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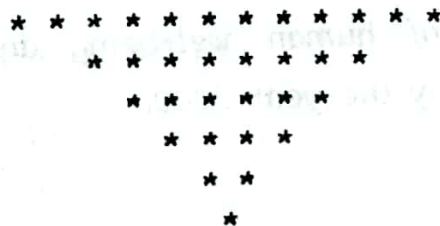
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HEALTH FOR ALL BY THE YEAR 2000

In September, 1978 an International Conference on Primary Health Care was held in Alma-Ata Capital of the Kazik Soviet Republic. The conference declared that the health status of hundreds of millions of people in the world today was unacceptable. A strategy was laid down to achieve health for All by the Year 2000. Health was defined as a stats of complete physical, mental and social wel-being and not merely the absence of disease or infirmity. A list of targets in support of achievement of Health for all strategy were laid down. In nut shell institutions of Primary Health Care would result in achieving this targets. This involves the application of social, biomedical and health services research and Public Health experience at community level. The main problems of community are tackled through providing promotive, preventive, curative and rehabilitative services. The primary health care centres would achieve health for all the providing safe water supply and basic sanitation, maternal and child health care, immunization against major infectious diseases, prevention and control of locally endemic diseases, appropriate treatment of common diseases and injuries and provision of essential drugs. A pivotal role involves community participation and appropriate health education. Health development cannot occur as isolated event. Related sectors such as agriculture, animal husbandry, food, industry, education, housing, public workers, communications etc. would require to be developed as an integral part of the process of human wel-being and attainment of the goal for health for all by the year 2000.

THE PATTERN OF VIBRIO EL-TOR EPIDEMICS IN PUNJAB, PAKISTAN

BY

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and

Dr. Syed Abdul Rashid²

SUMMARY

An epidemiological study of cholera in Pakistan from the year 1964 to 1979 with special focus on Punjab Province was carried out. It was concluded that classic cholera was not endemic in Punjab, Pakistan. On the other hand *Vibrio el-tor* introduced into Karachi in July, 1964 spread to various towns connected with Karachi by the highway and rail road entering the Punjab in the same month. A detailed study of Punjab indicated that Lahore, Multan and Sialkot where repeated epidemics occurred during sixteen years constitute the endemic areas. In Lahore the Mohallah wise distribution pin pointed nine endemic areas. More work is required for determining the role of contact carriers in the maintenance of a reservoir of infection.

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1. INTRODUCTION

The 7th pandemic of cholera caused by Vibrio cholerae bio-type el-tor started in Makassar in Sulawesi (Celebes), Indonesia in the year 1961. According to Tanamal (1959) this constituted an endemic area for Vibrio cholerae bio-type el-tor infection because of repeated isolations from the stools of persons with mild diarrhoea and of symptomless individuals. As quoted by Barua (1972), the history of the pandemic was as follows :-

El-tor cholera spread from the Celebes to Jawa in May, 1961. By August it spread to Kalimantan (Borneo), Hong Kong, and Canton. In September it entered Philippines and in November Sumatra. In 1962 Saba was infected in January, Taiwan in July and West New Guinea in October. In 1963 the pandemic spread to the main land of the Asian continent. Malaya was infected in May 1963 and by the end of December Singapore, Thailand, Cambodia, and Burma had been invaded. It entered Vietnam in January, 1964. Korea, Bangla Desh (at that time East Pakistan), India, and West Pakistan (Karach) were all infected by the end of 1964. There was a build up of the number of cholera cases in Bangla Desh, India and Pakistan and the subsequent progress of the pandemic became more dramatic in 1965. The pandemic entered Afghanistan, Iran, USSR and Iraq in 1966. As reported by Carter (1970) there was a stalemate in the reporting of cholera outbreaks to the World Health Organization because of the irrational restrictions placed by the new countries being invaded as it spread further westwards during the years 1967-1969. In 1970 there was a big spread of the pandemic to 24 new countries, 6 in Asia, 15 in Africa and 3 in Europe.

The objective of this paper is to bring on record various out breaks of Vibrio el-tor infections since its introduction into Pakistan in 1964 and to bring to light the areas where repeated outbreaks occurred.

2. MATERIALS AND METHODS

2.1 The laboratory of the Bacteriologist, Punjab Government constitutes the provincial reference centre for the bacteriological diagnosis of cholera epidemics. Before 1970 this laboratory was dealing with epidemic outbreaks in the whole of West Pakistan. In 1970 its area of jurisdiction was reduced to the Punjab province. The comparative study is therefore restricted to the Punjab. However, the epidemics investigated before 1970 include the entire area of West Pakistan.

Specimens for Vibrio el-tor investigation are received from the affected field areas by teams sent out by this laboratory till the epidemic is diagnosed. Subsequently they are sent to the laboratory through special messengers by District Health Officers of the area affected.

2.2 Specimen collection

Stool, vomit, soiled clothes were transported in liquid Monsur's medium (Monsur 1961). Where a stool specimen was not available rectal swab was taken. The swab was collected by the method advocated by Monsur and Rahman (1966). A wide bore stiff rubber tube which had been greased was introduced into the rectum. The swab was passed well up the rectum through the tube. The time of collection was mentioned on the specimens. Directions were given to ensure that specimens reached the laboratory within six hours of collection in Lahore and on the same day from out-stations. Specimens from cases which were positive were repeated and patients discharged from hospital if three consecutive stool specimens were negative for Vibrio el-tor.

In order to determine the vehicles responsible for the spread of the epidemic, samples of flies from the epidemic area, left over eatables which had been consumed during the last 24 hours, samples of water supply and sewage have also been tested in all epidemics.

Flies were transported in liquid Monsur's medium. Food samples were added to bottles of Monsur's transport medium so as to constitute a 1:1 mixture by volume.

Water samples were transported in large screw-capped media bottles. An equal volume of double strength liquid Monsur's medium was added to the water sample being collected. Volume of water collected was 100 ml. No refrigeration was done during transport but it was ensured that all specimens reached the laboratory in 24 hours.

Sewage samples were similarly collected. The volume of the sewage sample collected was 100 ml.

The Monsur's liquid medium not only constituted an effective transport medium but also acted as selective and enrichment medium. Growth of Vibrio el-tor would occur during transport and thus save the time of incubation.

All specimens reached the laboratory within 24 hours of collection from outstations and generally within 6 hours in Lahore.

2.3 Culture of Specimens

The specimens were processed immediately on reaching the laboratory. They were sub-cultured on Monsur's Medium and Modified Aronson's medium (Bokhari 1973) and a tube of alkaline peptone water (pH 8.5). Peptone water was sub-cultured on Monsur's medium after 6 hours incubation at 37° C.

2.4 Identification of Vibrios

The organism was identified by the following tests :-

- 2.4.1 Biochemical tests using sucrose, mannose and arabinose. (Heiberg 1934)
- 2.4.2 Cholera Red Reaction.
- 2.4.3 Haemolysin activity (Feeley & Ritman, 1963) Haemolysin production was studied by the cover layer method. A thin layer of nutrient agar poured in a plate. On this a layer of sheep's blood agar to which organisms had been added was poured. Results were read by the development of a halo of clearance around the colony.
- 2.4.4 V.P Test (Barritt, 1936).
- 2.4.5 Chick cell agglutination (Finkelstein and Mukerjee 1936).
- 2.4.6 Polymyxin 'B' Sensitivity (Gan and Tjia, 1963).
- 2.4.7 Phage sensitivity (Mukerjee 1963).
- 2.4.8 Serological typing. (Pollitzer & Burrows 1959) Sera were obtained from Seato Cholera Research Laboratory, Dacca, East Pakistan or from Difco Laboratories.

2.5 Sampling of epidemic outbreaks

Specimens were received from various parts of Pakistan/Punjab, during the summers of the years, 1964, 1965, 1966, 1968, 1969, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1978 and 1979. The total number of cases which occurred in any epidemic have not been documented. However, the number of cases which report for admission to various infectious diseases hospitals or cholera treatment camps mobilized during epidemics are being reproduced.

We are however, basing our reliance on cases yielding positive Vibrio el-tor. It may further be observed that only one stool is collected from the patient and the percentage of positive cultures from stool samples is 34-40% in our laboratory.

3. RESULTS

Vibrio el-tor serotype Ogawa was introduced in Pakistan in the year 1964.* The first case occurred in Liari (Karachi) in the Bengali Colony which was frequented by relatives from Bangla Desh. The epidemic spread to other cities along the rail road and highway entering Punjab. Ever since 1964 outbreaks of cholera have occurred in the Punjab every year.

Cases of diarrhoea and gastro-enteritis are admitted in various hospitals throughout the year. In the middle of summer when the number suddenly increases and cholera is suspected, the majority of cases were sent to Infectious Diseases Hospital or isolation camps. Admission figures of Infectious Diseases Hospital, Lahore, for the year 1964 to 1979 are available and are given in Figure-I.

Epidemics which occurred in Lahore were studied in detail whereas other parts of Punjab effected were studied to the extent that after a diagnosis of *Vibrio el-tor* infection in a case was confirmed further cases of gastro-enteritis were presumed to be *Vibrio el-tro* infections. The Lahore epidemics on the other hand were studied in greater detail and an area wise (Mohallah wise) occurrence of *Vibrio el-tor* infections has been worked out. This is enumerated in Table-I and Figure II.

CHOLERA EPIDEMICS, LAHORE: 1964-79

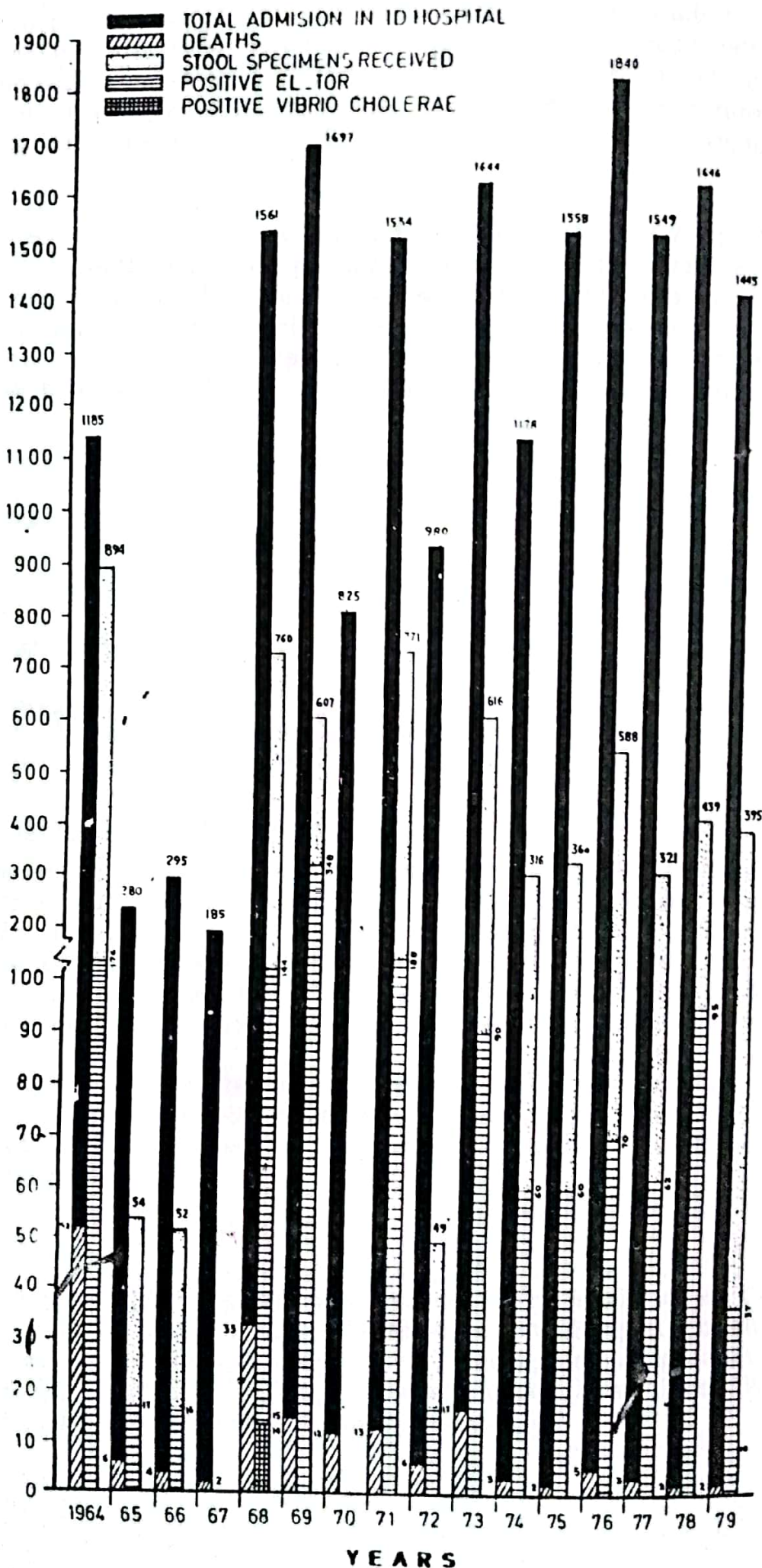
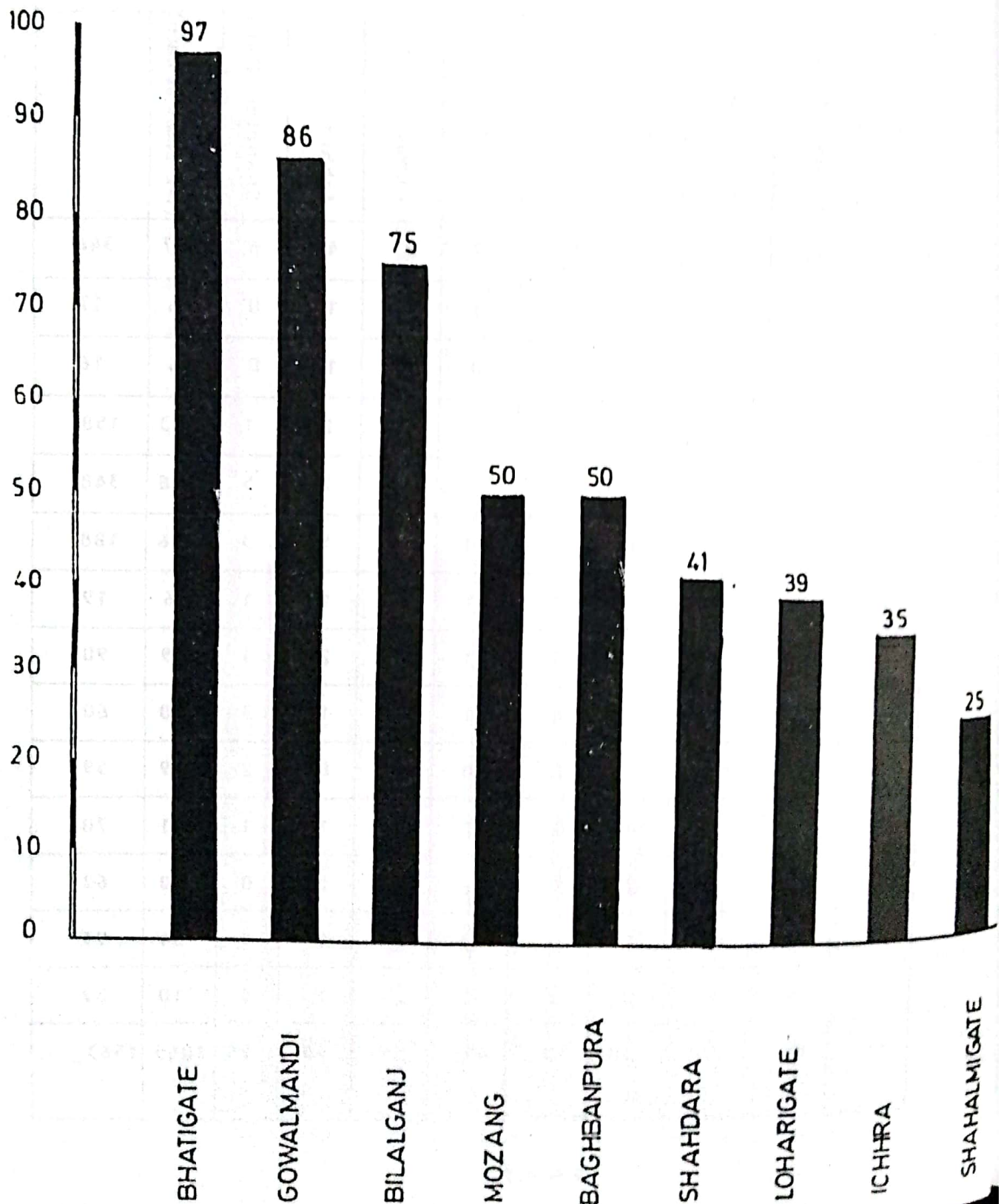


TABLE - I

NUMBER OF POSITIVE CASES (MOHALLAH WISE) OF VIBRIO
EL-TOR GASTROENTERITIS FROM 1964-1979
IN LAHORE CITY

Y E A R	M O H A L L A H S										TOTAL
	Bhati Gate	Gawal Mandi	Bilal Ganj	Mozang	Baghbanpura	Shahdara	Lohari Gate	Ichhra	Shahalmi Gate	Other Mohallahs (1-2 cases in each)	
1964	40	21	7	9	12	2	8	4	6	237	346
1965	1	2	3	1	1	1	1	1	0	6	17
1966	1	2	3	1	1	0	1	1	0	6	16
1968	4	18	3	2	1	1	3	2	1	123	158
1969	14	17	12	14	7	10	4	7	5	258	348
1971	9	6	9	11	9	0	4	11	3	126	188
1972	2	2	3	1	1	1	1	1	1	6	19
1973	4	9	3	3	3	3	3	2	1	59	90
1974	4	0	4	1	4	1	2	1	3	40	60
1975	2	4	5	4	0	0	3	0	2	39	59
1976	5	1	3	0	2	2	4	1	1	51	70
1977	4	1	0	2	4	8	1	2	0	40	62
1978	2	2	6	1	3	11	2	1	1	64	93
1979	5	1	14	0	2	1	2	1	1	10	37
Grand Totals	97	86	75	50	50	41	39	35	25	1065	1563

NO. OF POSITIVE CASES (Mohalla-wise)
OF VIBRIO EL-TOR GASTROENTERITIS
FROM 1964-1979 IN LAHORE CITY.



From these results it was found that vibrio el-tor epidemics occurred in Bhati Gate, Gowalmandi, Bilal Ganj, Mozang, Baghbanpura, Shahdara, Lohari Gate, Ichhra and Shah Almi Gate. These areas have been demarcated in the Lahore Map in Figure-III.

From the Lahore Map it is evident that Vibrio el-tor infections are occurring in the congested areas of Lahore where slum conditions prevail. There is a lack of an effective underground sewage system. There are open drains. Food vendors sell their produce in open shops or road side stalls. An item of common sale is cut fruits placed on hand driven carts. Another feature is leaking water pipes which may even pass through open drains. Water supply is also intermittent leading to suction of sewage water from drains or open collection of stagnant water.

