STUDIES ON LEISHMANIASIS
(KALA-AZAR)

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This thesis is submitted in partial fulfillment of the requirement for the Doctor of philosophy (Medical Sciences)

FACUL TY OF POST GRADUATE MEDICAL SCIENCES AND RESEARCH
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I hereby declare that this thesis is based on the work carried out by me and no part of it has been presented previously for a higher degree.

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LIST OF PUBLICATIONS OF PROF. DR. MD. SHAHJADA CHOWDHUY

1. Chowdhury Md. Shahjada: "Studies on aedes aegypti Survival after infection with Brugia pahangi Microfilariae (Buckley and Edeson 1956)" Journal of Preventive & Social Medicine. Bangladesh 1(2): 55-62, 1982.
2. Salahuddin AKM, Chowdhury Shahjada, Alam Sultanul, Ahmed Rafique Uddin: "Smoking Habit among workers of Adamjee Jute Mills Ltd. Narayanganj", Journal of Preventive and Social Medicine, Vol.3, No.1, 1984.
3. Chowdhury Mohd. Shahjada, Chemotherapy of Kala-azar at Primary Health Centre in Bangladesh", Bangladesh Medical Journal, Vol.19, No. 2, P. 51-55, April 1990.
4. Chowdhyr Md. Shahjada, Rahman AJM: "Study on Food Handlers as a Potential Hazard in Transmission of Pathogins in Dhaka; Medicine to day, Vol. 3, No. 1, January 1991.
5. Chowdhury Shahjada, Haque Farida, Abdullah, Al. Masum, Harith Abdulla and Enamul Karim; Positive Response to Sodium Antomony Gluconate Administration in Visceral Leishmaniasis Seropositive Patients; American Journal of Tropical Medicine and Hygiene, Vol/Issue 44/4, 390-393, April 1991.
6. Chowdhury Md. Shahjada, Ahmed Rafiquddin, Ahmed Touhiduddin, Enamul Karim and Hussain AM Zakir, Factors responsible for the Natural Control of Filariasis in the Northern Part of Bagladesh: Journal of Preventive and Social Medicine. December 1990-Vol. 4-9, No.2.
7. Masum MA, Chowdhury M.S., Ahmed Rafiquddin and Mia MAH; Outbreak of Visceral Leishmaniasis in Thakurgaon, Bangladesh: Journal of Preventive and Social Medicine, Dec.1990, Vol. 4-9 No.2.
8. Masum EL Abdullah M, Chowdhury M.S, Rahman MA; Bangladesh Medical Journal next issue accepted. Letter of acceptance enclosed.
9. Ahmed M.U., Miah, MAR, Chowdhury SMZ, Haider D., Hossain RM, Atypical Presentation of Post Kala-azar Dermal Leishmaniasis - A case Report. Journal of IPGM&R 1990, Vol. 5(1); 31-33.
10. Ahmed Touhid UTP, Joshi, Ahmed R.U. Dewan Z.U., Chowdhury M. Shahjada and Shireen Akhter, "Container Habitat Mosquitoes of Bangladesh, Journal of Zoology 1990, Vol. 5, 43-50 ISSN 1017-3765).

11. Chowdhury, Md. Shahjada, Karim E. Ahmed Touhid Uddin, Alam Rabiul, Soil transmitted Geohelminths in two areas of Dhaka city, Journal of Zoology, 1990,

Vol. 5, (7-16) ISSN 1017-3765.

12. Chowdhury Md. Shahjada, Hoq M.S. Waqar A. Khan, Kala-azar in Children of Bangladesh, Dhaka Shishu Hospital Journal Vol.7, 1991.
13. Chowdhury Md. Shahjada, Masum A.E., Hussain Zakir, Karim E., Alam Rabiul Md., Direct Agglutination Test for diagnosis of Kala-azar in Bangladesh. The Hygea, Vol. issue 4 April 1991.
14. Chowdhury Md. Shahjada, Masum AE, Karim E, Jahan Ysmin, Alam Rabiul, Micro ELISA for diagnosis of Visceral Leishmaniasis in Bangladesh, Medicine to day July 1991 Vol. 3, No. 1, 45- 47.
15. Chowdhury Md. Shahjada, Masum AE, et al "Post Kala-azar Dermal Leishmaniasis (PKDL) in Bangladesh, Journal of Preventive & Social Medicine, 1991.
16. Chowdhury, S, Haque, F., Masum, AL, Harith, A.E. and Karim E, 1991. Positive response to Sodium Antimony Gluconate administration in visceral leishmaniasis seropositive patients. Am. J. Trop. Med. Hyg. 44(4), 390-393.
17. Chowdhury M.S., Masum A, Harith AE, and Hussain Zakir "Post Kala-azar Dermal Leishmaniasis in Bangladesh 1991; JOPSOM 10(1):18.
18. Chowdhury. S., Harith. AE., Masum AL., Karim. E., and Rahman A. (1993) "Prevalence of Agglutinating Anti-Leishmania antibodies in two Multi-thousand Bengoli Communities, Parasitology Res. 79: 444-450.

ABBREVIATION OR SYMBOL

AT = Aldehyde Test
Ag = Antigen
Ab = Antibody
CIE = Counter Immuno Electrophoresis
DAT = Direct Agglutination Test
ELISA = Enzyme-linked Immunosorbent Assay
IFAT = Immuno fluorescent Antibody Test
BM = Bone marrow
SP = Spleen
Hb = Haemoglubine
MM = Water extract of mouse muscle
MW = Molicular weight
Na Doc = Sodium Deoxycholate
NCP = Nitro-cellulose paper
Nm = Nanometers
N.SS = Normal saline solution
P = Probability
PB = Sodium Phosphate Buffer
PBS = Sodium Phosphate Buffer Saline
PBS -T= PBS with 0.05% Tween in 20 washing buffer
PEG = Poly ethytline glycol
MP = Malarial parasite
m/hr = Man per hour
PH = Negative logerithim of hydrogen ion activity
DDT = Dichloro-diphenyl-trichloroethane
PMSF = Phenylmethyl -sulphonyl fluoride
PRPP = Phosphoribosyl phyrophosphate
RNA = Ribonuclic acid
S = Second
Sd = Significant difference
SD = Standard deviation
SDS = Sodium Dodecyle Sulphate
TB = Transfer Buffer

TC = Cytotxic T cell (s)
TDW = Triple Distille water
T_H = Helper T cell (s)
V = Volume
X = Arithmatic mean
yr = Year
VL = Visceral leishmaniasis
LD body = Leishman donovani (parasite)
SAG = Sodium Antimony Gluconate
BUN = Blood urea nitrogen
IR = Incidence Rate
ID = Incidence Density
GMRT = Geometric mean of reciprocal titere
Pts = Patients
THC = Thana Health Complex
THFPO = Thana Health & Family Planning Officer
SGOT = Serum glutamic oxaloacetic Transaminase
SGPT = Serum glutamic pyruvic transaminase

ABSTRACT

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Sero-prevalence rates were in Sirajgonj (6.60%) Mymensingh (3.86%), Tangail (0.63%) and Cox's Bazar (0.43%), which is correlated well with the official reports classifying Sirajgonj and Mymensingh districts as being highly endemic. Tangail moderately so Cox's Bazar as non-endemic. A slight difference was observed between the male (2.01% - 3.75%) and female (1.35% - 2.86%) population of the two districts. During evaluation and follow-up study 512 cases re-examined clinically & serologically for VI 515 remained positive in DAT of whom 312 developed typical symptoms of the disease or showing presence of LD body in bone-marrow.

On the other hand using antibody level (score) as the parameter three of the four tests investigated namely the ELISA, IFAT & DAT, do not significantly differ in their ability to assess the varying levels of endemicity of two kala-azar areas in high and low (P value for interaction of test x area = 0.7111 by = 0.353). Out of five independent variables investigated, three were shown to be statistically significant predictors of the antibody scores of populations from the two endemic areas studies - (a) type of serological test (b) age (c) level of endemicity of the area (P=0.0002, 0.0000, 0.0000, respectively). Among these three, the strongest predictor variable is age followed by area and then type of test (Beta=0.369, 0.155 and 0.131 respectively. Age and level of endemicity of the area account for 18.1% of the variation in the antibody scores (F ratio = 17.66, P value = <0.0001). The antibody score increases by 0.021 every year of increase in age same test from same area (+ = 10.372, P value < 0.0001).

The antibody scores of individuals from high endemic areas than those of low endemics for the same test and the same age (+ = 1.387, P = 0.0000), gender and parasite density do not have any significant influence of the variation in antibody score. P value > 0.05 for each variable. The criteria indicating sero-conversion increased 1: 1600 fold or more 1: 3200 fold or more and 1: 6400 or more dilution of different patient sera obtained between of 2 months of follow-up were set up and they were tested for agreement with the microscopic examination. It was concluded that the sero conversion the sensitivity and specificity of sero-conversion test against microscopic examination were 99% and 85.7% respectively. It was noted that annual average of kala-azar incidence was 15 per 1000 person-month, this means that, on the monthly basis 15 persons within 1000 population who lived in the area would suffered from kala-azar which indicates that it is an epidemics of kala-azar. The study revealed that the

low kala-azar transmission was shown during September to February by bone-marrow or spleen aspirated materials examination, on the other hand, sero-logical parameters such as seropositive rate (Level 3200 or more) and GMRT were also low during that period of time.

Local production of DAT was compared with reference antigen L. donovani, 1-5, titers obtained in all 33 VL (kala-azar) sera tested were equally higher (1:6400->1:51200 than in 35 out of 38 negatives control (<1:400-1:1600). The results signify the advantage of employing endogenous L. donovani isolates for further improvement of DAT sensitivity to detect early and sub-clinical kala-azar. DAT sensitivity was further improved, all 70 patients tested DAT showed strong reactivity against the PKDL strain than with the reference (L.donovani (1-s) currently in use) & no significant cross-reactivity was recorded in 60 other patients with mucocutaneous leishmaniasis (18) or leprosy from Algeria or Brazil. Evaluation of DAT for Kala-azar as an opportunity infection in HIV seropositive and AIDS patients was carried out in sera from 163 Europeans. In non of the Dutch (50) or Italian (94) patients having no history or clinical symptoms of VL did the DAT show cross-reactivity.

Study on different treatment schedule for SAG failure cases of VL (Kala-azar) revealed that 99% cases were responded in group D and no patients had an abnormal BUN during and after treatment 10 mg/kg body weight thrice daily for 7 days and 2nd significant result showed in group E with Pentamidine for 14 days. The result suggests that if 40 days of treatment, there is no response the either patient should be continued with antimony 10 mg/kg body wight thrice daily for 7 days or Pentamidine should be the 2nd choice of drug.

In another study 61 PKDL out of 5011 previously treated kala-azar cases when DAT showed very high titration were treated with SAG Sodium Antimony Gluconate (SAG) Pentostum (B. Welcome London) with dose schedule 20 mg/kg body weight not exceeding 850 mg/day I.V. for 120 days injection showed no complication and cured all the cases. In a study of 100 active kala-azar patients among the age group 1-12 years of old those who were showed both parasitologically and serologically DAT and curred SAG 20 mg/kg body wt. for 20 days and cured parasitologically & there is no record of reappearance of any signs and symptoms of kala-azar. Administration of SAG on early kala-azar cases 1273 including 45 PKDL cases were treated and not a single case developed PKDL from early case not develop any signs and symptoms of kala-azar and

the study revealed that SAG is still the 1st line of treatment of kala-azar pentamidine is the 2nd choice of drug.

In a cohort study on vector and kala-azar P. argentipes is still remain as a vector of kala-azar in Bangladesh and number of cases significantly reduced in the DDT sprayed area from 33.23% to .14% in 1989-90 & 0.02% in 1992. The vector density also decreased. Relation between these two was found significant (P value <005). Both the incidence of case & vector density were affected by DDT spraying.

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

Visceral leishmaniasis or so called Indian kala-azar is an insect born parasitic disease caused by protozoan organisms of genus leishmania and its sub-species which is conveyed from man to man or man to animal by infected female genus phlebutomus and its sub-species. The disease is of reticulo-endothelial system.

Kala-azar which had become an almost forgotten dreadful disease has again come to the forefront because of its dramatic and explosive reappearance in northern parts of Bangladesh as well as in the northern districts of Bihar or India. It caught the public and the medical profession as well as the administration totally unaware by virtue of the aware magnitude of the problem in the mid seventies. A resurgence of Kala-azar occurred in several countries like Bangladesh and India during the late seventies when large scale use of DDT spray was discontinued (Ahmed et al 1983). Historically it is known that it was present as early as 1869. In 1882 the Clerk of the Sanitation Commission drew public attention to the disease by reporting 100 cases from Garo Hills. The first noted epidemic in this country was in the adjacent Brahmaputra valley of Assam in 1880 (Rahman et al 1979) and epidemics of Kala-azar have occurred every 15-20 years ever since (Napier et al 1946).

Since then Kala-azar has been known to occur endemically in well defined areas in the eastern half of the Indian Sub- Continent, which includes Bihar, Assam, Tripura, West Bengal, certain areas of Bangladesh, Nepal, Sikim at the foot hills of Himalayas, Uttar Pradesh and Tamil Nadu. The last outbreak of Kala-azar commenced in Bihar in the late thirties, in Assam in 1940, in West Bengal including Bangladesh in 1942-43 and reached a peak in 1947 (Gupta S et al 1975).

The Malarial Eradication programme, both in India and Bangladesh reduced the incidence of Kala-azar to a medical rarity during the 1960s due to the collateral effect of residual spraying on the sandfly, the vector of Kala-azar (Thakur et al 1981, Rahman et al 1983, Das et al 1975). It was predicted that when the residual effect of antimalarial insecticides (DDT) became exhausted, vector density would rise and severe epidemics of Kala-azar would follow.

The prediction became true and a severe epidemic of Kala- azar occurred in Bihar, India in 1973 in those areas where DDT had been withdrawn. By 1977 the rise in

incidence reached an estimated figure of 100,000 cases with 4,500 deaths. In 1979-80, the infection spread to Malda district of West Bengal. The infection then crossed over to eastern Bangladesh and into Nepal where large areas are now effected by this disease (Chakrabarty et al 1982).

It is suspected that Kala-azar is endemic in Mymensingh, Pabna, Sirajgonj, Dinajpur, Rajshahi, Thakurgaon districts of Bangladesh. There are even fears of epidemics in those areas. However, on the basis of reports received from District Hospitals & Health Centres,15000 new cases per year in Bangladesh would not be an over-estimate, (Chowdhury et al 1991). A report in 1987-88 revealed that 31 out of 64 districts and 61 out of 460 thanas were affected by Kala-azar (Internal evaluation, 1988) and Kala- azar cases admitted in Rajshahi Medical College Hospital (in northern part of Bangladesh during the period 1980-89, showed a speedy increase in the number of cases and the number became alarmingly high since 1987 (Alam et al 1990).

The WHO expert committee expressed great concern about the increasing spread and occurrence of kala-azar (WHO 1984). Despite such a long association and epidemics, very little work has been done on the changing clinical patterns of the disease, especially in children. Though in different epidemics or even in same epidemics in different parts, clinical patterns are changing, a thorough evaluation of present features are essential. Since laboratory facilities are still very meager in most of our academic centers, we must have clear conception about changing features for early diagnosis, specially in most vulnerable pediatric age group where failure rate is very high.Early clinical diagnosis in an endemic area will facilitate reduction of reservoir density. Present study will also highlight present

clinical features of children with their predominance and thus help in identifying the differences with adult features in the future.

Although Bangladesh and the adjacent states of Bihar, West- Bengal and Assam in India, constitute the most important endemic area for VL (Chandler and Read 1949; Markell et al 1946; Bahr and Bel 1987), information on the prevalence of the disease is still lacking. Due to overpopulation, presence of various sandfly species and availability of both Kala-azar and post Kala-azar dermal leishmaniasis (PKDL), transmission of the disease at variable degrees including outbreaks is apt to occur(Sen Gupta and Mukherjee 1986; Elias et al 1989).

At present, VL is a major health problem in Bangladesh and with the exception of few districts in the north and south east, cases were reported from all over the country (Elias et al 1979). The available studies on VL prevalence in Bangladesh were mostly based on results obtained by the aldehyde test and presence of clinical indications of prolonged fever and splenomegaly. As in the case of leishmanin skin test, positivity in the aldehyde reaction, even if combined with symptomatology, does not necessarily indicate an on going VL transmission (Chorine 1937; Bahr and Bell 1987). Due to limited specificity in respect of tuberculosis, enteric fever and viral hepatitis results obtained by these two reactions may lead to overestimation of VL. Antibody detection techniques such as enzyme-linked immunosorbent assay (ELISA) and immuno-fluorescence antibody test (IFAT), (Quilici 1967) largely due to their complexity and high cost were applied in a more limited epidemiological survey studies (Chowdhury et al 1990 and 1991). Acknowledging the necessity of identifying an alternative epidemiologic indicator for VL having a similar feasibility for large scale application as the aldehyde and leishmanin skin test, it seems pertinent to assess the potential of the Direct Agglutination Test, Harith et al (1988). The reliability obtained by independent valuative studies (EL Safi and Evans 1989; Mengistu et al 1991) is expected to reveal a more realistic estimation of VL prevalence in two chosen endemic districts in Bangladesh. It is also intended to make local DAT antigen production thereby enabling coverage of the two multi- thousand endemic communities study was under taken.

On the other hand effective control of visceral leishmaniasis (VL) in highly endemic areas such as Bangladesh can be achieved by conducting regular surveillance programmes. Availability of reliable tools for VL active detection at rural level can contribute significantly to these control measures. Due to the known financial constraints, local production of reagents involved will allow regular application of these tools highly feasible. Also prompt intervention in sudden epidemics can easily be undertaken to avoid such devastating effects as those reported

recently in the Sudan (De Beer et al 1990). In addition to their availability, tools for VL detection should technically be less demanding in execution. Their stability under the less favourable storage conditions in rural settings is a prerequisite. The Direct Agglutination Test (DAT) seems to be applicable. Except for antigen processing, all

other steps in test execution were managed at a camp setting in Kenya (Dager et al 1989).

The objective of this study is to evaluate the possibility of DAT mass application in a rural health setting in Trishal Thana in Mymensingh district, a known endemic area for VL in Bangladesh. The study includes also took the efficacy of local antigen production at the Central Laboratory in Dhaka and evaluate homologous and authoctonous L. donovani isolates for reactivity in DAT.

In view of the variable responsiveness of Leishman donovani to pentavalent, antimonials as evident from different percentages of primary unresponsive cases (WHO 1984) one may presume differences in enzymatic profile, antigenic pattern or other molecular aspects in different isolates. Characterization by isoenzyme electrophoresis may indicate the similarity or disimilarity among different isolated strains from PKDL.

There has been a number of changes in the treatment schedule of Kala-azar in this part of the world. Recent work suggests that a prolonged period is not only unnecessary but may also increase the likelihood of induced drug resistance. It has also been shown that higher doses are well tolerated, (Thakur et al 1984). The latest trial on the dosage schedule of Sodium Stibogluconate, (Thakur et al 1988) also suggested a schedule of 20 mg/kg/day for at least 40 days. The requirement for prolonged daily parenteral therapy imposes a severe burden on the health care facilities of the developing countries and may also increase the dropout rate. A trial may be given with higher doses of Sodium Stibogluconate for shorter period in devided doses if one can rule out the toxicity factor.

The present study is also disigned to define the extent of public health problem due to kala-azar, characterize the Leishmania donovani isolates by using different serological tests with the objective of identifying one having field level applicability and effectiveness in early case detection the efficacy of a multidose short term regime of sodium stibogluconate therapy against the single dose newly recommended regime (40 days) will also be compared. Attempts will also be made to find newer antileishmanial agents.

Among the haematophagous insects phlebotomine sandflies occupy a position next to the mosquitoes in the transmission of arboviral and protozoan diseases to humen. Sand flies transmits visceral leishmaniasis (Kala-azar), Cutaneous

leishmaniasis, mucocutaneous leishmaniasis, papatasi fever (sandfly fever) etc to humans(Sinton 1924; Kaul et al 1976; Ashford 1983; Pandya 1983b; Sevice 1986). Sandflies may constitute a serious, but usually localized, biting nuisance. In previously sensitised people their bites may result in severe and almost intolerable irritations, a condition known in the Middle East as "Harara"

Sand flies are a serious pest to humans in the Indo-Pak sub Continent, especially in the Punjab and the north-west frontier Province. In hot weather they may cause much greater annoyance than mosquitoes, making the nights unbearable as the ordinary mosquito net does not provide any protection against them. Their bites produce intolerable itching and cause much scratching that abrasions are formed which, through secondary infection with pyogenic bacteria, give rise in many cases to chronic sores resembling impetigo contagious; these sores have been the cause of much invalidity and disability among troops operating on the Frontier during hot weather (Sinton 1924).

In Bangladesh the sandfly (P. argentipes) is responsible for the transmission of visceral leishmaniasis or Kala-azar. One of the means to fight the disease (Kala-azar) is to control its

vector, the sandflies. Thus it is necessary to know the habitat, seasonal prevalence, host preference etc. of sandflies for preparing an effective control measure against these insects. But such records are absent from Bangladesh.

Ahmed and Ahmed (1983) published a report on the basis of their entomological investigation in June and August, 1981 in the village of Madla,Shahjadpur Thana in Sirajganj district. Although their study provided some information about the species composition of that particular area, DDT susceptibility of the Leishmania vector, etc. but the study was conducted for only three months and in one village. Thus it was incomplete and fragmentary.

Knowledge about the host-biting habits of haematophagous insets provide much information about their role in transmission of a disease. So it is important to know the preferred host of a particular sandfly species. In the present study an attempt was also made to investigate this matter by using gel diffusion technique and Dot ELISA.

1.1 PRESENT STATE OF KNOWLEDGE IN THE PROPOSED RESEARCH FIELD

The most important procedure currently in use for the diagnosis, treatment and epidemiological studies of visceral leishmaniasis or kala-azar include:-

Clinical examination of patients for the presence of typical symptoms of the disease in combination with haematological and biochemical alternation (Harith et al 1986).

Demonstration of L. donovani parasites in the aspirates of visceral organs, bone-marrow or lymph nodes by direct examination or propagation in appropriate culture media (Harith et al 1987).

Detection of specific anti-leishmania donovan antibodies by serological methods of which the Immunofluorescence (IF) and Enzyme-linked Immunosorbent Assay (ELISA) are the most important (Harith et al 1988).

For epidemiological survey studies, the leishmania skin test and formolgel test, (Napier aldehyde test, AT) are used, (Napier et al 1927).

Failure or refractory cases to antimony remains as the best source of transmission, (Wijers, 1971, Zijlstra et al, 1991).

There is a strong correlation of the seasonal variation between vector density and VL prevalence.

1.2 JUSTIFICATION OF THE STUDY

In most areas endemic for VL clinical symptoms such as fever, hepatosplenomegaly, anaemia, lymphadenopathy and general malaise are also encountered with other disease, to mention: chronic malaria, tertiary syphilis (gumma), undulant fever, pulmonary tuberculosis, Shcistosomiasis with septicaemic salmonelosis, Chaga's disease and other bacterial and viral infections. Administration of antimonial drugs, if based solely on clinical symptoms, might therefore, be hazardous.

Demonstration of the parasite by aspiration techniques of organs requires skill and could be less safe in rural hospitals. In the early stages of kala-azar, the parasite population could be so low as to allow definite identification or successful multiplication in culture media. In addition, some L. donovani isolates are fastidious to culture and require special media ingredients. Specific detection of anti-leishmania donovani antibodies by the current serological tests. IFAT and ELISA, is hampered by the marked cross-reactivities in sera of other infections particularly African trypanosomiasis, malaria, typhoid fever, tuberculosis and autoimmune disorders. The complex nature of the two techniques requires purchasing of expensive reagents rendering their performance in developing countries not feasible. Their utility under field circumstances for diagnosis of kala- azar, epidemiological studies is extremely difficult or rather impossible.

Further improvements are required to render the potentiality simple methods as the leishmanin skin test and the aldehyde test (AT) specific for kala-azar.

In view of the variable responsiveness of leishmania donovani to pentavalent, antimonials as evident for different percentages of primary unresponsiveness cases, one may presume difference in enzymic profile, antigenic pattern or other molecular aspects in different isolates.

There has been a number of changes in the treatment schedule of kala-azar in this part of the world. Recent work suggested that a prolonged period is not only unnecessary but may also increase the likelihood of induced drug resistance. It has also been shown that higher doses are well tolerated, Thakur et al 1986 suggested a schedule of 20 mg/kg body weight for at least 40 days. The severe burden on the health care facilities of the developing countries and may also increase the drop out. Rees et al (1980) demonstrated that sodium stibogluconate is rapidly eliminated in the urine; 81.96% is excreted within first 6-8 hours and the blood level falls to around one percent of the peak within 16 hours. On the basis of this finding a multidose (10 mg/kg body weight thrice daily for 10 days) treatment regime for African kala-azar was completed with no relapse after one year and with no signs and symptoms of toxicity. This favours (12/8 hourly) short course multidose regime of sodium stibogluconate trial.

1.3 HYPOTHESIS

Indiscriminents use of antimonial drug to kala-azar cases increases unresponsiveness and test dosing may also contribute to unresponsiveness.

PKDL is known to be manifested due to Immunological factors first in clinically cured cases and even 5% of treated cases will show PKDL in one year time (Napier 1947). Relapse and PKDL cases may be associated with an inadequate course of treatment.

When the residual effect of antimalarial insecticides (DDT) becomes finally exhausted, vector density will rise and severe epidemics of kala-azar will follow.

Antibody titer remains high for a long period even after treatment of kala-azar patients.

Kala-azar case diagnosis depending on the agreement between DAT and microscopic diagnosis was complete up to 90% or more. In sera where the kala-azar transmission was low and persistent throughout the year, moreover, in the sub-clinical cases and in early cases, the detection of parasite (LD body) by classical microscopic examination might not be enough to give the actual situation (Harith et al).

Aldehyde test (Napier 1927) which is being widely used for diagnosis of kala-azar becomes positive 2-5 months after the onset of the disease. Compliment Fixation Test (CFT) (Sen Gupta et al 1969, Ridux et al 1962, Passive Haemagglutination Test (IHA), (Casio et al 1963) can not fulfill the easier, economical, reliable, criterion for early diagnosis of kala-azar.

OBJECTIVES

1.4 OBJECTIVES

1.4.1 To evaluate the applicability of DAT at clinical centres and to assess the kala-azar transmission by a mass screening programme involving 50.00 inhabitants.

1.4.2 To evaluate the reliability of DAT for detection of early kala- azar cases as a result of recent improvements introduced to the test system.

1.4.3 To compare prognostic study using the DAT as a follow-up tool on patients treated at the early phases of kala-azar and others who received chemotherapy after demonstration of the parasite. To study the probability of post kala-azar dermal leishmaniasis (PKDL) development in both patient groups.

1.4.4 Application of DAT on PKDL patient. L.donovani isolates from VL and PKDL will be compared for antigenic reactivities in the DAT to determine their serological relation.

1.4.5 To evaluate the recent simplifications introduced to the DAT by performing the test on whole blood samples collected by finger prick and adaptation of simple dispensing systems instead of volumetric pipettes.

1.4.6 To determine the optimum dose and treatment schedule of Sodium Antimony Gluconate (SAG). To find out the antimony refractory cases and to treat them with an alternate regime of treatment.

1.4.7 To find out the association of chemotherapy of kala-azar at primary health centres for treatment and evaluation with seroepidemiological surveillance in Bangladesh.

1.4.8 To evaluate the comparison of DAT with other sero-techniques (ELISA, IFAT, IHA) for diagnosis of Kala-azar at the field level.

1.4.9 To find out the relation of vector with VL (Kala-azar) & effect of DDT on its control in cohort study.

CHAPTER 2

REVIEW OF LITERATURE

2.0 Review of Literature

2.1 HISTORICAL REVIEW OF KALA-AZAR

History and present status of kala-azar in Bangladesh and other parts of the Indian sub-continent.

History: The cutaneous form was known by the INCAS, the Famous Middle Eastern Physician (Avicenna Died-1037) "Oriental sore" in Syria was first discovered by RUSSEL in 1756. Kala-azar was known in the Western literature dated from 1869. The parasites were simultaneously discovered by Leishman and Donovan in 1903. Ross gave the name Leishman to the parasite, Edmond Sergent, Etienne Sergent and Charles Nicolle of the Pasteur Institute of Algier and Tunis contributed to our knowledge of the epidemiology of leishmaniasis(1902-36). They demonstrated the dog to be the reservoir of visceral leishmaniasis. Oriental sore epidemic occurred in Delhi in 1941. Since the time of Aurangazeb it was called Aurangazebi Phora.

VISCERAL LEISHMANIASIS

Local name:

1. Jowr-Vikar 1824-25
2. Burdwan Fever 1866
3. Kala-dukh 1972
4. Kala-azar 1872
5. Sarkari bemari or Government disease 1875
6. Dum Dum fever 1903

History of Kala-azar in Indo-Bangladesh Sub-Continent

There was an outbreak of Jwor-vikar in Jessorein 1824-25 Characterised by relapse fever, progressive emaciation and hepato-splenomegaly. The so called Jowr-Viker first appeared in Mohammadpur under Jessore district.75,000 people died in that region. Then the disease was spread to Nadia and 24 Pargona in 1832, Hoogly district in 1857 and Burdwan district in 1857. While the disease appeared as an epidemic in Burdwan district in 1860, the disease was called Burdwan fever. Surprisingly, the disease disappeared in winter. But rainy season brought with it greater villages and become more general. The mortality was high at that time & the villagers called the disease Jowr-Vikar Natunjawar. The disease was spread to Dhaka in 1862 by the crew of a country boat who came from up country, through Dhaleshwari river. Subsequently Dhaka was affected and the disease spread to the neighbouring villages.

The mortality was so high that the dead were thrown into rivers. It was estimated that the disease spread to Bengal in 1875, from Garo Hills, which was caused by the direct extension of the disease from the Rangpur out break 1872. While opened the trunk roads along the Brahmaputra river. 95 percent of people of Assam died on Sarkari bimari as the disease was known to be Sarkeri bimari. Dr. Dodds Price detected so many Kala-azar cases among coolies in the tea-garden but he could not established the cause of kala-azar and compared with malaria. Subsequently kala-azar was also detected in other parts of India only Madras and the United province. Dr. Gile assumed the disease might be due to hookworm infestation but Dr. Dobson the Civil Surgeon of Assam, concluded that the black fever of Assam was only a pernicious form of malaria cachexia. However, the name **Kala-azar**, meaning black fever, came from the Garo Hills in Indo-Bangladesh border area, referring to the appearance of the victims (Lewis 1978). Kala-azar has long been known to be endemic in the north-eastern part of the Indo-Pak-Bangladesh sub-continent.

Kala-azar epidemics, before its resurgence, in this sub- continent can be summarised as follows:-

* 1854-1873: Burdwan and Dinajpur districts, West Bengal, India, Dinajpur and Rangpur districts, Bangladesh.
* 1882: Garo-Hills, Meghalaya, India.
* 1891-1901: Nowgaon district, Assam, India.
* 1917-1929: Entire Brahmaputra valley, Assam, India (Dhanda et al 1983).

In this subcontinent only two forms of leishmaniasis, namely Cutaneous Leishmaniasis (CL) and Visceral Leishmaniasis (VL) or Kala-azar are endemic. CL is restricted to the north-western part of India; VL is widespread in Eastern India.

Kala-azar was thought to have been eradicated from Bangladesh as a collateral effect of the malaria eradication programme on the sandfly vector. In India also, the disease had essentially disappeared in the 1960s. Now kala-azar has been reported to have appeared in epidemic form in different parts of India following suspension of spraying for malaria control. Evidently cessation of spraying for malaria eradication in Bangladesh has also resulted in a resurgence of kala-azar. Rahman and Islam (1983) expressed the danger that visceral or post kala-azar Dermal Leishmaniasis (PKDL) had reached or soon may reach a level which could provide a reservoir of a sufficient magnitude to spark a major epidemic in this country. Hossain and Rashid (1987) reported a few cases of kala-azar from the suburbs of Dhaka city.

2.2 CONTRIBUTION OF BRAHMACHARI AND OTHERS ON DIAGNOSIS

Rogers and G.C. Chatterjee cultivated the parasite LD Body in artificial culture media in 1904. Christophers, R.Row, Shortt, Mackie, Knowles demonstrated the morphology, cultural characters and other protozoological features of this parasite, Das Gupta, JC Roy and others wrote about the pathology in 1904. Rogers Muir, UN Brahmachari, Napier, and others clarified the more clinical picture of kala-azar. Dermal Leishmaniod was first discovered by UN Brahmachari a peculiar cutaneous sequel to kala-azar in 1922. Now known as post kala-azar Dermal Leishmaniasis (PKDL) was further studied by Short and Brahmachari, Acton and Napeir, Knowles, Das Gupta, Smith of Calcutta School of Tropical Medicine. The Globulin precipitation test was first described by UN Brahmachari in 1917 but it was not shown to be specific. Napier, in 1921 found in his observations in Kala-azar patients blood serum gave jellification while it was treated with formalines. Chopra and Gupta (1927), introduced the antimony test (Chopra),subsequently Napier standerdized the technique. Complement fixation test (CFT) in 1936 was known to be familiar with Witebsky Klienotein and Kuhn (WKK) antigen in Leishmaniasis. Spleen puncture syringe, sternum puncture needle for sternal biopsy in suspected kala-azar patients was introduced by Napier.

2.3 HISTORY OF TREATMENT: The disease first treated with quinine but was to be in effective, local irritation was found over enlarged spleen it was also useless. Alkalies were used as alkan is diminished in Kala- azar patient, but it had no value also. Sodi-bi-carbonate at strength of 2% I/V was used by Rogers. Injection of methylene blue or liquor aresenicalis or other preparations of arsenic slaverson proved no value. Vinna was the first to use antimony in the form of tartar emetic in the treatment of dermal leishmaniasis of South America with great value which was first advised by Manson for use of antimony. Kala-azar treatment with antimony established in 1918 in Assam. L.E. Napier and UN Brahmachari, introduced a new and less toxic pentavalent antimony preparation in 1920. Still, it has a value U.N.Brahmachari, 1922, prepared urea Stibamine i.e. Urea and Para-aminophenyl-stibinic acid. It was found very satisfactory and world wide assisted by Short and by Napier.

2.4 LITERATURE REVIEW OF IMMUNO-SEROLOGY: Srivastava et al 1980, evaluated different culture media for isolation of LD body from Bone-marrow of

suspected kala-azar patients and their efficacy was also evaluated by them. The media and culture LD/body used upto as follows:-

Modified Tobies medium, 10% defrinated rabit blood was added to the solid phase. The liquid phase constituted of two milliliter each of Lock's overlay on TC 199 overlay. Semi solid agar to which defibrinated rabit blood was added in noncuration of 10% and 15%. Semisolid agar in which 15% bovine calf serum (certain lab) was used instead of rabit blood. All the media were adjusted to PH 7.4 Penicillin 300 IU and streptomycin was mg per milliliter were added. Inoculated media was dispensed in 14 ml quantities in 403 Maccartney bottles. The inoculated media were incubated at 22^{O}C + 1^{O}C and the cultures were observed; the semisolid blood agar gave a high isolation rate 85% and the growth of promastigotes was detected earlier as compared to the other two media. On the other hand, growth of promastigote were observed up to 38% respectively with Lock's overlay and TC 199 whereas, semisolid agar with bovine calf serum gave a low recovery (33%).

Srivastava Lakshmi and Kumar Anil, 1983 estimated the immunoglobulin levels (IgG, IgM & IgA) of Kala-azar patients and they observed that IgG levels were raised in 85 percent of cases and 64 percent of treated individuals. Whereas the IgM levels were increased in only half 49.8 percent of cases and 35.4 percent of treated cases; IgA levels were within the normal limits in all the three groups, high IgG, IgM ELISA antibodies were observed in the diagnosed group (J.Com.Dis. 1980, 12(4); PP 188-191).

Srivastava et al 1984, conducted a comparison study of ELISA and indirect immuno-electrophorescence in sero-epidemiology of Kala-azar,they observed that ELISA was more sensitive than IFAT although IFAT was more specific and a good correlation was found between the two techniques. 327 sera samples belonging to different groups were tested for leishmania antibodies, out of 67 early cases of kala-azar, 45 were positive by ELISA and 22 by IFAT, while of 98 patients who had been treated successfully, 62 were positive by ELISA was found to be 85.1 and 81.8 percent respectively, whereas that sensitivity and specificity of ELISA of IFAT, 61.7 and 97.0 percent respectively. Srivastava et al 1984, in another study observed sero- diagnosis for kala-azar using Counter Immuno-Electrophoresis (CIE). Out of 111 sera from kala-azar cases 103 (92.7%) showed precipitin bands, 100% positivity in cases where LD bodies were demonstrable. However sera from kala-azar and healthy controls from epidemic (North Bihar) and non-endemic (Delhi) areas were examined by CIEP for leishmaniasis antibodies. In their opinion that the counter immuno-electrophoresis(CIE)

was shown to be a useful technique for detection of antibodies as it is easy to perform and much less time consuming.

Andrate et al (1987) undertook a small study to evaluate the reliability of Direct Agglutination Test (DAT) in the diagnosis of VL in Brazil. They observed 265 Brazilian human sera tested DAT using trypsinated L.donovani donovani antigen. Out of 265 sera 14 from kala-azar cases 10 with parasitological proven 4 with IFAT positivity results other samples were from patients with Chaga's disease (73). Schistosomiasis (5), Filariasis (18), Malaria (59), Mucocutaneous leishmaniasis (2), Hanseniasis (4), and from apparently healthy Brazilian donors (90). The titres found in this study with Brazilian Chaga's sera remarkably lower than those reported with African trypanosomiasis using the same antigen. The titres of the kala-azar sera were 51,200. No cross- reaction were observed with Chagasic patients sera (all below 1/400). In his opinion that the Direct Agglutination Test (Harith et al .....) can be of diagnostic value for American VL even in areas where Chaga's disease co-exists. As there is no such disease prevalence in Bangladesh, India, Nepal it can be employed for diagnosis of VL.

Amin et al (1985) conducted a study "ELISA" using intact promastigotes for immunodiagnosis of kala-azar. In their study they observed that using intact promastigotes,as antigen in comparison to sonicated promastigote antigen, the ELISA was able to detect specific antibodies in sera of kala-azar patient at a very high serum dilution. In this study they used 16 sera from healthy Dutch donors as negative controls, 25 sera of clinically and parasitologically confirmed kala-azar patient sera and 102 sera from other diseases. They observed that there was little difference in antibody binding to $5x10^6$ or to 10^7 promastigotes per well but that after that the reaction decreased and 105 was not sufficient to detect low titres of antibody in patients serum. They observed that not only did the promastigote antigen give a consistently higher reading at the low serum diluents but also that the binding of antibody decreased less rapidly upon further dilution then with the sonicated antigen. They also observed the stability of the antigen because it is used for the screening progress. This antigen is stable for up to 45 days at room temperature or -20^{o}& 4^{o}C. Regarding cross reaction they also observed that the toxoplasmosis and malaria leprosy and tuberculosis sera were negative even at low serum dilutions. Trypanosomiasis and schistosomiasis sera were positive at a low serum dilutions but most of the cross reaction could be diluted out if sera were diluted beyond 1/3000 while kala-azar sera were still largely positive. So to avoid cross-reactions in ELISA it is necessary to dilute the sera extensively. In their opinion using the intact promastigotes

in an ELISA to detect Anti-leishmania antibodies can be as good as or better than the soluble antigens and it is very much helpful in early diagnosis.

Harith et al (1986) in his study of "a sample and economical direct agglutination test for sero-diagnosis and sero- epidemiological studies of visceral leishmaniasis and observed that sera of 280 inhabitants of endemic area of Baringo, Kenya when treated cases were included the test showed a sensitivity of 100% and specificity of 99.3%. All recent kala-azar cases showed titres of 51,200 and higher, while in patients treated 4 to 14 months earlier, titres from 1:3200 to 1:51,200. All healthy European controls and those with disease unrelated to kala-azar had titres below 1:400 as did healthy Kenian and Kenyan patients with monsonian schistosomiasis. The results of brucellosis and malaria were 1:100 and 1:400 respectively. The predictive values of the negative and positive test in this population were 100% and 71.4% respectively. A titre of 1:3200 or greater in an area considered to be free of African trypanosoniasis is highly predictive of VL (recent and past). Harith claimed in his study that it was possible to distinguish active trypanosomiasis and kala-azar which could not be separated by ELISA (Voller et al, 1975).

Carolina et al 1986, evaluated the Dot ELISA for mucocutaneous leishmaniasis and comparison with Microplate Enzyme Immuno assay. The two assays were used to test 113 serum specimens from the normal individuals and patients with deep mycosis, toxoplasmosis, mucocutaneous leishmaniasis, visceral leishmaniasis, Chagas disease, malaria and schistosomiasis both tests exhibited cross-reactivity when testing specimens from cases of visceral leishmaniasis and Chagas disease. In their observations there were no significant differences in sensitivity between the Dot ELISA and the IgG ELISA. Due to high sensitivity and specificity, Dot ELISA should be given atrial in the field during sero-epidemiological surveys. They also observed that all mucocutaneous leishmaniasis patients and five deep mycosis patients had positive Montonegro tests in the skin. However,these five samples yielded negative results by ELISA, one of these specimens had a titre of 80, whereas the other four had titres of 20. Among the 28 mucocutaneous leishmaniasis sera only 2 were judged negative by the Dot ELISA. The authors claim that the advantages of Dot ELISA as due to their minimum amount of the antigen, antibody conjugate, and the chromogen solution required The method minimises the time as required at 3.5 hrs and it does not require spectrophotometric readings which are needed in conical plates or cuvette ELISA technique.

Khan and Desowitz, 1985 conducted a short study as a comparison of counter immuno-electrophoresis and indirect haemagglutination test (IHA) for the immunological investigation of kala-azar in Bangladesh. In their observation CIE was positive for all parasitologically confirmed cases whereas the IHA positivity was only 60%. The T. bruccei antigen was equally as good, if not better, than the L.donovani antigen for CIE. The CIE test was negative for all of 34 apparently healthy villagers but for the same group of individuals, 10(29%) were low titre IHA positive. Their finding suggest that CIE is the more reliable diagnostic test. The authors tested only with a small number of patients so conclusive results can not be given, but 10 of the 34 (29%) of the normal sera were positive at titres of 1:64 to 1:128.

The most conclusive evidence in diagnosis of a kala-azar is the demonstration of the parasite from bone marrow aspirate or spleen puncture material. The bone marrow aspirate examination may give negative results when the parasite is scanty where as in spleen puncture it gives positive results but it may lead to bleeding from the soft spleen.

In kala-azar, a sharp rise in gumma-globulin is found which is mostly nonspecific. A number of test eg Aldehyde test, Antimony test etc. are positive in kala-azar due to the excess of the non specific gumma-globulin. Specific antibody is also produced in kala-azar and antibodies are found in 1gG and 1gM compartment (Turk JK and Brvceson, 1971).

The first of the diagnostic serum tests, a globulin precipitation test was described by UN Brahmachari in 1917 and spackman observed in 1922. The opacity of kala-azar patient's sera if diluted formation is added. This test proved of value in to north China but in Bengal where there was so much malaria this test did not prove to be very specific. It was further studied and utilized for the diagnosis of kala-azar by Napier in 1921, 1922, 1923 and Published. Fax and Mackie (1921) independently also reported a similar series of tests and noted that in kala- azar, the blood serum when treated with formalin gave a peculiar reaction. This became the basis of Napier's aldehyde test for kala azar. This test has proved to be of great value in the diagnosis of chronic kala-azar. In 1927 Chopra, JC Gupta and David first described the antimony test. Subsequently, Napier standardized the technique of this test too. This test is of value in the diagnosis of the cases of kala-azar with small splenic enlargement and of duration of over two months. Chruick Shant et al 1975 observed that the aldehyde test (AT) has been found unreliable in the diagnosis of kala-azar in many countries and cross-reaction was also observed with chronic malaria, tuberculosis of Enteric fever, but in Indian sub- continent

the test is commonly used as a diagnostic test for kala-azar. Rahman and Islam et al 1979 also observed that aldehyde test becomes positive 3 months after the onset of the disease. The test is very simple, economical and can be performed in rural areas to diagnose the late cases, when AT gives almost 100% positive result, but the test has least value for mass screening for early detection of kala azar.

Complement Fixation Test(CFT): South American workers were pioneers to use this test for detection of kala-azar. A South American worker first noticed that a positive complement fixation test reaction was obtained with the Witebsky, Kingensteien and Kuhn (WKK) antigen in leishmaniasis. This finding was confirmed by Greval, lowe and their associates for Indian kala-azar. In 1939 Greval, Sengupta and Napeir developed a technique of complement fixation test of high degree of specificity and sensitiveness for kala-azar using the WKK antigen. Dharmendra showed that similar antigen could be prepared from various acid-fast saprophytic bacilli and this gave a positive reaction with kala-azar serum. The test was further worked out by Sengupta, (1944) and a modified technique was described by him next year (Sengupta, 1945). This test has proved to be of great value in the diagnosis of kala-azar and positive indication of kala-azar could be usually obtained as early as the third week of illness.

Dharmendra et al in 1946 cited from Monsur, (1956) isolated an antigen from Kedrowsky's bacilli (saprophytic acid-fast bacilli), using the same method as used for the preparation of WKK antigen. Both the antigens gave satisfactory results for routine diagnosis of kala-azar. The antigen prepared in this way is believed to be the alcohol-insoluble, pyridine-soluble and acetone-insoluble fraction of Kedrowsky's bacilli. In 1956, Monsur isolated an antigen from the same organism by simple alcoholic extract of the organism (less drastic procedure than the former) and the antigen was found as good as that isolated by Dharmendra et al in 1946. The alcoholic extract was further purified by acetone by Monsur and Khaleque (1957). They found this antigen (extracted from Kedrowsky's bacilli) 10 times more potent than WKK antigen. Rahman (1975) compared different saprophytic acid-fast bacilli as antigen for CFT for kala-azar and found the antigen extracted from Mycobacterium phlei as slightly more potent than the antigen prepared from Keydrowsky's bacilli, using the method described by Monsur and Khaleque (1957).

Graval et al (1939) found CFT to be fairly adequate for the diagnosis of visceral leishmaniasis. They found the test 100% sensitive. But the test gave a few false positive results with tuberculosis and leprosy cases. Dharmendra and Bose (1941)

compared the results of CFT using six different acid-fast bacilli as antigen. In their study, all seven sera of kala-azar cases fixed complement completely in 1:100 dilution of sera with all the six antigens. All the non-kala-azar febrile cases (except leprosy) gave negative result at 1:25 dilution of sera. In cases of 19 lepromatous leprosy cases, six to nine sera gave positive
results with CFT in 1:100 dilution of sera, using six different antigens. Out of 45 cases of neural leprosy, there were 80% (with Lieras bacillus) to 24.4% (with Mycobacterium tuberculosis) false positive results. Hommel et al (1978) found CFT easy to perform but unsuitable for large-scale epidemiological studies. The test become positive after 3 weeks of the onset of the disease (Chattarjee, 1982). But the sensitivity of the test varies with the source and method of extraction of antigen , the result of CFT also varies with different batches of the antigen (Aikat et al 1979). Rahman and Islam (1983) found the test 98.3% sensitive and 91.2% specific. Using homologous parasite antigen for CFT for kala-azar, Hockmeyer et al (1984) found the test almost 100% specific and 88% sensitive.

INDIRECT FLUORESCENT ANTIBODY TEST (IFAT) Immunofluorescence is an advanced technique of histochemistry or cytochemistry, which detects or localizes an antigen in a tissue or cell by specific antibody tagged with a fluorescent compound and is detected by fluorometric measurement (Stites et al 1987). Reniner (1930) and Heidelberger et al (1933) (cited from Kawamura, 1977) used the technique with azo dyes as tracer substance but could not achieve general use due to its low sensitivity. Coon et al (1941) succeeded for the first time to use fluorescence as a tracer substance and tagged it with antibody to detect a soluble Pneumococcal polysaccharide antigen in tissue sections of mice infected with Pneumococcus. In 1950, they used fluorescence isocyanate (FIC) to label globulin antibody. Later on Rigg et al (1958) (cited from Kawamura, 1977) introduced a more stable compound, fluorescence isosthiocyanate (FITC) which can be easily conjugated to globulin (Goldstein et al 1961; cited from Kawamura et al 1977).

The fluorescent antibody technique has been proved useful in determining antibody levels in malaria, Trypanosomiasis and Toxoplasmosis. It has also been used to demonstrate antigenic variations between races of Entamoeba histolytica and between various groups of malaria patients (Bray and Lainson, 1965).

Ingram et al (1961) and Voller (1962) used fluorescent antibody technique for measuring antibody to malaria. Using the technique, Voller and Bray in 1962 measured malarial antibody levels of a population in an endemic zone. Introduction of the technique in the study of infection due to flagellates was not until 1958, when McEntegart et al (cited from Shaw and Voller, 1964) differentiated Trichomonas foetus and Trichomonas vaginalis by fluorescent-labeled antisera. Fife et al (1959) used fluorescent antibody technique for serodiagnosis of Trypanosoma cruzi infections. Voller (1963) stained both the culture forms and blood forms of Trypanosoma cruzi by means of specific labeled antisera. In this experiment and in those of Shaw and Voller (1963) in which the indirect technique was used, cross-reactions were noted with other trypanosomes and leishmanias. Shaw and Voller (1964) studied the indirect fluorescent antibody technique with sera of a variety of infectious diseases using leishmanial and trypanosomal antigens and found that a group rather than a species specific antibody was demonstrated since antigen of T. cruzi and L. infantum fluoresced with kala-azar serum. It was concluded that the test is worthy of further consideration in the diagnosis and study of visceral leishmaniasis, except in regions where both trypanosomiasis and leishmaniasis are endemic. Duxbury and Sadun (1964) also proved the usefulness of the fluorescent antibody technique in the diagnosis of visceral leishmaniasis.

Shaw and Voller (1964) worked with leishmanial and trypanosomal antigens for fluorescent antibody test. Leishmania infantum grown in NNN medium was used as slide antigen. Both promastigotes and amastigotes (Indian strain) of L.donovani, Trypanosoma cruzi and L.braziliensis were used as antigens. The fluorescence intensity of the parasites were estimated and the serum dilutions at which they were just detectable was recorded as the endpoint. There was a certain degree of nonspecific staining when sera were used undiluted. The serum of kala-azar cases gave a strongly positive reaction with the amastigotes and promastigotes of L.donovani, L. braziliensis and with T. cruzi. Sera of healthy Europeans and those with malaria, syphilis and Cutaneous leishmaniasis gave a negative result. In the positive tests, the specific fluorescence was particularly located in the kinetoplast and around the edge of the organisms. There were a number of fluorescent areas within the cytoplasm but there was an absence of nuclear staining but the edge of this organelle appeared to stain. So, the test was considered as a group specific test, not species-specific and worthy of diagnosis of visceral leishmaniasis.

Bray and Lainson (1965) worked with 10 strains of leishmania to determine if there is any antigenic variation among the species of leishmania, which might be detectable by serial dilutions of antibody. Sera of rabbits artificially immunized by promastigotes of 10 strains of leishmania and human sera from patients infected with Mediterranean infantile kala-azar, South American kala-azar, South and Central American Mucocutaneous and Cutaneous leishmaniasis were tested against all the antigens. All the sera stained and all the antigen preparations at more or less the same intensity indicating a group-specific antigen antibody reaction. So no differentiation of strains was possibly by serial dilution of the test sera. Rezai et al in Iran (1977) used intact promastigotes of L. donovani as an antigen for IFAT to determine the diagnostic value of the test in kala-azar. They found the test 100% sensitive and specific and the diagnostic titre of IFAT (for kala-azar) was found to be 64 or above.

Behforouz et al (1976) found that by indirect fluorescent antibody test (IFAT), antibody can be detected as early as 12 days after infection as found in experimentally infected guinea pigs. They also found that sera of kala-azar cases reacted equally well with antigens prepared from L. donovani, L. tropica and L. enriettii. Using intact promastigotes of L. infantum, Edrission et al (1981) in Iran found the test (IFAT) highly sensitive, quite specific and more convenient in comparison to parasitological diagnosis of clinically suspected cases of kala- azar, in which parasite could not always be detected by routine parasitological examination. Enzyme-linked immunosorbent assay (ELISA) was first used by Voller et al in 1974 and 1975 for the serodiagnosis of malaria. Using the microplate method and antigen prepared from Plasmodium falciparum. Voller et al (1976) identified areas of malaria transmission and was able to evaluate malaria control operations.

Huldt et al (1975) and Schinski et al (1976) used the indirect ELISA for the detection of antibody in schistosomiasis. McLaren et al (1978) used ELISA test with microplate method for extensive field study of schistosomiasis and found the test very satisfactory. Indirect ELISA was used in chagas disease by Voller et al (1975) and in sleeping sickness by Ruitenberg et al (1977). An international comparison of all the serological tests for African trypanosinasis showed ELISA to be as good or better than any other methods (WHO Bulletin, 1976).

Voller et al (1977) used ELISA test for the detection of antibodies in chagas disease, sleeping sickness, malaria, schistosomiasis and invasive amoebiasis and

found satisfactory results. Voller et al in 1976 used ELISA for the serodiagnosis of leishmaniasis. Hommel et al in 1978 used ELISA technique for the serodiagnosis of visceral leishmaniasis and found the test 97% (35 out of 36 proven VL cases) sensitive and 99.45% (out of 22 sick control cases, 21 were negative by ELISA) specific. Subsequently, Edrission and Drabian (1979) and Anthony et al (1980) used the test for the serodiagnosis of Visceral and Cutaneous leishmaniasis. Edrission and Drabian (1979) compared the results of ELISA and IFAT in the serodiagnosis of leishmaniasis in Iran. The found ELISA a little more sensitive but some what less specific than IFAT. They considered ELISA as a good alternative test to IFAT, and described ELISA as a more practical and economical test especially for mass screening of kala-azar in field conditions. In both the tests (ELISA and IFAT) they found few nonspecific reaction with sera of malaria and larvamigrans. They concluded that those reactions did not greatly affect the validity of the tests in the serodiagnosis of kala-azar, because the leishmanial antibody levels in kala-azar patients were much higher, specially in IFAT, than the nonspecific antibody levels.

Ho M, et al (1983) evaluated ELISA technique for the serodiagnosis of visceral leishmaniasis and found the test 98.4% sensitive and 100% specific. Results of their study indicate that the ELISA has an accuracy comparable to that of parasite identification by splenic aspiration. Jahan and Diesfeld (1983) found ELISA as a 100% sensitive and specific test for the serodiagnosis of kala-azar. In their study, sera from nonendemic area showed no colour reaction but all the control sera from endemic area showed coloured reaction at low titre and were considered negative in their study. The differences observed between the control sera from endemic and nonendemic areas stresses the need to establish the parameters of the test under any given local epidemiological conditions before routine application of the test. Mohammed et al (1985) used intact promastigotes as an antigen in the ELISA technique for the serodiagnosis of visceral leishmaniasis and found that the use of intact promastigotes in an ELISA to detect anti-leishmanial antibodies can be as good as, or better, than the soluble antigens more commonly used. The preparation of ELISA plates with intact promastigotes is simple and reproducible, and the test is extremely sensitive. Such as ELISA could be very helpful in epidemiology or for early diagnosis of visceral leishmaniasis.

Harith et al (1987) in Kenya compared the results of iFAT and ELISA for the serodiagnosis of visceral leishmaniasis. Both the tests gave high rate of cross reactions with sera of African
trypanosomiasis and chagas disease. And it was concluded that the tests are (IFAT and ELISA) highly sensitive and specific for the diagnosis of kala-azar in areas where African trypanosomiasis and chagas disease are not present.

2.5 REVIEW OF EPIDEMIOLOGICAL INVESTIGATION OF KALA-AZAR

East Bengal Malarial Control Demonstration Team July, 1952, carried out a survey on the request of the Government for prevention and treatment of kala-azar patients. Two medical officers and two assistants were deputed from the Government and worked under the team. In the lst preliminary kala-azar survey from 12 January 1950 to 31 July 1950, the team examined 6,108 children below 15 years of age in 82 villages 6035 houses were visited and 2,718 children were found with spleen enlargement and out of these 510 children were found positive using the test for kala-azar, capillary tube method the patients were treated with Urea-stibine. Very interesting observation was observed by the team that 8.35% of these kala-azar affected children (in the DDT prayed area, but while 16.9% of the children with enlarged spleen were positive for kala-azar in the unsprayed area, 38% of the children with enlarge spleen gave positive reaction for kala-azar in the area prayed with DDT in July 1949. This observation indicates that DDT spraying produced a reduction in the number of spleen-positives due to malaria which caused the percentage of spleen positive due to kala-azar to rise.

In the second survey during October 1950 to January 1951 and 3,052 samples were examined and found negative for kala-azar among these population 899 were living in the area prayed since 1949, and out of them only one (0.11%) gave positive kala-azar test, but these boy was observed by the team that he was outside the DDT sprayed area (May - June) 2,153 were living prayed for first time in May-June 1950 and out of them 40 (1.85%) gave a kala-azar in the area that has been sprayed for the first time in May-June but it has not occurred in the area prayed since 1949. It was observed that DDT spraying seems therefore, to have checked kala-azar transmission.

Dye C, 1992 until recently, almost all studies of leishmaniasis epidemiology were qualitative and descriptive. But now that the natural history of many Leishmania parasites is quite well known, there is growing interest in quantitative analysis. In this paper I use mathematical models in conjunction with field data to try to answer a wider range of questions than has previously been possible with descriptive techniques, and to sharpen some of the outstanding questions for laboratory workers. This is done

with reference to the persistence and resilience of canine leishmaniasis, the maintenance of virulence polymorphisms in Leishmania populations and the possible existence of cycles of human kala-azar. In conclude by posing a set of problems under three headings: diagnosis of infection (as distinct from disease), natural immunity to Leishmania infection in the vertebrate host,and genetic variation in the parasite population. Some solutions from the laboratory can be found in the companion paper by Black well (1992).

Guan LR, 1991; Kala-azar, which was prevalent in the vast area of China that lies to the north of the Yangtze River from the 1920s to the 1950s, is now effectively under control as a result of strenuous intervention since the founding of the People's Republic of China in 1949. Apart from 15-20 new cases that occur annually in the Keshi plain, Xinjiang Autonomous Region, the achievements of control practiced in other former endemic areas in the plains have been significant and consolidated. In the mountainous areas in north-west China, where the vector, Phlebotomus chinensis, is abundant and canine visceral leishmaniasis is common, there are still sporadic cases of kala-azar. Also, in recent years, new infections have often occurred in the deserts of Xinjiang and western Inner Mongolia, although the reservoir of the infection has not been identified. Corridor A; Kreutzer RD; Test RB; Boshell J; Palau MT; Caceres E; Duque S; Pelaez D; Rodriguez G; Nichols S; et al, 1990 Mar; A total of 340 Leishmaniasis strains, isolated from humans, animals and sand flies from various regions of Colombia, were examined by isozyme electrophoresis. Seven different Leishmania species were identified. Leishmania panamensis and L. braziliensis were the most common, representing 53.8% and 30.3% of total respectively. Isolation rates of the other species were as follows: L. chagasi, 9.4%; L. guyanensis, 2.6%; L.amazonensis, 1.8%; L. mexicana, 0.8%, and a new species requiring additional study, 1.2%. Statistical analyses of representative L. panamensis and L. braziliensis isolates indicated that the populations of these 2 species are genetically very similar. L. panamensis may have a continuous distribution in Colombia west of the easter Andes Mountains and L. braziliensis may have a continuous distribution east of the western Andes Mountains. Information is given on disease manifestations of the parasites in human hosts and on isolation records from sand flies and animals.

