

PAKISTAN JOURNAL OF HEALTH

Vol. 26 1:2 1989 April.



COLLEGE OF COMMUNITY MEDICINE

6-BIRD WOOD ROAD LAHORE, PAKISTAN

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PAKISTAN *Lahore* JOURNAL OF HEALTH

Vol. 26 1:2 1989 April.



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EDITORIAL

Role of Health Services Research in achieving the goal of Health for all by the year 2000 is of paramount importance. The health delivery system should be dynamic involving community participation and active intersectoral collaboration. This can only be achieved if continuous planning, timely implementation and appropriate evaluation. Such a system will result in raising questions in the mind of health managers the solution of which will require health Services Research in order to find out the best, most economical and efficient method of a progressive delivery system.

In this aspect the College of Community Medicine can play a vital role in finding out answers to the questions raised by field workers. Keeping this in view a W.H.O. workshop was recently held at College of Community Medicine, Lahore. The recommendations of workshop were :

1. Health Services Research should be strengthened and encouraged.
2. It should involve all levels starting from basic health units to the Directorate level.
3. Doctors should be trained in Health System Research.
4. Results of the Health System Research should be incorporated in the developing phases of the planning process.

These recommendations would require the setting up of a research cell at the Directorate level which will cause awareness among the mid level managers of health delivery system. They will frame questions, which will be sent to the College of Community medicine, Lahore. The College has a battery of experts, who can perform analytical research to find the solutions to these problems.

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A SEROEPIDEMIOLOGICAL STUDY ON HUMAN IMMUNODEFICIENCY VIRUS (HIV INFECTION). BY

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SUMMARY

A total of 1601 serum samples including general population (1050) and high risk individuals for AIDS such as prostitutes (60), passive homosexuals eunuchs (30), blood donors (60) blood transfusion recipients (60) haemophiliacs (20), parenteral drug abusers (30), patients with persistent generalized lymphadenopathy (60) and expatriates/visitors of U.S.A. (200), Europe (30) and Bangkok (1), were tested for antibodies to human immunodeficiency virus.

Three commercial ELISA kits namely Flow Laboratories, Wellcome and Behring were used for HIV antibody detection for each serum sample. All ELISA positive sera by any of the above three kits were confirmed by the immunoblot assay (western blot).

No evidence of HIV infection was found in general population and high risk individuals for AIDS. Only one case was positive by three ELISA kits and was confirmed by the western blot and that was a case of AIDS imported from Qatar.

Out of 1601 serum samples tested, Flow Lab. Kit gave ten false positive results, Wellcome one and Behring kit gave no false positive

Behring kit gave no false positive result. The predictive values of ELISA positive tests by the above three kits for the detection of HIV antibodies were calculated. It was found that the predictive values of positive ELISA tested by Flow Lab., Wellcome and Behring kit were 9.09%, 50% and 100% respectively. The best kit in our study was the Behring kit.

INTRODUCTION

Human immunodeficiency virus (HIV) also known as lymphadenopathy associated virus (LAV) and human T-cell lymphotropic virus type III (HTLV-III) has been identified as the etiological agent of the acquired immune deficiency syndrome-AIDS (Clavie et al. 1987). The virus is known to be transmitted through (1) sexual contact (homosexual or heterosexual) with infected person either active or passive, (2) exposure of contaminated blood and blood products, (3) maternal transmission i.e., from mother to child before, during or shortly after birth through milk. Regardless of the area of the world studied, the mode of transmission are fundamentally the same (Mann 1986). HIV damages the immune system which results in fatal opportunistic infections and rare malignancies (Shahmanesh 1986). Infection begins when HIV

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enters the blood stream & stimulate the immune system & the development of antibodies. The presence of these antibodies indicate that infection has occurred. Antibodies to HIV can usually be detected 2-8 weeks after the initial infection (Landesman 1985), although in a small minority 6 months or more may elapse (Goedert 1985). The incubation period ranges from 6 months to 5 years or more (Malik & Farooq 1986). This long & often unrecognized period of asymptomatic infection, during which an infected person can infect others, complicates control of the virus (Mann 1987).

The first cases of AIDS were recognized & described in 1981 (Allen et al. 1988). Upto 4th June, 1988, a total of 96,433 AIDS cases were officially reported to W.H.O., from 138 countries (WHO Wkly Epidem. Rec. 1986). Three areas of the world (America, Europe & Africa) are highly endemic for HIV infection. America reported a total of 65,464 cases among which USA is at the top having 57,575 cases, Europe reported 10,851 cases wherein France is at the top reported 3,073 cases and Africa reported 10,639 cases, wherein Tunisia is at the top reported 2,369 cases. At present Asia is least affected area of the world reported 238 cases wherein Japan is at the top having 66 cases. (WHO Wkly Epidem. Rec. 1986). In Pakistan, since National Survey for AIDS has not been conducted, the evidence of HIV infection cannot be dogmatically stated. Khanani et al. (1988) documented the evidence of HIV infection in four individuals of

Karachi comprising a blood donor and three member of the family (husband, wife and a child). They collected a total of 500 sera from various hospitals and blood banks of Karachi and Lahore, the most populous and cosmopolitan cities in Pakistan. Sera were screened for the HIV antibodies by enzyme linked immunosorbent assay (ELISA) and the reactivity was confirmed by immunoblot assay i.e., western blot.

Enzyme immunoassays to detect HIV antibody were developed in 1984 and became commercially available in March 1985 for screening donated blood and plasma (Reesink et al. 1986). Antibodies to various structural proteins of HIV are easily measured by western blot assay. ELISA is a valuable test for screening blood and blood products (Vezinet et al. 1984) but since it is not so specific as it sometimes produces false positive results (Mendenhall et al. 1986). Therefore, ELISA alone is not valid for screening individuals (Carlson 1984), the seropositive cases by ELISA technique must be confirmed by more specific tests. Today, immunoblot assay enjoys a confirmatory status. Both the Public Health Service and W.H.O. recommend the western blot test to confirm a positive ELISA (WHO 1985, CDC 1985).

2. AIMS, OBJECTIVES & JUSTIFICATION

AIDS has established itself as one of the most deadliest disease of all times. It has struck the

human race in all continents of the globe and has become pandemic in a short span of time. This disease has undoubtedly generated anxiety and terror of unfathomed depth in the minds of humanity.

The number of reported cases of AIDS and of countries reporting AIDS cases have increased dramatically, as already discussed. As of 4th June 1988 a world total of 96,433 AIDS cases were officially reported to WHO from 138 countries including Pakistan (WHO Wkly Epidem. Rec. 1988). Pakistan reported one case of AIDS to WHO on 31st December 1987. Khanani et al. (1988) showed the evidence of HIV infection in Pakistan by quoting four cases of AIDS.

Reported cases are only a fraction of all cases; in some countries the disease may not yet be recognised or reported to national health authorities and some countries remain reluctant to speak openly about it. According to W.H.O. estimate, the actual number of global AIDS cases have been between 100,000 and 150,000 (Mann, 1988) and 5 to 10 million people are infected with AIDS virus with no symptoms (Liskin et al. 1987). It is estimated that for each actual case, there are likely to be an additional 50 to 100 virus infected people (Mann 1988). If past experience holds there will be 10 to 20 times more AIDS cases in the next five years.

AIDS seems always to be fatal, the virus can remain dormant

for years. HIV mutates rapidly. The most powerful weapon we have in the fight against AIDS is early diagnosis and prevention. The overall mortality of the seropositive cases is 43% but in patients with full blown disease it is nearly 100%.

In Pakistan, particularly in Punjab province, no work has been done on AIDS. In view of vast international travel, particularly in endemic areas for AIDS such as USA, Europe etc a continuous vigilance against the disease is most essential. So it is of vital importance to the country that research be carried on high risk groups from various sections of the society, so that effective preventive measures can be instituted before the problem assumes the epidemic proportion and also to formulate a policy regarding AIDS cases, their treatment, prevention and control.

The present study has been planned with the following objectives :-

1. To find out prevalence of HIV antibodies among high risk population for AIDS
2. To study HIV antibodies among travellers to endemic areas.
3. To detect HIV antibodies in general population other than high risk groups.
4. To compare the reliability and efficacy of the three available ELISA kits namely:-

- HIV TEK G (Flow Laboratories).
 - Wellcozyme anti-HTLV-III (Wellcome).
 - Enzygnost-Anti-HIV micro (Behring).
5. To compare the authenticity of the ELISA positive cases with the western blot test technique.

3. MATERIALS & METHODS

Antibodies to HIV has been studied in high risk groups for AIDS and in general population by enzyme linked immunosorbent assay (ELISA) with three different kits (as mentioned earlier) and confirmed by western blot assay.

3.1. STUDY POPULATION AND SAMPLING PROCEDURE

General Population

Subjects consisted of patients including both sexes and all age groups irrespective to the socio-economic status seen in the anti-rabies centre at the College of Community Medicine, Lahore. The simple random sampling procedure was adopted.

High Risk Population

Prostitutes

Heterosexual transmission is an important factor in the dissemination of HIV infection. The prostitutes residing in Shahi Mohalla were selected. Prostitution has been banned legally but the same group

has started singing and dancing to earn livelihood and heterosexuality is continuing under the garb of this entertainment. In order to assess seropositivity for HIV antibodies, simple random sampling was done on the basis of sample frame provided by the concerned Police Station.

Homosexuals

In our society nobody vouchsafes to be homosexual either active or passive due to social or legal restraint and at the same time eunuchs are reputed for indulging in homosexuality as passive partner. Taking this indicator in view, these people were included in homosexual group. Mostly eunuchs live in particular localities with their administrative chief (guro). These heads were contacted and motivated for cooperation. On the basis of list provided by these heads blood samples were collected from eunuchs living in different pockets of Lahore city by simple random sampling procedure.

Blood Transfusion Recipients

Patients receiving multiple transfusions have been thought to have a risk for AIDS if the blood is contaminated (Redfield, 1985).

The Medical Superintendents of Mayo Hospital and Services Hospital, Lahore were contacted for these patients and other high risk patients for AIDS attending the hospital. They circulated letters to Head of Department of all

Medical, Surgical Paediatric Units to take blood samples of these patients. Patients with haemoglobinopathies mainly thalassemia major, Leukaemia and post operative transfusion associated cases were sampled at random.

Blood donors

Donors are considered to be at high risk for transmission of HIV if they have AIDS, ARC or asymptomatic carrier stage i.e. HIV-antibody positive (Peterman 1985).

Professional donors from various private clinics and volunteer donors registered in different private blood transfusion associations were interviewed using a standardized questionnaire and examined for lymphadenopathy or skin lesions, and their blood samples were taken for HIV antibody.

Haemophiliacs

As stated by Sjamoeidin-Visser (1987) haemophilia patients are at an increased risk for developing AIDS and transmission occurs via blood and blood products such as factor VIII and IX preparations. Patients with haemophilia A and B treated with factor VIII and IX respectively were investigated for HIV antibodies from paediatric units of above mentioned hospitals, at random.

Parenteral drug abusers

Blood samples were obtained from drug abusers admitted in drug

addiction control centres of Psychiatry units of Mayo and Services Hospitals. They were longterm users or casual users who had developed addiction. Most of these people were using multiple drugs and had practice of using shared syringes.

Persistent generalized lymphadenopathy (PGL) group

As cited by Kaplon et al. (1987) unexplained generalized lymphadenopathy syndrome (LAS) is a condition clinically, epidemiologically and virologically linked to the acquired immunodeficiency syndrome. For the selection of study subjects, the following criteria for enrolment had been described previously:-

1. each had lymphadenopathy in two or more extra inguinal sites,
2. the duration of PGL was more than 3 months.

At the same time each individual was thoroughly examined for constitutional symptoms such as fatigue, cough, dyspnoea, diarrhoea, weight loss and night sweats. These subjects were selected from indoor patients at random.

Expatriates/visitors of endemic areas

Two areas of the world, America and Europe are highly endemic for AIDS. Upto 30th April 1988, the notified cases from America and Europe are 65,464 and 10,851 respectively (Wkly Epidem. Rec.,

1988) and these cases are increasing rapidly. The people coming from these areas play an important role in dissemination of disease in AIDS free zone. This group comprises those Pakistanis who are settled in various States of USA and Europe and came to their homeland for temporary visits.

Tourists visiting Europe and USA for short period of time may expose themselves to risk of acquiring infections and were also included in this group.

4. RESULTS

4.1 POPULATION SAMPLED FOR HIV ANTIBODY TESTING

Table I shows the population sampled for HIV antibody testing in this seroepidemiological study. The total number of individuals tested was 1601. Out of these, one thousand and fifty (65.58%) individuals belonging to general population were tested. From the high risk groups sixty (3.74%) prostitutes, thirty (1.87%) homosexuals eunuchs, sixty (3.74%) blood transfusion recipients, sixty (3.74%) blood donors including both volunteer and professional, twenty (1.25%) haemophiliacs, thirty (1.87%) parenteral drug abusers, sixty (3.74%) patients with persistent generalized lymphadenopathy, two hundred (12.49%) expatriates/visitors of U.S.A., thirty (1.87%) of Europe and one (0.06%) of Bangkok were included in the series for HIV antibody study.

4.2 PREVALENCE OF HIV ANTIBODY IN GENERAL POPULATION AND HIGH RISK GROUPS

Table II gives the prevalence of HIV antibody in general population and high risk groups. The high risk groups constitute prostitutes, homosexuals, blood transfusion recipients, blood donors, haemophiliacs, parenteral drug abusers, patients with persistent generalized lymphadenopathy and expatriates/visitors of the endemic areas such as U.S.A., Europe and Bangkok. Sera of all these individuals were tested for HIV antibody by three ELISA kits i.e. HIV TEK G (Flow Laboratories), Wellcozyme Anti-HTLV-III (Wellcome) and Enzygnost Anti-HIV micro (Behring) and western blot assay. As mentioned in Introduction and Material And Method, western blot assay is a standard test for HIV antibody detection so positive results by all ELISA kits were compared with it.

The above Table shows that Flow Lab., kit gave eleven positive results whereas Wellcome gave two and Behring one. All subjects included in this study were negative by western blot assay except the AIDS case (Late Mumtaz Begum). She has been included in blood transfusion recipient group since she had received multiple blood transfusions in Qatar. Her detailed history is described in Table III.

Out of 1050 sera collected from general population, two were positive by Flow Lab. kit and negative by Wellcome and Behring kits. Western blot also gave negative results.

TABLE I
POPULATION SAMPLED FOR HIV ANTIBODY TESTING

S.No.	G r o u p s	Number	Percent
1.	General Population	1050	65.58
2.	Prostitues	60	3.74
3.	Homosexuals (Eunuchs)	30	1.87
4.	Blood Transfusion Recipients	60	3.74
5.	Blood Donors	60	3.74
6.	Haemophiliacs	20	1.25
7.	Parenteral Drug Abusers	30	1.87
8.	Persistent generalized Lymphadenopathy	60	3.74
9.	Expatriates/visitors of endemic areas :-		
	U.S.A.	200	12.49
	Europe	30	1.87
	Bangkok	1	0.06
TOTAL:-		1601	

TABLE II
PREVALENCE OF HIV-ANTIBODIES IN GENERAL
POPULATION AND HIGH RISK GROUPS

G R O U P S	Number Tested	No. POSITIVE BY ELISA			No. Positive by Western Blot
		Flow Lab.	Well-Come	Behring	
General Population	1050	2	0	0	0
Prostitutes	60	2	0	0	0
Homosexuals (Eunuchs)	30	0	0	0	ND*
Blood Transfusion Recipients	60	1**	1**	1**	1**
Blood Donors	60	0	0	0	ND
Haemophiliacs	20	0	0	0	ND
Parenteral Drug Abusers	30	1	0	0	0
Persistent Generalized Lymphadenopathy	60	0	0	0	ND
Expatriates/Visitors of					
U. S. A.	200	4	0	0	0
Europe	30	0	0	0	ND
Bangkok	1	1	1	0	0
TOTAL:-	1601	11	2	1	1

* ND - Not done

** Case of AIDS

Out of 60 prostitutes two gave positive results on Flow Lab. kit and negative on the other two kits. They were also negative on western blot. One prostitute who showed positive response by ELISA on Flow Lab. kit, was suffering from fever with typical history of malaria. Her blood was examined for malarial parasite and Plasmodium falciparum was detected. To exclude cross reactivity due to malaria, blood sample was recollected after her recovery. This time serum was negative for HIV antibody even on Flow Lab. kit.