In order to pin point the areas from which the first case occurred in Lahore Table II was compiled. It mentions the date of occurrence of first case and the area in which it occurred. It is to be noted that four epidemics started in Bilal Ganj, three in Gowalmandi and Bhati Gate, two in Baghbanpura and one each in Ichhra and Shah Almi Gate.

TABLE - II
AREA WISE DISTRIBUTION OF 1st POSITIVE CASE IN
VIBRIO EL-TOR OUT-BREAKS IN LAHORE CITY
FROM 1964-79*

	1964	1965	1966	1968	1969	1971	1972	1973	1974	1975	1976	1977	1978	1979	TOTAL
Bilal Ganj			25.7			6.5		27.7						9.5	4
Gowalmandi	30.7	29.7		17.5											3
Bhati Gate					27.5		28.7			1.8					3
Baghbanpura									17.5				30.7		2
Ichhra												28.8			1
Shah Almi Gate											21.7				

* Specimens were not sent to this laboratory in the years 1967 and 1970.

FIG. III AREA WISE DISTRIBUTION
CHOLERA EPIDEMICS LAHORE



A study of the seasonal distribution of *Vibrio el-tor* gastroenteritis occurring in Lahore city was made and is expressed in Table III-A and III-B.

TABLE - III-A

SEASONAL DISTRIBUTION OF VIBRIO EL-TOR CASES IN LAHORE

YEAR	MAY	JUNE	JULY	AUG:	SEPT.	OCT	TOTAL
1964			16	277	53		346
1965			8	7	2		17
1966			6	9	1		16
1968	10	36	61	37	14		158
1969	5	63	141	131	8		348
1971	34	107	40	7			188
1972			8	11			19
1973			2	58	30		90
1974	7	18	20	15			60
1975				48	11		59
1976			2	20	48		70
1977				10	33	19	62
1978			26	46	21		93
1979	36	1					37

(Number of positive cases expressed monthwise)

TABLE III-B

SEASONAL DISTRIBUTION OF FIRST AND LAST*
POSITIVE CASE DURING THE EPIDEMIC

Y E A R	MAY	JUNE	JULY		SEPT.	OCTOBER
1964			30-7-1964		8-9-1964	
1965			29-7-1965		1-9-1965	
1966			25-7-1966		3-9-1966	
1968	17-5-1968				16-9-1968	
1969	27-5-1969				6-9-1969	
1971	6-5-1971			17-8-1971		
1972			28-7-1972	25-8-1972		
1973			27-7-1973		27-9-1973	
1974	17-5-1974			30-8-1974		
1975				1-8-1975	22-9-1975	
1976			21-7-1976		30-9-1976	
1977				28-8-1977		20-10-1977
1978			30-7-1978		27-9-1978	
1979	9-5-1979	5-6-1979				

*Last case is given in italics

Table III-A enumerates the number of cases occurring in each month where as Table-III B gives the date of occurrence of first and last case. It may be noted that five epidemics started in the month of May, seven in the month of July, and two in the month of August. One epidemic ended in June, three in August, nine in September and one in October. It may be concluded that the epidemic period starts from middle of May and lasts till the middle of October.

Table IV gives the age distribution of positive *Vibrio el-tro* cases. The largest number of cases occur in the age group 2-10 years and the incidence diminishes with increasing age.

TABLE - IV

AGE DISTRIBUTION OF POSITIVE VIBRIO EL-TOR
CASES IN LAHORE EPIDEMICS 1964-1979

AGE GROUP	*2-10	11-20	21-30	31-40	41-50	51-60	61 & above
No.	445	314	318	167	119	110	90
Percent-age	28.4	20%	20.3	10.6	7.6	7%	5.7

*This age group is from 2-10 years since cases below 2 years are not admitted in I.D. Hospital.

A study of occurrence of *Vibrio el-tor* infections in infants has not been made since these cases are not treated in Infectious Disease Hospital. However, in a study of bacterial causes of gastroenteritis in infants admitted in Ganga Ram Hospital, Lahore, it was found that *Vibrio el-tor* infections occurred in two infants below the age of six months. They were both bottle fed.

In the end of 1965, 423 water samples were tested for the presence of *Vibrio cholerae*. Samples were taken from waters of wells, hand pumps and electric tube-wells from the border areas of Lahore and Kasur districts effected by the 1965 War. Out of 423 water samples tested 171 yielded *Vibrio el-tor* serotype Ogawa.

In the year 1970 in the month of January and February 1160 pilgrims proceeding to Mecca for Haj were tested for carriage of *Vibrio el-tor* in their stools, this being a requirement for their entry into Saudia that year. The stool specimens tested were not obtained after a purgative since most people objected to purgation. Two persons out of 1160 were found positive for *Vibrio el-tor* serotype Ogawa. They had not suffered from gastroenteritis earlier and were other wise healthy.

In 1977 an outbreak of gastroenteritis occurred in two barracks of Kasur Jail. Eleven inmates out of 358 developed symptoms. They were living in two adjacent barracks in the jail. These barracks were drawing drinking water from a common hand pump which was communicating with the open sewage drain and was contaminated with *Vibrio el-tor* serotype Ogawa. The sewage was also positive for *vibrio el-tor*. A saline purge (Magnesium sulphate-20G) was given to all the inmates who had not suffered from gastro enteritis

or diarrhoea. Stool samples were thereafter collected. Seven persons of 347 tested yielded positive cultures of *Vibrio el-tor* serotype Ogawa (Holt 1982). It was concluded that the epidemic had been introduced by the into the barracks of a symptomless carrier.

VIBRIO EL-TOR OUT BREAKS IN OTHER DISTRICTS OF PUNJAB

Table V, Table VI and Figure IV enlist various districts of Punjab effected from 1964 to 1979. The town of Multan and Sialkot along with its neighbour villages, (Daska, Sambriyal, Pasroor, Talawan, Narang Mandi) are worth of special mention.

TABLE - V

VIBRIO EL-TOR OUT BREAKS IN VARIOUS DISTRICTS OF PUNJAB FROM 1964 - 1979

- 1964 Lahore, Gujranwala, Kasur and Sheikhupura.
- 1965 Lahore, Multan, Rawalpindi, Jhelum, Gujrat and Kasur.
- 1966. Lahore, Multan and Sialkot.
- 1967 *Lahore, Multan and Sialkot (Daska, Sambrial, Pasrur, Talawan).
- 1968 Lahore, Multan and Sialkot.
- 1969 Lahore, Multan, Sialkot (Daska), Rawalpindi, Jhelum, Gujrat, Gujranwala, Sheikhupura, Kasur, Faisalabad, Sargodha.
- 1970. *Lahore and Multan.
- 1971 Lahore, Multan, Sialkot, Sheikhupura, Kasur, Faisalabad and Sargodha.
- 1972 Lahore and Multan.
- 1973 Lahore, Multan, Gujrat, Gujranwala, Sheikhupura, Kasur, Mianwali Jhang.
- 1974. Lahore, Multan, Sialkot and Sheikhupura.
- 1975 Lahore, Multan, Attock (Campbellpur), Gujranwala, Sheikhupura, Rahim Yar Khan.
- 1976 Lahore, Multan, Sialkot, Gujrat, Sheikhupura, Sargodha and Rahim Khan.

- 1977 Lahore, Multan, Sialkot, Gujranwala, Kasur, Faisalabad and Sahiwal.
- 1978 Lahore, Multan, Sialkot, Attock (Campbellpur), Jhelum, Sheikhupura, Kasur, Sargodha, Faisalabad, Dera Ghazi Khan and Bahawalpur.
- 1979 Lahore, Multan and Sialkot.

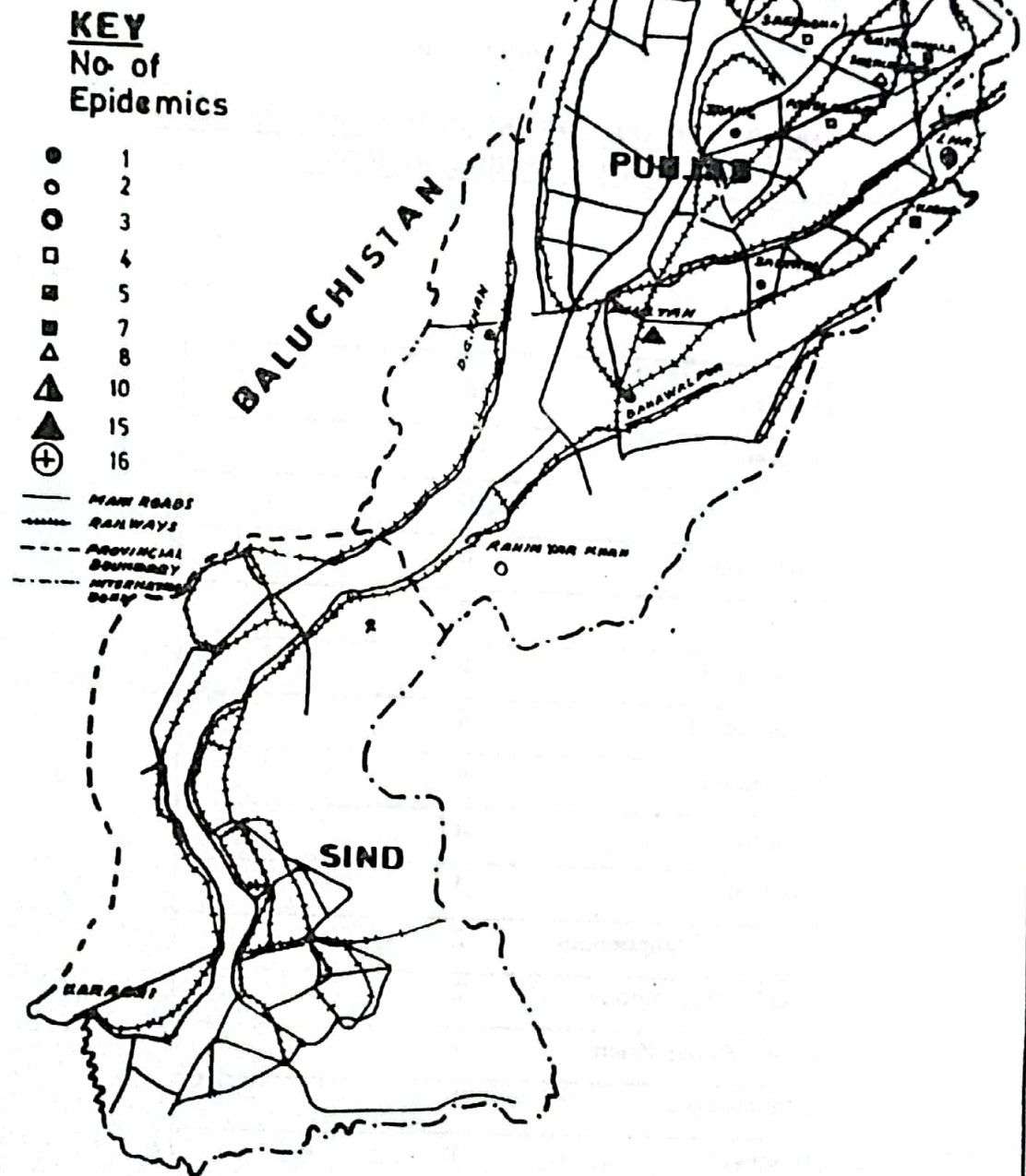
*Cases of gastro enteritis occurred but were not investigated bacteriologically.

TABLE - IV

NUMBER OF OUT-BREAKS IN DIFFERENT AREAS OF
PUNJAB DURING 16 YEARS.

Lahore	16
Multan	15
Sialkot	10
Sheikhupura	8
Kasur	7
Gujranwala	5
Faisalabad	4
Sargodha	4
Gujrat	4
Jhelum	3
Campbellpur	2
Rahim Yar Khan	2
Dera Ghazi Khan	1
Bahawalpur	1
Sahiwal	1
Jhang	1
Minawali	1

FIG. IV MAP SHOWING OUT-BREAKS IN
DIFFERENT AREAS OF PUNJAB
DURING 16 YEARS (1964-79)



Cases of gastro enteritis occurred in Multan practically every year. (Table VI). In an epidemiological study conducted in Multan in the year 1968, 1971 and 1977, the area revealing the highest endemicity was the interior of the city comprising Haram Gate, Dehli Gate, Daulat Gate and Pak Gate.

The 1968 epidemic in Multan was a dual epidemic. It effected 5017 persons. Out of these 69 deaths occurred. There were two epidemic peaks. The first occurred from 19th April to 6th July effecting 3090 persons with 54 deaths. The causative organism was classic Vibrio cholerae serotype Inaba. The second epidemic started in the end of July and lasted till the end of October effecting 1927 persons with 15 deaths. It was due to Vibrio el-tor serotype Ogawa. It is to be noted that this is the only incidence of classic cholera epidemic in the Punjab during the period under study. It was a limited outbreak and spread to Lahore and Sialkot. 14 cases occurred in Lahore and 6 in Sialkot.

In the 1968 Multan epidemic classic Vibrio cholerae was isolated from the open sewage drain as well as from different parts of the piped water supply. Water was not being continuously chlorinated. It was an intermittent supply and there were many points where the pipes were leaking. In 1971 and 1977 the same situation was found in Multan. It is for this reason that epidemics in Multan during these three years were extensive.

DISCUSSION

- 1.1 Vibrio el-tor serotype Ogawa epidemic first occurred in the Liari area of Karachi in May 1964. Although not proven bacteriologically there is evidence to denote that it was introduced from Bangla Desh (then East Pakistan).
- 1.2 Epidemics of classic cholera occurred in Pakistan in 1947, 1948, 1949, 1958 and 1960 (Ahmad et al, 1962) and 1968 (Khan and Gilani, 1969). Since 1968 there has been no outbreak of classic cholera and it may be presumed that Pakistan does not give any evidence of endemicity of classic cholera.
- 1.3 On the other hand Vibrio el-tor introduced in Pakistan in 1964 spread to various towns along the Rail road upcountry and epidemics have occurred every year since then.
- 1.4 From the present study covering the period 1964-1979 epidemics in Punjab occurred in Lahore every year, in Multan for 15 years, in Sialkot for 10 years, in Sheikhpura for 8 years, in Kasur for 7 years, in Gujranwala for 5 years, in Faisalabad, Sargodha and Gujrat for 4 years, in Jhelum for 3 years and for 1-2 years

in Campbellpur, Rahim Yar Khan, Dera Ghazi Khan, Bahawalpur, Sahiwal, Jhang and Mianwali (Table VI) out of sixteen years of study. There appears to be strong evidence that Lahore, Multan, and Sialkot constitute towns in which endemic foci exist.

- 1.5 The study on the area wise distribution in the Lahore city (Table I figure II & III) indicates that epidemics occurred in 9 mohallahs every year namely Bhati Gate, Gowalmandi, Bilal Ganj, Mozang, Baghbanpura, Shahdara, Lohari Gate, Ichhra and Shah Almi. On the other hand the remaining mohallahs did not have epidemics every year. In addition only 1-2 cases occurred in them whenever they were effected. These results indicate that the endemic foci exist only in the areas mentioned above and that cases occur in other areas through contact with cases or food products marketed in the endemic areas mentioned above.
- 1.6 The study in Kasur jail indicated that a number of symptomless contact carriers occurred in a closed community. The investigation of pilgrims proceeding to Mecca indicates that symptomless El-tor carriers with a negative past history of gastro enteritis also occur. Further study would be required to determine the total load of symptomless carriage in the population in the endemic foci in Lahore.
- 1.7 The present study contains information on all the positive cases occurring in Lahore during the years 1964-1979. These cases were admitted in Infectious Diseases Hospital and treated with antibiotics so that the chance of their becoming carriers is remote. However, their immediate contacts could be studied to determine the length of symptomless carriage among them.

A case could then be built as to how important a role these carriers play in constituting a reservoir of infection from which epidemics start every year. This study could further be augmented with a study of contact carriers developing in the next epidemic i.e. in the year 1980. The carriers detected during this study should be kept under surveillance and studied to arrive at the exact role which they play in initiating future epidemics.

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LITERATURE CITED

- Ahmed, N. 1962 Epidemiological observations based on cholera outbreaks in recent years in West Pakistan. Pak. J. Hlth. 12(2): 59-75.
- Barrit, M. M. 1936 The intensification of the Voges Proskauer reaction by the addition of naphthol. J. Pathol. Bacteriol 42 : 441-454.
- Barus, D. 1972 The global epidemiology of cholera in recent years. Proc. Roy. Soc. Med. 65 : 423-428.
- Bokhari, S. R. 1973 Culture media for Vibrio-cholerae. A Review. The Pakistan Journal of Health. 23(2): 1-12.
- Bokhari, S. R. 1982 Personal communication.
- Carter, I. D. 1970 Bilateral, Multilateral and international co-operation in cholera prevention. Bull. Wld. Hlth. Org. 34 : 371-378.
- Feely, J. C. 1963 Studies on the haemolytic activity of El-tor Vibrios. Bull. Wld. Hlth. Org. 28 : 347-456.
- M. Pitman.
- Finkelstein, 1936 Haemagglutination: A rapid method for differentiating Vibrio cholerae and El-tor Vibrios. Proc. Soc. Exp. Biol. Med. 112 : 355-359.
- R.A. S. Mukerjee.
- Gan. K. H. 1963 A new method for the differentiation of Vibrio comma and Vibrio El-tor. Amer. J. Hyg: 77 : 184-186.
- S. K. Tjia.
- Heiberg, B. 1934 Des reactions de fermentation chez les Vibrions. C. R. Biol. (Paris) 115 : 924.
- Khan, A. M. 1969 Laboratory observations on cholera in West Pakistan. Pak. J. Hlth. 19(1).
- S. Gilani.

- | | | |
|--------------------------------|------|---|
| Monsur. K. A. | 1961 | A highly selective gelatin-taurocholate-tellurite medium for the isolation of <i>Vibrio cholerae</i> .
Trans. Soc. Trop. Med. Hyg. 55 : 440-442. |
| Monsur, K. A.
Rehman. M. A. | 1966 | Training course in clinical Aspects, Epidemiology and control of Cholera, Dacca, East Pakistan. Page-18. |
| Mukerjee. S. | 1963 | The bacteriophage susceptibility test in differentiating <i>Vibrio cholerae</i> and <i>Vibrio El-tor</i> .
Bull. Wld. Hlth. Org. 28 : 333-336. |
| Pollitzer, R.W.
Burrows. | 1959 | Problems in immunology.
pp 202-372. In R. Pollitzer: Cholera. Monograph series, No. 43. W.H.O. Geneva. |
| Tanamel S.T.W. | 1959 | Notes on para-cholera in Sulawesi (Celebes).
Amer. J. Trop. Med. 8 : 72-78. |

A STUDY OF CHOLERA IN THE CITY OF LAHORE IN 1980

S. R. Bokhari¹ and S. A. Rashid²

SUMMARY

The history of El-tor cholera epidemics in Pakistan since 1964 has been reviewed. The 1980 cholera epidemic in the city of Lahore has been studied in detail.

