Original Articles.

SEASONAL VARIATIONS OF CHOLERA BACTERIOPHAGE IN NATURAL WATERS AND IN MAN, IN CALCUTTA DURING THE YEAR 1930.

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IN Bengal cholera seasons, as statistics clearly show, recur at the same time each year. In Calcutta there are two seasons, one of two months preceding, and another of two months following, the monsoon rains, that is, in April and May, and in November and December, respectively. The statistics of the last sixty years show very clearly that there is a marked abeyance of cholera activity during the rainy season. High temperature and humidity, stagnation of the air, great range in the diurnal temperature, changes in the subsoil-water levels, and various other explanations have been advanced to account for the seasonal prevalence of the disease. There is undoubtedly some relationship between the meteorological conditions and cholera activity, but the exact nature of this relationship has never been fully established.

Cholera, when introduced into an area, spreads rapidly, persists for a while and then rapidly subsides. d'Herelle and Malone (1927) suggest that the cessation of an epidemic of cholera is due to the spread of bacteriophage from convalescent cases. The virulent cholera vibrio no longer exists as such; a bacteriophage-contaminated avirulent vibrio takes its place and plays an important rôle in the spread of bacteriophage.

Cholera exists in Calcutta throughout the year, although there are two definite cholera seasons which are apparently determined by climatic influences. Conditions for localized epidemics, which d'Herelle and Malone studied and reported upon, do not exist here.

In order to obtain some idea of the prevalence of bacteriophage in Nature and its relationship to the incidence of the disease in Calcutta, samples of water from the river and from 'tanks' were examined throughout the year 1930. Certain tanks were selected with a view to having a representative collection of the natural waters. A uniform technique was employed throughout the year and altogether 385 samples were examined.

Source, frequency and methods of examination.

Samples were obtained mainly from the following places:—

(*i*) Baboo Ghat on the banks of the river Hooghly—a regular bathing ghat.

(*ii*) Dalhousie Square tank—situated in the midst of a modern business quarter; here bathing, etc. is strictly prohibited.

(*iii*) Cornwallis Square tank—situated in an Indian business and residential quarter —open to bathing.

(*iv*) College Square tank—situated in an Indian business and residential quarter —open to bathing.

(*v*) Campbell Hospital tank—situated in the compound of that hospital—bathing prohibited.

Waters from other sources were examined from time to time. Samples were collected by the senior staff of the laboratory in sterile Jena-glass bottles, all contamination being carefully guarded against. At least one sample from each source was examined every week.

On receipt at the laboratory, about a 100 cubic centimetres were added to 10 cubic centimetres of a 10 per cent. solution of peptone water in flat-bottomed flasks; these were incubated overnight. The surface growth was plated on bile-salt agar for vibrios, and the sample filtered through a Chamberland filter. In order to increase the amount of bacteriophage that might be present in the filtrate, a young peptone-water culture of a vibrio, known to be free from bacteriophage and capable of propagating all types of cholera 'phage, was added, and the flask again incubated. After filtration through a candle this second filtrate was tested for the presence of cholera 'phage. One centimetre of a four-hour-old peptone-water culture of a pure vibrio was added to 10 cubic centimetres of papain broth, varying amounts of the filtrate added, and the emulsion immediately spread on an agar plate. Samples showing the presence of bacteriophage were, after passage with a susceptible vibrio, tested for the type or types of cholera 'phage present.

Examination of cholera 'phage in man.

Patients admitted to the Carmichael Hospital for Tropical Diseases were examined for the presence of cholera 'phage in their stools. (No cholera cases are admitted into this hospital.) The stools in 403 cases were examined during the year and the technique employed was similar to that described above.

The results are given in the following table (I), and also in graphic form (figure I). The incidence of cholera is drawn from the monthly rate of admission into the Campbell Hospital (Indian cases) and the Presidency General Hospital (European cases). The meteorological data are from the Meteorological Office at Alipore, Calcutta.

