APPENDIX-I
View Letter

Date: 13 Dec 2013
To: "DrNarendraSrivastava"
    assoprofzoolbnmu@gmail.com,nssidhidatri1@yahoo.co.in
From: "PavitraTandon (SCLE)" pavitra.nasi@gmail.com
Subject: Your Submission SCLE-D-13-00039R3

Dear NARENDRA,

We are pleased to inform you that your manuscript, "SURVEY REPORT OF VISCERAL LEISHMANIASIS BASED ON SERODIAGNOSTIC TEST IN MADHEPURA DISTRICT OF BIHAR, INDIA", has been accepted for publication in National Academy Science Letters.

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With best regards,

Suresh Chandra, Ph.D.
Editor in Chief
# National Academy Science Letters

**SURVEY REPORT OF VISCERAL LEISHMANIASIS BASED ON SERODIAGNOSTIC TEST IN MADHEPURA DISTRICT OF BIHAR, INDIA**

---Manuscript Draft---

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<td>First Author:</td>
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<td>Order of Authors:</td>
<td>Dr Narendra Srivastava, Ph.D, FZSEI, CMLT, Brajesh Kumar SINGH, M.Sc (RESEARCH SCHOLAR) ARCHANA YADAV, M.Sc (RESEARCH SCHOLAR)</td>
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SURVEY REPORT OF VISCERAL LEISHMANIASIS BASED ON SERODIAGNOSTIC TEST
IN MADHEPURA DISTRICT OF BIHAR, INDIA
NARENDRA SRIVASTAVA, BRAJESH KUMAR SINGH1 AND ARCHANA YADAV1
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ABSTRACT
The paper is based on a population survey conducted for the analysis of epidemiological condition of VL from January 2010 to December 2011. The annual incidence (per 10000) was observed 8.98 for 2010 and 7.71 for 2011. The annual prevalence of disease was 26.92 in 2010 and 23.78 in 2011. The seasonal variation of incidence and its relationship with eco-climatic factors have discussed. The %incidence and temperature represented weak positive correlation with Pearson’s co-efficient value +0.269 (SER = 0.1892) and linear relationship by regression equation y=0.116 X + 4.895; whereas %incidence and humidity represented moderate negative correlation with Pearson’s coefficient value – 0.649 (SER = 0.1181) and linear relationship by regression equation y = – 0.154 X
+ 20.40. The retrospective analysis among epidemiological parameters, eco-climatic factors and vector population dynamics are discussed in present paper. It represents internal incubation period of leishmania is 6-8 month in the region under study.

**KEY WORDS**– Incidence, Prevalence, Eco-climatic factors, Visceral Leishmaniasis, Madhepura (Bihar).

**INTRODUCTION**

Bihar has experienced re-emergence of Visceral Leishmaniasis (VL) since 1990. The disease has been representing cyclic appearance particularly in Bihar and Assam since 1930’s (1,2). The 90% of the total global burden of disease has incorporated by India, Nepal, Bangladesh and Sudan (3). The disease is now endemic in 88 countries with a total 350 million people at risk (4). The earlier commitment of Government of India to eliminate Kala-azar by the year, 2010 (5) is a documented record only and yet to be fulfilled. In 2005, the Government of India, Bangladesh and Nepal agreed to participate in a regional VL elimination program to reduce the incidence of VL to one case per 10,000 populations by 2015. It has been reported that 96 contiguous districts of these three countries are affected by VL. In India, worst effected state is Bihar where 33 districts are endemic and contributed about 80% disease burden in country.
The disease is caused by an obligate intra-macrophage digenic parasite *Leishmania donovani* and only potential vector identified in India is *Phlebotomus argentipes*. The effect of environmental factors on the growth and population dynamics of *Ph. argentipes* has been reported by a number of workers (6,7,8,9). It has been suggested that poor socio-economic conditions of population, less accessibility to medical centers and poor surveillance of disease at population level are major obstacle in the control of disease. Some population based works suggested serious under reporting of patients by Government agencies (10,11). The poor surveillance has been also reported from Bihar (12).

**MATERIAL AND METHODS**

**RESEARCH DESIGN**

The study has based on the two issues; first to determine the actual disease burden of VL in district and second the general population risk by analyzing impact of econometric and environmental factors in incidence of VL. The actual disease burden was determined by both primary and secondary data. The primary data was based on the rK39 test outcomes whereas secondary data was based on records of local private and government hospitals. A team was constituted and trained for the purpose of collection of blood and econometric information through questionnaire. Some members were trained employees of PHC and voluntarily provided help in the present study. The blood was collected
after initial verification by a clinical or para-clinical trained staff to a person represented irregular fever, loss of appetite and weakness from panchayats (local administrative unit) of any block. The outcomes of survey were used for the analytical study of epidemiological conditions of VL in district and role of environmental and socioeconomic factors in control measure program. In present paper actual disease burden and impact of environmental factors on incidence of VL are considered.