In order to gauge the extent of cholera infection in the community two studies were carried out. The Bilal Ganj cholera contact study revealed that 45.5% house-hold contacts of cholera cases were cholera carriers. 80% of these presented as mild cases and 19.6% as symptomless carriers.

The Shahdara cholera study was performed in order to determine the load of El-tor cholera infection in a community where an outbreak of cholera had occurred. It was found that 40.2% of the general population were cholera carriers. Out of these 66.6% presented as mild cases and 33.3% as symptomless carriers.

Factors contributing to the spread of cholera were studied. It was found that flies infected with El-tor vibrios contributed to the spread of cholera and that professions involving close contact with faeces such as sweepers showed a high carrier rate.

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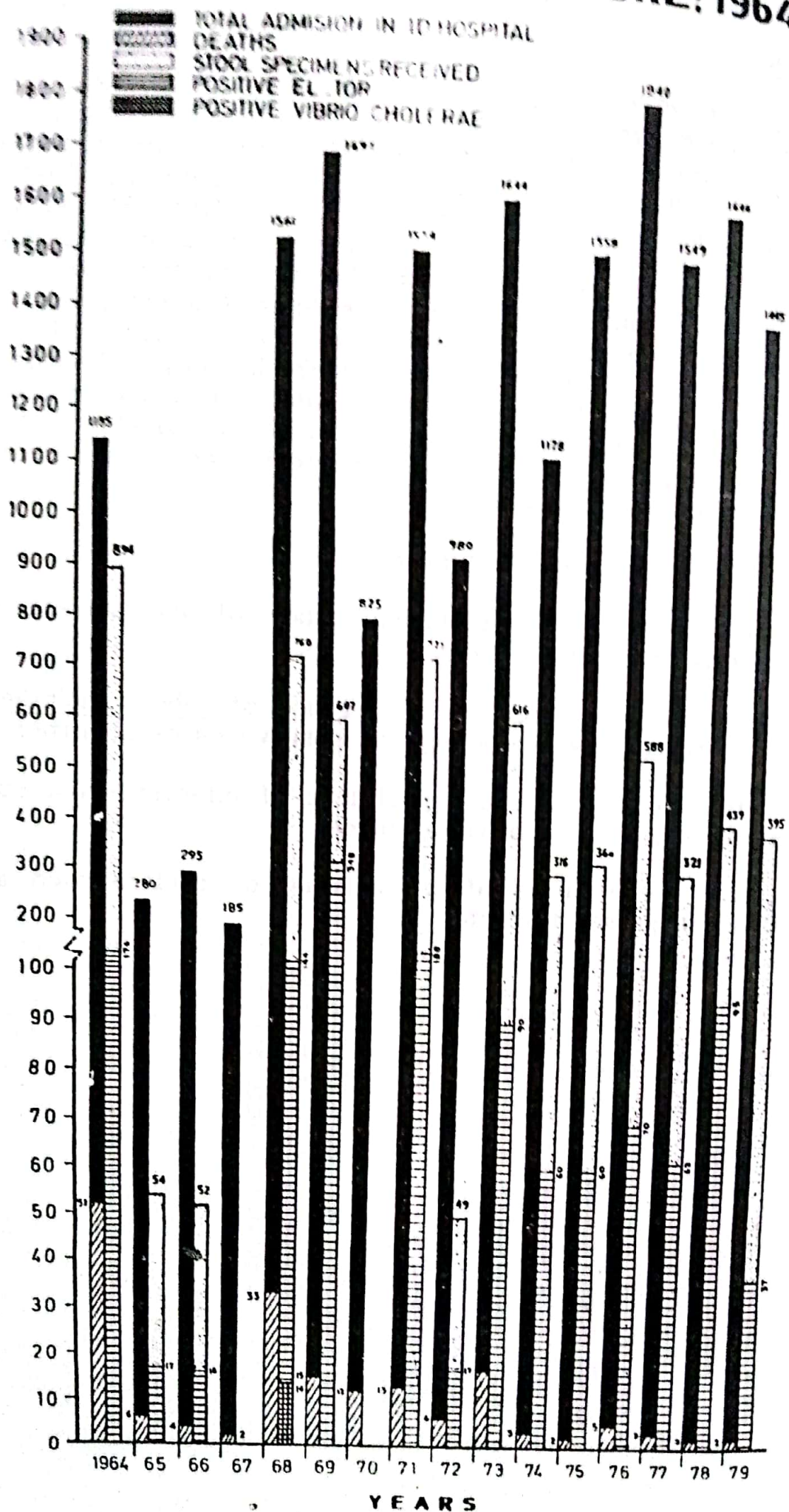
INTRODUCTION :-

Yaqoob (1944) while studying the endemicity of cholera in the Indo Pakistan sub continent did not consider the area covered by West Pakistan as endemic for classic cholera. Ahmed et al (1962) however described outbreaks of classic cholera in the years 1947, 1948, 1958 and 1960 in West Pakistan. Classic cholera epidemics did not occur after 1960 except for a small limited epidemic in Multan town in 1968 reported by Khan and Gilani (1969). Bokhari (1980) documented El-tor cholera epidemics which occurred repeatedly in Pakistan since 1964 when it entered Karachi as an extension of the pandemic which started in the Celebes in 1959 (Tanamel 1959). Although a number of towns were effected in the Punjab, Lahore, Multan and Sialkot had epidemics every years. A more detailed study was made in Lahore, and it was found that Bilal Ganj, Bhati Gate, Lohari Gate, Shahalmi Gate, Gawal-mandi, Mozang, Ichhra, Baghbanpura and Shahdara were the areas of the town which were effected every year (Fig: 1)

The objectives of the present study were

- to carry out an epidemiological study of the cholera epidemic in the city of Lahore in 1980.
- to determine the extent of occurrence of Vibrio cholerae biotype el-tor carriage among contacts of positive cases admitted in I.D.H.
- to determine the bacteriological load of infection in a community where a case of cholera had occurred.
- to study the vehicles of transmission of cholera such as water supply, sewage and flies etc.

CHOLERA EPIDEMICS, LAHORE: 1964-79



MATERIAL & METHODS

1. All cases admitted in I.D.H. were entered in the register with their addresses and age. Stool samples of cases with diarrhoea and vomiting and signs of dehydration were collected for laboratory investigations.

2. To determine the occurrence of Vibrio cholerae biotype El-tor carriage amongst contacts the following sampling technique was performed. Cases of cholera admitted in IDH from Bilal Ganj were treated as the universe from which a random sample consisting of eight patients was investigated. The total number of contacts of each case were bacteriologically tested for carriage of Vibrio cholerae. Number of contacts of the eight homes tested were 17, 10, 21, 34, 13, 17, 10 and 12 respectively, making a total of 134.

2.1 A history was taken of all the contacts. Those showing diarrhoea or diarrhoea and vomiting were labelled as mild cases. Others having no complaint and giving a history of passage of 1-2 normal stools a day were labelled as symptomless individuals.

2.2 Water, sewage and flies of each house were sampled. A clean container measuring 100 ml was used for collection of sewage. It was dipped in the sewage and brought out full to the brim. Its contents were immediately transferred to 100 ml double strength Monsur's medium (Monsur 1961) contained in a 500 ml screw capped bottle. This was transferred to the laboratory within two hours.

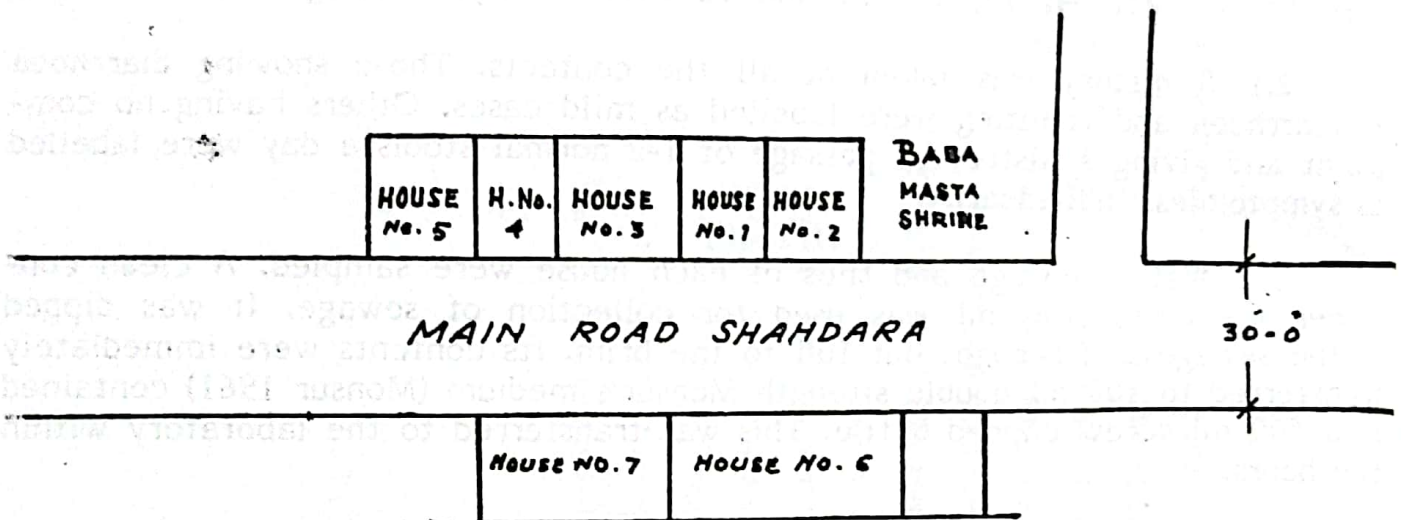
2.3 Flies were collected by killing them with a fly flapper and then transferring them to a bottle containing Monsur's medium. Fifteen to twenty five flies were collected from each house. Separate fly flappers were used for each house.

2.4 Water was being supplied by hand pumps or through pipes constructed by Lahore Development Authority (WASA). The mouth of the hand pump was flamed. It was then pumped for one minute and 100 ml of water sample was thereafter collected in a screw capped media bottle containing 100 ml of double strength Monsur's medium. In the case of WASA water supply the mouth of the tap was flamed. It was then opened and allowed to run for one minute and 100 ml of water then collected in the bottle mentioned above.

3. To determine the bacteriological load in a community where a case of cholera had occurred the following procedure was carried out. The Shahdara town was selected as the endemic area under investigation. A survey of the area was first made and it was considered suitable because it constituted a well defined community which could easily be demarcated and at the same time was representative of the prevailing social, economic, educational and environmental conditions prevailing in Lahore city.

3.1 One index case located in the centre of Shahdara was investigated. The houses were selected on the pattern laid down by the Statistical Division, Government of Pakistan for the Nutrition cell of the Planning Division (Expanded Programme of Immunisation 1978). A sight plan is given below :-

**SHAHDARA CHOLERA STUDY-SITE PLAN
OF SHAHDARA LOCALITY SURVEYED.**



From the index case house acting as the centre the adjoining five neighbouring houses (house No. 2, 3, 4, 5) were sampled. It is to be noted that there was one house on one side and three on the other. The reason for this is that the premises next to house number 2 was the Baba Masta shrine. The houses across the street which were selected were opposite houses number 1 & 3. The total number of houses sampled were seven. The entire population was sampled.

3.2. The water supply, sewage and flies were tested as enumerated in the Bilal Ganj study above.

CULTURE OF SPECIMENS

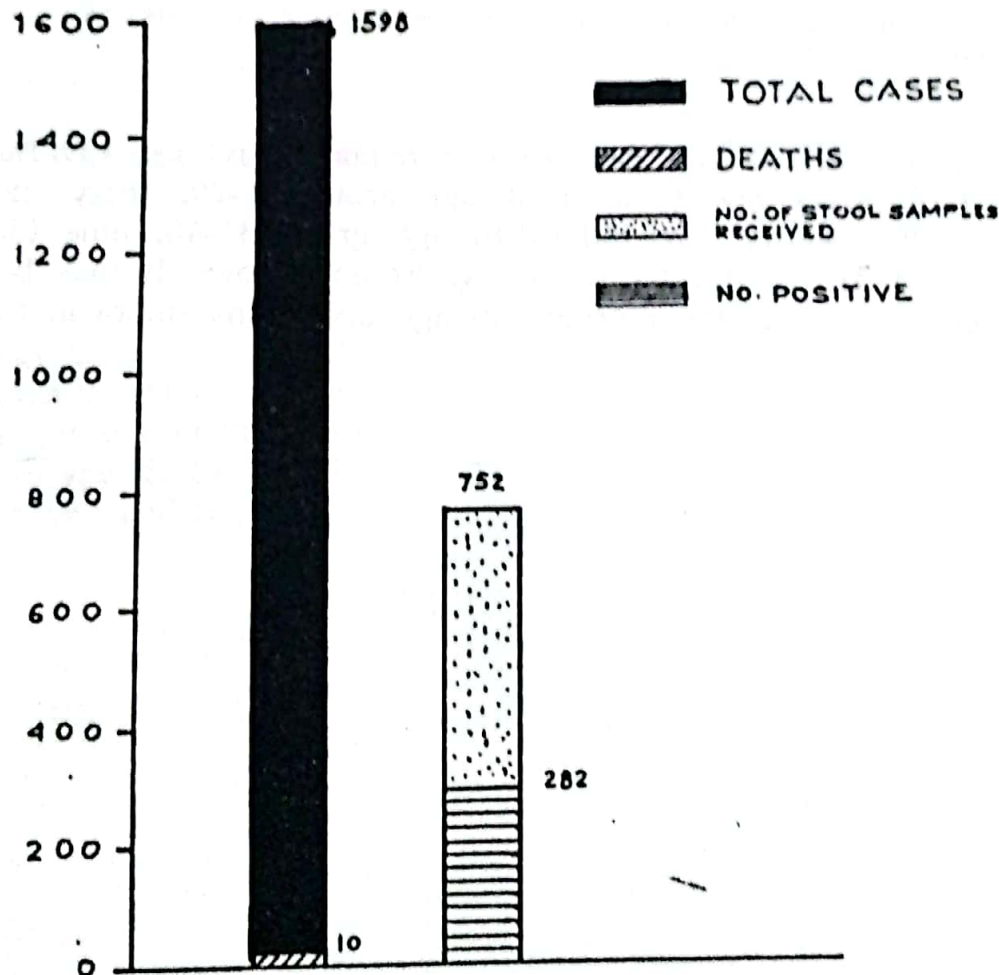
The stool tested were according to the method adopted by Bokhri (1980).

RESULTS

1. INVESTIGATION OF GASTRO-ENTERITIS CASES ADMITTED IN I.D.H.

1.1 A total of 1598 cases of gastro enteritis/diarrhoea were admitted in I.D.H. during 1980 epidemic commencing in July and ending in September. These cases were all treated, and there were ten (0.62%) deaths amongst them. 752 stool samples were sent to the Laboratory for investigation. 282 were positive for Vibrio cholerae biotype El-tor serotype Ogawa (Fig. II)

Fig. II - CHOLERA EPIDEMIC LAHORE, 1980.



1.2 The age distribution of positive cases of Vibrio cholerae biotype El-tor is given in Table - I.

TABLE - I

AGE DISTRIBUTION OF POSITIVE CASES OF VIBRIO CHOLERAEE
BIOTYPE EL-TOR ADMITTED IN I.D.H. IN THE 1980 EPIDEMIC

Age Group	2-10	11-20	21-30	31-40	41-50	51 and above
No.	110	61	46	25	9	31
%	39.0	21.6	16.3	8.8	3.1	10.9

* This age group is from 2-10 years since cases below 2 years are not admitted in I.D.H.

Out of a total of 282 isolates one hundred and ten (39.0%) belong to age group 2-10, sixty one (21.6%) of age group 11-20, forty six (16.3%) to age group 21-30, twenty five (8.8%) to age group 31-40, nine (3.1%) to age group 41-50 and 31 (10.9%) to age group 51 and above. It may be noted that incidence decreases with the increase in age and is maximum in the age group 2-10.

1.3 The seasonal distribution of positive cases is given in Table - II.

TABLE - II CHOLERA EPIDEMIC LAHORE, 1980

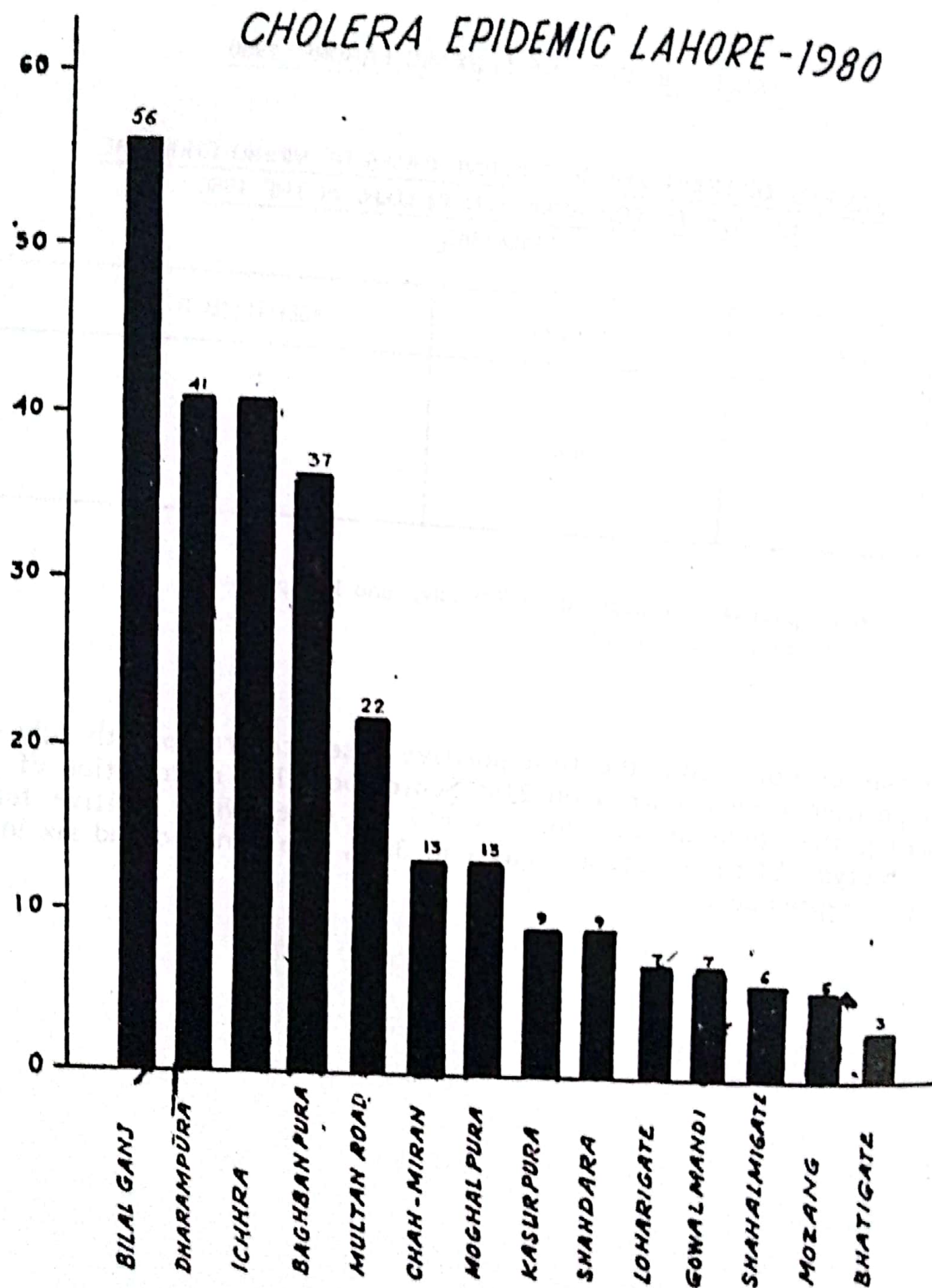
SEASONAL DISTRIBUTION OF POSITIVE CASES OF VIBRIO CHOLERAЕ
BIOTYPE EL-TOR ADMITTED IN LDJH. IN THE 1980
EPIDEMIC.

