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Diphtheria in Ceylon Review of Bacteriological Typing of 643 Cases

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Incidence

The incidence of Diphtheria in Ceylon shows a steady increase from 1 case in 1905 to 219 cases in 1949. Table 1 gives incidence by decades. In the earlier periods the increase was no doubt due to better investigation and reporting of cases, while the progressive increase in the later years could be attributed to the additional factor of over-crowding. The incidence is greatest in the Western Province (Table 2) chiefly in Colombo and its vicinity, where the population and over-crowding are most marked.

TABLE 1

Diphtheria incidence by decades.

1905–1914	1915–1924	1925–1934	1935–1944
68	174	474	1,544

The number of cases for the period 1945 January to 22nd February, 1950 is 983

TABLE 2

Diphtheria incidence in Western Province expressed as a % of total cases.

1942	1943	1944	1945	1946	1947	1948
69·2%	64·9%	71·2%	65·3%	79·1%	75·7%	77·3%

The highest incidence of Diphtheria is observed in the months April to August, (Table 3) which constitute the hottest and the most humid months of the year.

TABLE 3

Diphtheria incidence, average mean temperature and average degree of humidity by months.

Year	*Jan.*	*Feb.*	*Mar.*	*April*	*May*	*June*	*July*	*Aug.*	*Sept.*	*Oct.*	*Nov.*	*Dec.*
1945 ..	14	19	24	32	35	25	15	15	8	21	15	19
1946 ..	14	8	13	20	22	22	31	25	8	14	16	8
1947 ..	14	8	6	9	19	28	9	15	6	6	12	8
1948 ..	4	11	7	16	20	30	15	20	19	12	13	14
1949 ..	19	18	10	14	19	42	25	26	10	14	13	8
Total ..	65	64	60	91	115	147	95	101	51	67	69	57
Average Mean Temp. ..	79·2	79·7	81·0	81·9	82·2	81·4	80·9	80·8	81·0	79·9	79·4	79·0
Average Humidity % ..	70·	68·	68·	72·	77·	79·	78·	77·	76·	76·	76·	72·

Fatality Rates

This is fairly high and is probably due to several factors:—

1. Milder cases not being treated.
2. More serious cases seeking treatment late.
3. The prevalence of mitis strain in Ceylon which may be responsible for a high proportion of obstructive laryngeal diphtheria. H. Mason Leete (1945) found a relative increase of mitis infection in obstrucive cases in Hull.
4. The most important factor probably is the absence of immunity of the population.

TABLE 4

Fatality rate by years.

1940	1941	1942	1943	1944	1945	1946	1947	1948	1949
11·57%	31·20%	20·74%	16·06%	20·50%	19·00%	13·43%	22·14%	13·51%	13·69%

The fatality rate will remain at a comparatively high level until the immunity of the population is increased by artificial means.

Laboratory diagnosis

Primary Isolation.—Before 1939 inspissated serum medium alone was used for the primary isolation and diagnosis of diphtheria. In 1939 Clauberg's tellurite medium was introduced and ever since all swabs are first inoculated on inspissated serum and then on Clauberg's medium. The introduction of the Clauberg's medium as a primary culture medium has materially helped in the laboratory diagnosis of diphtheria, for while the inspissated serum aids in arriving at a speedy result—growth of many diphtheria strains being detected four or more hours before colonies on Clauberg's make their appearance, Clauberg's medium suppresses most of the throat commensals and gives characteristic well separated blue colonies with or without condensation of the blue. Inspissated serum on the other hand suffers from the disadvantage in allowing throat commensals to grow while a chance sporebearer reduces the surface rapidly to a fluid state; it also has the added weakness that certain strains of C. diphtheria would not grow on the serum medium—Goldie and Maddock (1943).

A comparative study of the serum and Clauberg's media for primary isolation in a series of 133 cases is shown in Table 5.

TABLE 5

Primary isolation on two media.

Inspissated serum	*Clauberg's Medium*	*Number of strains*	*Percentage isolated*
+	+	106	79·7
+	—	9	6·8
—	+	18	13·5

While dealing with the subject of primary isolations mention must be made of the presence of monilia in 155 cases in a series of 2,361 throat swabs, that were cultured. In 4 cases monilia was associated with diphtheria.

Typing.—Typing was started in 1946 and since then is being done as a routine measure. During the period under review January, 1946 to February, 1950, 643 strains were typed on Neill's (1937), tellurite medium.

This medium is an excellent selective medium for C. diphtheria and the advantage lies in its simplicity of preparation. It allows organisms of C. diphtheria to produce characteristic and distinctive colonies, the diphtheroids are easily distinguished from the diphtheria bacilli, while gravis, intermedius and mitis colonies are sharply defined.

