

## THE ROLE OF THE LABORATORY IN THE CONTROL OF DIARRHOEAL DISEASES\*

M JEGATHESAN MBBS, MRCPath\*\* AND GF de WITT PhD\*\*\*

### Summary

The laboratory can play a useful role in the control of diarrhoeal disease. Early diagnosis and the identification of the pathogenic agent will help in the institution of appropriate control measures. Ancillary tests such as phage typing will serve as useful epidemiological markers in the study of the disease. Laboratory backup is indispensable in maintenance of surveillance not only over the population but also in the monitoring of safety of food and water as well. Laboratory orientated research can contribute greatly towards further understanding of the pathogenic agents, their pathogenesis, host immunity and subsequently the development and trial of vaccines.

### INTRODUCTION

The transmission of any communicable disease will depend on a number of factors - namely, the presence of the pathogenic agent, a source of the agent, a susceptible host, and a suitable means of transmitting the pathogenic agent from the source to the susceptible host.

Communicable gastroenteritis is caused by a wide variety of micro-organisms and the mode of transmission is often through the so-called "faecal-oral" route either directly or through the medium of contaminated food or water. Preventive or control measures will have to be directed towards severing one or more links in this chain of transmission. This would be dependent on the rapid recognition of infection, the nature of the pathogen, the understanding of the mode of transmission, levels of infectivity and susceptibility and the ability to weigh the pros and cons of a variety of potential courses of action.

It will be the purpose of this paper to show how the laboratory can contribute towards some of these approaches to the control of diarrhoeal disease.

### NATIONAL PROGRAMME FOR CONTROL OF ACUTE DIARRHOEAL DISEASE

A National programme for the control of acute diarrhoeal disease should be developed. It would not be enough to take remedial measures when an outbreak of diarrhoeal disease has

occurred. A state of preparedness and continuous surveillance is essential if diarrhoeal diseases are to be brought under control. In cholera for example, it has been shown that in communities that are not prepared for it, the appearance of the disease is associated with case fatality rates as high as 50%. In a community that is prepared this rate is around 1% (unpublished data). This emphasises the need to take all appropriate measures in advance. This can best be ensured by developing a systematic and well co-ordinated National programme. This programme should have a number of components among which will be a good surveillance system and a reliable laboratory service.

### NATIONAL LABORATORY SERVICE

The laboratory service which would feature prominently in the control programme would need to have a country wide network at all levels culminating in a National Reference Laboratory. This laboratory would co-ordinate the efforts of all regional, state and peripheral laboratories, lay down policies and methodologies, provide training for laboratory personnel, run quality control and proficiency testing programmes and perform tests which are either too complicated or too laborious for the smaller laboratories to perform. This central laboratory should also be geared to provide additional manpower, materials and guidance to peripheral areas when sudden outbreaks have to

\*Paper presented at the 7th. SEAMIC Seminar on "Strategies for implementing Primary Health Care activities with special reference to the Control of Diarrhoeal Diseases" in Bangkok, 28th November - 4th December 1979.

\*\*Head, Division of Bacteriology, Institute for Medical Research, Kuala Lumpur (Address for reprint requests).

\*\*\*Director, Institute for Medical Research, Kuala Lumpur.

be investigated and controlled. This laboratory thus has to achieve a sophisticated level of proficiency.

On the other end of the spectrum, the small laboratory in the periphery need not be very elaborate but should at least be able to carry out simple field diagnostic tests and be in a position to collect and receive relevant specimens in appropriate transport media for transmission to more capable laboratories. A satisfactory system for despatching the specimens to the laboratory performing the test should be established.

#### LABORATORY DIAGNOSIS

Determining the etiology of a case of diarrhoea may not be all that important in the treatment of an individual case but it certainly has significant bearing in the epidemiology of the disease and is vital in instituting the appropriate control measures. In this regard microbiological surveillance of diarrhoea should be instituted. A tab must be kept on all diarrhoea cases occurring in a community and deviation from normal trends will signal the necessity for greater attention to be given. In this surveillance programme, specimens from as many diarrhoea cases as possible should be taken for laboratory investigation and the necessary bacteriological, virological, parasitological and serological studies carried out. Such activities will enable the start of an outbreak to be determined should it occur. Once it is known which organism is causing the outbreak, it may not be necessary or even practical for specimens from all cases to be studied.