	JULY *	AUGUST	*SEPTEMBER
No. of positive cases	26	206	50

*First positive case occurred on 9th July, and last positive case on 21st September.

It may be noted that the first positive case occurred on 9th July whereas the last positive case occurred on 21st September. The distribution of positive cases during the epidemic was that twenty six cases were positive for Vibrio cholerae biotype El-tor serotype Ogawa in July, two hundred and six in August and fifty in September.

1.4 Figure III gives a Mohallah wise (area wise) distribution of cholera cases.



In order of occurrence the areas affected are Bilal Ganj, Dharampura, Ichhra, Baghbanpura, Multan Road, Chah Miran, Moghalpura, Kasur Pura, Shahdara, Lohari Gate, Gowalmandi, Shah Almi Gate, Mozang and Bhati Gate.

Other areas of Lahore i.e. Gulberg, Cantonment and University Campus (Fig. IV) gave only 1-2 cases which were presumed to be cases which had occurred by contact with the cases of above mentioned areas since there was no out break & the disease remained limited to one or two individuals only. The Gulberg area is a large area comprising Gulberg, Shadman Colony, areas surrounding Jinnah Gardens, the Mall Road, and surrounding areas. The area of Lahore Cantonment which was free from cholera is shown in yellow colour in Figure IV. University Campus comprises the newly laid colonies of University, Iqbal Town & Faisal Town etc. A common feature of all these areas is an efficient under ground sewerage system and a well looked after deep tubewell water supply which is kept chlorinated throughout the year. These two features are lacking in the effected areas, where either the underground sewerage is absent or if present it is generally not in proper functioning order. Similarly the water supply is not chlorinated throughout the year. Gulberg, Cantonment and University Campus have a low density of population, so that there are fewer refuse heaps. Those present are generally surrounded by a wall and are evacuated daily which diminishes the fly population. There is also an effective fly proofing of houses.

2. BILAL GANJ CHOLERA CONTACTS STUDY

The objective of this study was to determine the extent of occurrence of Vibrio cholerae carriage among contacts of positive cases admitted in I.D.H.

2.1 Table-III gives the incidence of Cholera carriage amongst contacts of Index cases admitted in I.D.H. The total number of index cases investigated were 8. Stool samples of all the contacts i.e. 134 persons were examined. Sixty one (45.5%) were positive for Vibrio cholerae biotype El-tor serotype Ogawa. Analysis of the history of the positive cases revealed that forty nine (80.3%) were mild cases (suffering from diarrhoea or vomiting or both). They were not serious and therefore did not report to I.D.H. Twelve (19.6%) were symptomless carriers.

TABLE - III (BILAL GANJ CHOLERA CONTACTS STUDY
INCIDENCE OF CHOLERA CARRIAGE AMONGST CONTACTS OF
INDEX CASES ADMITTED IN I.D.H.

No. of index cases	No. of contacts	Positive for Vibrio Cholerae						
		Total		Mild cases		Symptomless carriers		
		No.	%	No.	%	No.	%	
8	134	61	45.5	49	80.3	12	19.6	

2.2 Table-IV gives the age distribution of cholera contacts.

TABLE - IV BHAI GANI CHOLERA CONTACTS STUDY

AGE DISTRIBUTION OF CHOLERA CONTACTS

Category	Total	No. Positive	%
Children 1-12 years	69	45	65.2
Adults 13 years and above.	65	16	24.6
TOTAL:-	134	61	45.5

Out of 134 contacts 69 were children in the age group 1-12 years and 65 adults of age 13 years and above. Forty five (65.2%) out of the 69 children tested were positive for Vibrio cholerae biotype El-tor serotype Ogawa and sixteen (24.6%) out of 65 adults tested were positive for Vibrio cholerae biotype El-tor serotype Ogawa. This indicates that there is a higher number of children affected as compared to adults. Difference is statistically significant at $p < 0.01$.

3. SHAHDARA CHOLERA STUDY

The objectives of the study as stated above were to work out the total load of infection in the general population in an endemic area.

3.1 Table V gives the incidence of cholera carriage in the community from which an index case of cholera was admitted in I.D. Hospital.

AREA WISE DISTRIBUTION OF CHOLERA
CARRIERS & CASES, LAHORE 1980.

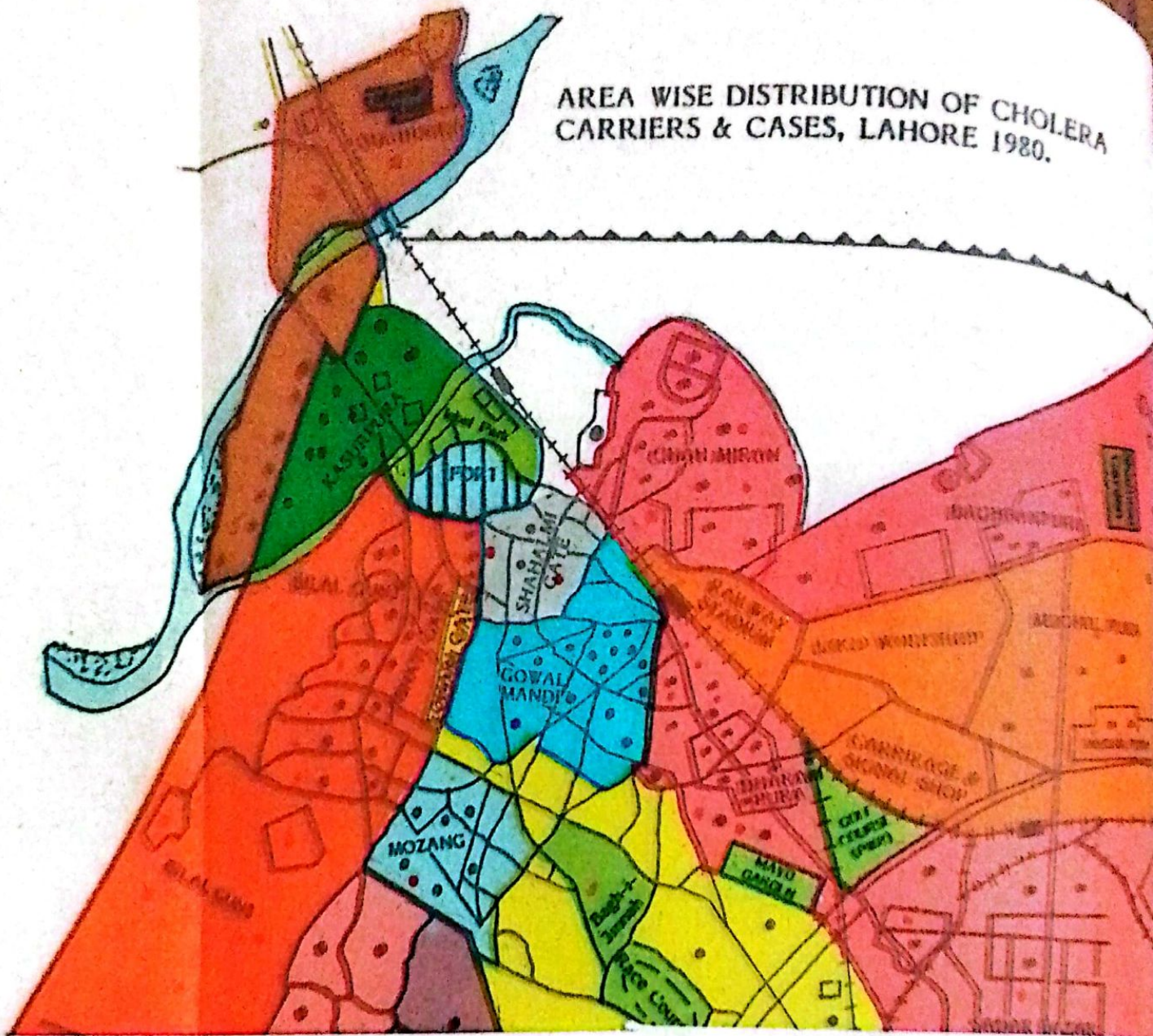


TABLE - V (SHAHDARA CHOLERA STUDY)

INCIDENCE OF CHOLERA CARRIAGE IN THE COMMUNITY
FROM WHICH AN INDEX CASE OF CHOLERA WAS
ADMITTED IN LD. HOSPITAL

House No.	Category	No. of Contacts	No. +tive	%	Mild cases			Symptomless carriers		
					No.	No. +tive	%	No.	No. +tive	%
1	Index house	34	29	85.2	34	29	85.2	-	-	
2-7	Neighbouring Pop.	82	33	40.2	35	22	62.8	27	11	23.4

The houses under study have been enumerated in the materials & methods. The number of contacts present in the house where the index case had been residing (labelled as House No. 1) were 34. This category is identical with the category of contacts studied in the Bilal Ganj study. All the 34 contacts gave a history of diarrhoea or vomiting or both and were therefore, considered as mild cases. Twenty nine (85.2%) of these were positive for Vibrio cholerae biotype El-tor serotype Ogawa. House No. 2-7 which constituted the neighbouring population had 82 inmates. 35 out of these were mild cases. Twenty two (62.8%) were positive for Vibrio cholerae biotype El-tor serotype Ogawa. There is a statistically significant difference in the two groups at $p < 0.01$ (i.e. the house hold contacts and neighbouring population). Forty seven inmates showed no symptoms. Out of these eleven (23.4%) were positive for Vibrio cholerae biotype El-tor serotype Ogawa.

3.2 Table VI gives the age distribution of the community with reference to cholera carriers in Shahdara Cholera study.

TABLE VI (SHAHDARA CHOLERA STUDY).

AGE DISTRIBUTION OF COMMUNITY WITH REFERENCE
TO CHOLERA CARRIERS

Category	Total	No. +tive	%
Children 1-12 years	55	39	70.9
Adults 13 years & above	61	23	37.7
TOTAL:-	116	62	53.4

Out of 116 individuals tested 55 were children in the age group 1-12 years and 61 adults of age 13 years and above. Thirty nine (70.9%) out of 55 children tested were positive for Vibrio cholerae biotype El-tor serotype Ogawa and twenty three (37.7%) out of 61 adults tested were positive. This indicates that there is a higher number of children affected as compared to adults, the difference being statistically significant at $p < 0.01$.

4. CORELATING FACTORS AND CHOLERA CARRIAGE.

4.1 Corelation of incidence of cholera carriage with recovery of Vibrio cholerae from flies. Isolation of Vibrio cholerae from flies in all the houses under investigation whether they belonged to those of cholera cases or the neighbouring population were carried out both in Bilal Ganj and Shahdara. Their results are expressed in Table VII.

35

TABLE - VII BILAL GANJ AND SHAHDARA CHOLERA STUDIES

INCIDENCE OF MILD CASES/SYMPTOMLESS CONTACT CARRIERS WITH
REFERENCE TO ISOLATION OF VIBRIO CHOLERAEE FROM FLIES
COLLECTED IN THE RESPECTIVE HOUSES

Result of fly culture	No. of inmates	No. positive	%	Mild cases		Symptomless carriers	
				No.	%	No.	%
Positive for <i>Vibrio cholerae</i>	161	107	66.4	91	85.1	16	14.9
Negative for <i>Vibrio cholerae</i>	89	16	17.9	9	56.2	7	43.7

Table VII gives the details of the incidence of mild cases/symptomless carriers with reference to isolation of *Vibrio cholerae* from flies collected in respective houses in Bilal Ganj and Shahdara studies. The houses which yielded positive cultures of *Vibrio cholerae* biotype El-tor serotype Ogawa from flies housed 161 inmates. Out of these one hundred and seven (66.4%) yielded positive stools for *Vibrio cholerae* biotype el-tor serotype Ogawa. Ninety one (85.1%) of these gave symptoms of mild cases and sixteen (14.9%) were symptomless carriers. The inmates of houses in which flies were negative were 89 in number. Sixteen (17.9%) of these had positive stools. Out of these nine (56.2%) presented as mild cases and seven (43.7%) as symptomless carriers. There is a statistically significant difference between the incidence of mild cases in houses with positive flies compared with those of negative flies. On the other hand, the difference between the incidence of symptomless carriers in the two groups of houses is not significant. This indicates that the presence of positive flies plays an important role in leading to mild cases whereas this is not so in the case of symptomless carriers. This is a well known fact that in order to cause infection, the load of the infecting dose has to be above 10^{11} organisms. (Hornick et al 1971). It appeared that a low dose of infection is all that is required for symptomless carriers and that some of the negative flies may be carrying a few organism missed by the isolation technique.

4.2 CORRELATION OF INCIDENCE OF CHOLERA CARRIAGE WITH
PROFESSION INVOLVING CONTACT WITH FAECES.

4.2.1 Table VIII gives the incidence of mild cases/symptomless carriers with reference to profession involving contact with faeces (Sweepers versus other professions) in Bilal Ganj and Shahdara studies.

TABLE VIII (BILAL GANJ AND SHAHDARA CHOLERA STUDIES)

INCIDENCE OF MILD CASES/SYMPTOMLESS
CONTACT CARRIERS WITH REFERENCE TO
PROFESSION INVOLVING CONTACT WITH
FEACES (I.E., SWEEPERS VERSUS
OTHER PROFESSIONS)

Profession	No. of contacts	No. +ive	%	Mild cases		Symptomless carriers	
				No.	%	No.	%
Sweepers	57	47	82.4	47	82.4	Nil	0
Other profession	193	76	39.3	53	69.7	23	30.2

Out of the 250 contacts investigated 57 were sweepers and 193 belonged to other professions (Milk Sellers, Grocers, Clerks, General Merchants, School Teachers, Plastic Workshop Mechanic and Tractor Mechanic). Forty seven (82.4%) out of the 57 sweepers were positive for Vibrio cholerae biotype el-tor serotype Ogawa. All the 47 were mild cases there being no symptomless carrier which indicates that the dose of infection to which the sweepers were exposed is so high that it invariably leads to symptoms of infection. Amongst the other professions out of 193 cases investigated seventy six (39.39%) were positive for Vibrio cholerae biotype el-tor serotype Ogawa. Among these fifty three (69.7%) were mild cases and twenty three (30.2%) were symptomless carriers. The difference in the infection rate in the two groups is highly significant at $p < 0.01$.

4.3 Corelation of incidence of cholera carriage with reference to income per capita. No corelation was found between incidence of cholera carriage with reference to income per capita.

4.4 (Corelation of incidence of cholera carriage with reference to number of rooms in the house. It was found that over crowding was not a contributing factor in cholera carriage.

4.5 Corelation of cholera carriage with recovery of Vibrio cholerae in water supply. The water supply of all the houses was tested and found

to be negative for Vibrio cholerae since the WASA had already super chlorinated the water supply. This correlate therefore could not be established.

DISCUSSION

1. Previous data on cholera studies

1.1 Vibrio cholerae biotype el-tor epidemic first occurred in the Liari area of Karachi in May, 1964. Although not proven bacteriologically there is evidence to denote that it was introduced from Bangla Desh (then East Pakistan).

1.2 Epidemics of classic cholera occurred in Pakistan in 1947 - 1948 - 1949 - 1958 - 1960 - (Ahmad et al 1962) and 1968 (Khan & Gilani 1969). Since 1968 there has been no outbreak of classic cholera and it may be presumed that Pakistan does not give any evidence of endemicity of classic cholera.

1.3 On the other hand, El-tor cholera introduced in Pakistan in 1964 spread up country to various towns along the Rail road and epidemics have occurred every year since then. Bokhari (1980) reports that epidemics of El-tor cholera occurred every year in the Punjab from 1964-1979. During this period, Lahore was affected every year whereas Multan was affected 14 years out of 16 under study. Sialkot had 10 epidemics. Sheikhupura, Kasur, Gujranwala, Faisalabad, Sargodha, Gujrat, Jhelum, Attock, Rahim Yar Khan, Dera Ghazi Khan, Bahawalpur, Sahiwal, Jhang and Mianwali had 1-8 epidemics. Because of repeated outbreaks in Lahore, Multan and Sialkot; Bokhari (1980) considered these areas to be highly suspicious of endemicity for El-tor cholera.

Bokhari's (1980) study of the area wise distribution in Lahore city (Figure 1) indicates that epidemics occurred in 9 mohallahs every year namely Bhati Gate, gowalmandi, Bilal Ganj, Mozang, Baghbanpura, Shahdara, Lohari Gate, Ichhra and Shahalmi. On the other hand, the remaining mohallas did not have epidemics every year. Moreover only 1-2 cases occurred in them whenever they were affected. These results indicate that the endemic foci exist only in the areas mentioned above and that cases occur in other areas through contact with cases or food products marketed in the endemic areas mentioned above.

2. 1980 Epidemic in Lahore City.

2.1 During the year 1980 Lahore epidemic there was 1598 admissions of gastro-enteritis in Infectious Diseases Hospital out of which 282 were confirmed for El-tor cholera. The age distribution revealed the maximum incidence in the age 2-10 and a diminution with increasing age. (Table I). In the 1980 epidemic the seasonal distribution of positive cases was July - 26 cases, August 206 cases and September 50 cases (Table II). The age and seasonal

distribution are in conformity with those reported by Bokhari 1980.