All passive homosexuals (eunuchs) were negative by three ELISA kits.

Only one transfusion associated AIDS case mentioned above who had received blood transfusion for Orthopedic Surgery in Qatar. She was positive by all the ELISA kits as well as by western blot. All other patients who had received multiple transfusions were tested and found negative.

Blood donors (professional and volunteer) were negative on serological testing for HIV antibody. Similarly haemophiliacs and patients with PGL were also negative by all three ELISA kits.

Out of 30 parenteral drug abusers one showed positive result on Flow ELISA kit and negative by other two kits and western blot as well.

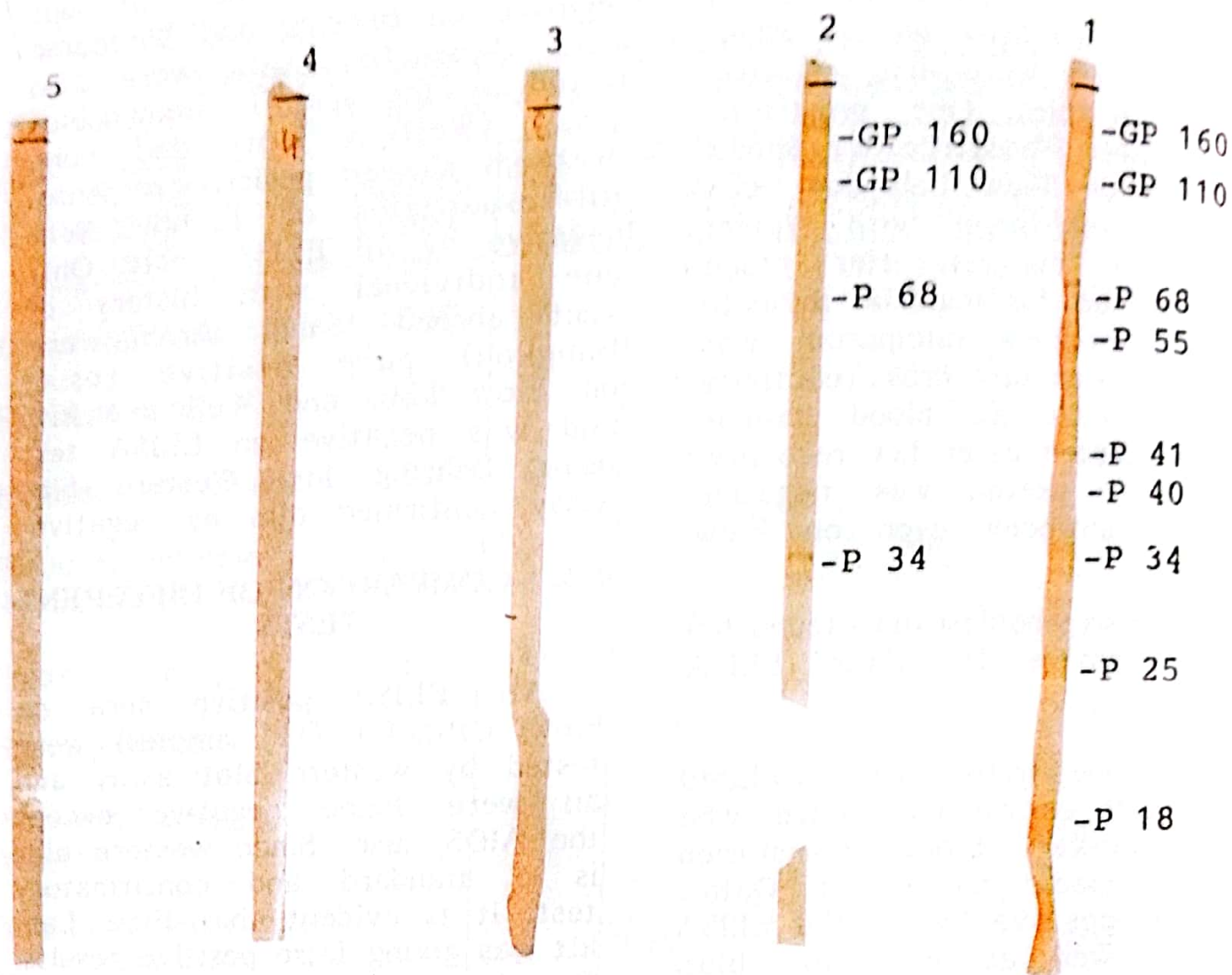
Four of the 200 sera of expatriates/visitors of U.S.A. were

positive by Flow Lab. kit but negative on Behring and Wellcome kits. These four sera were also tested by the specific immunoblot assay (Western blot) and none of them showed positive response. All expatriates of Europe were negative by all ELISA tests. Only one individual with history of visit abroad (Saudi Arabia and Bangkok) gave positive result on Flow Lab. and Wellcome kits and was negative on ELISA test using Behring kit. Western blot assay confirmed him as negative

4.3. COMPARISON OF DIFFERENT TESTS

All ELISA positive sera on Flow Lab. kit (11 samples) were tested by western blot assay and all were found negative except the AIDS case. Since western blot is a standard and confirmatory test, it is evident that Flow Lab. kit was giving false positive results. On the other hand Wellcome kit picked up only one false positive whereas Behring kit did not pick up any false positive test. This comparative study revealed that Behring and Wellcome kits give more reliable results as compared to Flow Lab. kit.

The sensitivity and specificity is determined by comparing the results of three ELISA kits with the reference test for HIV antibody i.e. western blot by the Fischeinger and Bolognesi method (Fischinger, and Bolognesi, 1985). As western blot is costly so we were unable to perform this test to all sera. Only ELISA positive sera by any of the three kits were tested by western blot.



WESTERN BLOT ASSAY OF SERUM FOR HIV ANTIBODIES TESTING

Brown coloured bands on the nitrocellulose membrane strips indicate the specific bands of viral proteins. These bands are of gp 160, gp 110, P 68, P 55, P 41, P 40, P 34, P 25 and P 18.

- Strip 1 = Positive Control
- Strip 2 = Case of AIDS
- Strip 3 = Test serum sample (ELISA +ve)
- Strip 4 = Test serum sample (ELISA +ve)
- Strip 5 = Negative control.

(LAV. BLOT Diagnostics Pasteur)

TABLE III
HISTORY OF HIV SEROPOSITIVE CASE OF AIDS

- Group category	blood Transfusion Recipient
- Age	36 years
- Marital status	Married
- Sex	Female
- Visit abroad	Qatar
- H/O blood transfusion	Yes-six and half year ago she was trans- fused blood after road side accident.
- Hepatitis (Jaundice)	N. A.
- Extramarital sexual contact	N. A.
- Drug abuse	No
- Stage of HIV infection	Symptomatic-full blown case of AIDS.
- Symptoms developed	July 1987
- Date of Expire	June 1988
- Duration of illness	11 months
- Date of serum collection	16 April 1988
- HIV antibodies detection by:-	
- Three ELISA kits (Flow Lab. Wellcome and Behring).	POSITIVE
- Western blot assay	POSITIVE

4.4 SEROLOGICAL TESTING OF AIDS CASE

A middle aged lady named Mumtaz Begum with clinical features of AIDS was admitted in Services Hospital, Lahore in April 1988. She has acquired HIV infection by blood transfusion in Qatar for Orthopedic Surgery following road side accident. Her serum was strongly reactive for HIV antibody by all three ELISA kits (Flow Lab., Wellcome and Behring). Her serum was also tested by the western blot. Brown coloured specific bands of viral proteins appeared on the nitrocellulose membrane strips. These bands were of gp 160 gp 110, p 68 and p 34 of viral proteins as described in Figure 1. These bands were specific for HIV viral proteins.

Table III gives history of HIV seropositive case of AIDS (Late Mumtaz Begum). Her discharge summary given below illuminates her history, clinical findings and course of disease. She has now expired after eleven months of illness. She was given a blood transfusion six and half years before the appearance of symptoms which would constitute the incubation period or the period of asymptomatic carrier stage of virus.

4.5. PREDICTIVE VALUES OF POSITIVE ELISA TESTS FOR HIV ANTIBODIES

Table IV gives the comparison of predictive values of positive ELISA tests for HIV antibodies by three kits. Flow Lab. kit gave ten false positive results and its predictive value of positive tests is 9.09%. The Wellcome kit gave one false positive and its predictive value is 50%. The Behring kit gave no false positive hence its predictive value of positive ELISA test is 100%.

5. DISCUSSION

5.1. PREVALENCE OF HIV INFECTION

AIDS could turn out to be the greatest medical challenge of this century. This worldwide pandemic is presenting more unique problems than we are able to cope with. Upto 31st January 1988, a total of 77266 AIDS cases were officially reported to W.H.O. from 138 countries but the actual worldwide number of AIDS cases has been between 100,000 and 150,000 (Mann 1988).

Khanani et al. (1988) documented the evidence of HIV infection in four individuals which include a blood donor and three members

TABLE IV

COMPARISON OF PREDICTIVE VALUE OF POSITIVE ELISA TESTS FOR HIV ANTIBODIES BY 3 KITS

K I T	FALSE POSITIVE	TRUE POSITIVE	PREDICTIVE VALUE OF POSITIVE ELISA TESTS (PERCENT)
Flow Lab.	10	1	9.09
Wellcome	1	1	50
Behring	0	1	100

of a family (husband, wife and a child) in Karachi, Pakistan. Their data indicates that HIV has been transmitted to the wife of the seropositive male, an intravenous drug abuser in the recent past. His wife passed the virus to her new born child transplacentally. The two seropositive males (blood donor and drug abuser) were diagnosed clinically as having persistent generalized lymphadenopathy, AIDS related complex and the child presented with developmental disorders and neurological manifestations.

John et al. (1987) working in Tamilnadu, India found overall prevalence rate (0.8%) of HIV infection in high risk population of India, wherein 2.9% female prostitutes, were positive (30 out of 1025).

The population sampled in our study belonged to high risk for AIDS such as prostitutes, homosexuals, blood transfusion recipients, blood donors, haemophiliacs, parenteral drug abusers, patients with persistent generalized lymphadenopathy and expatriates-visitors of U.S.A., Europe and Bangkok. In addition to high risk population, the general population was also included in our study. There were no positives in any high risk group or the general population. One case of AIDS which was imported from Qatar was only positive. It may be presumed that the Lahore population is presently free from AIDS. On the contrary Khanani's study of seropositivity in Karachi indicates

the presence of AIDS in Karachi which would require further studies of high risk groups.

5.2. EVALUATION OF THREE ELISA FOR THE DETECTION OF HIV ANTIBODIES

In Lahore, there was no established laboratory for the detection of HIV antibodies. In order to establish such a laboratory, the first step required was to carry out research on the comparative efficacy of various ELISA kits available, with a standard test. The available standard test was the western blot assay for HIV antibodies detection.

COMPARISON OF FREQUENCIES OF FALSE POSITIVE REACTIONS

Commercial ELISA kits became available in 1985 and developed quickly. When these kits were introduced, little was known about their quality. Initially five ELISA kits were evaluated in the United States but unfortunately evaluation of each kit used different set of serum samples, so that the study was not scientifically comparable (Reesink et al. 1986).

Mortimer et al (1985) evaluated five commercial ELISA kits but the sample size was too small to allow the statistical analysis of the sensitivity and specificity of various enzyme immunoassays.

Courouce (1986) evaluated eight ELISA kits for the detection of HIV antibodies. The kits used in

his study were; Litton, Behring, Wellcome, Dupont, Pasteur, Abbott and Organon. This study revealed that the Wellcome kit followed by the Pasteur kits are most specific of eight kits tested, the sensitivity of various kits was of the following order. Pasteur kit was the most sensitive. The Dupont kit was less sensitive than the Wellcome and the least sensitive was the Abbott kit.

Reesink et al. (1986) evaluated six ELISA kits for HIV antibody detection. The kits used in their study were: Abbott, Organon, Litton, Wellcome, Behring and

Pasteur. A total of 6488 samples of AIDS patients high risk population for were tested by these kits. The western blot assay was adopted as the confirmatory test. It was noted that Abbott and Wellcome kits identified all 163 immunoblot positive samples correctly whereas the other kits did not detect 1-3 samples.

The frequencies of false positive reactions in a "tricky" panel of samples from patients with autoimmune and acute viral disease and in a "blood donor" panel were:

TABLE V
COMPARISON OF FREQUENCIES OF FALSE POSITIVE REACTIONS
BY VARIOUS ELISA KITS IN TWO DIFFERENT STUDIES
(Reesink et al. 1986)

K I T	Reesink et al. study		OUR STUDY
	TRICKY PANEL	BLOOD DONOR PANEL	
Abbott	9.5%	0.42%	-
Organon	1.7%	0%	-
Litton	1%	0.4%	-
Behring	2.7%	0.06%	0%
Wellcome	0%	0%	0.062%
Pasteur	0%	0.02%	-
Flow Lab	-		0.62%

Abbot	9.5%	,	.42%
Organon	1.7%	,	0%
Litton	1%	,	0.4%
Wellcome	0%	,	0%
Behring	2.7%	,	0.06%
Pasteur	0%	,	0.02%

Table V gives the comparison of frequencies of false positive reactions by various ELISA kits in two different studies. It is noted that in Reesink's study, frequencies of false positive reaction by the Behring kit in "tricky" panel is 2.7% and "blood donor" panel 0.06% while in our study no false positive was detected by the Behring kit. In case of Wellcome, no false positive was seen in Reesink's series in contrast 0.062% in our study. The Flow Lab kit has not been used by Reesink's group. We used it in our study and found that it gave 0.62% false positives (10 out of 1601). This high rate is attributable to the fact that this is a "first generation" kit prepared from virus as such compared to Wellcome and Behring kits which are "second generation" kits and are prepared from purified proteins of the virus.

COMPARISON OF PREDICTIVE VALUES OF ELISA POSITIVE TESTS

In our study, three commercial ELISA kits (Flow Lab., Wellcome and Behring) have been evaluated for HIV antibody detection. A total of 1601 serum samples

of high risk population for AIDS and general population were tested by these kits. The western blot assay was adopted as the confirmatory test. The results are given in Table II.

It is to be noted that there are two methods of assessing the efficacy of various kits:

Sensitivity and specificity. This can only be done where the study includes a sizeable number of AIDS patients (Deinhardt 1987). In our study there is only one AIDS patient so we can not use this method.

Comparison of predictive values. The concept of predictive value can actually be applied to either positive or negative results. We applied it to ELISA positive results.

Table VI gives the comparison of predictive values of ELISA positive tests for the detection of HIV antibodies in our study and in Reesink's study. Two out of six kits in Reesink's series were used in our study. The predictive value of positive ELISA tests in our series by the Behring kit is 100% and the Wellcome 50%, which is somewhat different from those of Reesink's series (Behring 25%, Wellcome 100%). This difference is due to the fact that the study population of Reesink's series was entirely different from our series (Table VII).

