

## The Role of Non-agglutinable Vibrios in Diarrhoeal Diseases

by

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### INTRODUCTION

YAJNIK and Prasad (1954) described an epidemic of diarrhoea caused by non-agglutinable vibrios among pilgrims who attended the Kumbh Fair at Allahabad. The diarrhoea lasted 1-4 days. McIntyre, Feeley, Greenough III, Benneson, Hassan and Saad (1965) from East Pakistan reported that non-agglutinable vibrios produced fever in addition to diarrhoea.

Ceylon has been free from cholera since 1953 (Report, 1954). The organisms usually responsible for diarrhoeal diseases in Ceylon are salmonellae, shigellae and enteropathogenic *Escherichia coli* (Velaudapillai, Jayasundera and Nagaratnam, 1966). So far no work on the isolation of non-agglutinable vibrios from stools has been carried out in Ceylon. This paper deals with attempts to determine the presence of non-agglutinable (NAG) vibrios in Ceylon.

### MATERIALS AND METHODS

Most of the published reports on NAG vibrios have been on work done with fresh specimens of stools. At the Medical Research Institute, Colombo, practically all the specimens from diarrhoeal cases are received by post in Stuart's transport medium as modified by Rogers and Taylor (1961). Even when specimens were suspected to have vibrios, they were sent in this medium. Hence, initially an attempt was made to find out a single transport medium suitable for specimens containing vibrios as well as for pathogenic members of the enterobacteriaceae group.

The viability of salmonellae, shigellae, pathogenic *E. coli*, *Vibrio cholerae* and NAG vibrios in Stuart's medium and V. R. medium of Venkataraman and Ramakrishnan (1941) was tested. In Stuart's semisolid medium, pure culture of *V. cholerae* and NAG vibrios were viable for a period of over a week but when these organisms were mixed with faeces both survived for two days only. Similar results were obtained with the same medium even at pH values of 8.6 and 9.2. Trials with liquid Stuart's transport medium with these ranges of pH yielded the same type of results. In the V.R. medium, enterobacteriaceae mixed with faeces were killed in two days, while *V. cholerae* mixed with faeces were viable

for over a week and NAG vibrios for four days. Although the vibrios survived for two days in Stuart's medium, it was realised that owing to the postal delays, the specimens might not always reach the Medical Research Institute, within this period. It was therefore, decided to use both Stuart's and V.R. media. These two media are extensively used by workers abroad for enterobacteriaceae and *V.cholerae* respectively.

The swabs from the transport media were plated using the method suggested by Monsur (1961) and also on the older Aronson's medium and then inoculated into alkaline peptone water from which subcultures were made after incubating for six hours. Swabs from Stuart's medium prior to the inoculation into the peptone water were plated on Wilson and Blair's and S.S. agar media, tetrathionate and selenite broths for the detection of salmonella and shigellae. If the specimens were from children below two years, platings were done on MacConkey agar for pathogenic *E.coli*. Suspicious colonies were picked up from primary as well as secondary plates into a modified Kligler's medium (Velaudapillai, 1962) from which agglutination was done with polyvalent and factor sera. The identification of the members of the enterobacteriaceae group was done according to the methods described by Velaudapillai, Jayasundera and Nagaratnam (1966). The usual biochemical tests were carried out for the identification of the vibrios. They were the oxidase test as was done by Kovacs (1956), the string test according to Smith (1958), the chick cell agglutination test by the method of Finkelstein and Mukerjee (1963), the haemolysis test according to Feeley and Pittman (1963) and polymyxin sensitivity test as reported by Roy, Mridha and Mukerjee (1965). The pathogenicity test in the ligated guts of adult rabbits was done according to the method used by the WHO International Vibrio Phage Typing Centre, Calcutta.

#### RESULTS

A total of 601 faecal specimens from diarrhoeal cases in different parts of Ceylon were examined. The following places sent over 20 specimens: Amparai 65, Batticaloa 80, Hambantota 21, Koslanda 36, Kayts 22, Mannar 206 and Mullaitivu 21. However, positive isolations were confined to the coastal towns in the Northern Province, namely Kayts, Mannar and Mullaitivu.

All the vibrios gave positive reactions with the string test, oxidase test, chick cell agglutination test, haemolysis test, gelatin liquifaction test and V.P. test and negative polymyxin. The results of the other tests are summarised in Table I.

Vibrios isolated from patient 2678 agglutinated with polyvalent and Ogawa sera. This together with the other tests indicated that the organism was *V.cholerae* (Ogawa) bio type *EL TOR*. This strain was resistant to phage IV at the critical test dilution (CTD) but at one decimal strength higher than CTD, it was sensitive. Of the nine strains that were isolated, seven were from diarrhoeal cases, one from a contact and the other from a septic tank.

It was noticed that more isolations of vibrios were made from direct plating than from enrichment in alkaline peptone water.

TABLE 1

## Significant Reactions of Isolated Vibrios

No.	Place	Agglutination	Sensitivity to Mukerjee phage IV	Heiberg's group	Pathogenicity
P	Mannar	-ve	-ve	1	-ve
45	Mannar	-ve	-ve	1	-ve
46	Mannar	-ve	-ve	11	+
800	Kayts	-ve	-ve	11	-ve
2279	Kayts	-ve	-ve	11	-ve
2678	Kayts	+Ogawa	-ve	1	+
2925	Kayts	-ve	-ve	1	-ve
2280	Mullaitivu	-ve	-ve	11	-ve
3019†	Polonnaruwa	-ve	-ve	11	-ve

† 3019 was not from a case but from a septic tank.

Mukerjee's phage IV was cholera phage.