It is quite true that in studies on what appear to be infective diarrhoea cases the etiological agent is not found in a significant proportion of the cases.<sup>1</sup> This may be due to inappropriate specimens, the relative insensitivity of the method, inadequate range of tests, or to the fact that many infective agents of diarrhoea are hitherto unknown or unrecognised. Furthermore one will only find what one is looking for. However research is being conducted all the time to discover new etiological agents and better methods for detecting them.

The relatively recent recognition of agents such as the rotaviruses, *Campylobacter* and enterotoxigenic *E. coli* as causative agents of diarrhoea is a case in point.<sup>2</sup> With improved

knowledge and techniques concepts are changing all the time. Diarrhoea causing *E. coli* were at one time thought to be restricted to the so-called enteropathogenic serotypes.<sup>3,4</sup> It was subsequently shown that it was not the serotypes which mattered but whether the strain could produce enterotoxin or not.<sup>5</sup> Laboratory methods have now become available for demonstrating this enterotoxin production. The methods however are laborious and require specialised technique and thus not easily performed in any ordinary laboratory. The development of the ELISA technique for determination of the labile toxin of enterotoxigenic *E. coli*<sup>6</sup> will provide a simpler alternative to the traditional Chinese hamster ovary<sup>7</sup> and Adrenal Cell assays.<sup>1</sup> Research should be continued towards finding simpler methods for characterising enterotoxigenic strains so that even average laboratories may be capable of performing the tests.

#### ROLE OF THE LABORATORY IN THE INVESTIGATION OF OUTBREAKS

The laboratory is indispensable in the investigation and subsequent control of outbreaks of diarrhoeal disease. The questions to be answered will be: What is the organism involved? Its characteristic? Who are suffering from it? Who are the symptomless contacts or carriers? What is the source? How is it being transmitted? Is there any environmental source that is playing a significant role in the propagation of the outbreak?

Apart from confirming the etiology in patients with symptoms, stools and other relevant specimens have to be taken from contacts of cases who may be harbouring the organism but showing no signs of the disease. They have to be found as they play an important role in the continued propagation of the outbreak. In El Tor cholera there are very much more symptomless carriers than cases and the ratio can vary anything from 1:25 to 1:100.<sup>9</sup> The search for chronic carriers becomes particularly important in the control of typhoid. This is particularly so in the case of food handlers. In the investigation hundreds of people have to be screened and the laboratory is often hard pressed. The choice of persons to be screened therefore has to be done in a sensible manner with due regard to the capabilities of the lab-

oratory, the expected usefulness of the data obtained and cost benefit. This is particularly so in the non outbreak situation where it is debatable whether food handlers should be screened for bacterial enteropathogens as a routine. The cost of such testing is tremendous and isolation rates are low. Furthermore one stool examination done randomly at one point in time does not really rule out the presence of these enteropathogens in the individual both at that time and in the period between tests.<sup>10</sup> One way to get around this problem of having to screen a large number of people would be to test pooled sewage samples. This will at least help to localise the site where the pathogenic agent is present.

Non-human sources will also have to be investigated. Samples of water from wells, rivers and foods which are likely to be related to the outbreak have to be processed. In the examination of water it must be remembered that organisms even if present tend to be scanty in numbers and some enrichment or concentration technique is essential. In the investigation of typhoid outbreaks water can be collected into Selenite enrichment medium. Alternatively "Moore's swabs" can be used to concentrate the organisms found in running water.<sup>11</sup>

#### EPIDEMIOLOGICAL MARKERS

Typing tests can serve as extremely useful epidemiological markers in the investigation of diarrhoeal disease. Apart from this the different types of an organism will have different biological and ecological characteristics which will help to predict its behaviour in the community and also point towards clues as to its possible origin and modes of spread.