Corridor A et al 1989; Epidemiologic studies were conducted during the period 1986-1988 in a small rural community in Colombia (El Callejon) where visceral leishmaniasis is highly endemic. In this community of 185 people, 14 cases of infantile visceral leishmaniasis were diagnosed in the 9 years 1981-1988. Leishmanin skin testing of a sample of the human residents showed that prevalence of Leishmania

chagasi infection increased with age; overall, 51.2% of the subjects had a positive reaction. A canine surveillance program was instituted, using introduced sentinel dogs as well as the indigenous dog population. Eleven of 16 sentinel dogs were infected within 8 months of exposure; mean seroconversion time was 4.4 months. Eleven of 25 seronegative local dogs were also infected during the 26 month period; mean seroconversion time was 8 months. Parasites identified by isozyme electrophoresis as L. chagasi were recovered from 18 of 22 seropositive dogs. Collections of wild animals using baited live traps yielded mainly the neotropical opossum, Didelphis marsupials. Leishmania chagasi was recovered from 12 of 37(31.4%) opossums. Six of 681 female Lutzomyia longipalpis collected in the community has flagellates in their guts; cultures from 4 were identified as L. chagasi. These data confirmed that active parasite transmission occurred. The relatively high prevalence of L.chagasi infection found among D. marsupials captured near human dwellings suggests that these animals may be an important peridomestic reservoir.

Braga RR et al 1986; During epidemiological studies on an outbreak of visceral leishmaniasis in Santarem, Para State, north Brazil, isolates of Leishmania from two children, three dogs and six naturally infected specimens of the sandfly Lutzomyia longipalpis were compared, biochemically, by starch-gel enzyme electrophoresis. They have proved to be indistinguishable from each other and from a reference strain of Leishmania chagasi Ghana and Chagas, 1937 from a case of human visceral leishmaniasis from Bahia State, north-east Brazil, on their enzyme profiles for ASAT, ALAT, PGM, GPI, MDH and MPI. Lu. longipalpis is the principal, and possible the only vector to man in the Amazon Region of Brazil.

Navin TR et al 1975; Between 1975 and 1983, 53 patients with parasitologically proven visceral leishmaniasis (VL) and 16 patients with suspected VL were diagnosed in Honduras. The patients ages ranged from 3 months to 10 years, but 95% were younger than 3 years old. Since 1978, when 16 patients were reported, the yearly incidence has declined, and in 1982 only 4 patients were reported. We located and interviewed the families of 57 of the 69 patients. At the onset of illness, all 57 patients lived in rural areas, and 55 lived in southern Honduras. All the patients who were discharged from the hospital alive were still living at the time of the interview. A case-control study, using age-matched neighbors as controls, showed that patients were significantly more likely to have lived in poorly constructed, wood stick houses. We used an indirect immunofluorescence test to analyze blood samples for Leishmania antibodies from 218 family members of patients, 170 family members of controls, and 156 children living on the island of El Tigre, where 4 of the 5 most recently diagnosed

patients lived. Although 15 specimens gave a positive reaction to L. donovani antigen, each gave a stronger reaction when tested against tryparosoma cruzi antigen, suggesting that the reactions to L. donovani were false positives. A sero-surveyed of 279 dogs of cases and controls and from E1 Tigre showed that 24 had positive reactions to L.donovani antigen, but only 4 (1.4%) had higher titres to L. donovani than to T. cruzi.

Wijers DJ; Kiilu G, 1984; The epidemiology of kala-azar was studied in East Katangini, the area in Machakos District where the incidence of the disease had been highest during the epidemic years 1977-1979. A house-to-house survey showed that 19.3% of the homesteads had harboured kala-azar patients in the period 1977- 1980, while 3.2% of the people had suffered from the disease. Significantly more males had the disease than females and more children than adults, while the male patients came mainly from poorer homesteads. Significantly more kala-azar occurred in homesteads within 200 meters of a termite hill, while kala-azar seemed to occur particularly in homesteads near dry river beds. During a period of one year, sandflies were caught in a small focus of infection. They were still common in rock fissures, but were rare in other resting sites such as termite hills and huts. Particularly, the man-biting Phlebotomus martini was rare, as were other man-biting insects such as Anopheles gambiae. Very recently the farmers had begun to grow cotton which was prayed regularly with insecticides stored mostly in the farmers' homes. As a result, the number of new patients in 1980 fell to four, and the longer the people had stored insecticides in their compounds, the lower was the recent kala-azar incidence in these homesteads. Presumably the insecticide treatments killed many sandflies and other insects, while the storing of insecticides protected the people inside their huts, although some patients probably became infected outside, probably near termite hills.

2.7 Treatment of VL and PKDL: Post kala-azar dermal leishmaniasis (PKDL) is certainly a sequel to generalized infection with leishmania donovani as more than half of them have suffered from kala-azar about two years previously and have been given antimony treatment (Bahr, 1982). In case of kala-azar, visceral cure is followed by dermal resistance to new infection. After a variable period however, skin resistance is lost and a resurgence of old infection leads to PKDL while viscera remains unaffected (Sen gupta and Mukharjee, 1968). This viscerotropic character of L.D. body appears to change into dermatotropism possibly due to altered immune status of the host (Halder et al 1981). Manson and Bahar described this phenomenon as the result of the immune response on the part of the host.

Simultaneous visceral and dermal lesions suggest the possibility of involvement by multiple strains or an unusual immunological status of the patient (Heyneman, 1971). PKDL is not fatal but carries its importance in public health as a carrier source.

Treatment of V.L. using sb 15mg/kg body weight BID for 30 days

Sodium stibogluconate was administered to nine patients at a dose of 15 mg antimony/kg body weight twice daily for 30 days. No parasites was found in splenic aspirate smears immediately after treatment, in any of these patients. No serious side effect was observed. Sodium Stibogluconate at this dose seems to be an effective and safe drug for the treatment of visceral leishmaniasis. High daily doses of sb^5. might also reduce the relapse rate (Chunge et al 1986). A resurgence of the disease, including post kala-azar dermal leishmaniasis occurred, however, in several parts of Bangladesh during the late 1970s. When large scale of DDT spraying ceased.

A study was carried out in Greater Pabna district in Bangladesh from 1987 to December 1990. 5011 patients having a history or suspected history of kala-azar were under study. Sixty one were found to have cutaneous lesion suggestive of PKDL. Chemotherapy with antimony gluconate cured all cases except one who respond to pentamidine (Chowdhury et al 1990). A study was carried out in Thana Health Complex of Fulbaria, Trishal and Bhaluka of Mymensingh district of Bangladesh. A total number of 1273 kala-azar cases were treated with Sodium Antimony Gluconate (SAG) with the regimen of 20 mg/kg/day not exceeding 850 mg/day for 20 days. Out of 1273 cases 1187 (94.02%) showed immediate response. 44 (3.45%) showed delayed response for which additional 10-20 injections were required for cure and 11 cases did not response to SAG even with 40 injections. There were 45 cases of PKDL, which were cured with six courses of sodium Antimony gluconate with an interval of 10 days between 2 courses (Chowdhury et al 1990).

Visceral leishmaniasis (Kala-azar) if left untreated has a high mortality rate. In the territory that corresponds to present Bangladesh, the disease was an important public health problem during the pre-malaria eradication period in 1950s. But during 1960s it almost disappeared as a result of malaria control activities involving the wide spread use of DDT residual spraying. Although several epidemic outbreaks of kala-azar occurred in Bangladesh during premalaria eradication period, reliable information is very rare. In 1947 kala-azar survey were carried out mainly in the then Province of Asam, West Bengal and Bihar and East Bengal (Bangladesh). There were a few focus endemic for kala-azar, (WHO Bull.87 & 89).

In 1950, from January to July in Iswarganj, Mymensingh district 8.35% of children below 15 years were positive for kala- azar by Aldehyde Test. Average size of spleen of kala-azar children were 2.4 on the Hacket Scale. Furthermore in 41% of the families more than one person was infected with kala-azar (Gramiccia and Sacca, 1950). It is essential to note that in clinical response to chemotherapy in kala-azar cases many show slow or no response and other relapse after therapy (Chowdhury et a 1988) 1 9 8 8) .

Amphotericin B: Amphotericin B has selective toxicity for leishmania as it probably intercalates with the parasite episterol precursor of ergosterol in preference to host cholesterol (Berman et al 1986). Amphotericin B in the doses commonly used for fongal infection produces fever in 80% and anaemia in 75%. Temporary renal impairment occurs in virtually al patients, which may become permanent in 15% if the total dose in 75 mg/kg. Hypokalemia, hypomagnesemia, hypotension neurotoxicity and cardiotoxicity also occur (Hoeprich, 1992) Large VL trial series published (Misra et al 1992) randomised 120 VL patients unresponsive to sb^5 to receive either amphotericin B 0.5 mg/kg/day for 14 day or pentamidine isthionate 4 mg/kg on alternate days over 40 days. This study is of great interest because a very low total dose of amphotericin B (7 mg/kg) gave a high cure rate 98% with only minor toxicity. In comparison pentamidine cured only 77% of cases. **Drug delivery system** Macrophages in the liver and spleen remove particulate and vesicular drug carriers from the circulation. This can be used as a way to target drugs passively for the treatment of the VL. In 1977 three groups independently reported that liposomal encapsulation of sb^5 increased to drug efficacy in rodents in 300-700 fold. This is due to targeting of sb^5 to the infected cell and its increased retention within infected tissue. Toxicity is found in Monkey. Many other drugs and drug carrier have been studied in experimental VL.

In Kenya a study was designed to assess the effectiveness of parenteral aminosidine alone or combined with sodium stibogluconate in VL compared to treatment by stibogluconate alone. 53 patients were allocated to the 3 therapeutic regimes. At termination, clinical cures were achieved in all 53 patients with no difference between treatment groups. Spleen aspirates revealed the best parasitological results in patients receiving the combined treatment, with only 13% failure (partial cures + relapses) as opposed to 21% failures with aminosidine alone and 45% with stibogluconate alone. Treatment with aminosidine alone was the cheapest and safest regimen (Chunge et al 1990).

Comparison of 3 doses regimen of sb^5: A prospective randomized trial of three dosage regimen of sodium stibogluconate (pentostam, welcome Foundation London) to treat visceral leishmaniasis was conducted. Previously untreated patients were randomized to receive 31 doses of sodium stibogluconate (10mg sb/kg of body weight per dose) administered once daily for 31 days (group A) every 12 hours. 15 days (group B) or every 8 hours for 10 days (group C) of the 29 patients who completed treatment, seven of 10 in group B and all of the patients in group A and C responded to treatment and remained will for one year. One patient in group B completely failed to respond to treatment, and two others in group B initially responded to treatment but

relapsed six weeks after discharge. None of the treatment regimen was toxic. Parasites disappeared from splenic aspirates very quickly and Hb levels rose very rapidly in patients receiving sodium stibogluconate every 8 hourly. Treatment of visceral leishmaniasis in Kenya with sodium stibogluconate at a dose of 10 mg sb/kg every 8 hours for 10 days appeared to be a safe alternative to conventional treatment.

In Bihar, India, epidemic of kala-azar was in the highest peak in 1977 showing incidence rate of 5-9 per thousand. A study was carried out in Vaishali district, Bihar, India, in 750 parasitologically confirmed cases of kala-azar, Sodium stibogluconate, used as a first line drug, was effective in 92.6% of cases by increasing the course of antimonial therapy from 10 to 20 days the relapse rate was reduced to 0.5%. 86 cases unresponsive to sodium stibogluconate were given Pentamidine which was effective in 93.5%. Side effect with sodium stibogluconate were minimal but were common and serious with pentamidine. 20 case of PKDL were reviewed, 2 had no previous history of kala-azar. The relapse rate was higher in PKDL than in kala-azar, Thakur, (1989). In Khartum, Sudan from 1989 to 1990, six hundred twenty three cases were treated with sodium stibogluconate (Pentostam welcome UK) 10 mg/kg intravenous for 30 days. Later the treatment regimen was changed to 20 mg/kg for 15 days (70 cases) children received not less than 200 mg. At first the treatment was started gradually, reaching full dose in 6 days, this time was abandoned as no side effect of antimony is noted. The response to treatment was assessed clinically. However,in patients suspected of primary unresponsiveness or partial responders and relapse cases were given another course of antimony, 10 mg/kg for 30 days. Five patients with post kala-azar dermal leishmaniasis (PKDL) confirmed by biopsy were treated with 2 x 10 mg/kg intravenously 30 days. Six hundred and twenty three records were reviewed. Response to sb was rapid, fever subsided within 2 weeks with clinical improvement there was no side effect.

Three patients were given second dose due to persistence of parasite in lymphnode, bone marrow and spleen and for unsatisfactory improvement. Twenty four relapse case were recorded treated with 10 mg/kg sb I/V for 30 days within 2-11 months. Four patients relapsed after treatment with 2 x 10 mg/kg for 15 days with interval of 1-4 month. (Zijlstra et al 1990).

Trials are in progress with aminosidine 'Amnbisome', in India. In Africa, Brazil and Europe with 'Amphocil', with WR 6026 in Kenya. If successful and if supplied by WHO at a low cost then the next few years may see new drugs in use for VL at a cost not greater than current Sb^5 regimen.

In pre-antimonial era the mortality rate from kala-azar varied between 95 to 100 percent, (kala-azar commission, 1926, 1932). In one of the epidemics, 30 percent of the population of Nowgaon district in Assam died from this disease, (Paters W, 1981). Tartar emetic, a trivalent antimony compound first used by Viana in 1922 in South American leishmaniasis was introduced in India by Mc-combic young, 1924. Better tolerated analogue sodium antimony tartarate. Even with this toxic drug to active cure, a longer duration of treatment was suggested: for one to two months, (Ragers 1919). Napiere, 1924, Suggested that treatment spleen reduced to the level of the costal arch or by at least 4 inches and if the temperature came to normal by the 7th injection, 30 injections should be given, in cares where temperature fell to normal before the 10th injections, 35 injections should be normal after 16th injections, a total of 45 injections Brahmachari, an organic pentavalent compound, combining paraminophenyl stibinic acid with sera. This drug alone saved 3, 25,000 of patients between 1933 and 1936 in Assam only and the mortality rate from kala-azar was reduced to about 10% (Brahmachari U.N.(1928). But controversy started regarding the purity of this compound and the manufacture of the drug was discontinued. the newer pentavalent antimonials, Sodium Stibogluconate (Kikuth and Schmid, 1937) and maglumine antimoniate (Durand and his associate in 1946) became the standard first time of drugs for the treatment of leishmaniasis all over the world (Peters W.(1981).

Sengupta (1975) found that Sodium Stibogluconate was less effective drug compared to urea stibamine and meglumine antimoniate. In his series of Patients, 13% of patient relapsed and 4% were unresponsive 10 sodium stibogluconate when the drug was given in the dosage of Img/kg for 10 days (Sengupta PC 1975). The misconception about the difference in the efficacy of the two drugs was clarified by Eastro, (Castro RM, 1980) who found equal efficacy in daily dosage, 510mg sb in meglumine antimoniate and 600 mg sb in sodium stibogluconate in L. brasiliensis infection. They are chemically similar and have similar toxicity and efficacy in VL in relation to their context of pentavalent antimony (Sbr). Meglumine antimoniate solution contains about 8.5% Sbv (85mg/ml) where as sodium stibogluconate solutions contain about 10% sbv(100mg/ml).

DRUG ACTION BASES ON METABOLISM IN LEISHMANIA: Recently it has been shown that amastigotes as well as the promastigotes possess a surface coat,the glycocalyx,which contains a number of glycoproteins. Various glycopeptide exometabolites derived form surface coat form complexes within the parasitophorous vacuoles surrounding the parasites. This combination may provide a camouflage for

the parasite, or neutralise the host enzymes purine and pyramidine metabolism. Leishmanial promastigotes in culture require both folate and a pieridine for growth. The different species of parasites utilize these pathways in different degree depending upon their species, stage of life cycle and the availability for substrate.

The amastigotes use little glucose as its main source of energy, but instead, use proline which in first transaminated. The promastigotes use glucose in its early stationary phase of growth. Iron porphyrin is essential for the growth of leishmaniasis promastigotes and various porphyrin antagonists have been used as growth inhabitants. Evidences are scanty to pinpoint the action of sodium stibogluconate and pentamidine on the glycolytic mechanism. Marr and Bearns (1978) showed that allopurinol inhibited several steps in purine metabolism in leishmania. It has also been shown that both ethidium bromide and pentamidine inhibit biosynthesis of polyamine in promastigotes that are required for the completion of DNA and protein synthesis. All the three major pathways of glucose metabolism with some variations are utilised by different species of leishmania the anaerobic Embden-Meyerhof pathway which terminates at pyruvate, the Krebs by tricarboxylic acid (TCA) cycle and the pentose phosphate shunt. Recently it has been shown that sodium stibogluconate does influence anaerobic glycolysis, Janovy J. (1977).

Lipid metabolism: The antibiotic amphotericin B probably interacts with ergosterol associated with phospholipids in the cell membrane of leishmania.

Acid-Vescicle function: Leishmania organisms are protected from macrophage killing in the lysosome in part by their production of superoxide dismutase in contrast to toxoplasma, legionella, and nocardia. Leishmania actually benefit from the acidic environment of the lysosome, because their uptake of glucose and protein depends on it. Based on this absorption Ribonovitch et al 1986, use methyl or benzyl easters of leucine in vitro. First the easter is concentrated in the macrophages vescicle as a weak base. Then it is hydrolysed producing additional free acid and osmotic stress. Presumably, the drug kills the parasite by increasing the Ph of the macrophage lysosome (which is normally acid), Mukkada et al (1985).

The Present: The previous epidemic of kala-azar in Bihar (India) ended in late 1950's mainly as an incidental result of DDT spraying under the National Malaria Eradication Programme, Anonymous,(1978). The present epidemic of kala-azar started in 1970's. There were 100,000 cases in 1977 with 7% case fatalities, Wilcocks C. et al (1972).

The standard treatment was a 6-10 days course of sodium stibogluconate. The course was started was 1 ml IM or IV on alternate day and gradually increased. The rumour spread that 30% of Patients were unresponsive; any relapse after a 10 day course was probably taken as unresponsive. A committee of Indian experts recommended two courses of Sodium Stibogluconate with a break of 10 day in between. Starting with smaller dosages gap between the course and alternate day therapy did not seem logical. We therefore began giving the drug continuously for 20 days (Thakur CP 1984) with the regimen of treatment the relapse rate was reduced to 0.5% and 91.4% of patients were cured and only 86% of the patient did not respond. But it was realised later that if the drug could have been continued for longer, even more patients would have been cured.

In a randomised clinical trial, 62 out of 63 patients were cured with 20 days or more of treatment compared two only 54 patients in 20 day regimen, 8 patients of the later group relapsed. One patient in each did not responds to drug and were labelled as cases of primary unresponsiveness defined by WHO. Both the non-responders were cured with a course of 15 injections of Pentamidine. Side effects were minimum and patient tolerated even longer treatment (60 days) with Sodium stibogluconate, Thakur CP et al 1984). this result compared favourably with kenyan reports, Anabwani GM et al (1983). The better efficacy of even longer duration of treatment in Indian patients is being assessed.

Dosages and Administration:

Adults were given 6 ml I.M. daily in buttocks and children below 8 were given 20 mg per kg body weight. In the last 10 years only one patients complained of allergic type of reaction which improved with corticosteroids and the drug treatment was not discontinued. Then were studied to rationalise the treatment on the body weight basis and 6 regimens of treatment e.g. 10 mg/kg body weight for 20 and 40 days, 15 mg/kg body weight for 20 and 40 days and 20 mg/kg body weight for 20 and 40 days. They were studied in a randomise clinical treatment. 20 mg/kg body weight for 40 day regimen gave the best result.

High dosage antimony regimen: An injection of either sodium stibogluconate of meglumine antimoniate (sbv) is rapidly excreted in the urine, so that blood sb levels fall to less than 10 percent peak levels some eight hours after injection. There is a slight accumulation of sb reflected in a small rise in pre-injection blood levels, Ress W H et al (1980). In kenya it was found that patients unresponsive to usual dosage of antimony responded to 10 mg / kg body weight 3 times a day for 10 days,

Ress,P.H.,Kager PA (1980). With this regimen in 4 patients unresponsive to both sodium stibogluconate and pentamidine. Three of four developed heart failure and died and one surviving patient did not respond. These patients showed nonspecific S-T and T changes in ECG. There was no significant rise in serum enzyme (S.G.O.T and L.D.H); kidney and liver function tests were within normal limits. It was felt that some factor other than simple excretion of drug was responsible for the toxicity sodium stibogluconate, Thakur.C.P. (1986).

Penatamedine: Peak blood concentration of pentamidine after doses of 4 mg/kg are generally less than 1 mg but levels of 0.2 to 0.4 mg/kg body weight persists for at least 24 hours. The drug is slowly excreted in the urine over several weeks. The two salts of pentamidine- isethionate available as lomodine (Spacia) were used. Pentamidine (May & Beaker) and dimethanosulphonate available as lomodine were used. Pentamidine is available in powder form and lomodine in solution. Dosage was 4 mg /kg body weight on alternate day for 15 injection im or iv. In one of his series 68 out of 92 patients were cured (73.9 %). One patient did not respond and 2 patients relapsed who were finally cured with same drugs. Side effects were G.I.T disturbance, immediate hypoglycaemia, collapse, permanent diabetes and even death. Similar results were reported by T.K.Jha,. The diabetes in some cases was insulin dependent.

Allopurinol: It is given orally in the dosage of 21 mg/kg body weight. Experience in Kenya and India suggested that allopurinol was useful in a proportion of patients who did not responded to antimony. But it was not found very effective.

Antitubercular drugs: Peterset al, 1981, showed the effectiveness of INH plus rifampicin. It has been found effective against leishmania mexicana amastigotes in culture, Guradia J. (1981) found there was no effect Ethambutol alone or in combination with INH on patients with kala-azar.

Metronidazole: It was not effective in their series, of Pederson and Swaicks (1975).

Unresponsiveness and relapses: Unresponsiveness of drugs are quite common among kala-azar patients. Some patients did not respond to antimony from very beginning. They were labelled as cases of primary unresponsiveness if did not respond to a course of antimony given for 20 days. In the 70's, the percentage of

unresponsive patients was high as the drug was given for a short duration of 10 days: with the increasing duration of treatment with the sodium stibogluconate the percentage of unresponsive patients declined remarkably, Thakur et al (1986). They suggested that a course of 40 injections in all relapsed and unresponsive patients when they first come under observation. They have become unresponsive by taking inadequate dosage for inadequate duration. If after 40 days of treatment, there is no response , then either patient should be continued with antimony and allopurinol should be added for another 40 days or pentamidine should be added to antimony, they are getting better results with pentamidine and antimony combination.

Pentavalent antimonials remains the drug of choice but optimum duration of treatment with this drug is important. The shorter course of treatment is associated with higher rate of relapse and in unresponsiveness to the drug. The principle is that the treatment should be given until the aspirate is free of parasites and for 2 to 8 weeks longer depending on the speed of response. The children tolerate higher dosage and are usually given 20 mg/kg body weight. The cases of VL complicated with secondary infections, pulmonary tuberculosis, malnutrition, parasitic infections and anaemia should be concurrently treated for these conditions.

2.8 LITERATURE REVIEW OF SANDFLY POPULATION

Phlebotomize sandflies has a wide distribution, mainly in the tropics and subtropics. They are distributed over the whole continent of Africa, South and Central Asia and also occur in the northern part of Australia and New Guinea, Adler and Theodor (1957). Lewis (1978) recorded a total of 124 taxa (122 species and two sub species) in the Oriental Region. The Oriental sandfly fauna is represented by two genera, viz. Phlebotomus Rondani and Berte and Sergentomyia France and Paris.

Fauna of the Indian subzone is represented by 46 species which include important vectors of leishmaniasis, viz. P.argentipes (vector of VL) and P. salehi, P.papatasi and P.sergenti (vector of CL) Swaminath et al (1942), Das et al (1976), Lewis (1978); Lane et al (1990). There is no report, except one by Sen Gupta (1968), that the genus Sergentomyia play any role as a vector. This author mentioned that S. christophersy was suspected as a vector, but there seems to be no evidence for this, nor even a record for its biting a man. On the basis of this report, however, the species has been quoted as a habitual vector in a recent paper on North Africa, Lewis (1978).

P.argentipes is more common in the wet zone of eastern India, Papatasi P. salehi and P. sergenti are common in the arid part of north-west India, Kalra and Beng, (1988).

In Bangladesh, both the genus Phlebotomus and Sergentomyia were found Sinton (1932). In 1950-51, 134 specimens of sandflies were collected from Ishwarganj thana, Mymensingh (now Ishwarganj Thana of Netrokona district) and were identified as P.argentipes (1 male), P.(Prophlebotomus) squamipteuris var. indicus (8 male, 34 female), P.(Prophlebatomus) africans var. asiaticus (12 male, 48 female), P.(Prophlebotomus) shorttii (16 male, 15 female) Gramiccia and Sacca,(1953). Nasiruddin (1952) recorded four species of sandflies, namely P.africans. P.argentipes, P.minutus var. and P.theodori in Ishwarganj Thana of Netrokona district.

Ahmed and Ahmed (1983) reported P. argentipes (70.67%) to be the dominant species in Shajadpur Thana of Sirajganj. (Pabna) which was followed by P. malabaricus (15.03%) and P. minutus group (14.28%). They did not find any P. africans nor P.theodori in their study area. They reported P. argentipes to rest in large numbers in human dwellings and to bite man indoors. Kalra and Bang (1988) listed P. argentipes, P. papatasi barraudi S. pertuabans and S. shorttii from Bangladesh. Ahmed et al (1989) published a scientific note on the basis of some spot checks and longitudinal surveys on phlebotomus sandflies made in different parts of Bangladesh. They recorded 5 species of sandflies viz. P.argentipes. P. papatasi S. babu. S. barraudiand S. shorttii during their survey.

SEASONAL PREVALENCE OF SANDFLIES: Napier (1926) noted the following as the most favourable factors for sandfly growth:- Monthly mean maximum temperature below 37.8^{O}c and below 80% for at least three months, an annual rainfall or 1250 mm or more, with favourable altitude below 600 m, alluvial soil, high sub soil water level, and abundant vegetation. As these climatic conditions vary from area to area the seasonal prevalence varies accordingly. Lewis (1978) reported that in the tropics seasonal occurrence seems to depend on specific biology and local conditions. Some species occur throughout the year, and some flourish in the dry season when breeding places are not flooded, others are numerous in the rains, when high humidity may favour he adults and larvae of woodland species. Knudse et al (1979) reported that seasonal fluctuations of sandflies showed a negative correlation with rainfall. Kalra and Bang (1988) reported that sandflies are generally abundant during the warm part of the year. However, seasonal prevalence varies from year to year resulting from the complex interplay of biotic potential, physical, biological and environmental resistance. At Puna in Bihar state, India, Howlett (1909, 1913) noted that sandflies were common throughout the year but were most abundant in late September and early October. Sinton (1924) noted that Phlebotomus was found in the greatest numbers at times of excessive heat combined with high relative humidity. In the north-western region of India they may be absent for some months due to very cold winter and usually diminish in numbers during the hot dry months of the summers. In south and east India, Phlebotomus may occur during the whole year as the climate is moist and more equable.

The study of Sinton (1924) in the Pubjab and the North-West Frontier Province of India agrees with the study of Wimberly (1910), Robinson and Blackham (1912), and Harnett (1916) that sandflies, which appear in the spring, about the end of March, become very abundant at the end of April and during May, but as the weather becomes hotter and drier during June and July their numbers decrease, only to increase again in August, when the weather was moist. Sandflies were still numerous in September after which they gradually started to disappear until the beginning of November when they almost entirely disappeared. In Bombay, Powell (1909) found sandflies in March and April. De Mellow and Afonso (1921) recorded them as very common during the rainy months in Portuguese India.

Mackie (1914, 1915) found sandflies in Assam from February to November. Awati (1922) recorded S. Pertwibans throughout the year (this author mentioned S. Pertuibans and Phlebotomus pertuibans in his original paper). Raynal (1936a) reported

that in Indo-China sandflies were rare in the colder part of the winter from January to March.

Shields and Hull (1943) reported that in Florida there was a short and extremely heavy emergence of sandflies in the beginning of December which lasted until the middle of January and then fell rapidly. Another peak occurred in early April, dropped slightly during the next 30 days, then increased in early June, and remained fairly high through the middle of July. A sharp drop occurred in early August and continued until the middle of November. Parrot and Clastrier (1952) found that various species of sand flies at Phnom Penh in Cambodia disappeared in winter. Nasir (1958) found sandflies from February to November in Lahore, Pakistan, and George (1970) noted a sharp decline in October and November. Smith (1959) reported that in north-east India sandflies might vanish in the winter in December and January, and diminish again in mid summer. They became numerous after the monsoon, from August to October. In south India the number was lowest in the hot dry months. Dhanda and Modi (1971) pointed out that in Pakistan sandflies tend to vanish in winter and to appear in March and become numerous in April and in the damp month of August, whereas in Peninsular India they persist in the milder winter in Aurangabad (Decan) and Poona districts. Around Aurangabad sandflies were abundant throughout most of the year,where most numerous when the monsoon began in June and diminishwd in December.

Phlebotomus argentipes: P. argentipes, a wet-zone species of sandfly, is adapted to higher threshold levels of humidity, and are more numerous under optimal climate conditions (Kalra and Bang 1988). Although larvae were found at Gauhati in Assam adults of P.argentipes, were rare from late December to early February (Kala-azar commission, 1932). Brunetti (1912) recorded throughout the year at Calcutta, Sinton (1924) reported that sandflies might occur all the year round in southern and eastern India and that P.argentipes, was on the coast from Bengal to Sri Lanka. Basu and Ghosh (1955) found that P.argentipes, reached a peak in July and was least numerous in January, Pandya et al (1977) reported P. argentipes, in Surat city, Gujarat was not detected only during the post rainy and cold periods. Sanyal et al (1979) found P. argentipes, in Bihar rising from the month of July and peak in the September -October. During the peak period, they were detected in all roofed structures, but not in any outdoor situations.

Mathur and Rahman (1979) found no P. argentipes, during their study period (April 1979) in villages of Nowgong (Assam), India, which were worst affected with kala-azar during the previous epidemics. The total absence of this species in the area may be attributed to extensive and intensive DDT spray under NMEOP for more than two decades. Hati et al (1981) found P. argentipes, in maximum numbers in May in a village in West Bengal. Nasiruddin (1952) reported that in Bangladesh (Ishwarganj thana, Mymensingh) the highest incidence of Phtebotomus occurred in the months of March, April and May.

Gramiccia and Sacca (1953) during their study in 1950 at Ishwarganj thana, Mymensingh, Bangladesh found the Phlebotomus population increased in the pre-monsoon months (April-May) and there was a minor increase after the monsoon (November). They recorded the lowest Phlebotomus population in the monsoon (June-Sept). Ahmed (1987-1988) reported that P. argentipes were found throughout the year in Mymensingh, Sirajganj and Pabna. The peak density was found from July to November (highest in September).

Phlebotomus papatasi: Around Pusa, Howlett (1915) observed that the larvae of P. papatasi which hatched at the start of the cold weather, pupated in late February or early March according to temperature.

In north Bengal P.papatasi was common in April (Brunetti 1920), In Bombay P. papatasi seemed to diminish in the rain seasons (Young 1927), Craighead and Das (1928) reported that the species increased somewhat in the rains, in Poona, P.papatasi was common throughout the year (Mitra 1956), Pandya et al (1977) reported that P.papatasi was found throughout the year in Surat city, Gujarat, India.

The population of P. papatasi became very abundant at the endof April and during May. Their numbers decreased in June and July, and at the beginning of November they had almost disappeared (Robinson & Blackham 1912; Harnett 1916, and Sinton 1924). The periods of maximum prevalence of P. papatasi corresponded very closely with the prevalence of papatasi fever (Sinton 1924).

Sergentomeya spp.: Craighead and Das (1928) found S. indica around Pusa that increased somewhat in the rains. Raynal (1936a) reported that S. barraudi was an upland species in Indo-China disappeared from the northern low lands in summer. Pandya et al (1977) reported that Sergentamyia montana was detected in summer and winter months in Surat District, Gujarat. S.puryabensis was detected only

in the month of April (0.80 man - 1 hr. -1). November (1.26 man - l hr.-1), and in December (0 63 man - 1 hr.-1).

SANDFLY DENSITY IN DIFFERENT HABITATS: Rathnaswamy and Ramakeishnan (1954) reported that sandflies were common in human dwellings, mostly in the thatched roof and mud wall type; in pucca buildings sandflies were available only in very small numbers. This may be attributed to the favourable temperature and humidity conditions obtaining their in and the existence of breeding sites in and around thatched structures. Lane and Al-Taqi (1983) found that in general sandflies (both Phlebotomus and Sergentomyia) were only present in association with human dwellings. They also reported that sandflies were more abundant on the edges of towns or the city than in the central areas. Das and Mukherjee (1969) reported that cattle sheds provided the most suitable habitat for P. argentipes. They also collected P. argentipes from one living room adjacent to the cattle shed. Das et al (1979) found P. argentipes in the living rooms of houses both urban and rural areas of West Bengal. They also found that P. argentipes was more numerous in villages than in urban areas, and in urban areas this species favoured ground floor rooms of the masonry houses which were often dark and damp with a single small window and a door. Hati (1983) found P. argentipes to more numerous in cattle sheds than in mud huts in a village of West Bengal. He found 39.89 (mean man hour density) in mud huts.

Hati et al (1987) found the mean density of female P. argentipes collected in a village of West Bengal to be 13.1 man - 1 hr.-1 in cattle sheds and 7.5 man -1 hr. -1 in huts. Ahmed (1987-88) found that the density of P.argentipes in cattle sheds was 8-10 times more than from human dwellings in Mymensingh, Sirajganj and Pabna. In Dera,Ismail Khan in 1919-1920 P. papatasi was very common the cow sheds and rare in the dwelling houses while P. minutus was comparatively rare in cow sheds but common in dwelling houses (Sinton 1924). Mode et al (1977) reported that S. babu was equally distributed in all types of habitats such as human dwellings, cattle sheds, tree-holes, rock crevices, pig baited traps, and near pig sites.

Biting habits of sandflies: Most of the sandflies probably take plant sugars as their food (Lewis 1966, 1971; Kalra and Bang 1988). Kalra and Bang (1988) reported that, in general females of Phlebotomus feed on mammals and those of Sergentomyia on reptiles.

Phlebotomus argentipes: Das et al (1976) found P. argentipes as exophilic in some parts of West Bengal. Modi et al (1977) reported P. argentipes as endophilic in

Bankura district, West Bengal. Pandya (1983a) reported that P. argentipes was an endophilic species although a small number was also encountered in outdoor collection (2.82%). Lewis (1957) found P. argentipes attacked both cattle and human bait in West Malaysia. Lewis and Wharton (1963) reported that P. argentipes fed on cows but had rarely been found biting man. Lewis and Killick-Kendric (1973)) in Lane et al 1990 suggested that P. argentipes exhibits clinical variation in its biting preferences, being almost entirely zoophilic in the southern parts of its range, such as Sri Lanka and south-east Asia. Das et al (1976) found P. argentipes mainly as zoophilic which fed on man particularly in the absence of bovids. Modi et al (1977) found that P. argentipes showed a closer affinity for cattle sheds. Hati et al (1981) studied in West Bengal from September 1979 to August 1980 and reported that P. argentipes were greatly attracted to man. Dhanda and Gill (1982) and Hati (1983) found P. argentipes to be mainly zoophilic. Second choice of P. argentipes was found to be man. Hati (1983) found P. argentipes to feed on pigs and birds. His study revealed that the feeding habit of P.argentipes may be influenced by the different biotopes in different seasons. His study showed that when human and bovid baits were placed side by side, significantly greater numbers of P. argentipes were attracted to bovids, both indoors and outdoors. He also found 14.97% of P.argentipes in mud huts that took human and bovine blood simultaneously. This percentage increased to 25 in cow sheds. The study of Dhanda et al (1983) revealed that P. argentipes had fed mainly on cattle, when collected from cattle sheds, its anthropophilic index was zero. But when the flies were collected from human dwellings, the anthropophilic index was 66.7%. They also found that P.argentipes fed on multiple hosts. Of the four P. argentipes t 3 had mixed human + boved, and one had bovid + avian blood. The study of Dhanda and Gill (1982) found similar results in two villages near Pune. The observations of Addy et al (1983) differed with that of Smith (1959), Dhanda and Modi (1971), Dhanda and Gill (1982), and Hati (1983) in that P. argentipes were zoophilic, with man as the second choice. They showed that the flies bite whichever host was available with equal avidity.

Pandya (1985) found P. argentipes to be primarily a zoophilic species. Rehman et al (1986) in Kalra and Bang 1988 found P. argentipes in Nilgiri Hills to be mainly zoophilic. Lane et al (1990) found P. argentipes of zoopilic nature in Sri Lanka. The absence of VL in Sri Lanka is correlated with the zoophilic nature of P. argentipes. Since they were not recorded biting man. They reported the possibility the allopatric populations of P. argentipes in Sri Lanka were predominantly zoophilic in the low lands, but with more anthropophilic tendencies in the high lands.

Phlebotonous papatasi Modi et al (1977) reported P. papatasi as endophilic. Killick-Kendroc (1983) reported P.papatasi to be endophilic and others may be principally or wholly exophilic or, like P. ariasi exophilic but commonly endophagic. Entomological study of Dutta and Ghosh (1983) found P. papatasi to be exclusively endophilic. George (1970) showed that P. papatasi was primarily anthropophilic in Lahore and Peshawar areas but could also feed on birds, bovines, dogs and equines.

Dhanda and Modi (1971) found that P. papatasi fed mainly on man (95.25%). They also found that P. papatasi fed on cattle (2.16%), dogs (1.30%), birds (0.65%), and other mammals (0.43%). They found one P. papatasi to have mixed avian and human blood. Zivkovic et al (1973) found mixed blood protein of man, horse, pig, sheep, dog, rat and cow in P. papatasi from villages in Yogoslavia. Modi et al (1977) observed that P. papatasi was associated mainly with human dwellings. Dhanda and Gill (1982) reported that P. papatasi was mainly anthropophilic, but sometimes fed on cattle. They also noted that a significant percentage of P. papatasi (19.3%) had mixed blood of human and bovids, and one P. papatasi had mixed blood of human and rodent. The study of Dhanda et al (1983) indicated that P. papatasi mainly fed on man. The anthropophilic index was 97.7% when the flies were collected from human dwellings. He found one P. papatasi with mixed human and avian blood. Pandya (1985) found ubiquitous feeding habits of P.papatasi showing a multiplicity of hosts in a single blood meal. He called P. papatasi as an opportunistic feeder.

Sergentomuia spp.: Modi et al (1977) reported that S. batyi and S. puryobensis were mainly exophilic, inhabiting tree holes. Howlett (1913) reported that P. minutus fed on Cackos and bite man in summer at Pusa (P.minutus now belong to several species under the genus Sergentomyia), S. puryabensis and S. theodori feed on human or cattle but is frequently on some other host blood (the author mentioned these species as P. minutus in his original paper). Shortt and Swaminath (1913) found S, shorttii to bite geckos in a cage in India. Napier (1931) insisted that S. babu and S. shorttii could bite man in Calcutta (the author mentioned S. babu and S. shorttii as P. minutus group in his original paper). Raynal (1936b) found S. baraudi in Tonkin which had fed on man. Mitra (1956) found that S.babu and S. balyi to bite man readily in the laboratory. Alder and Theodor (1957) reported that S.babu attacked geckos in India and was said to bite man in Mauritius. Lewis (1957) reported that S. baghdadis was found on or near animal bait in Pakistan. The study of Dhanda et al (1983) noted that Sergentomyia species feed mainly on reptiles but Sergentanyia babu babu collected

from human dwellings had human blood. Pandya (1985) informed that Sergentomyia species generally prefer to feed on reptiles and mammals. He reported for the first time the anthropophilic nature of S.bailyi (28.57%). Ahmed (1987-1988) reported that P. argentipes was predominantly zoophilic preferring to feed on cattle with man as the second choice He also found P. papatasi as anthropophilic in nature. Lane RP; Iile MM; Amerasinghe FP, 1990. The visceral leishmaniasis (VL) vector Phlebotomus argentipes Annandale & Brunetti is widely distributed throughout the Indian sub- continent and S.E. Asia. The absence of VL in areas such as Sri Lanka has been attributed to the zoophilic nature of P. argentipes, since they were not recorded biting man. Field studies on P argentipes were undertaken in the central highlands of Sri Lanka, near Kandy, in May 1988. Male sandflies outnumbered females cows by 19:1, and were regularly spaced at all densities. This behaviors is considered analogous to swarming in other Nematocera. However, all-night human-biting catches show the biting rate to be similar (mean=8.4, range 2-25 bites per night over ten consecutive nights) to that in N.E. India where VL is endemic. This anthrophagy was maintained during laboratory colonization. Dhiman, Sen; (1991). found that some features like intra- macrophage habitat of the Leishmania parasites, rare availability of infected macrophages in peripheral blood for vector sandflies to such in, short flight range of sandflies, non-availability of an animal reservoir encountered in visceral leishmaniasis indicate, slow and limited transmission potential and even so epidemics occur every 15 to 20 year. To verify if these assumptions are true, the natural history of kala-azar was studied, using an endemic village in Bihar (India as an unit of study, over a period of 5 year (1984-1988). Village Jethuli is bound by the river Ganga on the north and separated from neighbouring endemic villages on other three sides by agricultural land is isolated entomologically (as regards sandflies). The village has a population of 3236 persons of different social status and depending on economic conditions have three types of dwellings, brick made with cement plaster, brick made with mud plaster and mud houses. The first case of kala-azar was reported in a migrant from district Vaishali on the other side of Ganga where kala-azar appeared in an epidemic form. Studies showed that the infection is built up slowly, first in the same house and then in the immediate neighbourhood. In this village, maximum number of cases occurred in 1984 and 1985, and they were treated by our Institute and cured. In subsequent years, only a few cases occurred (i.e. 6 in 1986 and 4 in 1987) while no case occurred in 1988. Gradoni L; 1991; As a part of a general survey on leishmaniases and sandflies of the Maltese islands, 22 Leishmania stocks were isolated from human visceral (1) and cutaneous (1) cases, dogs (16) and sandflies (4). They were characterized by the

analysis of 15 enzymes. The commonest Mediterranean L. infantum zymodeme, MON 78, was found to cause human and canine visceral leishmaniasis; L. infantum MON 78, which has so far been isolated only in Malta, was the agent of human cutaneous leishmaniasis. Both zymodemes were isolated from the same sandfly species, Phlebotomus perniciosus. Costa CH; Pereira HF; Araujo MV, 1990; The kala-azar epidemic in the State of Piaui 1980-1986 is analyzed on the basis of the data collected by SUCAM Piaui. The outbreak began in towns of central and northern Piaui in 1980. In contrast what has happened in endemic periods in which the disease occurred in areas of higher altitude and semi-arid climate, the epidemic developed in humid tropical river valleys in rural zones. The epidemic was worst in the towns. The state capital Theresina, hit in 1981, reached the epidemic peak in 1984 and accounted, for more than 60% of the 1,509 cases in the state. The epidemic was not substantial in those regions sprayed to combat malaria and Chagas disease. While control in Teresina was attempted through intensive use of insecticides, the outbreak gave way spontaneously in rural areas. Neither the number of cases nor the phlebotomize population of Teresina presented significant seasonal variations but were moderately correlated. There was greater prevalence in children of 5 years of age or less, especially during the peak epidemic years, and much lesser prevalence in adults over 40 years of age. The geographical distribution of the epidemic process and its beginning, concomitant with a prolonged drought with its accompanying migration of people and domestic animals from endemic to epidemic regions,suggests that migration unleashed the epidemic. The fact that the epidemic process spontaneously relinquished its hold in areas where no control was attempted, indicates that the end of the epidemic cannot be attributed solely to measures of control. An analysis of the coefficients of specific incidence within age groups sparks the discussion about the possibility that progressive reduction of susceptibility (determined by the great number of asymptomatic infections as well as by long lasting immunity) contributed to the extinction of the epidemic. Mutinga MJ; Basimike M. Kamau CC; Mutero CM; 1990. Host preference of wild caught phlebotomize sandflies was studied in Marigat, Baringo District, Kenya, an endemic focus for both Leishmania donovani Laveran & Mense and L. major Yakimov & Schokhov using precipitin test of blood meals. Sandflies of the Phlebotomus Rondani & Berte and Sergentomyia Franc & Parrot genera were encountered blood fed and resting in nine different habitats which were investigated. Analysis of their blood meals revealed a district hos.preference between the Phlebotomus and Sergentomyia generia. A distinction of host preference within species of each of the two genera was also observed. Furthermore, certain resting

habitats from which the blood fed sandflies were collected appeared to be favoured by specific sandfly species. It was observed that most of the wild hosts such as lizards and rodents except the hippopotamus, shared the same resting habitats with the sandflies. It was also observed that the man-biting sandflies preferred to rest outdoors after feeding. Domestic animals and man were the favoured hosts of vectors of both visceral and cutaneous leishmaniasis, thus introducing an element of zooprophylaxis.

Daoud W; Rioux JA; Delalbre-Belmonte A; Dereure J; Rageh HA; 1989. The systematic inventory and annual following of Phlebotomus population is established by the authors in a transmission area of visceral (human and canine) and cutaneous (human) leishmaniasis in the Yemen Arab Republic (province of Taez). Seven species of Phlebotomus and nine species of Sergentomyia are thus identified. Among them, four are considered as potential vectors: on the one hand, P. orientalis (s.g. Larroussius) and, probably P. arabicus (s.g. Adlerius) for L. infantum and L. donovani, in the other, P. sergenti and P. saevus (s.g. Paraphlebotomus) for L. tropica.

Dye C, 1988; This paper describes a compartmental model incorporating biological details of the transmission of canine visceral leishmaniasis in southern France. In contrast to earlier, empirical models (Jolivet, 1977; Rioux, Croset & Lanotte, 1977) the new model (1) predicts a threshold density of sandflies below which transmission cant be sustained, (2) suggests that, until better data become available, the maximum prevalence of infection obtained at high sandfly density should be considered an unknown quantity. Ryan L; Lainson RI Shaw JJ; Wallbanks KR; 1987. Lutzomvia furcata transmitted Leishmania chagasi to a hamster 10 days after being experimentally fed on an infected spleen. An individual female Psychodopygus carrerai that had fed on a hamster lesion caused by Leishmania mexicana amazonensis transmitted this parasite 6 days later to another hamster. Transmission electron microscopy of this fly's head revealed a small number of degenerate promastigotes in the foregut, but only a few were attached. Miscevic Z; Milutinovic M; 1986; The paper presents the results of faunistic, ecological and viral investigations concerning phlebotomize sandflies in an endemic focus of visceral leishmaniasis in Yugoslavia. These investigations were carried out in the period from 1969 to 1981. Strekova MV; Dergacheva TI; 1986; The authors succeeded in transmission of the visceral leishmaniasis agent (kazakh strain of Leishmania infantum) through the sand flies Phlebotomus longiductus, P. smirnovi and P. papatasi under experimental conditions. Infected P. longiductus and P. smirnovi are capable of preserving the agent and infecting the susceptible mammals after the cessation of the 1st and 2nd gonotrophic cycles. P. papatasi transmitted the agent of visceral leishmaniasis only after the cessation of the 2nd gonotrophic cycle. Under the

same conditions the transmission of visceral leishmaniasis agent through P. caucasicus failed. Informative value of characteristics of virtual vectors for differentiation of sandflies as carriers is analysed. A question of the necessity of obtaining additional data, which prove the role of P. caucasicus and P. papatasi as vectors of visceral leishmaniasis agent, is raised. Goncalves MD; Ryan L; Lainson R; Shaw JJ; 1985. A closed Lutozomyia longipalpis colony, from Ceara has been to transmitted Leishmania chagasi isolated from a fox in Para state. The last time this colony was successfully used in similar transmission experiments was eight years (64 generations) ago indicating that this colony of Lu. longipalpis has fully maintained its vectorial capacity in spite of such a long period of maintenance in the laboratory. Le Pont F; Desijeux P; 1985; A relatively high leishmanial infection rate was found in the phlebotomize sandfly Lutzomyia longipalpis collected from three villages of the Los Yungas region (Department of La Paz, Bolivia). 2,578 female sandflies were dissected. In three houses surveyed in Santa Barbara promastigote infection rates of Lu. longipalpis were 4.2, 2.2 and 3.2% respectively. Anatomical localization of the infection in the insect and biochemical characterization of the strains indicate that the parasite belongs to the Leishmania donovani complex. The geographical area and the biotopes of Lu. longipalpis are discussed in relation to the vector-parasite relationship.

CHAPTER 3

MATERIALS & METHODS

3.0 CHAPTER-3 : MATERIALS AND METHODS

3.1 MASS APPLICATION OF DIRECT AGGLUTINATION TEST (DAT) FOR VISCERAL LEISHMANIASIS (VL) IN BANGLADESH

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3.10 SERO-CONVERSION USING DAT AS COHORT STUDY IN BANGLADESH

3.0 CHAPTER - 3 MATERIALS AND METHODS

3.1 STUDY AREA :

A cross sectional study was carried out in 427 villages of 3 Thanas of Mymensingh district of Bangladesh, near the south bank of river Brahmaputra. A district is subdivided into several thanas. The union parishad, which is the administrative unit of thana has been sub-divided into some wards (a combination of some villages) and was used as the sampling unit of the project. Initially Fulbaria Thana of Mymensingh district was selected for the study. (As only the new confirmed kala-azar patients and not treated previously with antimony was the only criteria for selection of the patient in study area was taken too more health complexes). The area of Fulbaria is 154 Km, Trishal is 337.77Km, Bhaluka 275 Km with a total population of 863748. The total villages of the 3 thanas are 427. The study area is extended 35 Km to the south and 10 Km to the west of the district town, of which villages are within 30 Km. About 200 Km away from Dhaka city (Fig-2) which is connected by metalled road extending to the north of the city. The area is plain except a few hillocks and the village area is a low lying area and submerged by rain, every year. The kacha road passed through the eastern side of the village having ditches and pools along the side of it. A brick soling road connecting highway and thana health complex passed through north side of the villages. The clusters of houses are linked with linked road. The west bank portion of the canal of the villages are accessible throughout the year by road. Most of the villages are situated on each bank of the canal. Water remains in the canal even in the dry month of April. Winter is from November to February and temperatures usually range from a mean minimum of 57 degree F to a mean maximum of 79 Degree F. The dry summer is from March of June with mean temperature ranging from 71 to 91 degree F. The monsoon lasts from mid June until early October, the minimum temperature being 77 degree F and the maximum mean 90 degree F. The average yearly rainfall of 70" is received during this time. The average relative humidity ranges from 48% in March to 83% in July and August. During July 1987 to June 1988 the residents of the study area were listed by unit of house.

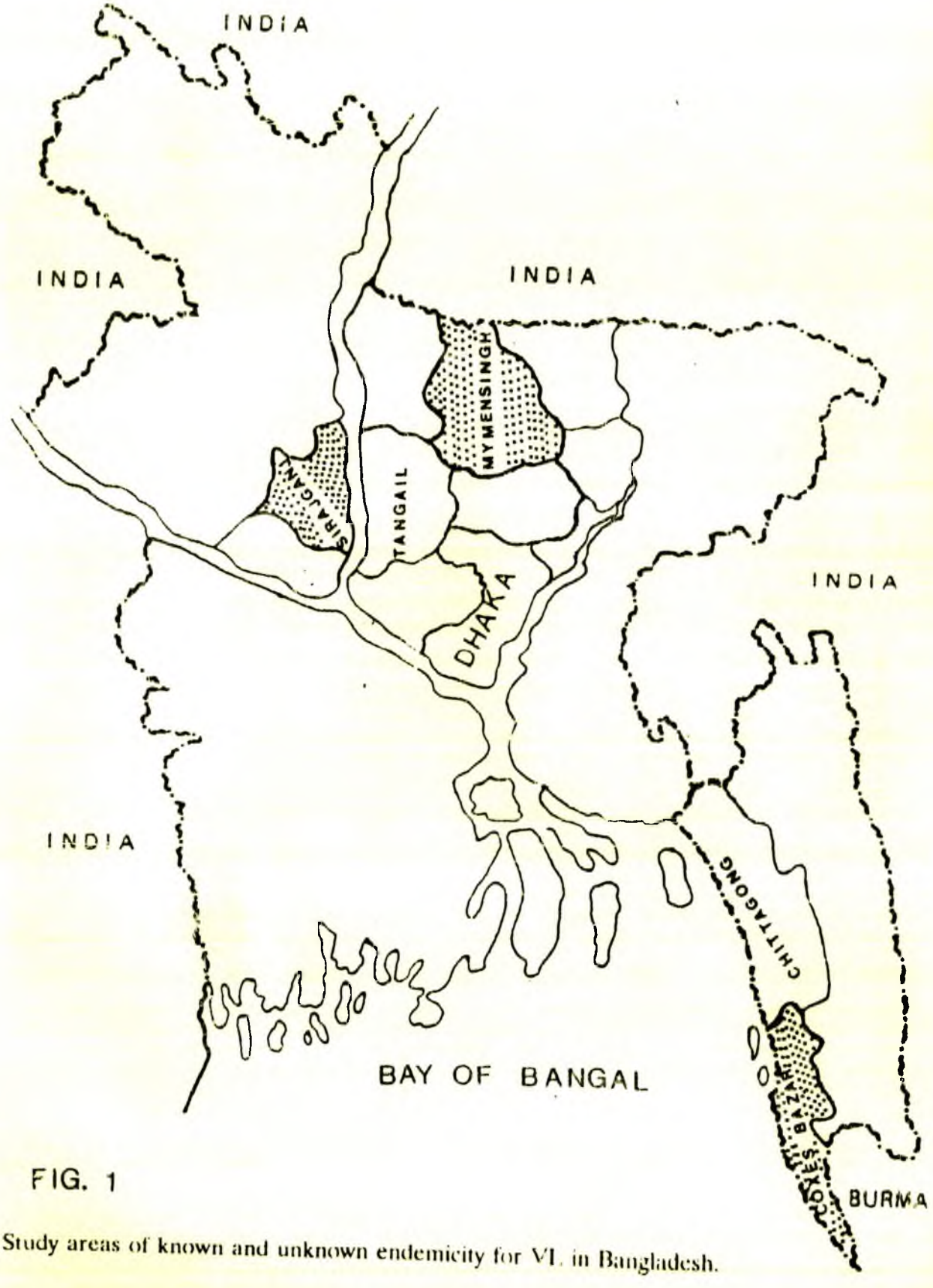


FIG. 1

Study areas of known and unknown endemicity for VL in Bangladesh.

3.1.1 MASS APPLICATION OF DIRECT AGGLUTINATION TEST (DAT) FOR VISCERAL LEISHMANIASIS (VL) IN BANGLADESH

Study areas and methods:

From the available records on VL occurrence in Bangladesh it was noticed that 27 out of 64 districts are endemic for the disease at variable levels. Mymensingh and Sirajganj are of high endemicity with respective registered cases of 2100 and 2659 during the period January 1987 to July 1991. Districts of moderate endemicity are those of Tangail and Gazipur having had 691 and 645 VL cases respectively. Chittagong and Coxe's Bazar are non-endemic for VL but highly endemic for malaria; in Cox's Bazar district only, 15539 cases were diagnosed during 1991.

In a 4 years study project, a target population of 50000 inhabitants residing in 2 or more of the endemic districts in Bangladesh are to be assessed for presence of anti-Leishmania antibodies. As a start, the study is intended to be cross- sectional for assessing VL point prevalence and eventually longitudinal having identified sero-positives for confirmation or follow-up studies otherwise. The campaign was started simultaneously in Trishal and Shahjadpur, two thanas (subdistricts of Mymensingh and Serajganj districts, by choosing 3 villages in each of them. To have a fair impression on VL prevalence in the chosen endemic areas. 2 villages in Teknaf thana (Cox's Bazar district) being non-endemic for VL were included (Fig.1).

House-to-House visits were conducted in all 6 villages and non descriminatively all family members available at the time of the visits were interviewed and examined. A questionnaire form included administrative and demographic issues, clinical symptoms such as fever, splenomegaly, anemia and personal or family history of VL was completed on each inhabitant. In total, 17826 inhabitants of different, socio-economical standard and age (< 1 - > 90 years) were interviewed and assessed (Table 1). The frequency of survey visits was about 10 times per month during the period September 1991 to April 1992. The adequate man-power and experience gained in previous campaigns for control of other diseases, made it possible it possible to complete this part of our study within the period assigned.

Sampling procedure:

Considering the size of population to be covered in this phase of our study, collection of venous blood for sera testing in DAT seemed impractical. Filter-paper

blood sampling though proved to be more easier, yet requires additional labour to quantify and prepare eluates for testing. Our previous studies in mice showed that DAT titres obtained with either whole blood, dried blood on filter-paper or plasma were highly agreeable (Harith et al 1988). Being more convenient in sampling and easier for performance of DAT, it was decided to employ whole blood sampling in this survey. After completion of the questionnaire, blood samples were obtained by finger-prick using sterile lancets.

A free-failing blood drop (+ 50 ul) was captured in screw cap glass vials containing 1 ml salt solution (0.9% Na C1). Assuming an approximate serum content of 40%, the start dilution of the sample collected was then 1:50. Following this procedure, blood samples were collected from all 17826 inhabitants involved in the survey (Fig.2).

Control Sample: To asses for reliability of using whole blood in current study, serum and finger prick blood samples collected as mentioned above from 10 VL (Treated) Patients and 10 (endemic) apparently healthy inhabitants were tested independently.

For quality control of antigen batches produced at large scale (3300 ml), 308 reference sera from our serum bank (UVA/Amsterdam) were used. The majority of those samples were described in details earlier and had been tested in DAT with the same antigen (L.donovani, 1-s) produced in miniature quantities of 100-500 ml (Harith et al 1986, 1987). The represented the following conditions.

- Visceral leishmaniasis: Bangladesh (20), Kenya (20), Somalia (20) and Algeria (23).
- Cutaneous leishmaniasis: Algeria (20).
- African trypanosomiasis: Ivory coast (14), Zambia (10) and Mozambique (10).
- Chagas' disease: Brazil (87).
- Auto-immune disorders: The Netherlands (32).
- Tuberculosis: The Netherlands (32).
- Toxoplasmosis: The Netherlands (10).
- European Heaith Controls: The Netherlands (9) and Portugal (10).

Table 1 **Total study areas and population covered in the survey from June 1991 to May 1993**

District	Number of upazilas	Total population covered	Sex	Age Group (years)				Total (M/F)	
				≤ 1-5	6-11	12-20	21-≥90	Number	%
Mymensingh	2	19130	M	1755	2098	1701	2867	8421	44.02
			F	1753	2213	1823	4920	10709	55.98
Sirajganj	1	10402	M	1163	1886	1280	1355	5684	54.64
			F	966	1311	944	1417	4718	45.36
Tangail	2	6079	M	346	831	907	1080	3164	52.05
			F	351	758	684	1122	2915	47.95
Coxe's Bazar	1	2343	M	218	360	326	344	1248	53.27
			F	163	315	216	401	1095	46.73
Total	7	37954	- (%)	6745 (17.8)	9772 (25.7)	7931 (20.4)	13506 (35.6)	37954	-

Large-scale antigen production:

Production of antigen had to be significantly intensified to enable execution of DAT on all 17826 inhabitants with a minimum number of batches to minimize possible variation in test results. The present instrumental capacity of our laboratory allows for a maximum production of 4000 ml at a time sufficient for screening of 6400 inhabitants. Antigen batches so produced were expected to comply with the required sensitivity and specificity experienced with earlier batches produced in miniature volumes.

In this study, adequate parasite culture for antigen preparation was established in a similar manner as in the initial procedures (Harith et al 1988). Seven litres GLSH-culture medium having had L.donovani promastigote density of 5-7x10/ml were obtained by continuous orbital shaking (140-150 rpm) at a constant temperature of 26-27 C for 60-72 hours. Promastigotes, mostly at the spindle form of growth (logarithmic phase) were harvested by centrifugation (4000 g) at + 4 C for 20 minutes. The procedures for washing, trypsin treatment and formaldehyde fixation remained unchanged except for the necessary adjustments proportional to the larger promastigote suspensions. Staining procedure was slightly modified by using 200 ml of 0.2% (w/v) Commassie Brilliant Blue versus the obtained promastigote packed cell volume of 7 ml. After several washings, promastigote were finally re-suspended in 3300 ml citrate-saline buffer supplemented with formaldehyde (0.43% w/v). Reliability of this antigen batch was assessed in DAT against reference sera (308) being analysed in earlier studies.

