The Central African
Journal of Medicine

Volume 20 AUGUST, 1974 No. 8

A Diagnostic Method for Cholera in the Field

BY

W. CRAIG and J. G. CRUICKSHANK

Department of Medical Microbiology, University of Rhodesia.

AND

B. P. B. ELLIS AND H. FARRELL

Ministry of Health, Rhodesia

When cholera occurs in its classical, clinical and epidemiological form laboratory diagnosis is only required on the index case. Thereafter diagnosis is essentially clinical and treatment and control measures are instituted on that basis.

Recently, however, in certain parts of Rhodesia cases have presented with very varied degrees of severity and in endemic rather than epidemic form — a case or two from one village and several days later one or two more from another village, and so on. In view of the general high incidence of gastro-enteritis, dysentery, typhoid and malaria it is essential to confirm the diagnosis in each and every case. Only then can such factors as prevalence, attack rate, carrier rate, etc., be properly assessed and appropriate control measures instituted.

A number of rapid methods have been advocated — direct microscopy of stool, inhibition of vibrio movement by antibody, examination of alkaline peptone water inoculum after a few hours by dark field microscopy, and others — but all require time, laboratory facilities and not inconsiderable expertise (Barna, 1970). Further, experience with these methods has indicated that results much depend on an individual observer. What is required is a method which is certain rather than probable, which can be performed in the field, which does not require special equipment or particular expertise and which will give a reasonably quick answer. Generally speaking, in a remote area accuracy of diagnosis is more important than speed and a 6 to 18 hour reading is adequate.

The following method has proved successful in one area where no laboratory facilities exist and is based on the ability of V. cholera to grow at low ambient temperatures. Requirements are rectal swabs, TCBS agar plates (Eiken Chem. Co. distributors Burroughs Wellcome), a plate writing pen and a thick plastic bag for disposal of swabs. Rectal swabs from cases or from contacts are plated immediately by cross-hatching onto half or whole plates of TCBS agar to within $\frac{1}{2}$" of the edge. The plates are put into a plastic bag and sealed with tape to prevent drying out. Incubation is at the local ambient temperature and has been carried out in the breast pocket, in the glove compartment of a Land Rover, on window ledges (sunny and shady), in an aircraft cabin, on a bedroom floor and indeed any place convenient to the moment. Obviously the higher the ambient temperature the more rapid the growth but at temperatures varying between 18° and 34°C strikingly luxurious growth is easily seen between 6 and 15 hours after plating. Vibrio cholera is readily identifiable by its bright yellow colour which develops first around the edges of the streaked area. When separate colonies are found they often appear colourless for a few hours after they are first discernable and then turn yellow. Other organisms do grow occasionally on the medium but their appearance is quite different.

Confirmation is necessary particularly from a confluent growth and is by slide agglutination. A slide, orange sticks and two small squeeze bottles (such as used for Widal reagents by Beckman diagnostics) one for saline, the other for polyvalent cholera antiserum, are all that are required. An emulsion is made of the culture in saline on the slide using the orange stick, and a drop of the antiserum is stirred in with another orange stick and agglutination looked for. Infected materials are discarded into large plastic bags for ultimate incineration.

The efficiency of the method compares well with incubation at 37° because (a) further incubation at 37°C of plates read as negative after 24 hours at room temperature produced *no* further positives and (b) parallel plates at

37°C and at room temperature with the same inoculum yielded the same average number of colonies. These tests were performed initially with the E1 tor biotype of V. cholera. When tests were run in parallel with classical V. cholera strains, visible growth took nearly twice as long to appear.

Since the cholera outbreak began in the S.E. of Rhodesia at the end of January 1974, all persons presenting with diarrhoea either to the field hospitals or in their kraals have been swabbed. Where possible the contacts were investigated at the same time. Over 40 clinical cases have been confirmed and two or three asymptomatic carriers detected. Since the vast majority of gastro-enteritis from this area turns out at the moment to be cholera, there are very few negatives to try to assess. However, indications are that this simple technique is both highly accurate and sufficiently sensitive to be of great value in the field.

REFERENCE

BARNA, D. (1970) *W.H.O. Public Health Paper No. 40* p. 47.

TCBS = *Thiosulphate citrate bile salts sucrose agar.*