, and generalised rash. He was admitted to hospital and had an episode of vomiting on the day of admission. Two weeks before the onset of his illness he had been bitten by his pet Alsatian whose fang tooth punctured the mucosal lining of his nostril. Apart from mild hypertension and an appendectomy 18 years ago, there was no history of serious illness.

On examination he had a temperature of 37.8°C, photophobia, but no neck stiffness or any localising neurological signs. There was an erythematous rash on the trunk but no lymphadenopathy. Chest, abdomen, respiratory, and cardiovascular systems were normal.

Haematological investigations showed a haemoglobin of 17.1 g/dl and white cell count of $14.5 \times 10^9/l$ with 87% neutrophils. Biochemical analyses including liver function tests were within normal limits, as were chest radiography and urine analyses. A lumbar puncture yielded cloudy cerebrospinal fluid that showed a white cell count of $1121 \times 10^9/l$ with 80% neutrophils, red cell count $80 \times 10^9/l$, protein 1.75 g/l and glucose 2.1 mmol/l (serum glucose 4.7 mmol/l). Gram stain of cerebrospinal fluid showed Gram negative bacilli. Parenteral treatment of chloramphenicol 900 mg six hourly and ampicillin 1 g six hourly was started intravenously. Clinical response was rapid and temperature returned to normal within 48 hours. Blood cultures taken on admission yielded an organism identical with that of the cerebrospinal fluid. After one week of treatment the patient was asymptomatic and discharged. He remained well when followed up in the outpatient clinic one month later.

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Growth of DF-2 on various agar media

Agar medium	Humidified incubator (5% carbon dioxide)	Anaerobic jar*
MacConkey agar (Oxoid)	NG	NG
Blood agar base No 2 (Oxoid)†	NG	+
Columbia agar (Oxoid)†	+	+
GC medium base with added Bacto-supplement B	NG	NG
Brain heart infusion (Oxoid)†	NG	+
Wilkins-Chalgren (Oxoid) with 5% horse blood	+	+
Tryptone soy agar (Oxoid)†	+	++
Aerobic Bactec medium 6B (solidified with Oxoid agar No 1 at w/v 2%)	+	+
Anaerobic Bactec medium 7D (solidified with Oxoid agar No 1 at w/v 2%)	+	+++

*Anaerobic jar: 10% carbon dioxide, 90% hydrogen. NG = no growth.

†No difference in growth obtained with the addition of 5% defibrinated horse blood (Tissue Culture Services).

Bacteriology

Initial attempts to culture DF-2 from cerebrospinal fluid on blood and chocolate agar (Tissue Culture Services, Slough) were unsuccessful despite the fact that pleomorphic Gram negative rods were seen in the Gram stained cerebrospinal fluid. Aerobic blood and chocolate agar plates were incubated in a 5% carbon dioxide moist atmosphere and anaerobic blood agar plates were incubated in 10% carbon dioxide and 90% hydrogen atmosphere at 37°C. No growth was observed on any of the plates after seven days' incubation. The isolate from blood culture collected on the day of admission was first detected radio-metrically at 48 hours in the aerobic Bactec 6B medium (growth index 36, positive threshold 25) and anaerobic Bactec 7D medium (growth index 55, positive threshold 35), which, on Gram staining, showed Gram negative rods with tapered ends similar to those seen in the cerebrospinal fluid. A sample of cerebrospinal fluid was therefore inoculated into an anaerobic Bactec 7D blood culture vial, which also recorded a positive growth index at 48 hours. Subsequent cultures passaged from both specimens in Bactec anaerobic medium yielded positive growth index at only 24 hours' incubation. Similarly, swabs of the gingiva and teeth of the patient's dog expressed in peptone water, which was in turn inoculated into anaerobic Bactec 7D medium, yielded the same organism.

In an attempt to culture the organism on a solid medium various agars were prepared and tested for their suitability to support growth of the organism. Their anaerobic and aerobic performances were also investigated (table).

A comparison of the results indicated that the media most supportive of growth were tryptone soy agar and solidified anaerobic Bactec 7D medium. The addition of horse blood did not improve growth and the organism also preferred an anaerobic atmosphere.