Following the same protocol, six antigen batches with volumes ranging 1120-4000 ml and sufficient for performing DAT on 1176- 6400 inhabitants were prepared. Before transportation to IEDC&R (Dhaka), each batches was quality controlled by full-out titration against reference sera from VL (10) and African trypanosomiasis (8) cases with previously known titres ranging 1:100 to > 1:52428800. As in the case of miniature batches, deviations in DAT results were not expected to exceed one titre reading.

Table 2 - DAT antigen batches prepared during the period July 1991-April 1994

Antigen batch N.	Mass culture Vol.(ML)	Antigen Vol.(ML)	Number of tests (1:102400)
31/07/91	4800	1400	2240
02/09/91	6000	3000	4800
08/10/91	5000	2500	4000
26/11/91	2000	1120	1176
17/12/91	7000	3300	5280
16/03/92	8000	4000	6400
15/06/92	4000	2200	3520
21/08/92	6000	2800	4480
23/09/92	7000	2700	4320
13/10/92	6000	3400	5440
19/11/92	3500	2800	4480
01/12/92	3000	2500	4000
01/03/93	6000	3500	5600
07/03/93	5000	2900	4640
11/08/93	2500	1600	2560
04/01/94	4500	2000	3200
17/03/94	4000	1800	2880
15/04/94	2000	800	1280
28/04/94	3000	1000	1600

Table 2A Study areas and population covered during the period June 1992 - M 1993.

District	Upazila (sub-district)	Sex	Age group (years) ≤ 1-5	6-11	12-20	21-≥ 90	Total (M/F) Number	%
Mymensingh (N=9511)	Trishal (N=5854)	M	520	702	476	848	2546	43.49
		F	491	723	552	1542	3308	56.51
	Bhaluka (N=3657	M	288	453	317	488	1546	42.28
		F	258	468	349	1036	2111	57.72
Tangail (N=6079)	Mirzapur (N=4998)	M	255	617	796	958	2626	52.54
		F	261	580	609	922	2372	47.46
	Kalihati (N=1081)	M	91	214	111	122	538	49.77
		F	90	178	75	200	543	50.23

Table 2B_ Study areas and population covered during the period June 1992 - May 1993.

District	Upazila (sub-district)	Sex	Age group (years) ≤ 1-5	6-11	12-20	21-≥ 90	Total (M/F) Number	%
Sirajganj (N=3074)	Shajadpur (N=3074	M	254	776	523	389	1942	63.18
		F	179	462	273	218	1132	36.82
Cox' Bazar (N=1464)	Teknaf (N=1464)	M	146	246	206	190	788	53.83
		F	113	205	116	242	676	46.17

Mass application of DAT:

After completion of the questionnaire on the administrative and clinical issues, blood samples collected as described above were either transported directly to IEDC&R in Dhaka or stored at +4 C for a maximum period of 24 hours until transportation was available. Execution of DAT on all 17826 blood samples collected in the field was according to the improved procedures (Harith et al 1988). As determined, a start dilution of whole blood sample below 1:1600 resulted in a concomitant erythrocyte sedimentation rendering reading of DAT rather difficult. Accordingly, 3 ul of the blood sample collected at the dilution indicated (1:50) were transferred to the second well of V-shaped microtitre plates containing 100 ul sample diluent (0.2% w/v gelatin + 0.9% w/v Nac1 + 0.78% v/v 2 mercaptoethanol) giving thus a start dilution of + 1:1600. After addition of the antigen and manual agitation, plates were incubated at ambient room temperature (25-35 C) for 18 hours. Reading of test results was as described by locating a sharp blue point similar to that in control well; the preceding dilution was considered as the titre of blood sample tested. Inhabitants with titres > 1:3200 were considered DAT positive for VL (Harith et al 1986).

Management of VL seropositive:

The obtained DAT titre on each inhabitant was entered into the relevant questionnaire form: inhabitants with positive test but having had no obvious symptoms of VL were considered for retesting and follow-up. Sero-positive inhabitants with typical VL symptoms such as persistent fever and splenomegaly were subjected to bone-marrow aspiration. Taking into account that absence of Leishmania parasites does not justify withholding of treatment (Abdel-Hameed et al 1989; Chowdhury et al 1991), Sodium Antimony Gluconate (SAG) was therefore administered to all parasitologically confirmed and unconfirmed sero-positive patients who had presented typical symptoms of VL.

3.2 APPLICABILITY OF DIRECT AGGLUTINATION TEST (DAT) AT A RURAL HEALTH SETTING IN BANGLADESH AND FEASIBILITY OF LOCAL ANTIGEN PRODUCTION

Study area and population:

Trishal is one of eleven thanas constituting Mymensingh district; a highly endemic area for VL in Bangladesh (Fig.6). Only in this district, 2100 cases were reported during the period 1987 to 1991.

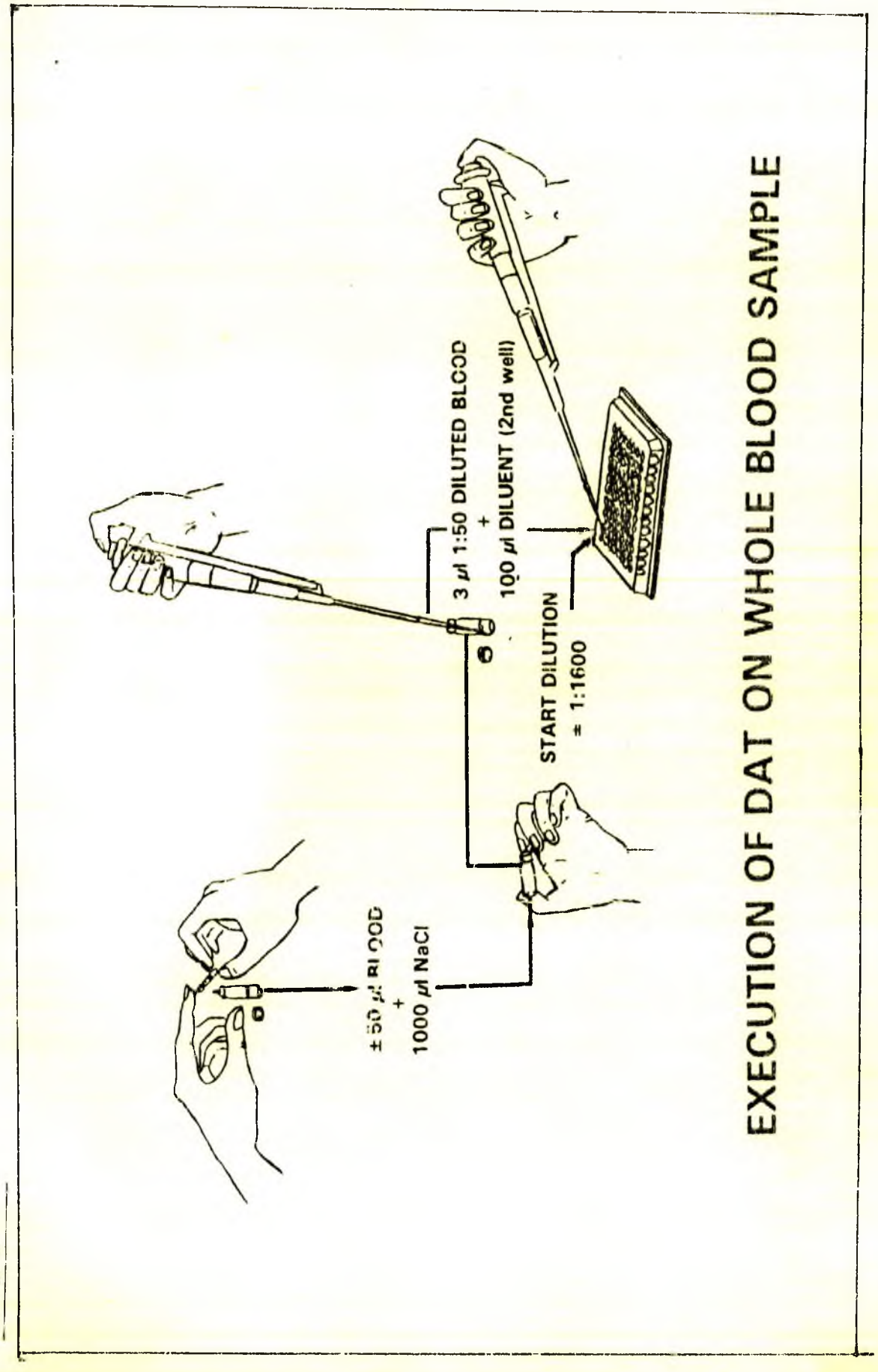


FIG 2

The existing health complex of Trishal is multi-functional and provide services such as birth and death registrations, management of simple casualties and complaints. The health complex has a capacity of 30 beds and the staff consists of a number of general practioners, health assistants and nurses. Cases requiring invasive diagnostic procedures are referred to well equipped hospitals in the district capital or to Dhaka city.

To determine for VL sero-prevalence in the inhabitants residing in this thana, a cross-sectional survey was conducted. Two populations were studied; the first (group A) consisted of 9619 inhabitants residing the following 4 villages: Bagan, Awaltia, guzium and Amirabari. Assessment of VL sero- prevalence in this population was carried out at the central laboratory (IEDC&R) in Dhaka during the period December 1991 to April 1992. In order to evaluate feasibility of DAT for mass execution at a rural setting, a second study population (group B) consisted of 5854 inhabitants from the neighbouring 7 villages of Sateropara, Selimpur, Porabari, Raymoni, Narayanpur, Bhawalipur and Konabari was assessed at Trishal Health Complex (THC). In this population, the survey was started on April 1992 and ended on October 1992. the relevant demographic data on either group are presented in Table-9.

Updating of DAT mass application in Bangladesh :

In the first [illegible] covering the period June 1991-May 1992,9619 inhabitants residing Trishal Upazila(Mymensingh district),7328 in Shahjadpur(Sirajganj) and 879 in Teknaf(Cox's Bazar)were assessed for VL sero-prevalence. To reach for the target population (50.000)stated in our initial project proposal, more inhabitants from other villages within the same upazila,other upazilas of the same district or from an additional district(Tangail)have been included in the study (Fig. 6 & Table 6).In the second phase of our project,18664 inhabitants were interviewed and assessed for VL;9511(Mymensingh district:Trishal 5854 and Bhaluka 3657),3074 (Sirajganj district:Shahjadpur),6079(Tangail district:Mirzapur 4998,Kalihati 1081).The demographic data on this population are presented in Table 6a & 6b . For further confirmation of DAT specificity,1646 inhabitants from Teknaf upazila(Cox's Bazar district),an area endemic for malaria but not for VL were also assessed for presence of anti-leishmania antibodies .

FIG 6

Survey & sampling procedures:

In both study populations, the survey was conducted through house-to-house visits and except for absentees, all family members available at the time of the visit were interviewed. The inhabitants comprising the two populations (15473 inhabitants) were of different age (< 1-90 years) and socio-economical standards; more than 90% can be considered as poor. A questionnaire form including the administrative, demographic and presence of VL symptoms was completed on each of the inhabitants. After completion of the questionnaire, blood sampling was done as described earlier (Chowdhury et al in press). A free-falling blood drop (+ 50ul) obtained by finger prick was captured in a glass vial containing 1 ml salt solution (0.9% NaCl). The approximate serum dilution in the sample collected was then 1:50.

The diluted blood samples were transported either to IEDC&R (Dhaka) as in the first study or to Trishal Health Complex (THC) in the second.

For evaluation of DAT performance with antigens prepared from a homologous India L.donovani (D88) or from an authoctonous VL isolate prepared locally (IEDC&R, Dhaka) 175 serum samples were employed: 86 from proven VL cases and 89 from endemic controls with conditions other than VL.

DAT antigen production:

Antigen suspensions employed for screening of both study populations (group A & B) were prepared at the Department of Medical Microbiology, University of Amsterdam (UvA) according to the standard procedures described (Harith et al 1988). The finished antigen suspension, after quality control testing was sent by air without special precautionary measures to IEDC&R in Dhaka. For execution of DAT in THC, the antigen suspension together with the essential material and accessories were transported to Trishal from Dhaka by road.

For preparation of a homologue DAT antigen at UvA (Amsterdam), a well characterized L.donovani strain (MHOM/IN/80/D88) from India was used. All steps including mass culturing, enzyme treatment, fixation, staining and preservation were exactly the same as with the reference strain (L.donovani 1- S).

At the first, local production of DAT antigen was demonstrated to researchers and technical staff members at the Department of Parasitology in IEDC&R (Dhaka). The Leishmania isolate used was from a bone-marrow aspirate of a 12 years male (Pabna district) with VL. Since 3 years it was maintained in NNN or RPMI-1640. For preparation of the antigen, adequate promastigote culture was raised in 200ml volumes of RPMI-1640 medium. Further procedures for antigen processing were the same as

mentioned above. In two other occasions, DAT antigen preparation was carried out independently at IEDC&R (Dhaka).

Execution of DAT at IEDC&R and THC:

Application of DAT on the blood samples collected (15473) was as in a previous study carried out in Bangladesh (Chowdhury et al 1993). From the diluted blood sample (+ 1:50), 3 ul were transferred to a 100 ul volume diluent in well 2 or a V-shapped microtitre plate in order to obtain an approximate start dilution of 1:1600. After addition of antigen and 18 hours incubation at room temperature (25 C - 35 C), the test was read against a back- ground. Titres > 1:3200 were considered indicative for VL.

As for the sera employed for comparison of antigen reactivities, the DAT was performed at a 1:100 start dilution. Further steps in test execution were essentially the same as for blood samples.

The identified sero-positive from group A and B were further assessed for VL. Those with obvious symptoms of VL were subjected to bone-marrow aspiration. Shortly after VL confirmation, sodium antimony gluconate (SAG) was administered according to a WHO regimen of 20mg/Kg Sb(1/m or 1/v) for 20 days (WHO, 1984). Inhabitants with positive DAT titre readings but manifesting no obvious symptoms of VL were scheduled for retesting and follow- up.

3.3 FOLLOW-UP STUDY OF VISCERAL LEISHMANIASIS (KALA-AZAR) SERO-POSITIVES DETECTED DURING MASS SCREENING.

During the period May 1993 and February 1994 after completion of the mass screening campaign for VL, a follow-up study was conducted for clinical assessment and DAT re-testing of 1134 sero-positive individuals identified. These were earlier advised to report to the clinical centres or district hospitals whenever signs or symptoms of the disease appear. Through collaboration with these health authorities in Mymensingh and Sirajganj districts, 542 sero-positives were physically examined for VL and blood samples were collected on filter-paper discs for DAT performance both in the Central Laboratory (IEDC&R, Dhaka) and the Department of Medical Microbiology (U.V.A. Amsterdam). Distribution of the sero-positives followed up was as follows: Mymensingh 324 (Trishal Thana, 291 and Bhaluka 33); Sirajganj (Shahjadpur Thana 218). The results obtained with DAT in the 324 individuals from Mymensingh showed titres indicative for VL in 311 (?1:3200). From the 218 sero-positives followed up in the District of Sirajganj, 204 wee also positive in DAT. VL clinical symptoms were

observed in 191 from the 324 re-examined in Mymensingh and 130 sero-positive from Mymensingh and in 48 of 218 from Sirajganj. SAG was administered in the majority of these patients and in others treatment is proceeding.

The follow-up included also 172 previously treated VL patients during the mass screening. All of these ex-VL patients although recovered from the disease their DAT titres were still in the positive range (1:3200 - > 1:102400). Also during this follow-up 28 sero-positives developed dermal lesions characteristic of post kala-azar dermal leishmaniasis (PKDL). Of those, 15 wee residing in Shajadpur (Sirajganj) and 13 in Trishal (Mymensingh) districts.

3. THE SAMPLE UNITS

There were five sampling units used in this study, they are: (i) Thana (ii) Union, (iii) Ward (iv) Household, (v) Hospital/Dispensaries. From each unit was selected one or more groups of respondents. The TH&FPO, M/Os, and health staffs of thana were the group from thana. At the union level we had the M/O & Medical Asstt. of sub-centre in thana, Chairman Union parishad and informal leader. From the ward level the H/A and finally from the household the healthy and suspected patients of kala-azar from endemic and non-endemic area of kala-azar were selected.

3. THE RESPONDENTS:

There were four groups of respondents of this study. From among the officials were taken (ii) the TH&FPO, (ii) M/O, (iii) H/I AHI, SI, (vi) H/Asstt. The union parishad Chairman, (ii) Informal leader and (iii) Members of Wards (iv) and four groups from (a) Parasitologically confirmed kala-azar pts (b) apparently healthy residents in kala-azar endemic areas, and (c) healthy residents in a non-endemic area (d) sick control-malaria, syphilis, tuberculosis and viral hepatitis. The last groups were from hospital/dispensary.

3.4 SEROLOGICAL STUDY ON POST KALA-AZAR DERMAL LEISHMANIASIS IN BANGLADESH

5011 patients those who have previous history of VL and treated with antimony were included as follow-up study in Pabna, Sirgajganj and Mymensingh to detect PKDL cases during June 1987 to 1990.

3.5 STUDY ON KALA-AZAR IN CHILDREN A STUDY OF 100 PATIENTS

100 clinical, parasitological or serologically positive kala-azar cases under 15 years who had attended the Dhaka Shishu Hospital during 1987-90 were also included in this series to assess the chemotherapy response in children.

3.6 STUDY ON SODIUM ANTIMONY GLUCONATE (SAG) ADMINISTRATION IN VISCERAL LEISHMANIASIS (KALA-AZAR) SEROPOSITIVE PATIENTS

In a prospective study while house to house survey was conducted during June 1987 - May 1988 in Trishal, Fulbaria and Bhaluka under Mymenshingh district, the resident of the study area was listed by unit of house. The total population was enumerated 47600 in 86545 houses among those 23600 were male and 24000 were female those who were screened for presence of fever. Of this population 1790 were selected for further evaluation. 1273 VL cases including 45 PKDL cases were under chemotherapy, 825 (64.8%) were male and 448 (73.2%) females. The vast majority (98.2%) were below 25 years of age.

216 healthy volunteers from Chittagong district where no previous VL cases were reported and laboratory confirmed cases of tuberculosis (50), malaria (77), enteric fever (50) viral hepatitis (51), leprosy (50) and syphilis (30) were taken as VL negative control.

3.7 STUDY ON DIFFERENT TREATMENT SCHEDULE FOR SAG FAILURE CASES OF VISCERAL LEISHMANIASIS (KALA-AZAR) IN BANGLADESH DISCUSSION

The disease (Kala-azar) is induced as reportable disease in Bangladesh since 1987. The rural health assistants were in charge of referring any suspected cases of kala-azar to the health centre. Personal data was collected as well as the exact place of residence. Established kala-azar cases were treated in the health centre. A house was considered as case if at least one of its members had the following characteristics:-

Presence of clinical signs suggestive of kala-azar no- previous history of attack. A positive smear or parasite growth in culture. The patient had resided at least two years in the houses.

SELECTION OF CONTROL The criteria for the selection of controls was geographical, only those houses within 100 m of the case house could be considered as potential controls. At the same time, a house had to have a resident of similar age to that of case (+ 3 years). This was determined by conducting an interview, revealing no history of kala-azar with signs and symptoms, sero-negative and no DDT spraying since 1965.

During the evaluation of DAT at the level of rural health setting in Trishal, Bhaluka under Mymensingh, Kalihati of Tangail and Shahjadpur of Sirajganj district of Bangladesh (study area) 1474 kala-azar cases were detected either parasitologically or serologically. Of these 340 were established both in clinical, parasitological and serologically. All 340 kala-azar cases were treated in the health center, splenic or none marrow) aspiration was done before and after a course of drug therapy. 55 (1617%) out of 340 showed primary and (3.63) secondary unresponsiveness to SAG. All the patients were received 20 days regimen of treatment with dose schedule SAG (Glaxo or B.Well Come). Albert David) in the strength or Antimony 100 mg/ml). 45 patients were from Trishal 10 from Shahjadpur of Mymensingh and Sirajganj. Some patients who were cured both clinically and parasitologically after the rural treatment with SAG had to be re-admitted within 60 days for relapse of kala-azar these were secondary unresponsiveness cases.

After admission to the study patients, were stratified with age and sex and assigned to one of the five treatment groups by a pre-determined schedule. Seriously complicated and dropout patient were excluded from the study. The patients, the physicians carrying for the study investigations evaluated the response to treatment were all aware of the treatment regimen used. All the patients remained in the hospital for 9 weeks after starting treatment and received the standard hospital diet. Response to treatment was evaluated according to standard plan of management of patients with kala-azar. Evaluation during and at the end of 20 days therapy the patients were assessed daily, weekly and the end of completion of therapy. History of fever and its duration and type height, weight and on examination, anaemia, Jaundice, oedema were assessed at the time of investigation during and after treatment. Spleen and liver were recorded as the costal margin. Bone marrow, spleen aspiration were done routinely for demonstration of parasite. Patients were -re-evaluated on clinical, haematological, bio-chemical and serological base line.

The apparently cured were assessed after 16 weeks, 3 months and 12 months. Evidence of drug toxicity was sought by a daily inquiry for new symptoms and fever and by weekly monitoring of haematologica' indices, liver function tests. Clinica' cure

was defined as:- 1) either negative splenic aspiration smear and culture or the absence of splenic aspiration smear or in culture or the absence of splenomegaly, 2) improvement of clinical and laboratory abnormalities, 3) primary unresponsiveness was defined as persistence of parasite in splenic aspirates and no improvement in clinical and laboratory parameters after treatment on the other hand relapse was defined as the reappearance of parasite after treatment on the other hand relapse was defined as the reappearance of parasite in splenic aspirates and recurrence of clinical and laboratory abnormalities after initial cure.

Cases of active kala-azar during cohort follow-up, Trishal, Myn.

3.8 STUDY ON VECTOR (SANDFLY) IN RELATION WITH VL (KALA-AZAR) AND EFFECT OF DDT ON ITS CONTROL

A cross-sectional study followed by longitudinal study was conducted in Trishal thana under Mymensingh district (Fig.). The area of Trishal thana was estimated about 337.77 km2. The land except a few hillock most of the area is low laying and situated to the north of Dhaka city on the bank of river Brahmaputra having 101 villages where there was sero-prevalence 3.46% during 1987-91.

There were 714 people registered in 102 families staying in 95 houses which composed of 374 males and 340 females. Followed up an survey conducted every month from April 1992 to April 1993.

Period of Survey

During June 1987 - May 1988, 2400 houses were visited both in intervention and control area. In July 1988 DDT sprayed in the same area. Entomological investigation was also being done for detecting sandfly especially P.argentipes before DDT spraying in the village Awaltia under Trishal thana. Similar entomological survey was carried out in control area during the period of June, '89 to May, '90. Another survey was conducted both in the intervention and control area where 1000 houses were visited in each area. 3rd survey was conducted in June 1992 to Dec. 1992 for detecting kala-azar cases and collecting sandfly.

3.8.1 ENTOMOLOGICAL INVESTIGATION

Collection of Sandfly

In the village of Awaltia of Trishal thana, sandflies were collected monthly by hand capture with aspirator during morning (07.00 hrs to 09.00 hrs) and evening (18.00 hrs to 20.00 hrs) from both human dwelling and cattle shed. Two hours spent for each type of collection thus total eight hours per month. Besides that night biting collections were carried out using two human baits, one in human dwelling and one in cattle shed. Half night collection done for 30 minutes in each hour. Searching was being made in resting places of sandfly such as cracks and crevices of walls of cattle sheds and human bedrooms and corridors. All the collected sandflies were identified under microscope and the blood fed ones were separated for observing host preference.

Larval search Scrapings from floor and mud wall of human dwelling and cattle shed, were collected once a month during lst survey to see the presence of larva. Scrapping samples were collected in earthen pots and kept for observation for 30 days. Water was poured regularly into them to keep the pots wet.

3.8.2 Host preference

Blood meal analysis was done using Dot ELISA for detection for human, bovine or other animal blood meal. The Dot ELISA kits were provided by the courtesy of WHO/TDR, Geneva. The abdomen of the blood fed sandfly was squashed on what man paper .91 filter paper. Identification number and date of collection were recorded in each sample. The filter paper was calibrated, punched and placed in 1ml PBS/Tween 20 for a period of 1 hour or over night. The test was carried out as per instruction provided by Biokema, Immunology department, WHO, Geneva, Switzerland. Interpretation was made by observing the diagnostic reaction on the anti-human and antibovine 1gG tracks in wells. Faint to strong band in the wells present on the upper part of the microtitre plate indicates human blood and faint to strong band in the wells present on the lower part of the microtitre plate indicates bovine blood. If no reaction observed insect fed on any animal other than human or bovine.

3.9 A COMPARATIVE SEROLOGICAL STUDY FOR MEASURING THE ANTIBODY OF LD BODY USING DAT, iFAT, ELISA & IHA IN HIGH AND LOW ENDEMIC AREA OF KALA-AZAR IN SELECTED AREA OF BANGLADESH

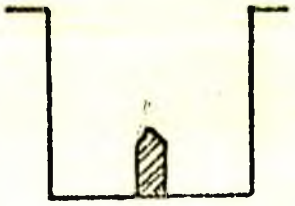
3.9.1 ANTIGEN

Antigen for Enzyme Linked Immuno Sorbent Assay (ELISA). The entire promastigote of local strain of L.D. Body was used as antigen for ELISA. The promastigote were grown in eagle's media, RPMI-1940 media and in NNN media. The growth was harvested on 7th to 10th post inoculation day by centrifugation at 550 for 10 minutes and washed thrice with PBS. The promastigote were killed with 4% formalin and adjusted to 5x10 ml in 0.05 M carbonate/bi- carbonate buffer pH9.6(in which further dilution were also made). They were used immediate or stored at -20 degree C until needed.

Figure The indirect ELISA for measuring antibody

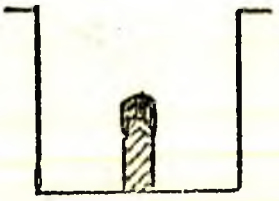
1. ANTIGEN ADSORBED TO PLATE

WASH



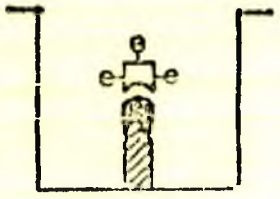
2. ADD SERUM ANY SPECIFIC ANTIBODY ATTACHED TO ANTIGEN

WASH

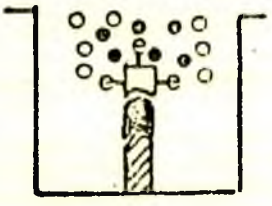


3. ADD ENZYME LABELLED ANTI-GLOBULIN WHICH ATTACHED TO ANTIBODY

WASH



4. ADD SUBSTRATE



AMOUNT HYDROLYSED = AMOUNT OF ANTIBODY PRESENT

(Reproduced from Voller et al., 1976, Bull. W.H.O., 53, 55-65)

Antigen for Direct Agglutination Test (DAT)

The antigen for direct agglutination test (A.E. Harith) which was prepared at Royal Tropical Institute (Harith et al) kindly provided by Dr. A.E. Harith through WHO (Geneva). The author is also using antigen, which is being prepared in the department of Parasitology, NIPSOM/IEDC&R.

Antigen for Indirect Haem-Agglutination Test (IHA)

The IHA test was done by using the supplied commercial cellognost leishmania kit (Lypilized sensitized cells positive and negative control sera)

3.9.2 METHOD FOR ELISA:

Polystyrenemicrotitre plates (Dynatech USA) were coated with whole promastigote as antigen Voller et al(1980) in 100 ul/well of 0.01 M sodium bicarbonate buffer pH9.6 after incubation at 37 degree C for 2 hours or the coating was allowed to proceed overnight as the antigen solution was decanted and the plates washed thrice for 3 minutes each at room temperature with 0.01M PBS + 0.05% tween pH7.2. Then 100ul of 1% BSA in PBS was added per well & the plates incubated for one further hour at 37 C and washed thrice as before. The test sera and control sera were added in a single dilution (1/20) and considered to be positive was above that of the maximum for the normal controls. They were incubated one hour at 37 C then washed thrice. After washing 10 ul of conjugate which was prepared by glutaraldehyde linkedalkaline phosphates (sigma) to antihuman IgG instead of peroxidase and after a further incubation at 37 C for one hour and then washing, the substrate P. Nitrophynyl phosphate (sigma) in diethylamine was added. After 30 minutes incubation at room temperature in the dark, the reaction was stopped with NaOH and the absorbance value were read by a micro-ELISA into reader (Dynatech MR 580), using 490 nm as test wave length and 405 nm as references.

3.9.3 METHOD FOR DOT ELISA

Supplied Dyana tech micro-titre plates were dipped in 15 ml PBS milk or wet in antibody side (marked), clamped the plate and incubated after filling with 100 ul PBS-milk is remained as negative control and another one as positive control 1-bovine control another 2-human serum positive control, incubated for 45 minutes at ambient temperature (22 c) then 5 drops of PBS were added to each well with a pasteur pipette and shake the contents. Next the plate wells was rinsed 4-x and disassembled the incubation manifold under gentle running tap water, washed 2x5 mins.antibody side facing down in the plastic box containing 15 ml PBS-milk, again incubated dot ELISA plate in the box containing 12 ml conjugated PBS milk mixtures. Washed 2x5 min in 15 ml PS. Incubated plate antibody facing down, in 15 ml peroxidase substrate

mixture. Maximum reaction is achieved after about 30 minutes. Rinsed in water then it was evaluated usually by observing the reaction on wet Dot ELISA Plate. Interpretation; were made by observing diagnostic reaction (on the anti human and anti-bovine IgG tracks in the Wells) Faint to strong band in upper part of the well. Insect fed on human showed faint to strong band in the lower part of the well. Insect fed on bovine, minimum and if no reaction was observed: insect fed on other animal.

3.9.4 METHOD OF DAT

The prepared trypsin-treated antigen was used for this test. The test was performed in 'V' shaped microtitre plate wells with a starting serum dilution of 1:100 in 1% of foetal bovine serum in physiological saline as well as distilled water on the other hand the same serum dilutions were used consisted of sodium chloride (0.9%), sodium Citrate (1.0%) dissolved in distilled water, to which Gelatin, instead of foetal bovine serum was added (0.2%) (W/V). The mixture was warmed (56 C for 10 mm) to dissolve gelatin and left to cool at room temperature. The titre level was observed almost same using either 0.2% (W/V) gelatin or 1.0% (V/V) FBS in the serum diluent. Then two fold dilution of all sera were made, well-1 was used as a negative control only 50 ul diluent was added, 50 ul of antigen suspension were then added to each well, after gentle shaking of the flask. The plates were left for 18 hours at room temperature after gentle shaken by hand on plain surface for 30 seconds. The test was read visually against a white back ground. The end point was estimated by a clear sharp edged as negative control. The used microtitre plate was reused after cleaning with warm tap water and 0.25% sodium dodecyl sulphate solution, rinsed with distilled water and dried.

3.9.5 METHOD OF IHA

The IHA test was done using the supplidcellognost-Leishmenia kit (lyophilized sensitive cell positive and negative control sera) according to the instructions supplied by the manufacturer, Bearing work diagnostics Frankfurt, Germany. For the economical point of view we used presumptive test (single dilution of 1:40) for healthy and sick control. The quantitative test was done on all the presumptive test positive sera.

3.9.6 METHOD OF ALDEHYDE TEST (AT): All the sera were tested by aldehyde test (Napier 1927). A drop of 40% formaldehyde was added to each serum sample and was observed for jellification and opacity. Jellification and opacity within 20 minutes was taken as strongly positive. But observed a jellification and opacity was formed even within 1 minute in same cases.

A comparative serological study for measuring the antibody of LD BODY using DAT, IFAT, ELISA & IHA in high and low endemic area of KALA-AZAR in selected area of Bangladesh: A single cross-sectional study of the anti LD body serological profiles of populations from two areas with varying levels of kala-azar endemicities i.e. high and low as determined by Chowdhury et al 1991.

A total of 862 specimens were collected from 2 villages Kaijuri and Madla of Shahjadpur under Sirajganj district of Bangladesh a well known endemic area since 1981 (Ahmed et al 1983). Sera were collected by finger-prick in heparinized microhematacrit tubes or with whatman paper 3 filter paper from the areas under investigation were assayed. 532 of these came from the high endemic area, while 330 were collected from the low endemic area. The collection of these sera was done by a single blood sampling survey was carried out during January 1990- 91. The samples were taken from all the residents in the study area where whose ages ranged from six months to 78 years. Fifty sera taken from healthy blood donors from a non endemic area (Chittagong) were used as controls for all the four tests. These were preserved in 50% glycerol and kept at - 20C until used. All normal control sera were, however used only as quality control in the laboratory and were not pooled in the analysis of the results. Antigen were used for those tests as mentioned.

3.10 SERO-CONVERSION USING DAT AS COHORT STUDY IN BANGLADESH

During 1991-92, 862 population were selected from high and low endemic areas of kala-azar village of Shahjadpur thana under Sirajganj district for the evaluation of the different serological techniques (DAT, IFAT, ELISA & IHA), currently used for diagnosis of kala-azar in other countries also by different authors in very limited scale or in the laboratory.

During April 1992 - April 1993, 714 peoples were registered in the cohort study in 102 families saying in 95 houses in Guzium and Radhakanai of Trishal thana which composed of 374 males and 340 females out of them 83 were in the group of migrates which were excluded for data analysis. The cohort study was conducted to observe the seroconversion and seasonal distribution of kala-azar.

3.1-10 QUESTIONNAIRES

There were 4 forms of questionnaires for interview the individual sample. Questionnaire form 1 (Annex.1) included the questions concerning.

* General information of individual sample.
* Migration and home village/district.
* Past history of kala-azar, Malaria, Enteric fever, Tuber- culosis and other auto-immune disorder diseases.
* Occupation in general.

This questionnaire was used for interviewing every people at the first enrollment as the studied subject both the local resident and the migrate.

Questionnaire form 2 (Annex.2) included the Questions concerning

* Monthly history of sickness especially kala-azar or fever (signs and symptoms).
* Previous history of treatment/previous medication.
* Self medication.
* Sleeping behaviour (time of sleeping, net).
* The body temperature of individual sample.
* Serological response - Results.
* Parasitological results.
* Prognosis of the disease by chemotherapy.
* Presence of sandfly.

These questionnaire was used for monthly interview to every study subjects.

Questionnaire form no.3 (Annex.3) included the questions concerning

* History of kala-azar.
* Evaluation of chemotherapy of different groups including control.
* Antimony resistance to LD body and alternate treatment.
* History of sickness/reaction.
* Body temperature.
* Serological response.
* Weight.

This form was used for follow-up the cases and to detect the PKDL.

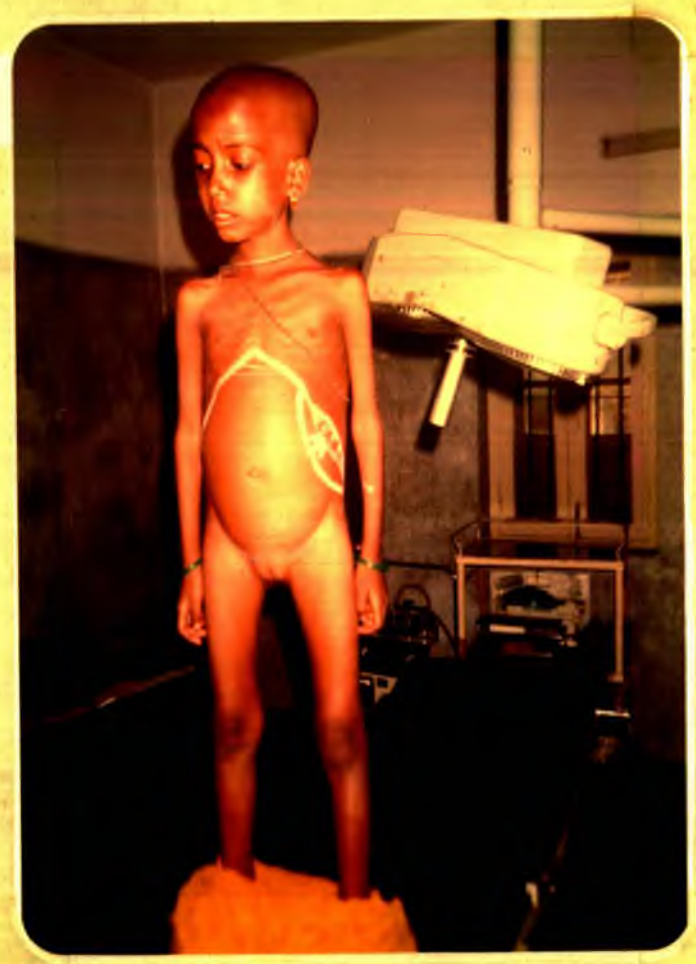
Questionnaire form no.4 (Annex.4) included the questions concerning

* Treatment schedule.
* Signs and symptoms.
* Body weight.

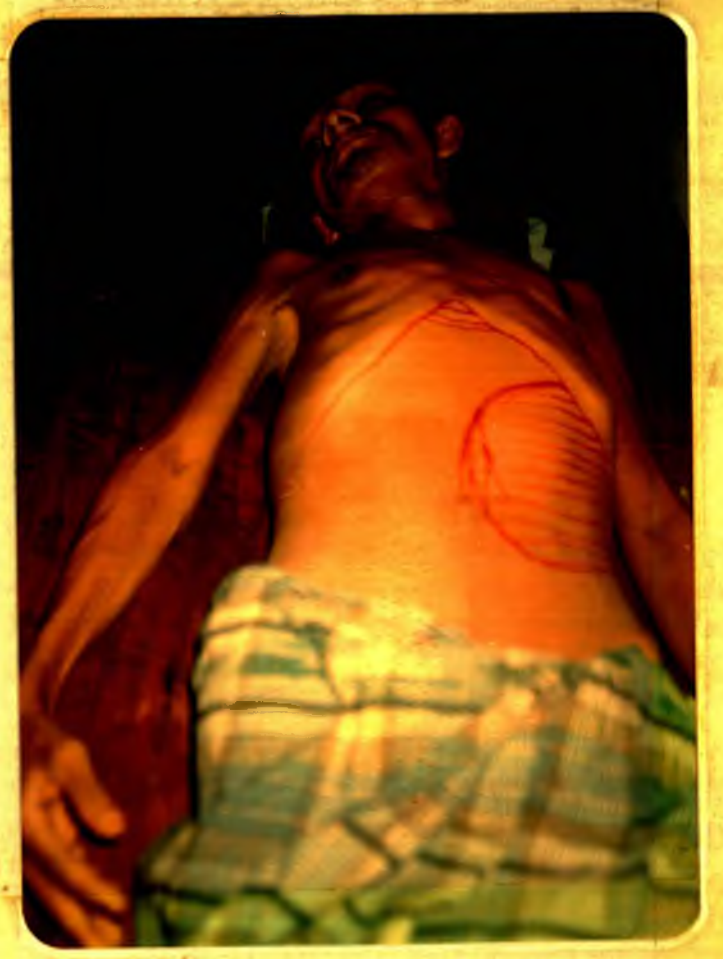
* Temperature.
* Haematological changes.
* Serological changes.
* Prognosis of the patients.

This form was used during and after treatment as follow-up for 2-3 years.

NB: METHODS OF STATISTICAL ANALYSIS AND INTERPRETATION ARE PRESENTED IN CHAPTER 4.9 & 4.10 WITH RESULTS.



Case No ..... Active Kala-azar case on admission, Miss Kamala Khatun, -10 yrs D/O Nabi Hussain, Trishal, Myn.



Case No..... Active Kala-azar case on admission Mr. Moin Uddin, - 38 yrs. S/O Mubarak Hussain, Guzium, Trishal, Myn.

CHAPTER 4

RESULTS

4.0 CHAPTER-4 : RESULTS

4.0 RESULTS

4.1 MASS APPLICATION OF DIRECT AGGLUTINATION TEST (DAT) FOR VISCERAL LEISHMANIASIS (VL) IN BANGLADESH

Production of DAT antigen in considerably large quantities up to 4000 ml as a single batch proved to be manageable. Performance of the antigen so produced was highly concordant with that obtained in previous studies with miniature quantities of 100-500ml. Reference VL negative sera (235) including those from patients with African trypanosomiasis (34), Chagas' disease (87) and auto-immune disorders (32) reacted with the antigen under study at significantly low titres (<1:800). In contrast, extremely high titres ranging 1:3200 - > 1:52428800, comparable to those obtained in previous studies, were obtained in all 73 VL sera tested (Table 4A). All 6 antigen batches prepared followed the same procedures had almost similar titres in quality control tests (Data not shown). Stability of the produced batches was evidenced by absence of auto-agglutination after transportation under the prevailing temperatures (22°C - 38°C) and storage for considerable periods (6-8 months) at +4°C. Collection of whole blood samples by finger-prick from the population under study (17826) proceeded without much difficulty and there was more willingness to cooperate than by venepuncture sampling. Application of DAT on the samples collected was more easier and time saving compared to filter-paper method which required qualification and 6-12 hours dilution steps. At the dilution employed (1:1600), presence of erythrocytes did not interfere with agglutination reactions and DAT results were easily read as with serum samples (Table 4B). At least 200 samples per day could be screened by a moderately trained technician. Primarily, due to availability of antigen in such large quantities, faster and convenient procedures of sampling and simplicity in DAT performance were we able to assess for VL sero-prevalence in this large population within the period assigned.

An anti-Leishmania antibody prevalence of 4.40% and 6.75% was obtained in Trishal and Shahjadpur thanas respectively (Fig.3 & 4). In these two endemic areas, antibody to the parasite was more prevalent (2.56%) - 4.5%) in < 1-20 years age group; those between 12-20 years were most affected. The lowest prevalence rate (0.12% - 0.78% was among younger children of < 1-5 years.

No significant difference in prevalence was observed between male (2.12%) and female (2.28%) populations of Trishal thana; in Shahjadpur however, a slightly higher rate of 3.70% was obtained in males if compared to 3.06% in female. Out of 879

inhabitants screened in the non-endemic thana of Coxes Bazar, only 3 (0.34%) inhabitants were found positive in DAT (Fig.5). From our interview with them, we could not exclude the possibility that exposure to Leishmania parasite was due to a stay in other districts. Naturally, even with the known high specificity of DAT, the possibility of cross-reaction with malaria or other infections should not be dismissed.

Clinic symptoms such as anaemia, fever and splenomegaly were frequently encountered with in this survey. However, DAT positivity together with presentation of at least one of the mentioned clinical symptoms suggested suspicion for VL at varying degrees. Among 379 inhabitants belonging to this group, 125 on grounds of severity of the symptoms were subjected to bone-marrow aspiration. L. donovani was demonstrated in 29 of them. Sodium antimony gluconate according to a WHO regimen, was started on all parasitologically confirmed (29) and the unconfirmed cases (96) on account of the obvious VL symptoms and DAT positivity; all 125 responded favourably to treatment. Seropositive inhabitants with mild (254) or no clinical symptoms (539) of VL were considered for re-testing and follow-up.

Updating of DAT mass application in Bangladesh:

In the first phase of study covering the period June 1991 May 1992, 9619 inhabitants residing Trishal thana (Mymensingh district), 7328 in Shahjadpur (Sirajganj) and 879 in Teknaf (Cox's Bazar) were assessed for VL sero-prevalence. To reach for the target population (50.000) stated in our initial project proposal, more inhabitants from other villages within the same thana, other thana of the same district or from an additional district (Tangail) have been included in the study (Fig.6 & Table 6). In the second phase of the study, 18664 inhabitants were interviewed and assessed for VL; 9511 (Mymensingh district; Trishal 5854 and Bhaluka 3657), 3074 (Sirajganj district; Shahjadpur), 6079 (Tangail district; Mirzapur 4998, Kalihati 1081). The demographic data on this population are presented in Table 6a & 6b. For further confirmation of DAT specificity, 1646 inhabitants from Teknaf thana (Cox's Bazar district), an area endemic for malaria but not for VL were also assessed for presence of anti-leishmania antibodies.

Table 3 **DAT antigen batches prepared during the period July 1991 - March 1993.**

Antigen batch Nº	Mass culture Vol. (ML)	Antigen Vol. (ML)	Number of tests (1:102400)
31/07/91	4800	1400	2240
02/09/91	6000	3000	4800
08/10/91	5000	2500	4000
26/11/91	2000	1120	1176
17/12/91	7000	3300	5280
16/03/92	8000	4000	6400
15/06/92	4000	2200	3520
21/08/92	6000	2800	4480
23/09/92	7000	2700	4320
13/10/92	6000	3400	5440
19/11/92	3500	2800	4480
01/12/92	3000	2500	4000
01/03/93	6000	3500	5600
07/03/93	5000	2900	4640

Table 4A **Reliability of DAT antigen produced at large scale as a single batch of 3300 ml.**

Clinical status	Number of sera tested*	Number of sera with the indicated DAT titre (between parenthesis)**
Healthy control	19	16 (≤ 1:200), 3 (1:400)
Toxoplasmosis	10	10 (≤ 1:200)
Tuberculosis	33	33 (≤ 1:200)
Auto-immune disorders	32	32 (≤ 1:200)
Chaga's disease	87	81 (≤ 1:200), 5 (1:400), 1 (1:800)
African Trypanosomiasis	34	32 (≤ 1:200), 1 (1:400). 1 (1:800)
Cutaneous Leishmaniasis	20	15 (≤ 1:200), 5 (1:400)
Visceral Leishmaniasis (VL)	73	1 (1:3200), 1 (1:6400), 1 (1:25600), 70 (1:102400 - ≥ 1:52428800)

* Serum samples previously tested against antigen batches prepared in volumes of 100-500 ml (Harith et al, 1986; 1987 & 1988)

** DAT cut-off titre is 1:3200

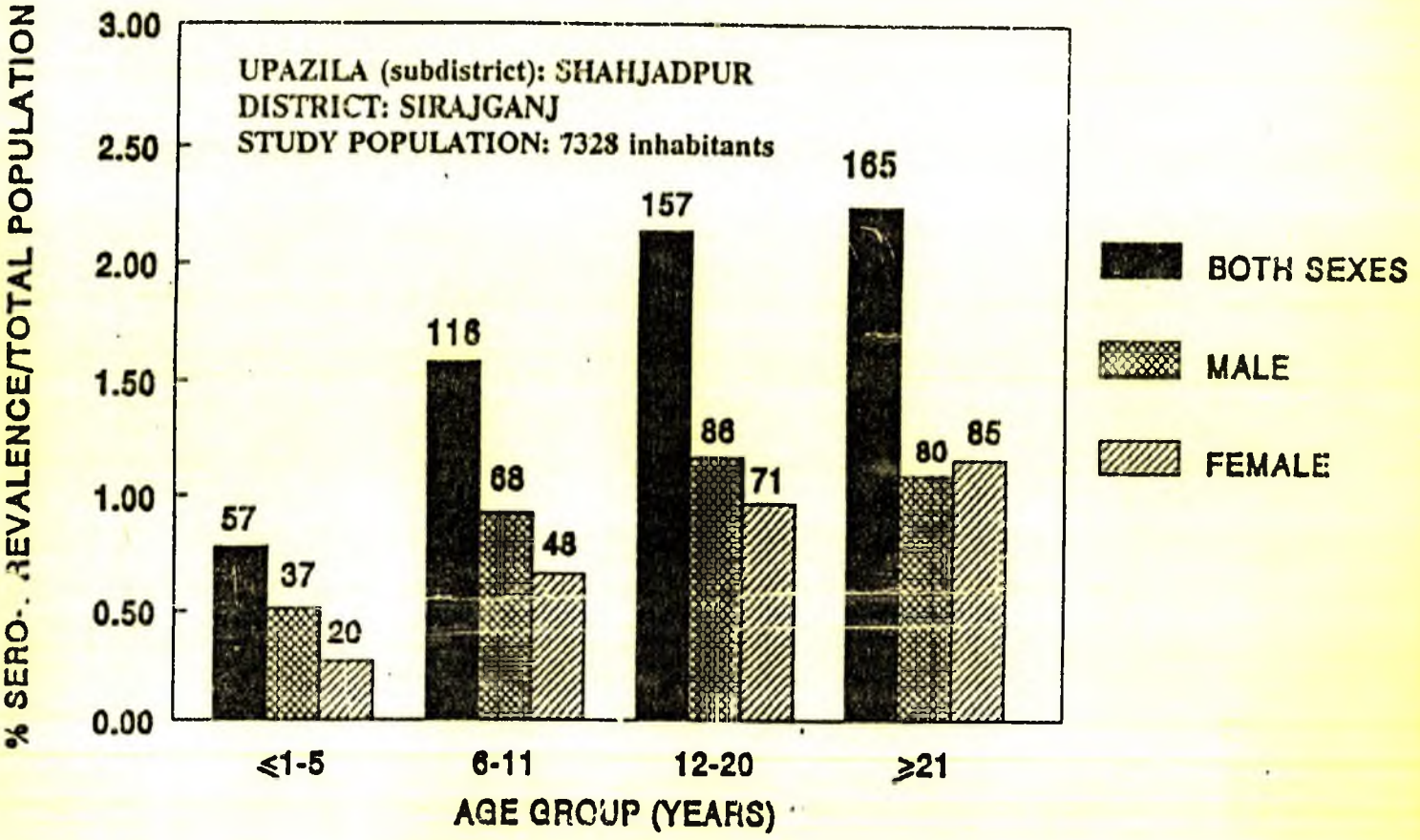
Table 4B **Comparison of DAT readings obtained in serum and whole blood samples collected from the same VL patients and apparently healthy inhabitants**

Laboratory code Number	DAT TITRES (FULL-OUT TITRATION)**		
	Serum sample reading	Whole blood sample reading	
		IERC&R (Dhaka)	UVA (Amsterdam)
T1	1:12800	1:6400	1:6400
T2	1:3276800	1:6553600	1:6553600
T3	1:3200	1:3200	1:3200
T4	1:1638400	1:1638400	1:1638400
T5	1:1:12800	1:3200	1:6400
T6	1:102400	1:51200	1:102400
T7	1:102400	1:102400	1:102400
T8	1:204800	1:102400	1:204800
T9	1:1600	1:1600	1:1600
T10	1:102400	1:51200	1:102400
H1	< 1:100	< 1:1600	< 1:1600
H2	< 1:100	< 1:1600	< 1:1600
H3	1:102400	1:102400	1:102400
H4	< 1:100	< 1:1600	< 1:1600
H5	< 1:100	< 1:1600	< 1:1600
H6	< 1:100	< 1:1600	< 1:1600
H7	1:800	< 1:1600	< 1:1600
H8	< 1:100	< 1:1600	< 1:1600
H9	< 1:100	< 1:1600	< 1:1600
H10	< 1:100	< 1:1600	< 1:1600

* T: Treated VL patients (Trishal, Mymensingh); H: Apparently healthy citizens (from the same endemic area)

** DAT cut-off titre is 1:3200 for either serum or whole blood sample.

FIG 5



Distribution of positive DAT titres according to age and sex in the study population of Shahjadpur upazila, Sirajganj district.

Table 5: Clinical status of sero-positive inhabitants identified in the survey

Upazila (sub-district)	sex	Sero-positives symptomatics	Sero-positives Asymptomatics	VL cases diagnosed	VL cases treated	sero-positives for re-testing and follow-up
Trishal	M	48	156	29	29	175
	F	46	173	21	21	198
Shahjad-pur	M	150	121	54	54	217
	F	135	89	21	21	203
Total sero-positives		379	539	125	125	793

Table 6A Study areas and population covered during the period June 1992 - May 1993.

District	Upazila (sub-district)	Sex	Age group (years)				Total (M/F)	
			≤ 1-5	6-11	12-20	21-≥ 90	Number	%
Mymensingh (N=9511)	Trishal (N=5854)	M	520	702	476	848	2546	43.49
		F	491	723	552	1542	3308	56.51
	Bhaluka (N=3657	M	288	453	317	488	1546	42.28
		F	258	468	349	1036	2111	57.72
Tangail (N=6079)	Mirzapur (N=4998)	M	255	617	796	958	2626	52.54
		F	261	580	609	922	2372	47.46
	Kalihati (N=1081)	M	91	214	111	122	538	49.77
		F	90	178	75	200	543	50.23

Table 6B Study areas and population covered during the period June 1992 - May 1993.

District	Upazila (sub-district)	Sex	Age group (years)				Total (M/F)	
			≤ 1-5	6-11	12-20	21-≥ 90	Number	%
Sirajganj (N=3074)	Shajadpur (N=3074	M	254	776	523	389	1942	63.18
		F	179	462	273	218	1132	36.82
Cox' Bazar (N=1464)	Teknaf (N=1464)	M	146	246	206	190	788	53.83
		F	113	205	116	242	676	46.17

Table 7 Total study areas and population covered in the survey from June 1991 to May 1993

District	Number of upazilas	Total population covered	Sex	Age Group (years)				Total (M/F)	
				≤ 1-5	6-11	12-20	21-≥90	Number	%
Mymensingh	2	19130	M	1755	2098	1701	2867	8421	44.02
			F	1753	2213	1823	4920	10709	55.98
Sirajganj	1	10402	M	1163	1886	1280	1355	5684	54.64
			F	966	1311	944	1417	4718	45.36
Tangail	2	6079	M	346	831	907	1080	3164	52.05
			F	351	758	684	1122	2915	47.95
Coxe's Bazar	1	2343	M	218	360	326	344	1248	53.27
			F	163	315	216	401	1095	46.73
Total	7	37954	- (%)	6745 (17.8)	9772 (25.7)	7931 (20.4)	13506 (35.6)	37954	-

Table 8A Distribution of DAT positives versus sex and age

SEX:

District	Total sero-positives	Sero-positive Males		Sero-positive Females	
		N=	(%)	N=	(%)
Mymensingh	739	385	52.10	354	47.90
Sirajganj	687	390	56.77	297	43.23
Tangail	38	20	52.63	18	47.37
Coxes Bazar	10	6	60.00	4	40.00

AGE:

District	Total sero-positives	Sero-positive ≤ 20 years		Sero-positive ≥21 years	
		N=	(%)	N=	(%)
Mymensingh	739	442	59.81	297	40.19
Sirajganj	687	478	69.58	209	30.42
Tangail	38	30	78.95	8	21.05
Coxes Bazar	10	6	60.00	4	40.00

Table 8B Sero-prevalence of anti-leishmania antibodies in the four districts studied in Bangladesh

District	Number of upazilas	Total population covered	DAT POSITIVES (≥ 1 : 3200)		Previous grading based on VL occurrence *
			Number	(%) of total population	
Sirajganj	1	10402	687	6.60	Highly endemic
Mymensingh	2	19130	739	3.86	Highly endemic
Tangail	2	6079	38	0.63	Moderately endemic
Coxe's Bazar	1	2343	10	0.43	Non-endemic

* According to reports from Ministry of Health and Family Planning (Bangladesh)

Table 8C Clinical status of DAT positive (titres ≥ 1 : 3200) inhabitants identified in the mass screening survey

Population covered	DAT POSITIVES (total = 1474)		Bone-Marrow Aspirate (symptomatics = 340)		Established VL diagnosis (n = 340)		Positive respose to SAG	Sero-positives for re-testing and follow-up
	Sympto-matics	Asympto-matics	Positive	Negative	Positive aspirate	Positive DAT + symptoms		
37954	594 (40.30%)	880 (59.70%)	180 (52.94%)	160 (47.06%)	180 (52.94%)	340 (100.0%)	340 (100%)	1134

Table 9A VL sero-prevalence versus sex in the two study populations of Trisha

Population surveyed	DAT titres		VL sero-prevalence				Positivity/ Total identified	
			MALE		FEMALE		MALE	FEMALE
	≤ 1:1600	≥ 1:3200	N =	(%)	N =	(%)	(%)	(%)
9619 (group A)	9196 (95.6%)	423 (4.4%)	204	2.1	219	2.3	48.2	51.8
5854 (group B)	5639 (96.3%)	215 (3.7%)	124	2.1	91	1.6	57.7	42.3

Table 9B VL Sero-prevalence versus age group in the two study populations of Trishal.

Total sero-positives	VL-sero-prevalence versus age group/total population (years)								Positivity/ total identified	
	≤ 1 - 5		6 - 11		12 - 20		21 - ≥ 90		≤ 1 - 20 years (%)	21 - ≥ 90 years (%)
	N=	(%)	N=	(%)	N=	(%)	N=	(%)		
Group A N = 423	23	0.24	84	0.87	139	1.45	177	1.84	58.15	41.84
Group B N = 215	30	0.51	54	0.92	48	0.82	83	1.42	61.39	38.6

Table 9C Confirmation of VL in the sero-positive symptomatic inhabitants identified in the two study populations of Trishal

Number of symptomatic sero-positives	Bone-marrow aspirates		Clinically & Serologically positives	Treated	Inhabitants for retesting and follow-up
	Performed	Positive			
94	50	15	35	50	44
215	215	151	64	120	64

Table 6A Study areas and population covered during the period June 1992 - May 1993.

District	Upazila (sub-district)	Sex	Age group (years)				Total (M/F)	
			≤ 1-5	6-11	12-20	21-≥ 90	Number	%
Mymensingh (N=9511)	Trishal (N=5854)	M	520	702	476	848	2546	43.49
		F	491	723	552	1542	3308	56.51
	Bhaluka (N=3657	M	288	453	317	488	1546	42.28
		F	258	468	349	1036	2111	57.72
Tangail (N=6079)	Mirzapur (N=4998)	M	255	617	796	958	2626	52.54
		F	261	580	609	922	2372	47.46
	Kalihati (N=1081)	M	91	214	111	122	538	49.77
		F	90	178	75	200	543	50.23

Table 6B Study areas and population covered during the period June 1992 - May 1993.

District	Upazila (sub-district)	Sex	Age group (years)				Total (M/F)	
			≤ 1-5	6-11	12-20	21-≥ 90	Number	%
Sirajganj (N=3074)	Shajadpur (N=3074	M	254	776	523	389	1942	63.18
		F	179	462	273	218	1132	36.82
Cox' Bazar (N=1464)	Teknaf (N=1464)	M	146	246	206	190	788	53.83
		F	113	205	116	242	676	46.17

Case No. ... Miss Rina during follow-up after treatment (SAG B. Wellcame, London) D/O Idris Ali, Vill. Awaltia, Trishal, Myn.

Case No. Mr. Bachu Mian, 16 yrs S/O Keramat Ali, Vill. Alahari, Trishal, Myn.