A number of typing mechanisms are available. This includes biotyping, serotyping, phage typing and colicine typing. Their respective epidemiological value depends on the organism. Biotyping is, for example, useful in *V. cholerae* where the classical and El Tor strains are so different in their behaviour as well as in the severity of the disease they cause. Serotyping is particularly relevant in salmonellosis where over a thousand serotypes are recognised. Knowledge of the serotype of the causative agent of an outbreak of salmonellosis has on many an occasion led to the discovery of a common source.<sup>12-14</sup> Serotyping is also performed for

*Shigella*, *E. coli*, *V. cholerae* and *V. parahaemolyticus*.

Phage typing is performed on *S. typhi*, *S. paratyphi* and *V. cholerae*. Because phage types are genetically stable they are useful markers in epidemiological investigation.<sup>15</sup> Knowing the phage types of strains implicated in an outbreak will allow some deduction to be made as to its possible source. This is enhanced by the fact that certain phage types are indigenous to certain areas. Not only are different countries known to have different patterns of phage types, different geographical locations within a country itself may have their own predominant phage types. This particularly becomes significant in this day and age when intra and inter country travel is becoming so prevalent. The value of the knowledge of a particular phage type will vary inversely with the degree of its distribution throughout the world. Thus cosmopolitan types will be less helpful in pointing the possible sources of outbreaks while more restricted phage types will give more significant clues.

The value of phage typing of a particular organism for epidemiological purposes is also related to the number of phage types that can be differentiated for that particular organism. For instance, in the case of *S. typhi* it often proves useful because almost 100 different phage types can be recognised. In the case of *S. paratyphi* A and B this value is naturally restricted because only a few phage types are currently recognised. However as new phage types are continuously being identified and characterised this situation may change.

#### ANTIBIOTIC SENSITIVITY

Monitoring antibiotic sensitivity of organisms responsible for diarrhoeal diseases is another important facet of the laboratory's work. This should be done regularly to see if there is a changing pattern in the susceptibility of the organisms to the useful and commonly available antibiotics. This has very practical considerations because of the increased emergence of late of antibiotic resistance strains. For instance, many of the *Shigella* strains nowadays are resistant to sulphonamides and other commonly used antibiotics such as chloramphenicol and tetracycline.<sup>16</sup> In some parts of the world a large proportion of strains of *S. typhi* which

are isolated are resistant to **chloramphenicol**.<sup>17</sup>

Interest in the antibiotic sensitivities of *S typhi*, other *Salmonellas* and other members of the enterobacteriaceae was stimulated since the report of a massive outbreak of chloramphenicol resistant typhoid in Mexico in 1972.<sup>18</sup> This resistance was found to be caused by carriage of a transferable resistance factor (R factor). R factors have been found in other enterobacteriaceae as well and many of them code for multiple resistance.<sup>19</sup> The serious implications of this cannot be emphasised enough.

#### QUALITY OF FOOD AND WATER

The provision of safe water supplies and food hygiene are **two factors** that **could** contribute significantly to the reduction of diarrhoeal disease.

Water from reservoirs will have to be monitored regularly by both **chemical as well as** microbiological analyses. For microbiological analysis indicator tests such as the presumptive coliform count and the *E coli* count are performed to keep a tab on evidence of **faecal pollution**. Standards are laid down which have to be complied with.

Microbiological standards for certain foods have been laid down with the **expectation that** such measures will reduce the bacteriological hazards that may be derived from such foods. It is not the **purpose of this paper to delve into** the merits and demerits of such standards but merely to point out the laboratory back-up required to implement and police such standards. In many countries such testing of **food** and water is part of the responsibility of a public health laboratory service. In countries which do not have a separate Public Health Laboratory Services this testing is done by existing **laboratories** in the Health and other allied departments.

#### RESEARCH

The laboratory will also feature prominently in research activities. The laboratory may itself conduct basic research involving studies on the causative organisms and immunity in the host or provide laboratory back-up to studies **involving** the epidemiological aspects of the problem. It is through such research that we have reached our Present **level of knowledge of the agents** and their behaviour. Much is left to be learnt.