2.2 The area wise distribution of positive cholera cases in Lahore as reported by Bokhari 1980 (Figure I) revealed that the areas where cholera cases occurred repeatedly were Bhati Gate, Gowalmandi, Bilal Ganj, Mozang, Baghbanpura, Shahdara, Lohari Gate, Ichhra and Shah Almi Gate in order of preference. In 1980, the areas affected were Bilal Ganj, Dharampura, Ichhra, Baghbanpura, Multan Road, Chah Miran, Moghalpura, Qasur Pura, Shahdara, Lohari Gate, Gowalmandi, Shah Almi Gate, Mozang and Bhati Gate in order of preference. (Figure III). Thus, El-tor cholera occurred in all the areas where it had been occurring previously. In addition there was a further spread to Dharampura, Multan Road, Chah Miran, Moghalpura, and Qasur Pura. This spread was due to the fact that during the epidemic season there were heavy rains in the months of June, July, August and September resulting in the accumulation of water in streets in these areas. The streets had underground sewerage system as well as open drains. The underground sewerage was originally to take the load of night soil for the flush latrines of the areas, but did not cater for the surface water. During the rains the surface water went into the gutters through man-holes and choked most of them with debris. This led to a back flow of sewage through man-holes into the rain water which had collected in the streets. The WASA water supply was not initially chlorinated and was intermittent. At many places, there were defective pipe lines through cracks of which the sewage water was sucked in resulting in sudden out breaks in these areas. It may also be noted from the Map (Figure IV), that the previous endemic areas showed extension in their boundaries. A comparison with Figure-I reveals these extensions.

3. Cholera contact Study:

3.1 The Bilal Ganj cholera contact study (Table III) gives the contact carrier rate. Carrier rate in Bilal Ganj was 45.5% out of which 80.3% were mild cases and 19.6% symptomless carriers. In the Shahdara study (Table V) however, the contact carrier rate is 85.2%. This may be explained as sampling differences since the single house in Shahdara which was sampled revealed the presence of Vibrio cholerae on the flies obtained from that house whereas eight houses examined in Bilal Ganj were a mixture of those with flies positive for Vibrio cholerae (5 houses) and those negative for Vibrio cholerae (3 houses).

3.2 In the Shahdara cholera study (Table V) the contact carrier rate is 85.2%, whereas the neighbouring population shows a carrier rate of 40.2%. In other words, the neighbouring population had half the carrier rate. Moreover all the contact carriers presented with symptoms whereas 62.8% of the individuals in the neighbouring population had symptoms. It may be noted that the carrier rate in the neighbouring population i.e. 40.2% has been estimated during the epidemic period, and that it may constitute a majority of transitory carriers lasting till the end of the epidemic. However, these cases would not receive any treatment. Further work would be required to determine

the length of cholera carriage and the persistence of the organism in these individuals in the non-epidemic period.

3.3 It may be noted from Table VII that the houses with positive flies had a high carrier rate (66.4%) than those with negative flies (17.9%). This difference is explained on the basis of load of infection. Flies are known as a vehicle of transmission of cholera. A point of practical use emanating from this study is that positive flies could be used as an index of the presence of cholera cases and carriers in houses in an endemic area.

3.4 Professions involving contact with faeces such as the sweepers as a high risk for cholera infection is evident from Table VIII.

From the present study, it appears that contact with faeces and positive flies as a means of transmission plays an important role in El-tor cholera. The role of contaminated water, however, could not be established because of super-chlorination of water supplies. In other words, the present incidence excludes the contribution of water spread under the controlled conditions prevalent in the town at the time of investigation.

LITERATURE CITED

1. Ahmad, N.
M. M. Ahmed,
M. S. Qureshi. 1962 Epidemiological observations based on cholera outbreaks in recent years in West Pakistan. Pak. J. Hlth. 12(2): 59-75.
2. Bokhari, S. R. 1980 The pattern of El-tor cholera epidemics in the Punjab, Pakistan (under publication).
3. E. P. I. 1978 Pakistan Data, Government of Pakistan, Ministry of Health and Population Planning. Expanded Programme of immunisation.
4. Hornick, R. B.
S. I. Music.
R. Wenzel.
R. Cash.
J. P. Libonati.
M. J. Synder.
T. E. Wood Word. 1971 The Broad Street pump revisited. Response of volunteers to ingested cholera Vibrios. Bull. N. Y. Acad. Med 47 : 1192-1203.
5. Khan, A. M.
S. Gilani. 1969 Laboratory observations on cholera in West Pakistan. Pak. J. Hlth 19 (1):.
6. Monsur. K. A. 1961 A highly selective gelatin - taurocholatetellurite medium for the isolation of Vibrio cholerae. Trans. Soc. Trop. Med. Hyg. 55 : 440 - 442.
7. Tanamel, S. T. W. 1959 Notes on para-cholera in Sulawesi (Celebes). Amer. J. Trop. Med. 8 : 72 - 78.
8. Yaqoob. M. 1944 Epidemiology of cholera in the Punjab, Indian Medical Gazette. 79 : 383.

A SUCCESSFUL ATTEMPT TO INCRIMINATE ANOPHELES STEPHENSI
AS MALARIA VECTOR IN RURAL AREA OF PAKISTAN

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INTRODUCTION:

Anopheles stephensi, though called malaria vector of Pakistan, yet has never been accepted so far as more than responsible for urban malaria. Following are the reasons for this belief :

1. At several occasions it has been found positive for sporozoites but always was found responsible for urban malaria epidemics e.g. Bombay, (1908; reported by Christophers, 1949); Lucknow, (1929; Banerjea, 1930), Baghdad, (1941; Krishnan, 1961); and Karachi (1966; Rehman and Muttallib, 1967).
2. It is not a homogeneous species and the possible existence of at least two races have been postulated by various investigators (Knowles and Basu, 1934; Mulligan and Bailey, 1936; Ramsay and MacDonlad, 1936; Sweet and Rao, 1937). Sweet et al. (1938) observed significant difference in the eggs, and classified this species into two races calling them as An. stephensi type and An. stephensi var. mysorensis. Visevanathan (1950) regarded the type form as an urban mosquito while he thought var. mysorensis inhabited the rural areas. Coluzzi and Co-workers (Coluzzi, 1972, Coluzzi et al, 1972, 1973, 1974, White, 1974) observed a relationship between egg size and behavioural characteristics and the presence or absence of the 2Rb inversion from the IRAQ colony.
3. Short life span, low anthropophily, lack of evidence of its completing sporogony beyond oocyst stage in rural area gives support to believe that "rural Anopheles stephensi" is innocent species. Var. An. stephensi was considered unimportant as malaria vector than the type form but senior White (1940) and Subba Rao and Appa Rao (1945) have observed it to be the chief rural malaria carrier in Vizagapatam.

Sinton (1917) found one mosquito with infected glands from Kohat out of 45 females dissected.

Present studies provide a strong evidence that Anopheles stephensi, belonging to rural area, is very much capable of completing sporogony. Both Plasmodium falciparum and Plasmodium vivax can develop in it.

MATERIAL AND PROCEDURES:

Fifty gravid females of Anopheles stephensi were captured from Gajju Matta (14 miles South East of Lahore) and brought to National Malaria Training Centre. These were kept under natural conditions in a cage 18" X 18" x 18" and allowed to lay eggs on 10, August. A day after hatching the larvae were thinned out in eight enamel pans; each containing 300 to 400 larvae and rest of them were discarded. These were fed daily on liver powder food till they pupated. By 22, August, we got first progeny adults of about three thousand mosquitoes.

Surprisingly the adult hatching was excellent. Mortality among larvae was very low only three larvae failed to fully develop and almost all larvae became adult.

On 22 August from above described mosquitoes two batches of 15 female about 60 hours old and hungry were taken to Gajju Matta to be fed on known malarial patients. A team collected 259 blood samples from fever cases. These smears were stained and examined in the field. 30 of them (6 for P. falciparum and 24 for P. vivax) were found microscopically positive for malaria parasites.

Two of them were selected whose blood revealed sufficient number of Plasmodium falciparum (892 Gametocytes/cumm) and Plasmodium vivax (1555 gametocytes/cumm) respectively.

Transported batches of female mosquitoes, mentioned above, were allowed to feed from the back of hands of the patients separately at about 10.00 A.M. Mosquitoes readily fed through the cotton gauze of captivity card board cups. These fed mosquitoes were brought to N.M.T.C. Seven mosquitoes were found to be fully engorged with blood of P. falciparum case and only five from the other patient. These were retained in separate paper cups and the others were discarded. Next morning all the females were noticed to have digested blood. They were fed on a volunteer (N.M.T.C. Entomologist). Following day the mosquitoes were found to be empty and were fed again. This exercise continued for 5 days. Later on 5% sugar bottle was provided in the paper cups for feeding. Mosquitoes were kept under almost natural conditions. Daily maximum room temperature during observation days was recorded between 34.5°C - 30°C minimum: 23.8°C-26.°C. Water soaked cotton pads (replaced thrice every day) might have lowered the temperatures by 1-2°C and kept relative humidity around 75% within the paper cups. Dead mosquitoes were separated and dissected for gut till 8th day and latter for glands/gut both.

Results are tabulated in the following pages:

In the second phase the studies were extended by feeding another 17 mosquitoes from the stock of above mentioned An. stephensi unfed but of unknown age). On the blood of N.M.T.C. Guard, detected positive for Plasmodium vivax (Parvez et al 1987) conditions and procedures were the same as explained for Phase-I studies.

However, some of these females developed eggs in about first 60 hours and oviposited in the paper cup provided with small plastic cup half full of water. Later on they were fed on sugar and did not develop eggs during the following study days.

RESULTS AND DISCUSSION:

Phase-I study mosquitoes daily survival is shown in the Chart-I. And results of the dissections of dead mosquitoes is tabulated in Table-I. One female An. stephensi on 08th day developed P. vivax gametocytes upto occyst stage which indicates the potential of this species as vector. Unfortunately the only survivor of this category was dissected on 8th day at the premature stage of sporogony.

However, one female of the other plasmodium species fed, completed sporogony on the 13th day after feeding. This confirmed that An. stephensi could develop sporozoite when fed on P. falciparum gametocytes.

Thousands and thousands of dissections of this species from rural area have never shown positive glands (MEP/MCP dissection records from 1969-1986). Anthropophilly studies also prove this species to be highly zoophilic. But circumstantial evidence strongly indicate it to be important vector in rural area. For example, there are localities having abundance of An. stephensi in the absence of An. culicifacies and active transmission was noticed. Secondly, Anopheline stephensi has developed resistance to Malathion whereas An. culicifacies still remains highly susceptible to it.

DAILY SURVIVING FEMALES THROUGH DAY-0 -13 CHART - 1

4-9-85					
OBSERVATION DAYS	13				
	12				2
	11				2
	10				3
	9				4
	8				4
	7		1		6
	6		1		6
	5		2		6
	4		3		6
	3		3		7
	2		3		7
	1		5		7
			5		7

5♀♀ FED ON P.V. Pt. ↑ ↖ 7♀♀ FED ON Pf Pt.

DISSECTION RESULTS TABLE - 1

DAYS AFTER FEDING	NO. OF MOSQUITOES DISSECTED	GUT	GLAND	REMARKS
3	2	<u>P. VIVAX FED MOSQUITOES</u>		2♀♀ CRUSHED & WASED
6	1	NEGATIVE	NEGATIVE	NATURAL DEATH
7	1	"	"	"
8	1*	Oocysts	"	ALIVE
<u>P.falciparum Fed Mosquitoes.</u>				
5	1	NEGATIVE	NEGATIVE	NATURAL DEATH
9	2	"	"	"
11	1	"	"	"
12	1	"	"	"
13	2*	"	SPOROZITES	ONE + VE ONE - VE

After spray An. culicifacies disappears altogether but malaria transmission is maintained in the presence of An. stephensi. Thus either An. stephensi or other available species like An. pulcherrimus, An. annularis are responsible for malaria transmission. But these species are usually found in very low densities.

In the second phase of the study, another attempt was made to observe P. vivax gametocytes fed, An. stephensi.

Chart-II and Table-II reflect the results.

Two females An. stephensi completed sporogony on 10th/11th day, which confirmed that this species could develop sporozoites of P. vivax also.

Suggestions for Future Studies

1. P. vivax fed mosquitoes seem to show shorter longevity. This may be further explored in comparison to control, batches.
2. Recent studies by N.M.T.C. at Narung, Okara and Gajju Matta indicate probable role of other species than reputed vectors. (An. culicifacies and An. stephensi). Thus similar studies are recommended to be extended to :
 - A. pulcherrimus.
 - A. annularis.
 - A. nigerimus.
3. Later, if indicated the observations may be expanded to study feeding habits, longevity etc.

CHART II DAILY SURVIVING FEMALES THROUGH DAY 0-11

30-9-85	11		2
	10		3
	9		7
	8		7
	7		8
	6		10
	5		13
	4		13
	3		13
	2		14
20-9-85	1		17

OBSERVATION DAYS

17♀♀ FED ON Pv pt ↗

DISSECTION RESULTS

P. VIVAX FED MOSQUITOES

TABLE-2

Days After	No Dissected	Gut	Glands	Remarks
2 21-9-85	3	NEGATIVE	NEGATIVE	NATURAL DEATH
3 22-9-85	1	"	"	"
6 25-9-85	10	"	"	"
7 26-9-85	2	"	"	"
8 27-9-85	1	"	"	"
10 20-9-85	1	"	SPORO-ZOITES	ALIVE
11 30-9-85	2	"	"	ALIVE ONE ♀ NEGATIVE

REFERENCES

1. Banergea, A.C 1930. Some observations on an unusual epidemic of malaria in the city of Lucknow. Ind. Med. Gaz. 65:149-153, 22-Alipur Road, Delhi, P-45-46.
2. Christophers, S. R. 1949. Relation of Malaria Endemicity to species of anopheline. In Malariology. M. F. Boyd (ed) W. B. Saunders Company, Ogekadekogua. 698 P. P.
3. Coluzzi, M. 1972. Inversion Polymorphism and adult emergence in Anopheles stephensi Science. 176:59-60.
4. Coluzzi, M. G. Canerini and M. Dideco. 1972. Polymorphismo Cromosomico E. Lunghazy Dell; noro in An. stephensi parassitologia. 15:261-266.
5. Coluzzi, M., M. Dideco and G. Cancerini. 1973 a. Chromosomal Inversions in Anopheles stephensi. Parassitologia 15 : 129-136.
6. Coluzzi, M. and M. Dideco and S. G. Montalenti. 1974. Cage experiments on homospecific and hetrospecific matings with females of Anopheles stephensi Carriers of different inversion Karyotypes. Accad. Naz. dei. Lincei Fis. Nat. 57:683-689.
7. Knowles, R. and Basu, B. C. 1934. Malaria Survey. Rec. Mal. Surv. Ind. 4. P. 291.
8. Karishnan, K. S. 1961. Vectors of Malaria. An. stephensi, Liston, 1901, Vectors of Malaria in India. The National Society of India for Malaria and other Mosquito-Borne Diseases. Delhi, 39-57.
9. Malaria Eradication/Malaria Control Unpublished data 1960-1986.
10. Mulligan, H. W. and Bailey, J. D., 1936. Vectors of Malaria. Rec. Mal. Surv. Ind. 6, P. 289.
11. Pervez S. D., Shah. I. H. and M. A. Rai 1987, Malaria in Model Town Lahore. Pak. J. Hlth. 24:44-47.
12. Ramsay, G. C., and MacDonald, G., 1936. The species control of anophelines in India. Med. Gaz. 699-710.

13. Rahman, M. and A. Muttallib., 1967. Determination of Malaria transmission in Central part of Karachi City and Incrimination of An. stephensi as the Vector. Pakistan J. Hlth. 17:73-84.
14. Senior White, R. 1940 a. An. stephensi in Calcutta. J. Mal. Ind. 3:349.
15. Subba Rao, D. and Appa Rao, M. Malaria Transmission in and around Vizagapatam. J. Mal. Inst. Ind., 6:95-96.
16. Sinton, J. A. 1917. Ind. J. Med. Res. 5:195.
17. Sweet W. C., Rao, B. A., 1937. Races of An. stephensi. Liston. Ind. Med. Gaz. 665-669.
18. Sweet W. C., Rao, B. A., and Subbarao, A. M. 1938. Egg difference of two forms of An. stephensi. J. Malaria. Inst. India. 1:149-154.
19. Visvanathan, D. K., 1950. Malaria and its control, in Bombay state. Aryabhusan Press, Poona.
20. White, G. B., 1974. Biological effects of Intraspecific Chromosome Polymorphism in Malaria Vector Populations. Bull. Wld. Hlth. Org. 50:299-306.

THE SUSCEPTIBILITY OF FALCIPARUM MALARIA PARASITES IN
GUJRANWALA DISTRICT, PUNJAB, TO CHLOROQUINE,
AND ITS MAJOR ALTERNATIVE DRUGS,
AMODIAQUINE AND FANSIDAR

BY

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INTRODUCTION

Chloroquine resistant falciparum malaria was first noted in Columbia, South America in 1960. In the Eastern Hemisphere, the emergence of chloroquine resistant strains of falciparum malaria was first reported from Thailand in the early 1960s. These strains gradually spread Westwards through Burma and into Eastern Bangladesh reaching the North Eastern States of India in 1973⁽¹⁾. Most recently, chloroquine resistant falciparum malaria has been reported in some of the East African countries with indication of rapid spread Westward⁽²⁾.

Beginning in 1975, the National Malaria Training Centre, Lahore, began field trials to detect the presence of chloroquine resistant *P. falciparum* in Pakistan. The existence of these resistant strains were fully confirmed in Sheikhupura, Jhang and Bahawalnagar districts of the Punjab in the late 1970s through early 1984⁽¹⁾. These chloroquine resistant parasites were also reported in Lahore, Sheikhupura, Kasur and Muzaffargarh districts of the Punjab by the Directorate of Health Services, Punjab in 1985.

Pakistan is situated in the tropical belt where irrigation has added considerably to the malariogenic potential of the area. About 80% of the population is living in the rural area where malaria is prevalent. In Pakistan, malaria continues to be a major health problem, which will intensify if *P. falciparum* strains resistant to chloroquine are allowed to spread unchecked. For this reason monitoring studies must be carried out on a continuous basis in order that resistant foci be identified quickly and remedial measures applied to eliminate such foci. This study was performed to meet the first objective. In addition, since the treatment regimens to counter chloroquine resistant falciparum malaria proposed by the Malaria Control Programme in Pakistan by using Amodiaquine and Fansidar as alternative drugs, this study was also intended to demonstrate the technical validity of the scheme by the evaluation of these alternative drugs.

MATERIALS AND METHODS

Time and place of study :

To minimise the possibility of re-infection with falciparum malaria the in-vivo tests were carried out during the low transmission season, from November to February.

After a review of the surveillance data of Gujranwala district, some of the localities with high malaria incidence were selected for carrying out the initial parasitological surveys to locate a trial site.

Selection of Test Subjects:

Mass blood surveys were carried out in each of the selected villages to detect *P. falciparum* cases. The *P. falciparum* cases with more than one thousand asexual parasite₃ per mm³ of blood were selected for in-vivo trial. Parasite density per mm³ of blood was determined by counting the number of parasites in 8,000 white blood cells (assuming an average WBC count of 8,000/mm³).