The chief facts to be noted are:—

(*i*) The maximum activity of cholera in 1930 was during the months of March, April, May and June, with an average of 290 cases per month. In July there was a rapid fall and

TABLE I.

Table showing the seasonal variations of cholera bacteriophages in water and in man in Calcutta during the year 1930, *together with the incidence of cholera, and the meteorological data.*

Month.	Water.		Man.		Cholera cases (Campbell Hospital).	Cholera cases (Presidency General Hospital).	Cholera mortality, per cent.	Meteorological data.			
	Number examined.	Cholera 'phages isolated, per cent.	Number examined.	Cholera 'phages isolated, per cent.				Mean of daily mean maximum temp. °F.	Mean of daily mean minimum temp. °F.	Rainfall in inches.	Daily mean humidities, per cent.
January ..	57	14	33	9	75	1	34.66	77.6	55.4	0.58	66
February ..	19	5	26	..	126	3	30.64	83.5	61.6	0.77	62
March ..	50	30	20	5	265	5	15.47	93.2	71.0	0.43	61
April ..	40	37	50	6	315	11	19.05	98.1	77.2	0.11	63
May ..	53	11	44	10	298	6	17.11	95.3	78.9	4.66	74
June ..	17	18	39	5	287	8	18.81	93.3	79.5	8.59	81
July ..	41	12	46	2	112	4	17.85	88.8	78.9	20.12	87
August ..	32	..	31	3	38	3	5.27	88.3	79.1	14.67	88
September	23	4	27	..	26	..	3.84	89.7	79.3	7.02	85
October ..	20	..	45	5	40	1	Nil	89.7	76.5	1.08	77
November	14	21	15	..	45	..	6.66	81.5	66.1	2.43	74
December	19	..	27	6	21	..	9.52	77.7	55.8	Nil	67

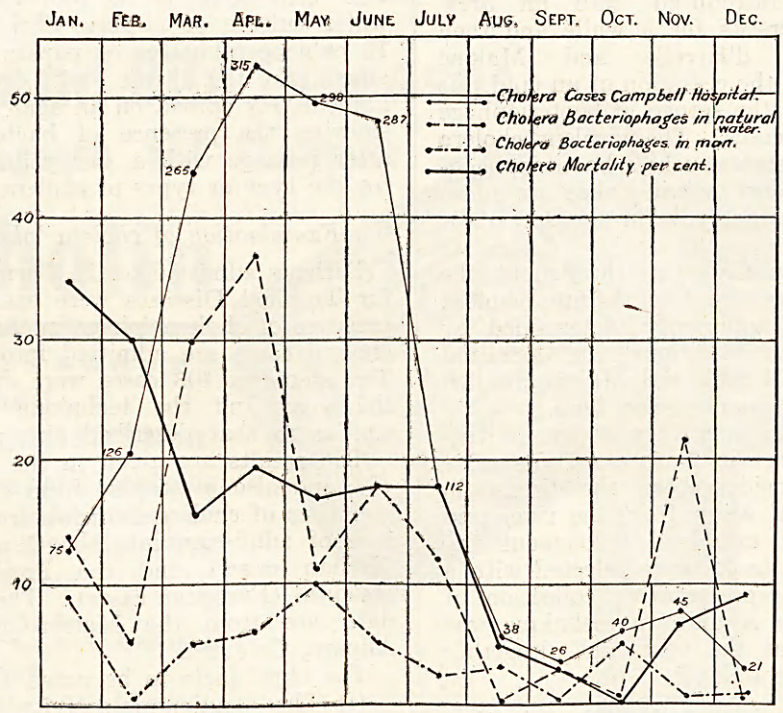


Fig. I.—The seasonal variations of cholera incidence and the percentage mortality, the percentage of waters containing cholera bacteriophages, and the percentage of healthy individuals passing cholera bacteriophages, in Calcutta during the year 1930.

in August and September there were only 38 and 26 cases, respectively. There was a slight increase in October and November and a fall again in December. The curve of cholera incidence is a typical one.