Areas of research which need to be pursued further include the identification and **characterisation** of agents responsible for diarrhoea, their life cycles, vehicles of transmission and pathogenesis; the development of quicker and simplified diagnostic procedures; the development of reliable serological and skin tests; the immunology of the gastro-intestinal tract and immunity to agents causing diarrhoea; the role of non-specific mechanisms **such as the** nutritional and physiological states in combating **diarrhoeal** disease and finally the development and trial of vaccines.

#### ACKNOWLEDGEMENT

Thanks are due to Mr. Thean Yit Sang for typing the manuscript.

#### REFERENCES

1. Jegathesan M, Singh RB, Kanaganayagy M, Lim ES. The bacterial causes of diarrhoea in Malaysia. *Med J Malaysia* 1976; 31: 46-56.
2. World Health Organization. Control of diarrhoeal diseases: WHO's programme takes shape. *WHO Chron* 1978; 32: 369-72.
3. Taylor J. Infectious infantile enteritis, yesterday and today. *Proc R Soc Med* 1970; 63: 1297-301.
4. Moffet HL, Shulenberger HK, Burkholder ER. Epidemiology and etiology of severe infantile diarrhea. *J Pediatr* 1968; 72: 1-14.
5. Sack RB. Human diarrheal disease caused by enterotoxigenic *Escherichia coli*. *Annu Rev Microbiol* 1975; 29: 333-53.
6. Yolken RH, Greenberg HB, Merson MH, Sack RB, Kapikian AZ. Enzyme-linked immunosorbent assay for detection of *Escherichia coli* heat-labile enterotoxin. *J Clin Microbiol* 1977; 6: 439-44.
7. Guerrant RL, Brunton LL, Schnaitman TC, Rebhun I, Gilman AG. Cyclic adenosine monophosphate and alteration of Chinese hamster ovary cell morphology: a rapid, sensitive *in vitro* assay for the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. *Infect Immun* 1974; 10: 320-7.
8. Donta S, Smith D. Stimulation of steroidogenesis in tissue culture by enterotoxi-

- genic *Escherichia coli* and its neutralization by specific antiserum. *Infect Immun* 1974; 9: 500–5.
9. **Benenson AS.** Cholera. In: Top **FH**, Wehrle PF, eds. Communicable and infectious diseases. Saint Louis: The CV Mosby Company, 1972: **142–51**.
  10. Jegathesan M. Is routine stool examination of food handlers worthwhile? *Med J Malaysia* 1976; 31: **113–4**.
  11. **Moore B**, Perry EL, Chard ST. A survey by the sewage swab method of latent enteric infection in an urban area. *J Hyg (Camb)* 1952; 50: 137–56.
  12. Jones HB, Farkas G, Hobbs BC. *Salmonella brandenburg*: an epidemiological study. *Monthly Bull Minist Health* 1964; 23: 162–77.
  13. Gilbert RJ. Cross-contamination by cooked-meat slicing machines and cleaning cloths. *J Hyg (Camb)* 1969; 67: 249–54.
  14. Sanford DA, Leslie DA, **McKeon JA**, Crone PB, Hobbs BC. *Salmonella senftenberg* in the Sunderland area. *J Hyg (Camb)* 1969; 67: 75–9.
  15. **Ohashi M.** Enteric fever eradication programme in Japan. Proc Second **SEAMIC** Seminar, Tokyo 1974 Dec 9–14: 16–47.
  16. Fukaya K. Clinical aspects of Shigellosis, **Salmonellosis**, Yersinia infection and enteropathogenic *E coli* infections. Proc Second **SEAMIC** Seminar, Tokyo 1974 Dec 9–14: 157–63.
  17. World Health Organization. Chloramphenicol resistant *S. typhi* in Vietnam and Thailand. *Wkly Epidemiol Rec* 1974; **35**: 295–7.
  18. **Anderson ES**, Smith HR. Chloramphenicol resistance in the typhoid bacillus. *Br Med J* 1972; 3: 329–31.
  19. **Anderson JD**, **Gillespie WA**, Richmond MH. Chemotherapy and antibiotic-resistance transfer between enterobacteria in the human gastro-intestinal tract. *J Med Microbiol* 1973; 6: 461–73.