Drug used and Dossage:

Nivaquine tablets, (chloroquine sulphate) (150 mg base) manufactured by May and Baker Pakistan, Ltd; Wah Cantt were used.

The chloroquine tablets were administered to the falciparum patients according to their body weight. The standard dosage consisted of 25 mg chloroquine (base) per kg body weight given in the following doses:

Day 0 : 1st Dose	-	10 mg/kg.
Day 1 : 2nd Dose	-	10 mg/kg.
Day 2 : 3rd Dose	-	5 mg/kg.

Amodiaquine tablets were provided by the UNICEF as 150 mg base. The standard test dosage of Amodiaquine was also 25 mg/kg body weight given over three days as per the following schedule:

Day 0 : 1st Dose	-	10 mg/kg.
Day 1 : 2nd Dose	-	10 mg/kg.
Day 2 : 3rd Dose	-	5 mg/kg.

Fansidar tablets used in the tests were manufactured by F. Hoffmann-La Roche & Co. Ltd Basle, Switzerland. Each tablets contains: sulfadoxine 500 mg, pyrimethamine 25 mg. The standard test dosage for adults was 2-3 tablets, given a single dose. Children's dose reduced according to their ages.

<u>Age Groups</u>	<u>Number of Fansidar tablets</u>
3 - 4 years	1/2 tablet.
5 - 6 years	1 tablet.
7 - 9 years	1-1/2 tablets.
10 - 14 years	2 tablets.

URINE TESTS:

Before the administration of chloroquine, the urine of each patient was tested with Dill-Glazko method to confirm the presence of chloroquine in the urine. Urine samples from pre-treatment and post treatment sample (day 2) of all patients were tested.

Follow up Method:

Blood smears from the subjects were prepared daily beginning on the first day of treatment (day 0) through day 3 then on day 5, 7, 10, 13, 16, 19, 22, 25, 28.

Use of Alternative Drugs:

Chloroquine resistant falciparum malaria cases were treated by using amodiaquine and those cases not cured by amodiaquine were then treated with Fansidar.

Smear Examination - Criteria for Negative Smear:

All blood slides were stained with 2.5% Giemsa for 30 minutes and parasite density was estimated by counting the number of asexual parasites against 9000 W.B.Cs. A smear was considered negative if no asexual parasite was found after searching 100 fields of a thick blood smear.

WHO Classification of in-vivo response:

- S : : Clearance of patent parasitemia by day 7 with no recrudescence during 28 day follow up.
- R I : Clearance of patent parasitemia by day 7 with recrudescence by day 28 or earlier.
- R II : No clearance of patent parasitemia by day 7 but parasitemia lower on day 7 than day 0.
- R III : No clearance of patent parasitemia by day 7; parasitemia higher on day 7 than day 0.

RESULTS

Parasitological Surveys:

Mass blood surveys were carried out in selected localities of Chindiala, Shergarh and Maha Chattha on 22 & 23 December, 1985. The result of these surveys was as follows :

BLOOD SMEARS SURVEYS IN GUJRANWALA DISTRICT:

<u>Date</u>		<u>Smears</u>	<u>Positive</u>	<u>P.v*</u>	<u>P.f.**</u>	<u>Mix.</u>
22-12-85	Chindiala	149	25	10	16	1
23-12-85	Shergarh	67	23	2	22	1
23-12-85	Maha Chatha	381	213	8	207	2
TOTAL:		597	261	20	245	4
		***	SPR : 43.7%	3.4%	41.0%	

*P.v : Plasmodium vivax.

*** SPR : Slide Positivity Rate. **P.f : Plasmodium falciparum.

After the surveys, 51 falciparum malaria cases were selected for in-vivo trial, in village Maha Chatha. The remaining cases not selected for study were given radical treatment for their infections.

Age and sex composition of selected 51 cases were as follows :

<u>Age Group</u>	<u>Male</u>	<u>Female</u>	<u>Total</u>
1 - 4 years	-	1	1
5 - 9 years	19	7	26
10 - 14 years	5	6	11
15 - above.	8	5	13
TOTAL:	32	19	51

Follow up result after chloroquine treatment:

Follow up was completed in all 51 cases. The examination of follow up slides showed that all the blood smears became negative by day 7 except in 3 cases, which remained positive up to day-7 (R-II resistant cases). In 29 cases, recrudescence of patent parasitemia was observed between day-7 to day-28. The pattern of recrudescence in these 29 R-I cases were as follows:

<u>Observation Day</u>	<u>Number of recrudescence cases</u>
Day - 7	2
Day - 10	8
Day - 13	3
Day - 16	2

Day - 19	7
Day - 22	4
Day - 25	2
Day - 28	1

As noted, approximately half of the R-I cases were recrudesced early, within the first 2 weeks and the other half, during the 3rd and 4th week.

The result of the chloroquine trial may be summarised as following:

THE INVIVO RESPONSE TO CHLOROQUINE

	No.	%
S	19	37%
R I	29	57%
R II	3	6%
R III	0	0
TOTAL:	51	100%

Amodiaquine was administered to 31 chloroquine resistant cases. The results of the examination of follow-up blood smears were as follows:

THE IN-VIVO RESPONSE TO AMODIAQUINE

	No.	%
S	19	61%
R I	7	23%
R II	5	16%
R III	0	0
TOTAL:	31	100%

As noted, of the chloroquine resistant falciparum infections treated with amodiaquine, 19 (61%) were cured.

The pattern of recrudescences in 7 R-I cases were as follows :

<u>Observation Day</u>	<u>Number of recrudescent cases</u>
Day 11	1
Day 12	2
Day 14	2
Day 15	2

As noted, all R-I cases recrudesced within 2 weeks.

Follow-up results after Fansidar Treatment.

Fansidar tablets were administered to 12 amodiaquine treatment failure and one chloroquine resistant falciparum infection (recrudescence of patent infection on day 28). The examination of follow-up blood smears showed that 12 of the 13 cases treated with Fansidar were sensitive, only one case was resistant, with asexual parasites.

A comparison of pre-treatment parasite density between the sensitive and resistant infections treated with the 3 respective drugs are summarized in (table-1). As indicated there was no striking difference in the pre-treatment parasite counts between the sensitive and resistant infections treated with chloroquine. In the case of the amodiaquine treated cases, the pre-treatment, parasitemias of the resistant cases were much higher than those from sensitive cases.

The in-vivo response of falciparum strains studied against chloroquine, amodiaquine and Fansidar are summarised in (table-2).

TABLE I

PRE-TREATMENT PATASITE DENSITY (mm^3 blood) RANGE AND (AVERAGE)

Drug	Sensitive	Resistant	
		R-I	R-II
CHLOROQUINE	1000/ mm^3 - 18000/ mm^3 (5136/ mm^3)	1014/ mm^3 - 12014/ mm^3 (3862/ mm^3)	6710/ mm^3 - 12171/ mm^3 (90002/ mm^3)
AMODIAQUINE	20/ mm^3 - 35000/ mm^3 (3159/ mm^3)	68/ mm^3 - 35892/ mm^3 (12107/ mm^3)	20/ mm^3 - 31375/ mm^3 (10413/ mm^3)
FANSIDAR	60/ mm^3 - 33469/ mm^3 (6807/ mm^3)	64/ mm^3	

TABLE - II

THE INVIVO RESPONSE OF P. FALCIPARUM STRAINS TO ANTIMALARIAL
DRUGS : CHLOROQUINE, AMODIAQUINE & FANSIDAR

Drugs	Total No. of Cases Evaluated	Response No. (%)		
		S	R = I	R = II
CHLOROQUINE	51	19 (37%)	29 (57%)	3 (6%)
AMODIAQUINE	51	19 (61%)	7 (23%)	5 (16%)
FANSIDAR	13	12 (92%)	1 (8%)	0 0

DISCUSSION:

The results of survey to select a site for this study show that in the part of December, 1985, malaria prevalence in Maha Chatha village was high with SPR of more than of 43%. In addition, falciparum malaria accounted for 97% of the positives. Such high infection rates emphasize the severity of the malaria problem at least in some of the localities in the Punjab at the time when this study was undertaken.

Compounding the malaria problem is the apparent rapid spread of chloroquine resistant strains which if allowed to spread unchecked will not only result in an increase in morbidity but more importantly, in mortality as well.

The results of this study confirm that chloroquine resistant falciparum strains are present in Gujranwala district, Punjab. In fact, of the infections evaluated, the majority (69%) demonstrated resistance against chloroquine in the in-vivo test.

The results of the evaluation of amodiaquine followed by Fansidar to treat chloroquine resistant falciparum strains support the basic soundness of the proposed use of these alternating treatments. The finding that more than half of the falciparum infections (61%) with confirmed resistance against chloroquine were susceptible to amodiaquine are in agreement with similar findings of others; that Amodiaquine is more effective than Chloroquine against chloroquine resistant falciparum strains. The fact that Fansidar cured 12 out of 13 (92%) infections resistant to both Chloroquine and Amodiaquine also confirm many other such observations. In addition, knowledge of the sensitivity of Fansidar against Chloroquine resistant falciparum strains in Pakistan is a source of great assurance that an efficacious drug which is simple to use operationally because of its single dosage is available to assist in helping solve the problem of Chloroquine resistant falciparum malaria.

Solutions to the problem of Chloroquine resistant falciparum malaria consist of at least the following measures:

1. Identify the resistant foci early by continuous monitoring to determine the presence and prevalence and degree of resistance in the resistant strains. To promote this objective, operational research must be intensified at every district. In this context, this study should be considered as only a beginning effort to prove useful data to guide the selection of suitable methods in a malaria control programme.
2. Once the resistant foci have been identified, attempts must be made to eliminate them by vigorous application of antivector measures such as house spraying with an insecticide and the elimination of resistant parasites by the efficient use of the proper drugs.

Presently, Fansidar is considered by the WHO as the most important alternative drug for the treatment of Chloroquine resistant falciparum malaria. The results of this study tend to support this contention. To ensure that the drug remains useful for as long as possible, steps must be taken to minimize its abuse by the medical profession. For this purpose the medical profession must be informed not only of Fansidar's benefits but also of its limitations.

These include :-

1. Resistance against Fansidar can develop readily.
2. Its usefulness against vivax malaria is uncertain since the susceptibility of the vivax parasites is dependent solely on the effect of the pyrimethamine component; if resistance of P.vivax against pyrimethamine is present, then the parasite will also be resistant to Fansidar.
3. Its use in people who are sensitive to sulfonamide can result in severe cutaneous skin reactions which may present as: erythema multiforme, Stevens - Johnson Syndrome or toxic epidermal necrolysis. (3)

The severity of these reactions may range from mild to death.

The best way to prolong the useful life of Fansidar as the treatment of choice for Chloroquine resistant falciparum malaria is to avoid its large scale and indiscriminate use. In particular, it should only be used when there are justifiable indications: in those cases of blood smear confirmed falciparum infection which have not been cured by the 3 day treatment with Chloroquine given in a total dosage of 25 mg base/kg body weight. Its use to treat clinically suspected malaria without smear confirmation or vivax cases diagnosed by blood smears is totally without justification. Continuation of such practices can only result in the increased exposure of parasites to this valuable drug and hasten the selection process for the resistant strains; the consequences of which predictably would be an increase in mortality due to falciparum infections acquired by the people of Pakistan.

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REFERENCES :

1. I. H. Shah and S. M. Muftaba, 1985.
CHLOROQUINE RESISTANT FALCIPARUM MALARIA IN PAKISTAN.
WHO-EM/MAL/208
2. C. C. Campbell, et al;
1979. CHLOROQUINE RESISTANT PLASMODIUM FALCIPARUM FROM
EAST AFRICA. CULTIVATION AND DRUG SENSITIVITY OF THE
TANZANIAN I/CDC STRAIN FROM AN AMERICAN TOURIST.
LAUCET, 2 : 1151-1154
3. Miller, K. D., Lobel, H. O., Satriale, R. F.,
Kuritsky, J. N., Stern, R., and Campbell,
C. C., 1986.
SEVERE CUTANEOUS REACTIONS AMONG AMERICAN TRAVELLERS
USING PYRIMETHAMINE - SULFADOXINE (FANSIDAR) FOR MALARIA
PROPHYLAXIS, Am. J. Trop. Med. Hyg.,
35 : 451 - 458.

MALARIA RISK IN
ISLAMABAD, PAKISTAN.

BY

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SUMMARY :

Islamabad Capital of Pakistan is receptive and vulnerable to malaria. Under favourable conditions (which are not usual) Malaria transmission can occur during May to September.

MALARIA RISK IN ISLAMABAD

1. INTRODUCTION:

Islamabad is located in hypoendemic zone. Epidemiological records of Rawalpindi, and Azad Kashmir, Jhelum, Attock and Abbottabad Districts around Islamabad, show low endemicity over the last many years.

Several malaria surveys in Islamabad have revealed high malaria parasite reservoir from time to time. However, epidemiological investigations do not provide direct evidence of malaria transmission in Islamabad. Directorate of Malaria Control arranged special blood/Entomological Surveys in Islamabad during end of April and early September, 1987 to assess malaria situation in the Capital. Present report is based on 1987 Surveys and tries to discuss malariogenic risk in Islamabad generally and this season especially.

2. SURVEY AREAS:

Four Katchi Abadies, well distributed in Islamabad, were chosen for our studies. They were located in G-7, G-8, G-9 and F-9 Sectors. These Abadies consist of Katcha houses and accommodate labour/poor homeless working class and their families. These abadies have emerged around streams/nullahs running in these Sectors. Each locality consists of 200-1500 houses and presents village like environment. Among these labour colony is the largest having a population of about ten thousand. Most of the families come from Districts of Peshawar, Mardan, Kohat and Hazara. Many of them visit their homes atleast once in one year. Other abadies are smaller, having population of 1000-1500 people, mostly christians belonging to Sialkot, Sheikhpura and Sahiwal Districts. Their visits to home villages are also common.

By law keeping of domestic animals is prohibited but it was noticed, especially in Labour Colony, that buffaloes, goats and dogs were quite common. In addition ducks, chickens and other pets were also not very un-common in all these abadies.

3. OBSERVATIONS:

3-1. Blood Surveys:

Following table shows the results of blood surveys conducted during April and September 1987.

Name of Locality	April 1987*			Sep. 1987**		
	Slides Exam.	P.v.	P.f.	Slides Exam.	P.v.	P.f.
G-7, 66 Quarters/ Tent Colony.	588	5 (0.85%)	0	102	2 (1.96%)	0
G-8, Charles Colony	223	15 (6.72%)	0	154	7 (4.54%)	0
G-9, Labour Colony.	257	4 (1.55%)	1 (0.38)	89.9	2 (2.24%)	0
F-4 France Colony	230	3 (1.3%)	1 (0.43%)	38	0 (2.63%)	1
Total:	1298	27 (2.08%)	2 (0.15%)	383	11 (2.87%)	1 (0.26%)

3-2 Case Histories of Positive Cases:

Two malaria cases out of five investigated suggested local infection during May this year.

* Include School Children also.

** Fever/fever history only.

3-3. Entomological Studies:

3-3-1. Anophelene Density

Indoor resting vector population was observed by flitting method. Results are tabulated below :

Name of Locality	Room Checked	A. cul.	Density/+v Room		
			A. step.	A. flu.	A. mac.
G-7, 66 Quarter	14	1.0	4.0	0.0	0.0
G-8, Charles Colony	9	1.0	1.0	0.0	0.0
G-9, Labour Colony	8	7.5	7.8	2.0	1.0
F-4, France Colony	11	0.0	2.0	0.0	0.0

3-3-2. Man Vector Contact.

- a) Baited net and direct catching of vector did not reveal any anopheles till 23.00 hrs. observed period. Due to rain study was abandoned afterwards.
- b) Freshly fed An. stephensi and An. culicifacies were smeared on filter paper for precipitation tests.

3-3-3 Three out-let window traps also did not record any anopheles till 23.00 hours.

4. DISCUSSION :

Slide positivity rate (S.P.R.) detected during 26 April-1st May and 7th Sep-9th Sep., 1987 shows little difference slightly higher SPR of Sep. may be attributed to strict selection of fever/fever history subjects, but the increase in cases is not so significant to reflect active transmission of malaria. However, case histories of two out of five investigated indicate no evidence of contracting malaria outside Islamabad.

Extensive flitting revealed the presence of An. culicifacies, An. stephensi, An. fluviatilis, An. maculatus. Among these first two species are confirmed vectors of malaria. They are normally highly zoophilic in rural areas. And as such. Low densities of these species is confirmed by out let window trap/man vector contact surveys also. Blood meal analysis of smeared mosquitoes reveal high zoophilic tendency among the recorded low densities of An. culicifacies and An. stephensi. However in the presence of low proportion of animals these two species could be diverted towards human being for feeding and even low densities could be dangerous.

CONCLUSIONS :

Ideal breeding places for mosquitoes are present all over Islamabad area. Malaria reservoir (imported mostly) are also always present; thus Islamabad appears to be receptive and vulnerable to malaria transmission. Low grade transmission can occur in favourable conditions. Favourable climatic conditions in long spells and high malaria parasite load in population can provide potential for malaria transmission.

However in the prevailing entomological conditions transmission of malaria during this season is almost nil.

REFERENCES

1. Districtric Malaria Control yearly (Unpublished) record 1980-86.
2. Rawalpindi District Malaria Control yearly (Unpublished) record (1964-1986). 1986).

ACKNOWLEDGEMENT

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A REPORT ON FIELD TESTING
OF
METHOPRENE (I. G. R.)
FOR
MOSQUITO CONTROL
(PAKISTAN)

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SUMMARY

A large scale field trial, under natural conditions was conducted to observe biological effects of methoprene on mosquito immature and mature stages. In addition Non-target invertebrates (N. T. Os) in the treated breeding places were also checked for any adverse effect.

One dose of Altosid Briquet formulation, one briquet per 100 Sq. Ft. and two dosages, 250 ml/hect. and 300 ml/hect. of liquid SR-10 methoprene were tested.

Negligible % (less than 1%) adult mosquitoes emerging from pupae were observed during the first week after treatment with briquets or 300 ml/hect. On 18th day of observation after treatment only 50% adults emerged from breeding places treated with 300 ml/hect. and same % from treated with briquets but on the 21st day after treatment.

Morphological abnormalities were noticed among the surviving adults, emerging between 6-15 days after treatment. Significant sex proportion distortion was also noticed among the apparent normal adults emerging between 3-12 days after treatment.

Both the formulations of methoprene were found to be very safe in handling and for N. T. Os.

1. INTRODUCTION:

Methoprene (ALTOSID) belongs to the third generation insecticides commonly referred to as I.G.Rs. (Insect Growth Regulators). I.G.Rs were offered in early 70s as a new class of mosquito larvicides. These compounds disrupt the normal cycle of development in insects and prevent maturation. Thus they control insects through interference with growth and development rather than through direct toxicity. These are grouped into ecdysoids and juvenoids. Former inhibit chitin synthesis following larval moults and the latter inhibit pupation (Mouchet 1980). Classification, mode of action and procedures of testing I.G.Rs was discussed at length during an informal consultation on I.G.Rs from 2 to 4 August, 1983. Proceedings are given in WHO/VBC/83.883 (1983) document.