(*ii*) The meteorological data illustrate the close relationship between cholera activity and rainfall. Once the rains have set in there is a very definite fall in the incidence of the disease. A study of the temperature curves will show that during the months of the greatest difference between the maximum and minimum temperatures cholera is active.

(*iii*) Bacteriophage. The curve of cholera 'phages in waters follows very closely the curve of cholera cases. If we disregard the fall in May (which might possibly be due to some trouble we had in our media during that month) the similarity of the curve is very marked. With the decline of cholera activity there is almost a complete absence of cholera 'phage in waters.

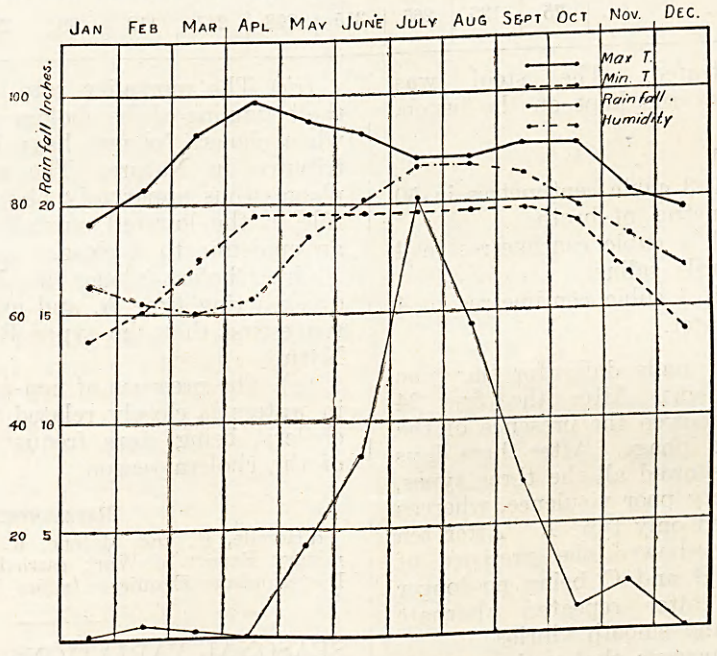
In man, the curve of cholera 'phage runs almost parallel with the incidence of disease, the 'phages present in Nature reaching the maximum a month after the maximum curve of cholera incidence.

(*iv*) The mortality rate. The percentage death rate is high in the beginning of the year, but falls rapidly in March when cholera 'phage becomes widely distributed in Nature. From March to July, although cholera incidence is maximum, the death rate remains low and falls with the decline in cholera incidence.

The cholera 'phages isolated from natural waters.

Out of 385 samples of waters examined during the year 57, or 14.7 per cent., showed the presence of cholera bacteriophage. The type of cholera 'phage present is shown in the following table.

It will be noticed that the majority of the cholera bacteriophages isolated from waters in Calcutta are of the quick-acting type A, which acts only on smooth strains of vibrios. Kind 62A (Asheshov) of type A is the commonest type of cholera bacteriophage present in Nature. Kind 64A type A cholera 'phage was isolated on rare occasions. Bacteriophages types B and C were isolated only in 3 out of the 385 waters examined, and experiments designed to increase any existing cholera 'phage types B and C by growth on rough strains of vibrios, which would propagate these two types and not type A, failed to demonstrate any cholera 'phages of these types in the original filtrates. All



Meteorological data.

TABLE II.

Analysis of the types of cholera 'phages isolated from 385 *samples of waters in Calcutta.*

Cholera 'phages.	Number of samples of water from which 'phage was isolated.	Percentage of waters containing cholera 'phages.	Percentage of each type of cholera 'phage.
Type A.			
Kind 61 A.	Nil	..	..
Kind 62 A.	52	13.5	90
Kind 63 A.	Nil	..	..
Kind 64 A.	2	0.5	4
Type B.	1	0.2	2
Type C.	2	0.5	4

types of cholera 'phages must have originally contaminated the waters; either types B and C die out, or our technique was not delicate enough to demonstrate their presence. The following experiment offers a possible explanation.