By way of comparison, horse and sheep blood, or

rabbit serum (final concentration 5% v/v), were added, respectively, to tryptone soy agar, brain heart infusion agar, or Columbia agar. None of the blood products was found to be superior to the others in improving growth, while some batches of blood totally inhibited the growth of the organism.

The organism was non-motile, catalase and oxidase positive, and did not grow on MacConkey agar. It was negative for indole, nitrate, citrate, and urea. Acid was produced from glucose, maltose, and lactose but not from sucrose, xylose, or mannitol. The organism was sent to Dr L R Hill at the National Collection of Type Cultures, in the Central Public Health Laboratory at Colindale, for further investigation and then to Dr Robert Weaver in the Center for Disease Control, Atlanta, Georgia, where it was confirmed to be DF-2.

During the course of isolation and identification of the organism, it was noted that satellitism occurred around contaminating colonies of *Staphylococcus aureus*, when incubated aerobically in 5% carbon dioxide. This phenomenon was also observed with coagulase negative staphylococci as well as with some coliforms. Further investigation showed that the organism did not require either X or V factor. Other substances tested included pyridoxal 6-phosphate, co-carboxylase, thiamine, D-L alanine, arginine monohydrochloride, asparagine, glutamic acid, leucine, methionine, proline, tyrosine, valine, ornithine, and L-cysteine. Of these, only the last amino acid showed slight enhancement of growth. Similarly, filtrates of staphylococcus broth, supernatants of centrifuged cultures, and sonicates of *Staphylococcus* did not exhibit growth enhancement. These results seem to suggest that the "growth enhancing factor" is extremely labile and is demonstrable only with active growth of *Staphylococcus*. An interesting observation from plates incubated over three days was the high incidence of large discrete colonies at the margins of the *Staphylococcus* streaks, which suggests that the "growth enhancing factor" is not essential but that its

presence contributes to the more rapid growth of DF-2. Further investigations on this "growth enhancing factor" are now in progress.

The organism was sensitive to discs containing penicillin (1 µg), erythromycin (5 µg), chloramphenicol (10 µg), and cefuroxime (30 µg) but resistant to gentamicin (10 µg), trimethoprim (1.25 µg), sulphafurazole (100 µg), and metronidazole (5 µg).

Serology

Indirect fluorescence antibody tests were carried out on smears of DF-2 freshly harvested from a three day culture with dilutions of the patient's acute and convalescent serum. After incubation the patient's serum was washed off and the slide was covered with optimal dilution of rabbit antihuman conjugate capable of staining human IgG or IgM. Smears of coliforms were included as negative controls. The acute phase serum collected on the day of admission showed an IgG titre of 1/160 to DF-2, irrespective of whether the isolate was obtained from the cerebrospinal fluid, blood culture, or the patient's dog. No IgM antibody was shown. Convalescent serum obtained two weeks later showed an IgG titre of 1/40. Five random serum samples from antenatal patients were used as negative controls and did not give a positive response at 1/20 dilutions. Serum from the patient's son, who developed fever and a rash on his face and shoulders at about the same time as his father but which settled spontaneously without medical treatment, showed an acute IgG titre of 1/80 and negative convalescent antibody.

Discussion

This is the first successful isolation of DF-2 from the oral secretion of the dog and from the cerebrospinal fluid and blood culture of the patient after dog bite: and it provides direct evidence that the oropharyngeal flora of the dog is a reservoir of this organism. The evidence is further supported by the serological findings, which show an appreciable difference in antibody titres between the acute and convalescent sera. The absence of IgM antibody is probably due to prior exposure of the patient to DF-2. Our patient did not have any history of splenectomy and should presumably have been quite capable of mounting a primary response with IgM antibody. The same explanation could be applied to the serological response of his son who presented with less severe and shorter lasting clinical features. It is well known that IgM antibacterial antibodies are far superior to those of IgG in bringing about bacterial agglutination and acting as opsonising agents, and deficiency of IgM is often associated with susceptibility to septicaemia.⁹

As the possession of IgG antibody did not seem to provide protection against infection by DF-2, it is perhaps fortunate that the organism seems to be of low pathogenicity, judging by its predilection for patients with impaired immune defences.¹⁰

Previous reports have not documented the state of health of the dogs implicated in the transmission of DF-2 infections. The study on the bacterial flora of oral fluids of dogs from which DF-2 has been isolated was carried out on animals with no apparent bacterial infections.⁵ The dog in our case did not seem to be ill. The organism seems likely to be part of the normal mouth flora in dogs.