The chemistry and biological activity of insect juvenile hormones and their analogs and mimics, to which class methoprene belongs, is well discussed by Menn and Beroze (1972). Among the various groups of insects against which insect growth regulators have shown some promise are several Diptera of medical and public health significance.

Jackob and Schoof (1971-72) Schoefer and Wilder (1972) and Steelman

and Schilling (1972) showed the efficacy of several I.G.Rs against mosquitoes. Wright (1972) evaluated these compounds against stable flies. Mulla et-al (1974) evaluated a number of I.G.Rs against several genera of aquatic midges in the laboratory, semifield plots and recreational lakes.

Chemical name of Methoprene (ALTOSID) is Isopropyl (2E, 4E)-11-methoxy-3, 7, 11 trimethyl-2, 4- dodecadienoate. Its chemical formula is C₁₉H₃₄O₃. The action of methoprene is selective. When applied at the recommended dose it does not harm wildfowl, amphibians, fish, crustaceans and other non-target invertebrates. Mirua and Takeshi (1974) have studied 35 species of non-target organisms (NTOS) including protoza, earthworms, leeches, water flees, shrimps, mayflies, damsel flies, water beetles, snails, tadpoles, mosquito fish and algae. They observed no effect or very little effect on them.

Nelson and others (1976) tested this compound against Culex pipens and found it to be very effective.

All toxicity studies conclude that methoprene, the active ingredient in (ALTOSID) I.G.Rs causes no hazard to man or the environment. It has been found to be a bio-degradable substance of very low persistence. In animal body it does not persist. Safety studies show that in animals the compound is metabolized quickly and eliminated via urine, faeces and expired breath. Neither wheat grown in treated areas showed any residual traces of methoprene, nor other plants.

Reported here are the results of Field Testing of OMS1697 Methoprene (ALTOSID) in the natural field conditions against culicine and anopheline target mosquitoes and other NTOs, aquatic invertebrates.

2. OBJECTIVES

2.1 The purpose of present studies was to determine the effectiveness and persistence of field applications of two formulations of (ALTOSID) in natural conditions against mosquitoes.

2.2 To propose dosage and regime of Altosid formulations for control of mosquitoes.

2.3 To observe effect on non-target aquatic organisms (NTOs).

3. SITUATION AND CONDITIONS OF TRIAL AREA:

3.1 Location:- Gajju Matta, a village 14 miles South-East from Lahore city, was chosen for this trial. Selection of this area promised the following advantages :-

3.1.1 Epidemiologically it is very sensitive area. There have been evidence of active transmission of malaria last year and conditions are always prone to serious spread of malaria.

3.1.2 There is variety of permanent and potential breeding places with almost perennial breeding of anopheles and culicine.

3.1.3. Last year a trial was conducted in this area, to assess Teknar and Dimilin larvicides and map of the area showing breeding places already existed to serve immediate start of the studies.

3.1.4 Short distance from NMTC and approach throughout the year provided additional advantages.

3.2 BREEDING SITES AND BREEDING.

3.2.1 Breeding Sites :- Ponds, ditches, ground depressions, seepage, hoof prints, irrigation channels and drains are the breeding places found in this area. Organic matter rich, polluted, clean and fresh water with vegetation or clear is the nature of different breeding places. They vary in size and depth and exposure to sunlight. During mapping such information was recorded for each site.

3.2.2 Mosquito Fauna :- Anopheles culicifacies, stephensi, pulcherrimus, annularis and neigerimus (hyrcanus) are the species usually captured during the regular entomological checking. An. subpictus, in very high densities, is also found during July-September. First two species are known vectors of the area.

A number of culicine species are also found in this area. Commonest being Cx. pipiens fatigans (= Cx. P. quingure fasciatus) Cs. tritaeniorhynchus, Mansonia uniformis and Ae. caspius etc Cx. pipiens fatigans is potential vector of Filariasis in Pakistan (Parvez 1974).

4. PROCEDURES AND METHODS

4.1 MATERIAL AND DOSAGES

4.1.1 Altosid Briquets :- One briquet per 100 Sq. Ft. or one upto 2' depth (75 Gal.) was tossed by hand in still water collections and in moving waters the dose was adjusted according to the flow of water by the following formula :-

$$\frac{\text{Allowable flow}^*}{\text{Actual flow}} \times 30 = \text{Adjusted treatment interval (days)}$$

(*Four Volume Changes)

4.1.2. ALTOSID SR-10

Two dosages i.e. 250 ml/hect. and 300 ml/hect. were finally selected for further observations from the tried five dosages. 100 ml/hect. and 150 ml/hect., doses were dropped since these failed to achieve 100% pupae mortality on first or second checking after 3rd and 6th day respectively.

4.1.3. Hudson X-pert Sprayers (10 litres capacity, hand operated, with 8002 SS Nozzle Tip), Pumps were operated with average operational pressure of 40 PSI with roughly double the speed that of normal indoor spraying rhythm in M.C.P. 100 ml/and 120 ml. ALTOSID SR-10 were added per sprayer (10 litres water) in order to get 250 ml/hect. and 300 ml/hect. dose. Since ALTOSID SR-10 tends to stay near the surface of water it was not felt necessary to adjust rates of application with various depths and thus depth was ignored as all the breeding places were not deeper than 2-3 feet.

4.2.1 Breeding Sites Mapping.

All the existing and potential breeding places were numbered and mapped on 10" to 1 mile map and detailed information as to the location, size, depth, nature and utility etc etc. of each breeding place was recorded.

4.2.2 Breeding Survey

Simultaneously, along with G. R. a team of two technicians checked all the existing breeding sites with the help of frying pan/scoop to assess extent of 4th instar larvae/pupae density per dip. Anopheline and culicine were recorded separately.

4.2.3. Division of Areas. See Map-1

Breeding places from number 1 to 29 were reserved for Briquet treatment. Total area was 7474 m² while 30 to 52 breeding sources (1965 m²) were treated at the rate of 250 ml a.i/hect; and the breeding sites from 53 to 80 comprising of 11175 m² with 300 ml/hect.

Upper part of breeding place number one was never treated and was kept aside to serve as check, and source of untreated larvae and pupae.

4.3 EVALUATION:

Efficacy evaluation of methoprene juvenile hormones (JH-I.G.R)

is different and difficult as compared to conventional larvicide. Normal assay procedures and criteria, e.g. larval density estimations or mortality counts etc, cannot be employed, since it does not manifest the acute toxic effects. So, it is not possible to measure its impact under natural conditions by traditional assessment techniques to evaluate its delayed effects against mosquitoes. It is necessary to design special evaluation methods. The following methods were used :-

4.3.1 Adult Emergence rate.

ALTOSID (J.H) is known to produce morphogenetic effects on mosquitoes rather than direct toxic effects. After the application at the recommended rate, absolutely no effect can be observed on the larvae. They continue their normal development and pupae. The pupae also appear to be normal but they eventually die and adults do not emerge. Because of this novel effect, the number of emerged adults could be considered as the prime criteria to assess control.

Five (5) breeding places, in each dose area were fixed for continuous observations. In addition "control breeding place" (untreated) was also checked regularly. Fourth instar larvae and pupae were collected from these observatory breeding sites and brought to insectary every 3rd day, separately in respective waters from the specific breeding place. Plastic cylindrical jars 6-1/2 "in length and 4-1/2" diameter were used for this purpose. Mouths of the jars were closed by cotton gauze and rubber band.

Pupae obtained from the field, described above, were kept in our insectary for observations of excuviation or mortality at this stage. Thus successfully emerged adults, looking normal (males and females) were counted. Incomplete emerged adults were counted towards "dead pupae." Deformed adults and adults that could not fly were recorded separately, but added to "dead pupae".

Fourth instar larvae were fed on liver powder and retained till they pupated. Every day pupae were picked up and kept in a jar for emergence. All the information described above for field pupae emergence was recorded for larvae also, as to how many died, pupated and successfully emerged into normal female/male adults.

Methoprene is known to stick to glass and organic matter therefore, it is ideal to use disposable containers for keeping larvae and pupae. since such disposable containers were not available we had to use plastic jars. These jars after each use were thoroughly washed by detergent and put under direct sunlight for two days before using them again.

4.3.2. Development of Morphological Abnormalities.

As mentioned above emerged adults were noted for morphological abnormalities and sex ratio. A record was maintained for such abnormalities during recording of the emerged adults.

4.3.3 Longevity of Emerged Adult Mosquito.

Counted males and females surviving treatment were kept in a wooden cage 10" X 10" X 8" which was draped with wet towels and the adults were provided with a sugar bottle (5%) cotton soaked. They were fed on rabbit/mice after third day onwards. Separate cages for each observed breeding place and control were maintained. These cages were kept under natural conditions in an un-controlled temperature. Each day, dead mosquitoes were removed and counted. Minimum and maximum room temperature was noted every morning at 0800 hours.

4.3.4. Ovicidal Effect.

Egg rafts from the treated breeding places were collected and held individually in plastic vials (with breeding places water) for two days. On the third day counting of hatched and unhatched eggs was made under a microscope.

4.3.5. Non-Reproductivity.

Emerged mosquitoes from treated places (apparently normal) and from "Check" breeding places obtained as described under Section 4.3.1. were observed in laboratory for :-

- (a) Number of egg batches laid by individual females. (from treated sites and un-treated site).
- (b) Number of eggs laid in each batch.
- (c) % of hatching of each batch.
- (d) % of development upto adult stage from each batch of eggs.

4.3.6. Initially five dosages of ALTOSID, 100 ml/hect., 150 ml/hect., 250 ml/hect. and 300 ml/hect. of SR-10 and briquet/100 ft² were tested in the field. Last three of them were finally selected since on the third day of observation emergence of adult mosquitoes from pupae collected was nil.

4.3.7. First round of treatment was given on the 8th April, 1985. But the area received heavy down pour (37 m.m rainfall) on April, 10, and evaluation on the following alternate days and onwards gave erratic results. However, we followed it up for two weeks and then retreated

the area on 25 April, 1985 with respective doses.

Results of this first treatment are not included in the regular evaluation but attached as Annexure-I.

4.3.8. Fourth instar larvae collected from the field and observed for pupation and adult emergence gave baffling, and non-consistent results. Thus evaluation on this basis was dispensed with and only conclusive observations are included here.

5. RESULTS AND DISCUSSION:

5.1. Observations on Field Collected 4th Instar Larvae and Pupae.

4th instar larvae and pupae collected from the field as described above were kept separately and observed for emergence into normal adults.

5.1.1 Field collected 4th instar larvae. As mentioned above, larvae collected from the field gave rather erratic results. However it was observed conclusively that :-

- (a) Some % (20-37%) 4th instar larvae did not develop to pupae when obtained from SR-LO treated breeding places. Mortality among anopheles species was much higher (60-80%). Twelfth day or latter collections usually 100% developed into pupae.
- (b) 20% larvae pupated after two days; another 50% on 4th day and remaining in other one or two days. Thus development process from 4th instar larvae to pupae seems to have delayed considerably. Most of the pupae collected from check breeding sites became adults in just two days.
- (c) Pupae developed from treated larvae emerged to adults; but % of emergence was highly variable. For example third day post treatment emergence rate was 80% and on day 6 it was 40% or less. Day 9 gave emergence of 10-100% from the same breeding site and same treatment round. Perhaps, sometimes larvae did not pick up enough dose and we brought them to laboratory for observations and they successfully developed to adult stage.
- (d) Pupae formed, some of them, were lethargic, with long and straighter abdomens, some others had flatter and longer cephalothorax. Among such pupae failure to emerge into normal adult mosquito was common. However, the percentage of such pupation was highly variable.

5.1.2 Observations on Field Collected Pupae :-

(a) ALTOSID BRIQUETS :-

In Table-I average % of adult emergence from five indicators breeding

sites is enlisted at different post treatment intervals. Following is the consolidated pooled average of adult emergence of 4 rounds of applications.

Post-treatment interval (Days)	No. of Observed Pupae.	Adult emergence %
3	1855	0.2
6	1723	0.87
9	1453	9.29
12	1410	17.16
15	1326	25.13
18	1427	36.37
21	1527	49.83

It will be observed that 3 days after treatment emergence of adult mosquitoes from treated breeding sites is 0.2%, almost nil. And it is 0.87 on the sixth post-treatment day.

Successful emergence of adult mosquitoes increases progressively and becomes almost 50% at post-treatment day 21. If we see individually the 21st day's observation of each round of treatment the emergence of adults is around 50% in each breeding site. Thus we can conclude that ALTOSID briquets when applied at the rate of one briquet/100 ft² is effective upto 21 days, if we fix the criteria of <50% adult mosquito emergence.

A deeper look on the more detailed Table-I.A, which gives information of individual breeding sites, reveals little association of the breeding site and effectiveness.

Breeding place No. 1 was a slowly moving drain with rich vegetation; it appears after 18 days the effect was wanning faster as compared to other confined breeding sites. This could also be due to heavy breeding in this water. This breeding site always recorded high densities of culicine larvae/pupae.

Breeding place No. 19 was a shallow drain with little vegetation and was favourite breeding site for An. stephensi and An. subpictus mainly. There appears pto be no significant difference of effect of effect as compared to other sites.

(b) Altosid 250 ml/hect.

Five sits in the area treated with 250 ml/hect. dose were selected for regular observations. The results of observations are given in Table-II and II.b

From Table-II following is the summary from all the four rounds :-

Post-treatment Interval (Days)	No. of Observed Pupae.	Adult Emergence %
3	1219	0.08
6	1145	2.88
9	981	9.38
12	1031	29.68
15	1017	46.21
18	926	57.66
21	763	73.92

This dose of SR-10 formulation appears to be more effective at the initial stage. On the 3rd day after treatment emergence is 0.08% as compared to briquets where the emergence was 0.2% but 6th day observation shows SR-10 has lost some of its effect and the emergence rate increases to 2.88% in comparison to 0.87% of briquets on the same day. On the 9th day effect seems to be the same. But afterwards SR-10 appears to loose its effect rapidly and on 15th post-treatment day 46.21% pupae give rise to adult mosquitoes.

Thus SR-10 is effective for 15/16 days when applied at the rate of 250 ml/hect.

Table-II b. which depicts data from each breeding place, does not reveal any significant difference of various breeding sites though nature of them was not identical. Breeding place No. 46 had anopheles breeding and the water was clearer. At this site effect appears to be a bit prolonged. And site No. 7 had more pollution and effect appears to decrease towards the end.

(c) ALTOSID 300 ml/hect

Following is the consolidated data from Table-III reflecting adult emergence % at every 3 days interval after 300 ml/hect. treatment of SR-10 ALTOSID.

Post-treatment Interval (Days)	No. of Observed Pupae.	Adult Emergence %
3	1965	0.15
6	1595	0.43
9	1301	13.06
12	1311	27.00
15	962	34.05
18	962	34.05
18	898	46.09
21	542	61.99

In comparison to ALTOSID briquets its initial effect appears to be stronger for the first six days. Afterwards briquets seem to have gradual deterioration in maintaining the control whereas SR-10 loses its effect more rapidly and can control only 50% emergence of adults at day 18 whereas briquets had slightly more control than this on day 21. Looking at Table-III.b a few interesting things are noticed.

(a) Breeding place No. 62 which was a small confined breeding site of An. stephensi seems to have controlled for much longer period especially during less hot months i.e. October and November. On day 18 after treatment emergence of adults is only 20% as compared to 40.3% 41.9% during April and May on the same time interval. And on 21st observed day it is 40.3% and 46.3% which indicates its longer effect on Anopheles stephensi.

(b) In the same breeding place at day 15 the emergence is much lower as compared to other places.

(d) 4th instar larval study also revealed highest mortality among Anopheles stephensi at larval stage and a good % (varying between 10-65%) did not pupate during 3-15 post-treatment days.

So we could conclude from the above discussion that this dose has the effect of controlling culicine larvae of this area upto 18 days and anopheles for more than 21 days. This dose has also pronounced killing effect on anopheles larvae at stage 4.

In general it appears ALTOSID briquets, at the recommended dose have 50% adult mosquito emergence control in any type of breeding place in this area for 21 days. These were easy to apply, without special equipment, and quicker to cover the area. There was no complain of irritation or itching what so ever among the workers who did this job for 2/3 hours continuously.

Altosid SR-10, 300 ml/hect., is better as compared to 250 ml/hect. dose in term of efficacy in control and longevity especially for An. stephensi control.

5.1.3. Aberrations.

4th instar larvae which died before pupation were noticed to be 50% which appeared continuing growth without becoming pupae. They

were extra-ordinarily long in size having exceptionally long necks.

About 10% dead larvae had unusual growth in body hairs and the body itself was very fluffy and pale in colour. This abnormality was more common in Anopheline larvae (sp when identified was An. step.)

Rest of the dead larvae appeared normal but had turned dark brown to almost black.

Fourth instar larvae took 2-5 days to become pupae. Amongst those which quickly pupated, mortality was high at the stage of pupae.

Field collected pupae also had some abnormalities e.g. very small size (especially Anopheline spp) and majority failed to become adult. Colour was pale to almost transparent.

Some pupae appeared very restive and most of the time ran about. 10% emergence was delayed to 3rd day.

5.2 Observations on Emerged Adults.

5.2.1 Morphological abnormalities among the adults emerging between 6-15 days after treat was common. Among some of these morphological abnormalities were :-

- (i) Left wing or right wing was extended or sometimes both of them.
- (ii) Wings too short and adult could hardly fly for a few seconds and hobbled around.
- (iii) Among anopheline (stephensi and subpictus) palpi were hardly half the length of proboscis.
- (iv) In one specimen of An. stephensi one antenna was found to be vestigial.
- (v) A couple of specimens of Cs. fatigans were noticed to have downwardly curved probosis.
- (vi) Sex ratio distrotrion :- Adults emerging between 3-12 days after treatment showed great sex proportion deviations. Number of males emerged was usually significantly low. (Sometimes the trend was reversed) See Table-I, II and III.

5.2.2 An attempt was made to observe genetical implications of such mutations. But all our efforts failed to get even first generation. Either the individuals survived for a few hours or they failed to mate even with normal males/females.

5.2.3 Longevity of treatment surviving adults :- Study was made on culex fatigans only. Emerging mosquitoes from one breeding place

on post-treatment day 12, 15 and 18 was usually not more than 8-10 male/females and pooling up of mosquitoes from various breeding sites was not considered suitable. However, it appears that there is no significant difference in longevity of treatment survivors as compared with control emerging mosquitoes.

5.2.4 Non-Reproductivity

During longevity studies, discussed above (5-2-3) observations were made for the number of eggs laid by each individual, % of hatching and number of egg batches laid in comparison to mosquitoes from control. But no appealing difference could be noticed in any of these aspects.

5.3 Ovicidal effect.

No ovicidal effect on field eggs was found on the hatching of culex eggs.