According to Mausner and

Bahn (1974), the predictive value is calculated by the formula $\frac{TP}{TP + FP}$ i.e. the predictive value of positive test is the ratio of true positive (TP) to the sum of true positives and false positives (FP) tests. In Reesink's study 71 AIDS patients and 800 seropositive individuals were included while in our study there was only one AIDS patient. So it is obvious that the results will be different when total number of positive cases will be large compared to a small number.

It has been proved that the tendency of false positive is more in those areas of the world where the prevalence rate of disease is low (Liskin et al. 1987).

6. CONCLUSION

The result of this study suggests that the AIDS is not prevalent in Lahore. The limitations of the study do not permit a decisive conclusion. Further study would be required on large sample size particularly in high risk groups for AIDS. Commercial preparations of

ELISA kits available for HIV antibody detection are not all suitable. The best kit in our study is the Behring kit. Since false positives do occur with various kits, ELISA alone is insufficient for screening. Therefore, all ELISA positive cases must be confirmed with the western blot assay.

7. RECOMMENDATIONS

7.1. THE NATIONAL AIDS INFORMATION CAMPAIGN

AIDS is becoming beyond doubt a major health problem for some developed and developing countries. There is yet no vaccine against HIV and no cure for AIDS. The key to reducing the spread of HIV infection is Health Education. A national information campaign against the spread of HIV infection should be launched. It includes four strategies:-

1. AIDS surveillance.
2. Education and social support of high risk people for AIDS.

TABLE VI
COMPARISON OF PREDICTIVE VALUES OF ELISA
POSITIVE TESTS IN TWO DIFFERENT STUDIES
 (Reesink et al. 1986)

K I T	POSITIVE PREDICTIVE VALUE IN PERCENT (Reesink et al. study)	POSITIVE PREDICTIVE VALUE IN PERCENT (Our study)
Abbott	5	—
Organon	100	—
Litton	5	—
Behring	25	100
Wellcome	100	50
Pasteur	50	—
low Lab	—	9.09

TABLE VII
COMPARISON OF STUDY POPULATION
IN TWO DIFFERENT STUDIES
 (Reesink et al. 1986)

PANEL	GROUP CHARACTERISTICS	N U M B E R	
		Reesink et al. study	Our study
A	AIDS patients	71	1
B	High risk people for AIDS	324	490
C	Seropositive individuals	800	—
D	Tricky	293	—
E	Blood donors	5000	60
F	General Population	—	1050
TOTAL:-		6488	1601

3. Information and education of general population.

4. Guidelines for preventing transmission of HIV in Health Care Workers.

7.1.1. AIDS SURVEILLANCE

Limiting the spread of HIV requires thorough and constant surveillance - reporting and record keeping on AIDS cases for scientific purposes. Disease surveillance programmes are important :-

- To find out how much prevalent infection is and who is at the greatest risk.
- To learn exactly how HIV is transmitted and how to prevent it.
- To plan for the care of people with AIDS.
- To keep the public aware of the dangers of AIDS.
- To plan for and evaluate the impact of prevention programmes
- To help generate government support for needed services and research.

7.1.2 EDUCATION AND SOCIAL SUPPORT OF HIGH RISK PEOPLE FOR AIDS

a. The following instructions should be followed by the high risk groups regarding

sexual practices :-

- Seek a mutually faithful relationship with one partner to reduce the chances of getting AIDS.
 - Do not have intercourse with someone who has had many sexual partners.
 - Remember that both vaginal and anal intercourse can spread AIDS.
 - Always use condoms or insist that your partner use them if there is any risk of infection. Protect yourself; protect your partner.
 - Remember that a person can look healthy but still spread AIDS.
- b. Do not share needles with anyone else. Do not use anyone else's needles. If this is not possible, sterilize the needles & syringes before using them.
- c. Do not donate blood, semen, or body organs.
- d. To be extra safe, do not share razors, tooth brushes, or other items that could be contaminated with blood.
- e. Think carefully before getting pregnant or getting your partner pregnant. An infected woman can give AIDS to her baby before it born.

7.1.3. INFORMATION AND EDUCATION OF GENERAL POPULATION.

The following basic information should be given to the general population :-

- AIDS is a fatal disease that cannot now be cured.
- AIDS is not spread by casual contact, routine social contact in workplace or public places, through water or food, eating utensils, coughing or sneezing, insect bites, toilets or swimming pools.
- AIDS is spread by sexual intercourse, by contaminated blood, and by contaminated hypodermic needles.
- An infected woman can give AIDS to her child during pregnancy and lactation.
- A stable, faithful relationship with one uninfected person is safest.
- Using condoms is a good protection against AIDS.
- A person can look and feel healthy and still be able to spread the infection that causes AIDS.

7.1.4. GUIDELINES FOR PREVENTING TRANSMISSION OF HIV IN HEALTH CARE WORKERS

- All needles, scalpels, and other sharp instruments should

be considered potentially contaminated and handled with extraordinary care.

- Disposable syringes and other sharp items should be placed in a puncture-resistant container located as close as possible to the treatment area. Later they should be destroyed to prevent reuse.
- To reduce needlestick injuries, needles should not be recapped after use.
- As workers may be exposed to possibly contaminated blood or other body fluids, they should wear gloves and for surgical procedures, gowns, masks, and eye coverings. After contact with blood, workers should wash immediately.
- Blood and other specimens from known patients or suspected to be infected with HIV should be labelled promptly with a special warning. Blood spills should be cleaned up immediately with a disinfectant (5-10% hypochlorite solution or 2% Glutaraldehyde).
- Mouthpieces, resuscitation bags, and other ventilation equipment should be available to minimize the need for mouth-to-mouth resuscitation.
- Pregnant workers should take special care of contacting the patients or infected material.
- The deadbody of AIDS patients

should be carefully disposed off with special plastic covering.

7.2. W.H.O. GLOBAL PROGRAMME ON AIDS (GPA)

The World Health Organization is directing the global battle against AIDS and is helping countries to develop their own national plans to combat the disease (Mann 1987). W.H.O. help should therefore be elicited at Federal Government level.

7.3 KEEPING BLOOD SUPPLIES FREE OF HIV

Screening of blood is the most effective way to prevent contaminated blood being transfused to normal individuals. W.H.O. recommends that hospitals and blood collection centres:-

- Educate potential donors about AIDS and encourage those at risk of HIV infection, not to donate.
- Screen all donated blood for HIV antibodies and discard any seropositive blood
- Inactivate HIV in concentrated blood products by heat treatment or other methods.

Our Provincial Government should provide blood testing facilities for HIV at Blood Transfusion Centres

7.4. MATERNAL AND CHILD HEALTH AND FAMILY PLANNING SERVICES

Maternal and child health and family planning services are particularly important for the prevention of transmission from mother to child. There will be a need for training as well as equipment and supplies in order to deal with tasks such as : education about AIDS, transmission of virus, risk for women and children as well as safe sexual practices, and the use of condoms.

7.5. INTERNATIONAL TRAVELLING

A certificate stating that a person is not infected with HIV should be required for international travel particularly those people who are coming from the endemic areas for AIDS such as U.S.A., Europe and some African countries. The Government should launch a policy of the requirement of a medical report certifying that a visitor is free of HIV infection. If he/she fails to produce such a certificate, there should be an arrangement for collection of blood samples and transporting the samples to the nearest established AIDS Research Laboratory by the Port Health Officer.

7.6 ESTABLISHMENT OF AIDS CARE CENTRE

Patients with HIV and AIDS remain well for long periods. The median incubation period from infection to the development of AIDS, has been estimated to be five years or may be longer (Mann 1987). Once AIDS is diagnosed patients may live for several years. After a patient develops

AIDS, acute illness will necessitate admission to the hospital.

At present we can offer neither an effective vaccine nor a cure to those suffering from AIDS, therefore, excellent nursing is of paramount importance. Nursing a patient with AIDS is not different from nursing any other ill patients, but sound knowledge, clinical skill and good interpersonal skills are essential for safe, effective care and treatment.

7.7. MANDATORY SCREENING OF PROSTITUTES

The Health Department, Government of Pakistan should formulate a policy for mandatory screening of prostitutes now called singing and dancing girls. Large scale testing for an individual Research Worker is not feasible. There should a permanent medical set up and HIV antibody testing for prostitutes every six months should be made compulsory. The Health Department should provide free condoms to the prostitutes.

7.8 FURTHER RESEARCH AND SCREENING PROGRAMMES

Khanani et al. (1988) documented HIV infection in Karachi. There is an urgent need to screen large number of high risk population in Karachi and Islamabad. The latter city is being suggested because of the presence of constant large scale travel of foreigners.

The Government should strengthen the already established AIDS

Research Centres with latest diagnostic facilities.

Our study revealed that Flow Lab. kit gives false positive results so that this kit should not be used for HIV antibody testing and the Behring kit should be used for such purpose as it proved its authenticity.

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A TRIAL OF BACILLUS THURINGIENSIS H-14 FOR MOSQUITO CONTROL

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ABSTRACT

Bacillus thuringiensis H-14 was tried to see its efficacy as a larvicide. It showed good initial toxicity but no residual effect. After 36 to 48 hours it did not kill the larvae and is therefore not recommended as a larvicide against mosquitoes for a country like ours unless further research is conducted to create a monomolecular layer on water surface for retaining the bacilli on the surface or else if some new strain is developed which has got longer residual effect.

INTRODUCTION

Insecticide resistance to mosquito vector species is continuously appearing worldwide. Therefore considering the worldwide increase in insecticide resistance and other related hazards to biotic flora and fauna, the WHO expert committee on vector biology and control discussed and suggested that counter measures including the biological control of resistant strains by using Bacillus thuringiensis H-14 should further be investigated and encouraged (W.H.O. 1980). When compared, the biological agents are always non-toxic to either fishes, field crops or man and other mammals.

Different species of both the culicine and anopheline mosquitoes i.e., Culex tritaeniorhynchus, C. fatigans, Aedes aegypti, A. albopictus, Anopheles culicifacies, An. fluviatilis and An. stephensi are found in high densities in Islamabad and its vicinities. Other mosquito species are also prevalent in low populations. Apart from having their role as vectors of different diseases like malaria, dengue, Japanese encephalitis, scrub typhus and west Nile virus etc., mosquitoes have always appeared as injurious and annoying due to their anthropophilic habits. Therefore in the present studies two well known biological pesticides, B. thuringiensis serotype H-14 and B. sphaericus which kill mosquito larvae were tested in field conditions to see their efficacy. This article is dealt only with Bti. The microbial pesticide used in the trial is briefly described here to present its characteristics.

It has got subterminal endospores & crystals. These spores and crystals have got delta endotoxins which acts as stomach poison for mosquitoes & other insects. Spores sometime germinate in the insects but further development is very rare. Parasporal crystals are not soluble in water and the sprays used are in the form of wettable powders or else it is used as dust Bti crystals

get dissolved in alkaline midgut of mosquitoes and other insects and endotoxins are released. Due to these toxins the epithelial gut cells get swollen and destroyed and the larvae die away. Bti is being used for more than 20 years against different types of insects and it has not shown any type of toxicity to other organisms and no resistance in the target insects. Bti H-14 is a variety of Bti which was isolated from soil samples from the breeding place of mosquitoes from the Negev Desert of Israel in 1977 (deBarjac 1978a, 1978b, 1978c, Hudson 1985) and it was named as israelensis deBar jac 1978. Bti is commercially available in the market under the trade names, Bactimos, Vectobac, Sandoz SAN-402 and Abbott ABG-8108.

MATERIALS AND METHODS

In Noor Pur Shahan 2.5 x 4 sq. m. artificial ponds were made around the stream for this purpose. Depth of water was 8 to 10 centimeters. The area was highly infested with different species of mosquito larvae. The larvae around the stream were trapped in these artificial ponds. This slow moving stream having nearly clean water with gravel bed serves as good breeding place for both anopheline and culicine larvae throughout the year except severe winter and drought. 1 ml and 2 ml per lit. of Bti was uniformly sprayed in the ponds by using small flit gun. Each concentration was replicated twice and a control was kept for comparison of the results.

Observations were recorded 2, 24, 48 and 72 hours and then one week after bacilli application. For taking observations larval dips were taken. The larvae were transferred to clean cloth, counted quickly and then again transferred back into the same pond.

For confirming the death of larvae due to microbes, 25 larvae from each ditch were picked randomly while taking the observations. The slide were made with these larvae and were stained with giemsa stain for 6 minutes and were then observed under oil immersion to see the presence of bacilli.

RESULTS

Two hours after spraying Bacillus thuringiensis large number of larvae were seen lying dead at the bottom. Before spraying the microbial pesticides average larval dip count in 1 ml per Lit. B. thuringiensis gave 45.1 larvae per dip and in comparison control average larval density was 44.8 larvae per dip. After 2 hours of Bti application the average larval count was found to be 6 larvae per dip in the treated site while in control average larval count was 45.4 larvae per dip. Larval density reduced by 86.79 per cent. After 2 hours of treatment larval density in the treated site was nil and in comparison control larval density was 43.6 larvae per dip. Larval density reduced by hundred per cent. After 48 hours of application the larval density in treated site was again

found as nil larvae per dip whereas the control density was 6 larvae per dip. The larval population had reduced hundred per cent. After 72 hours the average larval density in treated site was 50.4 and in the control site as 49.1 larvae per dip. The larvae were of first instar. Then after one week the

larval density again increased. The observations taken after one week showed larval density in treated and control sites as 52.5 and 51.2 larvae per dip respectively. At the end of one week no reduction in larval population was observed.

AVERAGE LARVAL DIP COUNTS

	<u>Bacillus thuringiensis</u>		Control
	1 ml.	2 ml.	
Before spray	45.5	48.9	44.8
2 hrs after	6	Nil	45.4
2 hrs after	Nil	Nil	43.6
8 hrs after	Nil	Nil	46
72 hrs after	50.4	47.6	49.1
1 wk after	52.5	48.4	51.2

Percent reduction in larval density

	Larval dip count.		larval dip		
	Control	1 ml	% reduction	2 ml	% reduction
Before spray	44.8	45.1	-	48.9	-
2 hrs after	45.4	6	86.79	Nil	100
2 hrs after	43.6	Nil	100	Nil	100
48 hrs after	46	Nil	100	Nil	100
72 hrs after	49.1	50.4	-1.84	47.6	4.26
After 1 week	51.2	52.5	-1.5	48.4	6.24

In the site for 2 ml per Lit. spray the average larval density count before spray was 48.9 and 44.8 larvae per dip in the treated and control sites respectively. After

2 hours of application the larval density in treated site had dropped to zero larvae per dip count in the treated site and it was 45.4 larvae per dip count in the control

pond larval density by hundred per cent. The observations after 24 hours of Bti application again gave larval density in the treated pond as nil larvae per dip and 43.6 larvae per dip count in the control site. There was a reduction in larval population density by hundred per cent when compared with the comparison control. After 48 hours of application larval density in treated site was again found as nil larvae per dip count while in the control site larval density was observed as 46 larvae per dip count. There was a cent per cent reduction in population density. After 72 hours average larval density in treated site was observed as 47.6 whereas in control site as 49.1 larvae per dip count. A reduction of 4.26 percent was observed which is negligible. The larvae collected were of early first instar which means that the egg laying occurred somewhere before 48 hours after bacilli application. After one week of Bti application the larval density was again high and it was found as 48.4 larvae per dip count in the treated site. In control place the density was 51.2 larvae per dip.

DISCUSSION

B. thuringiensis gave good initial toxicity towards different species of anopheline and culicine larvae. 2 ml per sq. m. of Bti gave comparatively better kill when compared with Bti as 1 ml per Lit. 2 ml per Lit gave complete kill of mosquito larvae after two

hours of application whereas with 1 ml per Lit. 86.79 per cent mortality was observed after two hours of application.