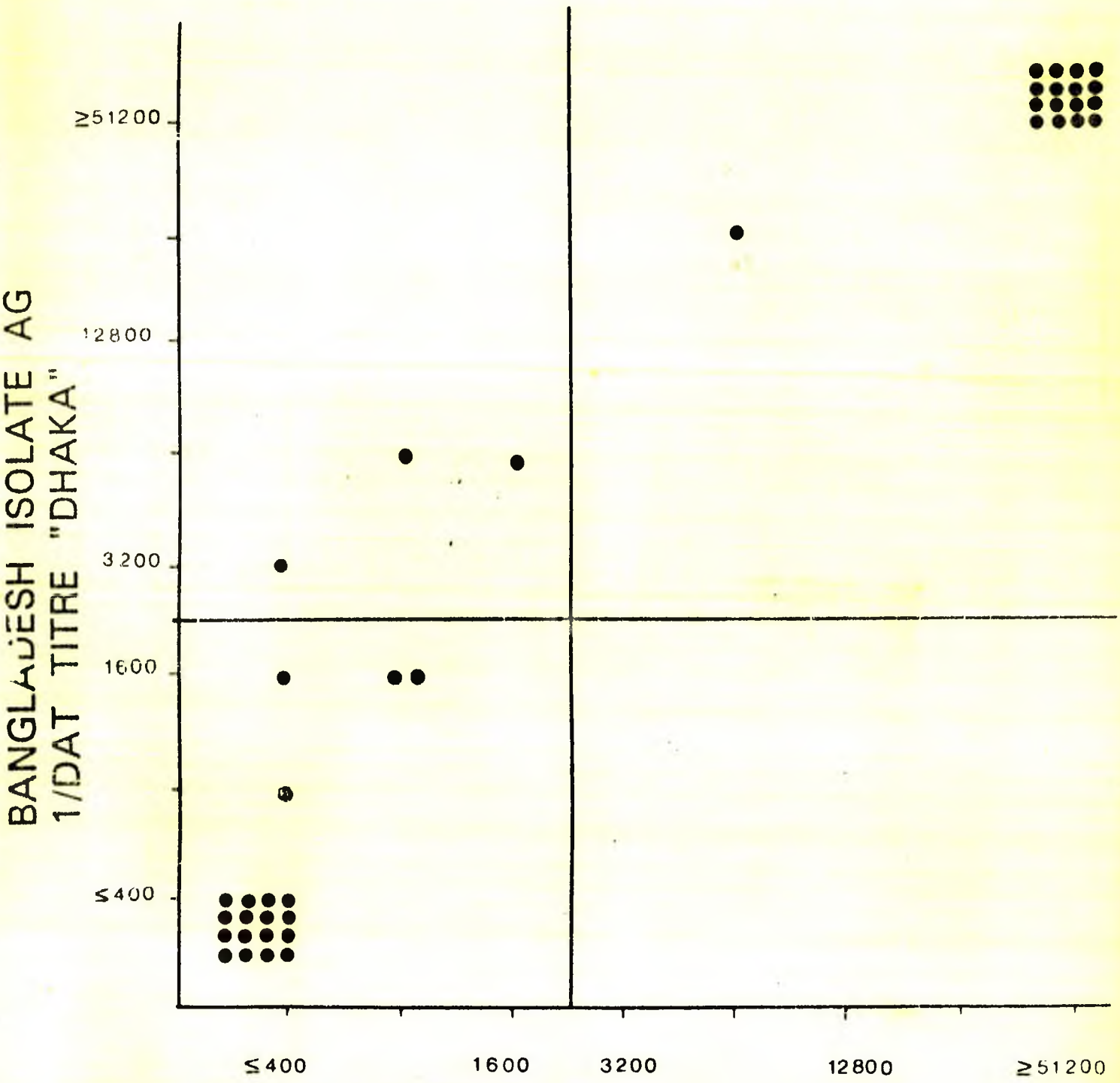
Case No.... Follow-up during treatment case control in Health complex, Trishal, Mymnshingh district.

4.2 APPLICABILITY OF DIRECT AGGLUTINATION TEST (DAT) AT A RURAL HEALTH SETTING IN BANGLADESH AND FEASIBILITY OF LOCAL ANTIGEN PRODUCTION

Execution of DAT at Trishal Health Complex proceeded without significant difficulties although availability of an accurate balance and water-bath could have had made the work more easier. However, prior weighing of ingredients at the central laboratory (IEDC&R) and use of fetal bovine serum had resolved the problem. Storage of the made antigen did not constitute a problem due to presence of a fridge. Further steps starting from blood sampling up to reading of DAT were equally manageable in both studies. Prevalence of agglutinatinating anti-leishmania antibodies in the two study populations (Group A & B) is presented in Table 9. In group A , the overall prevalence (4.40%) is somewhat higher than in group B(3.67%). While in the male population of both groups DAT positivity was similar(2.12%), in the female population of group A higher rate (2.28%) was obtained compared to group B(1.55%). Regardless of sex, inhabitants younger than 21 years in either population seemed to be more affected (2.25% - 2.56%) than those in the age group 21-90 years (1.42% - 1.84%).

In both study groups, inhabitants who revealed positive bone-marrow aspirates (166) or being clinically diagnosed as VL cases on grounds of symptoms (99), scored clearly positive titres (> 1:3200). Neither bone-marrow aspiration nor the manifested clinical symptoms independently was decisive for VL diagnosis (Table 10). DAT results however, indicated VL in all 309 including those with negative parasitology either in group A (35) or B (64). The immediate positive response to SAG observed in those patients indicated prior L. donovani infection. From the remaining sero-positives (108), 64 are under treatment and 44 undergoing re-testing or follow-up.

FIG 3

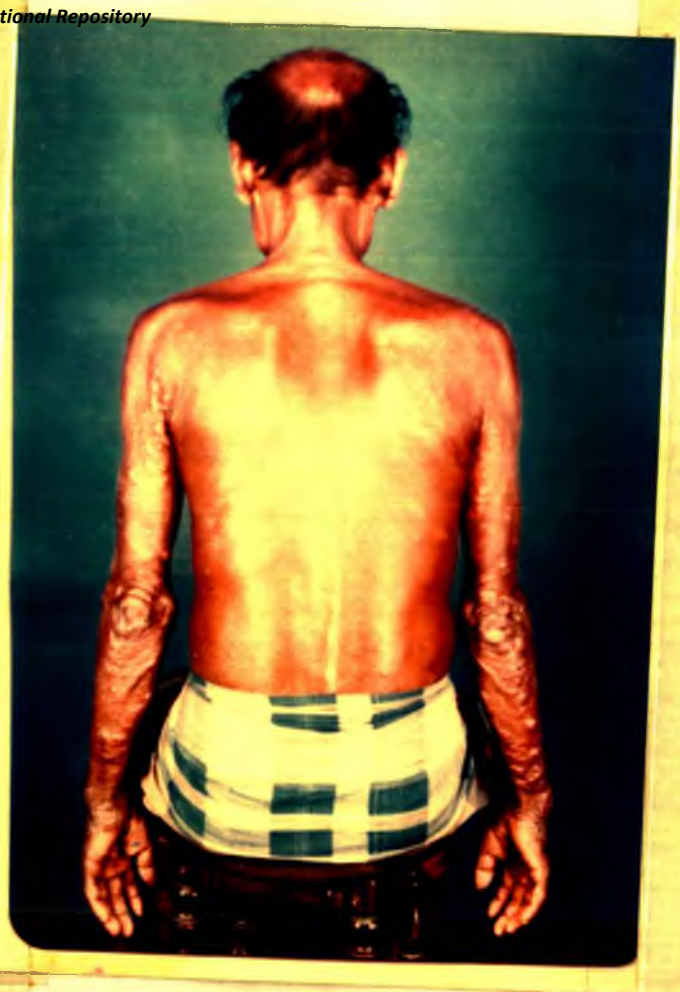


81 Ex

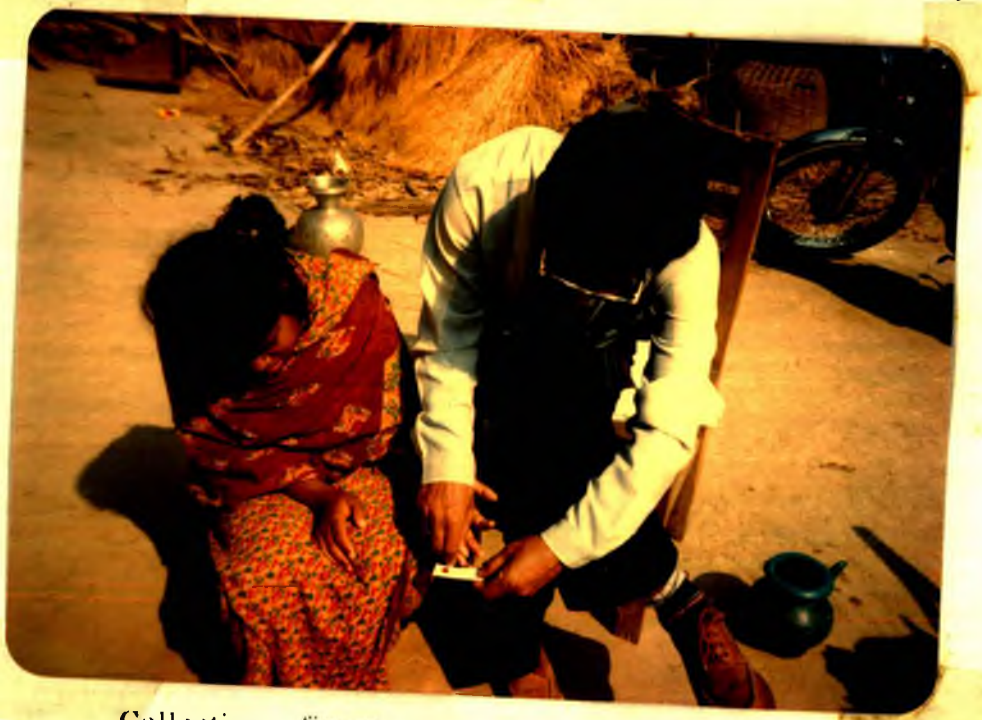
DAT TEST IN RURAL SETTING TRISHAL MYN.

Showing the collected ratus ratus from infected house and desected spleen and liver for LD body.

Case No. ... Miss Manjira Khatun, 25 yrs
D/O Mamataz Ali, Vill. Kaijury, Shahjabpur, Shirajganj.



Case No. ... Mr. Nurul Islam, 35 yrs. (P.K.D.L.)
S/O. Haji Tamijuddin, Trishal, Myn.



Collecting finger tip blood in whatman paper-3 for serological tests during mass screening.

Case No.... Miss Farida and others, 4 out of 6 members affected with Kala-azar.

Case No. ... Early case of P.K.D.L. (Hypopigmented)
Mrs. Rahima, Ramiza & Fatema in the same house D/O Mr. Abdul Bari Mandal. Awaltia, Trishal, Mymenshingh.

Showing the spleen biopsy from animal

Case No.... Early case of P. K. D. L

Cases of active Kala-azar during cohort follow-up, Trishal, Myn.

Preparation of DAT antigen at the central laboratory in Dhaka proved to be manageable. Despite the modest facilities for aseptic handling of Leishmania cultures and processing of antigen under constant lower temperatures (0-4_ C), quality of the antigen prepared in the two occasions (Fig. 7 & Table 11) was satisfactory. Comparable titres (1:6400 - > 1:51200) as with the reference antigen were obtained in all 33 VL cases. Remarkably, 7 endemic control sera in the first study (Fig. 2) scored higher titres (1:800 - 1:6400) against the autochthonous isolate antigen than with the reference (<1:400 - 1:1600). Reactivity of the characterized homologous L. donovani (D88) strain from India was almost similar to that of the autochthonous. All 53 VL sera had titres of > 1:25600 compared to <1:400 in 33 from the endemic controls (51). Again, titres as high as 1:800 - 1:3200 were found in 18 of the endemic control group (Table11)

Although stability of the antigen produced locally was not fully evaluated, reliable results were obtained after 2 months storage at +4_C. The maximum volume produced at IEDC&R (Dhaka) is about 500 ml which is sufficient for testing 75000 samples up to a 1:102400 dilution.

4.3 FOLLOW-UP STUDY OF VISCERAL LEISHMANIASIS (KALA-AZAR) SERO-POSITIVES DETECTED DURING MASS SCREENING.

Reactivity of Bangladesh VL and PKDL leishmania strains in DAT:

Two leishmania isolates, one from VL and another from PKDL diagnosed Bangladeshi patients were maintained by sub culturing for 5 times in brain-heart infusion medium. Promastigote mass culturing for DAT preparation was similar to the standard procedures for the reference strain (L. donovani, 1-s) except that cultures of the two strains under study wee harvested 96 hours after inoculation. Trypsin treatment, washing, fixation and staining procedures remained the same as for 1-s.

To evaluate for reactivity of the two authocthounous strains (VL&PKDL), the DAT was carried out with and without incorporation of 2-mercaptoethanol against sera from the following cases:

- African trypanosomiasis (11)
- Auto-immune disorders (12)
- Bangladeshi endemic controls (20)
- Confirmed Bangladeshi VL (20)

All sera were titrated to end-point reaction starting with 1:25 in the negative controls and 1:1600 in the confirmed VL samples. DAT reading was after 20 hours incubation as for the reference antigen (1-s).

From the results presented in Fig. 8 in can be observed that both authocthonous (VL& PKDL) antigens showed, to a greater extend, comparable titer readings against the serum samples tested. Without incorporation of 2-mercaptoethanol (2-

FIG 3+4

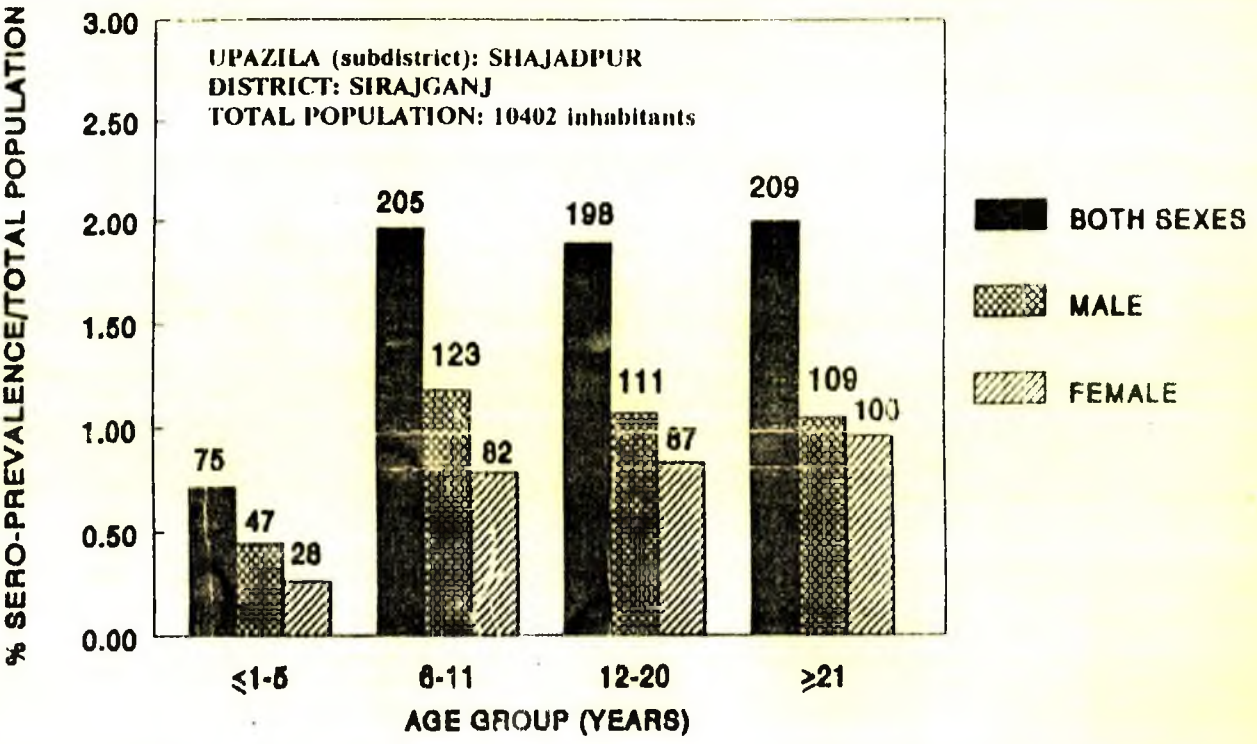


Fig-4

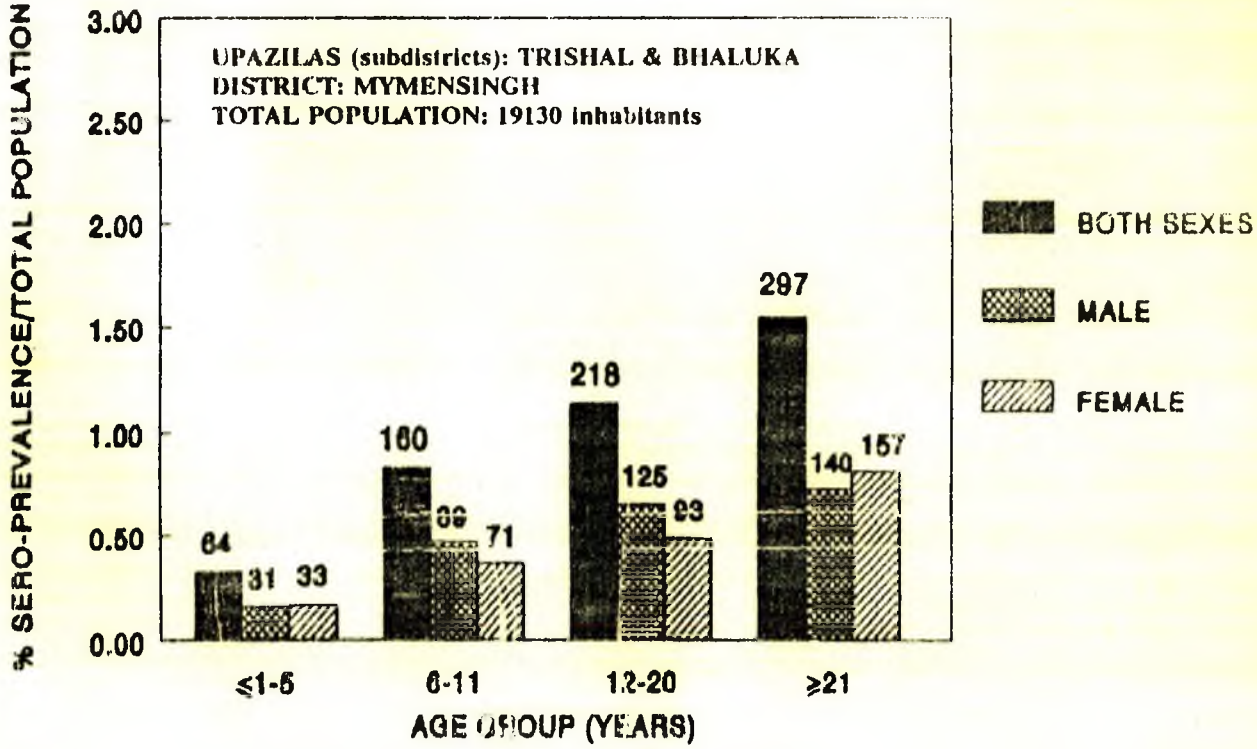
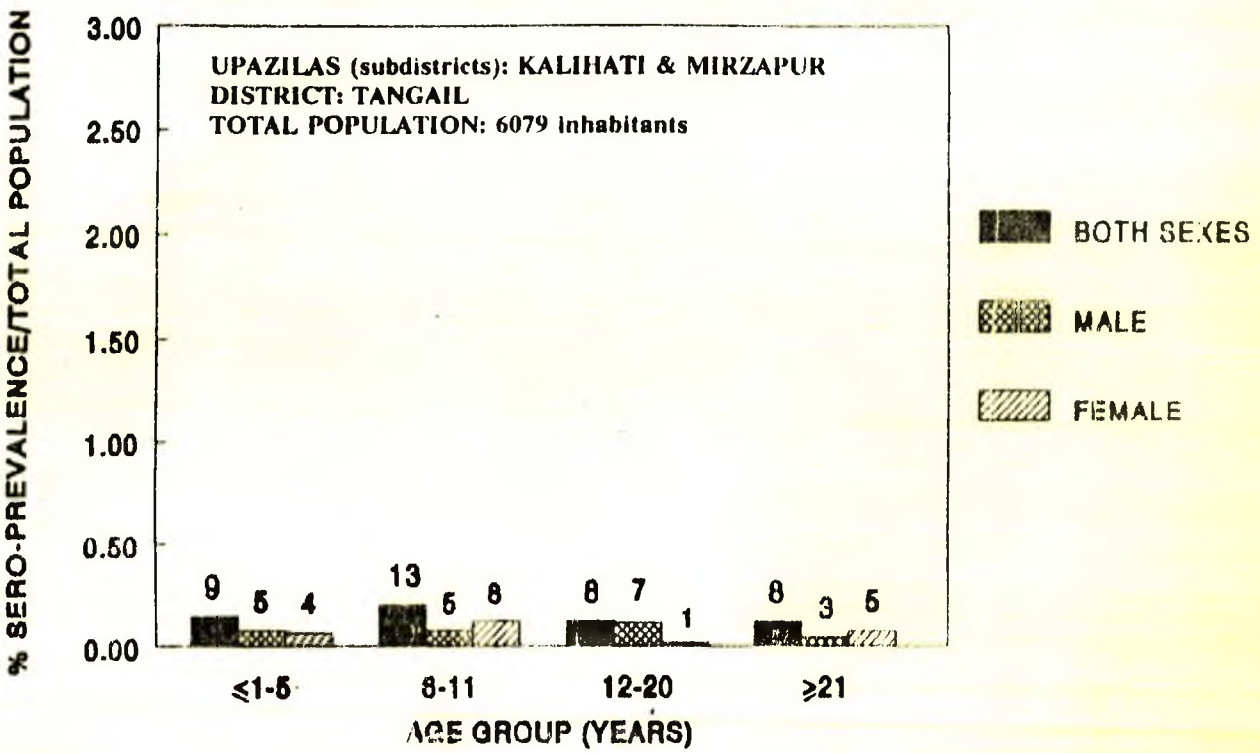
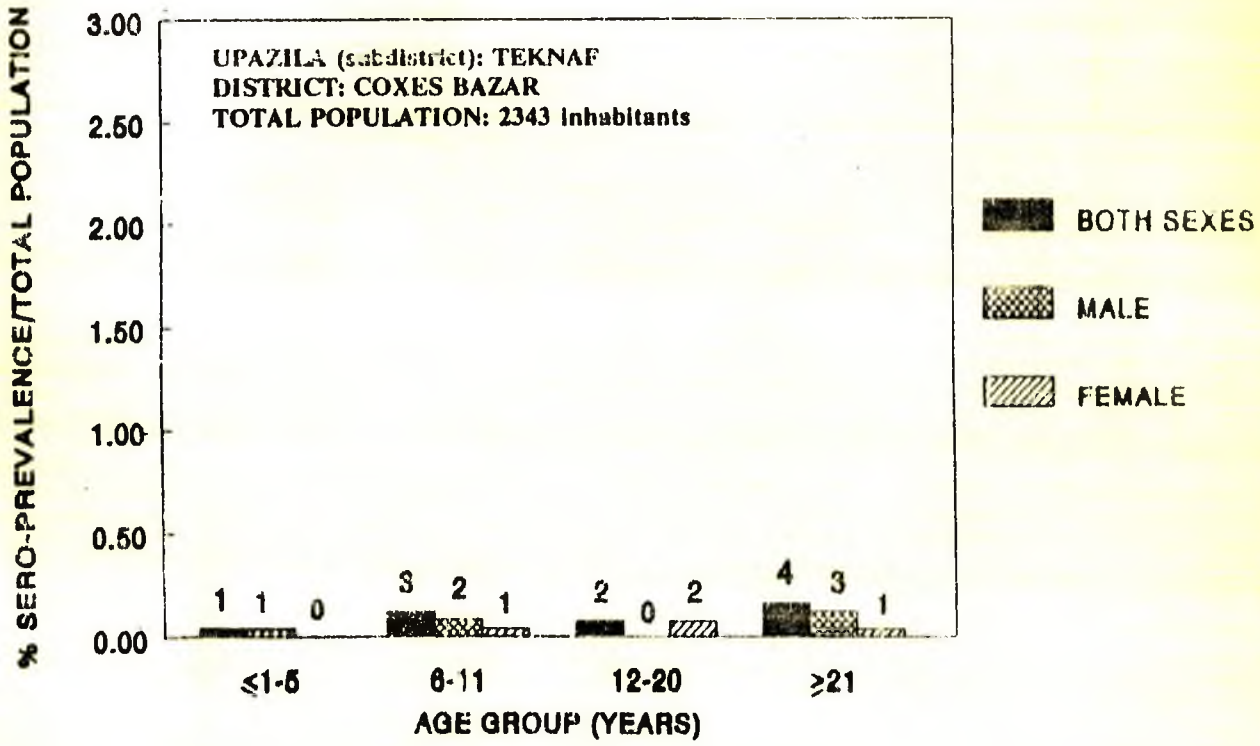


FIG 5





87B

ME) DAT Performance, both antigens scored significant high titres, due to non-specific antibodies (1:400 - 1:25600) in the negative control sera. By incorporating 2-ME in DAT performance on the same samples, specificity of the test was remarkably improved as expressed in the low titres levels (< 1:25 - 1:3200). In the VL serum samples, titres remained significantly high even after 2-ME incorporation irrespective of the nature of antigen used. The favourable reduction in the non-specific agglutination titre and thus the increase in DAT specificity versus challenging sera such African trypanosomiasis and auto-immune disorder is still obscure. However, it seems that 2-ME through splitting of I&M antibodies, allows specific reactions against IgH molecules highly available during the entire course of VL infection. It is worth mentioning that the favourable effect of 2-ME in DAT has been observed in our previous results using the reference antigen (L. donovani, 1-s). Incorporation of 2-ME is therefore essential for the desired DAT specificity even when local leishmania isolates are used for antigen preparation.

Improvement of DAT sensitivity for diagnosis of PKDL:

To further assess for the favourable effect in using homologous antigens in DAT, the PKDL patients. In this study, also the reference antigen was included to determine the degree of improvement for PKDL diagnosis.

From 70 PKDL Bangladeshi patients, all with previous history of VL and lesions suggestive of the disease, blood spots were collected and tested in DAT and IEDC&R (Dhaka) against the reference antigen (1-s). The duplicate of the same samples were mailed to Amsterdam for further confirmation. Elution of the samples was as described in earlier reports using physiological saline (0.9% NaCl) for _ 24 hours at +4_C. All 70 PKDL samples were tested against the authochtonous antigen as well as the reference (1-s) and the test reading was done 20-24 hours after incubation by two scientists independently. The results of this study are shown in Fig. 8.

In fifty out of the 70 PKDL samples tested, DAT titres were at least 2-fold higher with the PKDL antigen than the reference (1-s); in 19 samples both antigens scored equal titres and in only one did the reference antigen showed higher value. It may therefore be concluded that in order to achieve more sensitive detection of PKDL cases, a leishmania isolate from the corresponding infection should be used as antigen.

Specificity of the prepared PKDL antigen against other dermal infections:

Apart from being sensitive for PKDL diagnosis, the prepared homologous antigen should also evidence and acceptable degree of specificity. Due to possible confusion in clinical diagnosis of PKDL versus Leprosy and other dermal infections, availability of

specific homologous antigen, would be of great importance. To evaluate for this desired characteristic, the prepared PKDL antigen was challenged against serum samples from the following conditions:

- 32 cutaneous leishmaniasis (Algeria)
- 11 cutaneous leishmaniasis (Brazil)
- 5 mucocutaneous leishmaniasis (Brazil)
- 12 leprosy patients (Brazil)

Result obtained with the PKDL antigen on the above mentioned samples were compared with those previously recorded in the PKDL patients as shown below:

4.2.10 DAt results with challange group.

Diagnosis	Number of patients	Titre readings
Cutaneous leish. (Algeria / Brazil)	43	41 (_ 1:1600), 2(1:3200)
Mucocutaneous leish (Brazil)	5	5 (_ 1:1600)
Leprosy (Brazil)	12	11(_ 1:1600)
PKDL (Bangladesh)	70	70 (1:51200 - 1:26214400)

Considering the very low DAT titres obtained in sera from patients with other dermal infections by comparison with those being diagnosed as PKDL, it might be possible to differentiate between PKDL and leprosy patents on grounds of DAT results.

Application of DAT on European VL/HIV - positive patients:

Due to the scarcity and difficuity in obtaining sera from HIV- positive or AIDS patients, DAT performance in this important patient's group could not be evaluated earlier. The increasing number of these immuno-compromised patients who acquired VL infection and their importance in the epidemiology in all endemic areas necessitate availability of highly sensitive nonetheless specific detecting method. While serologic diagnosis appears to be insensitive considering the low antibody profile, direct demonstration of leishmania parasite is equally difficult and time consuming. Highly technical procedures such as immuno-blotting and the use of leishmania DNA specific probes and its amplification (PCR) is not always feasible in central laboratories of developing countries.

Acknowledging the strikingly high titre levels in the vast majority of immuno-competent VL patients in various endemic areas, it is expected that the DAT would monitor, to a reasonable extend, specific antibodies in these patients. Also, being simple and extremely economical, the DAT may therefore be a good alternative for high-tech methods in important VL endemic areas such as Bangladesh and India.

In this study, an antigen batch prepared from the reference L. donovani strain (1.s) was employed. Since the effect of 2-merecaptoethanol in DAT performance versus immuno-globulins in immuno-compromised sera was not determined earlier, it is decided to perform the test with and without incorporation of this reducing agent. In total, 163 serum samples collected from Dutch and Italian (Sicilian) HIV-positive and AIDS patients provided respectively by Dr. F. Wolff (Virology Dept., U.V.A. Amsterdam) and Dr. L. Negro (LSHTM, London). None of the Dutch patients (50) had history or clinical signs of VL while among the Sicilian patients, 9 were earlier diagnosed as having opportunistic VL infection.

The DAT was executed blindly in all 163 patients and the test results were compared with those of final diagnosis and immunofluorescence test (IFAT0 established earlier in Sicily and London (LSHTM).

Our results on the Dutch IHV-positives (50) confirmed the clinical findings that none of those patients had VL infection. It can be concluded thus that DAT for VL does not cross react with anti-HIV antibodies. From the Sicilian HIV-patient group (113), 5 had scored DAT titres ranging (1:6400 - > 1:102400); the remaining 4 patients were clearly

negative for VL (<1:100). In one out of 5 VL/HIV - positive, incorporation of 2-mercaptoethanol resulted in titre reduction from 1:6400 to 1:1600 while in the other 4 samples a significant improvement in test readings was observed (1:6400 to > 1:51200). It can therefore be recommended that in testing suspected VL/HIV-positive cases, DAT performance should be carried out with and without 2 mercaptoethanol incorporation. Compared to the immunofluorescent test (IFAT) which detected 4 out of the same 5 patients, it seems that the DAT is more sensitive. However, more evaluation and comparison with IFAT as well as other diagnostic techniques were evaluated in the continuous study of sero-responsive cases of VL.

Further improvement of DAT sensitivity for earlier VL detection:

With the exception of patients having immuno-suppressed system, the DAT in its present format is capable of monitoring VL patients with confirmed infection. Reliability of DAT to detect L. donovani infection at its inapparent or subclinical phase is still under evaluation. Enhancing sensitivity in DAT to establish diagnosis in this group of patients had earlier been attempted using an autochthonous (Bangladeshi) and a homologous (MHOM/IN/80/D88, India) strains of L. donovani. The results obtained were encouraging and showed superiority when compared with those by the reference leishmania strain (1-s). However, further exploration along this line is considered necessary to achieve the desired objective. Apart from detecting early VL infection, achieving optimal sensitivity may also result in an incubation than the one currently indicated (18-20 hours).

Recent studies (King & Turco, 1988) had evidenced that the presence of lipophosoglycan (LPG) layer on leishmania promatigote surface masks antigenic binding sites. L. donovani strains from India and Sudan were found to be poor agglutinators as they expressed abundant amounts of LPG. A specific mutant (R_2D_2) leishmania strain laking LPG layer is therefore expected to show higher reactivity in DAT if compared to the reference (1-s) or the homologous strains from Bangladesh or India. Accordingly, the sensitivity of the DAT can be expected to improve in respect of the low reacting subclinical VL cases.

The first evaluation using the above mentioned LPG mutant strain (R_2D_2) was carried out with a corresponding antigen prepared by Dr. D. Evans (LSHTM, London). The serum samples employed in this study were collected from North Pakistan from 42 human and 20 canine hosts by Dr. Rab (LSHTM, London). In addition to mutant (R_2D_2) strain, the DAT was carried out at LSHTM using the reference 1-s antigen to assess for the expected difference. Results obtained with both antigens are expressed in the table presented below:

Number of sera tested	DAT titres versus Antigens		Diagnosis
	LPG mutant (R_2D_2)	Reference antigen (1-s)	
29	≤ 1:1600	≤ 1:1600	other conditions than VL
5	≤ 1:1600	1:6400-25600	3 treated VL
1	1:3200	1:3200	suspected VL
4	1:3200	1:3200-≥102400	3 treated VL
1	1:6400	≥ 1:102400	undetermined
2	1:12800	1:12800-≥102400	1 treated VL
1	1:25600	1:6400	undetermined
4	1:25600	≥ 1:102400	2 treated VL
2	1:51200	≥ 1:102400	1 treated VL
15	≥ 1:102400	≥ 1:102400	10 treated VL

Cultures of the LPG mutant were obtained from Dr. D. Evans (LSHTM, London) and the strain is currently being maintained at the laboratory of Medical Microbiology (U.V.A. Amsterdam). Two attempts were made to raise mass cultures of the strain for preparation of DAT antigen. Unlike the reference strain (1-s), R_2D_2 grew very poorly in RPMI medium and promastigotes showed tendency to formation of large clumps. The antigen batches prepared from this strain so far were considered as invalid for DAT execution as they showed unacceptable degree of auto-agglutination.

4.4 SEROLOGICAL STUDY ON POST KALA-AZAR DERMAL LEISHMANIASIS IN BANGLADESH

In the present study 61(1.21%) out of 5.011 of visceral leishmaniasis cases had cutaneous lesions suggesting post kala- azar dermal leishmaniasis (PKDL). Amastigotes were detected in 42 smears examined from 61 PKDL patients. About 98% patients has more than one lesion and as many as 50 to 100 nodules were counted on some of them (Table 1).

Table 4.4.1: Age-wise distribution number and type of PKDL cases

Age Group	No. of lesion		
Years	Total No.	Single	Multiple
0-5	1	-	1
6-10	6	-	6
11-20	14	-	14
21-30	20	-	20
30+	20	1	19
Total	61	1	60
%	100	163	98.37

The commonly affected sites in the group of patients were the face (83.33%) and the upper limbs (8.19%) followed by the lower limbs (6.55%) (data note shown). The youngest patient in the series was of 5 years of age and the oldest was 85. The majority of the cases were in the age group of 21 to 30 years (Table - 2).

Table 4.4.2: Distribution of the PKDL cases by age and sex.

	Age (in years)					
Sex	0-5	6-12	13-20	21-30	31+	Total
Male	1	8	12	20	4	45
Female	-	4	3	8	1	16
Total	1	12	15	28	5	61

All the patients (100%) had thermal and tactile sensations intact over their lesions (Table-3). Among the three who came from the Middle-East one had cutaneous leishmaniasis and the other two seemed to have muco-cutaneous leishmaniasis.

Table 4.4.3: Presence of LD body and sensation on the lesions by the type of the lesion

	Sensation		LD body in skin		
Type	No	Absent	Present	Absent	Present
Hypo	11	0	11 (100)	7 (11.48)	4 (6.56)
Eryth	19	0	19 (100)	8 (13.11)	11 (18.03)
Nod	25	0	25 (100)	3 (4.92)	22 (36.07)
Ulcer	6	0	6 (100)	1 (164)	5 (8.20)
Total	61 (100)	0 0	61 (100)	19 (31.15)	42 (68.85)

(Percentage figures are given in parenthesis).

Table 4 shows the results of chemotherapy 98.38% of the patients responded to an IV or IM regimen of the drug in dosage mentioned in the text. 1.64% i.e. only one patient needed pentamidine to get a cure.

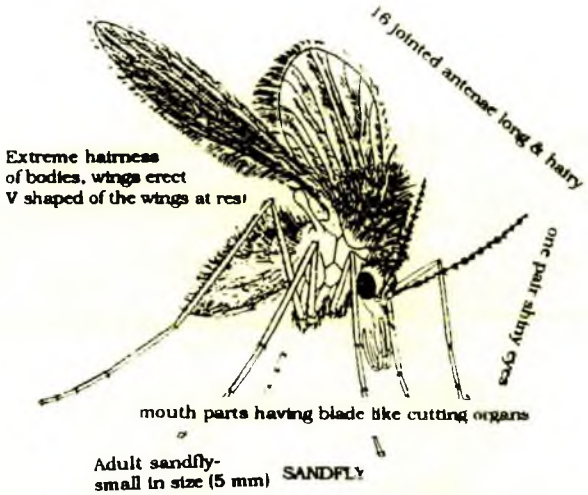
Table 4.4.4: Result of chemotherapy on PKDL.

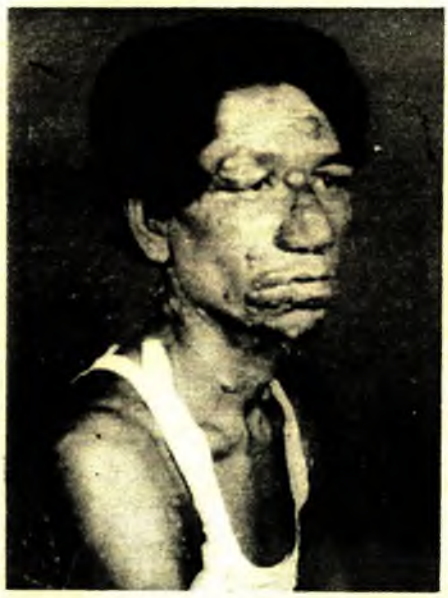
Type	No	Response to stibatin (Glavo Lab) Immediate	Delayed	No response
Hypo	11	3 (4.92)	8 (13.11)	-
Eryth	19	12 (19.67)	7 (11.48)	-
Nod+Ulcer	31	18 (29.51)	12 (19.67)	1 (1.64)
Total	61 (100)	33 (54.10)	27 (44.26)	1 (1.64)

(Percentage figures are given in parenthesis)

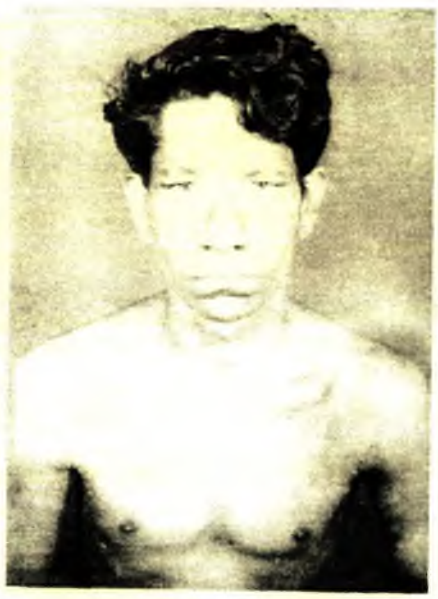
Key note: Immediate = Response with one course of antimony Delayed=Response with three courses of antimony. No response=No response even after 6 courses of antimony. (LD present on skin biopsy but no parasite could be cultured). No extension or change in skin lesion was observed.

Antibody titres remained high in 11 PKDL patients after treatment when tested by DAT and IFAT and 98.38% of them could be detected by ELISA (data not shown). Serological results showed 100% sensitivity for DAT and IFAT, and 86.88% for ELISA (Table 5).





Post kala-azar dermal leishmaniasis
Before treatment



Post kala -azar dermal leishmaniasis
After treatment

Table 4.4.5: Result of the serological test by the type of the lesion.

Type	Type of serological test			
	No	DAT	IFAT	ELISA
Hypopigmented	11	11	11	10
Erythematous	19	19	19	17
Nodular	25	25	25	20
Ulcerative	06	06	06	06
Total	100	61	61	53
	(100)	(100)	(100)	(86.88)

Key note: DAT = Direct Agglutination Test with a cut of serum antibody titre of 1:3200

IFAT = Immunofluorescent Test

ELISA= ELISA at a cut off absorbency of 0.30

4.5 STUDY ON KALA-AZAR IN CHILDREN A STUDY OF 100 PATIENTS

Amongst the 100 cases of Kala-azar the age of the patients varied between 1-12 years. The highest incidence was found in age group of 9-12 years. Only one patient of 1 year age with demonstrable LD bodies was found. 31(46.95%) of patients were of 9-12 years, 25 (37.87%) of 6-8 years, 7 were 0-5 years of age amongst the male group. There were 66% male and 34% female Table 1 (Fig 1) Male female ratio was 1.94:1. The duration of illness of the disease was calculated from first onset of the symptoms to the date of admission to the hospital. Duration varied from 1 week to 2

years, average period was 6 months and the median range was 4-6 months. Table 2 and 3 showed the symptoms and signs of Kala-azar patients in this study. Table 3 showed the types of fever. 100% had rise of temperature above 37 C. The types of fever as observed, were continuous in 50% of patients, intermittent in 20% and 30% patient had remittent type of fever and 10% of patient had fever associated with chills and rigors. None of these patients gave history of convulsion. Weight loss was noted in all cases, although most patients did not mention it. However, weight gain was observed after three months of therapy.

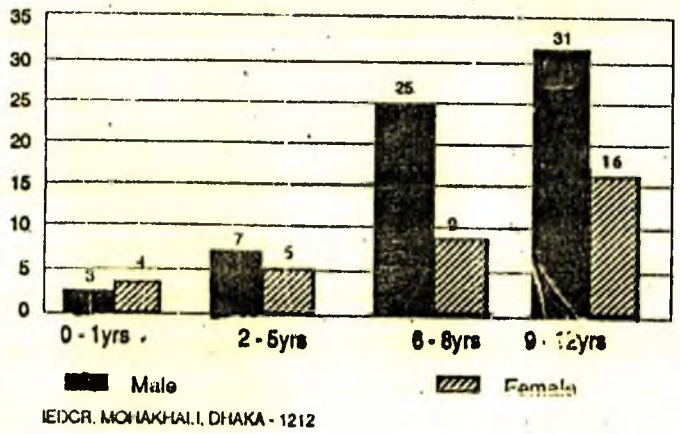
Anaemia was prominent feature in all cases of which 2% had mild type, 41% had moderate type and 57% had severe type. Appetite was normal in 70% but increased in 28% and reduced in 2%. (Table 2) Dry irritating and nonproductive cough was prominent feature in 36% of patients. Acute abdominal pain located either in left hupochondrium or umbilical region was the presenting complaint in 20% of patients. Oedema was found in 6% patients. Haemorrhagic manifestations were found in 2% patients of which 8% presented with epistaxis and 2% with haemoptysis. There were 4% patients with loose motion and another 4% with bloody mucoid stool associated with tenesmus. Table-3 showed that Kala-azar patients in the present series also associated with bronchopneumonia (3%), malaria (1%) and tuberculosis (1%). Splenic enlargement was found in all the patients while hepatomegaly was seen in 54% (Fig-2).

Table - 4.5.1

Age and Sex distribution of patients (N = 100)

Sex	Age Group				
	0-1 yr	2-5 yr	6-8 yr	9-12 yr	Total
	N%	N%	N%	N%	N%
Male	3(4.5)	7(10.6)	25(37.87)	31(46.95)	66(100)
Female	4(11.76)	5(14.70)	9(26.47)	16(47.65)	34(100)
BOTH SEXES	7	12	34	47	100%

Fig - 1
Age and Sex Distribution Among Patients In Childen



IEDCR, MOHAKHALI, DHAKA - 1212

Fig - 2
Distribution of enlargement of Spleen and Liver Amony the Patients

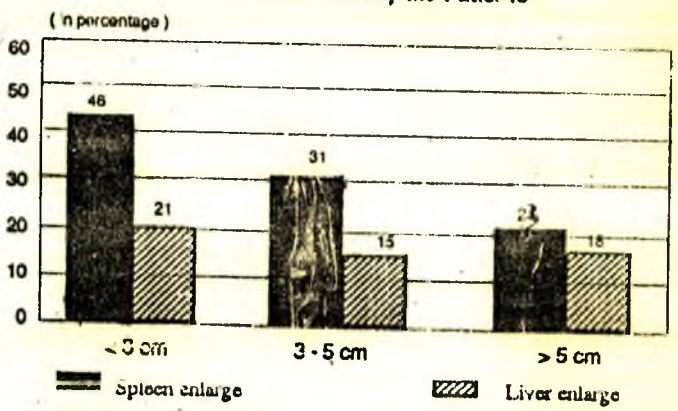


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Table - 4.5.2

Presenting symptoms of Kala-azar patients

Sl.No.	Symptoms	Total Number of patients = 100
1.	Pyrexia	100%
2.	Weight Loss	100%
3.	Appetite	
	a. Normal	70%
	b. Reduced	2%
	c. Increase	28%
4.	Anaemia	
	a. Mild 9-11 gm/dl	2%
	b. Moderate 6-9 gm/dl	41%
	c. Severe 6 gm/dl	57%
5.	Loose motions	4%
6.	Bloody mucoid stool	4%

4.6 STUDY ON SODIUM ANTIMONY GLUCONATE (SAG) IN VISCERAL LEISHMANIASIS (KALA-AZAR) SEROPOSITIVE PATIENTS

Leishmania donovani parasites were demonstrated in Giemsa- stained smears or by culture in 715 bone-marrow aspirates of the VL suspected population (1,273) (Table 1). With the exception of two all others (638) from the confirmed cases tested in DAT (640) had titers indicative of VL (1:3,200). Of the remaining 558 L. donovani negative cases, the DAT revealed positive results in 547. Leishmania donovani parasites were also demonstrated in all 45 PKDL suspected cases; 43 of those patients had DAT titers 1:3,200 (Table 1).

Of the confirmed and suspected VL cases treated (1,273), 1,197 (94.02%) showed immediate response to a 20 day treatment course. In more than 90% of the patients, fever subsided during the first 3-5 days of treatment and within 2-3 months post-treatment there was significant weight gain regression in spleen size and improvement in hemoglobin concentrations and total leukocyte counts (Table 2). All bone-marrow aspirates and skin snip/nodule biopsies collected from the treated VL and PKDL patients were negative for L.donovani and no relapses or PKDL signs were observed during the follow-up period. In 44 (3.46%) of the VL treated cases (1,273), SAG administration was extended to periods varying from 40 to 60 days as those patients did not show desirable improvement during the initial course of treatment (20 days). All 44 cases were eventually cured and no relapses were registered during the follow-up period. In the total population treated (1,273), only 11 (0.86%) showed no signs of improvement even after administration of SAG maximum dose for 60 days; they did, however, respond positively later to pentamidine treatment. Twenty one patients (1.65%) died as a result of malnutrition (15) or before completion of therapy against intercurrent pulmonary tuberculosis.

Based on the number of parasitologically confirmed cases (640) or those with a positive response to specific anti-leishmania chemotherapy (558), the sensitivity of the three serological methods were as follows: DAT (99.6%). ELISA (85.8%) and IHA (80.3%); DAT specificity was 97.7%).

Of the 45 PKDL cases treated as described. 36 (83.5%) showed no side effects. However, in nine patients, epistaxis (1), body ache (3) and abscess formation (5) were observed.

6.1

AGE AND SEX DISTRIBUTION AMONG KALA-AZAR CASES IN ONE CALANDER YEAR 1991

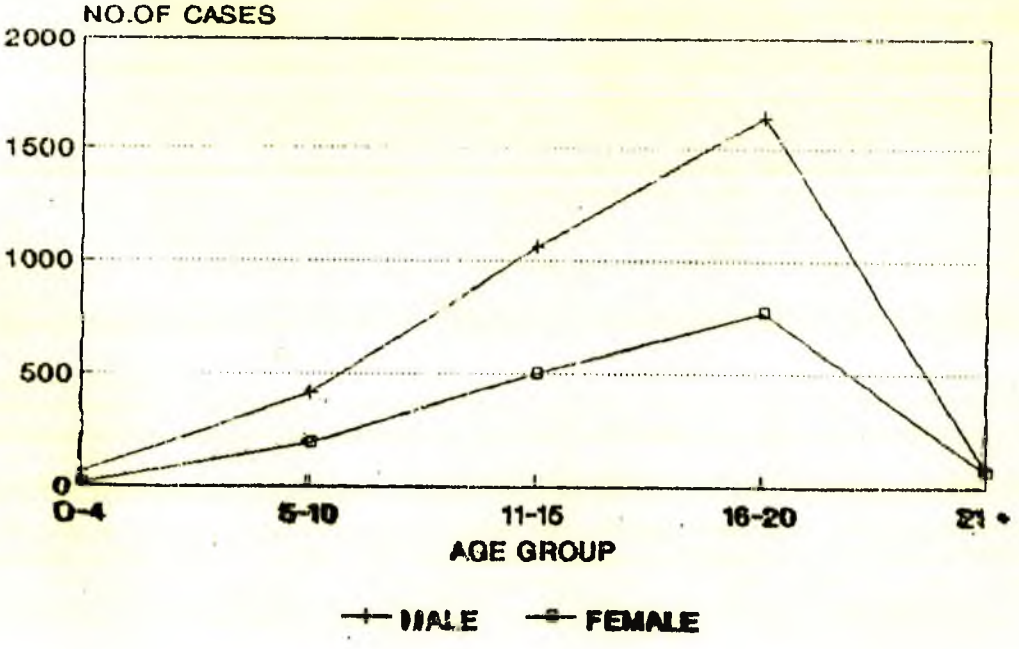


TABLE - 4.6.1

Serologicalresults in parasitologically confirmed and unconfirmed visceral leishmaniasis (VL) patients

Diagnosis	Number	Serodiagnostic Test*	Serological results		
Unconfirmed VL		DAT	<1:1,600	1:3,200-25,600	<1:5,120
			11	175	372
				ELISA absorbance	
	558	ELISA	<0.29	0.30-0.60	<0.61
			79	364	115
				IHA titre	
		IHA	<1.64	1:128-1:256	<512
			110	309	139
				DAT Titre	
			<1:1,600	1:3,200-1:25,600	<1:5,120
Confirmed VL	715**	DAT	2	45	593
PKDL	45	DAT	2	15	28
Treated VL	272	DAT	0	79	193
Endemic cont.	200	DAT	187	13	0
Other control	524	DAT	520	4	0

Direct Agglutination Test (DAT), Enzyme-Linked Immunosorbent Assay (ELISA) and Hemagglutination Test (IHA) **DAT was performed on 640 VL patients.

TABLE - 4.6.2

Clinical response to sodium antimony gluconate (SAG) administration in parasitologically or serologically diagnosed visceral leishmaniasis patients

Clinical	Status*	Assessment of clinical criterion criterion				
		Increase in spleen size (inches)				
		0	1-2	3-4		5
Splenomegaly	B.T.	115	549	415		194
	A.T.	1.247	7	13		6
		Duration of fever (weeks)				
		2-4	4-12	13-24		25
Fever	B.T.	48	221	495	358	151
	A.T.	0	0	0	0	11
		Hemoglobin concentration(gm/100 ml)				
		4	5-8	9-12	13-14	14
Anemia	B.T.	195	894	150	33	1
	A.T.	18	135	995	110	15
		Leukocyte count (cells/CMM)				
		2000-2500	2501-3000	3001-3500	3501-4500	>4500
Leukopenia	B.T.	269	495	390	79	40
	A.T.	0	0	198	650	425

*Before start of treatment (B.T.) and after treatment (B.T.) during the follow-up period (3-12 month).

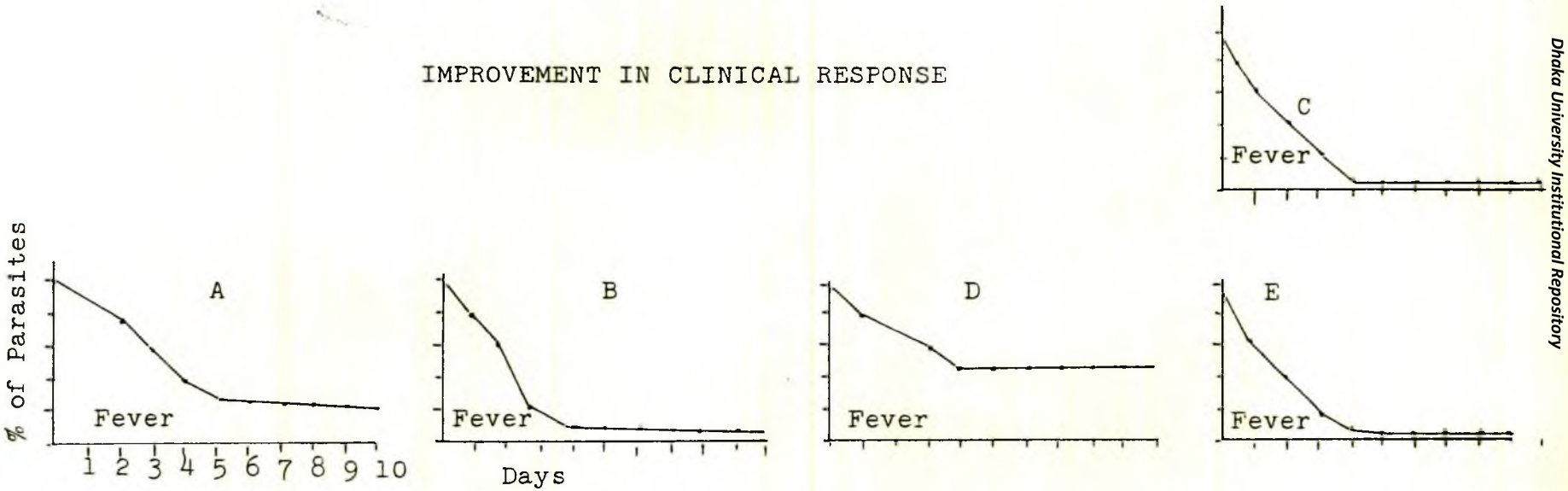
4.7 STUDY ON DIFFERENT TREATMENT SCHEDULE FOR SAG FAILURE CASES OF VISCERAL LEISHMANIASIS IN BANGLADESH

Out of 55 SAG unresponsive cases to antimony 10 were group A, 10 in group B, 15 in group C, 5 in group D and 14 in group E. Response to different treatment schedule were summarized in Table 1 and the findings of group A revealed that 86% of cases with 30 days of SAG showed parasitological cure. 60 percent cases were responded to group B on the other hand 100% cases responded to group C and 99% in group E. One patient from group C and 4 from group B were responded to group C. 5 patients from those who did not respond to group D were also responded to group C. One patient relapsed during followed-up a 15 year old boy in group A who responded to group E. The follow-up examination six weeks, 3 months and 12 months revealed that spleen axis was reduced haematological level gradually increased in group A, B, C and D, but rapidly in group C. Results of the one year follow-up examination showed that the haemoglobin level, leukocyte count had risen to 14.5 g/dl, 10,000/cum. gradually increased in group A and group D but one had 1 cm spleen even after 1 year. Serum albumen levels gradually returned to normal by follow-up examination of three weeks but hyperglobulinemia persisted for one year in some patients, although at lower levels (4.4 g/dl than during active cases. No patient had an abnormal BUN before and or during treatment. Only five cases of VL developed epistaxis (2.62%) and gastroenteritis (6.3%) as an immediate complication. There were also other complications like pain and abscess (.35%) in injection site, bodyache (2.62%). Delayed complications were anaemia (11.4%), oedema (6.3%), cough (3.5%). All of them were given symptomatic treatment. 2 cases where VL associated with other diseases like malnutrition and corrected by symptomatic treatment and diet therapy.

Randomised controlled study was held to evaluate of different treatement Schedule SAG failure cases of Kala-azar.

Total Number (N) cases-55

IMPROVEMENT IN CLINICAL RESPONSE



Amastigotes in spleen aspirated materials in SAG failur cases of kala-azar in different treatment schedule

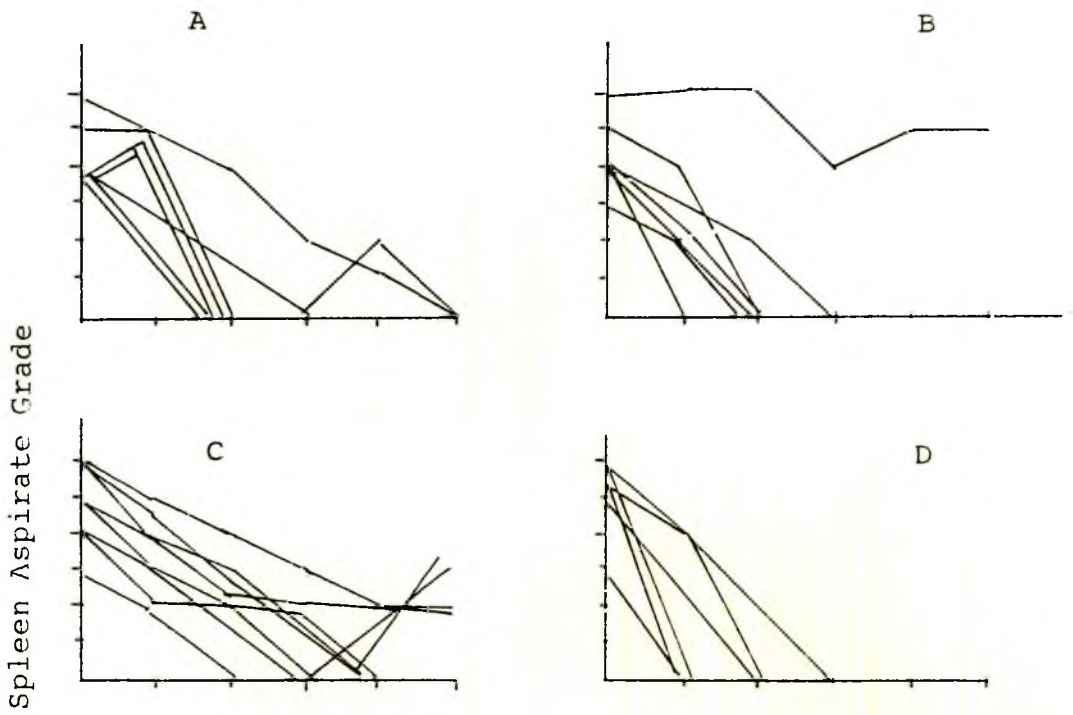


Table-1 Different Treatment Schedule for SAG Failure Cases of Visceral Leishmaniasis in Bangladesh

Group	Different Treatment Schedule
A	SAG 20 mg/kg body weight not exceeding not more than 850 mg/day, IM, IV for 30 days
B	SAG 10 mg/kg body weight twice daily, IM or IV for 10 days
C	SAG 10 mg/kg body weight thrice daily for 7 days
D	ALLOPURINOL + SAG. Allopurinal 20 mg/kg body weight orally daily for 9 weeks inaddition to 20 mg/kg SAG daily inj.for 3 weeks (4th to 6th weeks).
E.	PENTAMEDINE - 4 mg/kg body weight every alternals day I.V. for 14 days.

Note: At the end of therapy as per 6 treatment schedule, the result was considered "success' meaning absence of parasite or 'failure' meaning persistence of parasite from splenic or bone marrow aspiration.

Table-2 Results of Different Treatment Schedule for SAG Failure Cases of Visceral Leishmaniasis in Bangladesh

Treatment	Schedule Days		No.of	Success	%	Failure Remarks	Cases
A. One daily inj.SAG	30	10	8	80	2	1 responded to group C and another 1 G	
B. Two daily inj.SAG	10	10	6	60	4	all 4 responded to group C	
C. Three daily inj.SAG	7	15(1)	15	100	0	1 responded from group A & 4 from B	
D. Allopurinol & SAG	63	05	0	0	5	5 from D	
E. Pentamidine inj	14	15(1)	14	99.33	1	1 responded from Group A	
Total	55(1)	44(2)	12	12 out of	55 cases had multiple treatment regime		

Note: No. of secondary unresponsive cases are given in parenthesis.

4.8 STUDY ON VECTOR (SANDFLY IN RELATION WITH VL (KALA-AZAR) AND EFFECT OF DDT ON ITS CONTROL

Kala-azar case and vector density

During the first survey 5,500 samples were examined in 1400 houses (Table-1). Out of that 1828 kala-azar cases were detected. Of which 715 cases were serologically and parasitologically positive, 1068 serologically positive and 45 were PKDL cases. 1273 were given treatment according to WHO 1984 schedule and 555 cases were put under follow up. Sandflies were collected in the village Awaltia for one year during first survey (Table 2). A total of 1395 sandflies (5.82 m/hr) were collected by hand catch. Out of 1395 sandflies 559 (40.07%) were **P.argentipes**, the known vector of Indian subcontinent. Correlation between the number of kala-azar case and vector density (m/hr) was calculated using SPSS/PC+ and the relation was found significant ($p<.025$).

TABLE 1. EFFECT OF DDT ON SANDFLY (VECTOR) AND INCIDENCE OF KALA-AZAR IN SPRAYED & UNSPRAYED AREA.

