2 Sonoda S, Nakayama T. Detection of measles virus genome in lymphocytes from asymptomatic healthy children. *J. Med. Virol.* 2001; **65**: 381–7.
3 Kawashima H, Mori T, Takekuma K *et al.* Polymerase chain reaction detection of the hemagglutinin gene from an attenuated measles vaccine strain in the peripheral mononuclear cells of children with autoimmune hepatitis. *Arch. Virol.* 1996; **141**: 877–84.
4 Afzal MA, Armitage E, Ghosh S, Williams LC, Minor PD. Further evidence of the absence of measles virus genome sequence in full thickness intestinal specimens from patients with Crohn's disease. *J. Med. Virol.* 2000; **62**: 377–82.
5 Kawashima H, Mori T, Kashiwagi Y, Takekuma K, Hoshika A, Wakefield A. Detection and sequencing of measles virus from peripheral mononuclear cells from patients with inflammatory bowel disease and autism. *Dig. Dis. Sci.* 2000; **45**: 723–9.

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Dear Editor

DIFFICULTIES IN THE DIAGNOSIS AND MANAGEMENT OF INFANT BOTULISM

In reply to the instructive case report 'Instructive case: A taste of honey',[1] we would like to make a number of comments. First, we ask the authors to list the electrophysiological features and the results of toxin detection in serum by mouse bioassay in their case. Second, we wish to discuss the need to pursue other diagnostic methods for this condition, the significance of early stool collection, the role of other clinical information in the face of equivocal laboratory data, the marked variability in the clinical course of infant botulism, and the availability of botulism immunoglobulin (BIG) and equine antitoxin in Australia, based on the following case.

A five-month-old boy presented on 2 September 2000 with lethargy, constipation and poor feeding. He lived on a cattle property in central Queensland, with the herding yards adjacent to the house. He required ventilation due to respiratory failure secondary to global flaccid paralysis. Initially, the only helpful diagnostic procedures were nerve conduction studies, which showed reduced compound muscle action potential amplitudes, absent F-waves, normal motor conduction velocities and distal motor latencies, and short-duration low-amplitude motor action unit potentials suggestive of infantile botulism. An incremental response in compound muscle potential amplitudes with rapid repetitive nerve stimulation at 50 Hz was not elicited. *Clostridium botulinum* type A was cultured in the stool collected on 4 October 2000. More definitive results were never achieved, as toxin detection in stool or serum by mouse bioassay was equivocal. Exposure to raw honey had occurred, but later environmental testing of food in the home was negative for *C. botulinum* spores. The child showed clinical features typical for infant botulism, except for a remarkably slow resolution of the paralysis. He required ventilatory assistance for 99 days and hospital admission for 120 days. No BIG or antitoxin was given to this infant.

Our case demonstrates that mouse bioassay results are not always conclusive. The mouse bioassay is considered to be the most sensitive technique for the diagnosis of infantile botulism. Unfortunately, it is technically demanding, and takes up to 4 days.[2,3] There are other diagnostic techniques available, such as antibotulinum toxin monoclonal antibody in an enzyme-linked immunosorbent assay, and polymerase chain reaction nucleic acid amplification, for toxin and organism detection, respectively.[3,4] These tests achieve results approaching the sensitivity of the mouse bioassay, with much shorter time requirements. However, public health laboratories in the USA and UK still rely on the mouse bioassay test for a definitive diagnosis of infant botulism. The yield of toxin detection in serum by mouse bioassay is lower when compared with faeces samples. It is recommended that in inconclusive cases with ambiguous samples, another reference laboratory should repeat the assays.

Stool collection is paramount to detection of the *C. botulinum* organisms and toxin. The paralysis often leads to delayed gut transit and passage of stool. This was certainly a factor in the delay of diagnosis in this child. The difficulty in toxin detection in this case is in contrast to the majority of reports regarding infant botulism, which suggest that the period of toxin and organism excretion in the faeces is prolonged.[5] To facilitate collection of a diagnostic sample from a constipated infant early in the illness, all fluid from an enema or rectal wash using sterile water should be collected. The gathering of other supportive evidence from electrophysiological studies is also important, although electromyography can often generate false-negative results or inconclusive testing.

Our case had a prolonged admission. Most published cases of infant botulism have admissions between 26 and 37 days in length.[2] This may be a significant individual variation found in response to botulinum toxin, although a prolonged duration of paralysis is known in severe cases (R Schechter, Infant Botulism Prevention Program, Californian Department of Health, pers. comm.). Alternatively, as mentioned by McMaster *et al.*,[1] aminoglycoside use may have contributed to the slow convalescence of this patient. Before the diagnosis was made, this child received 7 days of gentamicin for an intercurrent *Klebsiella oxytoca* urinary tract infection.