Two ml per Lit. gave complete mortality of different anopheline and culicine larvae upto approximately 36 to 48 hours because the first instar larvae were again observed after 72 hours of Bti application. Bti as 1 ml per sq. m. also gave kill for almost similar period. (William et al. 1987) has reported 12 to 76% reduction in larval population by using B. thuringiensis H-14 and they also did not observe good residual effect. According to them the larvae of *C. pipiens* and *Aedes* reinfested the breeding places after 48 hours of exposure with Bt. H-14 but Lee and Cheong (1987) report that Bt. H-14 when used as plaster of paris and sand granules preparations remained effective for 8 weeks in earthen jars and automobile tyres for a trial against larvae of A. (stegomyia) albopictus.

The residual longevity of Bti was observed as very short and gave up its toxic effect when the observations were recorded 72 hours after pesticide application. The larval dip counts in both the treated and untreated comparison control almost equalled and there was a nonsignificant difference in larval density. The results obtained are in accordance with the reports of Hudson (1984) who has reported that most probably the parasporal crystals of Bti settle down at the bottom and that better

results could be obtained by developing floating and slow releasing formulations of Bt. H-14.

It has appeared from the present findings that Bti has got good initial toxicity but less residual effect. The larval mortality was high and lots of larvae were found dead at water bed. However further studies will still be conducted

on the possibility of increasing the residual effect of Bti by mixing in a substance like wheat and maize flour etc. which enables us to create a monomolecular layer on the surface of water and retains Bti on water surface. This will help in increasing the residual effect of Bacillus thuringiensis.

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CARRIAGE OF ENTEROPATHOGENIC BACTERIA BY DIFFERENT SPECIES OF COCKROACHES

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SUMMARY

A total of 500 cockroaches of different species were collected from various sites of the Services Hospital, Lahore, to study the pattern of infestation of the hospital premises and the carriage of the bacteria belonging to the family Enterobacteriaceae. Three species of cockroaches namely Periplaneta americana (American cockroach), Blattella germanica (German cockroach), and Blatta orientalis (Oriental cockroach) were identified. The cockroaches were collected from five sites of the hospital viz. Gynaecology and Obstetrics ward, Medical ward-I, Surgical ward-I, Kitchen and Medical store. American cockroaches were the most common in all the

sites accounting for 70.4%, German cockroaches were second with 24% and Oriental cockroaches third with 5.6% of the infestation.

One thousand twenty seven bacteria belonging to family Enterobacteriaceae were isolated. These constituted 15 bacterial genera Salmonella, Shigella, Escherichia, Klebsiella, Enterobacter, Serratia, proteus, Morganella, Providencia, Citrobacter, Edwardsiella, Alkaligenes, Acinetobacter, Pseudomonas and Bacillus. The Salmonella isolated were Salmonella typhi, Salmonella arizona and Salmonella enteritidis. Three serotypes of Salmonella enteritidis, namely, Salmonella paratyphi B, Salmonella typhimurium,

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Salmonella barielly were identified. The Shigella isolated were Shigella flexneri and Shigella sonnei. In addition, 20 organisms belonging to the genus Candida were isolated.

INTRODUCTION

The cockroaches are among the most primitive of all winged insects and have been around for a long time (Burgess 1978'. More than dozen species have some degree of medical importance but the following six species all with worldwide distribution are the principal vectors (Smith 1973):

1. Periplaneta americana
(American Cockroach).
2. Blatella germanica
(German Cockroach).
3. Blatta orientalis
(Oriental cockroach).
4. Periplaneta australasiae
(Australian cockroach).
5. Spella longipalpis
(Brown banded cockroach).
6. Leucophaea maderae
(Medeira cockroach).

The close association of cockroaches with man has brought its pest status at several levels including social and medical (Wood et al 1981). The filthy habits and cursorial nature of cockroaches make them ideally suited for the mechanical spread of certain pathogenic organisms but contaminating food stuffs and fomites

through contact with the infected appendages (Janssen & Wedber 1952).

The workers in several parts of the world have isolated pathogenic organisms such as Salmonella and Shigella (Okafor 1981, Burgess and Chetwyn 1981, panthora et al 1981), Staphylococcus Faecalis, Poliomyelitis virus and Entamoeba histolytica (Roth and Willis 1957), E.coli, Enterobacter aerogenes, Serratia marcescens, proteus Vulgaris and Klebsiella pneumoniae (Fernandez and Zarror 1972), Pseudomonas aeruginosa, Moraxella and Actinomyces (Hiro and Fuji 1966), Campylobacter jejuni (Umannabuike 1986) and Bacillus spp, Acinetobacter and Micrococcus (Bergess et al 1969). The pathogenic helminths that are found associated with cockroaches have been listed by Burgess (1985' and they are Enterobius vermicularis, Trichiuris trichiura, Ascaris lumbricoidis, Ancylostoma duodenale and Necator americanus.

Hospitals throughout the world provide an ideal site for cockroaches and they are commonly found in hospital kitchens, canteens and dinning rooms and in wards pantries store rooms etc. The hollow supports of benches and trolleys provide an excellent method of disseminating cockroaches throughout the hospital bed frames (Leib). They often gain access to hospitals through defective drains and are frequent inhabitants of sewerage system (Burgess 198).

Information on the identification

and occurrence of cockroaches in the hospitals and dwellings, their role as vector of pathogenic bacteria in Pakistan has not been reported except a study conducted by Zuberi et al (1972) and Razia and Jafri (1986). Therefore the present research has been carried out with the following objectives:

1. To find out the different species of cockroaches which inhabit the hospital environment.
2. To find out what types of the bacteria belonging to family Enterobacteriaceae are present on cockroaches which might contaminate hospital articles.

MATERIALS & METHODS:

Collection of Cockroaches:

The samples of various species of cockroaches were collected from different sites of the Services Hospital, Lahore: The sites were:

Gynaecology & Obstetrics ward.

Surgical ward-I.

Medical ward-I.

Kitchen.

medical Store.

In all 500 cockroaches were collected.

LABORATORY INVESTIGATION

The species of cockroaches were

identified after being caught. They were then collected and transported from all these sites of the hospital in sterilized bottles containing Cary Blair medium (Difco 1984 g). These bottles were shaken for two minutes on the vortex shaker in order to suspend bacteria present on the surface of the cockroaches. The suspension was then cultured on the following media :

- Salmonella Shigella agar (Difco 1984 a).
- Bismuth Sulphite agar (Difco (1984 b).
- Xylose Lysine Deoxycholate agar (Difco 1984 c).
- Selenite broth (Difco (1984 e)).
- Tetrathionate broth (Difco 1984 f).

Isolation and Identification of the Bacteria

S S agar, B S Agar, XLD agar were streaked by inocula taken from the bottles containing cockroaches. These media were incubated at 37°C for 24 hours. After 24 hours, growths from Selenite broth and tetrathionate broth were subcultured on MacConkey agar (Difco 1984 d). The identification of bacterial isolates was carried out by:

- Colonial morphology.
- Grams Staining.
- Motility.

Biochemicals reactions enumerated in the table used by Cowan and Steel (1975)* and Koneman et al (1983)**

Serological tests: Slide and tube agglutination tests were performed for identification of *Salmonella* and *Shigella* (Difco 1984 g & r).

The following biochemical reactions were carried out:

- A Growth in KCN broth (Difco 1984 i).
- B Citrate as C source (Difco 1984 j).
- C. Carbohydrate fermentation tests. (Difco 1984 k).
- D MR and VP Tests (Difco 1984 l).
- E. Indole Test (Difco 1984 m).
- F. Gelatin Liquefaction Test (Koneman et al 1983 b).
- G. Urease Test (Cruickshank 1968 c).
- H. Phenylalanine Deaminase and Malonate utilization Tests (Difco 1984 n).
- I Triple Sugar Iron Agar Test (Difco 1984 o).
- J. Aminoacids decarboxylase Tests (Difco 1984 p).

K. Oxidase Test (Koneman et al 1983 c).

L Catalase Test (Koneman et al 1983 d).

Serological tests were performed for *Salmonella* with the aid of Kauffman-White Scheme *Salmonella* polyvalent sera with Difco number 2264-47-2 and individual antisera with Difco number 2892-32-9 using factor 1, 4, 5, 6 and 9.

For *Shigella flexneri* and *Shigella sonnei* *Shigella* antisera poly group B with Difco number 2835-47-2 and *Shigella* antisera poly group D with Difco number 2835-47-9 were used respectively.

RESULTS:

GENERAL DISTRIBUTION OF HOSPITAL COCKROACHES

A total of 500 cockroaches of different species were collected to determine their distribution in various sites of the hospital and to know the enterobacteriaceae carried by them.

Table-III summarises the distribution of various species of cockroaches collected from Service Hospital, Lahore.

Of the 500 cockroaches collected, 352 (70.4%) were *Periplaneta americana* (American cockroach).

* Table I

** Table II

cockroaches), 120 (24%) Blattella germanica (German cockroaches) and 28 (5.6%) Blatta orientalis (Oriental cockroaches).

species of cockroaches collected from various sites of the hospital.

DISTRIBUTION OF COCK-ROACHES BY HOSPITAL SITES

Table IV shows the different

TABLE III

DISTRIBUTION OF DIFFERENT SPECIES OF COCKROACHES IN HOSPITAL

Species of Cockroaches	No	Percentage
1. <i>Periplaneta americana</i>	352	70.4%
2. <i>Blattella germanica</i>	120	24.0%
3. <i>Blatta orientalis</i>	28	5.6%
Total	500	

TABLE - IV

DISTRIBUTION OF DIFFERENT SPECIES OF COCKROACHES IN VARIOUS SITES OF HOSPITAL

P L A C E	Old or New Building	Floor	SPECIES OF COCKROACHES						Total
			Periplaneta americana		Blatella germanica		Blatta orientalis		
			No.	Percentage	No.	Percentage	No.	Percentage	
Gynaecology and Obstetrics ward	Old	Ground	157	78.89%	39	19.59%	3	1.5	199
Medical ward I	Old	Ground	57	57.58%	42	42.42%	0	-	99
Surgical ward I	Old	Ist.	4	40.0%	0	-	6	60.0%	10
Surgical ward I	New	2nd	0	-	0	-	0	-	0
Kitchen	Old	Basement	100	68.96%	37	25.5%	8	5.5%	145
Medical Store	Old	Basement	34	72.34%	2	4.25%	11	23.40%	47
TOTAL:			352		120		28		500

Approximate percentage at some places is due to the calculations made by calculator. The same may be noted in some other Tables.

GYNAECOLOGY AND OBSTETRICS WARD

(Situated in old building on ground floor).

A total number of 199 cockroaches were captured from the Gynaecology and Obstetrics ward. The number and percentage wise distribution of various species of cockroaches were as follows

American cockroaches 157
(78.89%)

German cockroaches 39 (19.59%)

Oriental cockroaches 3 (1.5%)

The figures show that the most common species found was the American cockroaches. The large number of cockroaches may be correlated with the poor sanitary conditions prevailing in the ward. Broken window frames and their loose and faulty fittings and the cracked walls and floor favoured the large population of cockroaches to breed and infest the ward.

MEDICAL WARD-I

(Situated in old building on ground floor)

A total number of 99 cockroaches were collected from the medical ward I, their species wise distribution was as follows:

American cockroaches 57
(57.58%)

German cockroaches 42 (42.42%)

No oriental cockroaches were found in the ward. The American and German cockroaches were collected from the toilet and kitchen of the general ward respectively. Sometimes the cockroaches were collected from the toilet of the Doctor's office. Statistically the difference of association of various species of cockroaches to Gynaecology and Obstetrics ward and Medical ward I was found significant. P. O. 001.

SURGICAL WARD-I

(Situated first in the old building and then shifted to new one)

Only 10 cockroaches were collected from Surgical unit-I, the species wise distribution was as follows.

Oriental cockroaches 6 (60%)

American cockroaches 4 (40%)

After two initial collection the ward was shifted to new building. Several times attempts were made to collect cockroaches from new surgical ward, but it was not found infested with any cockroach. No prediction can be made as to when the ward will become infested since the place is new and it may take sometime for the cockroaches to establish themselves.

KITCHEN

(Situated in old building at basement)

A total of 145 cockroaches were collected from the kitchen,

their species wise distribution was as follows

American cockroaches 100
(68.96%)

German cockroaches 37 (25.5%)

Oriental cockroaches 8 (5.5%)

The kitchen was situated at the basement and it was a damp and dark place, moreover improper sanitary conditions and unserviceable oven (tandoor) produced the favourable conditions for cockroach breeding. Piles of trash were seen in some places which caused infestation.

MEDICAL STORE

(situated in old building at basement)

A total of 47 cockroaches were collected from the medical store of the hospital, their species wise distribution was as follows

American cockroaches 34
(72.34%)

Oriental cockroaches 11 (23.40%)

German cockroaches 2 (4.25%)

From the table IV it may be noted that whereas 199 cockroaches were found in Gynaecology and Obstetrics ward, 99 in Medical ward and 10 in Surgical ward (Old building), no cockroaches could be found in new surgical ward. The reason is obvious since surgical ward I was a new building.

Cockroaches in medical store although small in number (47) collected during the study time, were found due to the fact that the building was old and situated in basement. Although no food material was available there and the medical store was kept clean, it was possible that cockroaches could have entered from the infested kitchen which was also situated in the basement or the cockroaches may have entered through the W.C. of the toilet which was connected with the manhole by sewage pipes. Statistically the difference of association of the various species of cockroaches to kitchen and medical store was found significant to the level of $P < 0.001$.

This distribution of the cockroaches in Gynaecology & Obstetrics ward was analysed & it was found that there were higher number of American cockroaches in this ward. The ratio between American and German cockroaches was about 4:1, whereas the oriental cockroaches were in small number 3 (1.5%).

The analysis of the data of the medical ward on the other hand shows that number of American cockroaches was not too high in comparison to German cockroaches; the ratio between them was about 3:2. The Blatta orientalis was not found in medical ward, the probable reason being that it is rare species in tropical countries. An analysis of the data related to the kitchen and medical store as well as gynaecology and obstetrics ward and the medical ward indicates that Blatta orientalis

was more likely to occur in basement in comparison to the upper floor. The figures in respect of surgical ward I (old building) however, is contradictory to this statement, but may be explained because of the fact that the total number of cockroaches obtained was very small and therefore could not be true representative sample for drawing valid conclusion.

The association between the various sites of the hospital and the prevalent species of cockroaches was tested by applying Chi square test (X^2). A significant difference was found between the sites of the hospital and the species of cockroaches $P < 0.001$.

PREVALENCE OF ENTERIC MICRO-ORGANISMS IN THE COCKROACHES

All the cockroaches studied were found to carry one or more micro-organisms.

Table V shows the prevalence of micro-organisms in 500 hospital cockroaches. From the cockroaches studied, 1027 bacterial isolates and 20 fungal isolates were obtained. The bacteria could be classified into 15 genera while all the fungi belonged to only one genus.

The distribution of the bacteria by genera and species is detailed as follows:

SALMONELLA

This genus was observed in

88 (17.4%) of the 500 cockroaches examined, Salmonella enteritidis was the leading species isolated as it appeared in 62 (12.4%) Salm. typhi was isolated in 21 (4.2%) and Salmonella arizona in 5 (1%) of the insects.

SHIGELLA

This genus was isolated from 37 (7.4%) of the insects. The shigella species isolated were, Sh. flexneri 23 (4.6%) and Sh. sonnei 14 (2.8%).

OPPORTUNISTS:

The rest of the cockroaches 375 (75%) were found to carry the opportunists.