5.4 Effect on N.T.Os.

Breeding place No. 1 (Treated with Altosid Briquets) was very rich in aquatic organisms. Similarly, breeding sites No. 47 and No. 66 in areas treated with 250 ml/hect Altosid SR-10 and 300 ml/hect Altosid SR-10 were also selected for observations on N.T.Os.

During the study period frequently found aquatic organisms were: Chlorophyta (green algae) and spirogyra along with common grass and some unidentified weeds. Among invertebrates Buena sp. (back-swimmers) water beetles and may fly larvae were commonly found.

Other ciliates, Araneae (spiders) small molluscs, fresh water snails of class Gastropoda, shrimps and other crustaceans, odonata nymphs, corixidae, hydophilids, notonectids and dytiscids and occasionally frog tadpoles were also encountered.

Some of them were brought to laboratory along with larvae and pupae from the field for observations. But no effect was found on them. Neither any decrease per dip was noticed during larval surveys after treatment.

Hydrophytae also showed no signs or adverse effects at all.

6. Conclusions.

6.1 Altosid briquets, when applied at the rate of one briquet per 100 Sq. Ft, controls emergence of mosquitoes around 50% upto 21 days. These are easy to apply and pose no difficulty like itching or irritation.

6.2 Altosid SR-10, 250 ml/hect. application controls mosquito emergence by 50% till 15/16 days only.

6.3 Altosid SR-10 300 ml/hect has the same effect as briquets but have longer efficacy for anopheles spp.

6.4. Altosid briquets or SR-10 formulations (300 ml/hect) in our typical field conditions in which studies were conducted can be applied as a part of some integrated programme of mosquito control for culicine as well as Anopheles mosquitoes. Three weeks interval round shall effectively control all types mosquitoes of the area.

6.5 Both the formulations are very safe to handle and have no effect on common non-target aquatic organisms usually present in our water collections.

7. SUGGESTIONS

7.1 This field study clearly shows high biological activity against all the culicine and anopheles mosquitoes of the area. And there is appreciable residual effect also. It is therefore suggested that a practical evaluation of large scale may be conducted to observe its impact on malaria transmission and in suppression of other mosquito nuisance.

A trial on two/three village basis can demonstrate its usefulness against our prevailing insecticide resistant mosquitoes.

7.2 Altosid briquets are too heavy and go down to the bottom in mud etc. which makes its life shorter. Or sometimes animal's movement in certain type of breeding places makes it ineffective. It is therefore suggested that a formulation be prepared which remains submerged in water and does not go deep to the bottom. This shall surely enhance its effectiveness in terms of residual effect.

7.3 National Malaria Training Centre has conducted several field trials with various larvicides and has always realized that appropriate dose application of these expensive compounds is not a simple proposition, particularly in practical use of normal field operations (other than specially designed field trials) and without special equipment. Altosid Briquets, during this study were found to be a good device which could check over/under dose to a large extent and were easy to apply. But we noted out of 86 breeding sites that we plotted, 25% of them were small enough that 1/2 a briquet (a.e) would have been sufficient for recommended dose. Similarly previous experience of city area trials also show that most of the breeding sites were 50 Sq. Ft. in size and were shallow (a few inches in depth). Thus Altosid briquet containing 1/2 a.i. would be more appropriate for economic application.

TABLE 1

OBSERVATIONS ON PUPAE

COLLECTED FROM TREATED AND CHECK SITES

BRIGUET 1/100 FL²

COLLECTED FROM TREATMENT

DAYS AFTER TREATMENT		No. of Pupae Observed	♀ EMERGED + ABNOR.*		♂ EMERGED + ABNOR.*		Σ EMERGED ♀ + ♂	TOTAL NORMAL EMERGED		X ²	REMARKS
								♀	♂		
2nd TREATMENT 25-4-1985	3	680	5	1	1	0	0.86	5	1	2.66	* MORPHOLOGICALLY ABNORMAL ADULTS COUNTED TOWARDS DEAD MOSQUITOES:
	6	514	8	2	1	1	1.75	8	1	5.44	
	9	423	15	3	7	0	5.20	15	7	2.90	
	12	489	63	5	19	2	16.97	64	19	24.39	
	15	470	65	1	71	0	28.93	65	71	0.26	
	18	648	104	0	117	0	34.10	104	117	0.76	
	21	819	206	0	209	0	50.67	206	209	0.02	
CONTROL		401	193	0	189	0	95.26	193	189	0.04	
3rd TREATMENT 20-5-1985	3	502	3	0	1	0	0.79	3	1	-	
	6	505	3	3	1	1	0.79	3	1	-	
	9	378	23	4	11	2	8.99	23	11	4.23	
	12	429	47	3	14	1	16.55	47	24	11.25	
	15	377	46	1	54	2	26.52	46	54	0.64	
	18	286	54	1	57	0	38.81	54	57	0.03	
	21	363	86	0	88	0	47.93	86	88	0.02	
CONTROL		389	180	0	189	0	94.85	180	189	0.21	
4th TREATMENT 7-10-1985	3	425	0	0	0	0	0.00	0	0	-	
	6	437	2	1	0	0	0.45	2	0	-	
	9	411	31	3	17	1	11.67	31	17	4.08	
	12	302	27	2	13	0	13.24	27	13	4.90	
	15	325	51	3	46	2	26.76	41	46	0.28	
	18	356	67	0	65	0	37.35	65	66	0.02	
	21	345	88	0	84	0	50.00	88	84	0.09	
CONTROL		631	310	0	303	0	97.14	310	303	0.07	
5th TREATMENT 27-10-1985	3	248	0	0	0	0	0.00	0	0	-	
	6	267	0	0	0	0	0.00	0	0	-	
	9	241	19	1	12	0	12.86	19	12	1.58	
	12	190	26	4	22	2	25.26	26	22	0.33	
	15	154	28	1	29	0	37.01	28	29	0.01	
	18	137	28	0	28	0	40.87	28	28	0.00	
	21										
CONTROL		409	193	0	199	0	95.84	193	199	0.09	

TABLE 1. A.

BREEDING PLACE WISE

ADULT MOSQUITOES EMERGENCE OBSERVATIONS

DAYS AFTER TREAT- MENT	ALTOSID.				BRIQUETS 1/100 ft2				CONTROL			
	Br. Pl. No: 1				Br. Pl. No: 13				Br. Pl. No: 11			
	Obs EMERGED				Obs EMERGED				Obs EMERGED			
	P	♀	♂	%	P	♀	♂	%	P	♀	♂	%
3	260	2	0	0.8	97	0	0	0.0	63	0	0	0.0
6	199	1	0	0.5	103	3	0	2.9	79	2	1	3.8
9	140	0	1	0.7	93	6	2	8.6	83	4	2	7.2
12	240	37	16	22.0	113	12	6	15.9	78	9	4	16.7
15	190	30	32	32.6	89	12	9	23.6	53	6	8	26.4
18	250	31	33	25.6	110	23	22	40.9	62	10	12	35.5
21	250	60	75	54.0	121	29	26	45.5	71	19	16	49.3
3	172	2	1	1.7	49	0	0	0.0	93	0	0	0.0
6	183	0	0	0.0	63	2	1	4.8	73	0	0	0.0
9	109	6	3	8.25	71	7	3	14.0	82	3	1	4.9
12	133	18	7	18.8	69	8	4	17.4	75	10	6	21.3
15	111	20	15	31.5	75	8	13	28.0	81	7	13	24.7
18	99	20	19	39.4	53	9	10	35.8	47	8	14	46.8
21	121	30	32	51.2	67	14	16	44.8	72	20	18	52.8
3	190	0	0	0.0	57	0	0	0.0	65	0	0	0.0
6	159	2	0	1.2	75	0	0	0.0	78	0	0	0.0
9	153	13	7	13.0	69	4	2	8.7	53	5	2	13.2
12	82	8	4	14.6	71	6	2	11.3	60	4	3	11.7
15	91	12	16	30.8	72	10	11	29.2	57	5	5	17.5
18	104	23	20	41.3	85	13	14	31.8	62	14	13	43.5
21	89	26	27	59.5	75	18	20	50.7	51	14	12	51.0
3	37	0	0	0.0	53	0	0	0.0	59	0	0	0.0
6	67	0	0	0.0	49	0	0	0.0	53	0	0	0.0
9	46	3	2	10.8	58	3	1	6.9	52	6	4	19.2
12	56	7	5	21.4	36	4	3	19.4	22	3	4	31.8
15	44	7	7	31.8	22	4	4	36.4	26	5	5	38.5
18	31	7	7	45.2	43	7	8	34.9	16	4	3	43.7
21												
3												
6												
9												
12												
15												
18												
21												

* BREEDING PLACE WITH ANOPHELES.

TABLE-2
OBSERVATIONS ON PUPAE

COLLECTED FROM TREATED AND CHECK SITES

		ALOTOSID SR-10						250 ml a/hect				
DAYS AFTER TREATMENT		NO. OF PUPAE OBSERVED	♀ EMERGED		♂ EMERGED		EMERGED		TOTAL NORMAL EMERGED		X ²	REMARKS
			+ ABNOR.		+ ABNOR.		♀ + ♂		♀ + ♂			
SECOND TREATMENT 25-4-1985	3	425	1	2	0	0	0.23	1	0	-	MORPHOLOGICALLY ABNORMAL ADULTS	
	6	408	5	2	2	2	1.71	5	2	1.28		
	9	355	21	3	10	2	8.73	21	10	3.90		
	12	353	49	2	51	1	28.32	49	51	0.04		
	15	388	93	0	98	0	49.22	93	98	0.13		
	18	353	106	0	112	0	61.75	106	112	0.16		
	21	340	123	0	127	0	73.52	123	127	0.06		
	CONTROL	401	193	0	189	0	95.26	193	189	0.04		
THIRD TREATMENT 20-5-1985	3	247	0	0	0	1	0.00	0	0	-	COUNTED TOWARDS DEAD MOSQUITOES	
	6	244	6	1	2	0	3.27	6	2	2.00		
	9	222	13	2	6	0	8.55	13	6	2.57		
	12	175	33	3	21	1	30.85	33	21	2.66		
	15	199	52	2	49	1	50.75	52	49	0.08		
	18	153	47	0	46	0	60.78	47	46	0.01		
	21	155	53	0	58	0	71.61	53	58	0.22		
	CONTROL	389	180	0	189	0	94.85	180	189	0.21		
4th TREATMENT 7-10-1985	3	305	0	0	0	0	0.00	0	0	-		
	6	277	6	1	1	0	2.52	6	1	3.57		
	9	182	19	3	13	1	17.58	19	13	1.09		
	12	245	46	1	33	2	32.24	46	33	2.06		
	15	237	52	0	50	3	43.03	52	50	0.03		
	18	233	58	0	73	1	56.22	58	73	1.71		
	21	268	95	0	108	0	75.74	95	108	0.83		
	CONTROL	631	310	0	303	0	97.14	310	303	0.07		
5th TREATMENT 28-10-1985	3	242	0	0	0	0	0.00	0	0	-		
	6	216	8	2	3	3	5.09	8	3	2.27		
	9	222	20	2	10	1	13.51	20	10	3.33		
	12	258	42	4	31	0	28.29	42	31	1.65		
	15	193	37	1	39	0	39.37	37	34	0.12		
	18	187	47	0	45	0	49.20	47	45	0.04		
	21											
	CONTROL	409	193	0	199	0	95.85	193	199	0.09		

TABLE 2. B
BREEDING PLACE WISE.
ADULT MOSQUITOES EMERGENCE OBSERVATIONS

ALTOSID SR-10																				250 mls/hect.									
Days after Treat- ment	Br.Pl.No: 50				Br.Pl.No: 42				Br.Pl.No: 46*				Br.Pl.No: 39				Br.Pl.No: 47				CONTROL:								
	Obs. EMERGED				Obs. EMERGED				Obs. EMERGED				Obs. EMERGED				Obs. EMERGED				Obs. EMERGED								
	P	Q	♂	%	P	Q	♂	%	P	Q	♂	%	P	Q	♂	%	P	Q	♂	%	P	Q	♂	%					
3	83	0	0	0.0	97	0	0	0.0	67	1	0	1.5	91	0	0	0.0	87	0	0	0.0	0	26	30	93.3					
6	91	0	0	0.0	83	1	1	2.4	73	0	0	0.0	86	2	1	3.48	75	2	0	2.7	60	27	30	95.0					
9	73	4	2	8.2	75	5	2	9.33	51	2	1	5.9	78	5	2	8.9	78	5	3	10.3	50	26	22	96.0					
12	79	12	11	29.1	53	7	3	18.8	69	9	10	27.5	83	12	15	32.5	69	9	12	30.4	55	28	25	96.3					
15	81	20	23	53.1	67	18	14	47.8	74	14	16	40.5	89	23	26	55.1	77	18	19	48.1	50	24	22	92.0					
18	63	21	18	61.9	58	18	19	63.8	71	21	17	53.5	82	22	30	63.4	79	24	28	65.8	60	32	26	96.6					
21	60	20	24	73.3	63	25	24	77.8	75	20	30	66.7	87	38	30	78.2	55	20	19	70.9	66	30	34	96.9					
3	40	0	0	0.0	31	0	0	0.0	42	0	0	0.0	71	0	0	0.0	63	0	0	0.0	60	28	30	96.6					
6	38	2	1	7.9	45	1	0	2.2	46	1	0	2.2	63	0	0	0.0	52	2	1	5.8	55	29	25	98.2					
9	31	3	1	12.9	37	1	1	5.4	39	3	1	10.3	58	3	1	6.9	57	3	2	8.8	58	25	29	93.1					
12	27	6	4	37.1	17	2	2	23.5	31	5	3	25.8	41	9	4	31.7	59	11	8	32.2	53	23	27	94.3					
15	33	7	9	48.5	23	6	5	47.8	37	9	11	54.1	43	12	10	51.2	63	18	14	50.8	57	27	26	92.9					
18	29	10	9	65.6	28	7	9	57.2	28	10	8	64.3	26	8	6	53.8	42	12	14	61.9	50	20	28	96.0					
21	31	12	14	83.9	19	6	6	63.2	21	7	9	76.2	35	13	11	68.6	49	15	18	67.3	56	28	24	92.8					
3	53	0	0	0.0	71	0	0	0.0	51	0	0	0.0	49	0	0	0.0	81	0	0	0.0	101	47	51	97.0					
6	49	2	0	4.1	63	1	0	1.6	45	0	0	0.0	47	0	0	0.0	73	3	1	5.5	97	48	47	97.9					
9	44	4	4	18.2	28	4	2	21.4	25	1	1	8.0	45	3	2	11.1	40	7	4	27.5	83	42	38	96.4					
12	66	16	10	39.4	48	11	9	41.7	36	4	4	22.2	72	9	6	20.8	23	6	4	43.5	90	46	42	97.7					
15	58	13	13	44.8	44	9	7	36.4	40	10	10	50.0	56	10	10	35.7	39	10	10	51.3	87	42	41	95.4					
18	51	15	15	58.8	52	12	14	50.0	38	8	10	47.4	47	11	12	48.9	45	12	22	75.6	89	47	40	97.7					
21	57	21	24	78.9	73	28	32	82.2	39	12	12	61.5	50	12	13	50.0	49	22	27	100.0	84	38	44	97.6					
3	49	0	0	0.0	41	0	0	0.0	38	0	0	0.0	43	0	0	0.0	71	0	0	0.0	85	42	39	95.3					
6	39	3	1	10.3	35	0	0	0.0	43	0	0	0.0	60	3	1	6.7	39	2	1	7.7	68	33	32	95.6					
9	48	3	1	8.3	45	1	1	4.4	40	5	2	17.5	45	5	3	17.8	44	6	3	20.5	59	26	31	96.6					
12	41	8	6	34.1	56	6	3	16.1	37	2	2	10.8	44	12	5	38.6	80	14	15	36.3	60	25	32	95.0					
15	40	8	7	37.5	41	7	7	34.1	29	5	8	44.8	31	7	8	48.4	52	10	9	36.5	70	33	35	97.1					
18	33	9	10	57.6	40	11	8	47.5	18	4	3	38.9	33	8	8	48.5	63	15	16	49.2	67	34	30	95.5					
21																													

* BREEDING PLACE WITH ANOPHELES.

TABLE 3
OBSERVATIONS ON PUPAE

COLLECTED FROM TREATED AND CHECK SITES

ALTOSID										300 mls/hect	
DAYS AFTER TREATMENT	NO. OF PUPAE OBSERVED	♀ EMERGED		♂ EMERGED		EMERGED		TOTAL NORMAL EMERGED		X ²	REMARKS
		+ ABNOR.		+ ABNOR.							
		♀	♂	♀	♂	♀	♂	♀	♂		
SECOND TREATMENT 26-4-1985	3	526	1	0	0	0	0.19	1	0	-	* MORPHOLOGICALLY MORPHOLOGICALLY
	6	411	2	0	1	1	0.72	2	1	-	
	9	305	31	2	11	1	13.77	31	11	9.52	
	12	518	82	3	54	2	26.25	82	54	5.76	
	15	356	62	2	60	1	34.26	62	60	0.03	
	18	258	61	0	58	0	46.12	61	58	0.07	
	21	328	101	0	103	0	62.50	101	103	0.02	
CONTROL		401	193	0	189	0	95.26	193	189	0.05	ABNORMAL ADULTS COUNTED ADULTS
THIRD TREATMENT 21-5-1985	3	453	1	0	0	0	0.22	1	0	-	
	6	375	3	1	1	1	1.06	3	1	-	
	9	314	25	3	8	2	10.50	25	8	8.75	
	12	301	51	4	21	2	23.92	51	21	12.50	
	15	260	44	1	43	0	33.46	44	43	0.01	
	18	287	67	0	70	0	47.73	67	70	0.06	
	21	214	61	0	71	0	61.68	61	71	0.75	
CONTROL		389	180	0	189	0	94.85	180	189	0.22	TOWARDS COUNTED DEAD TOWARDS
4th TREATMENT 8-10-1985	3	402	0	0	0	0	0.00	0	0	0.00	
	6	345	0	6	0	1	0.00	0	0	0.00	
	9	369	35	2	19	3	14.63	35	19	4.74	
	12	285	50	4	31	1	28.42	50	31	4.45	
	15	218	40	2	42	1	37.61	40	42	0.04	
	18	234	71	1	72	0	61.11	71	72	0.006	
	21										
CONTROL		631	310	0	303	0	97.14	310	303	0.07	DEAD MOSQUITOES. MOSQUITOES
5th TREATMENT 27-10-1985	3	584	1	0	0	0	0.17	1	0	-	
	6	464	0	0	0	0	0.00	0	0	-	
	9	313	30	3	18	2	15.33	30	18	3.00	
	12	207	42	2	23	1	31.40	42	23	5.55	
	15	128	22	0	19	0	32.03	22	19	29	
	18	119	24	1	25	0	41.17	24	25	0.02	
	21										
CONTROL		409	193	0	199	0	95.84	193	199	0.09	