Finally, the general comment by McMaster *et al*. that equine antitoxin is 'more readily available' requires clarification.[1] They mention the use of both BIG and equine antitoxin for the treatment of infantile botulism. Equine-derived antitoxin is used in the USA for the treatment of food-borne and wound botulism, while BIG is a Food and Drug Administration (FDA)-approved Investigational New Drug for the treatment of infant botulism.[6,7] The use of BIG outside the USA is restricted

by limited inventory (R Schechter, pers. comm.). Equine antitoxin has rarely been used in infant botulism because of the risk of inducing lifelong hypersensitivity to equine antigens and a lack of evidence of its benefit.[6]

Equine antitoxin is available in Australia, although no mention of it is made in the current Australian Immunisation Handbook.[8] The product is manufactured by a variety of sources, including Aventis-Pasteur (Canada), Chiron-Behring (Germany), and the USA military.[7] A total of six vials (treatment for two children or adults), imported by the Australian Commonwealth Department of Health and Aged Care from Chiron-Behring, at a cost of approximately AU$18 300, are stored at the Commonwealth Serum Laboratories, North Ryde, New South Wales (24 h telephone 02 9887 4433). Use of equine antitoxin is controlled under the provisions of the Special Access Scheme of the Therapeutic and Goods Administration. Side effects include transient elevations in body temperature, allergic reactions, acute anaphylaxis and serum sickness.[9] However, the titre of equine botulinum antitoxin greatly exceeds what is needed for treatment. The number of vials (Aventis-Pasteur, 10 mL per vial, 5500–8500 IU) used per case of food-borne or wound botulism in the USA has decreased from four to one over the last several decades.[7] As the product is available in Australia, we request that the Technical Advisory Group on Immunisation of the Commonwealth Department of Health and Aged Care list the product in subsequent editions of the Immunisation Handbook to alert physicians about its proper use, dosage and availability.

A double-blind placebo-controlled treatment trial of BIG has been performed.[10] Use of BIG reduced mean hospital stay per case from approximately 5.5 weeks to 2.5 weeks ($P < 0.001$), and reduced mean hospital stay costs per case by approximately US$70 000 ($P < 0.001$). Considering the reported effectiveness of BIG, the side effects of equine antitoxin, and the fact that we are now aware of three cases of infantile botulism in Australia in the last two years (the third case, from Victoria, is unpublished), we also request that the Technical Advisory Group on Immunisation investigate the possibility of obtaining access to BIG from the FDA for the future treatment of infantile botulism in Australia.

REFERENCES

1 McMaster P, Piper S, Schell D, Gillis J, Chong A. A taste of honey. *J. Paediatr. Child Health* 2000; **36**: 596–7.
2 Midura TF. Update: Infant botulism. *Clin. Microbiol. Rev.* 1996; **9**: 119–25.
3 Shone C, Wilton-Smith P, Appleton N *et al.* Monoclonal antibody-based immunoassay for type A *Clostridium botulinum* toxin is comparable to the mouse bioassay. *Appl. Environ. Microbiol.* 1985; **50**: 63–7.
4 Szabo EA, Pemberton JM, Gibson AM, Eyles MJ, Desmarchelier PM. Polymerase chain reaction for detection *of Clostridium botulinum* types A, B and E in food, soil and infant faeces. *J. Appl. Bacteriol.* 1994; **76**: 539–45.
5 Paton JC, Lawrence AL, Steven IM. Quantities of *Clostridium botulinum* organisms and toxin in feces and presence of *Clostridium botulinum* toxin in the serum of an infant with botulism. *J. Clin. Microbiol.* 1983; **17**: 13–15.
6 American Academy of Pediatrics. *Botulism in Red Book, Report of the Committee on Infectious Diseases, American Academy of Pediatrics*, 25th edn. American Academy of Pediatrics, Elk Grove Village, IL, 2000.
7 Shapiro RL, Hatheway C, Swerdlow DL. Botulism in the United States: a clinical and epidemiological review. *Ann. Intern. Med.* 1998; **129**: 221–8.
8 National Health and Medical Research Council. *The Australian Immunisation Handbook*, 7th edn. Technical Advisory Group on Immunisation, NHMRC, Commonwealth Department of Health and Aged Care, Australian Government Publishing Service, Canberra, 2000.
9 Queensland Health Public Health Unit Networks. *Botulism in Control of Communicable Diseases Protocol Manual*, 2nd edn. Queensland Health, Brisbane, 1998.
10 Arnon SS. Infant botulism. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases*, 4th edn. WB Saunders, Philadelphia, 1998; 1570–77.

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Dear Editors

REPLY

May *et al*. have provided another instructive case with further useful comments on the management of infant botulism. In reply to their request for details of the neurophysiology findings, we would happily provide anyone interested with a copy of the complete report, but will add the following summary of relevant details for comparison with other possible future cases.

The nerve conduction velocities were borderline low/normal and latencies normal. The amplitudes were all reduced. Repetitive stimulation resulted in no increment at 2, 20 or 50 Hz, nor was any decrement seen at 2 Hz. The electromyogram showed insertional activity and, at rest, profuse fibrillation-like potentials. On effort, the compound motor action potentials were of short duration. The picture was consistent with denervation, as in infant botulism, but equally with other anterior horn cell pathologies and many other diseases.

Typical electromyography (EMG) findings in infant botulism are not specific to the illness; therefore, EMG cannot serve as a definitive diagnostic study. The brief, small, abundant motor-unit potentials often observed in infant botulism, which may also be found in other conditions, were explained and given the acronym BSAP by W King Engle at the National Institutes of Health in the USA many years ago.[1]

Definitive diagnosis in our patient was made by the mouse neutralization assay, which identified botulinum toxin type A in the faecal extract; *Clostridium botulinum* was also cultured from the faeces. None of the honey samples yielded organisms resembling *C. botulinum.* We agree with May *et al*. regarding the need for early stool samples in order to establish the diagnosis. The inability to identify toxin in faeces in their case