**STUDY AREA**

The district is situated at longitude between 25034’ to 26007’ and latitude between 86019’ to 87007’ in North-Eastern Bihar. It surrounds 1787 Km2 area with total population of 1904140 as per record of District Health Department (2010 – 2011). It is divided into 13 administrative blocks, 170 panchayats and 449 villages. All blocks were included in the study.

**EPIDEMIOLOGICAL DATA**

In each month suspected persons were screened for the test on the basis of preliminary symptoms such as mild and irregular fever from one/two months, loss of appetite, weakness, weight loss and hepatomegaly. The blood was collected in blood collection tube and serum was separated and stored at – 20oC till further use. A continuous survey of population was carried for two years, from January 2010 to December 2011. The total persons included in study were 1843 in 2010 and 1599 in 2011. The number varied from 22 to 595 in each block per year. The screening of
population was fundamentally based on rK 39 dipstick test, provided by “In Bios International”, Inc Seattle, Washington, DC, USA. The total rK 39 positive patients recorded were 1706 in 2010 and 1470 in 2011 (Table-1). The sensitivity of the test is reported as 98 – 100 %, although 15 % of healthy population of endemic areas may have a positive test (13). The person represented positive test was advised for further clinical confirmation of disease and treatment.

During survey period four PKDL cases were also recorded (secondary data). The reports were analyzed statistically for the derivation of inferences. Both incidence and prevalence were calculated per 10000 individuals of defined area. The epidemiological terms were used as defined and established by standard text (4,14). The statistical analysis of observed data was based on standard book (15). The calculations were carried in MS – Excel 2010 program of Microsoft.

**CLIMATIC DATA**

The humidity and temperature were recorded from effected area of blocks with the help of Coconut Development Board, a research unit of ICAR, New Delhi. The mean temperature and humidity of each month were used for the statistical analysis.
RESULT
The annual prevalence and incidence of VL were calculated 26.92 and 8.98 respectively for 2010 whereas these values were marginally decreased in 2011 as 23.78 for prevalence and 7.71 for incidence. The VL cases were recorded throughout the years represented month-wise variation (Table–2). In 2010, the maximum and minimum values of incidence were observed in April (1.11) and October (0.41) respectively. In 2011, the maximum value of incidence was observed in April (0.82) whereas minimum value of incidence was observed in December (0.33).
The prevalence rate in 2010 is maximum in June (2.89) and minimum in October (1.5), whereas in 2011 the value is maximum in May (2.37) and minimum in December (1.3). Seasonally, the incidence and prevalence were maximum in pre-monsoon and minimum in post-monsoon.
The two climatic factors namely humidity and temperature were recorded for both 2010 and 2011 for the retrospective analysis. The monthwise fluctuations of both these climatic factors are presented in Table -3 (represents mean value). The temperature of area varies from 7oC to 37oC (mean month temperature 19.22 – 36.7 0C). The highest temperature was observed in April-May and lowest in December-January. The humidity varies from 54.13 to 91.13 (RH %). The maximum humidity was observed in August-September and minimum in the month of April-May.
The results for statistical interrelationship between fluctuation in % incidence of VL and climatic factors were calculated as follows –

Temperature Vs % Incidence
Humidity Vs % Incidence

1. Pearson coefficient \( r = + 0.2695 \) \( r = - 0.6491 \)
2. Standard Error (SEr) = 0.1892 (SEr) = 0.1181
3. Regression line \( y = 0.116 X + 4.895 \) \( y = - 0.154 X + 20.40 \)
4. \( t \) – significance value \( | t | = 1.3128 \) \( | t | = 4.0026 \)

(non-significant at 0.05; df =22) (Significant at 0.05; df = 22)