Cholera stool No. 1325 collected on 9th July, 1931, from a case of cholera on the second day of the disease—agglutinating vibrios present in the stool. The original filtrate of the stool showed type A cholera 'phage only, but after passage with a susceptible vibrio all three types could be demonstrated. The stool was distributed into flasks and kept on the bench, as follows:—

(*i*) Original stool.
(*ii*) Original stool, 3 cubic centimetres in 50 cubic centimetres of broth.
(*iii*) Original stool, 3 cubic centimetres in 4 litres of sterile saline.
(*iv*) Original stool, 3 cubic centimetres in 4 litres tap water.

Examinations were made daily for the type of bacteriophage present. After the first 24 hours all the flasks showed the presence of the three types of cholera 'phage. After three days flasks 1 and 2 still showed all the three types, B and C being of very poor virulence, whereas flasks 3 and 4 showed only type A. After ten days all the flasks showed the presence of type A only, types B and C being no longer demonstrable, even after repeated alternate transfers on rough and smooth vibrios.

This experiment suggests that under natural conditions types B and C cholera 'phages die out.

The cholera 'phages from healthy individuals.—Out of 403 samples examined during the year, 17, or about 4 per cent., showed the presence of cholera 'phage; all were of type A (kind 62A, Asheshov) cholera 'phage, and were all poor races of bacteriophage. We found only 3 vibrio-passers in this series. We could find no association between those passing bacteriophage and those passing vibrios.

The vibrios isolated from water.—We isolated non-agglutinating vibrios from 59 out of 366 samples of waters examined. The following table gives the percentage of water samples containing vibrios each month, and for comparison the cholera incidence taken from table I is also given.

It will be seen that the presence of non-agglutinating vibrios in waters follows very closely the incidence of the disease.

Summary.

(*i*) Cholera in point of prevalence is very intimately related to and dependent upon the climatic and seasonal influences. The heavy rainfall during the monsoon checks the activity of cholera.

(*ii*) Cholera 'phages in Nature vary with the incidence of the disease. It is rare to isolate cholera 'phages from waters during the non-cholera season.

Table III.

	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Percentage of waters containing vibrios.	13	37	46	17	17	6	5	Nil	Nil	10	21	Nil
Cholera cases	75	126	265	315	298	287	112	38	26	40	45	21

(*iii*) The mortality rate, which is high at the beginning of the cholera season, falls rapidly when cholera 'phages have become widely distributed in Nature. The spread of bacteriophages thus apparently plays a very important rôle in the lowered mortality, and in bringing an epidemic to a close.

(*iv*) Cholera 'phages in Nature are of the quick-acting type A, and evidence is presented suggesting that the types B and C die out in Nature.

(*v*) The presence of non-agglutinating vibrios in waters is closely related to the incidence of cholera, being more frequent at the beginning of the cholera season.

Reference.

d'Herelle, F., and Malone, R. H. (1927). A Preliminary Report of Work carried out by the Cholera Bacteriophage Enquiry. *Indian Med. Gaz.*, LXII, 614.

SEASONAL VARIATIONS OF DYSENTERY BACTERIOPHAGES IN NATURAL WATERS AND IN MAN, IN CALCUTTA DURING THE YEAR 1930.

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Samples of waters from the river Hooghly and certain tanks were examined throughout the year for the presence of bacteriophages active against *B. paradysenteriæ* (Flexner) and *B. dysenteriæ* (Shiga).

The source, frequency of examination, and the technique employed were similar to that fully detailed in our report on the variations of cholera bacteriophages in Nature, which will be found elsewhere in this number of the *Gazette*.