CANDIDA:

The fungus candida was carried by 20 (4%) cockroaches.

TABLE - V
PREVALENCE OF MICROORGANISMS IN 500 HOSPITAL COCKROACHES

Sr. No.	GENERA	No.	Percentage	SPECIES	No.	Percentage
1.	Salmonella	88	17.6	Salm. typhi Salm. enteritidis Salm. arizona	21 62 5	4.2 12.4 1.0
2.	Shigella	37	7.4	Sh. flexneri Sh. sonnei	23 14	4.6 2.8
3.	Escherichia	171	34.0	E. coli	171	35.0
4.	Klebsiella- Enterobacter Serratia.	272	54.4	K. pneumonia K. ozaenae K. oxytoca Ent. aerogenes Ent. cloacae Ent. agglomerans Ser. marcescens	120 16 15 34 15 39 33	24.0 3.2 3.0 6.8 3.0 7.8 6.6
5.	Proteus Morganella Providencia	113	22.6	Pr. vulgaris Pr. mirabilis Prov. rettgeri Prov. alcalifaciens Morg. morganii	45 18 17 20 13	9.0 3.6 3.4 4.0 2.6
6.	Citrobacter	81	16.2	Cit. freundii Cit. diversus	38 43	7.6 8.6
7.	Edwardsiella	17	3.4	Ed. tarda	17	3.5
8.	Alkaligenes	23	4.6	Alk. faecalis	23	4.6
9.	Acinetobacter	50	8.0	Acinetobacter	40	8.0
10.	Pseudomonas	136	27.2	Ps. aeruginosa & species.	136	27.2
11.	Bacillus	49	9.8	B. subtilis	49	9.8
12.	Candida	20	4.0	Candida	20	4.0

COCKROACH SPECIES AND THEIR CARRIAGE OF MICROORGANISMS

Periplaneta americana (American Cockroach)

Out of 352 American cockroaches examined, 13 (4%) were carrying Salm typhi, 50 (14%) were carrying Salm enteritidis and 4 (1%) were carrying Salm arizona. Shigella flexneri type 1-5 was isolated from (14.4%) & Shigella sonnei from 10 (3%) of the insects. The Escherichia were recovered from 119 (34%) of the cockroaches. A total of 191 (54%) cockroaches were found to carry the organisms belonging to the Klebsiella-Enterobacter-Serratia group. 74 (21%) of the cockroaches carried the Proteus-Morganella - Providencia group of the organisms. Citrobacter & Edwardsiella were isolated from 61 (17%) & 11 (3%) of the insects respectively. There were 14 (4%) cockroaches positive for Alcaligenes. Acinetobacter were harboured by 27 (8%) of the insects. A large number of insects carried Pseudomonas, as 92 (26%) were found positive for it. Bacillus was also quite prevalent as 35 (10%) cockroaches were found positive for it. The fungus, Candida was isolated from 16 (4.5%) of the insects.

Blattella germanica (German cockroach)

Salmonella typhi was recovered from 8 (7%) of the 120 German cockroaches studied. Salmonella enteritidis and Salm arizona were isolated from 10 (8%) and 1 (0.8%) of the insects respectively. The cockroaches found positive for Shigella flexneri type 1-5 were

8 (7%) while Shigella sonnei were observed in 4 (3%) of the insects. A large number of cockroaches 85 (70.7%) carried the opportunistic group of organisms. Their distribution was as follows:

Escherichia = 37 (31%)

- Klebsiella-Enterobacter-Serratia group of organisms = 65 (5%).
- Citrobacter 16 (13%).
- Edwardsiella = 5 (4%).
- Alcaligenes = 8 (6.6%).
- Acinetobacter = 9 (7.5%).
- Pseudomonas = 3 (2.8%).
- Bacillus = 11 (9%).
- Candida = 4 (3%).

Blatta orientalis (Oriental cockroach):

Salmonella enteritidis was the only species isolated from 2 (7%) of the 28 cockroaches whereas Salm. typhi and Salm. arizona were not recovered. Only one (3%) cockroach carried the Shigella flexneri type 1-5 whereas Shigella sonnei were not found on any of the cockroach. The rest of the cockroaches 25 (89.3%) carried the opportunists. Their distribution was as follows:-

- Escherichia = 15 (53%).
- Klebsiella-Enterobacter-Serratia group = 15 (53%).
- Proteus - Morganella - Providencia group = 8 (28%).

- Citrobacter = 3 (11%).
- Edwardsiella = 1 (3.5%).
- Acinetobacter = 4 (14%).

- Pseudomonas = 10 (36%).
- Bacillus = 3 (11%).
- Alcaligenes and Candida were not found on these cockroaches.

The next data shows that the number of German cockroaches 8 (7%) carrying Salm. typhi were more than the number of American cockroaches 13 (4%). Statistically this difference was not found

significant $P > 0.05$. Similarly there was no significant difference between American and German cockroaches in relation to the carriage of Salm. enteritidis.

TABLE VI
DISTRIBUTION OF MICROORGANISMS CARRIED BY DIFFERENT SPECIES OF COCKROACHES

Species of Cockroaches	No Tested	NAME OF MICROORGANISMS														
		Salm. typhi.	Salm. enteritidis.	Salm. arizona.	Sh. flexneri (1-5)	Sh. sonnei.	Escherichia.	Klebsiella - Enterobacter Serratia.	Proteus - Morganella Providencia.	Citrobacter.	Edwardsiella.	Alcaligenes.	Acinetobacter.	Pseudomonas.	Bacillus.	Candida.
Periplaneta americana (American cockroaches)	352	No. 13 & % 4%	No. 50 (14%) & %	No. 4 & % 1.4%	No. 14 & % 4%	No. 10 & % 3%	No. 119 & % 34%	No. 191 & % 54%	No. 74 & % 21%	No. 61 & % 17%	No. 11 & % 3%	No. 14 & % 4%	No. 27 & % 8%	No. 92 & % 26%	No. 35 & % 10%	No. 16 & % 4.5%
Blattella germanica (German cockroach).	120	8 & % 7%	10 & % 8%	1 & % 0.8%	8 & % 7%	4 & % 3%	37 & % 31%	65 & % 54%	30 & % 25%	16 & % 13%	5 & % 4%	8 & % 6.6%	9 & % 7.5%	34 & % 28%	11 & % 9%	4 & % 3%
Blatta orientalis (Oriental cockroach).	28	0 & % -	2 & % 7%	0 & % -	1 & % 3%	0 & % -	15 & % 53%	15 & % 53%	8 & % 28%	3 & % 11%	1 & % 3.5%	0 & % -	4 & % 14%	10 & % 36%	3 & % 11%	0 & % -

CARRIAGE OF MICROORGANISMS BY COCKROACHES ACCORDING TO THE SITES OF THE HOSPITAL

Gynaecology & Obstetrics ward:

Out of 199 cockroaches collected from this ward, 6 (3%) were positive for Salm typhi, 30 (15%) were positive for Salm. enteritidis and 3 (1.5%) were positive for Salm arizona. Shigella flexneri type 1-5 and Sh. sonnei were carried by 1 (5%) and 6(3%) of insects respectively. The opportunists were isolated from the rest of the cockroaches 144 (72.36%). They were distributed as follows,

- Escherichia = 70 (35%).
- Klebsiella Enterobacter Serratia group = 100 (50%).
- Proteus - Morganella - Providencia group = 37 (18%).
- Citrobacter 34 (17%)
- Edwardsiella = 7 (3.5%).
- Alcaligenes = 10 (5%).
- Acinetobacter = 19 (9.5%).
- Pseudomonas = 49 (24%).
- Bacillus = 18 (9%).
- Candida = 13 (6.5%).

Medical Ward-I

This ward provided 99 cockroaches of which 10 (10%) were carrying Salm. typhi & 17 (17%) Salm. enteritidis. None of the cockroach carried the Salm. arizona. Shigella flexneria type 1-5 were recovered from 6 (6%) of the insects, while Sh. sonnei were recovered from 3 (3%) of the

insects. The remaining cockroaches 63 (63.3%) were found to carry the opportunists and their distribution was as follows.

- Escherichia = 32 (32%).
- Klebsiella-Enterobacter Serratia group = 52 (52%).
- Proteus - Morganella - Providencia group = 24 (24%).
- Citrobacter = 18 (18%).
- Edwardsiella = 8 (8%).
- Alcaligenes = 3(3%).
- Acinetobacter = 3(3%).
- Pseudomonas = 25 (25%).
- Bacillus = 9 (9%).
- Candida = 4(4%)

Surgical ward-I:

Out of 10 cockroaches collected none was found to carry pathogenic enterobacteria (i.e. Salmonella and Shigella). All the cockroaches carried the opportunistic group of organisms.

Kitchen:

Out of 145 cockroaches 5 (3.4%) were carrying Salm. typhi, 12 (8.3%) Sal enteritidis and 2 (1.3%) Sal arizona. The shigella flexneri type 1-5 and Sh. sonnei were carried by 6 (4.1%) and 4 (2.7%) of the insects respectively. The opportunists were harboured by the insects respectively. The opportunists were harboured by the

of 18 (8%) of the cockroaches.
The distribution was as follows

- *Escherichia* = 44 (30%)
- *Klebsiella*-*Enterobacter* *Serratia* group = 33 (55%)
- *Proteus* - *Morganella* - *Providencia* group = 34 (23%)
- *Citrobacter* = 18 (12%)
- *Edwardsiella* = 5 (3%)
- *Alcaligenes* = 9 (6%)
- *Acinetobacter* = 12 (8%)
- *Pseudomonas* = 40 (27%)
- *Bacillus* = 19 (13%)
- *Candida* = 3 (2%)

Medical Store:

Salmonella enteritidis was the only species which was isolated from 3(6.4%) of the 47 cockroaches. *Salmonella typhi* and *Salm arizona* were not carried by these insects. *Shigella flexneri* type 1-5 and *Sh. sonnei* were carried by 1 (2%) cockroach each.

The 42 (89.4%) cockroaches were found to carry opportunitis. Their distribution was follows.

- *Escherichia* = 20 (42%)
- *Klebsiella*-*Enterobacter* *Serratia* group = 29 (62%)
- *Proteus* *Morganella*-*Providencia* group = 16 (34%)

- *Citrobacter*, 7 (15%)
- *Edwardsiella* = 3 (6%)
- *Alcaligenes* = 1 (2%)
- *Acinetobacter* = 1 (2%)
- *Pseudomonas* = 16 (34%)
- *Bacillus* = 3 (6%)
- *Candida* were not carried by these insects.

The difference in the prevalence of *Salm. typhi* between Gynaecology and Obstetrics ward and Medical ward-I was found to be statistically significant, $P < 0.05$.

The reason for this difference could probably be that typhoid patients get admission in Medical ward and their excreta provide infective material for the cockroaches.

From the above figures it is shown that the units having inpatient facilities i.e. Gynaecology and Obstet. ward and Medical Ward-I were found to harbour higher proportion in comparison to Services units i.e. Kitchen and medical store which carried a lower proportion of pathogens. This difference was found statistically significant, $P < 0.01$.

DISTRIBUTION OF MICROORGANISMS CARRIED BY COCKROACHES ACCORDING TO
DIFFERENT SITES OF THE HOSPITAL

Site of Hospital	No. of Cock roaches	NAME OF MICROORGANISMS															
		No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %
Gynae. and Obstet Ward	199	Salm. typhi 6 3%	Salm. enteritidis 30 15%	Salm. arizona. 3 1.5%	Sh. flexneri 91-5) 10 5%	Sh. sonnei. 6 3%	Escherichia. 70 35%	Klebsiella-Enterobacter-Serratia. 100 50%	Proteus Morganella - Providencia. 37 18%	Citrobacter. 34 17%	Edwardsiella. 7 3.5%	Alcaligenes. 10 5%	Acinetobacter. Acinetobacter. 19 9.5%	Pseudomonas. 49 24%	Bacillus 18 9%	Candida 13 6.5%	
Medical ward I	99	1 10%	17 17%	0 -	6 6%	3 3%	32 32%	52 52%	24 24%	18 18%	8 8%	3 3%	3 3%	25 25%	9 9%	4 4%	
Surgical ward-I	10	0 -	0 -	0 -	0 -	0 -	7 70%	2 20%	2 20%	3 30%	0 -	0 -	0 -	6 60%	0 -	0 -	
Kitchen.	145	5 3.4%	12 8.5%	2 1.3%	6 4.1%	4 2.7%	44 30%	85 58%	34 23%	18 12%	5 3%	9 6%	12 8%	40 27%	19 13%	3 2%	
Medical Store.	47	0 -	3 6.4%	0 -	1 2%	1 2%	20 42%	29 62%	16 34%	7 15%	3 6%	1 2%	1 2%	46 34%	3 6%	0 -	

SEROTYPES OF SALMONELLA ENTERITIDIS

Table-VIII summarises the distribution of the serotypes of *Salmonella enteritidis* carried by cockroaches.

from 14 (22.58%) of the insects.

iii. *Salmonella barielly*:

This serotype was isolated from 31 (50%) of the cockroaches.

TABLE - VIII
DISTRIBUTION OF SEROTYPES OF SALMONELLA ENTERITIDIS

No. of cockroaches	SEROTYPES OF SALMONELLA ENTERITIDIS					
	Salmonella paratyphi B		Salmonella typhimurium		Salmonella barielly	
	No.	%	No.	%	No.	%
62	17	27.42%	14	22.58%	31	50%

Serotyping of *Salmonella enteritidis* isolated from 62 (12.4%) of the 500 cockroaches was done by using antisera.

The following serotypes were identified.

i. *Salmonella paratyphi* B (Schotmuelleri)

This serotype was isolated from 17 (27.42%) of the cockroaches

ii. *Salmonella typhimurium*:

This serotype was isolated

SHIGELLA FLEXNERI AND SHIGELLA SONNEI AND THEIR SEROTYPES:

Table IX elucidate the serological identification of the *Sh. flexneri* and *Sh. sonnei* and their serotypes.

Shigella flexneri type 1-5 was isolated from 23 (4.6%) of the 500 cockroaches. These organisms were identified as positive by using *Shigella* antiserum poly group B.

Shigella sonnei type 1 was carried by 14 (2.8%) cockroaches. *Shigella* antiserum poly group D was employed for the identification of this species.

DISCUSSION

GENERAL DISTRIBUTION OF DIFFERENT COCKROACH SPECIES

In the present study 500 cockroaches were collected to see their distribution and carriage of enterobacteriaceae. This study revealed the high prevalence of American cockroaches.

Our study also shows that American cockroach was the leading species in four out of five sites of the hospital. The reasons should be that there is a large network of sewer system and the American cockroach inhabits the sewers preferentially. Due to extensive ramification of the sewage lines cockroaches gain an access to various sites easily.

In the present study the highest number (199) of cockroaches were found in Gynaecology & Obstetrics ward which could be correlated with the unsanitary conditions prevailing in this ward. This is borne by the fact that the building is an old structure with broken window frames, having loose and faulty fittings, cracked floor, over crowding of the patients, dirty and old beds with blood stained mattresses.

The kitchen was the second common place to show the high number (145) of cockroaches. Since it was situated in the basement, the dampness, darkness and dingy surroundings provided chance for cockroach breeding and infestation. Moreover insanitary conditions, availability of food material parti-

cularly the bread, chapati and flour bag caused attraction to these insects. Although cockroaches consume a wide variety of foods but they prefer starchy foods, particularly bread which was readily available in the kitchen. This feeding habit has been supported by Reiersen (1977) who carried out experimental study and found that large number of German cockroaches were attracted to white bread and bread extract in the presence of many other foods.