YEAR/ PERIOD	Intervention Area where DDT sprayied in July 1988 after 1st surveillance						
	No. house visited	No. Examined	Kala-azar cases detected				P.argentipes (S.fly) (m/hour.8 hrs)
			Sero LD. body	Cl. Sero -ve DAT	PKDL	Total	
1st survey Jun'87 -May'88 DDT sprayed Jul'88	1400	5500	715 13	1068 19.41	45	1828 33.23	5.82
2nd survey Jun'89 -May'90	1000	4800	2 0.04	5 0.1	0	7 0.14	0.25
3rd survey Jun'92 -Dec 92	880	3500	0	1 0.11	0	1 0.11	0.18
INTERVENTION AREA							
1st survey Jun'87 -May'88 DDT sprayed Jul'88	1400	7000	5 0.07	12 0.17	1	18 0.25	1.56
2nd survey Jun'89 -May'90	1000	4200	35 0.83	77 1.83	3	115 2.73	2.76
3rd survey Jun'92 -Dec 92	880	3100	15 0.48	60 1.93	2	77 2.48	2.86

Note : Figures within parenthesis are percentage of row total.

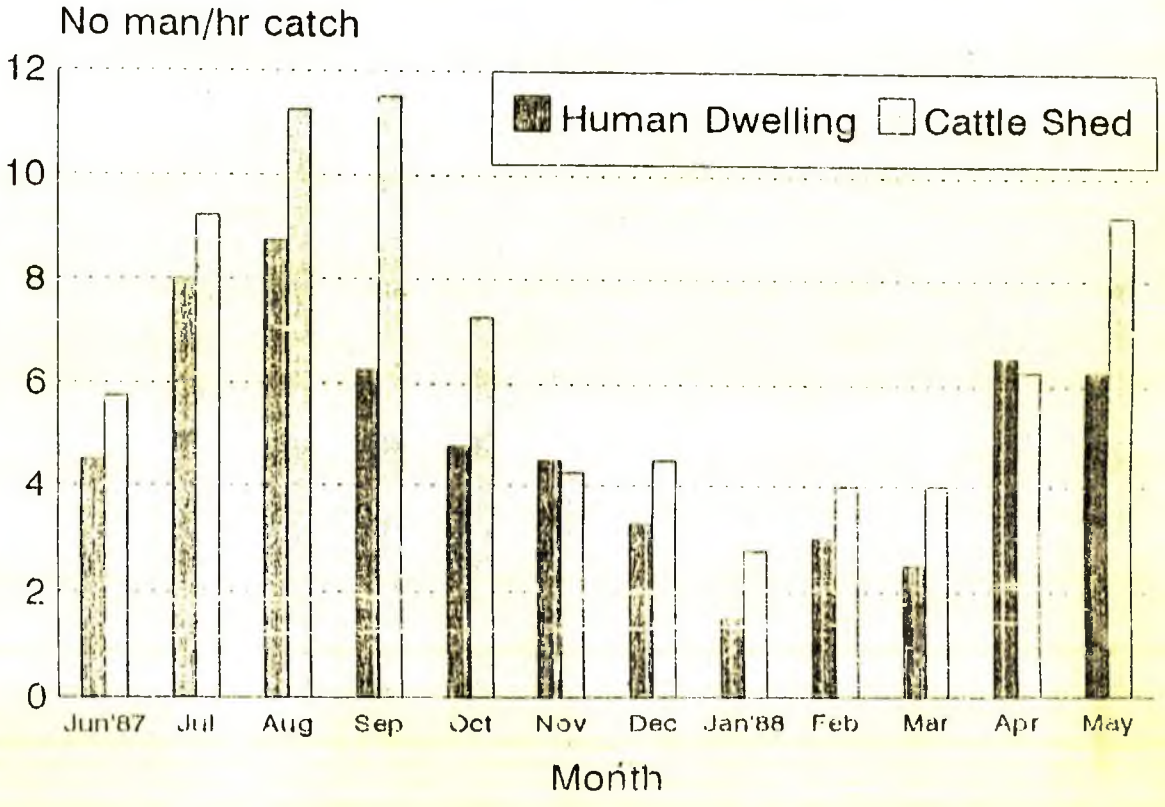
Table 2: Species composition of sandfly collected by hand catch. during June '87 - May '88.

Month	Total S.fly	P.argentipes	P.papatasi	S.babu babu	S.shorti	S.baraudi
1987 June	102	41	8	50	2	1
July	163	69	15	66	9	6
Aug	202	80	10	102	7	3
Sept	185	71	5	108	0	1
Oct	114	48	3	60	2	1
Nov	102	35	7	52	7	1
Dec	79	31	5	35	6	2
1988 Janu	45	17	0	28	0	0
Feb	58	28	3	24	3	0
Mar	75	26	6	39	2	2
Apr	111	51	10	42	4	4
May	157	62	18	71	2	4
	1395	559 (40.07%)	90 (6.45%)	677 (48.53%)	44 (3.16%)	25 (1.79%)

Table 3: Showing the comparative results between sandfly density and incidences of Kala-azar during survey 1987-88 and 1991-92 in Mymemsingh district of Bangladesh. (Control area where there was no intervention i.e. where no DDT spraying was done since 1987 till to date in Trishal, Fulbaria and Bhaluka under Mymensingh.

June, 1987-May, 1988 Control area, No DDT spraying					June, 1991-May, 1992, Control area, No DDT spraying			
Year Month	No.Captured	Instances in which Phlebotomus found	m/hr. Collected 8 hrs.	No. Kala-azar detected	No.Captured	Instances in which Phlebotomus found	m/hr. Collected 8 hrs.	No. Kala-azar detected
1987 June	11	3	0.37	0	37	2	0.25	1
Jul	15	5	0.62	0	41	6	0.75	4
Aug	24	9	1.12	1	44	9	1.12	6
Sept	36	10	1.25	1	55	19	2.37	8
Oct	40	5	0.62	0	57	4	0.5	1
Nov	51	6	0.75	0	71	2	0.25	2
Dec	65	3	0.37	1	70	4	0.5	2
1988 Jan	58	7	0.87	0	85	9	1.12	5
Feb	69	11	1.37	1	91	18	2.25	10
Mar	60	23	2.87	0	112	51	6.37	13
Apr	81	35	4.37	0	130	60	7.5	29
May	85	33	4.12	1	210	81	10.12	31
Total	595	150	1.56	5	1003	260	2.7	112

SEASONAL DISTRIBUTION OF SANDFLY COLLECTED FROM HUMAN DWELLING & CATTLE SHED ('87-'88)



SEASONAL DISTRIBUTION OF Pargentipes IN THE VILLAGE AWALTIA,TRISHAL ('87-'88)

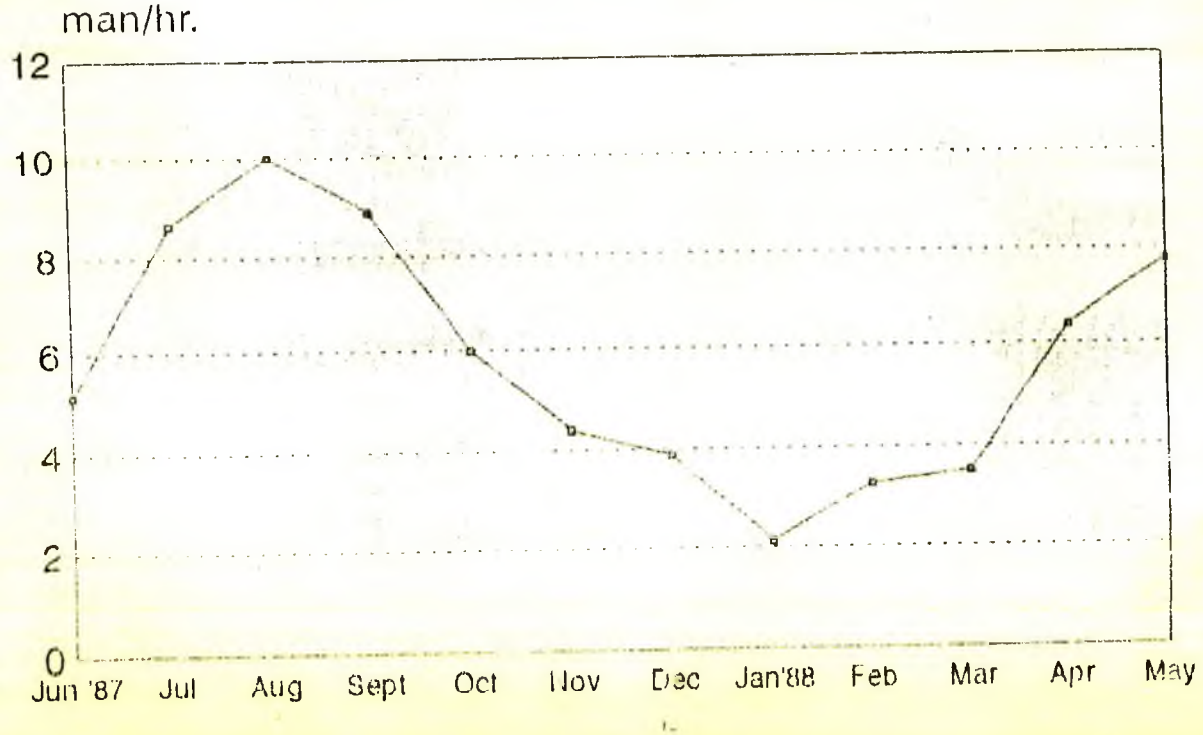


Table 1 shows the effect of DDT in sprayed and unsprayed area. It was observed that during lst survey where 45 PKDL cases were detected, during 2nd survey one year after DDT spraying not a single PKDL case was detected though 7 (0.14%) new kala-azar cases were found. During 3rd survey only 1 (0.028%) kala-azar case was detected serologically and no PKDL or parasitologically positive case was found.

Simultaneously vector density was also reduced, 0.25 m/hr and 0.18 m/hr during 2nd and 3rd survey period. On the other hand in the unsprayed area (control) during the three surveys 0.24%, 2.73% and 2.48% kala-azar cases were detected and the vector density were 1.56, 2.76 and 2.86 m/hr respectively.

Sandfly Fauna:

A total of 1395 sandflies were collected during one year and out of that 559 (40..07%) were P. argentipes. Other species were P.papatasi (6.45%), S.babu (48.53%), S. shortii (3.16%) and S. barraudi (1.79%) Table 3.

Larval Habitat

24 larval collection were performed during the lst survey period, 12 from human dwelling and 12 from cattle shed. 4(33.33%) out of 12 collection from human dwelling and 7(58.33%) from the cattle shed were found positive.

Host preference

Blood meal analyses were done for 236 smears and they showed 95(40.25%) were from human, 123 (52.11%) from bovine and 18 (7.63%) from other hosts.

Seasonal Distribution

A seasonal distribution of P. argentipes was found in it two peaks, one between July and September and other between April & May and lowest in January (Fig. 1). Kala-azar cases were also high in the post monsoon months. After DDT spraying in July '88 in the study areas, during the 2nd and 3rd surveys, 24 and 18 P.argentipes were collected. No trend can be shown with so few numbers sandflies. Kala-azar incidence was also reduced from 1828 during Ist survey to 7 and I case in 2nd and 3rd survey respectively (Table 1).

It was again observed that the density of **.argentipes** is higher in the cattle shed compared to the human dwelling both in morning and evening resting collection throughout the year (Fig.2).

4.9 A COMPARATIVE SEROLOGICAL STUDY FOR MEASURING THE ANTIBODY OF LD BODY USING DAT, IFAT, ELISA & IHA IN HIGH AND LOW ENDEMIC AREA OF KALA-AZAR IN SELECTED AREA OF BANGLADESH

METHODS OF STATISTICAL ANALYSIS

Methods of Analysis and Data Analysis:

A pair specimen of each individual was examined both smeared slide/culture examination and DAT Test. The measure of association namely kappa coefficient (Cohen, 1960) was used to measure the degree of agreement of that association. Following, the other parameters were sensitivity and specificity of seroconversion diagnosis and the probability of agreement of positive and negative diagnosis.

1. 2 x 2 Table of Match Pair Analysis

	Slides/culture Positive	Negative		Total
Seroconver-sion	Yes	a	b	pl
	No	c	d	ql
	Total	P^2	Q^2 N	

where:

a = Slide +ve and seroconversion positive
b = Slide negative but seroconversion positive
c = Slide +ve but no sero-conversion positive
d = Slide negative and no seroconversion positive
N = All specimens

Parameter of Data Analysis:

1. Sensitivity of seroconversion: defined as the proportion of positive reactors, serological positive, among the individuals with patient parasitemia by bone-marrow examination.

$$\text{SENSITIVITY} = \frac{a}{a+c}$$

2. Specificity of seroconversion: defined as the proportion of Negative reactors,serological negative,among the individuals without patient parasitem a by bone-marrow examination.

$$\text{SPECIFICITY} = \frac{d}{b+d}$$

3. The probability of agreement of positive diagnosis (Pa)

$$Pa = \frac{2a}{2a+b+c}$$

4. The probability of agreement of negative diagnosis (Pd):

$$Pd = \frac{2d}{2d+b+c}$$

5. Kappa coefficient $= \dfrac{2\,(ad - bc)}{p^1q^2+p^2q^1}$

DATA ANALYSIS:

Data obtained from this study will be analysed using five statistical tests, namely, the chi-square test, student's t test, one-way analysis of variance (Anova) with multiple range test, Bartlett's test, and step wise multiple linear regression analysis (Kleinbaum and kupper, 1978; Duncan and Trapp, 1989; Hassard, 1990), SPSSPC+ and LOTUS statistical computer packages will be used where appropriate, Significant alpha levels for all analysis will be set at 0.05.

A. Data Analysis for qualitative Variables:-

1. The chi-square test for multiple independent proportions (2/k) will be done to test for the:-

1.1 difference in the distribution of the samples in the high and low and non endemic control areas among the four tests.

1.2 difference in the distribution of the area of residence of the study population among the three tests.

1.3 difference in thesex distribution of the study population among the three areas and among the three tests.

1.4 difference in the frequency of distribution of the antibody scores of the study population for each type of test among the three areas.

2. Bartlett's chi-square test will be used to test for the homogeneity of variance of the age distribution among the three tests. It will be used to indicate whether the one - way ANOVA assumption of homogeneity of variance will be violated or not.

Data Analysis for Quantitative Variables: Bivariable comparison of the means of the antibody scores obtain from each test among the three study areas will be carried out using the ANOVA test following by multiple range test.

For bivariable comparison of the means of the antibody score, student's t test and one way ANOVA for comparison of two means or more than two means respectively will be used. The multiple range test (LSD approach) was done when the ANOVA test

will be revealed a significant difference to identify which pair(s) of means will be different. For multi variable analysis, the multiple linear regression will be used.

Comparison of the mean ages among the three study groups will be accomplished using the ANOVA test following by the multiple linear regression test (when appropriate).

The Antibody Scoring System:

The parameter used to assess the efficiency of the serological methods under investigation to differentiate between high and low endemic areas was the antibody levels of the population in the respective study areas. Since the inherent units of measure used to express the antibody levels for each method differed, i.e. 'titre' for the two types of IFA's and optical density (O.D.) for the standard ELISA, it was decided to transform the original values obtained for each method into a unit of continuous measurement which would make them uniform and thus, statistically comparable. A scoring system was, therefore, devised where the original values were coded and given an equivalent score which ranged from one to four. The process of coding that was done detailed the following steps:-

FOR ELISA & IFAT

1. Preliminary transformation of the original results (i.e. in "titers") to their equivalent logarithmic values (log to the base 10).

This was done to restore the extremely skewed distribution of data set to normality, inasmuch as this is one of the fundamental assumptions that must be kept for results of the parametric analyses that were carried out in this study to be considered valid and meaningful (Dawson - Saunders and Trapp, 1990; Hassard, 1991).

The titers of the samples tested for IFAT ranged from five to 1280. All samples with titers less than five were given a value of 1.25, since this is the four - fold dilution titer preceding the starting working dilution of 1:5 For IFAT the minimum titer was 20 and values of less than 20 were entered as 10, inasmuch as this is the titer equivalent to the two fold dilution less than the initial working dilution of 1:20. The maximum titer obtained was 10240.

2. Determination of the arithmetic mean and median of the logarithmic transformations of the respective titers of the data set for each test.

As shown in Table 1, the logarithmic transformation was not as effective as it was hoped it would be since the distribution of the data set still tended to be skewed to the right (i.e. the mean values were larger than the median values) (Dawson - Saunders and Trapp, 1990) although to a lesser extent than if the original titer values were used.

3. Construction of the scoring system:

The scoring system was constructed using the inter quartile ranges based on the arithmetic median of the $\log_{10}$ titer values for each test (Dawson-Saunders and Trapp, 1990). The inter quartile ranges and their respective equivalent antibody scores are given below:

Inter quartile Ranges of Data Set	Antibody Score
Minimum value - 25th percentile value	1
>25th percentile value - median value	2
>median value - 75th percentile value	3
>75th percentile value - maximum value	4

Using this system, the antibody scores for the IDAT ranged from 1 to 4 with a mean of 2.38 + 1.15 S.D.; while those of the DAT were from 1.5 to 4 with a mean of 2.10 + 1.01 S.D. The lowest score for the DAT was set at 1.5 instead of 1, since the minimum up to the median values obtained for the test were the same (i.e.0.097), as shown in Table 3. The minimum score of 1.5 for DAT was derived from the average of the equivalent antibody scores of the first two inter quartile ranges (i.e. 1+2 divided by 2 = 1.5).

The blueprint for this scoring system is presented in **Table 2.**

b. **ELISA**

The recording process for ELISA differed from that of DAT and IFAT in that the original results (O.D.) were not transformed into logarithmic values since the data set did not have a markedly wide range.

The lowest O.D. value obtained was 0.060 and the highest was 1.39. The median was 0.460. Direct transformation of the O.D. values to antibody score showed that the minimum score was 1 and the maximum was 4 (mean = 2.46 + 1.12 S.D.) as shown in Table 2.

4.9.1 Descriptive Statistics of the Data Set for DAT, IFAT & ELISA.

Descriptive Measure (arithmetic)	Test		
	DAT (Titer $\log_{10}$)	IFAT (Titer $\log_{10}$)	ELISA (O.D.)
Minimum	0.097	1.000	0.060
25th percentile	0.097	1.602	0.280
Median	0.097	2.204	0.460
75th percentile	0.699	3.107	0.680
Maximum	3.107	4.010	1.390
Mean	0.749	2.326	0.498
Std. Deviation	0.715	0.921	0.274
Variance	0.512	0.848	0.075

4.9.2 Blueprint of the Antibody Level Scoring System Adapted for the Comparison of the Results of DAT, IFAT & ELISA

Test	Range of $\log_{10}$ transformed titers	Range of optical density (o.D.)	Equivalent antibody score	Mean of antibody	+ S.D. score
DAT	0.000-0.097			1.5	
	0.098-0.699			3	2.10 + 1.01
	>0.699			4	
IFAT	1.000-1.602			1	
	1.603-2.204			2	.37 + 1.15
	2.205-3.107			3	
	>3.107			4	
ELISA	0.060-2.280			1	
	0.281-0.460			2	2.46 + 1.11
	0.461-0.680			3	
	>0.680			4	

Results of the comparison of distribution of the 826 serum samples in the high and low endemic areas following random allocation to the three serological tests are presented in 4.9.3

4.9.3 Comparison of sample distribution in the High and low Endemic areas among the three serological tests.

Study population group No.	Serological Method	Number of samples (%)		Total
		High Endemic area	Low Endemic area	
Group 1	ELISA	131(60.4%)	86(39.6%)	217
Group 2	DAT	132(60.6%)	86(39.6%)	218
Group 3	IFAT	134(61.8%)	83(38.2%)	217
Group 4	IHA	134(63.8%)	76(36.2%)	210
Total :		531(61.6%)	331(38.4%)	862

As shown a greater proportion (61.6%) of the sera came from the high endemic area; while 38.4 percent were taken from the low endemic area. However, this difference was shown to be not significant (P value = 0.88), indicating that such high proportion observed from the data were due to chance variation. Except for IHA where only 210 samples were assigned, the number of sample allocated to the other tests was more or less equal, i.e. 217 for ELISA, 218 for DAT and 217 for IFAT (P value=0.949). In addition as a result of random allocation the distribution of the samples from the respective endemic area among the four tests is apparently comparable as shown in Table-3.

4.9 SERO CONVERSION USING DAT AS A COHORT STUDY

Out of the 862 serum samples collected only 15 (1.7%) were positive on bone marrow slide film examination & in culture (Ten 1.2%) of these came from the high endemic area, while, 5(0.05%) were taken from the low endemic one. Sixty percent (6/10) of the slide positive samples or growth in culture from the high endemic area were infected with LD body while 401 (4/10) growth in culture.

ELISA	COMPARISON OF DAT	IFAT

A. DEMOGRAPHIC PROFILE OF STUDY THE POPULATION

Statistical analysis (ANOVA, Chisquare) of the demographic characteristics of the study population (i.e. age, sex, place of residence) assigned to each test showed that the three groups were homogeneous and comparable in age and sex distribution, as well as in the distribution of area of residence.

B. AGE DISTRIBUTION

The overall age distribution for the three study groups ranged from six months to 78 years (N=652), with a mean of 24.8 + 17 years (95%) confidence interval: 22.56 - 26.20 years). The minimum age for group 1 was one year and the maximum was 78 years. For group 2, the age range was from 0.5-70 years; whereas,

for group 3, it was from 1-73 years. The variance of the age distribution among the three texts was found to be homogeneous (Bartlett's square test = 1.695 (df = 2), p=0.428. As shown in Table 4, there was no significant difference in the mean ages of the study population in the three tests (F=0.9904), suggesting that random allocation was successful in controlling for the extraneous effect age.

4.9.4 Comparison of mean Ages Among the three study groups

Group No.	Total No.	Mean Age(yrs)	SD	SE	95% C1 For Mean	Ratio	F Rati	F Prob
1	217	24.76	16.54	1:12	1.12	22.54-26.97	0.0096	.9904
2	218	24.98	18.01	1:22	1.22	22.57-27.18		
3	217	24.92	16.89	1:15	1.15	22.66-27.18		
Total:	652	24.88	17.13	0.67	0.67	23.57-26.20		

Sex Distribution

Comparison of the sex distribution of each study population among the three tests revealed that males and females were relatively equally distributed within each test and between test (X^2 = 1:36 (df = 2), P value = 0.5067) (Table 5), again indicating that random allocation was able to control for the potential confounding effect of gender.

4.9.5 : Sex Distribution of the study population among the three tests.

Sex	Test			X(2) value	P value
	ELISA	DAT	IFAT		
Male	117 (50.92%)	107 (49.08%)	117 (53.92%)	*1.36	0.5017
Female	100 (46.08%)	111 (50.92%)	100 (46.08%)		
Total:	217 (33.30%)	218 (39.4%)	217 (33.30%)		

* df = 2

Distribution of the Area of Residence

It was shown that most of the subjects included in each test came from the high endemic area and there was no significant difference in the distribution of the respective study groups from high and low endemic areas among the three tests (x^2 = 0.102

(df=2). P value = 0.95).

4.9.6 : Distribution of the study population in the two study Areas Among the three tests.

Area	Test			X^2 value	P value
	ELISA	DAT	IFAT		
Low endemic area	86 (39.63%)	86 (39.45%)	83 (38.25%)	*.102	.94985
High endemic area	131 (60.37%)	132 (60.55%)	134 (61.75%)		
Total:	217 (33.30%)	218 (33.4%)	217 (33.30%)		

* df = 2

B. Comparative Analysis of the Ability of the three Tests to Discriminate varying levels of kala-azar endemicity

Results of the overall frequency distribution of the antibody scores among the study populations for each test are presented in Tables 7 and 8.

4.9.7: Frequency Distribution of Antibody Scores obtained for ELISA among the populations in the two study areas.

Antibody Score	Number of samples %		Total	*P value
	High endemic area_Low endemic area			
1.5	87 (66.4%)	71 (82.6%)	158	
3	12 (9.20%)	5 (5.8%)	17	0.030
4	32 (24.4%)	10 (11.6%)		
Total:	131	86		217

*p value of x^2 test; df = 2

4.9.8. Frequency Distribution of Antibody Score obtained for DAT and IFAT among the populations in both study areas.

Test Area	Antibody score				Total value	*P
	1	2	3	4		
DAT High	33 (25%)	22 (16.7%)	37 (28%)	40 (30.3%)	132	0.000
Low	40 (46.08%)	17 (19.8%)	23 (26.7%)	6 (6.9%)	86	
IFAT High	42 (31.3%)	22 (16.4%)	35 (26.1%)	35 (26.1%)	134	0.001
Low	15 (18.1%)	33 (39.8%)	19 (22.9%)	16 (19.2%)	83	

*p value of x^2 test; df (Std DAT=3; df (IFAT)=3

As shown in the Tables 7 and 8, there was a statistically significant difference (P <0.05, x^2) in the frequency distribution of the antibody scores between the high and low endemic areas for each type of test.

Comparison (ANOVA) between the overall mean antibody scores obtained from the three tests (tables 9 and 10) likewise, revealed that at least, one of the three tests was significantly different from the others (P value =0.0021). To determine which of the tests differed significantly from the others, a multiple comparison test or multiple range test (LSD approach) of the mean scores obtained for the three tests was, thus, performed. Results showed that the difference in the mean scores between DAT and IFAT were not significantly different from each other (P value, 0.05); but were significantly different from that of ELISA data not shown).

The antibody scores of all the fifty normal control sera for the respective tests used in this study were demonstrated to be as follows: ELISA=1.5, DAT=1, IFAT=1

4.9.9 . Comparison of the overall Mean Antibody Score Among the three Tests (ELISA, DAT, IFAT) from Both study areas.

Test	Total No.	Mean score	Std Deviation	Std Error	95% confidence interval for mean
ELISA	217	2.1014	1.0144	.0689	1.9657 - 2.2371
DAT	218	2.3761	1.1543	.0782	2.2221 - 2.5302
IFAT	217	2.4562	1.1177	.0759	2.3067 - 2.6058

4.9.10 : Anova Table : Comparison of variances of the mean scores of ELISA, DAT and IFAT

Source of variation	Degree of freedom	Sum of square	Mean squares	F Ratio	F Prob.
Between groups	2	15.0364	7.5182	6.2454	.0021
Within	649	781.2596	1.2038		
Total	651	796 2960			

From the narrow range of the confidence limits shown for each test (Table-9) one could conclude with 95% confidence) that the estimates of the true mean antibody score in the population are relatively precise. Moreover, if also gave us a rough idea as to which test differed from the others (in this case ELISA being different from DAT and IFAT which was estimated by the multiple range test).

To reach the general objective of this study, the key question that had to be answered was, which of the three tests shows the best correlation between antibody score and the known travels of endemicity of the respective study areas ? To address this issues, a stepwise multiple linear regression analysis (bawvion - saundess and Trapp. 1990) of the data was performed. The choice of this method of statistical analysis essentially rested on the awareness of the existence of a vast number of variables that could possibly influence the outcome (i.e.
antibody score) of the tests. There were two basic steps that were carried out in the regression analysis. The first step was to include all the explanatory variables that were thought to be biologically related to the outcome in to the regression equation. Then those variables whose regression coefficients were found to be not statistically significant were eliminated from the equation and the regression equation was recalculated by using only the variables whose regression coefficients were demonstrated to be statistically significant.

The basic equation formula that was used for the calculation is given below (klein baumn and Kupper, 1978; Duncan and Trapp, 1989; Hassard, 1090).

$$Y=a+b^1,x^1 + b_2x_2 + b_3 X_3 \ldots\ldots b_1x_1$$

Where

Y= antibody score (outcome variable)

a= intercept term.

b_1, b_2, b_3 .....b_1 = the regression coefficients of the study factors (explanatory variables) that were included in the equation.

x_1, x_2, x_3 ..... x_1 = the explanatory or independent variables that may affect the outcome.

A total of three multiple linear regression models were formulated and the various independent variables included in each model are summarized below:

Model 1 (Step 1): DAT, age, area, parasite density, sex ELISA.

Model 2 (Step 2): Interaction effect between type of test and level of endemicity of area (interaction), test, age, area.

Model 3 (Step 3): Test, age, area.

Results of the sequential processes that were involved in the analysis are presented in Tables 11-16. These results show the respective regression coefficients the respective standard errors and statistics of the independent variables included in each models, as well as the results of the respective tests for significance of each regression equation (ANOVA) Table II and 12 for regression model 1, Tables 13 and 14 for regression model 2, and Tables 15 and 16 for regression model 3).

4.9.11 Regression coefficient of DAT, age area, sex ELISA against the antibody score of IFAT (Regression)

Model No.1: Antibody score = 1.615 - .082 x DAT + .024 x Age +
0.352 x Area - 2.475 E-04 x density + 0.066 x Sex -
0.345 x ELISA).

Explanatory variable (x^1)	Regression coefficient b^1	Std error (Sf b^1)	Standardized Regression (Beta)	t value	p value
DAT(x^1)	-.082	.096	-.035	-.851	.3952
Age (x_2)	.024	.002	.370	10.385	.000
Area(x_3)	.352	.081	.156	4.358	.0000
Density(x_4)	-2.475	E-04	4.222 E-04.021	.586	.5580
Sex (x_5)	.066	.079	.030	.831	.4061
ELISA(x_6)	.345	.096	.117	3.581	.0004
Intercept(a)	1.615		.108	14.995	.0000

Data coding ELISA = DAT = 2 IFAT = 31 age (in year) density (Number of parasite i.e. body per field), sex (Male=1, Female=0)

4.9.12 : Analysis of variance (ANOVA) Table for antibody score

Regression on DAT, Age, Area, Parasite density,Gender and ELISA (Regression Model No.1)

Source of variation	df	sum of squares	Mean squares	F	Signif F	Multiple R	R	Std Errol
Regression	6	145.69	24.28	24.072	.0000	.42774	18296	1.0034
Residual	645	650.61	1.01					
Total	651	796.30						

As indicated in Table 11, only three of the independent variables included in the regression model no.1, namely age, area and ELISA, were found to have genuine influence (P value of 0.0000, 0.0000 and 0.0004, respectively) on the antibody score using IFAT as the reference test. Parasite density and gender were proven to be insignificant prediction of the antibody score (P value 0.5580 and 0.4061). DAT was likewise, shown to have no real influence on the estimated antibody score (P value = 0.3952) using IFAT as the reference.

Having identified parasite density and gender as statistically insignificant prediction of antibody score, they were then deleted in the second regression model. In addition, two new terms (or explanatory variables) were introduced into the equation. These were a) "Test" which was a dummy variable coded 1 for ELISA and 0 for IFAT or DAT and b) "Interaction" which represented the explanatory variable of the effect of interaction between of test and level of endemicity of area on the antibody score. This term was introduced into the equation to determine whether the relationship between type of test and antibody score is dependent on the level of endemicity of the area or not.

The reason why DAT and IFAT were collapsed into just one term, i.e. "test", was because the predicted scores for these two tests (when all the other five variables in the first regression model were held constant) were found to be not significantly different from each other **(P value = 0.3952) Table II.**

4.9.13 : Regression coefficients of Interaction effect between (3) Test (Regression Model No.2: Antibody score=1.617+

0.056 test x area + 0.024 x age + 0.335 x area -0.341 x test).

Independent variable (X_1)	Regression coefficient (b_1)	Std error (b_1)	Standard regression (beta)	T value	P value
Interaction (test x area (x_1)	.056	.171	.020	.326	.7444
Age (X_2)	.024	.002	.369	10.369	0000
Area (X_3)	.335	.099	.148	3.386	0008
Test (X_4)	-.341	.133	.145	2.565	.0106
Intercept	1.617	.095		17.030	.0333

Data coding age (in years), Area (high=1, Low=0) Test (ELISA=1, DAT/IFAT = 0)

4.9.14 ANOVA Table for antibody score regressed against interaction effect between Test and Area, Age, Area & Type of Test (Regression Model No.2)

Source of variation	df	Sume of squares	Mean square	F	Sig F	Multiple R	R	Std Error
Regression	4	144.059	36.015	35.726	.0000	.42534	.18091	1.404
Residual	647	652.237	1.008					

As shown in Table 13, age, level of endemicity of the area, and the type of serological test used were still found to exert a strong influence on the antibody score (P values <0.05) even after exclusion of parasite density and sex from the equation. Moreover, it was shown that there was no interaction between type of test used and level of endemicity of the area (P=0.7444), indicating that these two factors operate independently of each other in their effect on the antibody. Further more, it indicates that the difference in the mean antibody score between the high and low endemic areas using ELISA is equal to the difference in the mean antibody score between the two endemic areas using either DAT or IFAT. In the other words, it reveals that there was no significant difference in the ability of the three tests to distinguish between the high and the low endemic areas when all the other variables included in the equation are held constant.

4.9.15: Regression coefficients of Type of Test Age and Area Regressed Against Antibody Score(Regression Model No.3)

Independent variable (X_1)	Regression coefficient (b_1)	Std error (b_1)	Standard regression (beta)	T value	Sig T
Test (x_1)	-.307	.083	-.131	-3.686	.0002
Age (X_2)	.024	.002	.369	10.372	0000
Area (X_3)	.353	.081	.156	4.387	0000
Intercept	1.606	.089		18.065	.0000

Data coding: Test (DAT/IFAT = 0, ELISA=1), Age (in years), Area (high-1, Low = 0).

4.9.16 ANOVA Table for antibody score regressed against Type of Test, Age and Area, (Regression Model No.3)

Source of variation	df	Sume of squares	Mean square	F	Sig F	Multiple R	R	2 Std Error
Regression	3	143.952	47.981	47.66	4 .0000	.4252	.18078	1.0034
Residual	648	651.344	1.007					

In the final analysis only three of the independent variables tested in this study were shown to exert a significant influence on the predicted antibody scores of the study population from two endemic areas investigated. These were ages, type of serologic test employed and the inherent level of endemicity of the area. The final regression model that was, thus, adapted to analyze the results of this study was as follows:-

$$Y=1.606-.307\ X_1 + 0.024\ X_2 + .353\ X_3$$

Where,

Y = Predicted antibody score

1.606 = intercept

X_1 = Test (a dummy variable coded

1=ELISA, 0=DAT or IFAT)

0.307 = regression coefficient test (b_1)

X_2 = Age (in years)

0.024 = regression coefficient for age (b_2)

X_2 = Area (a dummy variable coded

1 = high endemic area

0 = Low endemic area

0.353 = regression coefficient for area (b_3)

From this statistical equation three conclusions were drawn: First, is that when age and area are held constant, the predicted antibody score for ELISA will be .31 less than that of DAT or IFAT. Secondary it tells us that the antibody score is estimated to increase by 0.02 for every year of increase in age for the same test and area. Thirdly, it indicates that the predicted antibody score in the high endemic area will be 0.35 higher mean that of the low endemic area where the age and test remain constant. The intercept is not interceptable in this design.

Results of this test for the significance of the overall regression equation (Table 16) revealed that 18.1% (R2=.18078) of the variation in the predicted the antibody score is significancy explained (F ratio 47.984, P value = 0.0000) by the combined influence of the three independent variable included in the model. As indicated in Table 14, of these three, age was found to exert the greatest influence on the variation in the antibody score (Beta = .369) followed by level of endemicity of area (Beta = .156) then by type of test (Beta = -.156).

A tabulated summary of the predicted mean antibody scores for ELISA and DAT/IFAT for each age group are shown in Table 17 and 18 bellow. As can be seen in Table 17 and 18, the differences in the predicted mean antibody scores for same test (and age) between the high and low endemic areas are the same (i.e. 0.35) regardless of whether the test employed in ELISA or DAT/IFAT. Since this difference in the mean antibody scores between the two areas was shown to be statistically significant (P value = 0.000) (by in Table 15), then it clearly indicates that the efficiency of the three tests to discriminate between high and low endemics areas, based on the antibody scores of the populations in the respective areas are equal or the same.

The difference in the antibody scores obtained for the same area between tests was likewise, shown to be equal, (i.e. 0.307). In other words, the antibody score obtained for ELISA will be 0.307 less than that of DAT or IFAT, regardless of whether the blood samples is taken from the high endemic area or from a low endemic one.

4.9.17: Comparison of the Predicted Mean Antibody Scores obtained for ELISA between the High and Low Endemic Areas for Each Age Group.

Age (yrs)	Antibody Score		Age (yrs)	Antibody Score	
	High Endemic Area	Low Endemic Area		High endemic Area	Low Endemic Area
1 yr	1.676	1.323	41 yrs	2.636	2.283
2 yrs	1.7	1.347	42 yrs	2.66	2.307
3 yrs	1.724	1.371	43 yrs	2.684	2.331
4 yrs	1.748	1.395	44 yrs	2.708	2.355
5 yrs	1.772	1.419	45 yrs	2.732	2.379
6 yrs	1.796	1.443	46 yrs	2.756	2.403
7 yrs	1.82	1.467	47 yrs	2.78	2.427
8 yrs	1.844	1.491	48 yrs	2.804	2.451
9 yrs	1.868	1.515	49 yrs	2.82	2.475
10 yrs	1.892	1.539	50 yrs	2.82	2.499
11 yrs	1.916	1.563	51 yrs	2.76	2.523
12 yrs	1.94	1.587	52 yrs	2.9	2.547
13 yrs	1.964	1.611	53 yrs	2.924	2.571
14 yrs	1.988	1.635	54 yrs	2.948	2.595
15 yrs	2.012	1.659	55 yrs	2.972	2.619
16 yrs	2.036	1.683	56 yrs	2.996	2.643
17 yrs	2.06	1.707	57 yrs	3.02	2.667
18 yrs	2.084	1.731	58 yrs	3.044	2.691
19 yrs	2.108	1.755	59 yrs	3.068	2.715
20 yrs	2.132	1.779	60 yrs	3.092	2.739
21 yrs	2.156	1.803	61 yrs	3.116	2.763
22 yrs	2.18	1.827	62 yrs	3.14	2.787
23 yrs	2.204	1.851	63 yrs	3.164	2.811
24 yrs	2.228	1.875	64 yrs	3.188	2.835
25 yrs	2.252	1.899	65 yrs	3.212	2.859
26 yrs	2.276	1.923	66 yrs	3.236	2.883
27 yrs	2.3	1.947	67 yrs	3.26	2.907
28 yrs	2.324	1.971	68 yrs	3.284	2.931
29 yrs	2.348	1.995	69 yrs	3.308	2.955
30 yrs	2.372	2.019	70 yrs	3.332	2.979
31 yrs	2.396	2.043	71 yrs	3.356	3.003
32 yrs	2.42	2.067	72 yrs	3.38	3.027

33 yrs	2.444	2.091	73 yrs	3.404	3.051
34 yrs	2.468	2.115	74 yrs	3.428	3.075
35 yrs	2.492	2.139	75 yrs	3.452	3.099
36 yrs	2.516	2.163	76 yrs	3.476	3.123
37 yrs	2.54	2.187	77 yrs	3.5	3.147
38 yrs	2.564	2.221	78 yrs	3.524	3.171
39 yrs	2.5888	2.235			
40 yrs	2.612	2.259			

4.9.18: Comparison of the Predicted Mean Antibody Scores obtained for DAT/IFAT between the High and Low Endemic Areas for Each Age Group.

Age (yrs)	Antibody Score		Age (yrs)	Antibody Score	
	High Endemic Area	Low Endemic Area		High endemic Area	Low Endemic Area
1 yr	1.983	1.63	41 yrs	2.943	2.59
2 yrs	2.007	1.654	42 yrs	2.967	2.614
3 yrs	2.031	1.678	43 yrs	2.991	2.638
4 yrs	2.005	1.702	44 yrs	3.015	2.662
5 yrs	2.079	1.726	45 yrs	3.039	2.686
6 yrs	2.103	1.753	46 yrs	3.063	2.71
7 yrs	2.127	1.774	47 yrs	3.087	2.734
8 yrs	2.151	1.798	48 yrs	3.111	2.758
9 yrs	2.175	1.822	49 yrs	3.135	2.782
10 yrs	2.199	1.846	50 yrs	3.159	2.806
11 yrs	2.223	1.87	51 yrs	3.193	2.83
12 yrs	2.247	1.894	52 yrs	3.207	2.854
13 yrs	2.271	1.918	53 yrs	3.231	2.878
14 yrs	2.295	1.942	54 yrs	3.255	2.902
15 yrs	2.319	1.966	55 yrs	3.279	2.926
16 yrs	2.343	1.99	56 yrs	3.303	2.953
17 yrs	2.367	2.014	57 yrs	3.327	2.974
18 yrs	2.391	2.038	58 yrs	3.351	2.998
19 yrs	2.415	2.062	59 yrs	3.375	3.022
20 yrs	2.439	2.086	60 yrs	3.399	3.046
21 yrs	2.463	2.11	61 yrs	3.423	3.07
22 yrs	2.487	2.134	62 yrs	3.447	3.094
23 yrs	2.511	2.158	63 yrs	3.471	3.118
24 yrs	2.535	2.182	64 yrs	3.495	3.142
25 yrs	2.559	2.206	65 yrs	3.519	3.166
26 yrs	2.583	2.23	66 yrs	3.543	3.19
27 yrs	2.607	2.254	67 yrs	3.567	3.214
28 yrs	2.631	2.278	68 yrs	3.591	3.238
29 yrs	2.655	2.302	69 yrs	3.615	3.262
30 yrs	2.679	2.326	70 yrs	3.639	3.286

31 yrs	2.703	2.35	71 yrs	3.663	3.31
32 yrs	2.727	2.374	72 yrs	3.687	3.334
33 yrs	2.751	2.398	73 yrs	3.711	3.358
34 yrs	2.775	2.422	74 yrs	3.735	3.382
35 yrs	2.799	2.446	75 yrs	3.759	3.406
36 yrs	2.823	2.47	76 yrs	3.783	3.43
37 yrs	2.847	2.494	77 yrs	3.807	3.454
38 yrs	2.871	2.518	78 yrs	3.831	3.478
39 yrs	2.895	2.542			
40 yrs	2.919	2.566			

4.10 SERO-CONVERSION USING DAT AS A COHORT STUDY

The cohort and its community

The community in Trishal Thana under Mymensingh district of Bangladesh is rather compact. Most of the people built their houses along banks of the river (Brahmaputra).

There were 714 people registered in 102 families staying in 95 houses which composed of 374 males and 340 females. The studied population was classified in two group. They were the group of local residents or owners and their relatives which composed of 631 people and the group of migrants or labourers and their relatives which composed of 83 people (Table-1). These two groups were considered that they were different regarding the risk of kala-azar infection. To some extent, the group of local residents lived in the endemic areas for longer time. They were suspected as the group of immuned population. Those migrants came from the different areas in the south or Bangladesh. Where kala- azar was scarcely distributed and they had been commonly lived in this community not more than one to two months, then they were suspected as the non-immuned population. For the sake of data analysis and evaluation of the serological response, the data of migrants must be excluded so the data of local residents or owners and their relatives were only used in analysis of this study.

4.10.1 The population of 714 people in Gazipur of Trishal were grouped by sexes and different status.

Local residents		Migrants	Total
Male	321	53	374
	45.00	7.4	52.4
Female	310	30	340
	43.40	4.2	47.6
Total:	631	83	714

Note: Fig. within parentheses are percentages of row totals.

Surveillance of seasonal epidemic of kala-azar in the group of local residents:

The incidence density was calculated as the rate of the number of new attacks or monthly incidence and the number of person - months. Denominator of incidence density or man - months was estimated from the summation of the inhabited month of individual which was followed up in every month under the assumption that the local resident who lived in the village in each month had a risk of kala-azar through the month. The monthly incidence density demonstrated the epidemic pattern of kala-azar. The annual incidence density per 1000 person-months of kala-azar in the group of local residents and their relatives were (Chowdhury et al 1993 at press) 7.7 and 7.7 respectively and they were added up to be 15.3 incidence.

The epidemic pattern of kala-azar among the local residents was demonstrated that there was an epidemic were occurred during May to August with the variation of the monthly incidence from 12.6 to 17.1 per thousand person - months. In the rest of this year the monthly incidence densities were not so high. They varied from nil to 3.4 per

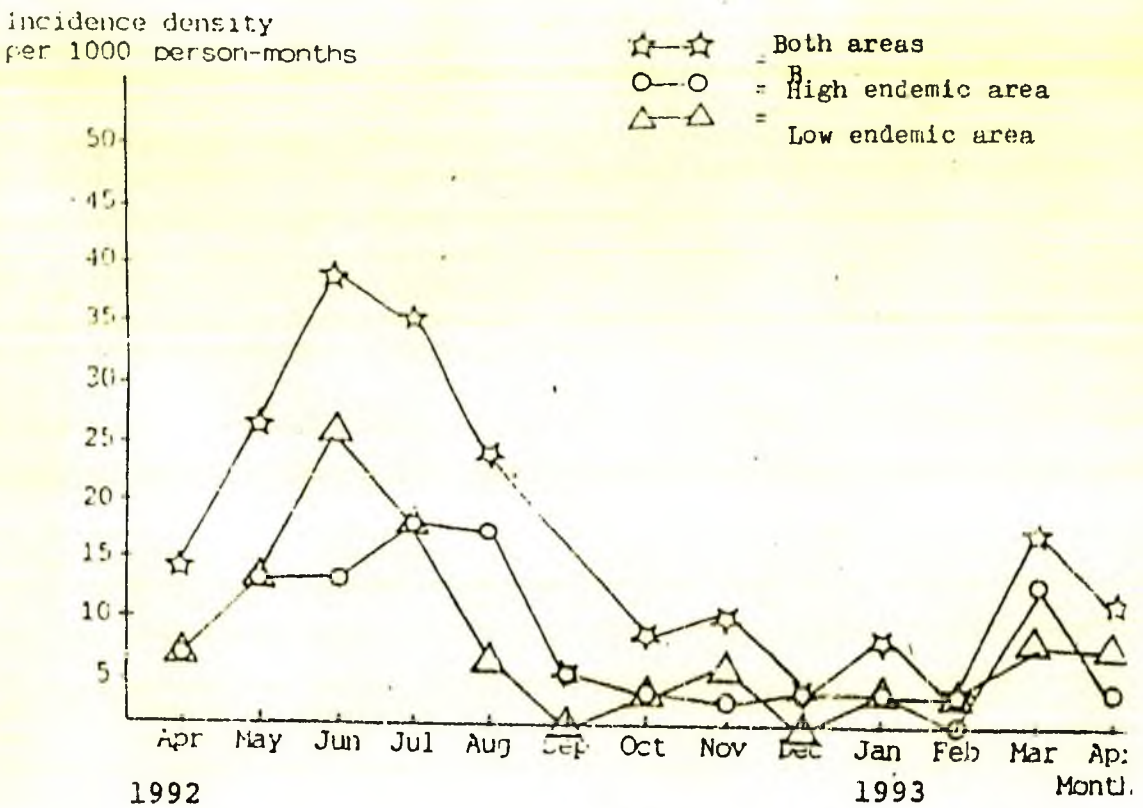
1000 person - months except in March when the incidence density swang up to 10.2 per 1000 person - months. Statistical analysis was tested for heterogenicity of the monthly distribution of kala-azar incidence densities. The outcome revealed that they were statistically significant ($P<0.05$). Then it was explained that the transmission of kala- azar is minimum during September to February. The period of high peak began on May and last to July with variation of monthly incidence densities from 13.0 to 25.2 per 1000 person - months. But during September to February the monthly incidence density varied from zero to 3.1 per 1000 person - months. The rest of period seemed to be the inter period phase with the incidence densities from 6.3 to 7.6 per 1000 person - months.

4.10.2 Distribution of monthly DAT titers in terms of geometric mean of reciprocal titer (GMRT) and seropositive (>1:3200) rate in the group of local residents from April 1991 to April 1991 in Guzium of Trishal Thana.

Month/ Year	High endemic Kala-azar area		Low endemic Kala-azar area		Total case +ve/person -month
	LD body +ve/ Person-month	ID	LD body +ve Person-month	LD	
Apr'91	2/284	7	2/284	7	4/234
May	4/308	13	4/308	13	8/308
Jun	4/318	12.6	8/318	25.2	12/318
Jul	6/350	17.1	6/350	17.1	12/350
Aug	5/302	16.6	2/302	6.6	7/302
Sep	2/323	6.2	0/327	0	2/323
Oct	1/249	4	1/249	4	2/249
Nov	1/331	3	2/331	6	3/331
Dec	1/295	3.4	0/295	0	1/295
Jan'92	1/325	3.1	1/325	3.1	2/325
Feb	0/377	0	1/377	2.7	1/377
Mar	4/394	10.2	3/394	7.6	7/394
Apr	1/318	3.2	2/318	6.3	3/318
Total:	32/4074	7.7	32/4074	7.7	64/4094

Note: Number of persons in the group of local residents (owners highly endemic and low endemic) of bone-marrow/spleen aspirated materials examination was equal 620 persons from 631 persons.

Figure 15 Monthly incidence density (per 1000 person-month) of total cases(Kala-azar) among the rgow local resident from April, 1992 to April, 1993 in Mymensingh district.



4.10.3. Distribution of monthly DAT titers in terms of geometric mean of reciprocal titer (GMRT) and seropositive (>1:3200) rate in the group of local residents from April 1991 to April 1992 in Guzium of Trishal Thana.

Month/ Year	DAT TITRE							Total	Sero +ve	GMRT
	>1:1600	3200	6400	12800	15600	51200	102400			
Apr'91	061	36	23	22	22	21	18	203	70	9.1
May	100	30	30	29	28	28	21	266	62.4	32.7
Jun	88	18	16	14	14	12	31	215	59.1	37.5
Jul	104	32	26	22	21	12	41	277	62.5	38.0
Aug	80	21	16	12	11	10	13	196	59.2	27.6
Sep	99	19	20	19	17	15	26	236	58.1	35.2
Oct	121	40	14	12	11	9	12	248	51.2	18.7
Nov	104	39	28	25	25	21	32	290	64.1	37.5
Dec	125	17	26	20	18	16	28	266	53	31.2
Jan'92	102	33	24	29	16	11	28	277	63.2	39.2
Feb	158	15	35	24	29	25	31	338	53.3	30.0
Mar	132	43	39	29	30	25	27	325	59.4	28.8
Apr	105	26	38	30	34	28	48	299	64.9	50.8
Total:	1379							3436	59.9	33.6

Figure 16 Graph of distribution of monthly GMRT titres in terms of geometric mean of reciprocal titre (GMRT) and seropositive (≥ 1 :3200) rate in the group of local residents and their relatives from April 1992 to April 1993

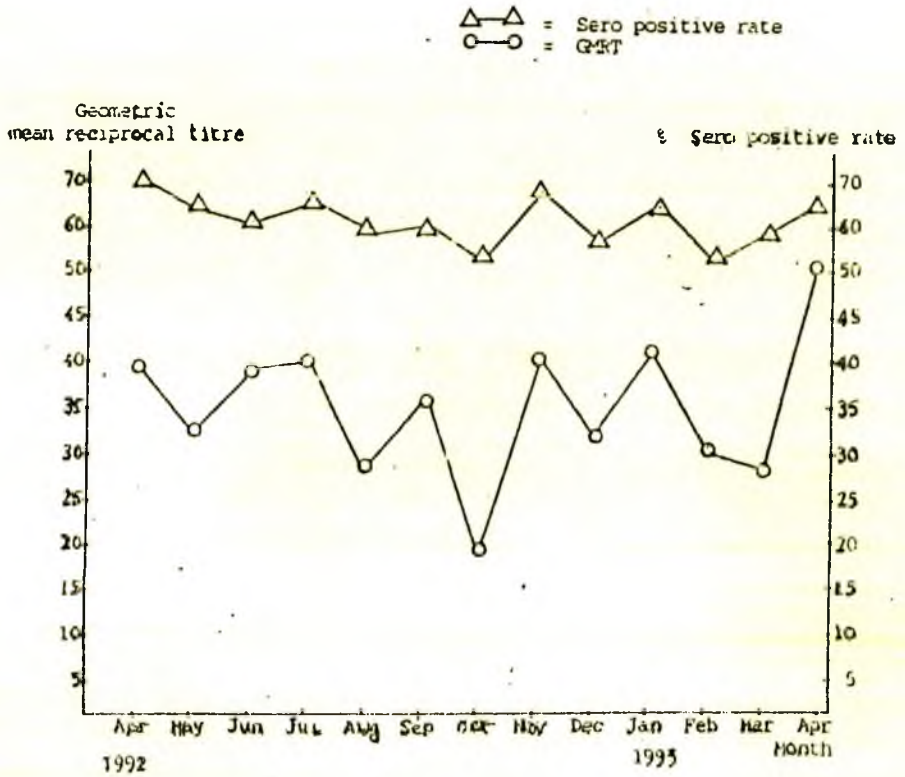


Figure 17 Graph of distribution of monthly increased titre at least 1600 fold dilution in the group of local residents and their relatives from April 1992 to April 1993

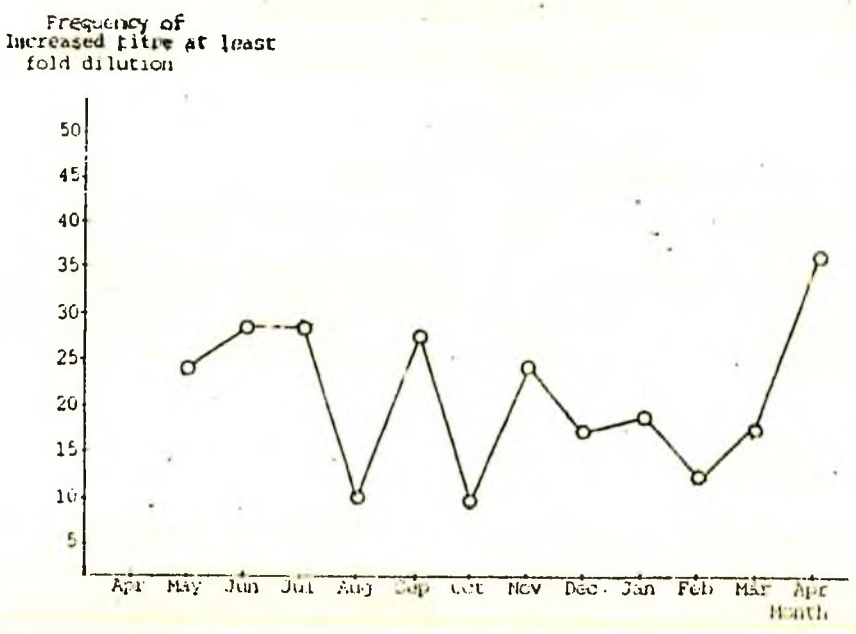
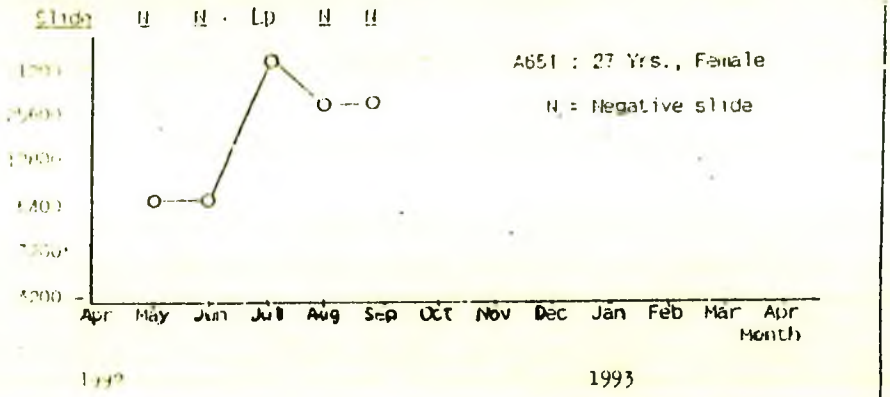
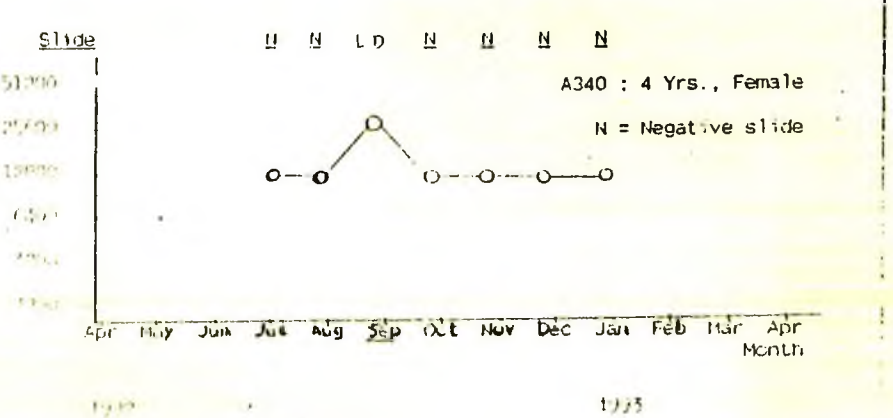


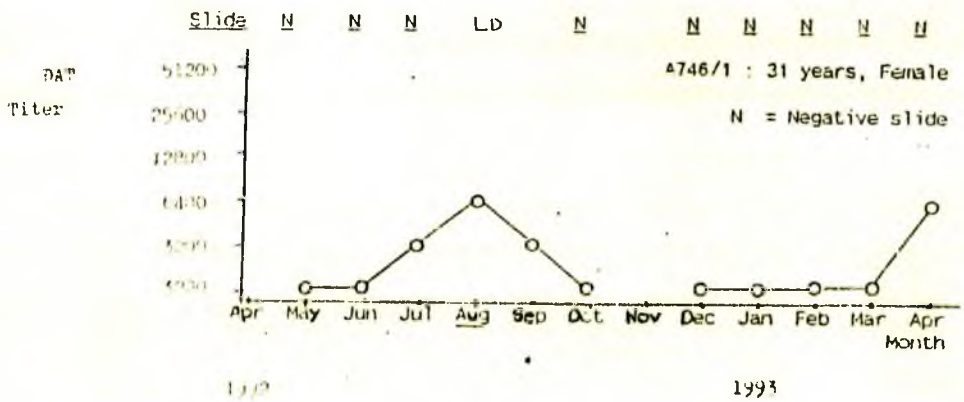
Figure 19 : Pattern of serological changes with in one or two months before the same month of slide positive.

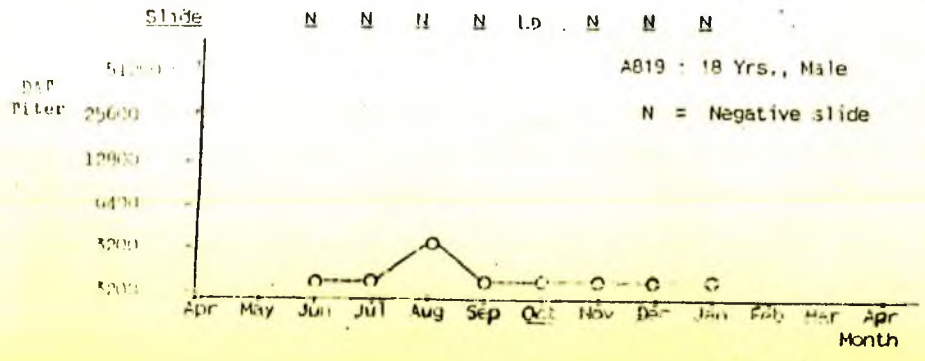




Pattern I

Figure 18 : Pattern of serological changes with in one or two months before side positive.