DISCUSSION

The findings suggest that Madhepura is endemic zone for Kala-azar (14) and a ten folds reduction is necessary to achieve the goal as determined the Government of India by 2015. Simultaneously the present work indicates that the official notification of Government of Bihar to eliminate the diseases by 2010 is not realistic rather far away from the realization. The observed values are even high than the available Government record of annual incidence in epidemic years 1991-92 (6.9 / 10,000 persons) and 1996 (3.68 / 10,000 persons) (12). The disease is prevalent throughout the year with maximum number of patient between March - August. The similar nature with minor variation has been reported by other workers with high degree of incidence and prevalence from May to October (16). The seasonal variation of prevalence and incidence represents the respective peak of disease in summer season, which starts downward trend in rainy season and comes at lowest level in winter season (Graph –
Likely a quarterly analysis from Bangladesh showed a trend for more cases from July to September and fewer cases from January to March. The variation in present study may be observed due to variation in ecoclimatic factors (17). The ecoclimatic factors actually provide fluctuation in population dynamics of vector sand flies and eventually to the appearance of disease after a lag of internal incubation period of parasite *L. donovani*. The available literatures on longitudinal studies carried out on the seasonal prevalence of *Ph. argentipes* in the Kala-azar endemic states of India revealed that sand fly density was found its highest peak during the post monsoon months and a gradual decline in sand fly population was observed from November onwards and reached at its lowest ebb during extreme winter months (16,18,19).

The mean humidity above 70 % (80-85 RH %), a temperature range of 15°C -38°C (24 – 26°C), subsoil water (present in Kosi river catchment zone), alluvial soil and abundant vegetation are favorable condition for growth of parasite – vector and transmission of Kala-azar (7,9,20). The geographical distribution and development of insect vectors are strongly related with climatic factors like humidity and temperature. The retrospective analysis represents that the % incidence of diseases is inversely related with the humidity (Graph -2). In the present study, the degree of correlation between humidity and % incidence of VL is moderately negative (r = - 0.65, SEr = 0.12, t –test = significant at 0.05 level). The linear relationship is represented by regression equation y = -
0.154X + 20.40 with reverse slope in graph (Graph -3). The degree of correlation between temperature and % incidence is weak positive (r = +0.27, SEr = 0.19, t – test = non-significant at 0.05 level) (Graph-4) and linear relationship is represented by regression equation \( y = 0.116X + 4.89 \) (Graph- 5). It has been reported that leishmaniasis is associated with drier conditions whereas sand fly vectors need high humidity and moderate temperature (21). The humidity and damp surface are favorable for the eggs and larvae of fly (22). The temperature has direct correlation with the insect metabolic rate, egg production, pre-imaginal stages, adult longevity and frequency of blood feeding as well it accelerates both growth of vector and multiplication and migration of promastigotes; hence; the probability for the transmission of disease is also increased (23).

The careful observation represents that the environmental factors suitable for the growth of vector (Aug-Sep) is lagged each year by 6-8 months from the maximum value of incidence rate (March-April). The season is cyclic in India. The observed climatic characteristic of the area and available literature suggests that the temperature (28-300C) and humidity (80-85 RH %) of August are most suitable for the highest population of \( Ph. argentipes \) (21,24). The higher sand fly density provides higher probability of successful transmission of subject to an infective host and position of eco-environmental conditions (25). The similar findings have been reported from Nepal and Haryana.
(26). It has been analyzed that the relationship between environmental factors and incidence of disease is existing due to internal incubation period of leishmania by 6-8 months and cyclic season. The workers have no common view on the intrinsic incubation period and reported differently from time to time (27,28).

**CONCLUSION**

The area is endemic zone for VL. It is very hard to achieve National goal of VL incidence rate 1/10000 by 2015 without considering the scientific methods for the control measure at grass-root level.

**RECOMMENDATION AND SUGGESTIONS**

The Kala-azar endemic or epidemic zones are varied from one to another area in the microeco-climatic condition; hence a scientific planning is necessary to achieve the state or national goal. Actually the maximum incidence rate of disease is predetermined by the earlier activity of sand flies. So a study on vector population dynamics is suggested for formulation of effective control measure in each zone. The problem is further aggravated by the development of DDT resisted vector population in some districts of Bihar, earlier included as endemic area for leishmanial infection like Samastipur, Bhakhtiyarpur, Vaisali, Sitamarhi etc. The resistance has developed due to reluctant use of insecticide.

The best methods for the control of VL at population level is to reduce man-vector contact either by reducing or eliminating the population of
vector or preventing men to come in contact with vector. In both of these processes, former provides actual and long term control. The present epidemiological study suggests that the “Two time spraying” method is necessary for the control. First spraying is recommended in the month of September-October to decrease the population of vector in post monsoon season and eventually the contact between Man and vectors.

The second spraying is recommended in the month of February-March, when increase of environmental temperature makes active to vectors. However for the final formulation of planning it is necessary to do entomological and epidemiological survey in each effected districts for fruitful outcome of control program.