Medical ward-I was the place where the highest number of German cockroaches (42.42%) was found. The kitchen of this ward provided food material to the cockroaches. The unwashed utensils, sugar and milk stained counters, food stained stoves and burners caused attraction to this species. In comparison to Gynaecology and Obstetrics ward the American cockroaches were less in number. This is conformity with the fact that the sewage ramifications surrounding this ward were less in number.

Medical store, although situated in basement, was found to be less infested. Here the sanitary conditions were better than all the other sites. The other reason for the low occurrence could be explained by the fact that no food material was available for their existence.

The Surgical ward-I, before shifting to new building, provided 10 cockroaches. The number of insects was so small that it could

DISTRIBUTION OF SHIGELLA SPECIES & ITS SEROTYPES ISOLATED FROM
HOSPITAL COCKROACHES, IDENTIFIED BY USING POLYVALENT
ANTISERA

TABLE -IX.

Shigella species	Serotypes identified by Polyvalent antisera.	No. of cockroaches.	Cockroaches positive for	
			No.	Percentage.
<i>Shigella flexneri</i> .	1 - 5	37	23	62.6 %
<i>Shigella sonnei</i>	1		14	37.4 %

not be a true representative sample. However out of 10 insects, 6 (60%) were Oriental cockroaches and 4 (40%) American cockroaches. Had the number of insects been high, the results might have been different. After two initial collections the ward was shifted to the new building where, after many attempts no cockroaches could be collected. This is an important aspect of this study showing that new structure of the hospital with improved engineering skills can reduce the cockroach nuisance.

From the above explanation it is evident that significant differences were observed in the prevalence of cockroaches between various sites of the hospital.

ROLE OF COCKROACHES IN THE CARRIAGE OF ENTEROBACTERIACEAE.

The present study revealed that three types of the Salmonellae were isolated from 88 cockroaches. Salmonella typhi was recovered from 21 cockroaches. These organisms find their way to the hospital through the agency of the patients who pass these bacteria in the toilets. Since the cockroaches live in the sewers, they get bacteria from faeces present in the sewage. The presence of Salmonella typhi on these insects is of public health significance since it is an important pathogen. Further studies would be required to establish the role played by cockroaches in acting as vehicles of transmission in nosocomially acquired typhoid fever.

In our study 62 cockroaches

were found to carry Salmonella enteritidis, and isolation of its serotype have the public health importance as these are associated with diarrhoea, septicaemia, food poisoning and other ailments of man and animals. The results of the present study are in conformity with the result of Mackerras and Mackerras (194) who recovered Salmonella enteritidis serotype bovis-morbificans from cockroaches in an Australian hospital in a gastroenteritis outbreak

Cockroaches habitually feed on infected material found in lavatories, latrines, refuse areas and drainage system of the hospital and then crawl over and feed on kitchen equipments and utensils. Not only utensils but many other belongings of the patients such as tooth brush, tooth paste, towels, napkins etc, can also be infested with these insects. In the kitchen of the ward they might feed on prepared food such as rice, custard, soup, gruel etc, left overnight on tables or in cupboard. The mugs, spoons, milk packs and sugar pots left unwashed on benches, trolleys and side tables could be the ideal sites for the cockroach infestation. This sort of situation prevailed in Gynaecology and Obstetrics ward on a large scale, but to a lesser extent in the Medical ward and least in the new Surgical ward.

In our study, 1% cockroaches were found to carry the Salmonella arizona. There has been no mention about these organisms in available research literature. This organism however was isolated from cock-

roaches in our study. According to Wilson et al. (1984) it causes diarrhoeal diseases in man and animals.

In the present study genus Shigella was isolated from 7.4% of the cockroaches. The species of Shigella isolated were Shigella flexneri and Shigella sonnei. Both of these organisms can survive on the appendages of the insects for quite a long time as has been shown in a study carried out by Artyukhina and Edokimova (1973) in which Shigella flexneri and Shigella sonnei were recovered from the artificially infected Blattella germanica for 3 and 8 days respectively. The same result has been verified by Svec et al (1975).

The data from Table-V shows that the highest number of cockroaches 171 (34%) were found to carry Esch. coli on their external surface. The presence of Esch. coli on the cockroaches indicate that the cockroaches came in contact with patient's faecal matter. Our results are identical with those of Frishman and Alcamo (1977), Burgess (1979) and Cornwell and Mendes (1981). All of these workers isolated Esch. coli from the hospital cockroaches.

Relation of the various genera of pathogenic and opportunistic organisms to the species of cockroaches have been studied in the present research. It was found that no significant difference was observed between the bacterial genera and various species of cockroaches. The data suggests that the difference in the level of

carriage, for example of Salmonella and Shigella by American and German cockroaches was just incidental. There was no predilection of one or other species of cockroach to a particular bacterial genus. It suggests that whatever types of bacteria contaminate the hospital environment they will be picked up by various types of cockroaches almost equally. However there was significant difference between the American and Oriental cockroaches in relation to the carriage of Salmonella and Esch. coli, but this difference was not carrying much weightage since the number of Oriental cockroaches was very small in comparison to other species. The basis for the low occurrence of Oriental cockroaches in hospital is that its breeding may be low due to tropical environment in Pakistan. No significant difference was found between the three species of cockroaches and their carriage of pathogens (Salmonella and Shigella together) and opportunists. The evidence provided thus is convincing to conclude that all the species of cockroaches could be equally involved in terms of their carriage of pathogens and opportunists.

After studying the genera the organisms were distributed into species. No significant difference was found between the American and German cockroaches in terms of the Salm. typhi and Salm. enteritidis carried by them. It also provided the evidence that both the species of cockroaches picked up these Salmonellae just incidentally. None of the species

had preference for any type of the Salmonella.

The present study revealed that 7 (1.98%) American cockroaches carried genus Salmonella exclusively in comparison to 1 (0.83%) German cockroach. This difference was not found significant statistically, indicating that both species were almost equally capable of carrying the genus Salmonella exclusively. It appeared from the data that Oriental cockroach was least responsible for carrying pathogens while the other two types had almost the same potential of carrying the pathogens.

This study shows (Table-VII) that the genus Salmonella was carried by 19% of the cockroaches in Gynaecology & Obstetrics ward in comparison to 27% of Medical ward-I. Statistically this difference was not found significant. Similarly no significant difference was found between kitchen and the Medical Store in terms of the genus Salmonella carried by cockroaches. It may be concluded therefore that from all above mentioned sites cockroaches had equal chance to carry the microorganisms.

The results of this study, in terms of opportunists, were similar to that Fernandez and Zerror (1972) who isolated some members of Enterobacteriaceae from cockroaches captured in Maternity and Gynaecology ward of John. Kennedy Hospital, Valdivia, Chile. The organisms isolated were Esch. coli, Ent. aerogenes, K. pneumoniae, Pr. vulgaris, Pr. mirabilis, Serratia marcescens

and Pseudomonas aeruginosa.

Our data suggests (Table-VII) that higher percentage of cockroaches (10%) from Medical ward-I carried the Salmonella typhi in comparison to 3% of the Gynaecology and Obstetrics ward. This difference was found to be statistically significant at level of $P < 0.05$. The reason for this difference could probably be that typhoid patients get admission in Medical ward and their excreta provide infective material for cockroaches. Further studies are required to investigate this sort of relationship.

The present study revealed that among the various sites of collection, Medical ward-I carried the highest proportion of pathogens exclusively and in combination with opportunists (Table-VII). When it was compared with Gynaecology and Obstetrics ward no significant difference was found. However when a comparison was made between units having inpatient facilities (i.e. Gynaecology and Obstetrics ward and Medical ward-I) and service units (i.e. Kitchen and Medical store), a significant difference was found at the level of $P < 0.01$. The explanation for this difference could probably be that inpatient units received cases of different bacterial diseases which could contaminate the ward environments and cockroaches while crawling in that contaminated environment got the microorganisms on their surfaces. On the other hand since service units do not deal with the patients therefore cockroaches carried less pathogens.

CONCLUSION AND RECOMMENDATIONS

The result of the study suggests that the cockroach infestation is a public health problem in Services Hospital, Lahore. The high prevalence of American and German cockroaches indicate the low standard of hygienic conditions. The presence of Salmonella and Shigella indicate that cockroaches act as vehicle of transmission of hospital cross infection. Further studies are required to establish the role played by cockroaches in the transmission of nosocomially acquired infections.

Health education towards the hazards and control of the

cockroaches can cause awareness among the Doctors, Nurses, Ward boys, Sweepers, patients and their attendants.

Attention should be given towards ward sanitation along with correction of faulty fittings and repair work.

Strict measures are required for the cleanliness of the kitchen, food should not be left out at night, refuse and scraps should be cleared daily, dustbins should be placed and emptied daily.

After correcting the faults and improving sanitation cockroach control can best be achieved by using insecticides.

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- | | | |
|---|---|------|
| a | - | 765 |
| b | - | 132 |
| c | - | 1128 |
| d | - | 546 |
| e | - | 839 |
| f | - | 946 |
| g | - | 1008 |
| i | - | 476 |
| j | - | 864 |
| k | - | 203 |
| l | - | 543 |
| m | - | 655 |
| n | - | 665 |
| o | - | 1019 |
| p | - | 268 |
| q | - | 784 |
| r | - | 851 |
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b - 180
c - 100
d - 279.
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EFFECT OF MELIA AZADIRACHTA L. LEAVES EXTRACT AGAINST ENTEROBIUS VERMICULARIS L.

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ABSTRACT

Effect of Melia azadirachta crude leaves extract was observed against Enterobius vermicularis and leaves extract completely controlled worm infections.

INTRODUCTION

Neem tree Melia azadirachta L family meliaceae is found in almost all over Baluchistan, Punjab and Sind provinces of our country. Its local name is Nim and in Urdu it is called as neem. Its english name is margosa tree or Pakistani lilac. The tree is large and ever green. Flowering takes place in the months of March to May. Fruits ripe during July August. These trees are mostly used as shade trees and firewood in rural areas of our country.

The seeds and leaves are used in indigenous medicine on a very small scale by some people in rural areas in our country. Butterworth and Morgan (1968) reported the occurrence of a highly oxidized terpenoid, Azadirachtin in Melia azadirachta seeds. According to Parker (1918) and Zaman and Khan (1970) neem seed oil contains nimbin, nimbinin and nimbidnin. The blossoms contain

glucoside nimboosterin and a highly pungent essential oil, nimbosterol, nimbecetin and fatty acids. Flowers contain a bitter substance and an irritant bitter oil. Fruits contain bitter bakayanin and trunk yields nimbin; nimbidin and an essential oil. According to them the bark is bitter and is used as tonic, leaves are antiseptic and are used in ulcers, eczema and as poultice to boils. The gum is used as tonic in catarrhal affections. The oil is, stimulant, antiseptic and is used in rheumatism and in skin diseases. The berries are used as purgatives and anthelmintics. Henderson et al (1964) and Connally et al (1968) reported the occurrence of Triterpenoids and tetranortriterpenoids (Selanin, meldonin and nimbidinin) in neem seed oil. Chopra et al (1958) reported the use of nim leaves against variety of forms for external applications against ulcers and skin diseases. Shaikh and Wahidy (1983) studied the effect of leaves extract of Melia azadirachta against fourteen different species of pathogenic and non-pathogenic bacterial species and they found the leaves extract as active against Sarcina lutia, all six strains of Staphylococcus

coccus aureus used in the studies, Bacillus anthracis, Bacillus cereus and Bacillus subtilis.

Small fresh branches of the tree are also used as tooth brushes (miswak) by a fraction of the people and for the past many years experience it has appeared that these chewing sticks or tooth brushes have not shown any visible toxic effect on man. Khan, Khan and Mahmood (1985) worked on the effect of these chewing sticks against different bacteria Bacillus subtilis, B. pumilus, B. thuringiensis, B. megaterium, Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa, Proteus vulgaris, Sarcina lutea, Staphylococcus aureus and the fungi Aspergillus niger and A. flavus. They observed variable microbial activity against all microbes except S. lutea and B. pumilus. Both the leaves and seeds have very strong bitter taste. Present studies were therefore planned to confirm the effect of Neem leaves extract against Enterobius vermicularis.

MATERIALS AND METHODS

250 grams of nim tree Melia azadirachta (family meliaceae) leaves were washed in clean tap water and were then macerated. These leaves were then boiled in 1 liter of tap water until 100 ml crude extract was left. This extract was strained and leaves were discarded. The extract was cooled and considering the information of Zaman and Khan (1970) the effect of this extract was studied against a patient having pinworm, Enterobius vermicularis

L infections The patient was given 4 tablespoons of leaves extract initially and afterwards as 2 tablespoons three times a day after meals for four consecutive days. The effect on pinworm infection was observed by making slides of the patient from perianal area, by observing excretion of worms in the faces and by enquiring from the patient till 5 days after treatment.

RESULTS AND DISCUSSION

After 1 hour of giving four tablespoons of leaves extract patient told that he was feeling comfortable from the itching due to worms. Upto 2 days after treatment he expelled worms in the bowel and the swabbings from perianal region revealed eggs but on 3rd day till 5th day after treatment neither eggs, nor worms were detected from the patient. The leaves extract also did not show any toxic effect on the treated patient.

From our studies it has appeared that neem tree leaves extract is completely non-toxic to man. The patient having pinworm infection when treated with neem leaves extract upto four days was relieved from pinworm infections and he did not complain any other problem. No eggs in the perianal swabbings or worms in the bowel were seen three days after treatment and no complaints or toxic effect was observed on the patient. Zaman and Khan (1970) has described the effect of nim tree leaves extract against helminth worms and our studies has also

confirmed their findings. Small fresh branches of this tree are also used as tooth brushes (miswak) for cleaning teeth in our country. This tooth brush (miswak) is commonly being used as long before and the practice is a very good proof of its harmlessness to man. Khan, Khan and Mahmood (1985) worked on the effect of these chewing sticks against different bacteria and found these chewing sticks as having antimicrobial effects. They did not find any toxic effect due to these chewing sticks. According to the studies of Butterworth and Morgan (1968) the nim seed contains an enzyme azadirachtin but according to the informations available this azadirachtin has not so far been tried for helminth control in Pakistan or anywhere else. According to the studies of Shaikh and Wahidy (1983) the leaves extract of Melia

azadirachta L is very active against Sarcina lusia, all six strains of Staphylococcus aureus used in the studies, Bacillus anthracis, Bacillus cereus and Bacillus subtilis. These workers have reported that nim tree extract either from bark, leaves or fruits has got medicinal value and our studies have confirmed the effect of nim tree extracts on Enterobius vermicularis. The extracts are therefore very valuable in having anthelmintic effects. The question remains in conducting further studies on dose determination and its toxic effects if any so that it can be used on large scale as an anthelmintic and as antibiotic in man. The dose required for application against different diseases in man will certainly be low and it required further studies on different bacterial and parasitic pathogens.

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PERIODONTOL DISEAS ASSOCIATED WITH PREGNANCY

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HISTORICAL BACKGROUND

Periodontal disease constitutes a major problem in modern dental practice. Periodontology is of more academic interest to the present-day practitioners. There is evidence of the existence of periodontal disease in the Jaws of unearched human skeletal remains, but earliest historical record shows, that there was an awareness of periodontal disease and the need for treating it in the past.

Periodontal disease has been described as the commonest of all diseases in the embalmed bodies of the Egyptians, 400 years ago. Dental Hygiene was not only practiced by Sumerians of 3000 before Christ, but also by our old mothers, in the near past who used to have the golden/silver toothpicks for their interest in cleanliness of the mouth. Similarly Babylonians and Assyrians have suffered from Periodontal diseases and have used clay tablets for the gingival massage combined with various herbal medications as a treatment.