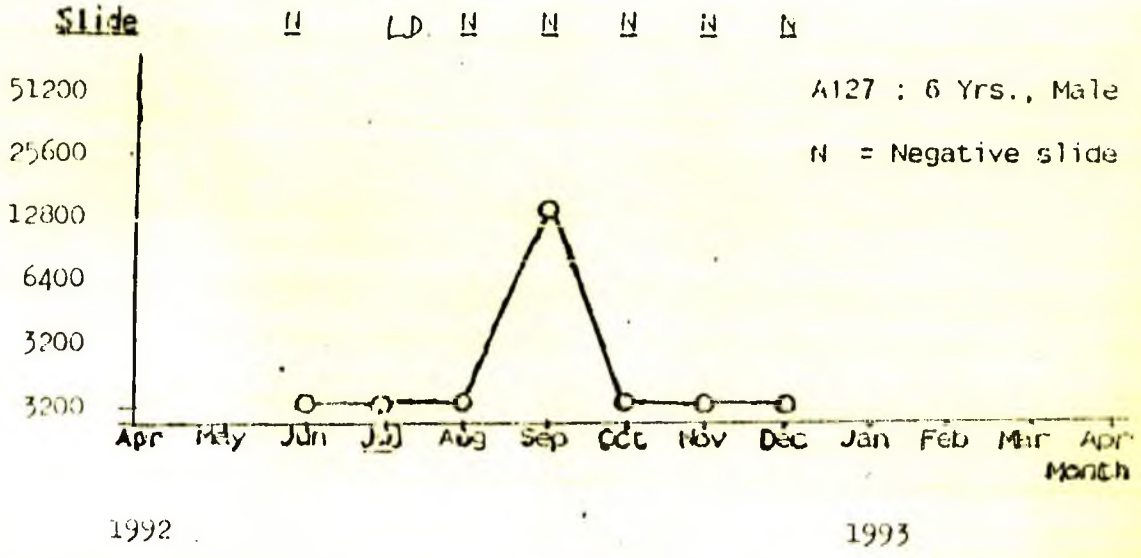


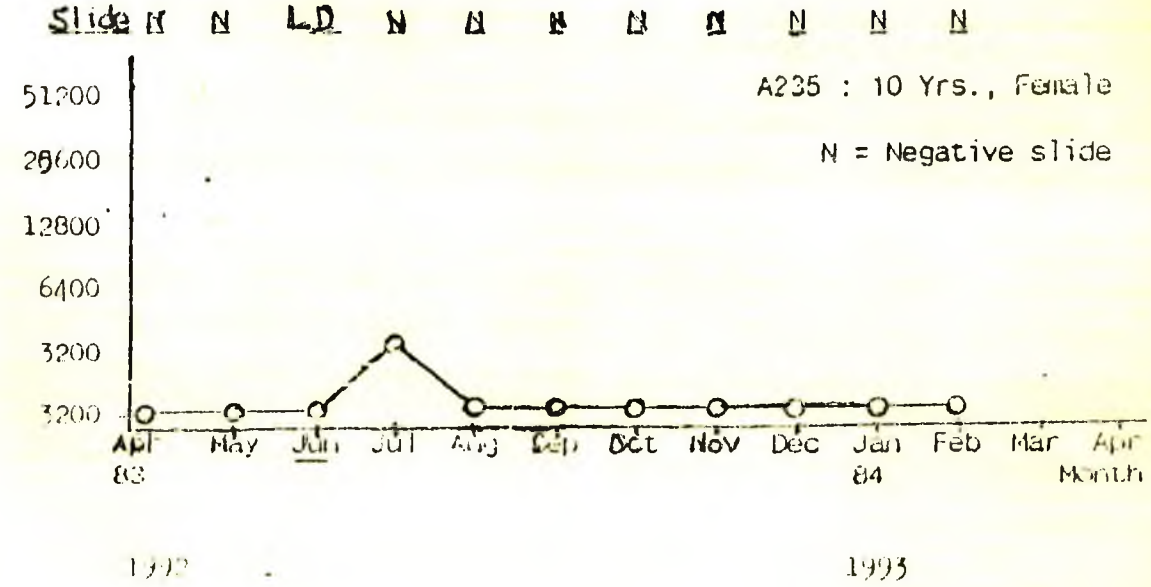


155 B

Pattern III

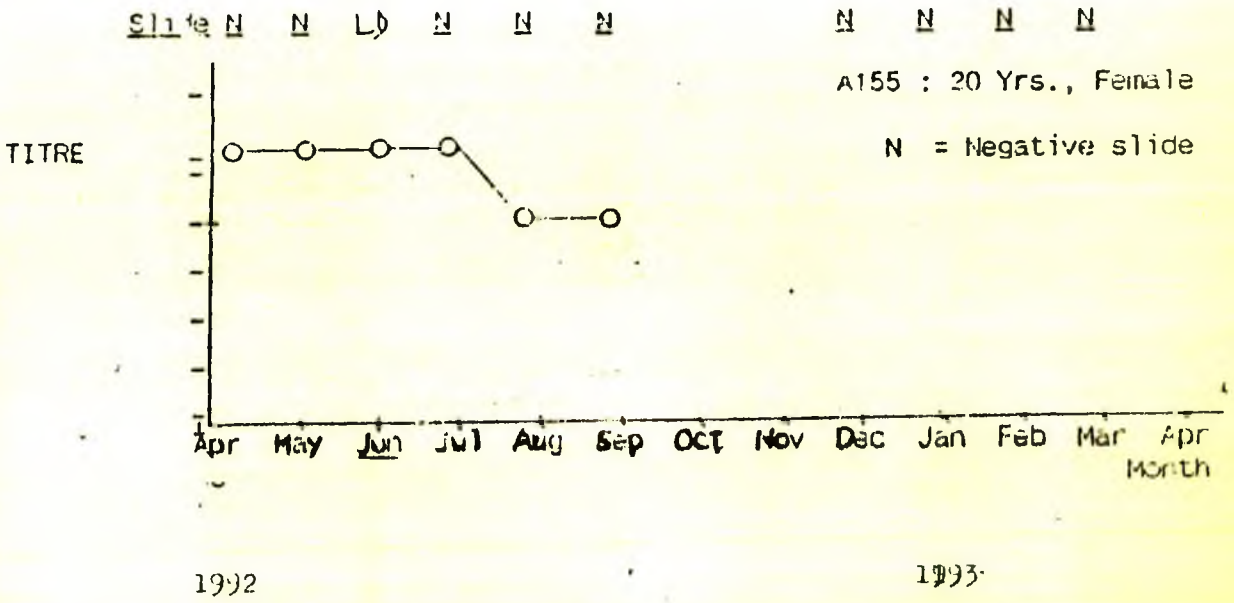
Figure 20 : Pattern of serological changes one or two months after slide positive.

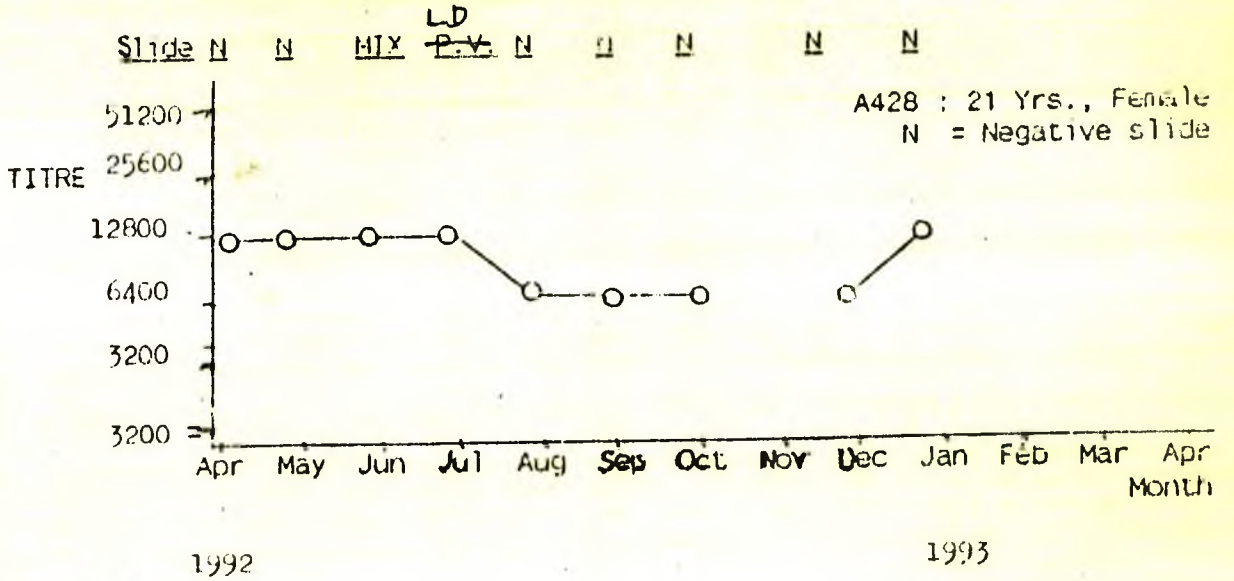




Pattern IV

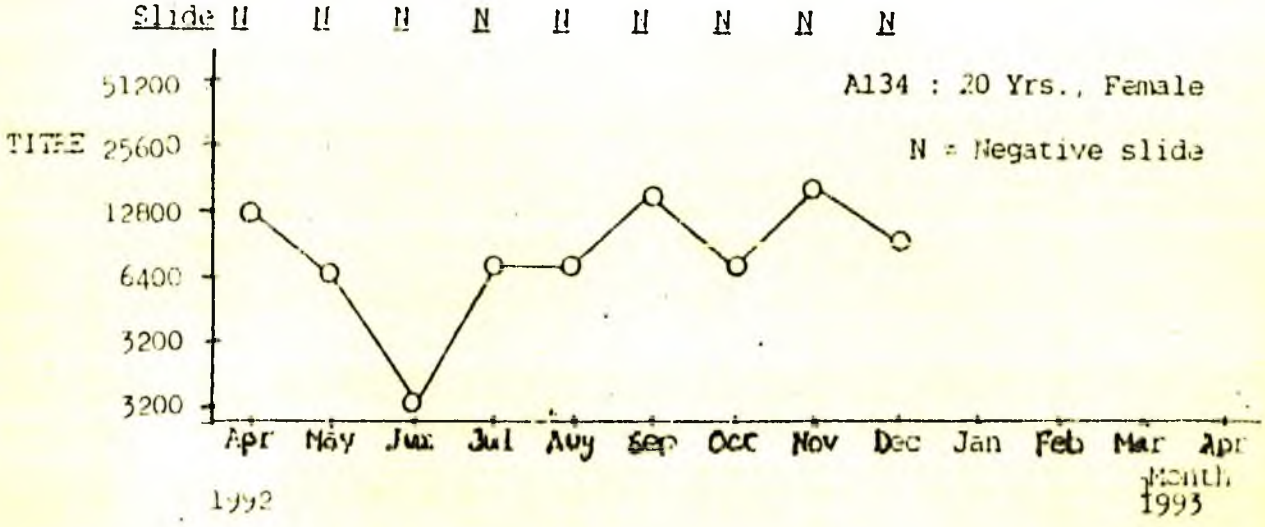
Figure 21 : Pattern of no serological changes among those negative slide turned to positive.

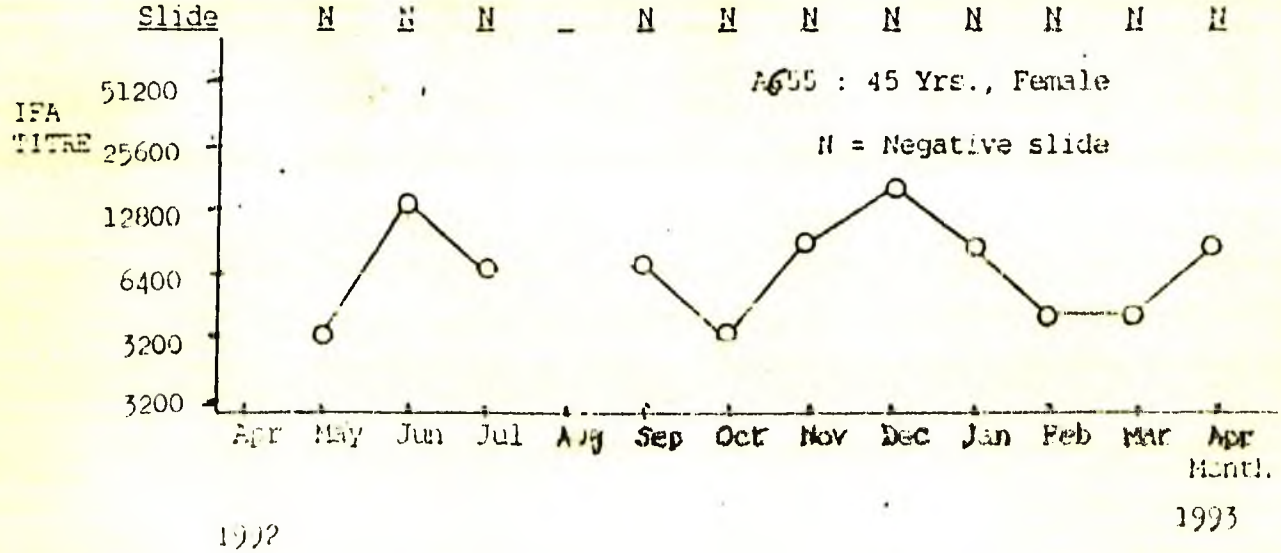




Pattern V

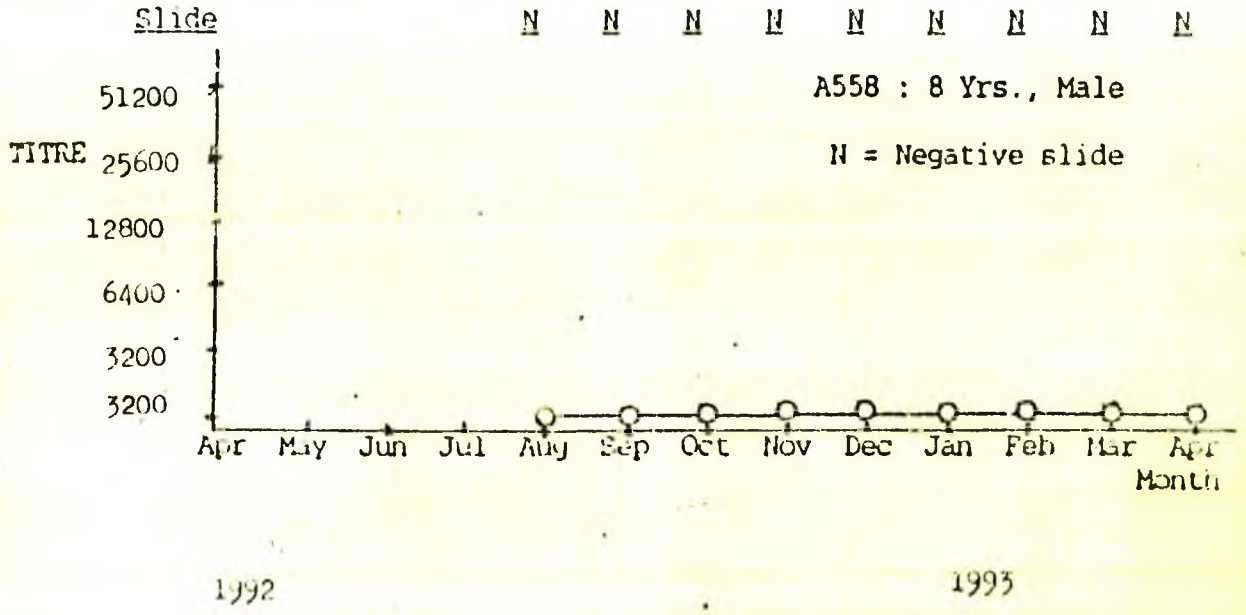
Figure 22 : Pattern of srological changes among those whose slide remained negative.

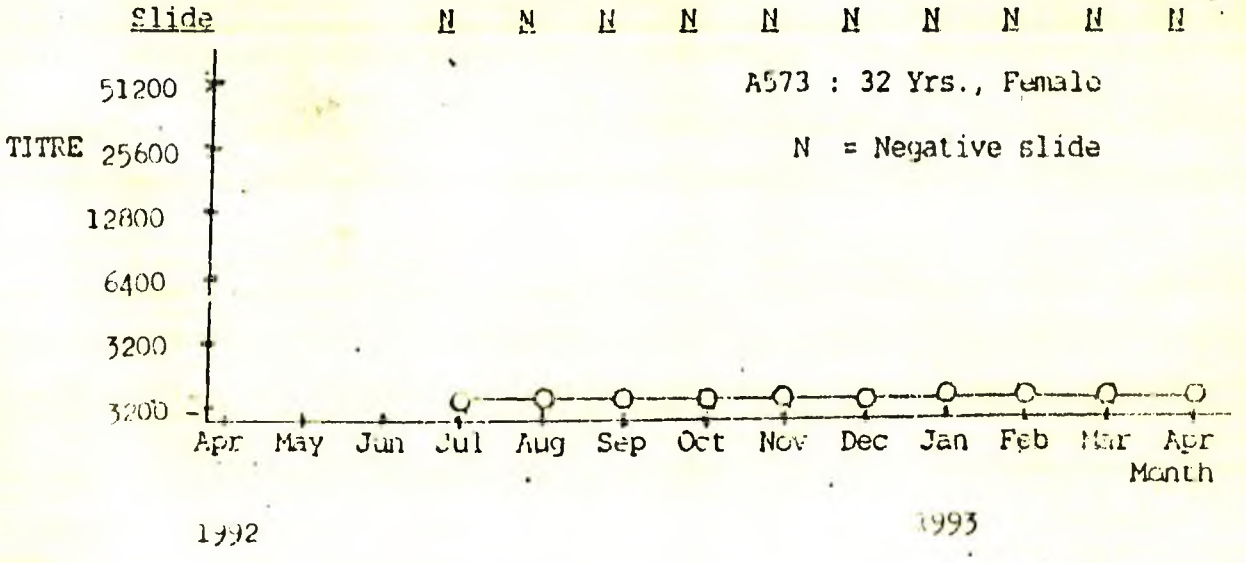




Pattern VI

Figure 23 : Pattern of no serological changes among those whose slide remainded negative.





Distribution of DAT titer in the group of local residents and their relative from April 1992-April 1993 in Guzium in village,
Trishal Thana.

From the total 3,436 samples obtained from 596 persons of local residents, there were 2,057 samples or 59.9% of total samples had the titre equal or more than 1:3200 which were considered as seropositive samples, within these 2,057 seropositive samples were collected from 445 persons. The monthly variation of the seropositive rates and the Geometric Mean of Reciprocal Titre (GMRT) were illustrated in the graph (Fig.5) and the Table-5. Anova test was done to evaluate the heterogenicity of the monthly GMRT. The outcome revealed that they were significantly different especially the GMRT in October when GMRT was significantly lowest statistical analysis was applied the assess of the monthly variation of seropositive rate. It gave the important outcomes that the seropositive rate measured in October, December and February were significantly different from other. The seroconversion rate was supposed to be the best parameters using to diagnose the new attack of kala-azar. However the monthly variation of this rates were more fluctuated. The lower level occurred in August and October. The lower trend appear on the end period of the vector density came below 1 man/hour December to February with the variation of seroconversion from 17.9 to 12.8%.

Reproducibility of the test:

The overall of studied serum samples from population of 672 in Guzium village were 3645, of which 859 (23.6%) were randomly selected for replicate testing. The type of reproducibility used in this part of analysis was test - retest reliability. Test -

retest reliability was determined by a comparison of the two titers obtained on the 859 replicated serum samples. Table (5) showed the number of samples with the same titers and with 1600 fold, 3200-fold and 6400-fold differences in titers on the two determinations. From the test - retest reliability, 90.8% of the serum specimens showed a 1600-fold differences between the two tests 1.3% showed a 3200-fold difference between the two tests. The difference as much as 6400 fold dilutions of the test in the study was 0.1%. From this test of differences of both test-retest titer, the distribution of outcomes had a mean and standard deviation of 39.8+ 3.05 and 40.02 + 3.5 respectively. There was no differences of the two means (P>0.05). For titer of second reading from retest which had difference from titer of first reading titer of first reading would be used in analysis of result of DAT test become comparison titer of first reading and 2nd reading only wanted reliability of DAT titer reading.

4.10.5 Percentages of titer changed groups

Reproducibility	No.of serum samples	%	*1st> 2nd	lst	*2nd>
With exact agreement on both titre		78	90.8	-	-
With a 1600 fold difference between titre		67	7.35	35	32
With a 3200 fold difference between titre		11	1.3	3	8
With >6400 fold difference between titre		1	0.1	1	-
Total samples tested:	859	100	39	40	

Foot Note: Value of statistical test.

SD= 3.05 tc = 0.047, P>0.05

(t.05 = 1.96)

*lst = Titer of lst reading

*2nd = Titer of 2nd reading

The serological response

The outcomes of frequent episodes of seroconversion made a great controversial decision of positive diagnosis of kala-azar because some of them had occurred in every two months for a particular period without definite relation with parasitological diagnosis. On the other hand, the number of asymptomatic kala- azar were commonly found. There were 69% of the kala-azar cases without signs and symptoms of kala-azar (Chowdhury et al 1993). Moreover, many cases produced very low parasitemia. It was possible to conclude that some of low parasitemia cases were misdiagnosed. The possible outcome might be considered in other phenomenon. During the early parasitemia condition, the density of parasite was very low until it was undetectable by routine Bone marrow examination but the serological response was positively examined later on the density of the parasite increased than it was detected by slide examination or in culture and vice versa. From those consideration, the pattern of serological response might be classified into six variations in relation with the appearance of parasitic in bone marrow/spleen aspirated material examination as follows:-

1. Pattern 1 - Increase in seroconversion of at least 1:3200 fold dilution occurred within 2 months before negative slide becomes positive (Fig.18).

2. Pattern II - Increase in seroconversion of at least 1:3200 or more fold dilution occurred at the same month when a negative slide became positive (Fig.19).

3. Pattern III - Increase in seroconversion of at least 3200- fold dilution occurred within 2 months after the positive slide (Fig.20).

4. Pattern IV- No response in seroconversion occurred among the negative bone marrow/spleen aspirated slide that become positive within 2 months before and after a positive slide (Fig.21).

5. Pattern V - Increase in seroconversion of at least 3200 fold dilution occurred among individuals with negative slide (Fig.22).

6. Pattern VI - No increase in seroconversion occurred among individuals with negative slide (Fig.23).

The frequency distribution of serological responses related to the bone marrow /spleen aspirated slide patterns was shown in Table 5. It was observed that the majority (69%) were pattern of increase in seroconversion of at least 3200 fold dilution from the previous baseline occurred among those negative slide (Pattern V) only 4.8% were in the pattern of increase in seroconversion occurred at the same time as slide become positive III. which had increase in seroconversion within 2 months after slide positive were 2.5% which 0.75% were pattern 1 (increase in seroconversion of at least 3200 fold dilution occurring with in 2 months before negative slide became positive). Pattern of no increase in seroconversion among the negative slide that become positive with in 2 months before and after positive slide (pattern IV) were 1.25%. And pattern of no increase in seroconversion among the negative slide (Pattern VI) were 21.8%. From this knowledge it could set criteria of the time of positive seroconversion when had slide positive in following result of this study. And all cases would further be grouped up for the test of association of serology and parasitology. Fig.24 demonstrated that there was one case that increase in seroconversion was found two months before the parasite would be positive examined. Two cases showed that increase in seroconversion appeared one month before appearance of

parasitaemia. Twenty cases could be diagnosed by both methods within the same month. Eight and four cases were diagnosed by the microscopic method had increased in seroconversion of at least 3200 fold dilution within 2 months after a positive slide (Fig.24).

6) MATCHING OF DATA

The sample data were selected as matched pair according to the six patterns of the relative response of both the seroconversion and the microscopic blood examination. The match pairs in the pattern I, II, III were combined together as a group "a" or the group of cases who were diagnosed as positive by antigen and DAT detection. Since the assumption was followed the reason that the seroconversion might be detected with in the period of 2 months before and after the antigen was detected.

The match-pairs cases who produced the relative pattern IV of seroconversion and the blood examination were classified as group "c". The group "b" and "d" were the cases who showed the response to both methods of diagnosis in pattern "V" and "VI" respectively.

In this study, Criteria for seroconversion of group "b" or pattern "V" were as follows :

a) a group which had an increase in seroconversion of at least four-fold dilution without any previous pattern of a positive titer.

b) a group which had a second change to a four fold dilution when there was no increase in seroconversion for two months after the peak of a previous conversion.

Validity of test

In the context of this study, the validity of the test with LD body the antigen in comparison with the microscopic bone marrow slide examination had been conducted. The validity of the test was indicated by the sensitivity and the specificity of the parameters where the sensitivity of the test was defined as the true positive of the proportion of the number of paired data group "a" which were the number of the cases who possessed positive diagnosis of kala-azar by both methods and the number of all cases who were positively examined by microscopic method or the adding number of the data in group "a and c". The specificity was defined as the true negative which is the proportion of the number of paired sample group "d" and group "b+d" where from the number of cases who were negative diagnosed by both tests and the summation of the mentioned one and the number of cases who were positive with seroconversion but negative with microscopic test respectively.

Since the reliability of conclusion of serodiagnosis by seroconversion is the controversial consideration then the validity would be done on the basis of three different criteria firstly the seroconversion was positive when the titre of DAT increased 2 folds of serially dilution or more,others were 1:3200 folds or more and 6400 folds or more respectively. The results showed that the sensitivity and the specificity of the validity test when the seroconversion 1:1600 folds or more of dilution was considered as the positive test on the basis of microscopic examination as gold standard test were 99 and 85.71 percent respectively (Table 8).

It means that the seroconversion gave rather good outcome which closed to the outcome of microscopic of microscopic test while the sensitivity equals to 99 percent and on the other hand negative outcome of the seroconversion was agreeable with the microscopic test since the specificity was 85.71 percent.

By both equation, the probability of agreement of positive and negative diagnosis were calculated and the out come were 0.99 and 0.25 respectively. These indicated that both methods of diagnosis gave good out comes of agreement. Kappa is another measure of agreement. It was measured that equals to 0.14 and with the probability 0.20 of the Kappa test. These measures gave the same conclusion that the results of both methods had agreement of diagnosis even though the sensitivity of the test is rather high.

If the condition of diagnosis of positive seroconversion changed to be rising >3200 fold dilution the sensitivity and specificity were 96 and 71 percent respectively. As well as the probability of agreement of positive and negative diagnosis were .97 and 0.5 respectively (Table-9). It meant that the seroconversion gave still rather good positive outcome that close to outcome of microscopic test while specificity equals to 71 percent. Value of Kappa was 0.5 with the probability 0.7 of Kappa test It maned that the results of both method had good agreement of diagnosis as same as the results of increasing titre at least 3200 fold dilution.

Again if the criterion of diagnosis of seroconversion was increased to 6400 or more fold dilution, the sensitivity and specificity were still 95.5% and 71.4% respectively as well as the probability of agreement of positive and negative diagnosis were 0.97 and 0.28 respectively (Table 10). It maned that the seroconversion coat diagnosed the case as better as the microscopic as well as code diagnose the negative cases as well as the microscopic examination. Value of Kappa was 0.72 and 0.89 of the Kappa tests. It

also maned that the results of both method has good agreement of diagnosis as same as the results of increasing titre at least 1:3200 fold dilution. These maned that the result gave the same conclusion that the results of both method had agreement of diagnosis.

4.10.7 Pattern of serological responses was relation with the bone-marrow/spleen aspirated

Type of Pattern	No.	%	Frequency in 2x2 Table
Pattern I (Seroconversion 1-2 months before slide positive)	356	68.66	_
Pattern II (Seroconversion at the same month of slide positive)	77	14.80	a
Pattern III (Seroconversion after 1-2 months of slide positive)	79	14.42	_
Pattern IV (No seroconversion among those slide positive)	1	0.19	c
Pattern V (Seroconversion among slide negative)	1	1.15	b
Pattern VI (No seroconversion among slide negative	1	1.53	d
Total		520	100%

4.10.8 Matching Data

2x2 Table or pair Matched Table

LD body		Parasite by microscopic examination +ve	-ve	Total
DAT	Increased sero conversion	512	6	518
	Titre Not Increase	1	1	2
	Total	513	7	520

1) Sensitivity = 99%
2) Specificity = 85.71%
3) The probability of agreemen: on positive diagnosis = 1.05%
4) The probability of agreement on negative diagnosis = 0.22
5) Kappa = 0.28
6) Probability of Kappa = 0.69

4.10.8 : Validity of criterion of increased 1600 fold dilution asseroconversion related to the blood slide examination.

LD body		Parasite by microscopic examination +ve	-ve	Total
	Increased fold dilution Titre	508	6	514
	Not Increase	5	1	6
	Total	513	7	520

1) Sensitivity = 99%
2) Specificity = 85.7%
3) The probability of agreement on positive diagnosis = 0.99%
4) The probability of agreement on negative diagnosis = 0.25
5) Kappa = 0.14
6) Probability of Kappa = 0.89

4.10.9 : Validity of DAT test criterion of increased 3200 fold dilution as seroconversion related the bone-marrow examination.

	Parasite by microscopic examination +ve	-ve	Total
Increased >3200 fold dilution Titre	493	5	498
Not Increase	20	2	22
Total	513	7	520

1) Sensitivity = 96%
2) Specificity = 71%
3) The probability of agreement on positive diagnosis = .97%
4) The probability of agreement on negative diagnosis = 0.2
5) Kappa = .5
6) Probability of Kappa = 0.89

4.10.10: Validity of DAT LD body criterion of increased >64 related to the bone-marrow examination.

LD body	Parasite by microscopic examination +ve	-ve	Total
Increased > 64 fold dilution Titre	490	5	495
Not Increase	23	2	27
Total	513	7	520

1) Sensitivity = 95.5%
2) Specificity = 71.4%
3) The probability of agreement on positive diagnosis = 0.97
4) The probability of agreement on negative diagnosis = 0.28
5) Kappa = 0.72
6) Probability of Kappa = 0.89

8) Factors influencing on the Agreement of diagnosis of kala-azar by seroconversion and microscopic examination.

It was observed that within 520 paired specimens diagnosed by seroconversion and microscopic examination of either bone- marrow or spleen aspirated materials.

The agreement of both diagnostic outcomes might be varied according to some categories of the factors. When the paired data was stratified according to the categories of sex, the sensitivity and specificity of seroconversion of male and female were 98.05 and 75, 99.60 and 66.66 percent respectively (Table 12). Probability of agreement of positive and negative of both genders were 0.99 and 0.4, 0.99 and 0.33 for female. The Kappa and the probability of test were 0.74 and 0.95, 0.33 and 0.98 of female paired data respectively. From the analysis, there was no difference in sex. Therefore, sex was the factor that made no difference of agreement.

The paired data of the different outcomes of both methods of diagnosis were stratified again by different age groups of sample. The samples were classified into 3 age groups, < 14, 15- 29 and > years old. The probability of agreement was calculated for sensitivity, specificity, agreement of positive diagnosis (Dice, 1945), agreement of negative diagnosis, Kappa of over all agreement and probability of Kappa test. The sensitivity and specificity of the seroconversion in 3 groups were 97% and 50%, 98.8%and 66.66%, 98.85% and 50% respectively (Table 13). This indicated that the highest sensitivity and specificity of serological investigation were in the group of young adult 15-29 years old. The probability of positive agreement in 3 groups were 0.98 and 0.25, 0.98 and 0.28, 0.99 and 0.60 respectively. This also indicated that the agreement of positive diagnosis was higher in the groups of children <15 years old than other groups. The probability of agreement of negative diagnosis was also higher in the group

of children < 15 years old. They were 0.54, 0.25 and 0.08 in those 3 groups. The over all Kappa could conclude that the agreement of both methods of diagnosis in the group of 15-29 years old was the best because there was better agreement in both positive and negative diagnoses. The overall Kappa could conclude that the agreement of both methods of diagnosis among the all group 15-19 year group was the best because there was better agreement in both positive & negative diagnosis.

The past history of kala-azar in individuals was considered as an important factor. The sample was classified into two groups, the ones who had past history of KA attack and the ones who did not have. The sensitivity and the specificity seroconversion examinations among the group of having no experience KA were 99.64 and 75% respectively. These indicators explained that the serological examination gave higher with microscopic examination in the sample who have no history of KA experience. The probabilities of positive and negative agreement in the two classes without and with KA experience were 0.99 and 0.5, 0.95 and 0.25 respectively. This method of assessment gave the outcome which indicated that sample without past history of KA had probability of positive agreement less than the sample with past history of experience but had higher the probability of positive agreement.

The Kappa of those people without past history of kala-azar was 0.25 with the probability of Kappa-test of 0.34. The Kappa of people with past history of Kala-azar was 0.24.This maned that the over all agreement of the sample without past history of KA was same with past history of kala-azar so DAT can detect even previous attack of kala-azar.

At this point of analysis, it yields that the diagnosis by microscopic examination and the serological diagnosis could be alternative used. A few factors might be

attributed to the error of the serological examination. The less of these factors, the more accuracy of diagnosis could be acquired.

4.10.11 2x2 table of total paired data of LD body parasite by microscopic examination and seroconversion.

		LD body parasite by Microscopic Examination		
		+ve	-ve	Total
Seroconversion	* +ve	508	6	514
DAT (Titre)	* -ve	5	1	6
	Total	513	7	520

1) Sensitivity = 99%
2) Specificity = 85%
3) The probability of agreement on positive diagnosis = 0.99%
4) The probability of agreement on negative diagnosis = 0.25
5) Kappa = 0.14
6) Probability of Kappa = 0.69

Note:
+ve - increase in seroconversion at least 1600 fold dilution
-ve - not increase in seroconversion

4.10.12 Stratification of the paired data of LD body parasite by microscopic examination &seroconversion according to the sex difference.

MALE

LD body parasite by Microscopic Examination

		+ve	-ve	Total
Seroconversion	* +ve	252	3	255
DAT (Titre)	* -ve	5	1	6
	Total	257	4	261

1) Sensitivity = 98.05%
2) Specificity = 75%
3) The probability of agreement on positive diagnosis = 0.99%
4) The probability of agreement on negative diagnosis = 0.4
5) Kappa = 0.74
6) Probability of Kappa = 0.99

Note: +ve - increase in seroconversion at least 4 fold dilution
-ve - not increase in seroconversion

FEMALE

LD body parasite by Microscopic Examination

		+ve	-ve	Total
Seroconversion	* +ve	253	2	255
DAT (Titre)	* -ve	3	1	4
	Total	256	3	259

1) Sensitivity = 99.60%
2) Specificity = 66.66%
3) The probability of agreement on positive diagnosis = 0.99%
4) The probability of agreement on negative diagnosis = 0.4
5) Kappa = 0.33
6) Probability of Kappa = 0.98

Note: +ve - increase in seroconversion at least 1600 fold dilution
-ve - not increase in seroconversion

4.10.13 Stratification of the paired data of microscopic examination and seroconversion according to the age group difference.

LD body parasite by
Microscopic Examination

		+ve	-ve	Total
Seroconversion	* +ve	282	3	285
DAT (Titre)	* -ve	1	1	2
	Total	283	4	187

1) Sensitivity = 99.64%
2) Specificity = 75%
3) The probability of agreement on positive diagnosis = 0.99
4) The probability of agreement on negative diagnosis = 0.5
5) Kappa = 0.24
6) Probability of Kappa = 0.89

Note: +ve - increase in seroconversion at least 1600 fold dilution

-ve - not increase in seroconversion

4.10.15 AGE GROUP OF 30 YEARS OLD OR MORE

		LD body parasite by Microscopic Examination		
		+ve	-ve	Total
Seroconversion	* +ve	174	1	175
DAT (Titre)	* -ve	1	1	2
	Total	175	2	177

1) Sensitivity = 98.85%
2) Specificity = 50%
3) The probability of agreement on positive diagnosis = 0.79%
4) The probability of agreement on negative diagnosis = 0.60
5) Kappa = 0.50
6) Probability of Kappa = 0.68

Note: *+ve - increase in seroconversion at least 1600 fold dilution

-ve - not increase in seroconversion

4.10.16 Stratification of the paired data of parasite by microscopic examination and seroconversion according to difference of the history of previous infection of kala-azar.

The group of no previous kala-azar infection history

		LD body parasite by Microscopic Examination		
		+ve	-ve	Total
Seroconversion	* +ve	182	3	285
DAT (Titre)	* -ve	1	1	2
	Total	183	4	287

1) Sensitivity = 99.64%
2) Specificity = 75%
3) The probability of agreement on positive diagnosis = 0.99
4) The probability of agreement on negative diagnosis = 0.5
5) Kappa = 0.24
6) Probability of Kappa = 0.89

Note: * +ve - increase in seroconversion at least 1600 fold dilution
-ve - not increase in seroconversion

4.10.17 The group who had previous KA infection

		LD body parasite by Microscopic Examination		
		+ve	-ve	Total
Seroconversion	* +ve	25	2	227
DAT (Titre)	* -ve	4	1	6
	Total	230	3	233

1) Sensitivity = 97.78%
2) Specificity = 66.66%
3) The probability of agreement on positive diagnosis = 0.98
4) The probability of agreement on negative diagnosis = 0.25
5) Kappa = 0.34
6) Probability of Kappa = 0.98

Note:

* +ve - increase in seroconversion at least 3200 fold dilution

-ve - not increase in seroconversion

CHAPTER 5

DISCUSSION

5.0 CHAPTER-5 : DISCUSSION

5.1 MASS APPLICATION OF DIRECT AGGLUTINATION TEST (DAT) FOR VISCERAL LEISHMANIASIS (VL) IN BANGLADESH

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RELATIONSHIP BETWEEN INCIDENCE OF KALA-AZAR FACTOR

CHAPTER-5 : DISCUSSION

5.1 MASS APPLICATION OF DIRECT AGGLUTINATION TEST (DAT) FOR VISCERAL LEISHMANIASIS (VL) IN BANGLADESH

Prevalence of anti-Leishmania antibodies as determined by DAT in this survey was higher (7.70%) in Shahjadpur thana by comparison to Trishal to Trishal (4.40%). Teknaf (Coxes Bazar district) being non-endemic had significantly lower sero- prevalence rate of 0.34%. Among the younger population (< 21 years) studied in both thanas of Trishal and Shahjadpur, those between 12 - 20 years of age were most affected (1.45% - 2.14%). Our results appear to agree with those obtained earlier by others in Mymensingh district where the highest VL prevalence was found in children below 15 years (Elias et al 1989). Other comparable data were those reported in the neighbouring Bihar state (India) where children between 10-20 years of age had the highest incidence of VL (Bahr and Bell 1987). The lower sero-positivity found in the adult population (> 21 years) is concordant with the results of aldehyde test obtained in Sirajganj district (Elias et al 1989). However, a different pattern of VL prevalence versus age was reported in other endemic areas. In the mediterranean littoral positivity in leishmanin skin test was shown to be highly correlating to progress in age reaching up to 46% in inhabitants of 60 years and older (Marty et al 1992). Nevertheless, the vast majority of the diagnosed VL cases in these areas, was among children (< 20 years) (Markell et al 1986). During the recent VL epidemic in the Sudan incidence rates of 23% to 40% were found by IFAT and DAT among inhabitants of 15 years of age and older (De Beer et al 1991; Perea et al 1991). A suspectability to Leishmania infection in male as high as four times by comparison to female has been reported (Bahr and Bell 1987). In this study however, only a slight difference in sero- positivity was found in the male population (2.12% - 3.70%) when compared to the female (2.28% - 3.06%). The profound susceptibility reported in males could not be supported by data obtained in Bangladesh or other endemic areas. In fact, the country has been reported in Southern Sudan where VL prevalence was significantly higher in females particularly those above 15 years (Perea et al 1991). In essence, the current results do confirm the ministerial report covering the period January 1987 to July 1991 attesting that Mymensingh and Sirajganj districts are important endemic areas for VL in this country. Credibility of the prevalence rates found here can further be supported by the contrasting low sero-positivity (0.34%) obtained in Coxes Bazar, a district known to be highly endemic for malaria but not for VL. Earlier laboratory evaluation had shown that

the DAT is highly reliable in differential diagnosis of VL versus clinically alike infections such as tuberculosis, enteric fever and viral hepatitis prevalent in Bangladesh (Chowdhury et al 1991). Being so far the only cross-reacting disease in DAT, trypanosomiasis is not occurring in this country, a fact which may further support liability of the obtained prevalence rates. Due to its higher sensitivity, the DAT may have picked up a number of subclinical VL cases most probably could have been missed by the leishmanin skin test (Bahr 1961; Markhel et al 1986). Whether these asymptomatic cases will manifest clinical disease in future is subject to further information still to emerge from the on- going follow-up.

Considering the results obtained, we think that the DAT can be an alternative field indicator for VL prevalence in large endemic communities with a similar practicability as that of the leishmanin skin and aldehyde tests. If compared to DAT, the predictive value of the former test for active VL is rather poor and genuine cases may thus be overlooked (Zijlstra et al 1991). Also due to high reactivity versus other infections, results obtained by this test on the other hand may lead to over estimation of VL (Hahr and Bell 1987). Although reactivity in the aldehyde test is a consequence of significant dysproteinanemia mostly occurring in late VL, a negative test is not necessarily an indication for absence of VL (Chorine 1937). Early and subclinical VL cases, due to the unaltered albunin/globulin ratio, may therefore be missed contributing to a lower estimate of VL in the endemic area under study.

Production of antigen at such a large-scale did not interfere with sensitivity and specificity of DAT for detection of VL. As with the miniature batches (100 ml-500 ml) prepared previously, the antigen under study (3300 ml) did react as desired (1:3200 - > 1:52428800) with all 73 VL reference sera. None of the challenging samples (235) particularly those from trypanosomiasis patients (121) showed reactivity at the cut-off the field regarding all 29 genuine VL cases and 96 others with unconfirmed infection but who positively responded to anti-Leishmania chemotherapy.

Not merely because of antigen availability at such a large-scale do we consider the DAT as a feasible field indicator but also as for leishmanin skin test, merits related to applicability were realized. The sampling procedures were both convenient and time saving and performance of DAT was extremely simple precluding thus sources for possible technical errors. The low cost involved related to large-scale production, encourages its regular application in developing countries. Reading of test results was direct and DAT not include a second visit which is obligatory in application of leishmanin test. As experienced in this survey by spontaneous monitoring of 125 affected inhabitants, the DAT in contrast to leishmanin test, can contribute in detection

of active VL. Should the remaining 539 sero-positives identified in this study develop clinical disease during the on-going follow-up, further conclusion can be drawn regarding early diagnosis and prompt administration of chemotherapy.

In the light of information on the spreading of acquired immune deficiency syndrome (AIDS) throughout this area, the cellular response characteristic of leismanin test does default arguing therefore the necessity of exploring other means for VL survey studies. Although we haven't sufficiently evaluated the DAT in this respect, our impression Although we haven't sufficiently evaluated the DAT in this respect, our impression considering the astronomical titre levels, is that the test can pick up anti-leishmania antibodies at extremely low humoral response (De Korte et al. 1990).

On grounds of these and other results reported earlier we may conclude that the developed DAT beside being a useful diagnostic test is also a feasible epidemiological indicator for regular surveillance of VL in large endemic communities.

5.2 APPLICABILITY OF DIRECT AGGLUTINATION TEST (DAT) AT A RURAL HEALTH SETTING IN BANGLADESH AND FEASIBILITY OF LOCAL ANTIGEN PRODUCTION.

Evaluation of the various serological tests for VL diagnosis is best carried out under conditions prevailing in the affected area. The DAT in its present format evidenced high potential for applicability at the rural health setting in Trishal thana (sub-district). This is largely due to the minimal technical requirements for its execution. Except for a cooling device to maintain antigen stability for considerably long periods up to one year, preparation of the sample diluent, performance of the test and reading of results are not essentially dependent on an electric supply. As a result of latest improvements introduced (Harith et al 1988) the use of fetal calf serum as protein source in the serum diluent is no longer an obstacle for DAT applicability in the field. Being a denature acted protein, gelatin as powder is very stable at the maximum ambient temperatures of Bangladesh (34 C - 38 C). Also, storage of 2 mercaptoethanol does not require special condition provided that the bottle is kept tightly closed. Should the test be carried out in adequately aerated space, no hazards are to be expected. Our efforts are continuing to identify an alternative reducing agent of no unsavoury odour.

Among the very few serological tests employed for VL diagnosis under field conditions, ELISA appeared to be more practical (Jahn and Diesfeld, 1983). The assay was performed under comparable conditions and blood collected on filter-paper instead of serum were used for assay performance. In the current study, the DAT was applied on whole blood samples collected by finger-prick method. This method is significantly time saving particularly when dealing with a VL survey of such a magnitude. Reliability of this method proved to be adequate when compared to results obtained with serum samples from the same individuals (Chowdhury et al 1993, in press).

In both populations from Trishal screened either at the central laboratory in Dhaka (group A) or locally at the health Compiles (group B), inhabitants in the age group <-20 years had the highest VL sero-prevalence rate compared to others (>21 years). Also, in either of the two groups, inhabitants who revealed positive bone-marrow aspirate (166) or who were finally diagnosed on clinical grounds (99) as VL were clearly DAT positives at the start of the survey. Generally, these results are concordant with those of the aldehyde test as performed earlier in Bangladesh (Elias et al 1989). However unlike DAT, in addition to inhabitants harbouring VL, patients with other infections showed positivity in the aldehyde test. A part from being non-specific the aldehyde test also requires veno-puncture blood which is rather inconvenient and time consuming. Although the Leishmanin skin test is more easier and less demanding in application than the aldehyde, its specificity for detection of and less demancing in application than the aldehyde, its specificity for detection of active VL is known to be very poor.

The success of using an indigenous L.donovani isolate from Bangladesh or its Indian homologue as antigen in DAT settle the dispute as to whether the current reference L.donovani (1-S) is the only suitable one for optimal DAT performance. Following the standard procedure for antigen preparation, both the indigenous.

5.3 FOLLOW-UP STUDY OF VISCERAL LEISHMANIASIS (KALA-AZAR) SERO-POSITIVES DETECTED DURING MASS SCREENING.

Improvement of DAT sensitivity for diagnosis of PKDL:

To further assess for the favourable effect in using homologous antigens in DAT, the PKDL patients. In this study, also the reference antigen was included to determine the degree of improvement for PKDL diagnosis.

From 70 PKDL Bangladeshi patients, all with previous history of VL and lesions suggestive of the disease, blood spots were collected and tested in DAT and IEDC&R (Dhaka) against the reference antigen (1-s). The duplicate of the same samples were mailed to Amsterdam for further confirmation. Elution of the samples was as described in earlier reports using physiological saline (0.9% NaCl) for _ 24 hours at +4_C. All 70 PKDL samples were tested against the authochtonous antigen as well as the reference (1-s) and the test reading was done 20-24 hours after incubation by two scientists independently. The results of this study are shown in Fig. 8.

In fifty out of the 70PKDL samples tested, DAT titres were at least 2-fold higher with the PKDL antigen than the reference (1-s); in 19 samples both antigens scored equal titres and in only one did the reference antigen showed higher value. It may therefore be concluded that in order to achieve more sensitive detection of PKDL cases, a leishmania isolate from the corresponding infection should be used as antigen.

Specificity of the prepared PKDL antigen against other dermal infections:

Apart from being sensitive for PKDL diagnosis, the prepared homologous antigen should also evidence and acceptable degree of specificity. Due to possible confusion in clinical diagnosis of PKDL versus Leprosy and other dermal infections, availability of specific homologous antigen, would be of great importance. To evaluate for this desired characteristic, the prepared PKDL antigen was challenged against serum samples from the following conditions:

- 32 cutaneous leishmaniasis (Algeria)
- 11 cutaneous leishmaniasis (Brazil)
- 5 mucocutaneous leishmaniasis (Brazil)
- 12 leprosy patients (Brazil)

Result obtained with the PKDL antigen on the above mentioned samples were compared with those previously recorded in the PKDL patients as shown below:

Considering the very low DAT titres obtained in sera from patients with other dermal infections by comparison with those being diagnosed as PKDL, it might be possible to differentiate between PKDL and leprosy patents on grounds of DAT results.

Application of DAT on European VL/HIV - positive patients:

Due to the scarcity and difficulty in obtaining sera from HIV- positive or AIDS patients, DAT performance in this important patient's group could not be evaluated earlier. The increasing number of these immuno-compromised patients who acquired VL infection and their importance in the epidemiology in all endemic areas necessitate availability of highly sensitive nonetheless specific detecting method. While serologic diagnosis appears to be insensitive considering the low antibody profile, direct demonstration of leishmania parasite is equally difficult and time consuming. Highly technical procedures such as immuno-blotting and the use of leishmania DNA specific probes and its amplification (PCR) is not always feasible in central laboratories of developing countries.

Acknowledging the strikingly high titre levels in the vast majority of immuno-competent VL patients in various endemic areas, it is expected that the DAT would monitor, to a reasonable extend, specific antibodies in these patients. Also, being simple and extremely economical, the DAT may therefore be a good alternative for high-tech methods in important VL endemic areas such as Bangladesh and India.In this study, an antigen batch prepared from the reference L. donovani strain (1.s) was employed. Since the effect of 2-merecaptoethanol in DAT performance versus immuno-globulins in immuno-compromised sera was not determined earlier, it is decided to perform the test with and without incorporation of this reducing agent. In total, 163 serum samples collected from Dutch and Italian (Sicilian) HIV-positive and AIDS patients provided respectively by Dr. F. Wolff (Virology Dept., U.V.A. Amsterdam) and Dr. L. Negro (LSHTM, London). None of the Dutch patients (50) had history or clinical signs of VL while among the Sicilian patients, 9 were earlier diagnosed as having opportunistic VL infection.

The DAT was executed blindly in all 163 patients and the test results were compared with those of final diagnosis and immunofluorescence test (IFAT0 established earlier in Sicily and London (LSHTM).

Our results on the Dutch IHV-positives (50) confirmed the clinical findings that none of those patients had VL infection. It can be concluded thus that DAT for VL does not cross react with anti-HIV antibodies. From the Sicilian HIV-patient group (113), 5 had scored DAT titres ranging (1:6400 - > 1:102400); the remaining 4 patients were clearly negative for VL (<1:100). In one out of 5 VL/HIV - positive, incorporation of 2-mercaptoethanol resulted in titre reduction from 1:6400 to 1:1600 while in the other 4 samples a significant improvement in test readings was observed (1:6400 to > 1:51200). It can therefore be recommended that in testing suspected VL/HIV-positive cases, DAT performance should be carried out with and without 2 mercaptoethanol incorporation. Compared to the immunofluorescent test (IFAT) which detected 4 out of the same 5 patients, it seems that the DAT is more sensitive. However, more evaluation and comparison with IFAT as well as other diagnostic techniques were evaluated in the continuous study of sero-responsive cases of VL.

Further improvement of DAT sensitivity for earlier VL detection:

With the exception of patients having immuno-suppressed system, the DAT in its present format is capable of monitoring VL patients with confirmed infection. Reliability of DAT to detect L. donovani infection at its inapparent or subclinical phase is still under evaluation. Enhancing sensitivity in DAT to establish diagnosis in this group of patients had earlier been attempted using an autochthonous (Bangladeshi) and a homologous (MHOM/IN/80/D88, India) strains of L. donovani. The results obtained were encouraging and showed superiority when compared with those by the reference leishmania strain (1-s). However, further exploration along this line is considered necessary to achieve the desired objective. Apart from detecting early VL infection, achieving optimal sensitivity may also result in an incubation than the one currently indicated (18-20 hours).

Recent studies (King & Turco, 1988) had evidenced that the presence of lipophosoglycan (LPG) layer on leishmania promatigote surface masks antigenic binding sites. L. donovani strains from India and Sudan were found to be poor agglutinators as they expressed abundant amounts of LPG. A specific mutant (R_2D_2) leishmania strain

laking LPG layer is therefore expected to show higher reactivity in DAT if compared to the reference (1-s) or the homologous strains from Bangladesh or India. Accordingly, the sensitivity of the DAT can be expected to improve in respect of the low reacting subclinical VL cases.

The first evaluation using the above mentioned LPG mutant strain (R_2D_2) was carried out with a corresponding antigen prepared by Dr. D. Evans (LSHTM, London). The serum samples employed in this study were collected from North Pakistan from 42 human and 20 canine hosts by Dr. Rab (LSHTM, London). In addition to mutant (R_2D_2) strain, the DAT was carried out at LSHTM using the reference 1-s antigen to assess for the expected difference. Results obtained with both antigens are expressed in the table presented below:

Diagnosis	Number of Sera	ELISA Readings (range) Absorbance		Titre reciprocal	
		(-)Trypsin	(+)Trypsin	(-)Trypsin	(+)Trypsin
Visceral Leishmaniasis	8	0.4 - 1.3	0.5-1.0	6400-102400	102400
African trypanosomiasis	5	0.2 - 0.4	0.1-0.5	200 - 3200	100 - 800
Auto-immune disorders	5	0.2 - 0.9	0.1-0.4	400 - 3200	100 - 800
Oncogenic patients	5	0.2 - 0.6	0.1-0.2	200 - 800	100 - 400
AIDS patients	4	0.2 - 0.6	0.1	200 - 800	100 - 200

Although automation of ELISA reading is presently considered as decisive for establishing test results, in our experience the visual reading (expressed as titre) is consistent and easy. In addition, as ELISA reader is rather expensive to purchase and maintain in laboratories of developing countries. The validity of visual reading in this

ELISA version is further evidenced by the high concordance obtained with DAT and parasitological diagnosis.

The modified ELISA proved also to be easily applicable in the central laboratory (IEDC&R) in Dhaka. The test was carried out there using the trypsinated 1-s antigen following the procedures described above and the reading was done visually. The results obtained in the tested serum samples (VL and negative controls) are compared with those of DAT as shown below:

Number of Sera	Titre dilution		Diagnosis
	ELISA	DAT	
6	_ 1:1600	_ 1:102400	Visceral Leishmaniasis
1	1:3200	_ 1:102400	"
1	1:25600	_ 1:102400	"
1	1:51200	_ 1:102400	"
23	_1:102400	_ 1:102400	"
9	_1:100	_1:100	Other clinical conditions
2	1:100	1:100	"
1	1:200	1:200	"
2	1:100	1:100	"
1	1:100	1:100	"
1	1:3200	1:3200	"

Considering the low titres obtained VL patients in this study, further standisation is needed to render the test sensitive for routine diagnosis. Due to the stability of the trypsinated and formaldehyde-fixed antigen, this ELISA version could highly be applicable in Bangladesh as well as in other endemic areas. It's routine application together with DAT will help improving diagnosis of VL especially in doubtful cases.

The same trypsinated formaldehyde-fixed antigen is currently under evaluation in an immunofluorescent antibody test for VL diagnosis.

5.4 SEROLOGICAL STUDY ON POST KALA-AZAR DERMAL LEISHMANIASIS IN BANGLADESH

In the present series amastigotes were detected in smear examination of 42 out of 61 patients. Those 19 patients who had early signs and symptoms with hypo-pigmentation gave positive reaction by serological tests. All the PKDL cases were from old foci of visceral leishmaniasis and majority were from Mymensingh district of Bangladesh. One patient had been identified to have cutaneous leishmaniasis who had earlier returned from the Middle East. PKDL is commonly found between the age of 20 to 30 years.

In our observation the age varied between 5 and 85 years although the highest prevalence was still noted to be in the age group of 21 to 30 years. Male cases were predominantly higher than the female cases as has been noted else where. All the patients (96.72%) except 2(3.27%) had a past history of kala-azar. Most of the younger patients who had hypo-pigmentation had a past history of receiving subtherapeutic doses of antimony. In the present series LD bodies. Specific antibodies to failed to depict LD bodies. Specific antibodies to leishmania antigen could be detected in PKDL patients by the DAT, IFAT and micro ELISA tests. These results compare well with those already obtained in kala- azar by others. Antibody titre ranges in PKDL sera are noted to be lower than those of kala-azar patients but in this series, except for micro ELISA antibody,titration remains high in DAT and iFAT. Thus the absence of definite correlation between DAT, IFAT and ELISA suggests that at least some of the relevant antigens involved in these tests are different, which agrees with the earlier results obtained with kala-azar.

5.5 STUDY ON KALA-AZAR IN CHILDREN A STUDY OF 100 PATIENTS

Kala-azar is an insect born protozoal disease which affects all age groups with profound immuno-suppressive effect. WHO expert committee mentioned it as a threat to the health of children in endemic areas, as this age group is more vulnerable. In the present study all patients belongs to 1-12 years of age and the most vulnerable group appears to be between in ages of 9- 12 years in both sexes. Though in previous studies young adults and tanagers were the main victims of kala-azar but in recent epidemics in India it was shown that there was a shift to the left effecting more paediatric patients. Results of a study in Malda District in West Bengal observed that

there was prepondence among paediatric age group (54.26%) C.P. Thakur found 18 patients under 10 years of age amongst his 30 patients in a single village. Aikat in his study in Bihar, India, observed male: female ratio 1:1 which differs with our findings of 1:94:1. No correlation was found between the duration of illness and splenic size. Aikat in his study also showed that the duration of illness was from 15 days to 1 year, average being 6 months. A hospital based study showed that majority of Kenyan patients gave history of 1 to 6 months illness. Their findings corresponds to ours.

High continuous fever with short duration illness of less than 4 weeks, slight splenomegaly and leukopenia may lead to confusion with enteric fever. Fever with chills and rigor may be confused with UTI and Malaria. Fever was present in 100% cases in this study. Thakur also found in 98.1% & Kager et al found in 73.77% & Chowdhury in 100% cases. From the type of temperature alone we could not ascertained the diagnosis of Kala-azar. However, weight loss was very conspicuous in the present series. Poor nutritional status and chronic disease process itself maybe the contributory factors.

In the present series 100% cases showed anaemia which was a striking feature and parents brought their children to the hospital for medical advice Degree of anaemia was related to chronicity of the disease but not all chronic cases are associated with severe anaemia. Heart failure due to anaemia was not observed in our series. Cough was next important presenting symptoms which was dry irritating and often very distressing to the children leading reluctant to take food. Vomiting was present in 36% cases. Thakur found in 5.5% and Kager et al found in 40.98% of Kenyan patients. Abdominal pain w.s. the next prominent symptom. It was usually felt in the left hypochondrium or in the umbilical region and confuse with acute abdomen probably due to progressively enlarged spleen stretching the capsule. Twenty percent of the patients presented with abdominal pain in the present study, Kager et al found in 54% cases but Thakur did not encounter such symptoms during his study in Bihar. Haemorrhagic manifestation were found in 12% cases. Out of them 8% presented with epistaxis, 2% with haemoptysis and 2% with patchy haemorrhagic manifestation was also found by Kager et al in 3.2% and Thakur in 7.6%. Oedema was seen in 4% of cases but Kager et al found in 19.67% cases and Thakur found in 29.1% cases. Malnutrition and anaemia may be the two crucial factors for the development of oedema. In 4% of cases respiratory complication was seen. Physical and radiological evidence confirms the diagnosis of bronchopneumonia and tuberculosis. This may be

attributable to secondary infection due to immunosuppressive effect of the disease. All the patients received antimony (stibamine) and were cured and did not come to the hospital with relapse.

5.6 STUDY ON SODIUM ANTIMONY GLUCONATE (SAG) ADMINISTRATION IN VISCERAL LEISHMANIASIS (KALA-AZAR) SEROPOSITIVE PATIENTS

The successful treatment achieved in 97.49% of the VL cases studied including those with negative parasitological results (45.66%) points out the necessity of employing reliable serodiagnostic tests for detection of prepatent and early Leishmania infections. Of the three tests performed, the improved DAT evidenced higher potential as 98.03% of those unconfirmed cases showed unequivocally positive results. Although it is difficult to conclude from these data how early it would be possible to diagnose VL using the DAT, it can be assumed that the parasitic load in those 547 unconfirmed cases was so low even to be demonstrated by inoculation into three different culture media. Our current follow-up studies will reveal further information in this regard.

Knowing that VL transmission in the Indian subcontinent is largely dependent on the availability of PKDL cases, detection of early infections followed by prompt application of antimonial chemotherapy should contribute to the control of the disease in this area. The toxicity experienced with these compounds, and the need for prolonged therapy in VL and PKDL relapses, urge specific and early diagnosis of VL. From this and previous studies in a limited number of patients in the Sudan and in naturally infected dogs in some of the mediterranean areas, it can be argued that chemotherapy should be administered in clinically suspected cases showing positive DAT results, regardless of the parasitological findings.

Out of 558 early suspected kala-azar cases 98.02% in DAT. 80.20% in IHA. 85.84% in ELISA and 34.94% in AT showed positive results whereas out of 640 parasitologically proven cases 99.69% in DAT, 89.85% in IHA, 99.06% in ELISA and 76.41% in AT were positive. Almost all recent kala-azar cases showed titres from 1:3200 - 1:52100 in DAT and all the healthy endemic and non- endemic and sick control sera showed 1:1600 titer except only 1 case of viral hepatitis 1 case of TB and 1 case of malaria showed titration value of 1:3200 on the other hand IHA was positive in 18% cases of Enteric fever, 9.8% for V.hepatitis. 77.3% for malaria, 7% for syphilis

and 12% of tuberculosis case. The ELISA absorbancy showed significant higher value in endemic than non- endemic sera. On the other hand DAT was positive in 100% of treated patients sera within 1 year after treatment whereas ELISA showed only 4.77% in same patients sera although the cross reactivity is reported in one of sick control sera showed in DAT more than the borderline (i.e. 1:3200). AE Harith et al 1987 also observed the same in his study. Sensitivity of DAT is higher than IHA (89.84%).