Some of the organisms that were isolated were positive for oxidase and string tests, but their morphology and biochemical reactions such as gas in glucose proved that they belonged to the aeromonas group.

## PATIENT STUDIES

Case P: A male 38 years old from Mannar had profuse diarrhoea, resembling cholera. On admission he was severely dehydrated. The specimen of stool sent to the Medical Research Institute for culture had the typical rice water appearance. NAG vibrios were isolated in pure culture. The patient was given antibiotics and rehydration therapy and recovered in a weeks time. Subsequent specimens of stools were negative for vibrios.

Case 45: A male 60 years old from Mannar. His diarrhoea was not severe.

Case 46: The third case from Mannar was also a male 75 years. Here too the diarrhoea was not of a severe nature.

All these three isolations were made within a period of one week of each other.

Case 2280: A male 36 years old from Mullaitivu. He had chronic diarrhoea. He was treated three times at the hospital in March, April and June, 1967 respectively. On his first admission to the hospital, he gave a history of having had 7-8 motions a day.

Case 800: A male of 70 years from Kayts. On admission he had diarrhoea of 8 days duration with no fever.

Case 2279: A male 70 years from Kayts. He had diarrhoea of 8 days duration with significant dehydration but no fever.

Case 2678: This patient was also a male from Kayts. He was 58 years old and had diarrhoea of one month duration. According to the history given, he had as many as 10 motions on certain days. *V.cholerae* biotype *EL TOR* was isolated from this case. He had never been to India.

Case 2925: A female 60 years old was a contact of case No. 2678 from whom *V.cholerae* biotype *EL TOR* was isolated. NAG vibrios were isolated from this case. She did not give any history of diarrhoea in the immediate past.

In all these cases no other pathogens were isolated. On making a close study of these cases, what strikes one forcibly is that, except in the case P, the majority belonged to 58 and 75 age groups and, case 2678 the diarrhoeas were not severe.

#### DISCUSSION

Although Venkataraman and Ramakrishnan (1941) stated that their medium was not suitable for the isolation of NAG vibrios, in the present investigation, all the nine strains of vibrios were isolated in this medium. No isolations were made from Stuart's medium. During the closing stages of this work it was found that McIntyre and Feeley (1965) had isolated this type of vibrio by using Monsur's (1963) liquid transport medium. In our preliminary study, one strain of NAG vibrio was found to be viable for four days in V.R. medium, but it is not possible to say whether all strains of NAG vibrios would have been viable in it. Considering the work of McIntyre and Feeley (1965), a trial with Monsur's liquid transport medium would be helpful in determining the extent to which NAG vibrios are present in diarrhoeal cases and in normal people.

Patient P had typical symptoms of cholera but the rabbit pathogenicity test was negative. In addition there were two positives and five other negatives to this test in this series. Therefore, much reliance cannot be placed in this test.

Abou-Gareeb (1967) reported that NAG vibrios were isolated from the first case of a cholera epidemic in Afghanistan. From the subsequent cases *EL TOR* vibrios were isolated. He also stated that all the people who were affected had no contact with the people who came from other countries. In Ceylon, although no epidemic of cholera occurred in Kayts in 1967, the first two diarrhoeal cases had NAG vibrios and the third had *EL TOR* and his contact had NAG vibrios. All these cases were continuously residing in Ceylon, and had no contacts with people from other countries prior to detection of vibrios. It is of interest to mention that in the last outbreak of cholera in Ceylon in 1953, cases were confined to Jaffna in the Northern Province and several of them were from Kayts.

Pollitzer (1957) in his review of the work of Pillay and others stated that when true cholera vibrios were consumed by fish the vibrios might mutate into NAG vibrios. In this form they would have been kept viable for a longer time. It is possible that when these organisms were passaged through human intestines they mutated back to true cholera

ABOU-GAREEB  
FEELEY  
FINKEL  
KOVACH

vibrios. Kayts is closer to India where cholera is endemic. The fish from the Indian coastal waters might have swallowed true cholera vibrios and excreted them as NAG vibrios along the northern coastal waters. It might also be possible that cholera vibrios from 1953 epidemic in Ceylon might have been maintained as NAG vibrios by the fish in the northern sea coast.

The agglutinable vibrios from case 2678 belonged to Heiberg's group I into which the majority of cholera vibrios fall. The NAG vibrios from the contact of this case also belonged to the same group. Further NAG vibrios from case P from Mannar which gave a typical clinical picture of cholera also belonged to this group. Some of the other NAG vibrios belonged to group II into which a small proportion of true cholera vibrios including *EL TOR* vibrios fall.

Since both true cholera vibrios and NAG vibrios fall into groups I and II, the non-agglutinability of NAG vibrios may be due to mutational change. These mutational changes may affect not only agglutinability but also other properties such as biochemical reactions as shown by the differences between NAG and *EL TOR* vibrios on the one hand and the classical cholera vibrios on the other, in tests like the polymyxin sensitivity, phage-resistance, sheep cell haemolysis and chick cell agglutination.

From the public health point of view, it is most desirable to examine, faecal specimens from all diarrhoeal cases for vibrios especially in the North and North Central Provinces. Since cholera is not endemic in Ceylon, a continued study in this country may help to show whether NAG vibrios could change into agglutinable forms and *vice versa* under certain natural conditions.

#### SUMMARY

1. Non-agglutinable vibrios were isolated from diarrhoeal cases. Most of the isolations were from elderly people.
2. *V. cholerae* biotype *EL TOR* was isolated from a diarrhoeal case who was continuously residing in Ceylon.
3. All the vibrios isolated from stools were from people residing in the northern coastal towns of Kayts, Mannar and Mullaitivu.
4. Only one strain of NAG vibrio isolated from a diarrhoeal case was pathogenic to adult rabbits.

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