In the oldest Chinese medical work, before 2500 B.C. the oral disease was divided into three types:

- a. Fong Ya - Inflammatory conditions
- b. Ya Kon or diseases of the soft investing tissues of the teeth
- c. Chong Ya or dental caries.

Gingival inflammations, periodontal abscesses and gingival ulcerations are described in accurate detail by Hwang-fi. Herbal remedies were prescribed for the treatment of these conditions, as it is practiced, now a days in our country in the rural areas. Chinese have used the chew stick as a tooth brush or tooth pick for the purpose of massage of the gingival tissues and to keep the mouth as it is being practiced now, by the majority of the Pakistanis in the form of sunna of Holy Prophet Muhammad (p.b.u.h.)

Talmudic writings show the importance of oral bygiene recognized by the early Hebrews. Egyptian medicine predominantly was mystic, however, reference is available of gingival disease and prescriptions for strengthening the teeth, there is also reference that shows the presence of a specialist for the care of teeth. Hippocrates of Cos (460-335), one

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of the ancient Greeks and a father of modern medicine, discussed the function and eruption of the teeth and also the etiology of periodontal disease. According to his belief, the inflammation of the gums was produced by accumulations of calculus, and gingival hemorrhage occurring in the cases of persistent disease.

Among the Romans, Aulus Cornelius Celsus (1st Century A.D.) mentioned that looseness of the teeth is caused by flaccidity of the gums. The Romans were interested in oral hygiene and for treatment red hot iron (Cautry) and honey was used for massage. Because gingival massage was an integral part of oral hygiene

Rhazes (850-923) Arabian Physician, recommended opium oil, rose and honey in the treatment of periodontal disease. To strengthen the loosened teeth, he recommended astringent mouth wash and dentifrice powders. He described a procedure of scarification of the gingiva, and strong counterirritants in the treatment of disease of the gums.

Avicenna (980-1037) wrote extensively on diseases of the gingival such as ulcers, suppuration, recession and fissures. Abulcasis (936-1013) stressed upon cure and treatment of the supporting structures. He recognized some interrelation between tartar and disease of the gums. Abulcasis (936-1013) referred to the treatment of the periodontal disease as follows. "Sometimes on the surface of the teeth, both inside and outside, as well as under

the gums, are deposited rough scales of ugly appearance, which are black green or yellow in colour, thus corruption is communicated to the gums.

A set of instruments was designed by Abulcasis for scaling the teeth. These instruments were very crude, but their role in the heritage of modern periodontal instrumentarium is quite apparent.

In the fifteenth century, Valescus of Montpellier (1382-1417) stated that in order to treat diseases of the gums tartar must be removed little by little either with iron instruments or with dentrifices.

With the beginning of the eighteenth century, dentistry assumed the early signs of scientific curiosity, which developed into present day research disciplines. Pierre Fauchard (1678-1761) the father of modern dentistry, discussed many aspects of the subject of periodontology. He described chronic periodontal disease as a kind of scurvy which attacked the gums, the alveoli and the teeth.

John Hunter, the eighteenth century English physiologist, and surgeon, published two books on dentistry in which he discussed diseases of the alveolar processes which he believed to be the site of suppurative periodontal disease. The nineteenth century brought new names and developments to the periodontal field. Such as Glickmann and his surgical measures for the treatment of periodontal disease.

The observation that periodontal disease is the major cause of tooth

loss in individuals beyond the age of forty is a significant one. However, it does not mean that periodontal disease is limited to that age group. Periodontal disease in chronic condition should be the concern of the dentist and patients of all ages of sex. Early treatment is simpler and the results are more gratifying. More important than early treatment is prevention.

Periodontal care is a comprehensive approach toward the maintenance of the health of all the periodontal tissues, in relation to the teeth of all patients. Periodontal care is required for healthy as well as disease tissues.

The concept of "Periodontal care" inculcates a periodontal consciousness into the practice of dentistry, a need for eliminating all potential causes of periodontal disease in all patients and caution against creating conditions which induce periodontal disease.

Islam in its profile has laid emphasis for oral hygiene. Cleanliness of mouth has become compulsory for 5 times prayer in a day. Thus mouth wash, use of finger and chewing stick, does not allow affliction of disease to occur because it does not allow plaque, to deposit. Holy Prophet Muhammad (peace be upon him) recommended use of Miswak" from the gums towards the teeth" and also asked for chewing it. He, The prophet Muhammad (peace be upon him) said "if I don't feel a burden on my followers, I would have made compulsory use of Miswak on each Muslim. He, too has recom-

mended and used tooth picks of bitter wood and discarded the tooth pick made up of gold or metal. The Holy prophet, have been promptly using the wooden stick and it was his concern, that he had a desire for chewing of Miswak at the last moment of his life.

OUTLINE OF INFORMATION

Pregnancy can easily accentuate pre existing local factors to cause an increase amount of bleeding and inflammation in the gums. The gingiva, during pregnancy becomes red, due to increased vascularity. The microscopic changes have been observed in the gingiva in pregnancy. It reveals epithelial hyperplasia with accentuated retepegs and some alteration in surface keratinization, marked vascularity, inflammatory cellular infiltration with edema and associated secondary generative changes in the epithelium and connected tissues.

The changes during the pregnancy, in gingiva sometimes is termed as pregnancy gingivitis. There is also tendency for the teeth to become more mobile during pregnancy. And, in some women localized enlargement of the gingiva may occur. These enlargements, called "Pregnancy Tumors" they often originate from the inter dental area with bright red mulberry surface and bleed easily.

Finding these changes and a great deal of controversy over, whether gingivitis in pregnancy is the cause of estrogen deficiency,

or clinical picture or local causes are responsible. It has evolved me for prevalence of the disease, and other changes 100 pregnancy the following studies have been carried out:

MATERIAL AND METHOD

Forty two women were selected and 33 found with hyperplasia of gums during pregnancy, 9 were

free of hyperplasia but there was slight congestion and redness at the marginal borders of the mucosa.

The patients with hyperplasia of gums had the experience of several pregnancy i.e. 3rd, 4th and 8th para (Table No. 1).

TABLE NO. 1

No. of cases examination	Total pregnancies	Hyperplasia	Free
6	3	4	2
9	4	7	2
3	5	3	..
3	6	3	-
9	7	7	2
12	8	9	3

It was observed that the pregnant mothers from 4 to 6 months and 6 to 8 months of pregnancy, had shown more marked hyperplasia as compared to those in early and late pregnancy. No case in this study was found with "pregnancy tumor".

The common complaint of such patients was the inflammation, congestion and tendency for bleeding from the inter dental papila and gum margins. Local examination showed bacterial plaque, and calculus deposits, were in various degrees and in certain cases pus-pockets were found. Most of the patients could not carry out the oral hygiene discipline. The study was observed from the different strata of the

society and average was from the higher middle social class. All of them had good physique except women who were anaemic.

In another study, 47 pregnant mothers were examined for dental problems. The age group was 20 to 40 years and according to this study 23% of the cases were free of D.M.F. and the rest of them were computed for each group separately. Table which shows higher number decay missing teeth with number of the pregnancy.

Overall, 23 per cent cases were free of D.M.F. and for the rest, cases range (Mean = L.S.R.) have been computed for each age group separately.

TABLE 2

Age groups	Percentage of cases free of D.M.F.	DECAYED, MISSING AND FILLED			
		Mean	S.E.	Confidence Ranges 95 per cent level	
				Lower	Upper
20-24	11.1	2.22	0.43	1.33	3.11
25-29	28.2	1.87	0.39	1.09	2.65
30-34	20.4	2.98	0.34	2.32	3.64
35-39	28.6	3.34	0.70	2.01	4.85
44-40	20.0	5.00	1.19	2.56	7.44
Overall:	23.00				

Age and D.M.F. are significantly correlated ($r = 0.337$ at $P < .05$ and the linear trend $D = 0.171(A) - 2.338$ (Where D = Expected D.M.F. at any age (A) given and expectation at any age preferably 20-45 years because it is a quite safe region for prediction.

Oral Cleanliness and Social Status.

Among 402 pregnant mothers,

16.6 per cent were illiterate or primary pass, 42.9 percent had received education upto Middle and Matric standard and 40.5 percent to that of College and University level (details given in Table 3 & 3A). The means of cleanliness was toothbrush, tooth powder, miswak. Several women had never used any mean of cleanliness. Higher number of the women have used powder. The toothbrush and Miswak, was equally used by women having 7 to 8 pregnancies.

TABLE 3 (A)

Educational Status	Percentage persons
Illiterate	7.1
Primary	9.5
Middle	12.7
Matric	30.2
College	23.0
University	17.5
TOTAL	100.00

TABLE 3B
MEDIUM OF CLEANLINESS

Pregnancy Groups	Tooth Brush	Powder	Miswak	Nothing
1-2	9.5	61.9	2.4	26.2
3-4	9.7	54.8	12.9	22.6
5-6	26.6	46.7	20.0	6.7
7-8	40.0	13.3	40.0	6.7
9-14	25.0	37.5	45.0	12.5

Mean oral hygiene with pregnancy has got a correlation and

significant picture has come out.

TABLE 4

Pregnancy Group	Free Cases (per cent)	ORAL HYGIENE INDICES			
		Mean	S. E.	Ranges at 95 per cent level	
				Lower	Upper
1-2	-	2.03	0.24	1.54	2.52
3-4	-	2.84	0.13	2.57	3.11
5-6	-	3.28	0.13	3.03	3.53
7-8	-	3.88	0.32	3.24	4.52
9-14	200	3.16	0.54	3.05	3.27

Correlation between pregnancy and OHI ($r = 0.73$) is significant even at $P < .01$ and prediction

equation is $\hat{OHI} = 0.195(P)$

Similar gingivitis in relation to the pregnancy has been observed.

TABLE 5

Pregnancy Groups	Free cases (per cent)	GINGIVITIS SCORES			
		Mean	S. E.	Ranges at 95 per cent level	
				Lower	Upper
1-2	22.00	1.41	0.15	1.11	1.71
3-4	33.0	2.23	0.21	1.81	2.65
5-6	32.3	2.92	0.21	2.50	3.34
7-8	-	3.90	0.44	3.00	4.80
9-14	11.1	3.78	1.15	1.41	6.15

G.I. are directly and significantly related to pregnancies, $r = 0.565$ and prediction of G.I. at any age can be worked out from the linear regression $G.I. = 3.45 + 0.353 (P)$

DISCUSSION

Hyperplasia of gums during pregnancy was described by Ziskin, Blackberg and Stout (1933). Ziskin (1938) injected folliculating into normal female monkeys and found condition as in pregnancy gingivitis. He also in 1937 investigated hormonal therapy for hyperplasia of gums.

Gonadotrophic sex hormones are being secreted by two anterior lobes of pituitary gland. Prolan A has effect on ovaries for activating graffianfollicles. Root and Root (1923) believed that the basal metabolic requirements of women increase from 20 to 21 per cent. Lintz (1931) quoted by Weintraub (1932) made a study of 229 cases, 179 pregnant and 50 non-pregnant. His results showed more looseness of teeth and greater increase of dental carries in the pregnant than in the other group. Gohen et al (1969) had made longitudinal investigation during pregnancy and he has repeated again (1971). The Lindhe, et al in (1967) have observed increase gingival exudation during pregnancy. It is a common feature that during pregnancy, the body is under various changes and is of low resistance and some times calcium depletes from alveolar bone. The deficiency or variation in the source of dietary protiene has

an effect on the supporting tissues of the mothers and in respect to Calcium and Vit-D deficiency leads to low response of the bone structure. Waerhaug (1958) has observed the effect of deficiency of Vit-C on supporting structure of the teeth. During pregnancy the prophylaxis are not observed and vitamin supplementation is not carried out, therefore there is possibility of more initiation of periodontal disease. During pregnancy and in under nutrient mothers, the agent becomes more virulent as compared to the host and therefore, the periodontal disease and other clinical inflammatory changes are liable to occur. In our observation the gingival crevices increase in pregnancy and according to Mann (1963) increased gingival fluid has been correlated with gingivitis. The local immunoglobulins complement in the crevicular fluid cannot show activity to overcome the attack of the gingival infection. Egelberg (1964) has shown gingival exudate measurements for evaluation of inflammatory changes of the gingiva. Soofi and Soofi (1970) made a study on pregnant sheep and observed hyperplastic activities during pregnancy. Therefore, the periodontal disease is liable to be present during pregnancy because of:-

- Sex hormone changes in the body.
- Immunoglobine complement changes in the gingiva.
- Increase of gingival exudate during pregnancy.

- d. Due to diet inconsistency during pregnancy.
- e. Less prophylaxis on dental side.

CONCLUSION

1. The 7.4% of the mothers have neglected oral hygiene thus more periodontal problems were faced by them.
2. Decayed, Missing, Filled teeth (D.M.F. Index) is directly significant by the age and number of pregnancies.
3. The incidence of gingival disease was higher, with mothers having several pregnancies, than those with few.
4. The pregnancy, do influence the gingiva of the women.

5. The clinical gingival inflammation of pregnant mothers was observed, inpared with nutritional status.

THANKS

Thanks are to Mr. M.R. Qureshi, statistical expert of Live Stock Department, Government of the Punjab and Mr. Muhammad Sarwar of Health Department for providing respectively, and Dr. Fakharul-Islam of Mayo Hospital for correction.

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ROLE OF ANOPHELES STEPHENSI

AS

MALARIA VECTOR

IN

RURAL AREA OF PAKISTAN

BY

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DR. I. H. SHAH

I. NEED OF THE STUDY.

Towards mid eighties there were frequent reports of An. stephensi developing resistance to Malathion, insecticide being used to control malaria in Pakistan. However, Anopheles culicifacies remained susceptible to Malathion. Thus initially An. stephensi resistance to Malathion was ignored because this species was considered (and confirmed) as urban malaria vector only. In rural areas its vectorial capability was doubted on the following basis.

a. Anopheles stephensi is not a homogeneous species and the possible existence of at least two races have been postulated by several investigators (Knowles and Basu, 1934, Mulligan and Baily, 1936, Ramsay and McDonald 1936; Sweet and Rao, 1937). Sweet and others (1938) observed significant difference in eggs, and classified this species into two races naming them as An. stephensi type, as an urban mosquito and var myso-rensii inhabiting rural conditions Colluzi and co-workers (Colluzi, 1972, Colluzi et al 1972, 1973, 1974; White, 1974) observed a relation between egg size and behavioural characteristics and the presence or absence of the 2RB Inversion from Iraq colony.

Afridi and others (1958) concluded that prevalence of An. stephensi in Pakistan is restricted to myso-rensii only which is not able to transmit malaria.

b. At several occasions An. stephensi was found involved in malaria epidemics in urban cities and rarely in rural areas e.g Bombay 1908; reported by Christophers, (1949); Lucknow, (1929) and Karachi, 1966 (Rehman and Muttalib, 1967).

c. Short life span, low anthropophily, lack of strong evidence of its completing sporogony beyond oocyst stage in rural areas gives further support to Afridi and others (1958) belief "rural An. stephensi" myso, prevalent in Pakistan, does not play any role in the transmission of malaria.

Further wide spread high resistance An. stephensi to Malathion and the general feeling about epidemiological situation after spray (especially in relation to the absence of An. stephensi) warranted to seriously review the status of An. stephensi as malaria vector. In this regards, recently an attempt was

made to determine the capabilities of An. stephensi as malaria vector. In 1985 laboratory studies were conducted in which, without indulging in the debate of races, it was found that village strain of An. stephensi is able to complete sporogony of Plasmodium falciparum as well as Plasmodium vivax. It was decided to extend these studies to field area in order to confirm/assess vectorial capacity in our field conditions and to determine the races of An. stephensi in Pakistan.