Our observation was also same as lakshmi Srivastova et al (1988). She observed in her comparative study of healthy endemic and non-endemic sera with that of kala-azar and sick control. The values obtained in early cases as well as established cases were significantly higher than value obtained in normal healthy individuals in the same sera. Hommel et al (1976-78) observed 35 out of 36 patients sera of VL were positive. However, it is interesting to have the idea that the absorbency value of ELISA was significantly higher in the endemic sera than non-endemic sera. However, none of these sick control sera showed higher titration than 1:3200 in DAT. Though the cut off point was determined 1:3200 in DAT, 1:64 in IHA and 0.30 absorbency in ELISA. On the other hand ELISA showed highest absorbency of >1:256 in 1 case of TB, 1 of malaria, 1 in leprosy and 6 in V. hepatitis. Abdulla-El-Harith et al (1978) observed in his study that a higher sensitivity (95.6%) the specificity was 94.7% for DAT, while incorporated in the test, 2 merceptoethanol (2ME) treatment of chagasie sera had no influence on antibody titre, although chagas, disease not existing in Bangladesh.

The simplicity and reliability of DAT and the findings of the study suggested, that the test (DAT) may be applicable for mass screening in the rural set up. However, before applying the test in the field it is necessary to repeat the experiment and evaluate it in the field condition in Bangladesh.

(Edrissian et al 1979) observed in his findings that in detection of leishmanial antibodies ELISA is a little more sensitive, but somewhat less specific and economic it could be considered as a good screening for kala-azar in field survey.

Although L. donovani antigens nonspecific reactions occurred with some other infectious diseases (Hommel et al 1978) observed in his first experiments that the test to be positive in 35 out of 36 patients of visceral leishmaniasis he also suggested that the micro ELISA test may be used in epidemiological investigations. The specificity of

the test in distinguishing from, Tuberculosis, viral hepatitis, syphilis enteric fever has been etc. demonstrated. In out finding it is interesting to see that the serum level of antibodies have been significantly higher in apparently healthy individuals in Mymensingh endemic area in contrast to similar value in Chittagong. Mymensingh is an epidemic area. Whereas Chittagong known to be a non endemic VL area which indicates exposure of population to the infection. The study revealed that the considerable value of ELISA in the serodiagnosis of Visceral Leishmaniasis.

Regarding incidence by age,(Sanyal et al 1979)in his study of 42 cases observed that disease affects all age group viz. 0-9 years (25.6%) 10-19 yrs. (20.9%) 20-29 yrs. (30.2%) 30-39 yrs. (20.9%) and 40 yrs (2.3%). This study revealed the following age incidence Figure-1 viz. 0-5 yrs (5.8%). 6-10 yrs (16.6%), 11-15 yrs (34.5%), 16-20 yrs (23.6%), 21-25 yrs (.5%) and above 25 yrs (13.09%).

There has been a number of changes in the treatment schedule of kala-azar. It was believed that the Indian strain was of less virulent type and responded well to SAG with a dosage schedule of 10 mg/kg body weight for 10-15 days. As a matter of fact that was quite popular regimen as the duration of treatment was short and less painful and gave immediate clinical response in most of patients. It is presumed that due to the above mentioned dose schedule relapse or PKDL developed.

Inadequate treatment showed a failure rate of 13% in India (Sen Gupta 1953). By prolonging the duration of treatment relapse rate reduced from 13% to 0.5% (Thakur et al 1984) used 20 mg/kg body weight for 20 days for adult and in case of children 10 mg/kg body weight/day for 20 days and found a failure rate of 7.4%. When the dose was raised 20 mg/kg body weight for 20 days but not exceeding 850 mg per day the rate of failure was reduced to 0 (zero). However, in a subsequent study, (Thakur 1988) recommended a duration of 40 days treatment as with 20 days schedule.

In the present study 94.02% cases showed complete cure with 20 mg/kg body weight for 20 days regimen. In remaining cases cure was achieved by giving a total of 30-40 injections at the same dosage. The side effects of SAG were minimum and in no case
treatment had to be stopped. Cases of PKDL need prolonged treatment of 120 injections of SAG which constitutes the main source of infection. These patients should be searched out and motivated for prolonged treatment.

Our study revealed that the WHO recommended dose schedule of SAG is quite effective. As already been emphasised by WHO and recently reconfirmed by our study in Bangladesh the standard regimens of SAG as a first line of treatment for VL should be 20 mg/kg body weight not exceeding 850 mg/day for 20 days and for PKDL cases 6 courses of same dose schedule with an interval of 10 days in between 2 courses. If this regimen is followed, the relapse rate and rate of PKDL development will get reduced and the patient morbidity and fatality rate will show reduction.

5.7 STUDY ON DIFFERENT TREATMENT SCHEDULE FOR SAG FAILURE CASES OF VISCERAL LEISHMANIASIS (KALA-AZAR) IN BANGLADESH DISCUSSION

In the past the standard treatment was a 6-10 days course of sodium stibogluconate. The course was started of 1 ml or IV on alternate day and gradually increased and observed 30% of patients were unresponsive, any relapse after a 10 day course was probably taken as unresponsive. A committee of Indian experts recommended two courses of sodium stibogluconate with a break of 10 days in between, (Thakur et al 1984). Starting with smaller dosages, gap between the courses and alternate day therapy did not seem logical, Thakur et al 1981 & 1984) conducted a trial giving the drug continuously for 20 days and observed the relapse rate was reduced to 0.5% and 91.4% of patients were cured and only 0.86% of the patient did not respond. Chow et al 1991, also observed that Sodium Stibogluconate 20 mg/kg body weight not exceeding 850 mg/day as a first line of treatment responded 94.7% of VL cases with minimum side effect. The study revealed that the 55 primary unresponsive cases from different places of Bangladesh were under trial and rendomised selected grouped, Group 'C' responded the 100% cured 10 mg/kg body weight for 7 days and second highest responded in Group 'E' pentamidine group (99%). An injection of Sodium Stibogluconate of meglumine antimoniate (Sbv) is rapidly excreted in the urine, so that blood sb level fall to less than 10% peak levels some eight hours after inj., Ress. PH et al (1980).

In Kenya it was found that patients unresponsive to usual dosage of antimony responded to 10 mg/kg body weight 3 times a day for 10 days, Ress, P.H. et al (1984 & 1980). This study agree with them. Unresponsiveness to drugs are quite common among kala-azar patients. Some patients did not respond to antimony from very

beginning. They were labelled a cases of primary unresponsiveness if did not respond to a course of antimony given for 20 days. We also agree if after 40 days of treatment, there is no response, then either patient should be continued with antimony 10 mg/kg body weight thrice daily for 7 days or pentamidine should be given alternate suggestion or second choice.

5.8 STUDY ON VECTOR (SANDFLY) IN RELATION WITH VL(KALA-AZAR) AND EFFECT OF DDT ON ITS CONTROL.

The first explosive epidemic of kala-azar of this sub- continent was recorded in Garo-hills and the adjacent Brahmaputra valley of Assam in 1880 (Rahman et al, 1979). Although it was reduced below the level of Public Health importance, the disease was never totally eradicated. Cases of post kala-azar dermal leishmaniasis (PKDL), a suspected reservoir of infection and occasional cases of active kala-azar continued to be seen in the clinic and different hospital of Medical College (Khan 1977).

The factors responsible for the apparent disappearance of the disease were supposedly related to successful chemotherapy of all cases, acquired protective immunity in the population and decrease in vector density following mass indoor residual insecticidal spray (DDT) under National Malaria Control Programme followed by the National Malaria Eradication Programme (NMEP).

Following withdrawal of DDT spraying operation as a strategy of the NMEP, there was gradual increase in the vector population. Study revealed that out of 64 districts, active kala-azar cases were detected in 34 districts. Presence of vector was also observed in all the microfoci of 34 districts in Bangladesh. An alarming degree of anthropophilism in **P.argentipes** was demonstrated by Ahmed and Ahmed in Sirajganj districts in 1980. Further a gradual increase in the number of active cases and susceptible human population was also observed (Islam 1982, Rahman & Islam 1983, Hossain & Rashid, 1987).

During the first survey when there was no DDT spraying in the study area for the last 15 years, 1828 cases were detected serologically and only 7 and 1 cases were detected in the same area during our second and 3rd survey which were after DDT

spraying. Simultaneously sandflies were collected in the village Awaltia within our study area and 559 P.argentipes (5.82 m/hr) were collected in 1987 - 88. On the other hand after DDT spraying only 24 and 18 p.argentipes (0.25 and 0.18 m/hr) were collected in the same area during survey.

Malaria Demonstration Team (WHO/UNICEF) with the participation of the national staff during their survey in 1950 observed that transmission of kala-azar occurred to a great extent in the non-sprayed area. In some area where spraying was done in May - June 1950, transmission has become to a very limited extent. Current finding also agree with that, while DDT spray even in low concentration (lgm/m2) can interrupt kala-azar transmission by controlling the vector. We also observed that correlation between incidence of kala-azar and vector density is significant ($p<025$). It may be concluded that residual house- spray of insecticide could interrupt kala-azar transmission.

In the study of sandfly fauna only 5 species were found. The prominent species was s.babu babu (48.53%) and next was p.argentipes (40.07%). Recent workers, Ahmed et al (1988), Masum et al (1990) and Hossain et al (1993) found the same 5 species (P. argentipes, p.papatasi, s.babu babu, s.shortii and s. barraudi). In addition to these 5 species also reported to more other species (S.bagdadis and S. ameeni, Hossain et al 1993). Out of these p.argentipes is the vector of eastern part of Indian subcontinent (Swaminath et al 1942) Lewis 1978 published his opinion that in addition to p. argentipes, p.papatasi in also a vector in India. No confirmatory study was conducted for identification of vector in the country. So further study is needed to identify the confirmed vector of kala-azar in Bangladesh.

Larval collection from floors of human dwelling and cattle shed showed that sandfly larva could be found more in cattle shed than human dwelling. It also coincides with the collection by hand catch where more sandflies were collected by from cattle shed than human dwelling (Fig.2).

Blood meal analysis for host preference using DOT ELISA, P. argentipes gave highest positive reaction to bovine (52.11%), next to human (40.25%) and very little to other animal. Hati et al, 1981 of course differs with this opinion and he observed in his study that p.argentipes is more attracted to human that bovine and other. Ahmed and Ahmed 1983, recorded that p.argentipes was found to be more zoophilic that anthropophilic. May be the feeding behavior depends on the availability of host in

different situation. P.argentipes was found to be present throughout the year with different density. The highest number (10 m/hr catch) was found in August and lowest (2.12 m/hr) in January. If the seasonal trend is drawn it shows two peaks, July - Sept. and April-May (Fig.1).

Bashu and Ghosh (1954) made a sandfly collection in and around Calcutta city and found the highest peak in July and lowest in January, which is in keeping with the findings from the study. In Bihar **P.argentipes** density falls to almost nil in the winter months and with the advent of warm climate, density increases and becomes highest in post monsoon (Sanyal et al 1978). A different finding was observed by Napier, who showed that in the Southern part of India where there was no cold season, density fluctuated in different season and was lowest during hot dry month (Napier and Smith, 1926).

From the seasonal prevalence it is found that the density becomes maximum in pre and post monsoon period. In conclusion though sandfly is very susceptible to DDT and there is correlation with kala-azar cases but alternate control measure to be find out without DDT intervention due to its other hazardous effects.

5.9 A COMPARATIVE SEROLOGICAL STUDY FOR MEASURING THE ANTIBODY OF LD BODY USING DAT, iFAT, ELISA & iHA IN HIGH AND LOW ENDEMIC AREA OF KALA-AZAR IN SELECTED AREA OF BANGLADESH

* THE COMPARATIVE ANALYSIS OF THE RESULTS OF * ELISA, DAT IFAT

The statistical approach adapted in this study has apparently provided us with some information which, to the best our knowledge has not yet been reported particularly here in Bangladesh. It has not only indicted that any one of the three serologic tests investigated can be aptly used to stratify or categorize levels of kala-azar endemicity with equal efficiency; but, has also given us new insights into the conduct of epidemiologic surveillance studies for kala-azar in the future. In addition, it has presented us with a more detailed description of the magnitude of the impact of certain factors, such as, age and level of endemicity that have long been known to influence the antibody levels of populations exposed to kala-azar to wee out those factors which do not expert any real influence on the level of antibody, but has also

enabled us to describe the joint influence of the various independent variables that have genuine effect on the antibody level in one integrated package rather than as a series of separate analysis (as what has usually been done in previous studies). Furthermore, and even more important, the method has enabled us to measure the effect of each independent variable on the antibody score that the was uniquely attributable to the influence of that independent variable alone and which is not the possible result of other independent variables. Last, but not least, it has enabled us to determine the order of the degree of influence exerted by each of the significant predictor variables of the antibody levels of the study populations.

A considerable number of studies have amply documented the age-dependent increase in the antibody titers of individuals from endemic areas using the IFAT, Hommel, Vollar (McGregor et al, 1965; Collins et al, 1967; Thomas and Dissanaikeer al, 1977; Cornille - Brogger et al 1978), the conventional ELISA (Voller et al 1980) and type of serological test are held constant). The significance of this more precise quantification of the age dependent increase of the antibody titer in kala-azar surveys or in the kala-azar control programmes could, however, not yet be envisioned at this point.

The finding that the antibody scores of the study population in the high endemic area was higher than those of the low endemic area by 0.35 (for the same kind of test and same age) was rather interesting. This is because of the observation that, though this difference was found to be significant enough (p value < 0.0001) for us to conclude that the efficiency of the three tests to detect the difference in the levels of endemicity between the two study areas were the same, such difference does not really seem to be adequately large enough to justify the categorization of these two study areas into "high" and "Low" endemic areas.

As mentioned earlier, the levels of endemicity of the two study areas, were defined by the previous study on the basis of the annual parasite incidence in high and low areas as these two terms one would have expected a much higher degree of difference in the antibody scores between the two study areas than what has been demonstrated. With this finding one would be tempted to speculate that perhaps the endemicity levels of the two areas studied may in fact, just fall within the same range, but with one area having a relatively higher level of endemicity that the other (ergo, a probable case of mis-classification).This speculation would, certainly, be not far -

fetched if one takes into account the geographic proximity of the two study areas (i.e. only five kms away from each other). It would therefore, be interesting to conduct a study to compare the antibody scores of serum samples collected from areas with known, clearly defined levels of endemicity if the two areas investigated in this study have indeed been appropriately labeled as "high" and "low" endemic areas.

More importantly, a study (particularly a longitudinal one undertaken in different geographical areas with different levels of transmission), which is aimed at comparing the antibody scores of populations from endemic areas of varying levels could perhaps lead one into the possibility of devising a novel system of classifying or stratifying levels of kala-azar endemicity on the basis of antibody score alone. A system of classification which utilizes this purely serological approach would, particularly, be useful in endemic communities where the level of parasitemia of the population does not reflect the true picture.

2

The observation that only 18.1 percent ($r = .1809$) of the variation in the antibody score is accounted for by the three significant predictor variables tested, i.e. age, area, and test, suggests that there are many other factors that could potentially affect the antibody score, which have not been included in the regression model (equation) used in this study. Inclusion of these factors would not have only filtered out the confounders, but more importantly, would have greatly increased our capacity to understand exactly how these various other explanatory variables could affect the outcome of the antibody score. Hence, it would have enabled us to create a more comprehensive and refined picture of the relationships involved. Unfortunately, we could not have access to some of these important data.

A considerable amount of evidence has been presented to suggest that the following factors could influence the level of antibodies developed against the LD bodies. 1) history of kala- azar; 2) history of travel to high transmission area; 3) season (ie. rainy vs. dry). In addition, some of the variables that have not yet been explored, but are speculated to influence the sero-reactivities of populations from endemic areas would include the socio-demographic and behavioral factors as well as attitudes and perceptions that favour the risk or exposure to kala-azar. Among these risk factors following are deemed to exert a significant influence on the antibody profiles of endemic communities: occupation, place and type of residence, duration of stay in

the area, social and work - related activities, population movement or migration knowledge and perceptions about the transmission and prevention of kala-azar.

The finding that there is no significant difference in the efficiency of the three tests investigated in this study to discriminate different levels of endemicity would indeed reduce the burden of choosing which test to employ in VL sero- epidemiological surveys. Emphasis for the guidelines on the choice of the serologic method to use would now be focused on the objective of the study, the simplicity of the technique, the cost, capacity for automation, specificity, sensitivity and applicability for large scale field operations.

One of the most important findings in this study is the revelation that the use of the "antibody score" (which does not have any unit) to measure the level of anti LD body sero-reactivities of the two endemic communities has provided us with a way of standardizing the results of three serological tests which inherently differ not only in their respective units of measure, but in the types of specific anti LD body antibody detected and procedures as well. By using this "standardized Unit" it has made the results of the three tests statistically comparable.

The significance of the system of standardization used in this study could take on a much broader perspective if one was to apply it to sero-epidemiological surveys conducted in different places using different types of reagents (i.e. for the same test) or different types of serological methods. Expectedly, the results of these surveys would vary considerably from lab to lab. However, regardless of the method chosen, if this scoring system is adopted, then, the differing results between laboratories could still be comparable. Hence, the current problem of standardization of inter lab. variations of results, particularly for DAT (which has been recommended for use in sero-epidemiologic studies) can perhaps, be partly overcome.

The impact of this approach in overcoming the standardization problems of serological tests can even be magnified if the existing problems of standardization of antigens, reagents, equipments, positive and negative reference sera, cut-off points have been surmounted. Meanwhile, in the absence of a solution to these priority problems then the adoption of this antibody scoring system could serve as an alternative solution in the present situation. On the other hand, if the solution to these

priority problems has been found (in the future), then, this system could still be used to amplify the validity of the interpretation of data from different laboratories.

The results of this study could, therefore, serve as a nucleus for a series of future studies that can be conducted (either retrospective or prospective, preferably longitudinal) which are ultimately aimed at devising a novel system of classification of kala-azar endemicity based on antibody score. This implies that the process would entail the collaborative and networking efforts between individual investigators from established institutions in various geographic locations. Results of these studies can then, be collated and integrated into one "data bank" from which the antibody scoring system can subsequently, be constructed.

On the basis of these results a longitudinal study was being conducted using DAT for classify the endemicity.

5.10 SERO-CONVERSION USING DAT AS COHORT STUDY IN BANGLADESH

The goal of serological investigations was to obtain data on the levels and patterns of antibodies to specific antigens in the sera of population in relation to relevant variables, and thus to contribute to the knowledge of epidemiology of the disease (Paul and white, 1973).

As the objective of the present study focussed on the serological response to kala-azar infections of the endemic area, therefore only the data from local residents were at analysed. Due to the mobility of the local residents in and out of the village, epidemiological method for data analysis used in the condition the accurate results. It was noted that the annual kala-azar incidence density per thousand person month in Mymensingh district was 15.3. This finding was rather unexpected. It was due to patient from attending the health complex where medicine and other diagnostic facilities were available in which the distribution of kala-azar infection was not similar as other areas of Bangladesh.

In general, there were two peaks of kala-azar transmission during the year. The first peak of transmission was normally occurred around May - July, beginning of the rainy

> 6400 fold dilution as the positive seroconversion were practical because in the two criteria the antibody titers after infection had to be risen and our observation was also same as the higher titer as expected, Harith et al also observed in his observation in simple and economical agglutination test showed a sensitivity in the hospital setting when a titer of 1:1600 was considered as indicative of visceral leishmaniasis where infection of African trypanosomiasis is not expected, a specially specificity of 100% would be attained. Applied to sera collected in the field survey, the test showed sensitivity of 100% and specificity of 99.3%. The predictive values of the negative and positive in this study were 100% and bearing on the serological response of follow up individuals to kala-azar infection, six different pattern of serological responses in relation with the microscopic examination results were obtained. Two months before slide positive and two months after slide positive it was noted that there was a strong correlation among them p value = .05 and sensitivity and specificity were 99% and 85.71% respectively, probability of agreement on positive diagnosis were 0.99 and probability of Kappa coefficient also 0.98.

DAT can detect even after treatment of kala-azar patient. We also observed that in the follow-up of early diagnosed treated cases did not show any PKDL on the other hand those who were chronic and treated with SAG have developed PKDL, Chowdhury et al (1993 at press).

Though there were not significantly variation which crossed over the possible due variation due to the variation of the individual immuned response and of laboratory study both parasitological and serological examination. It was well understood that the individual's immune response was affected by several factors such as age, immunological competence, cumulative exposure to LD body antigen in terms of frequency, length and intensity of the infection and the kind and amount of specific therapy includes the length and frequency of treatment, Harith et al (1986), and this factors would produce the dynamic serological responses. For laboratory examination in the present study, microscopic examination and DAT test were used. The variation in slide examination appeared to be less than the DAT test. The sources of minor variation were incorrect technique of sample collection improper staining the slide and in reading the stained slide. The variation of slide examination was rather high when parasitemia was very low. Conversely DAT was proposed to be useful in the situation of low parasitemia sub-clinical infection, Harith et al (1986). However in the study on serological responses of follow-up individual, one should cautiously be sure aware of

the variations on both human and laboratory technique as well as clerical errors. An attempt to assess the association between the outcomes of DAT diagnosis in terms of seroconversion increased >1600 folds dilution from previous titer and microscopic examination as well as the factors which might influenced the above association was carried out. The association mentioned above was assessed by the following parameters on the basis of using microscopic examination as the gold standard.

- Sensitivity and specificity of seroconversion.
- Probability of agreement on positive diagnosis.
- Probability of agreement on negative diagnosis.
- Kappa and probability of Kappa.

Bearing on the factors influencing the association by DAT test and microscopic examination the following factors were investigated.

- Sex : male, female
- Age group: 1-14 years, 15-29 years & >30 yrs.
- History of previous kala-azar infection - Yes/No

On the basis of probability of Kappa it was shown that no significant results were found in the study factors except in the age group 15 - 29 yrs. which had the probability of Kappa = 0.50. This maned that the association between the outcomes of DAT and microscopic examination was existed in the age group 15 - 29 yrs. old.

The methods of analysis plays the significant role upon the interpretation of the data, consideration on the test of agreement between those methods in this study regarding the influence of sex, age, and the history of kala-azar experience. The sensitivity in male sample. Children >14 years old and the people who had past history of kala-azar was >98% respectively. But all other methods including Kappa that the degree of agreement indicated the strong agreement of seroconversion method than microscopic examination. These analysis emphasised very much on the reliability of the various methods of assessment.**The new method might enhance us to significantly very good outcome.**

In the present study direct agglutination test (DAT) was positive 512 being the sensitivity and specificity 99% and 87.71% respectively our results agree with that of Mengistu et al (1990) who found all the visceral leishmaniasis cases positive and control cases (sick and control healthy) negative by DAT. Harith et al (1986) in Kenya

also found the test (DAT) 100% sensitivity and 99.3% specific. However when the cases of African Trypanosomiasis were included in the control group the specificity was decreased. In another study Safi and Evans,(1989) found that all (100%) of 25 parasitologically positive kala-azar cases and 9 (69.2%) out of 13 clinically suspected cases gave positive results with DAT. Thus the test is 100% sensitive although in our longitudinal study showed 99% sensitivity and 85.71% specificity. But in case of patient with African trypanomiasis cross-reactivity persisted to over come this problem they used a high cut off value viz.1:12800. But this high cut off value decreased the sensitivity of this test, Harith et al (1986). In our study DAT was considered positive at titre >1:1600 because all the parasitologically positive cases showed a titer >1:1600 and control group sick and healthy showed the titer <1600. The titer in positive cases ranged from >1600 to 1:102400. Our study correlated with that of other worker. Mengistu et al (1990) in Addis Ababa found the same titer as our >1:1600 to 102400 in all the serologically positive cases,control cases showed the titer <1:1600, Harith et al (1986) in Kenya and found the titer, from 1600 to 1:51200 in cases of confirmed kala-azar cases. In case of healthy and sick control cases the titer always below 1:1600. However, in a study of Safi and Evans (1989) in UK the titer was 1:3200 or above for positive VL cases, they demonstrated that DAT at least as good as ELISA. Harith et al (1987) have shown that DAT to have a diagnosing performance of IFAT. Thus DAT compares favourable in several respects with ELISA & IFAT but ELISA & IFAT require either costly reagent or expensive apparatus and trained personnel. On the other hand DAT neither requires costly reagent nor expensive apparatus and it does not require trained personnel. In our series it was observed that in a rural set up (Trishal Health Complex) DAT was performing by laboratory technician under the guidance of Prof. M. S. Chowdhury and compared with the same sera by the Department of Medical Microbiology, University of Amsterdam, The Netherlands. In our study the antigen of DAT was used up to 4 to o 6 months after preparation kept at 4 C and was found stable. Harith et al (1988), Mengistu et al (1987), Chowdhury et al (1993) also found that the antigen stable for 3 weeks at 45 C and 10 months at 4 C respectively our prepared antigen from local strain of LD body showed also the same result.

5.11 EPIDEMIOLOGY OF KALA-AZAR IN BANGLADESH

The available information on leishmaniasis in Bangladesh is rather fragmentary. Th standard country profile adopted in the disease profile was not been included since

1987. It all concerns VL since there has been no reference to CL.in the literature consulted. From the history it appears that it was present as early as 1869. The first explosive epidemic of kala-azar of this sub-continent was recorded in Garo-hills and the adjacent Brahmaputra valley of Assam in 1880, Rahman et al (1979). Clark of Sanitation Commission draw public attention by reporting 100 cases from Garo-hills. The last outbreak of kala-azar started in Bihar in late thirties, which spread to Assam, West Bengal including other parts of India and the then East Pakistan (now Bangladesh) reaching peak in 1947. Some districts of the then East Pakistan reported 1500-4000 VL cases annually between 1950- 1960, majority of whom were in the age group of 3-15 yrs.

In 1951-1953 a plan of operations involving the Government, UNICEF and WHO was formulated to increase the treatment services of VI infections among children and mothers in those regions information has been made on VL; the world health statistics report (1968) only quoted the data of the above mentioned trial under East Pakistan (Bangladesh) M Rahman (1980) presented a paper and he observed that the incidence of kala-azar during the epidemic years of 1959-1963 as 15000-17000 per year in East Pakistan (Now Bangladesh and Pakistan).

Although it was reduced below the level of Public Health importance, the disease was never totally eradicated. Cases of Post Kala-azar dermal Leishmaniasis (PKDL), a suspected reservoir of infection for sandflies, and occasional cases of active kala- azar continued to be seen in the clinic and different hospital of Medical colleges (Khan 1977).

The factors responsible for the apparent disappearance of the disease were supposedly:

a) Successful chemotherapy of all cases;
b) Acquired protective immunity in the population and
c) Decreased in vector density following mass indoor residual insecticidal spray (DDT) under National Malaria Control Programme followed by the National Malaria Eradication Programme (NMEP).

Following withdrawal of the DDT spray operation as a strategy of the NMEP, there was gradual increase in the vector population. An alarming degree of anthropophilism

in P.argentipes was demonstrated by Ahmed and Ahmed 1982, Further with the declining trend in transmission, there was a gradual increase in the susceptible human population. Thus, the stage was almost set for a large scale transmission to start. A forecast of such a possibility came from chowdhury et al 1991, he estimated that 15000 new cases per year in Bangladesh. A report of 1987 revealed that 31 districts out of 64 and 61 out 460 thanas of Bangladesh were affected by kala-azar.

It started from small unit from Sirajganj, Mymensingh. Kala- azar cases, admitted in Rajshahi Medical College Hospital and Thana Health Complex situated in Northern part of Bangladesh showed a steady increase in the number of cases and the number became alarmingly high since 1987, N M Alam et al, 1990.

On the basis of the recommendation of Inter country consultative group meeting organized by WHO held at the WHO regional office, New Delhi from 9-13 Dec. 1985, realized the urgent need for the development of trained manpower to assess the magnitude of the problem of kala-azar in Bangladesh.

5.12 GEOGRAPHICAL DISTRIBUTION OF KALA-AZAR IN BANGLADESH

Though it is difficult to plot accurately the present geographical distribution of the leishmaniasis and to determined their prevalence in man. Information available on kala-azar in Bangladesh is based on poor recording and reporting of cases, so false assessment of prevailing condition arise. Whatever information available is mainly based on paper works, even few studies done on kala-azar are also either clinical or laboratory based very recently Chowdhury et al (1988) conducted a field based study on sero-epidemiology and chemotherapy on kala-azar in Bangladesh followed by longitudinal study. The study revealed that a sharp increase of kala-azar cases in Rajshahi Fig.-- Pabna--Fig----. Shirajganj, Mymensingh, Tangail and Gazipur.

Endemic foci of visceral leishmaniasis of the world are characterised rarely on the epidemiological groups. On the basis of regional epidemiological peculiarities, the known foci viz. (i) Mediterranean and Chinese kala-azar, (ii) East African Kala- azar and (iii) Indian Kala-azar can be identified on the following criteria (Table I).

TABLE I

Epidemiological characteristics of Indian Mediterranean and East African Kala-azar

Epidemiological entity	Indian	Mediterranean	East African
i)Distribution	India	Mediterranean Middle Asia and China	Sudan Kenya
ii)Age group	Mostly older children & young adults (10-20 yrs)	Mostly infants and younger children (1-10 yrs)	Older Children & young adults
iii)Animal	Absent as primary reservoir of infection.Parasite available in cutaneous lesions of animals.	Dogs,Jackals & Fox	Different sps of rodents(parasite available in biopsy.
iv)Causative agent in human host.	Availablein blood & in post kala-azar dermal leishmanoid.	Man is biological terminal.Parasite not available in blood.	Parasite available in blood.
v) Post-kala azar dermal leishmaniasis.	Prevalent	Absent	Frequently present.
vi)Drug response to antimony compounds.	Susceptible	Resistant	Resistant
vii)Epidemics	Frequent	Rare but small outbreaks occur	Mainly endemic

From Table 1, it is apparent that Indian Kala-azar has become completely "Anthroponosis" and Man maintains itself as an efficient host principally on two considerations viz. (i) presence of parasites in peripheral blood in sufficient numbers to produce a high infection rate in P.argentipes (Knowles et al 1924) and (ii) Appearance of post kala-azar dermal leishmaniasis (PKDL) where LD bodies can easily be detected in blood smears in majority of the cases.

INCIDENCE BY AGE

Sanyal et al (1979) in his study of 42 cases observed that disease affects all age group viz. 0-9 years (25.6%) 10-19 year (20.9%), 20-29 years (20.2%), 30-39 years (20.9%) and 40 years (2.3%). This study revealed the following age incidence (Table-3) viz. 0-5 years (5.8%), 6-10 years (16.6%), 11-15 years (34.5%), 16-20 years (23.6%), 21-25 years (7.5%) and above 25 years (13.09%).

INCIDENCE BY SEX

Sanyal et al 1979 and Hati et al 1985 observed that incidence was three times higher in males than in females i.e. 38.5% among females against 61.5% among males respectively. The study also revealed that the male female ratio was about 3:1. It is of great epidemiological significance, as it indicates that males of all age groups are more at risk than females. This raises the possibility of outdoor transmission, as males remain predominantly outdoor towards evening as compared to females who keep busy in cooking or other indoor activities. This may be due to female are heavily clothed as compared to male and hence protected from sandfly bites. Unequal incidence could only be explained by extradomiciliary transmission.

DISTRIBUTION OF CASES IN THE COMMUNITY

Sanyal et al (1979) and Hati (1985) observed in North Bihar and West Bengal multiple cases in three huts. While rest of 34 were single cases per hut. Sanyal also observed that earliest cases in 1977 and those detected in 1979 occurred in the peripheral part of the village. Hati observed one infected person per hut no clustering of cases.

In our observation we found the clustering of cases in the villages where PKDL were detected in the village Kushmail, Radhakanai and Amirabad of Fulbaria and Trishal Thana of Mymensingh district (study area). The villages were also affected with kala-azar during 50's decade.

TRANSMISSION OF DISEASE

Considerable epidemiological data has been generated during the current episode of VL now ravaging, Rajshahi, Dinajpur, Thakurgaon, Pabna, Sirajganj and Mymensingh in the epidemic form, Epidemiological and entomological features as emerged during these studies are briefly summarized below for comparison purposes with erstwhile known features of kala-azar in Bangladesh (Fig.____).

A sudden outbreak of VL occurred during 1979-1980 in one village Madla of Sirajganj district which was investigated both entomologically and serologically Touhid et al 1982 and followed up and total number of cases recorded in the outbreak was 45 (30 adults 6 children less than 3 yrs old 9 aged three to six years).

5.13 SEASONAL DISTRIBUTION OF KALA-AZAR AND SANDFLY IN BANGLADESH AND RELATIONSHIP BETWEEN INCIDENCE OF KALA-AZAR FACTOR

In a longitudinal study revealed that the maximum number of kala-azar cases occurred during March to June highest peak in April and May and minimum in September and October in the year 1988, 89, 90 (Fig.-----) Chowdhury et al 1992. During this work it observed that the density population of sandfly was also high in March to June (highest in May) there is strong relationship between sandfly (vector and kala-azar while vector density increased incidence of kala-azar were also increased.

There were approximately 25000 cases of kala-azar treated in various hospitals of the country. Since the 1980s, there have been reports of a resurgence of kala-azar following suppression of insecticide spraying in India and Bangladesh. The number of VL and PKDL cases in creased sharply and reached a level which could provide a

reservoir of sufficient magnitude to spark off a major outbreak. Kala-azar and PKDL are prevalent in many areas of Bangladesh. Between 1980 and 1985 447 cases were reported from the 5 districts but since 1985 and up to today, cases have been registered from 61 thanas of 27 dist icts. During hour to house survey from July 1987 to June 1988 in Mymensingh district covering 3 thanas 1427 villages) 1273 cases of VL including 45 cases of PKDL were diagnosed parasitologically or serologically and treated. The highest incidence was in the 11-15 year age group. The highest death rate was 6.4% cases occur mainly within families, especially PKDL cases. In 1988 kala-azar cases were 3548 with 8 death, 2526 cases with 26 deaths in 1989. 3334 cases with one death in 1990, 3039 cases in 1991 and 6818 cases in 1992.

Circulation of parasite in blood: Chowdhury et al (1988-93) treated 1273 cases in primary health centre and found positive for LD bodies either by bone- marrow and or by splenic puncture. No parasite could be isolated from the peripheral blood. Similarly Thakur 1993 giving an account of 670 cases treated in Patna Medical College found positive for Ld bodies either by none marrow and or by splenic puncture. However, no parasite was found in the peripheral blood.

Similarly workers of RMI, Patna and NICD Unit on Kala-azar have failed to confirm parasite in the circulating blood of the kala-azar patients so far.

Post-dermal Kala-azar (PKDL): PKDL is known to be a manifestation due to immunological factors in a clinically cured case. Napier (1931) reported that 5 per cent of treated cases will come up with PKDL in one year time. Chowdhury et al 1993 observed 75 cases i.e. 5.5% cases have been developed PKDL even after treatment. Dutta et al 1983 reported that incidence of PKDL in present episode in Bihar was only 1.98%. In our study also revealed that the same as (Chowdhury et al, 1990).

Association of cases with professions: No study was attempted to find any correlation of cases with professions of the community. However, casual observations in Mymensingh and Sirajganj districts indicated that most affected person 90% were affected within low socio-economic group of population. Some of the tribe in Bhaluka related to prevalence to P.argentipes which was very low in huts, but in high density in adjoining Muslim village without any case of kala-azar. Low socio-economic status of these two professions and labourers on agriculture.

Clinical profile: Lymphadenopathy which is very common characteristics of Mediterranean kala-azar (50 to 65 %) was uncommon in Indian kala-azar and hence the fatality of lymph gland puncture as ancillary diagnostic techniques is well known, Moskovakis and Southgate,1971. However, Chowdhury(1983) in 24 Pargana West Bengal recorded as high as 80 % generalized lympnadenopathy and 78 % biopsies of lymphnode were positive by impression smear and/ or histopathology. However, lymphadenopathy was also in Malda and in Bihar, but was generally less prominent and Dr. Nandy from Calcutta Tropical Institute, could not established parasite by lymphnode biopsy of 20 kala-azar patients in Bangladesh.

Chemotherapy: Sodium Antimony Gluconate 20 mg / kg body weight not exceeding 850 mg per day for at least 20 days (WHO 1982) was used as a first line of treatment. C.P Thakur(1984) observed 92.6% of cases improved by providing the above schedule of treatment, in his 750 cases study. Our study also revealed that antimony was effective in 94.7% of cases by the above regime of treatment with minimum side effects. Sengupta(1983) also observed that in long course regime, the relapse rate would fall from 15% to 0.5%. Only two cases failed to respond. Although all cases responded well to antimony during sixties decade, the number of case increased and the drug was indiscriminently used by local doctors and Quacks so the number of unresponsiveness increased and also test dosing (1 c.c.) may also contributed to unresponsiveness. Irregular, sub-therapeutic and therapy on alternate days were also unscientific and in our observation most physicians used low dose, In PKDL cases 20 mg/ kg body weight for 20 days- six courses with 10 days interval between two courses showed 100 % cured.

DRUG RESISTANCE: Strain of LD body detected in the current episode has shown high degree of resistance to antimony compound. L.S.Prasad (1977) who himself a clinical of eminence from Bihar and Ex- Director of RMI, Patna, recorded failure of antimony treatment in high percentage of cases. He also recorded cases of double resistance in L.donovani to antimony compound and Pentamidine Drug resistance has also been confirmed by Sanyal and Arora (1979) who found that 45.7% cases from Bihar in 1975 and 1976 admitted in school of Tropical Medicine Calcutta were resistance to antimony. Though there is no record in Bangladesh, our observation shows out of 571 treated patients with (antimony) Sodium Stibugluconate by 20 mg/kg body wt. for 20 days, resistance is 0.87%.

Animal Reservoir Studies: Chowdhury et al 1992 screened number of animals viz. dogs (12), Cats (2), Rats (55), Rabbits (07), Cows (55), Goats (37), Sheeps (13), Fox (2) and Bird (10), with negative results. However, Srivastava and Chakravarty (1984) detected leishmanial antibodies in five out of 226 Bandicoota benglaneis in Bihar.

Entomology: Prevalence of P.argentipes in areas of resurgence

P.argentipes the classical vector of Indian Kala-azar was found prevalent in Shahjadpur, Mymensingh and other places which prevalent indoors in affected and non affected villages of Bangladesh.

Specially traditionally known to be an endophilic was also encountered in tree holes and near rodent burrows in peridomestic situations in Bihar indicating that species also rest outdoors (Kaul et al 1979).

CHAPTER 6

SUMMARY

6.2.i SUMMARY

CHEMOTHERAPY OF KALA-AZAR AT PRIMARY HEALTH CENTER IN BANGLADESH

After the resurgence of kala-azar since 1981 in Bangladesh, 61 out of 460 thanas of 31 districts out of 64 districts reported cases till November, 1988.

The incidence of kala-azar in Mymensingh, Sirajganj and Pabna was 0.33, 0.41 and 0.51 respectively (June '87 to December '88). The male/female ratio was 3:1 and about 60% of cases were between 11-20 years. Out of 2577 detected cases of kala-azar including PKDL, 1273 cases were assessed and evaluated for chemotherapy and serology. About 94.02% showed immediate clinical response after providing sodium stibogluconate 20 mg/kg body weight not exceeding 850 gm per day IM/IV for 20 days. Only 11(0.86%) cases showed no response. All 45 (100%) cases of post kala-azar dermal leishmaniasis (PKDL) tolerated and responded well to six courses of treatment with an interval of 10 days in between 2 courses. Side effects were minimum.

6.2.2. POST KALA-AZAR DERMAL LEISHMANIASIS IN BANGLADESH

5,011 patients having history or suspected history of kala- azar were studied for post kala-azar dermal leishmaniasis (PKDL) in two districts of Bangladesh from June 1987 to December 1990. Among these patients, 61 were found to have cutaneous lesions suggesting of PKDL. None of their lesions had last either thermal or tactile sensations. Chemotherapy with antimony gluconate cured all cases except one who responded to pentamidine serological tests showed 100% sensitivity for DAT and 86.88% for ELISA. Antibody titer ranges in PKDL sera are noted to be lower than those of kala-azar patients but in DAT showed the higher titration in sera of the same PKDL opinion is that the developing of PKDL not due to strain variation but might to due to incorrect or subtherapeutic treatment.

6.2.3 KALA-AZAR IN CHILDREN A STUDY OF 100 PATIENTS

100 of patients of kala-azar between the ages of 1-12 years of old were studied. Age, sex history, clinical and physical findings were analysed. Out of 100 cases 78% were parasitologically proven and 72% were serologically positive 47% patients were in 9-12 years in age group and male female ratio was 1.94:1. All the patients had fever, anemia and spienomegally. Hepatomegally was present in 54%. Appetite was normal in 70% increased in 28% and reduced in 2% response to chemotherapy was excellent (100%). It was observed that those who had malnutrition they had developed more signs and symptoms.

2.4 POSITIVE RESPONSE TO SAG ADMINISTRATION IN VISCERAL LEISHMANIASIS

In a prospective study conducted in Mymensingh district of Bangladesh 1273 patients were assessed for the presence of visceral leishmaniasis (VL). Sodium antimony gluconate (SAG) was successfully administered to 715 patients with parasitologically confirmed infection. In the remaining 558 although there was clinical indication of VL leishmania donovani parasites could not be demonstrated. Administration of SAG in this group was on the grounds of the prevailing symptoms, exclusion of malaria and a positive direct agglutination test (DAT) significant improvements in the clinical and hematological parameters were obscured in 547 (98%) of the unconfirmed of VL cases. On the basis of the parasitological findings or positive response to specific anti-leishmania chemotherapy, the sensitivity and specificity for diagnosis of VL at levels below that of parasitological detection. On the other hand by statistical analysis a high correlation and coefficient was found among the test AT, ELISA, IHA, *DAT techniques (P=001). All recent kala-azar cases showed 99.6% sensitivity and 97.7% specificity on the other hand all the health control in endemic and non endemic, sick control showed the below 1:1600 titers in DAT i.e. negative result for kala- azar. Although IFAT was negative after six months of treatment in most cases (7% cases positive after treatment) ELISA was the next who could identify the cure after 1-2 years of treatment but DAT titers remained high in the longer period even after treatment.

2.5 DIFFERENT TREATMENT SCHEDULE FOR SAG FAILURE CASES OF VISCERAL LEISHMANIASIS

The comparative study of different treatment schedule for the failure cases has been carried out in this series. The sensitivity of leishmania to drugs can be tested only in sophisticated laboratories by macrophage culture. It could not be employed routinely on patients but assessed clinical, parasitological and serological techniques.

During the evaluation of DAT at the level of rural health center in Trishal, Bhaluka under Mymensingh, Kalihati of Trishal and Shahjadpur of Sirajganj districts of Bangladesh. Out of 1474 kala-azar cases 340 (13.3) were established both clinical, parasitological and serological. All 340 kala-azar cases were treated in the health center, splenic or bone marrow aspiration was done before and after a course of drug therapy (SAG) out of these 55 (16.17%) showed primary and 3.63% secondary unresponsiveness to SAG. All 55 cases, the patients were received 20 days regimen of treatment with dose schedule SAG (Glaxo,Albert David and B.We come) in the strength of Antimony 100 mg/ml. Primary and secondary unresponsive cases were stratified with age and sex and assigned to one of the five treatment groups by a pre-

determined randomized schedule. All the patients remained in the hospital for 9 weeks, group A revealed that 86% case with 30 days of SAG showed parasitological cure. Out of 10 cases 60% cases were responded to group B on the other hand 100% cases responded to group C and 99% in group E. No patients had an abnormal BUN before and after or during treatment 10 mg/kg body weight thrice daily for 7 days showed an excellent result and 2nd significant result showed in group E with pentamidine for 14 days. The result suggest that if after 40 days of treatment, there is no response, then either patient should continued with antimony 10 mg/kg body weight thrice daily for 7 days or pentamidine should be given alternate suggestion or second choice.

6.2.6 PREVALENCE OF AGGLUTINATION ANTI-LEISHMANIA ANTIBODIES IN TWO MULTI-THOUSAND BENGALI COMMUNITIES

For control of visceral leishmaniasis (VL) in large endemic communities, a feasible epidemiological indicator capable of monitoring an on-going transmission rather than mere exposure to the parasite is required. Having evidenced the desired reliability for laboratory diagnosis of VL, she direct agglutination test (DAT) is employed here to estimate VL sero- prevalence in the endemic thanas of Trishal and Shahjadpur appendant to Mymensingh and Sirajganj districts of Bangladesh. DAT antigen production was duly maximized to allow coverage of a study population comprising 17826 inhabitants of whom 9619 resided in Trishal, 7328 in Shahjadpur and 879 in Teknaf of Cox's Bazar a known leishmania-free district in Bangladesh. Despite large scale production, all DAT antigen batches processed in quantities of 1120-4000 ml and sufficient for screening of 1176- 6400 inhabitants per single batch, performed as desired in quality control tests for sensitivity, specificity and stability. Whole blood by finger prick instead of venipuncture or filter paper collection methods was employed reaching for more convenience in sampling and handiness in execution of DAT at such a magnitude. The cross-sectional survey revealed VL point prevalence of 4.40% in Trishal and 6.75% in Shahjadpur by caparison to an extremely low rate of 0.34% in the non-endemic Teknaf. In both endemic thanas (Trishal and Shahjadpur) VL was more prevalent (2.56%) in <1-20 years age group than in those of 21 years and older (1.84% - 2.25%). Of 918 recorded as seropositive, 539 were asymptomatic and 379 were symptomatic at various degrees of suspension to VL. Diagnosis of VL was established in 125 symptomatic seropositive either on grounds of Leishmania amastigote demonstration (29) or positive DAT results combined with presentation of typical VL signs (96). All diagnosed patients responded favourably to sodium antimony gluconate

administration; re-testing and follow-up procedures were started on the remaining 793 sero-positives detected. With comparable feasibility merits for VL survey in large endemic communities, the DAT can be considered as an appropriate alternative epidemiological indicator to the leishmanin and aldehyde tests.

4.2.7 SANDFLY DENSITY AND KALA-AZAR INCIDENCE IN BANGLADESH

Three periodic surveys were conducted both in an intervention and a control area in each of Fulbaria, Trishal and Bhaluka thana under Mymensingh district.DDT was sprayed after the first survey in the intervention area. The purpose of the study was to compare the prevalence of visceral leishmaniasis and the vector, Phlebotomies argentipes, in relation to DDT spraying and seasonal distribution of vector. 1828 (33.23%) Kala-azar cases were detected from the study area during first survey. Vector density (5.82 per man hour) was correlated with the prevalence of kala-azar. Relation between these two was found significant (P value <.025). Both the incidence of case and vector density were affected by DDT spraying. Number of cases significantly reduced in the sprayed area from 1928 (33.23%) to 7 (0.14%) in 1989-90 and 1 (0.02%) in 1992. The vector density also decreased. During fauna survey five species of sandfly, phlebotomus argentipes (40.04%) P. papatasi (5.45%), Sergentomyiua babu babu (48.53%), S.shortii (3.16%) and S. barraudi (1.79%) were collected. Resting behaviour and blood meal analysis suggested the zoophilic nature of P. argentipes. The vector was found to be present in all seasons but with two peaks, one in July - September and other in April - May.

4.2.8 A COMPARATIVE SEROLOGICAL STUDY FOR MEASURING THE ANTIBODY OF LD BODY USING DAT, IFAT, ELISA & IHA IN HIGH AND LOW ENDEMIC AREA OF KALA-AZAR IN SELECTED AREA OF BANGLADESH

The empirical findings of this study are summarized as follows:

1. Using antibody level (score) as the parameter, three of the four tests investigated, namely the ELISA, IFAT and DAT, do not significantly differ in their ability to assess the varying levels of endemicity of two kala-azar areas, i.e., high and low (P value for interaction of test x area = 0.7111, b3 = 0.353).

2. Out of five independent variables investigated, three were shown to be statistically significant predictors of the antibody scores of populations from the two endemic areas studies. These were, a) type of serological test, b) age and c) level of endemicity of the area (p = 0.0002, 0.0000, 0.0000, respectively). Among these three,

the strongest predictor variable is age followed by area and then type of test (Beta = 0.369, 0.155 and -0.131, respectively).

3. The combined influence of the type of test, age and level of endemicity of the area account for 18.1 percent of the variation in the antibody scores (F ratio = 17.66, p value <0.0001).

4. For individuals of the same age and coming from areas with the same level of endemicity, the predicted antibody score for DAT will be 0.307 less than that of standard ELISA or IFAT
(t value = 3.686, p value = 0.0002).

5. The antibody score increases by 0.021 for every year of increase in age, if the same test is employed on individuals from the same area (t = 10.372, p value <0.0001).

6. The antibody scores of individuals from high endemic areas will be higher than those from low endemic areas by 0.353 for the same type of serological test and the same age (t=1.387, p=0.0000).

7. Gender and parasite density do not have any significant influence on the variation in antibody score (p value >0.05 for each variable).

8. The IHA test system carried out in this study cannot be used as a sero-epidemiological assay to differentiate between areas of varying levels of endemicity and even between normal and immune sera.

6.2.9 SEROCONVERSION USING DAT AS A COHORT STUDY

Series of cross sectional studies followed by longitudinal studies had been carried out in an area of low persistent kala- azar transmission in Bangladesh in Awaltia and Guzium villages of Trishal thana in Mymensingh district of Bangladesh were selected as the study areas where the whole local residents of 672 persons had been regularly followed up. The DAT was used in the study by using local strain of LD body. Out of a total 3,645 serum samples, 23.6 % were randomly selected for replicated testing and 90.8% reproducibility with the same titer were obtained. Three criteria indicating sero-conversion increased 1600 fold or more 3200 fold or more and 6400 or more dilution of different paired sera obtained between of 2 months of follow-up were set up and they were tested for agreement with the microscopic examination. It was concluded that the seroconversion of increasing 3200 fold dilution or more was the appropriate parameter on the basis of increasing 3200 or more as seroconversion the sensitivity and specificity of seroconversion test against microscopic examination were 99% and 85.7% respectively. It was noted that the annual average of kala-azar incidence was 15 per 1000 person-months. This means that, on the monthly basis, 15 persons within

1000 population who lived in the area would suffered from kala-azar which indicates that it is an epidemic of kala-azar. The pattern of seasonal variation of kala- azar transmission in the area was measured by parasitological and serological study. The low kala-azar transmission was shown during September to February by bone marrow or spleen aspirated materials examination, on the other hand, serological parameters such as seropositive rate (titer 1:3200 or more) and GMRT were also low during that period of time. This was probably due to an effect of low density of sandfly, the sandfly collection had been conducted during September - December in the area, the most common pattern (68.66%) was the group of having seroconversion without positive diagnosis by microscopic examination. Nearly 14.80% were the pattern which seroconversion occurred in the same month when the slides became positive and 14.42% were the pattern of no seroconversion and the slides were negative.

The parameters: Sensitivity and specificity of DAT test, probability of agreement of positive and negative diagnosis by seroconversion and microscopic examination and Kappa coefficient and the probability of Kappa were used to assess the association between DAT and microscopic examination. It was found that there was a weak association between DAT test and microscopic examination.

Further analysis on the factors influencing on the association between DAT and microscopic examination such as sex, age (1-14, 15-29, 30 yrs. old or more) and past history of kala- azar infection was done. To some extent association between the outcomes of DAT test (seroconversion) and parasitological examination was found more in the age group 15-29 years old. However, in a low degree it was postulated that the association between DAT and microscopic examination was distorted by the high level of kala-azar antibody.

6.3 LIMITATIONS OF THE STUDY AND RECOMMENDATIONS

1st cross-sectional study was conducted to assess the base line data and to have the magnitude of kala-azar in Bangladesh. Initially the study was conducted during August 1987 to July 1988 followed by longitudinal studies 1989-93. First 6 months were used for organizational and preparatory phase 2-3 months for active case detection rest of the period to show the efficacy of chemotherapy including refractory cases and serodiagnosis including evaluation of different sero-techniques.

Longitudinal studies were conducted from July 1988-89, 1989- 90, 1990-91, 1991-92, 1992-93 for vector bionomics and to observe the effect of DDT on kala-azar transmission. Alternate treatment schedule was being carried out on SAG failure

cases. Mass application of the DAT and assessment of kala-azar transmission in two multi-thousand endemic areas in Bangladesh.

Mymensingh, Tangail, Gazipur and Cox's Bazar were conducted during 1989-93. Another study for sero-conversion using DAT as a cohort study was also being conducted to determine the endemicity, seasonal prevalence of kala-azar in Bangladesh from April, 1992 - April, 1993.

1. In as much as the conclusions made above were drawn from the results of a statistical model which did not take into account the sensitivity and specificity of the serological tests investigated, the findings of this study should, therefore, be taken with some caution. This is because it has been advocated that when statistical analysis is applied to serological data, these two factors, plus the possible fading of antibodies in a proportion of the population should be accounted for sensitivity and specificity tests were not carried out primarily because of the unavailability of a test system that could be used as the "gold standard" in lieu of the bone marrow or spleen aspirated materials smear. So that as it may it is, therefore, suggested that the following be done:

1.a. Sensitivity and specificity studies be carried out on the same set of blood samples to increase the validity of the results obtained.

Since it is felt that the BM/SA smear is no longer reliable for use as a "gold standard" in the Bangladesh situation as it can not give 100% diagnosis a suggested alternative reference material for these sensitivity and specificity studies are sera obtained from hospital patients with B.M. slide - positive confirmed B.M. smear (LD body).

1.b. A modification of the study design can be attempted where all the four tests are run on the same batch of serum samples (randomly selected) and the results compared with those of this study.

2. Since this study is a cross-sectional one it fails to provide us with a description of the temporal sequence of the relationship between the development and magnitude of the antibody levels and the inherent level of endemicity of the respective areas. Thus, the causal relationship between exposure of the study population to the two kala-azar areas with different levels of endemicity and the antibody level could not be established. A longitudinal study would have been more informative in this case. Nevertheless, a follow-up study carried out during the low transmission period to see whether the pattern of the level of antibody score changes, i.e., drops, as what has been shown in other studies (Chowdhury et al, 1993) could be done. Although this will not provide us with any information regarding the causal relationship between antibody

score and exposure to the area it can give us a perspective of the seasonal fluctuations of the antibody levels among the study population.

3. In as much as only 18.1% of the variation in the antibody score is explained by the combined influence of age, area and type of serological method used, then there is a need to add the other variables that have been speculated to potentially influence the antibody score into the regression model to obtain a more precise picture of the explanatory power of each independent variable, as well as a more comprehensive perspective of the interrelated factors that affect the outcome of the antibody score.

4. Considering the greater proportion (81.9%) of the potential explanatory variables that have not been accounted for in this study and the finding that area is a significant predictor of the antibody score of the population then, the results of this study can be applied only to areas with a similar setting as that of the study areas. The need to conduct more studies in various other geographic regions with a different setting or population profile is, therefore, emphasized if one is to devise a standardized system of classifying levels of kala- azar endemicity based on antibody score.

Based on the regression model adapted in this study, it was shown that, using antibody score as the parameter, there was no significant difference ($p>0.05$) in the ability of three of the serological assays investigated, i.e. DAT, ELISA, and IFAT to detect the difference in the levels of endemicity between the two study areas and to categorize them accordingly (i.e. high and low) as defined by the (Chowdhury et al 1991). The difference in the antibody scores between the two areas (for the same age group) was found to be 0.353 regardless of the method used. The equal efficiency of the three tests investigated in this study to detect even this apparently minimal difference in the antibody scores of the population from the two study areas commends them for use in sero-epidemiological investigations of the level of endemicity of kala-azar (perhaps especially so after sensitivity and specificity studies have been done), particularly in areas where parasitemia tend to be subpotent.

Inasmuch as only 18.1% of the variation in the antibody score of the study population is significantly explained by the combined influence of age, area and type of method used, then there is a need to conduct more studies that could identify and define the other explanatory variables that would account for the remaining 81.9% of the variation in the antibody score. A more comprehensive picture of the factors that would influence the outcome of the antibody score would provide for a better and more refined interpretation of results. These studies should preferably be longitudinal ones and carried out in endemic areas from different geographic locations with clearly defined varying levels of endemicity. Moreover, because of the apparent multiplicity of

complex factors that could potentially influence (either singly or jointly) the anti LD body serologic profiles of populations from endemic, areas, it is suggested that the data obtained from these studies be analyzed using the same statistical method (i.e. stepwise multiple linear regression analysis) as was done in this study.

6.4 SIGNIFICANCE OF THE STUDY

1. The results of this study have apparently provided us with an empirical data which could serve as a guiding principle in the choice of the appropriate sero-epidemiologic method that should be used in epidemiological kala-azar surveillance and control studies. Since the three methods have been found to be relatively equal in their efficiency to categorize the level of kala-azar endemicity, then the primary considerations that have to be taken in the choice of the method to be used would be the objective of the epidemiologic study and the applicability of the test for large scale field investigations.

2. The information obtained from the study could also serve as a preliminary or base line data for future longitudinal studies that are aimed at determining the serological profiles of populations from areas with varying levels of endemicity. The results of these studies could then, be used as an information base for the formulation of strategies and policies to improve or maximize the existing surveillance and control schemes for kala- azar.

3. Results of the study have also underlined the issue of the unreliability of the use of the parasitological method to assess the level of endemicity of kala-azar in areas where parasitemia tend to be subpotent. As such it challenges the justification of the continued use of the DAT as the basis for classifying kala-azar endemicity in the country.

4. Results have, likewise, opened up areas for future research directions.

5. Perhaps, one of the most significant findings in this study is the possibility of having come up with a system that could not only be used as an alternative solution to the current major problem of standardizing the interlaboratory variations of results of serological tests but, which could also serve as a basis for a conceptual framework for devising a novel system of classifying and categorizing levels of kala-azar endemicity, particularly in endemic areas where the use of the annual incidence rate is no longer deemed to be reliable as in Bangladesh and perhaps, in other South-East Asian countries as well.

6.5 CONCLUSION

In conclusion, we have to mention that method of diagnosis and confirmation of the disease with an assessment of cell mediated immunity can only be done in a well equipped hospital. It is quite different from the diagnosis of the case at primary health center where we have to mainly depend on correct clinical interpretation of the signs and symptoms of the disease and probably has no facilities of diagnosis of VL only. It is also difficult to diagnose an early case who is a casual visitor to endemic area or an irregularly treated case without classical clinical feature than a clear-cut case coming from an endemic area having irregular fever for more than 3 months not responding to usual antibiotic or antimalarial treatment, hepatosplenomegaly with moderate anemia with leucopenia, neutropenia and high erythrocytic sedimentation rate (usually more than 100 mm per hour) with positive aldehyde test. The later case leaves no doubt about the diagnosis of VL. For confirmation, demonstration of parasites either in bone marrow aspiration or splenic puncture followed by culture of these materials in blood agar media in hospital environmental where these facilities are available is essential. A negative tuberculin or leishmanin test during the active stage of the disease is a corroborative evidence which however become positive after 6 months of successful treatment. The role of serological tests are mainly to point out the disease either present or past and are useful in epidemiological survey. These tests have also some importance in longitudinal studies of the patients in assessing the cell mediated immunity. Serial studies give an idea about individual response of patients to treatment. It has also been found in some places that the number of positive serological cases exceeds that of kala-azar cases. Some of these positive serological cases deny any history of attack of VL either at present or past though they may have splenomegally. These cases may be the 'sub-clinical' cases of VL and may act as potential source for the spread of the diseases. The PKDL cases may also serve as reservoir of parasites in their skin and may also help in the spread of VL. More attention must be given to PKDL cases which may be about 10% of VL cases. As they are ambulatory and have no disability except the skin changes they usually do not come to doctors for proper diagnosis. Moreover they usually do not like to lose their daily earnings by admitting themselves in a hospital for diagnosis and treatment. So they should be searched for by domiciliary visits to villages, diagnosed by skin aspiration or biopsies and full coarse of treatment should be instituted from primary health center or by domiciliary visits by health assistants.