The colony appearance together with the fermentation tests enable the typing of most strains.

Procedure of typing.—Morphologically and culturally positive colonies on Clauberg's medium are picked and sub-cultured of serum. An emulsion of 24 hour growth is made in broth and a drop plated on Neill's medium. After 48 hours incubation the plates are studied using a hand lens or plate microscope when necessary. This technique gives well separated colonies showing characteristic colonial appearance for the three types of C. diphtheria.

The diphtheria types with the exception of six fall within the classification of gravis, intermedius and mitis as described by McLeod and his co-workers (Anderson Cooper, Happold, McLeod and Thompson, 1931, 1933).

Mitis was found to be the prevalent average strain of diphtheria bacillus 88·5% of the series falling into category of mitis.

TABLE 6

Result of typing of 643 strains.

Types	*Number of Strains*	*Percentage*
Gravis	20	3·1
Intermedius	54	8·4
Mitis	569	88·5

The six atypical strains which differed from the normal in their reaction on starch are as follows :—

4 Mitis-like colonies which fermented starch.

1 Intermedius-like starch fermenting colony.

1 Colony with Gravis-like colony characters that failed to ferment starch.

0·93% or 6 in 643 atypical strains met with in these series, is in keeping with the findings of Carter in Glasgow, who found them to the extent of 0·25% in 1,600 strains (McLeod 1943). Wright in South Africa in a small series, observed 13% of such strains.

Fermentation tests

A set of serum peptone water tubes is inoculated from a pure culture on serum medium. The four serum water tubes are (1) 1% glucose, (2) 1% Dextrin, (3) 1% Saccharose, (4) 0·5% Starch. They were observed after 48 to 72 hours.

The indicator used is Bromo-Thymol Blue (1% of a 0·2% Alcoholic Solution) which gives a good three colour range, blue on the alkaline side, yellow on the acid side with green in between.

A reference has to be made to 15 Saccharose fermenting C. diphtheria strains that were met with. The colony characteristics were typical of mitis in 14 and 1 was of the intermedius type. No virulence test was done on these strains. Mause Evelyn and Keown Margret, J. (1946) isolated 8 strains of C. diphtheria from the throat which were typical and virulent except for production of acid in saccharose.

Haemolytic activity.—The Haemolytic activity of the organism was tested on freshly prepared ox and rabbit blood agar slopes. 84·3% of mitis strains was haemolytic on both ox and rabbit blood while lysis was variable with the remaining mitis and the intermedius and gravis strains examined.

Virulence.—Virulence was tested in the usual way by intradermal inoculation of guinea pigs. A series of 135 strains, 127 of which were mitis was tested for virulence. The gravis strains were not tested. The 8 intermedius strains tested proved to be all virulent. The gravis-like atypical strain that failed to ferment starch referred to above, was tested and found to be virulent. The investigation revealed that 57·5% of the mitis strains was virulent.

TABLE 7

Results of virulence test on 135 *strains.*

Type	*No. Tested*	*Virulent*	*Avirulent*
Mitis	127	73	54
Intermedius	8	8	0

Note—It is regretted that the diphtheria strains of the series are not available.

Summary

1. There is a steady increase in the incidence of diphtheria in Ceylon.

2. The fatality rate is high.

3. Primary inoculation was originally done with serum alone; since 1939 Clauberg's tellurite medium is used in conjunction with serum.

4. Comparative study of serum and Clauberg's as media for primary isolation showed that 79·7% could be isolated from both, 6·8% on serum alone and 13·5% on Clauberg's medium only.

5. Monilia was found in 155 throat swabs examined.

6. Typing started in 1946, is done on Neill's tellurite medium. 88·5% of a series of 643 strains was found to be mitis.

7. Virulence Test revealed that 57·5% of the mitis strain was virulent

References

1. ANDERSON, J. S., HAPPOLD, F. C., MCLEOD, J. W. and THOMPSON, J. G (1931), *J. Path. Bact.*, 34, 667.
2. ANDERSON, J. S., COOPER, K. E., HAPPOLD, F. C. and MCLEOD, J. W. (1933), *J. Path. Bact.*, 36, 169.
3. D.M. and S.S., Ceylon Annual Administration Reports.
4. GOLDIE, W. and MADDOCK, E. C. G. (1943). *Lancet*, 1, 285.
5. MCLEOD, J. W. (1943), *Bact. Rev.*, 7, 1.
6. MASON LEETE (1945), *J. Hyg.*, 44, 192.
7. MAUSE EVELYN and KEOWN MARGRET, J. (1945), *J.Sc. Sept.* 13. 252-3.