2 ANOPHELES STEPHENSI IN THE LIGHT OF LITERATURE (AS MALARIA VECTOR).

Knowles and Basu (1934) made several sets of studies to observe survival and susceptibility of An. stephensi to Plasmodium infections. Their major findings were that infections were heavy in conditions similar to those during the monsoon, post monsoon and cold weather. They found temperature factor playing more important role than humidity. Higher survival percentage was noticed at lower temperatures with high relative humidity. Spring and hot weather conditions were found to be most unsuitable for survival and infection development. The ineffectivity of mosquitoes was also determined by the maturity and density of gametocytes in the donor's blood.

and others (1979) studied natural and induced infection with human plasmodia in Tamil Nadu. They observed that 50 percent An. stephensi became infected whereas all An. stephensi failed to complete sporogony.

In 1985 we also conducted plasmodium infection experiments and found that An. stephensi (rural strain) was capable of developing sporozoites of Plasmodium falciparum and Plasmodium vivax. Observations were made in early September in natural room conditions, and was found that An. stephensi developed sporozoites of Plasmodium falciparum in 13 days and that of Plasmodium vivax in 10 days (Parvez and colleagues 1988). It was also noticed that mortality among the P. vivax fed An. stephensi was higher.

Extensive dissections have been made in several cities and villages of Pakistan and India some of the positive results are tabulated below in the Table-I.

Table - I

FIELD INFECTIONS RECORDED IN AN. STEPHENSI

Locality	Number dissected.	No of Infections.		Reporter & year.
		Gut.	Gland.	
Bombay City.	1,228	91	30	Covell 1927 (Bentley 1911)
" "	671	17	12	Covell 1928
Kohat.	45	-	1	Sinton(1917)
Khairpur.	250	6	-	Covell & Baily (1934)
Quetta.	719	5	2	Mulligan & Baily (1936)
Fort Sandeman.	152	1	0	Bruce & Jacob, 1947
Culcutta.	1,052	4/678	6/1052	Siddons, 1946
Bijapur.	1,272	-	1	Godbole, 1948.
Deccan.	4,706	-	1	Viswanthan, 1950.
Dirgaipur.	1,613	-	1	Neogy & Sen, 1962.

An. stephensi is known to be an important vector under rural conditions in Western and North Western India, Persian Gulf and under urban conditions in Bombay, Bangalore, Lucknow, Culcutta and in Pakistan, Karachi.

An. stephensi mysorensis was considered less important as a vector than the type form but Senior White (1940) Subba Rao and Appa Rao (195) have shown it to be the chief rural carrier in visakhapatnam (Vizagapatam).

Work in Bombay state, reported by Viswanathan (1950) and in Karnataka (Bhaskar Rao et al 1946) has shown that An. stephensi was vector of some importance in rural areas of Deccan. It had, however, a comparatively low infection rate, but it made up for it by occurring in large numbers. In this area it shares the transmission with An. culcificies and An. fluviatilis. The form in this area was mysorensis as recognized by egg morphology.

3. OBJECTIVES

3.1. Present study was organized to thoroughly investigate the status of An. stephensi as malaria vector in natural field conditions of our rural areas.

3.2 Further, to investigate the prevalence of biracial/sibling species of An. stephensi.

This report covers the findings regarding first objective while work on races is reported separately.

4. WORK PLAN

4.1. Experimental mosquitoes infection: 100 females from a colony raised by Orrarra (District Kasur) were used in this set of experiments. Other methods and procedures were the same explained by Parvez and Shah (1988). Infective mosquitoes before dissections were isolated individually for ovum measurement.

4.2 At Indicator Villages. Three villages in Lahore District and two in Kasur District were selected for regular monthly studies. These villages were picked up on the basis of 1986-87 malaria positivity and An. stephensi dominance.

a. Day time resting population of anopheline mosquitoes was estimated (to note seasonal variations in the An. stephensi population).

b. July onward An. stephensi were dissected for parity to determine appropriate time for gland dissection, in conjunction with the results of blood examination of fever cases.

c. Blood samples of fever cases concentrating on 2-9 years age group and infants, were examined. Confirmed malaria cases were investigated to detect transmission time for dissections of mosquitoes.

d. Extensive gut and gland dissections of An. stephensi and An. culicifacies were done during August to October.

e. All other anopheline mosquitoes (An. pulcherrimus, An. nigerrimus and An. annularis) landing on man for feeding were also dissected for glands.

4.3. Spot Checks. At random every month some villages, other than above mentioned fixed localities in Lahore district, Kasur district and Okara district were surveyed for adult mosquito density and other activities according to the situation. During 1988, instead of Okara district, Sheikhupura was substituted for spot checks. Although this district is An. culicifacies dominant area but is highly malarious as compared to other studies area of 1987.

In the third week of September, 1988 we got the clue from BHU Bahtal about "fever cases" in Tahliwala village district Sheikhupura, and arranged further studies in the last week.

5. RESULTS & DISCUSSION:

5.1. 1987 Results. 19 villages (35 visits) in Lahore district and 12 villages (15 visits) of Kasur district and 7 villages of Okara district were surveyed from March to September, 1987.

Above mentioned localities were selected mainly on the basis of 1986-87 malaria positivity and An. stephensi dominance. According to recent records of the district most of these localities did not indicate active transmission of Malaria. Therefore, we conducted limited (strictly fever cases) blood survey

extensive gland dissections of An. stephensi and An. culicifacies during July, August and September. 2437 An. stephensi and An. culicifacies dissected were all negative for sporozoites agreeing with parasitological results of our blood surveys as well as district office surveys. In fact during 1987 there were few rains and perhaps insignificant malaria transmission occurred in our study districts.

5.2.1988 FINDINGS: Preliminary Survey: On the 24th and 26th September we conducted first blood and Entomological Surveys at Tahliwala (Sheikhupura District). It was revealed that the village is in the grip of malaria epidemic.

Table-2
SLIDE POSITIVITY AT TAHLIWALA

Dates	Slides Examined	P.v	P.f	Mixed	Total.
24-09-1988.	30	01	14 15	1	17 (56.7%)
26-09-1988	93	11	54	1	66 (71.0%)
04-10-1988	52	06	25	3	34 (65.4%)
09-10-1988	21	01	14	0	15 (71.4%)
Total	196	19	108	5	132 (67.34%)

Overall parasite rate being 67.3% with 81.8% Plasmodium falciparum 4.4% Plasmodium vivax and 3.8% Mixed infection.

3. Entomologically, we found the following anopheles species per positive room.

Table 3
ADULT ANOPHELES DAY TIME RESTING POPULATION
(Per +ve room)

Sp	24-9-1988	26-9-1988	4-10-1988	6-10-1988	7-10-1988
<u>An. culicifacies.</u>	3.60	4.25	4.0	4.25	4.25
<u>An. stephensi</u>	1.00	2.25	2.0	2.0	2.25
<u>An. pulcherrimus</u>	1.50	1.00	1.00	1.00	1.50
<u>An. annularis</u>	1.00	1.50	2.00	2.00	1.50
<u>An. subpictus</u>	2.00	2.00	2.50	2.25	3.00
<u>An. nigerrimus</u>	1.00	0.00	0.00	0.00	1.00

5.4. **ANOPHELES STEPHENSI DETECTED SPOROZOITE POSITIVE IN THE FIELD (ON THE SPOT DISSECTION).**

Due to floods our field studies were interrupted till 3rd October. From 4th to 7th October extensive dissections of An. stephensi and An. culicifacies were carried out. Knocked down mosquitoes were processed in the field while hand captured were kept in the insectary for "delayed dissections".

On 6th October three An. culicifacies and one An. stephensi were found gland positive with sporozoites, on immediate dissections among the flitted mosquitoes. Details of these dissections are given in Table-4

Table 4
IMMEDIATE FIELD DISSECTIONS

Date	<u>An. stephensi</u>		<u>An. culicifacies</u>	
	No. Dissected	Gland +ve.	No. Dissected	Gland +ve.
4-10-1988	39	0	68	0
6-10-1988	40	1	87	3
7-10-1988	27	0	63	0
Total:	106	1 (0.94%)	218	3 (1.31%)

As mentioned earlier hand captured alive mosquitoes were kept under insectary conditions for "delayed" dissections. Results of this study are tabulated below:

Table 5
RESULTS OF GUT/GLAND DELAYED DISSECTIONS OF
AN. STEPHENSI AND AN. CULICIFACIES

Date	Days after collection	<u>An. stephensi</u>			<u>An. culicifacies</u>		
		No. dissected.	Gut positive	Gland positive	No. dissected	Gut +ve	Gland +ve.
28-09-1988	2	6	0	0	29	2	0
29-09-1988	3	8	0	0	30	0	0
02-10-1988	6	20	0	0	24	2	0
08-10-1988	4	5	0	1	10	0	0
10-10-1988	6	19	1	0	42	2	2
11-10-1988	7	14	2	1	37	3	1
12-10-1988	5	9	0	0	22	0	1
15-10-1988	8	12	2	0	29	2	1
Total		93	5 (5.4%)	2 (2.1%)	223	11 (4.9%)	2 (2.24%)

Thus, in the field, 1.5% (3/199) An. stephensi were with sporozoite positive glands; and 1.81% (8/441) An. culicifacies.

Below table reflects the results of dissections of other species.

Table 6

**GUT/GLAND DISSECTION RESULTS OF AN. NIGERRIMUS
AND AN. PULCHERRIMUS**

Date	<u>An. nigerrimus</u>		<u>An. pulcherrimus</u>		Remarks
	No dissected	Gut/Gland positive.	No dissected.	Gut/Gland positive.	
<u>Immediate dissection.</u>					
06-10-1988	22	0	9	0	Landing on man for feeding.
07-10-1988	31	0	11	0	
Total:	53	0	20	0	-
<u>Delayed dissection.</u>					
06-10-1988	11	0	5	0	Trap collection. 2 days after collection
07-10-1988	14	0	3	0	Trap collection 3 days after collection
08-10-1988	19	0	7	0	Trap collection. 4 days after collection
09-10-1988.	20	0	3	0	Trap collection. 6 days after collection
10-10-1988	3	0	3	0	Trap collection. 8 days after collection.
Total:	67	0	21	0	-

A few (28) An. annularis (all sources) were also dissected, but any role in the transmission of malaria by these three species was not revealed.

5.6. **Man/Animal Vector Contact:** During our study period at Tahliwala from 24-9-1988 An. stephensi and An. culicifacies density, resting indoor was not high (see table 3) 2 - 2.25 and 4 - 4.5 females/+ve room respectively. Thereby indicating that An. stephensi density was 50% less as compared to An. culicifacies. But contact of An. stephensi with man is equal or higher than An. culicifacies (one night observation). We recorded 3 culicifacies per man against four An. stephensi landing on man on the observed night during direct catching from human bait. Human baited net gave negative results.

In contrast An. culicifacies was attacking more on animal. Direct catches from animals recorded 8 females of An. culicifacies and 5 An. stephensi females

during the night per bait observed. In animal baited net we recorded 8 An. culicifacies and 3 An. stephensi females throughout the night/bait.

These observations confirm low density of An. culicifacies and An. stephensi. Overall An. stephensi density is 50% less than An. culicifacies but it comes in more contact with man and appears to be more anthropophilic as compared to An. culicifacies.

5.7. Vector Density and N/P Ratio: An. culicifacies and An. stephensi density was low during our study period (Table 3) at Tahliwala; high parous ratio (An. stephensi 78%, An. culicifacies 83%), however, indicates that breeding had slowed down due to cooling weather but survival of adult was high. (On 6-7 October, 1988 Maximum temperature was 28.0°C and minimum 16.0°C to give an idea about the weather at the time of study.)

5.8. Window Trap Studies: Locality Tehliwala was unsprayed and objective of operating out let window traps was to observe resting habit and density of mosquitoes. Density of An. culicifacies and An. stephensi is low (An. culicifacies 5 and An. stephensi 3 per trap/night). Gravid proportion coming out in O.W.T. and flitting capture clearly shows that An. culicifacies and An. stephensi both are endophilic.

5.9. Experimental Infections.

5.9.1. An. stephensi

We made four attempts to observe experimental infections of An. stephensi with Plasmodium vivax and Plasmodium falciparum. Results are given below:

Date of feeding Plasmodium.	No. of <u>An. stephensi</u> fed.	<u>An. stephensi</u> surviving 10 days.	Gland +ve.	<u>An. stephensi</u> surviving 13 days.	Gland +ve.
<u>22-8-1985.</u>					
<u>P. vivax</u>	5	0	0	-	-
<u>P. falciparum.</u>	7	4	0	2	One
<u>20-9-1985</u>					
<u>P. vivax</u>	17	3	1	2	0
<u>P. falciparum</u>	-	-	-	-	-
<u>26-9-1988</u>					
<u>P. vivax</u>	-	-	-	-	-
<u>P. falciparum</u>	100	83	0	69	56

11-10-1988					
<u>P. vivax</u>	50	11	0	8	3
<u>P. falciparum</u>	50	40	0	28	25

Repeated successful laboratory experiments confirm beyond any doubt that rural strain of An. stephensi is capable of completing sporogony with Plasmodium falciparum as well as Plasmodium vivax. Careful look at the results indicate that An. stephensi has some special association with Plasmodium falciparum. In each set of experiments at least 50% An. stephensi survived and became infective by 13th day, on the contrary just one out of 17 gametocyte (vivax) fed An. stephensi became infective and in another attempt 8 An. stephensi survived till 13 days and only 3 became infective.

This leads us to formulate a hypothesis that An. stephensi has special association with Plasmodium falciparum and needs testing.

5.9.2. **Malaria Induced to Human Volunteer:** Sporozoite induced infection in a human volunteer was attempted to further confirm the role of An. stephensi as a vector and to carry the experimental infections to their logical conclusion. Three females which had survived for 13 days after feeding on a gametocyte carrier of P. falciparum was allowed to feed on a human volunteer on 10-10-1988. After feeding, all three were dissected. One was heavily positive for sporozoites while another was completely negative while the third having moderate density of sporozoites. On 24th October, 1988, the human volunteer came down with fever and his slide showed moderate parasitaemia (.1313/mm.)

5.10. **Case Histories:** Some of the cases investigated revealed that all of them contracted malaria locally and onset of fevers to the most of cases was during third/fourth week of August and onwards. Recent most cases were of last week of September also.

6. CONCLUSION

The role of An. stephensi as malaria vector in the rural areas is not less important than the role of An. culicifacies. And if the hypothesis formulated herein during these studies (5-9-1) proves to be correct that An. stephensi has some association with Plasmodium falciparum then this species has to be given special importance as malignant malaria vector. In this regard please refer to report "Consequences of Malathion resistant Anopheline stephensi on P. falciparum malaria in Pakistan" by Shah and Parvez (1989).

ACKNOWLEDGEMENT

Hard work and technical assistance of Messrs. Mohammad Sarwar, Javed Ahmed and Wadood-ul-Haque is highly appreciated. They worked with us day and night for three years to complete this assignment.

We wish to express our gratitude to Dr. W. Chin, US AID Advisor on Malaria to the Government of Pakistan, who exhibited practical keen interest in this important project and always encouraged us at every step during his stay in Pakistan.

We are also thankful to Dr. Nabil S. Al Tawil, W.H.O. Senior Advisor, Mr. A. A. Mujahid, Director, Malaria Control Programme, Dr. Rifaq (US AID) who were all interested in this work and always emboldened us.

We dedicate this work to late Dr. S. M. Mujtaba (Director, National Malaria Control, from 1973 to 1985; till his death.) who showed concern in 1983-84 about this important malaria vector and wished someone to work on it to reveal its role in malaria transmission in rural areas of Pakistan.

The authors are thankful to Mr. M. Shahid Amir, Stenographer to Director, who typed this manuscript nicely and accurately.

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