ACKNOWLEDGEMENT

We are thankful to employees of Primary Health Centre and ASHA workers of Madhepura. We are also thankful to Prof. Anant Kumar, Head of the Department of Zoology for providing necessary lab facilities.

REFERENCES


9. Picado,A; Murari, L.D; Vijay,K; Diwakar, S.D; Suman, R; Shri, P.S; Pradeep, D; Marc, C; Marleen, B and Clive, D (2010): Phlebotomous argentipes seasonal pattern in India and Nepal. *J.Med.Entomol*. 47:283 – 286


TABLE. – 1.
Represents Block-wise Raw data of suspected, rK 39 positive cases and total diseased person in District Madhepura for years 2010 and 2011.

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TABLE – 2.
Represents month-wise variation in number of Total Patients (TOT), number of new patients (rK 39+ve), Incidence (INCI) and Prevalence (PRE) of Visceral Leishmaniasis in District Madhepura (Bihar) for years 2010 and 2011.

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TABLE – 3.
Represents month wise variation in average temperature (TEMP), average humidity (HUMI) and % incidence of Visceral Leishmaniasis in District Madhepura in years 2010 and 2011.

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<tr>
<td>HUMI’10</td>
<td>89.97</td>
<td>87.93</td>
<td>66.66</td>
<td>58.99</td>
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<td>78.48</td>
<td>84.1</td>
<td>81.92</td>
<td>83.88</td>
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<td>85.79</td>
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<td>TEMP’11</td>
<td>19.58</td>
<td>24.82</td>
<td>29.87</td>
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<td>32.64</td>
<td>34.06</td>
<td>32.38</td>
<td>32.45</td>
<td>32.5</td>
<td>31.45</td>
<td>26.86</td>
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<tr>
<td>HUMI’11</td>
<td>79.22</td>
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<td>68.14</td>
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<td>54.13</td>
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<td>81.95</td>
<td>81.1</td>
<td>74.62</td>
<td>81.51</td>
<td>81.25</td>
<td>89.04</td>
</tr>
</tbody>
</table>

Graph – 2: Represents month wise variation of humidity (upper line) and %incidence of VL (lower line) from January to December 2010 (1 to 12) and 2011 (13 to 24).

Graph.3-Represents scattered graph and linear relationship between humidity and %incidence after considering all observation in 24 months of 2010 and 2011.
Graph 4: Represents month-wise variation of temperature (upper line) and %incidence of VL (lower line) from January to December in 2010 (1 to 12) and 2011 (13 to 24).

Graph 5: Represents scattered graph and linear relationship between temperature and %incidence after considering all observation in 24 months of 2010 and 2011.
National Seminar on
RECENT TRENDS IN TEACHING
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on 150th Birth Anniversary of Gurudev Ravindra Nath Tagore

Ph. D. THESIS OF BRAJESH KUMAR SINGH
tests. The field of population ecology often uses data on life history and matrix algebra to develop projection matrices on fecundity and survivorship. This information is used for managing wildlife stocks & setting harvest quotas.

**Conclusion:**- Population are essential units of ecology to complete the series or chain of ecosystem as from primary consumers to decomposers.

#### MORBIDITY OF VISCERAL LEISHMANIASIS IN MADHEPURA DISTRICT (BIHAR)

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**Abstract**

As per the available literature and Government records, kala-azar epidemic zone is situated in plain of North Bihar including Tirhut, Purnea and Kosi Commissionery. Efforts to control this neglected disease have recently gained momentum for the Government of India's. Commitment to eliminate kala-azar by the year 2010. Keeping the above view a survey was conducted in Madhepura district to study the morbidity of disease. The survey was conducted by the method of stratified random sampling. The infection was determined by serological assay used rK 39 stripe.

The samples were collected from January 2010 to December 2011. The prevalence and incidence were determined per 10,000 population. The prevalence and incidence of kala-azar in 2010 is observed as 26.94 and 9.04 respectively. The rate of prevalence and incidence is marginally decreased in 2011 and recorded as 23.75 and 7.72 respectively. The observation is very high and it is very difficult to achieve goal as determined by Government.
of India as 1/10,000 by 2012. The month-wise analysis reveals that the prevalence and incidence are maximum in the month of April-May and minimum in November-December.

The available literatures have been suggested that the researches are varied on the point of incubation period of Leishmania donovani from 10 days to 2 years. The retrospective analysis of epidemiological (present study) and entomological data (reported records) and their correlation with eco-climatic factors suggests that the entesic incubation period of Leishmania in Madhepura is 6-8 months.