Our patient differs from most of the other reported cases in that he was healthy and had no history of any serious underlying illness. The presentation with meningitis and septicaemia is also unusual: in a review of the literature only three of the 28 reported cases had meningitis while all had septicaemia. The wide spectrum of clinical presentations includes cellulitis, endocarditis, arthritis, Waterhouse-Friderichsen syndrome,³ and necrotising eschariform skin lesions.⁴

It has been suggested that the widespread use of penicillin (to which DF-2 is sensitive) for the treatment of dog bites may prevent this infection from being recognised.¹⁰ This, coupled with the difficulties in culturing the organism from blood cultures on to agar media as well as its slow growth on the latter, as shown in our experience and those of others,¹¹ may have resulted in it being missed on routine subculture of blood culture. A high level of awareness on the part of the clinicians when confronted with febrile patients with a recent history of dog bites or dog contacts and prompt consultation with the microbiologists for the possible presence of this fastidious Gram negative bacillus in the blood culture may facilitate the diagnosis of DF-2 septicaemia.

The antibiotic sensitivities of our isolate agree with those previously reported.^{1 10 12} Interestingly, in common with another Gram negative pathogen, *Pasteurella multocida*, which has been cultured from 17% of wounds after dog bites,¹³ is resistant to the aminoglycosides but sensitive to penicillin, which seems to be the antibiotic of choice for DF-2 infection.

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References

- 1 Bobo RA, Newton EJ. A previously undescribed Gram-negative bacillus causing septicemia and meningitis. *Am J Clin Pathol* 1976;65:564-9.

- 2 Ofori-Adjei D, Blackledge P, O'Neill PP. Meningitis caused by dysgonic fermenter type 2 (DF-2) organism in a previously healthy adult. *Br Med J* 1982;**285**:263-4.
- 3 Rubin SJ. DF-2: a fastidious fermentative Gram-negative rod. *Eur J Clin Microbiol* 1984;**3**:253-7.
- 4 Kalb R, Kaplan MH, Tenenbaum MJ, Joachim GR, Samuels S. Cutaneous infection at dog bite wounds associated with fulminant DF-2 septicemia. *Am J Med* 1985;**78**:687-90.
- 5 Bailie WE, Stowe EC, Schmitt AM. Aerobic bacterial flora of oral and nasal fluids of canines with reference to bacteria associated with bites. *J Clin Microbiol* 1978;**7**:223-31.
- 6 Martone W, Zuehl RW, Minson GE, Scheld WM. Post-splenectomy sepsis with DF-2; report of a case with isolation of the organism from the patient's dog. *Ann Intern Med* 1980;**93**:457-8.
- 7 Hinricks JH, Dunkelberg WE. DF-2 septicemia after splenectomy; epidemiology and immunologic response. *South Med J* 1980;**73**:1638-40.
- 8 Chaudhuri AK, Hartley RB, Maddocks AC. Waterhouse-Friderichsen syndrome caused by a DF-2 bacterium in a splenectomised patient. *J Clin Pathol* 1981;**34**:172-3.
- 9 Hobbs JR, Milner RDG, Watt PJ. Gamma-M deficiency predisposing to meningococcal septicaemia. *Br Med J* 1967;**ii**:583.
- 10 Butler T, Weaver RE, Ramani TKV, et al. Unidentified Gram-negative rod infection. *Ann Intern Med* 1977;**86**:1-5.
- 11 Lennette EH, Balows A, Hausler WJ, Truant JP. *Manual of clinical microbiology*. 3rd ed. Washington, DC. American Society for Microbiology, 1980.
- 12 Schoen RT, Wohlgelemler D, Barden GE, Swartz TJ. Infection with CDC group DF-2 gram-negative rod: report of two cases. *Arch Intern Med* 1980;**140**:657-8.
- 13 Lee MLH, Buhr AJ. Dog bites and local infection with Pasteurella septica. *Br Med J* 1960;**i**:169-71.

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