Although reliable morbidity and mortality data are lacking from many countries like our, the disease is responsible for high morbidity and mortality and suffering on five continents. On a global basis it constitutes a major public health problem and imposes an extra burden on countries which already have serious economic difficulties. The prolonged and expensive treatment required for visceral leishmaniasis patients can overburden, and occasionally overwhelm, medical facilities and impair the ability to provide other essential medical services.

Relapses and non-responsiveness to treatment are seen in all areas and indicate that improved treatment is needed, particularly second line drugs. This study indicated that it can be overcome if proper treatment with shorter period with three divided doses of antimony for primary unresponsive cases, pentamidine is the second choice of drug for secondary refractory case of antimony.

NEED FOR THE FUTURE STUDY

A critical appraisal of foregoing epidemiological and entomological evidence would indicate that epidemiological features viz. i) high incidence among 0-10 age group.

ii) non availability of parasite in the circulating blood.

iii) high drug resistance.

iv) high rate of lymphadenopathy and

v) low rate of PKDL are highly suggestive of circulating strain of leishmania to be more nearer to Ld infantum, which has different host, parasite-vector system. Absence of natural infection of promastigote in P.argentipes population in epidemic areas in spite of large scale dissections cast double about role of man as reservoir and that of P.argentipes as transmitter at least in the initial stages of build-up of the epidemic till such time PKDL.

Absence of multiple cases and non clustering phenomenon of the disease, preponderance of infection in males, close association of the disease with particular profession those who are low socio-economic group of population. Therefore, there is a urgent need to undertake in depth co-ordinated epidemiological and entomological studies not only to prove or disapprove the much believed mechanism of man to man transmission through P.argentipes of Indian kala-azar either in the initial stages, and or in maintenance stages, but also to define and explain the role of rodents in a synanthropic foci (in view of detection of leishmanial antibody in rodents) and the possibility of extra domiciliary transmission to explain the above observation.

Epidemiological information obtained will only enable to formulate cost effective control strategy for control of kala- azar in the region.

CHAPTER 7

REFERENCES

REFERENCE

1. Alam. M.N. Chowdhury, M.A.I. Rafiquddin, A.K.M., Hussain, A., Rahman, M.H, Siddiquy, A.B. Nazir, F.H. and Rahman, K.M., Kala-azar in Bangladesh. Bangladesh Journal of Medicine. 1(1):5-8(1990).

2. Ahmed. T.U. & Ahmed, R.U. An entomological investigation of kala-azar focus in Bangladesh. Journal of preventive and social medicine, Bangladesh. 2:31-36(1983).

3. Abdullah RE, Serodiagnosis of Visceral Leishmaniasis in an endemic area of the Sudan, Annais of Tropical Medicine and Parasitological 1980; 74(4), 415-419.

4. Ahmed T.U., Masum M.A., Ahmed R.U., Akhter S. (1989), Phlebotomine sandflies (Diptera:psychcdidae) of Bangladesh Journal of Zoology, 4:61-64.

5. Abdulla RE, Immunodiffusion, counterimmunoelectrophoresis and immunofluorescence in diagnosis of Sudan Mucosal Leishmaniasis. The American Journal of Tropical Medicine and Hygiene 1977; 26(6), 1135-1138.

6. Abdullah SH, Kasily FG, Weatherall DJ, The coombs direct antiglobulin test in Kenyans, Transactions of the Royal Society of Tropical Medicine and Hygiene 1983; 77(1), 99-102.

7. Aikat BK, Shegal, S. Mahjan RC, Pathania AGS, Bhattacherya PK, Shahaya S, Chowdhury AB, Pasricha N, Prasad LSN, The role of counter-immunoelectrophoresis as a diagnostic tool in kala-azar, Indian Journal of Medical REsearch 1979; 70, 592-597.

8. Aikat BK, Sahaya S, Pathania AGS, Bhattacharya PK, Desai N, Prasad LSN, Mishra S, Jain S, Clinical profile of cases of kala-azar in Bihar, Indian Journal of Medical Research 1979 (Oct); 563-570.

9. Anthony RL, Christensen HA, Johnson CM, Micro enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of World Leishmaniasis. American Journal of Tropical Medicine and Hygiene 1980; 29(2), 190-194.

10. Abdel-Hameed AA, Harith AE, Abdel-Rahim IM (1989); Potential of a direct agglutination test (DAT) for detection of visceral leishmaniasis in a known endemic area in Sudan.

11. Badaro R, Reed SG, Carvalho EM, Immunofluorescent antibody test in American Visceral Leishmaniasis: Sensitivity and specificity of different morphological forms of

two Leishmania species, American Journal of Tropical Medicine and Hygiene 1983; 32(3), 480-484.

12. Behforouz N, Rezai HR, Gettner S, Application of immunofluorescence to detection of antibody in Leishmaniasis infections, Annals of Tropical Medicine and Parasitology 1976; 70(3), 293-301.

13. Belding DL, Textbook of Parasitology 3rd edition, Meredith Publishing Company New York, USA; 1965; 202-229.

14. Bhatt KN, The evaluation and improvement of ELISA system and agglutination system for the diagnosis of leishmaniasis, Dissertation for the Master of Science degree of the London School of Hygiene and Tropical Medicine, University of London 1987.

15. Bray RS, Lainson R, The immunology and serology of Leishmaniasis; the fluorescent antibody staining technique, Transactions of the Royal Society of Tropical Medicine and Hygiene 1965; 59(5), 536-544.

16. Bray RS, Bryceson ADM, Cutaneous Leishmaniasis of the Guinea pig; Action of sensitised lymphocytes on infected macrophages, The Lancet 1968; ii, 898-899.

17. Brito TD, Hoshino-Shimizu S, Neto VA, Duarte IS, Penna DO, Glomerular involvement in human kala-azar, a light, immunofluorescent and electron microscopic study based on kidney biopsies, The American Journal of Tropical of Medicine and Hygiene 1975; 24(1), 9-18.

18. Broeckaert-Van Drshoven A, Micheilsen P, Vandepitte J, Fatal Leishmaniasis in renal-transplant patient, The Lancet 1979; ii, 740-741.

19. Brown R, Poxton IR, Wilkinson JF, Centrifuges, colorimeters and bacterial counts, In: Mackie and McCarthey Medical Microbiology vol-2, 13th edition, Collee JG, Duguid JP, Fraser AG, Marmion BP (Editors), Churchill Livingstone London UK 1989; 240-247.

20. Bryceson ADM, Immunological aspects of clinical Leishmaniasis, Proceedings of the Royal Society of Medicine 1970; 63, 1056-1060.

21. Bryceson ADM: Leishmaniasis in Weat theall D.J. Ledingham J.G.G. Warrd DA (Eds) Oxford Test Book of Medicine ELBS Oxford 2nd Ed. 1987; 5:530.

22. Cachia EA, Fenech FF, A review of kala-azar in Malta from 1947 to 1962, Transactions of the Royal Society of Tropical Medicine and Hygiene 1964; 58(3), 234-241.

23. Chandler AC, Reed CP, Introduction to Parasitology 10th edition, Wiley Eastern Private Limited New Delhi, India; 1970;11-130.

24. Chatterjee KD, Parasitology (Protozoology and Heminthology) in relation to clinical medicine, 12th edition, Chatterjee Medical Publishers Calcutta India 1982; 53-69 and 208-223.

25. Cheesbrough M, Principles of Immunity, Serological Diagnosis of Microbial Diseases, In: Medical Laboratory Manual for Tropical Countries, volume II, First Edition, Tropical Health Technology, Butterworths, England 1984; 70-99.

26. Cheesbrough M, Preparation of Reagents, In: Medical Laboratory Manual for Tropical Countries, Vol. II, First Edition, Tropical Health Technology, Butterworths England 1984; 432-449.

27. Cheesbrough M, Microscopy, In: Medical Laboratory Manual for Tropical Countries, vol.I, Second Edition, Tropical Health Technology. Butterworths England 1987; 67-85.

28. Chulay JD, Adoyo MA, Githure JI, Leishmania donovani parasitaemia in Kenyan Visceral leishmaniasis, Transactions of the Royal Society of Tropical Medicine and Hygiene 1985; 79, 218-222.

29. Chulay JD, Bryceson ADM, Quantitation of Amastigotes of Leishmania donovani in smears of splenic aspirates from patients with Visceral Leishmaniasis, American Journal of Tropical Medicine and Hygiene 1983; 32(3), 475-479.

30. Coons AH, Creech HJ, Jones RN, Immunological properties of an Antibody Containing a Fluorescent Group, Proceedings of the Society of Experimental Biology and Medicine 1941; 47, 200-202.

31. Coons AH, Kaplan MH, Localization of antigen in tissue cells, Journal of Exploratory Medicine 1950; 91, 1-13.

32. Cruickshank R, Duguid JP, Marmion BP, Swain RHA, Medical Microbiology: The practice of Medical Microbiology, Twelfth Edition, vol.2, Churchill Livingstone, Edinburgh London UK 1975; 96-150, 139-144, 494-509, 551-552.

33. Chandler AC, Read CP (1949). Introduction to parasitology. 10th edn. John Wiley & Sons Inc. New York, London PP 120- 129.

34. Chorine V. (1937). Les reactions serologiques dues aux euglobulines. Ann Inst Pasteur 58: 78-124.

35. Choudhry A, Guru PY, Saxena KC (1990) Enzyme-Linked immunosorbent assay in the diagnosis of kala-azar in Bhadohi (Varanasi) India. Trans R. Soc Trop Med Hyg 84: 363-366.

36. Chowdhury S, Haque F, Al Masum A, Harith AE, Karim E (1991). Positive response to sodium antimony gluconate administration in visceral leishmaniasis sero-positive patients. Am J Trop Med Hyg 44:390-393.

37. Chowdhury Mohd. Shahjada, Chemotherapy of Kala-azar of Primary Helath Centre in Bangladesh", Bangladesh Medical Journal, Vol. 19, No. 2, P. 51-55, April 1990.

38. Chowdhury, S. A. E. Harith, Al-Masum, A., Karim, E. and A. Rahman. 1993. Prevalence of agglutinating anti-Leishmania antibodies in two multi-thousand Bengoli communities. Parasitol. Res. 79, 444-450.

39. Chowdhury S., Masum A., Karim E., Semiao-Santos S., Rahman K.M., Ar-Rashid H. and Harith A. EL (1993).

Applicability of direct agglutination test (DAT) at a rural health setting in Bangladesh and feasibility of local antigen production.
IV EURO-LEISH Workshop, Tunis 15-21 May 1993

40. Chowdhury Md. Shahjada, Hoq M.S. Waqar A. Khan, Kala-azar in Children of Bangladesh, Dhaka Shishu Hospital Journal Vol.7, 1991.

41. Chowdhury Md. Shahjada, Masum AE, Karim E, Jahan Yasmin, Alam Rabiul; Micro ELISA for diagnosis of visceral Leishmaniasis in Bangladesh, Medicine to day July 1991 Vol. 3, No. 1, 45-47.

42. Chowdhury Md. Shahjada, Masum AE, et al "Post Kala-azar Dermal Leishmaniasis (PKDL) in Bangladesh, Journal of Preventive & Social Medicine, 1991.

43. De Korte PM, Harith AE, Dereure J, Huigen E, Faucherre V, Van der Kaay H.J. (1990). Introduction of an improved direct agglutination test for the detection of Leishmania infantum infection in Southern France. Parasitol Res 76: 526-530.

44. Dacie JV, Lewis SM, Practical Haematology 6th edition, Churchill Livingstone, Edinburgh London New York 1984; 118-123.

45. Dey NC, Dey TK, Medical Parasitology, 4th Edition, Messrs Allied Agency, 36 Dr. Sundari Mohan Avenue Calcutta India 1972: 42-57.

46. Dharmendra, Bose R, Complement fixation in Leprosy with antigens prepared from various acid-fast bacilli, Indian Journal of Medical Research 1941; 29(1), 7-15.

47. Douglas SD, Microscopy, In: Manual of Clinical Microbiology, 4th edition, Lennettee EH, Ballows A, Hausler Jr. WJ, Shadomy HJ, American Society for Microbiology, Washington D.C. 1985; 8-13.

48. Duxbury RE, Sadun EH, Fluorescent antibody test for the serodiagnosis of Visceral Leishmaniasis, American Journal of Tropical Medicine and Hygiene 1964; 13, 423-529.

49. Elias M, Mizanur Rahman AJM, Khan NI (1989); Visceral leishmaniasis and its control in Bangladesh. Bulletin of the World Health Organization. 67: 43-49.

50. El-Safi SH, Peters W, Evans DAT (1989); A comparison of the direct agglutination test and enzyme-linked immunosorbent assay in sero-diagnosis of leishrnaniasis in the Sudan. Trans R Soc Trop Med Hyg 83: 334-337.

51. Edrissian Gh.H, Darabian P, zovein Z, Seyedi-Rashti MA, Nadim A, Application of the indirect fluorescent antibody test in the serodiagnosis of cutaneous and visceral leishmaniasis in Iran, Annals of Tropical Medicine and Parasitology 1981; 75(1), 19-24.

52. Edrissian Gh.H. Darabian P, A comparison of enzyme-linked immunosorbent assay and indirect fluorescent antibody test in the sero-diagnosis of Cuteneous and Visceral Leishmaniasis in Iran, Transaction of the Royal Society of Tropical Medicine and Hygiene 1979; 73(3), 289-292.

53. Encyclopaedia Americana, International Edition, vol. 17, American Corporation, International Headquarters New York USA 1969; 246.

54. Encyclopaedia Britannica vol. 13, Jerez Dela (editor), William Bentom Chicago USA, London UK 1964; 894-897.

55. Engvall E, Perlmann P, Enzyme-linked Immunosorbent Assay (ELISA), III. Quantitation of Specific Antibodies by Enzyme-labled Anti-immunoglobulin in Antigen-coated Tubes, The Journal of Immunology 1972; 109(1); 129-135.

56. Fife EH, Muschel LH, Fluorescent-antibody Technic for Serodiagnosis of Trypanosoma cruzi infection, Proceedings of the Society for Experimental Biology and Medicine 1959; 101, 540-543.

57. Ghose AC, Haldar JP, Pal Sc, Mishra BP, Mishra KK, Serological investigation on Indian kala-azar, Clinical and Experimental Immunology 1980; 40, 318-326.

58. Greval SDS, Sengupta PC, Napier LE, Serological reactions in kala-azar: Complement-fixation false wassermann reaction, and high anti-complementary titre, Indian Journal of Medical Research 1939; 27(1), 181-189.

59. Harith AE, Kolk AHJ, Leeuwenburg J, Muigai R, Huigen E, Jeslma T, Kager P (1988). Improvement of a direct agglutination test for field studies of visceral leishmaniasis. J Clin Microbiol 26: 1321-1325.

60. Harith Ae, Kolk AHJ, Kager PA, Leeuwenburg J, Faber FJ, Muigal R, Kiugu S, Laarman JJ, Evaluation of a newly developed direct agglutination test (DAT) for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis: comparison with IFAT and ELISA, Transactions of the Royal Society of Tropical Medicine and Hygiene 1987; 81, 603-606.

61. Ho M, Leewenburg J, M Bugua G, Wamachi A, Voller A, An enzyme-linked immunosorbent assay (ELISA) for field diagnosis of visceral leishmaniasis, American Journal of Tropical Medicine and Hygiene 1983; 32(5), 943-946.

62. Hommel M, Peters W, Ranque J, Quilici M, Lanotte G, The micro-ELISA technique in the serodiagnosis of visceral leishmaniasis, Annals of Tropical Medicine and Parasitology 1978; 72(3), 213-218.

63. Hommel, M. Enzymoimmunoassay in leishmaniasis, Transactions of the Royal Society of Tropical Medicine and Hygiene 1976;15-16.

64. Hockymeyer WT, Wellde BT, Sabwa CL, Smith DH, Rees PH, Kager PA, A compliment fixation test for visceral leishmaniasis using homologous parasite antigen 1, Annals of Tropical Medicine and Parasitology 1984; 78(5), 489-493.

65. Hudson L, Hay FC, Practical Immunology, Second Edition, Blackwell Scientific Publications, Oxford, London UK, Boston USA, Victoria Australia, 1980; 142-155, 328-340.

66. Huldt G, Lagerquist, Phillips T, Draper CC, Voller A, Detection of antibodies in schistosomiasis by enzyme-linked immunosorbent assay (ELISA), Annals of Tropical Medicine and Parasitology 1975; 69(4), 483-488.

67. Ingram RL, Otken LB, Jr., Jumper JR, Staining of malaria parasite by the Fluorescent Antibody Technic, Proceedings of the Society for Experimental Biology and Medicine 1961; 106, 52-54.

68. Jahn A, Diesfeld HI. Evaluation of a visually read ELISA for serodiagnosis and sero-epidemiological studies of kala-azar in the Baringo District, Kenya, Transactions of the Royal Society of Tropical Medicine and Hygiene 1983; 77(4), 451-454.

69. Kafiluddin AKM, Director, Bangladesh Research Council, Mohakhali, Dhaka 1989; Personal communication.

70. Kagan IG, Serodiagnosis of parasitic diseases, In: Manual of Clinical Laboratory Immunology, 3rd Edition, Rose NR, Friedman H, Fahey JL (Editors), American Society of Microbiology, Washington DC 1986; 467-487.

71. Kawamura A, Jr. (Ed), Fluorescent antibody techniques and their application, 2nd edition, University of Tokyo Press, University Park Press 1977: 3-12.

72. Khaleque, K.A. 1965, Complete Fixation Test for kala-azar with an Antigen prepared from Acid Fast Bacillius PJMR. July 65. Pp 234-240.

73. Latif BMA, Al-Shenawi FA, Al-Alousi TI, The indirect fluorescent antibody test for diagnosis of kala-azar infection in Iraq, Annals of Tropical Medicine and Parasitology 1979; 73(1), 31-35.

74. Lioyd RB, Paul SN, Serum changes in kala-azar, Indian Journal of Medicine Research 1928; 16, 203-218.

75. Mahmoud AAF, Protoza, In: Immunological Diseases vol. 1, 3rd edition, Samter M (Editor), Talmage DW, Rose B, Austen KF, Vaughan JH (Section Editor), Little Brown and Company USA 1978; 738-751.

76. Manson-Bahr PEC (1961); Immunity in Kala-azar. Trans R Soc Trop Med Hyg 55: 550-555.

77. Markell EK, Marietta V, John D (1986); Medical Parasitology 6th edn. Saunders Company, Philadelphia, pp 121-130.

78. Manson-Bahr PEC, Bell DR, Manson's Tropical Diseases, 19th edition, Bailliere Tindall, London Toronto Sydney Tokyo 1987; 87-113 and 1318-1321.

79. Mansur KA, Alcoholic extracts or Kedrowsky's Bacillus as antigen for complement-fixation tests in kala-azar, Transactions of the Royal Society of Tropical Medicine and Hygiene 1956; 50(1), 91-96.

80. McLaren M, Draper CC, Roberts JM, Goebloed MG, Lighthart GS, Teesdald N.@sK.S Immunosorbent assay (ELISA) test for Schistosoma mansoni infections, Annals of Tropical Medicine and Parasitology 1978; 72(3), 243-253.

81. Mohammed EE, Wright EP, Kager PA, Laarman JJ, Pondan KW, ELISA using intact promastigotes for immunodiagnosis of kala-azar, Transactions of the Royal Society of Tropical Medicine and Hygiene 1985; 79, 344-350.

82. Musumeci S, Agata AD, Schiliro G, Fischer A, Studies of the neutrophenia in kala-azar: results in two patients, Transactions of the Royal Society of Tropical Medicine and Hygiene 1976; 70(5/6), 500-503.

83. Mansur, K.A. and Khaleque, K. A. 1957: Trans. Ray. Soc. Trop. Med. Hyg. 51,527.

84. Napier LE, A new serum test for kala-azar, Indian Medical Research 1922; 9, 830-846.

85. Neva FA, Leishmaniasis, In: Cecil Textbook of Medicine, vol.2, 18th edition, Wyngaarden JB, Smith Jr. LH (Editors), W.B. Saunders Company, Jovanovich Inc., Philadelphia 1988; 1969-1975.

86. Pampiglione S, Manson-Bahr PEC, Placa ML, Borgatti MA, Musumeci S, Studies in Meditteranean Leishmaniasis 3. The Leishmanin skin test in kala-azar, Transactions of the Royal Society of Tropical Medicine and Hygiene 1975; 69(1), 60-68.

87. Pampiglione S, Manson-Bahr PEC, Giungi, Giunti G, Parenti A, Trotti GC. Studies in Mediterranean Leishmaniasis 2. Asymptomatic cases of Visceral Leishmaniasis, Transactions of the Royal Society of Tropical Medicine and Hygiene 1974; 68(6), 447-453.

88. Phillips T, Draper CC, Detection of antibodies in Schistosomiasis by enzyme-linked immunosorbent assay (ELISA), Annals of Tropical Medicine and Parasitology 1975; 69(4), 483-488.

89. Rahman KM, Use of different saprophytic acid-fast bacilli in eomplement-fixation test for kala-azar, Bangladesh Medical Research Council Bulletin 1975; 1, 32-39.

90. Rahman KM, Islam N, Resurgence of Visceral Leishmaniasis in Bangladesh, Bulletin of the World Health Organization 1983; 61(1), 113-116.

91. Rassam MB, Al-Mudhaffar SA, Comparative diagnostic study of Bkala-azar, Annals of Tropical Medicine and Parasitology 1980; 74(3), 283-287.

92. Sengupta PC, Mukherjee AM (1968), Recurrence of kala-azar associated with post kala-azar dermal leishmaniasis. Journal of the Indian Medical Association 50: 1-7.

93. Smith ROA, Halder KC, Ahmed J (1941); Transmission of kala-azar, Indian Journal of medical Research 28: 575-591.

94. Swaminath CS, Shortt HE, Anderson LAP (1942); Transmission of Indian kala-azar to man by the bites of Phlebotomus argentipes.

95. Thakur C.P. Kumar. M. Kumar, P. Misra B.N. Pandy A.K. Treatment of PKDL with sodium stibogluconate British Med. J. 1987; 295: 886-87.

96. Thakur C.P. Kumar. M. Kumar, P. Misra B.N. and Pandey AK Rationalization of regimen of treatment of kala-azar with sodium stibogluconate in India, a randomized study. Brit Med Jou 1988; 296: 1557-1561.

97. Voller A, Bidwell DE, Bartlett A, Edwards R. A comparison of isotopic and enzyme-immunoassays for tropical diseases, Transactions of the Royal Society of Tropical Medicine and Hygiene 1977; 71(5), 431-437.

98. Voller A, Bartlett A, Bidwell De, Enzyme-immunoassays with special reference to ELISA techniques, Journal of Clinical Pathology 1978; 31, 507-520.

99. Voller A, Bidwell D, Enzyme-linked immunosorbent assay. In: Manual of Clinical Laboratory Immunology, 3rd Edition, Rose NR, Friedman H, Fahey JL (Editors), American Society of Microbiology, Washington DC 1986; 99-109.

100. Walton, BC, Brokks WH, Arjona J, Serodiagnosis of American Leishmaniasis by indirect fluorescent antibody test, The American Journal of Tropical Medicine and Hygiene 1972; 21(3), 296-299.

101. Weir DM, Young H, Immunological and serological methods in microbial infections, In: Mackie and McCartney Medical Microbiology vol 2 13th edition, Collee JG, Duguid JP, Fraser AG, Marmion BP (Editors), Churchill Livingstone London UK 1989; 182-203.

102. WHO 84, WHO Technical Report of WHO expert committee report series no.701, 1984: 01-140.

103. WHO 90, Report of WHO expert committee Tech. Report 1990; series 793 p-9.

104. Yang J, Kennedy MT, Evaluation of enzyme-linked immunosorbent assay for the serodiagnosis of Amebiasis, Journal of Clinical Microbiology 1979; 10(6), 778-785.

105. Yanjia - A Review of Kala-azar in China from 1949 to 1959 Trans. R. Soc. Trop. Med. Hyg. 1982; 76(4): 531-37.

106. Zijlstra E.E., M. Siddiq Ali, A.M. El-Hossain, Islam A. El. Toum, Maria Satti, H.W. Ghalib, Kala-azar in displaced people from Southern Sudan: Epidemiological clinical and therapeutic finding Trans Roy So Trop Med and Hyg. 1991; 85:365-369.

107. Zovein A, Edrissian Gh.H, Nadim A, Application of the indirect fluorescent antibody test in serodiagnosis of cutaneous leishmaniasis in experimentally infected mice and naturally infected Rhombomys opimus, Transactions of the Royal Society of Tropical Medicine and Hygiene 1984; 78, 73-77.

108. Zijlstra EE, Siddig MA, El-Hassan AM, El-Toum IA, Satti M, Ghalib HW, Kager PA (1991). Direct agglutination test for diagnosis and sero-epidemiological survey of kala-azar in the Sudan. Trans R Soc Trop Med HYg 85: 474-476.

Annexure 1

BANGLADESH AT A GLANCE

OFFICIAL NAME : The people's Rupublic of Bangladesh.

LOCATION :
Bangladesh which lies on the north part of South Asia between 20° 34' and 26°39' North latitude and 88fl00 & 92°41' East longitude.

AREA : 1,43,998 sq. km.

BOUNDARY :

The country is bounded by India on the west and north and Mayanma (Burma) on the east. The Bay of Bengal is on the south. Except for the hilly regions in the north east and south east, high land in the north and western parts, the country consists of low and fertile lands made up of alluvial soil. A network of rivers Padma, Brahmaputra, Meghna etc. run throughout the country, finally entering the Bay of Bengal.

	CITY		POPULATION
CAPITAL :	DHAKA	:	61,105,160 (1991)
OTHER CITY :	CHITTAGONG	:	2,040,663 (do)
	KHULNA	:	877,388 (do)
	RAJSHAHI	:	517,136 (do)

POPULATION : Total 108.0 million on 11th March, 1991 (Census)

0-14 years	-	46.7%
15-39 years	-	35.4%
40-64 years	-	14.5%
65 years	-	3.4%

STATE LANGUAGE : BANGLA

CLIMATE	:	The main seasons :		Winter (Nov. - Feb)
				Summer (March - June)
				Monsoon (July - Oct.)
TEMPERATURE	:	Maximum	:	34° C, Minimum : 8° C
RAINFALL	:	Higest	:	13", Lowest : 47"
HEALTH	:	Hospital	:	875 (including thana and RHC)
		Bed	:	33376
		Reg. Physicians : 19387.		

Annual Birth Rate :

Average per capita per day cal. intake			:	2215 k. cal. (1988-89)
Sea - Ports	:	Chittagong and Mongla		
Air-Ports	:	International	:	Dhaka and Chittagong
		Domestic	:	Sylhet, Saidpur, Rajshahi and Cox's Bazar.

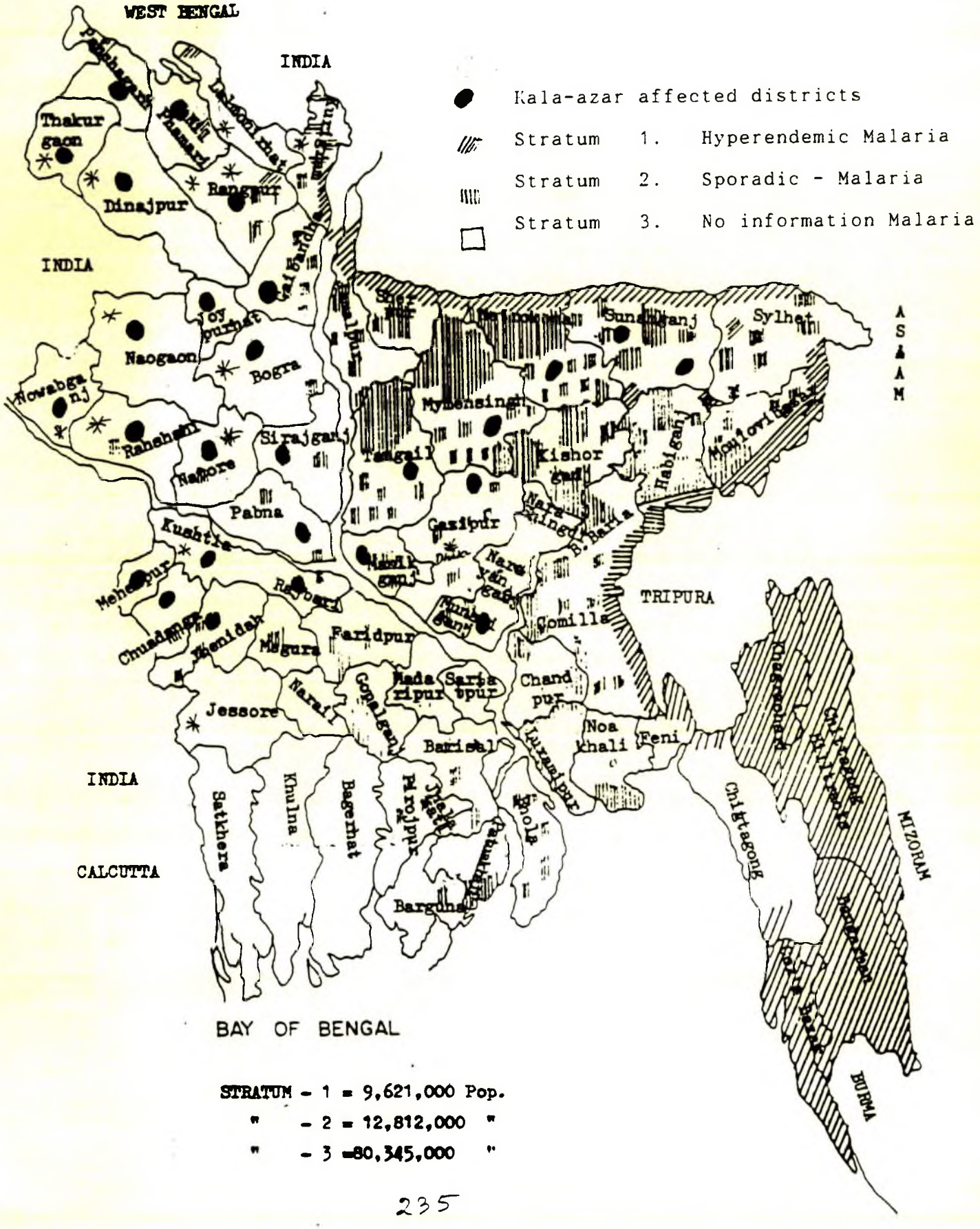
Various studies and observations made over the decades regarding the genesis of epidemics have shown various factors which facilitate epidemics;

An altitude of less than 600 meters above minimum sea level, heavy annual rainfall which exceeds 121 cms with mean humidity above 70% alluvial soil, mean diurinal temperature range of 11° c and abundant vegitation with sub-soil water and rural settings.

All these conditions prevailed in Bangladesh where the disease remained rapant. The number of cases and deaths due to visceral leishmaniasis in different districts of Bangladesh have been shown in Fig-

BANGLADESH

Annexure No 1



STRATUM - 1 = 9,621,000 Pop.
" - 2 = 12,812,000 "
" - 3 = 80,345,000 "

Annexure - No 3

A COLLABORATIVE RESEARCH (1991-95)

APPLICATION OF DAT IN MASS SCALE FOR DIAGNOSIS OF VL (KALA-AZAR) IN BANGLADESH

Department of Parasitology
Ntional Insititute of Preventive & Social Medicine (NIPSOM), Dhaka-1212, under Faculty of Post Graduate Medicine and Research, Dhaka University.

Date _____ Sample No. ☐☐☐☐☐

District : _____ ☐ Upazila : _____ ☐

Mouza/Village : _____ ☐ Household No. _____ ☐☐

1. Name :
2. Age : ☐☐
3. Sex : Male 1 ☐ Female 2 ☐
4. Occupation (Main) : Service 1 ☐ Business 2 ☐ Agriculture3 ☐ Labour 4 ☐ Student 5 ☐ H/W 6 ☐ Others 7 ☐
5. Monthly Income : Low 1 ☐ Med. 2 ☐ High 3 ☐
6. Marital Status : Married 1 ☐ Unmarried2 ☐ Divorce 3 ☐

CLINICAL EXAMINATION

7. Fever : Yes 1 ☐ No 2 ☐
8. Emaciation : Yes 1 ☐ No 2 ☐
   শীর্ণকায়
9. Anoroxia : Yes 1 ☐ No 2 ☐
   ক্ষুদামন্দা
10. Cough : Yes 1 ☐ No 2 ☐
11. Haemoptysis : Yes 1 ☐ No. 2 ☐
    রক্ত মিশ্রিত কফ
12. Oedema : Yes 1 ☐ No. 2 ☐
    শরীরে পানি

13. Anaemia : Yes 1 ☐ No 2 ☐
রক্ত শূন্যতা

14. Spleen (cm) : N P 1 ☐ 1—2 cm 2 ☐ > 2cm 3 ☐

15. Liver (cm) : N P 1 ☐ 1—2cm 2 ☐ >2cm 3 ☐

16. Ascitis : Yes 1 ☐ No 2 ☐
পেটে পানি

17. Skin condition : Normal 1 ☐ Dry 2 ☐ Rough 3 ☐
চর্মের অবস্থা Hypopigmentation 4 ☐ Erythomatous 5 ☐
চামড়ার রং পরিবর্তন
Nodular 6 ☐
গুটি

18. Hair : Normal 1 ☐ Brittle 2 ☐
স্বাভাবিক ভঙ্গুর

19. Tongue : Normal 1 ☐ Coated 2 ☐ Clean 3 ☐

20. History of Kala-azar : Yes 1 ☐ No 2 ☐
পূর্বে কালাজ্বর হইয়াছিল কিনা?
If yes
Treated 1 ☐ Not treated 2 ☐
If treated, How long ago
6 months 1 ☐ 1 year 2 ☐ >1 year 3 ☐

21. History of Malaria : Yes 1 ☐ No 2 ☐
ম্যালেরিয়ার ইতিহাস
If yes
Treated 1 ☐ Not treated 2 ☐

22. History of typhoid : Yes 1 ☐ No 2 ☐
টাইফয়েডের ইতিহাস
If Yes
Treated 1 ☐ Not treated 2 ☐

23. History of TB : Yes 1 ☐ No 2 ☐
যক্ষা রোগের ইতিহাস
If yes
Treated 1 ☐ Not treated 2 ☐

24. D A T titre : 1 : 1600 1 ☐ 1:3200 2 ☐ 1: 6400 3 ☐

25. Seropositivity of VL : Yes 1 ☐ No 2 ☐
or PKDL (≥ 1 : 1600)* :

N.B. In sampling X will be indicated for every family followed by subcode i.e X1 father, X2 mother and children according to age starting with the eldest.

* Individuals with titres ≥ 1: 1600 should be titrated to ≥ 1 : 102400

Signature of Interviewer
Date

Signature of Parasitologist/Doctor
Date

Annexure - No 4

APPLICATION OF DAT IN MASS SCALE FOR DIAGNOSIS OF KALA-AZAR IN BANGLADESH.

Department of Parasitology 788) BETWEEN ICDDR, DHAKA, BANGLADESH AND UNIVERSITY
Ntional Insititute of Preventive & Social Medicine (NIPSOM),Dhaka-1212,
under Faculty of Post Graduate Medicine and Research, Dhaka University.

Questionnaire Form 2 : Monthly Questionnaire and Re-screening.

Date of interview: ___/___/___/ ☐☐☐☐☐☐

01. Interviewer ID : _____ ☐

02. Object's ID : _____ ☐☐☐☐☐

PART-A

07. What is your status in the house. ☐
    1= Owner, 2= Owner's relative
    3= Working hand or their relatives

08. What type of house(by observation)? ☐
    1= Thatched 2= Mud+ straw
    3= Tin shed 4= Building

09. How many family members live in this house? ☐☐
    Number :- _____

10. Is there a cow-shed, if so ☐
    1= No,
    2= attached with the dwelling house
    3= Cow living inside the living room

11. Do you use bed nets in the house? ☐
    1= Nobody in the house
    2= Some person(s) use
    3= All persons use

12. Where do you sleep? ☐
    1= Floor
    2= Cot

13. Do you sleep ☐
    1= In the room or
    2= Outside

14. What time do you go to bed? ☐
    1= Before Esha prayer/azan
    2= After Esha prayer/azan

15. Are you staying here for ☐
    1= permanently (If 1 move to 13)
    2= Temporarily but I do not know the date
    3= Temporarily but know the date last visit here

Case definition

Malaria:- High fever, Intermittent type of fever with chills, anaemia, sweating confirmed by Microscopy - MP present.

Kala-azar:- Fever continuous, low grade temperature or double rise temp. Within 24 hours. Splenomegaly, confirmed by demonstration of L.D body or seropositive.

16. Did you have any experience like this in your life?
1= No, 2= Yes Malaria, 3=Yes Kala-azar,
4= Yes Malaria+Kala-azar(If 1 move to Q.16) ☐

17. How long was the duration of your last attack?
1= Do not know, 2= 7 days, 3= 1-4 week
4= 1-3 months, 5= 4-12 months,6= >12 months ☐

18. During the past illness did you have any medication
1 = No, 2= yes (If 1 move to 16) ☐

18.1 Analgesic	1=No, 2=Yes	☐
18.2 Anti malarial responded	1=No, 2=Yes	☐
18.3 Anti malarial not responded	1=No, 2=Yes	☐
18.4 Anti tuberculars- not responded	1=No, 2=Yes	☐
18.5 Anti-tubercular responded	1=No, 2=Yes	☐
18.6 Antibiotics- not responded	1=No, 2=Yes	☐
18.7 Antibiotics- responded	1=No, 2=Yes	☐
18.8 Anti Kala-azar 10- 14 days	1=No, 2=Yes	☐
18.9 Anti Kala-azar 20 days - responded	1=No, 2=Yes	☐
18.10 Anti Kala-azar(21-30 days responded	1=No, 2=Yes	☐
18.11 Anti Kala-azar not responded	1=No, 2=Yes	☐

19. At the time of mass screening did you have fever
1= No, 2= yes ☐

Annex. 4

Questionnaire Form 2 : Monthly Questionnaire and Re-screening.

Date of interview: ___/___/___/ ☐☐☐☐☐☐

01. Interviewer ID : _____ ☐

02. Object's ID : _____ ☐☐☐☐☐☐

PART-B

20. At the present time are you sick and have the fever ☐
 1 = No, (If no move to 21)
 2 = Yes, I am sick but do not know I have fever or not
 3 = Yes, I am sick and have the fever

21. What type of fever do you have? ☐

 21.1 Period
 1 = Intermittent fever with double rise
 2 = Continuous fever
 3 = Intermittent fever

 21.2 Type
 1 = With chills ☐
 2 = With rigors
 3 = With out chills and rigor

22. Date of onset : ☐
 1= Don't know
 2= Know - date / / . (If 2 move to 20) ☐☐☐☐☐☐

23. Duration of the fever ☐
 1 = 1 - 2 weeks, 2 = 3 - 4 weeks
 3 = 1 - 2 months 4 = 3 - 6 months
 5 = 6 - 12 months 6 = 1 - 2 years
 7 = 3 - 5 years.

24. Did you visit a doctor and you know the diagnosis? ☐
 1 = No or Don't know 2 = Malaria,
 3 = Kala-azar, 4 = Other

25. How many family members in your family have been suffering from kala-azar. ☐☐
 Number =_____ . (If unknown then 99)

26. Emaciation 1=No, 2=Yes ☐

27. Cough 1=No, 2=Yes ☐

28. Haemoptysis 1=No, 2=Yes ☐

29. Other Bleeding tendency 1=No, 2=Yes ☐

30. Oedema 1=No, 2=Yes ☐

31. Anaemia 1=No, 2=Yes ☐

32. Jaundice 1=No, 2=Yes ☐

33. Pulse per min. _____ ☐☐

34. Spleen by palpation: Spleen measured from the costal margin at left axillary line to tip. ☐
1 = Not palpable, 2 = Just palpable,
3 = 1 - 2, 4 = 3 -5 5 = Above (in cm.)

35. Liver by palpation: Measured from the costal margin at right mid clavicular line to edge. ☐
1 = Not palpable, 2 = Just palpable
3 = 1 - 2 , 4 = 3 - 5, 5 = Above (in cm.)

36. Ascites 1 = No 2 = Yes ☐

37. Tongue, condition:- ☐
1 = Moist and clean, 2 = Dry & coated

38. Condition of hair: ☐
1 = Normal, 2 = Brittle

39. Skin condition: ☐
1 = Normal (If 1 move to 38)
2 = Hypopigmentation 3 = Erythematous
4 = Nodular(Single) 5 = Nodular(Multiple)
6 = Nodular+ Ulcerated 7 = Rough
8 = Nodular+ Hypopigmentation

40. Skin sensation: ☐
1 = Absent, 2 = Present

41. Site of the pigmentation in the body, ☐
1= Nose, 2= Trunk,3= Upper extremities,4= Whole body
5= Lower extremities,6= Nose+Trunk,7= Trunk+Upper ext.

42. Do you have loose motion? ☐
1 = No (if 1 move to question 41) 2= Yes

43. How many time you have loose motion in a day? ☐
1 = One time, 2 = two time
3 = three times, 4 = more than three/day

44. At present time do you have any medication? ☐
1 = No, 2 = Antibiotic
3 = Analgesic drug, 4 = Antimalarial drug
5 = Anti Kala-azar, 6 = Other drug (show)

PART-C

Laboratory observation:

45. Demonstration of L.D body ☐
1 = absent
2 = Present Microscopic in bone marrow
3 = present microscopic in splenic aspiration
4 = present microscopic in skin scrapping

46. LD body in Culture: ☐
1 = Absent 2 = Present

Haematology:

47. Haemoglobin in g/100 ml,
1 = <4, 2 = 4 - 7.9, 3 = 8 - 12, 4 = >12 ☐

48. Total leukocyte count/cu.mm of blood
1 = <2500, 2 = 2501 - 3000, 3 = 3001 - 4000,
4 = 4001 - 5000, 5 = 5001 - 7000,
6 = 7001 - 10,000 7 = >10,000 ☐

49. Differential count
49.1 P = _____ % ☐☐
49.2 L = _____ % ☐☐
49.3 E = _____ % ☐☐
49.4 M = _____ % ☐☐
49.5 B = _____ % ☐☐

50. Blood slide for MP 1 = Absent, 2= Present ☐

51. H. bacilli 1 = Absent, 2 = Present ☐

52. W. test 1 = Negative, 2= Positive ☐

53. Sputum for AFB 1 = Negative, 2= Positive ☐

54. Hbs Ag 1 = Negative, 2= Positive ☐

55. Urine- Albumin 1= Absent, 2= Present ☐
Serology:

56. AT 1= Negative, 2= Positive ☐

57. DAT ☐☐
1 = 1: 100, 2 = 1:200, 3 = 1:400, 4 = 1:800,
5 = 1:1600, 6 = 1:3200, 7 = 1:6400, 8 = 1:12800,
9 = 1:25600, 10 = 1:51200, 11 = 1:102400,
12 = >1:102400

58. IFAT ☐
1 =1:4, 2 =1:8, 3 =1:16, 4=1:32,
5 =1:64, 6=1:128, 7 =1:256

59. ELISA - direct absorbency Value : _____ ☐☐

60. The measurement of body temperature/minute exact ☐☐☐☐☐
_____ °F.

61. Weight in Kg. _____ ☐☐

62. Height in cm. ☐☐☐

Signature
Date
Name
Designation

Signature
Date
Name
Designation

Annexure - No 5

EARLY DIAGNOSIS OF KALA-AZAR (VISCERAL LEISHMANIASIS). DETECTION OF SUB-CLINICAL INFECTION AND FOLLOW-UP OF ANTIBODY TITRE OF TREATED KALA-AZAR CASES USING DIRECT AGGLUTINATION TEST (DAT).

Department of Parasitology
Ntional Insititute of Preventive & Social Medicine (NIPSOM),Dhaka-1212 under Faculty of Post Graduate Medicine and Research, Dhaka University.

District _____ **Thana** _____ **Date** _____

Village _____ **House No.** |_|_|_|

1. **Name** _____ 2. **Age** |_|_|
3. **Sex:** |_|
Male - 1,
Female- 2

S/O, D/O, C/O _____

4. **Occupation :** Service-1, Business-2, Cultivation-3, Day Labour-4, House wife-5, Student-6. Other. |_|

5. **Marital status :** Married-1, Unmarried-2, Divorced-3 |_|

6. **Economic status:** Rich-1, Upper middle class-2, Lower middle class-3, Poor-4, Very poor-5. |_|

7. **Are you suffering from fever ?** Yes-1, No-2 |_|
If yes, How long : 1 . . . . . 52 Weeks.

8. **Spleen:** Not palpable-1, Palpable-2 |_|
If palpable then how far it extends from costal margin (in finger/cm./gradition) |_|_|

9. **Liver :** Not palpable-1, Palpable-2 |_|
If palpable then how far it extends from costal margin (in finger/cm.) |_|_|

10. **Anaemia :** Not anaemic-1, Mild-2, Moderate-3, Severe-4 |_|

11. **Tongue:** Normal-1, Coated-2, Clean & moist-3 |_|

12. **Hair :** Sparse-1, Brownish-2, Normal (black)-3 |_|

13. Skin : Normal-1, Hypopigmented-2, |_|_|_|_|_|
Erythematous-3,
Raised from normal skin surface-4,
Nodular-5.

14. Did you suffer from Kala-azar ? Yes-1, No-2 |_|

If yes, how long ago : 1 . . .b. 100 (in months) |_|_|_|

15. Who made the diagnosis of Kala-azar? |_|

THC-1, Survey-2, Health Worker-3, General practitioner-4
Other-5.

16. Did you take anti-Kala-azar treatment : Yes-1, No.2 |_|

If yes, Where : Village-1, THC-2, Dist. Hospital-3 |_|
Medical College Hospital-4.

|_| How long: <10, 10, 20, 30 days |_|_|

(If he/she is able to recapitulate the daily dose of SAG then write the amount in cc/ml)

17. Other complaints if any.

18. Blood examination :

Hb %

DAT results : (after every 1 or 2 months interval)	1	5	9
	2	6	10
	3	7	11
	4	8	12

N.B: In case of kala-azar patients, blood samples are to be collected once in two months and from healthy persons samples are to be collected once in a month for one year.

Remarks :

Sign. of the Interviewer: Sign. of the Medical Officer

FOLLOW-UP & OBSERVATION SHEET

Annexure No 6

(GROUP _____)

IEDCR, Mohakhali, Dhak

Department of Parasitology
Ntional Insititute of Preventive
& Social Medicine (NIPSOM), Dhaka-1212,
under Faculty of Post Graduate Medicine
and Research, Dhaka University.

NAME OF HEALTH FACILITY _____

SL. No.	Sample No.	Name & address with House No	Age	Sex	Date test	DAte treat.	Follow-up & observation			
							1st date	2nd date	3rd date	4th date
						Temp				
						Wt.				
						Spleen				
						Liver				
						Hb%				
						TC.				
						A.T.				
						DAT				
						ELISA				
						IFAT.				
						OTHER				

Name of the patient :

S/o, D/o, W/o, C/o :

Villoge :

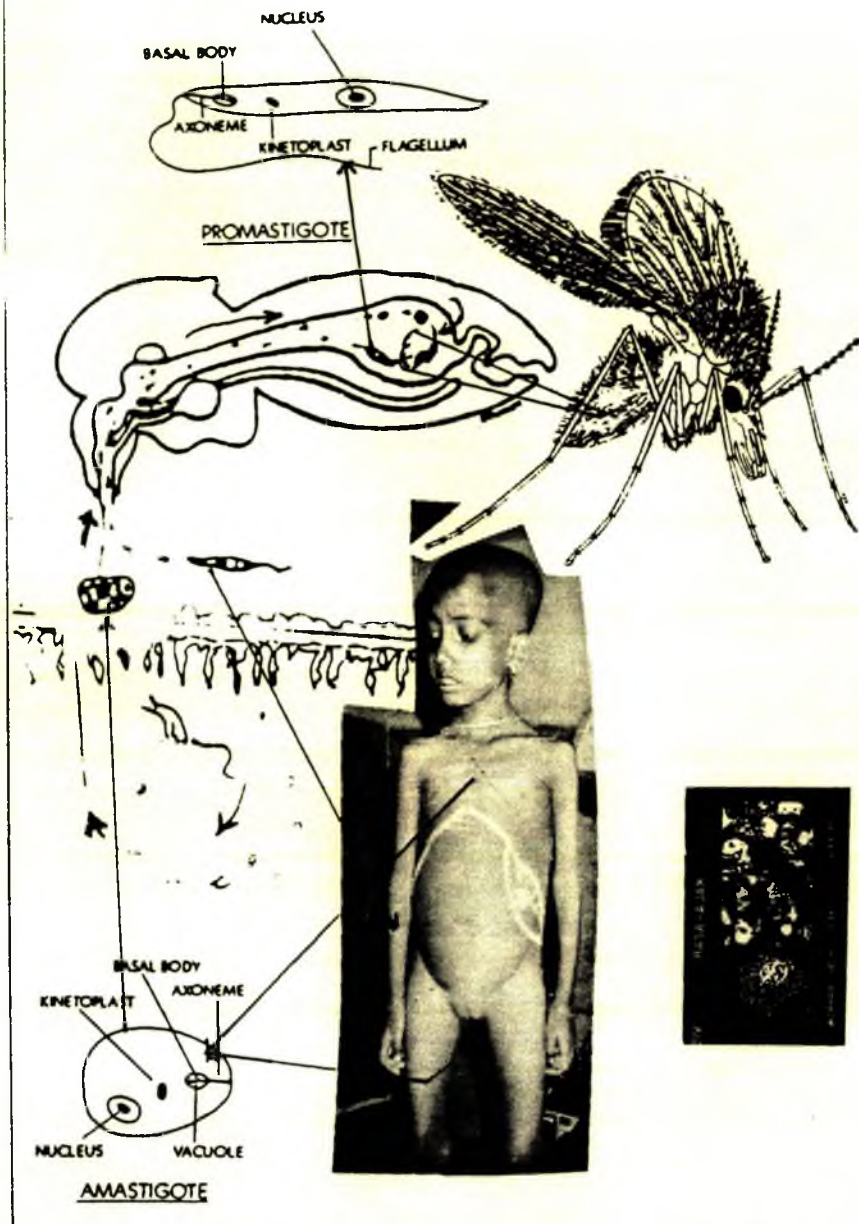
Post office :

House No. :

REMARK :

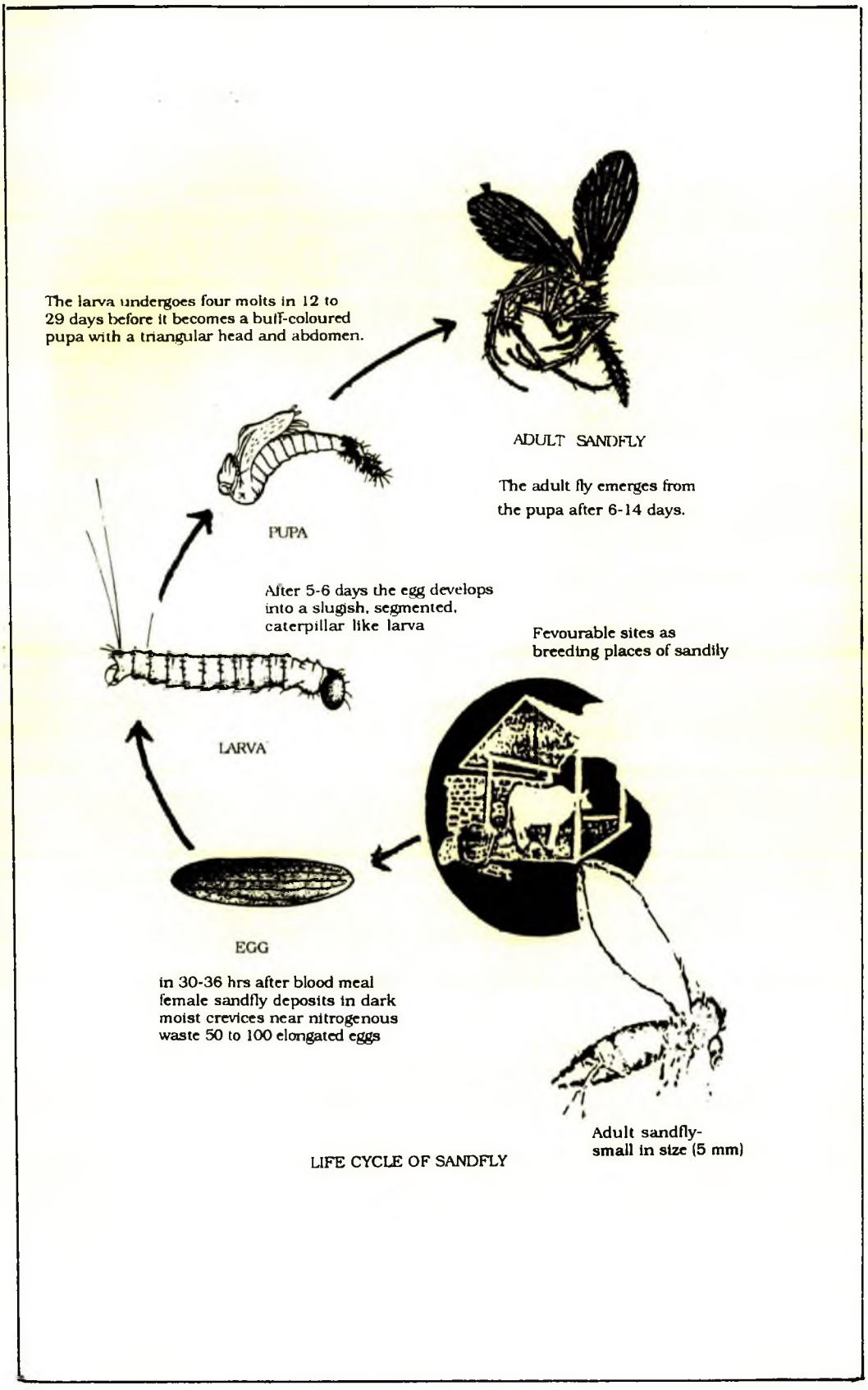
Reverse

Annexure No - 7



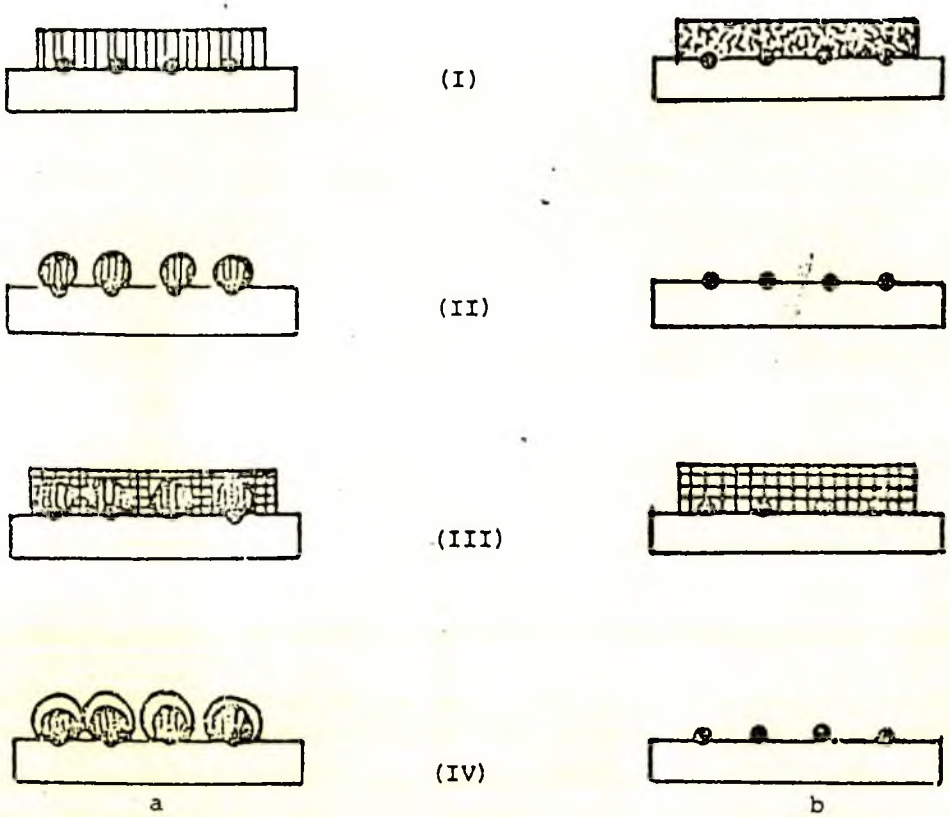
LIFE CYCLE OF PARASITE

Annexure No. 8



LIFE CYCLE OF SANDFLY

Figure . Principle of the indirect fluorescent antibody test (IFAT)



(I) Parallel preparations a and b which contain antigen (black discs) are treated respectively with specific antiserum (hatched) and non immune serum (stippled).

(II) The immune serum combines with the antigen in a while the non immune serum is removed from b by washing.

(III) The fluorescent labelled specific antiserum (chequered) is applied.

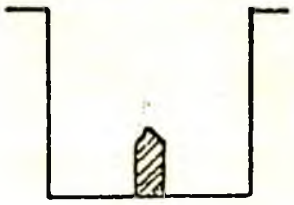
(IV) The fluorescent antiglobulin has combined with the antibody attached to the antigen in a and is washed away from b.

(Reproduced from Nairn, R.C., 1962 ch 6 in Fluorescent Protein Tracing)

Figure . The indirect ELISA for measuring antibody

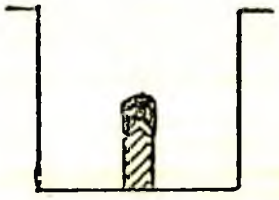
1. ANTIGEN ADSORBED TO PLATE

WASH



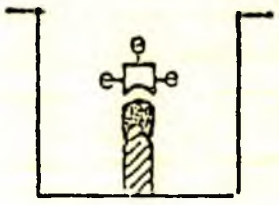
2. ADD SERUM ANY SPECIFIC ANTIBODY ATTACHED TO ANTIGEN

WASH

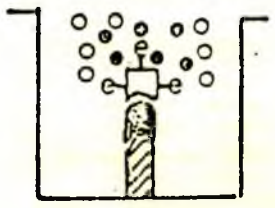


3. ADD ENZYME LABELLED ANTI-GLOBULIN WHICH ATTACHED TO ANTIBODY

WASH



4. ADD SUBSTRATE



AMOUNT HYDROLYSED = AMOUNT OF ANTIBODY PRESENT

(Reproduced from Voller et al., 1976, Bull. W.H.O., 53, 55-65)

Diethanolamine Buffer pH 9.8

97 ml diethanolamine

800 ml distilled water Adjust to pH 9.8 with Im HCl

0.5mM $MgCl_2$ - 101 mg $MgCl_2.6H_2O$

0.2 g NaN_3

Phosphatase Substrate

Add one 5 mg tablet p-nitrophenyl phosphate (Sigma) tO 5.0 ml diethanolamine buffer.

Peroxidase Substrate (Stock)

100 mg orthophenylene diamine

10 ml absolute methanol

Mix thoroughly

Store the sample in the dark at $4^{\circ}C$.

Stable for 1-2 weeks.

Peroxidase Substrate (working solution)

50 ml distilled water

24.7 ml 0.1M citric acid

25.3 ml 0.2M Na_2HPO_4

1.0 ml stock OPD

0.05 ml 6% H_2O_2

Substrate Inhibitors

3M NaOH for phosphatase 8N H_2SO_4 for peroxidase

(Omit NaN_3 when using peroxidase)

Annexure - No 12

IFA test ..... Date .....

Ag ..... of .....De Hb ..... Fix .....

Conjugate .....Dil ..... E.L.....

Inc.sera .....Wash ..... Inc.conj.....Wash.....

Acetone ..... Serum dils.....

Serum	Dil.	Slide	Result	Serum	Dil.	Slide	Result
		1				20	
		2				21	
		3				22	
		4				23	
		5				24	
		6				25	
		7				26	
		8				27	
		9				28	
		10				29	
		11				30	
		12				31	
		13				32	
		14				33	
		15				34	
		16				35	
		17				36	
		18				37	
		19				38	