It has been analysed and suggested that two time spraying of insecticides (first in February and second in October) is more suitable for control measure.

Keywords: visceral leishmaniasis, morbidity and incubation period.

STUDY ON THE QUALITY OF DRINKING WATER IN FIVE PANCHAYATS OF DISTRICT MADHEPURA

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Abstract
The safe drinking water is basic necessity for a good health. Keeping the same view a survey was conducted in five panchayats viz., Madhuban, Madanpur, Manikpur, Tulisbari, Gauripur. The parameters selected for study were pH, Iron, fluoride and nitrate. The fluoride, nitrate and pH are within permissible limit whereas the Iron is maximum in all panchayats under study. The Iron concentration (mg/l) is observed as 0.84 ± 0.20 (Madhuban), 0.80 ± 0.20 (Madanpur), 0.84 ± 0.20 (Manikpur), 1.03 ± 0.21 (Tulisbari) and 2.57 ± 0.38 (Gauripur). The effect of Iron on human health has been discussed in paper.
Section II: Animal, Veterinary and Fishery Sciences

EP P-41

DEVELOPMENT OF BIVOLTINE DOUBLE HYBRID OF SILKWORM, BOMBYX MORI L. FOR HILLY CONDITIONS

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Keywords: Bombyx mori, bivoltine silkworm, double hybrid

The objective of this breeding study was to develop and identify superior bivoltine hybrids possessing the desired targeted traits on productivity along with relatively shorter larval duration suitable for hilly conditions. Amalgamation of desired traits on fitness, productivity, fibre quality and shorter larval duration was attempted. This had resulted in the development of six new breeds. Utilizing these breeds, one new double hybrid (SLD2 x SLD4) (SLD8 x SLD9) with superior productivity traits and shorter larval duration (one day 18 hours less) than the control, (CSR2 x CSR27) x (CSR6 x CSR26) was developed.

EP P-42

SURVEILLANCE REPORT OF VISCERAL LEISHMANIASIS BASED ON SERODIAGNOSTIC TEST IN MADHEPURA DISTRICT OF BIHAR, INDIA

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Keywords: Incidence, prevalence, eco-climatic factors, visceral leishmaniasis, Madhepura

A population survey was conducted from January 2010 to December 2011 in Madhepura district of Bihar for the analysis of epidemiological condi-
tion. The report is based on RK 39 dipstick sero-diagnostic test. The incidence and prevalence was calculated per 10000 of population. The annual incidence of 2010 was observed as 8.98 and in 2011 it was observed as 7.71. The prevalence of disease was 26.92 in 2010 and 23.78 in 2011. The seasonal variation has been studied and the effect of eco-climatic factors on the disease incidence has been discussed. The incidence of disease is positively associated with temperature \( y = 0.122 \times + 4.795 \). The incidence of disease is negatively related \( y = -0.161 \times + 20.97 \). The retrospective analysis among epidemiological parameters, eco-climatic factors and vector population dynamics are discussed in present paper. It represents internal incubation period of leishmania is 6-8 months in the region under study.

EP P-43

A BRIEF NOTE ON THE BIOLOGY OF FURCOCERCOUS CERCARIA

Neelam Panwar, Kamal Kumar Saxena and Renu Chaudhari
Pest and Parasite Research Laboratory, Department of Zoology,
Bareilly College, Bareilly, Uttar Pradesh

Keywords: Furcocercous cercaria, reserve food material, cercarial activity

Cercariae as a rule do not feed during their free life. Their activities predominantly depend upon the reserve food material which they accumulate during their parasitism while inter-molluscan stage. It is seen that the main reserve substances present in the cercarial body are glycogen and fat. Glycogen are found in some quantities in the form of large, rounded stellate cells with bubble like nucleus located on either side of caudal excretory canal. However, accumulation of lipid takes place in parenchymatus cells in the form of refractile granules. The amount of glycogen and fat varies from species to species. This present communication deals with the study of biology of furcocercous cercariae. The observations are concentrated around the amount of reserve food material, life span, nature of movement, circadian rhythms of larval emergence.
The Indian Science Congress Association
14, Dr. Biresh Guha Street,
Kolkata – 700 017

Paper Presentation Certificate

This is to certify that Prof./Dr./Shri/Smt. Brajesh Kumar Singh,
Research Scholar
has presented a Paper (Oral/Poster) entitled "Surveillance and control of visceral
toxoplasmosis in the Section of Animal veterinary and
tropical Science..." during
the 100th Indian Science Congress held at Calcutta University, Kolkata from
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