REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad

1. **Name and Designation of ICMR-IF:** Dr. Diwakar Singh Dinesh, Scientist D

2. **Address:** Division of Vector Biology and Control, Rajendra Memorial Research Institute of Medical Sciences(ICMR), Agamkuan, Patna-800007, India

3. **Frontline area of research in which training/research was carried out:** Biomedical Sciences: "Transmission dynamics of Leishmaniasis"

4. **Name & Address of Professor and host institute:**

   Prof. Alon Warburg, The Kuvin Center for the study of Infectious & Tropical Diseases, The Institute of Medical Research, Israel-Canada, The Faculty of Medicine, The Hebrew University of Jerusalem, Ein Kerem, Jerusalem 91120, Israel

5. **Duration of fellowship:** 15 days (28.10.2015 to 11.11.2015)

6. **Highlights of work conducted**

   i. **Technique/expertise acquired:**

      a. **Sampling method of sandflies:**
      The latest technologies for sampling of sandflies from different habitats was learnt under hands on programme like
         i. using CO_2,
         ii. use of chemoluminescence light,
         iii. use of indigenous light trap
         iv. Coloring technique of sandflies with food dye to scruitinise sandflies coming from different biotopes

      b. **Scruitinisation:** Simple Indigenous technique for scruitinizing wild sandflies was learnt

      c. **Dissection:** The technique was learnt to dissect sandflies under sterile conditions for identification, taxonomy, blood meal analysis and natural infection from single sandflies and practiced with the samples brought together.

      d. **Natural infection:** Detection for the presence of *Leishmania* parasite in sand flies applying different molecular techniques; namely: ITS-PCR and kDNA quantitative real time PCR was learnt.

      e. **Blood meal analysis:** Evaluation of blood meal taken by sand flies; was practiced with Dr. Abbasi with the samples brought with me. The encouraging results were obtained worth to be published in a good journal.

f. **Culture of parasite**: The discussion was made how to culture the parasite detected from microscope for maintenance of strain which is not available in Indian context.

ii. Research results, including any papers prepared/submitted for publication

Some experiments were conducted with the samples of sandflies brought together during training programme in parallel. These included mostly molecular diagnostic techniques for detecting and identifying *Leishmania* parasites in sand flies (quantitative real-time k-DNA PCR, real-time ITS-1 PCR followed by restriction cutting using HAE-*III* to separate different Leishmania species simultaneously to identify the source of the blood-meals of sand flies cytochrome *b* PCR was conducted followed by Reverse-Line Blotting (RLB). The techniques learnt by me shared from Alon in cutaneous leishmanisis will be of immense help in studying the transmission dynamics of Kala-azar in different biotopes among highly endemic countries like India, Nepal and Bangladesh along with the critical density of sandflies responsible for transmission of the disease in different ecological conditions with their rate of infectivity and susceptibility status against different insecticides to be used under control program.

Results:

DNA was extracted from different sand flies and ITS-PCR and applied for specific amplifcation of *Leishmaia* parasite DNA. Sand flies numbre: 5, 12, 13, and 16; showed a faint band of ITS PCR product indicated the presence of *Leishmania* parasite. Attempts were done to obtain sequence information for species identification. The expected PCR amplified band is 330bp

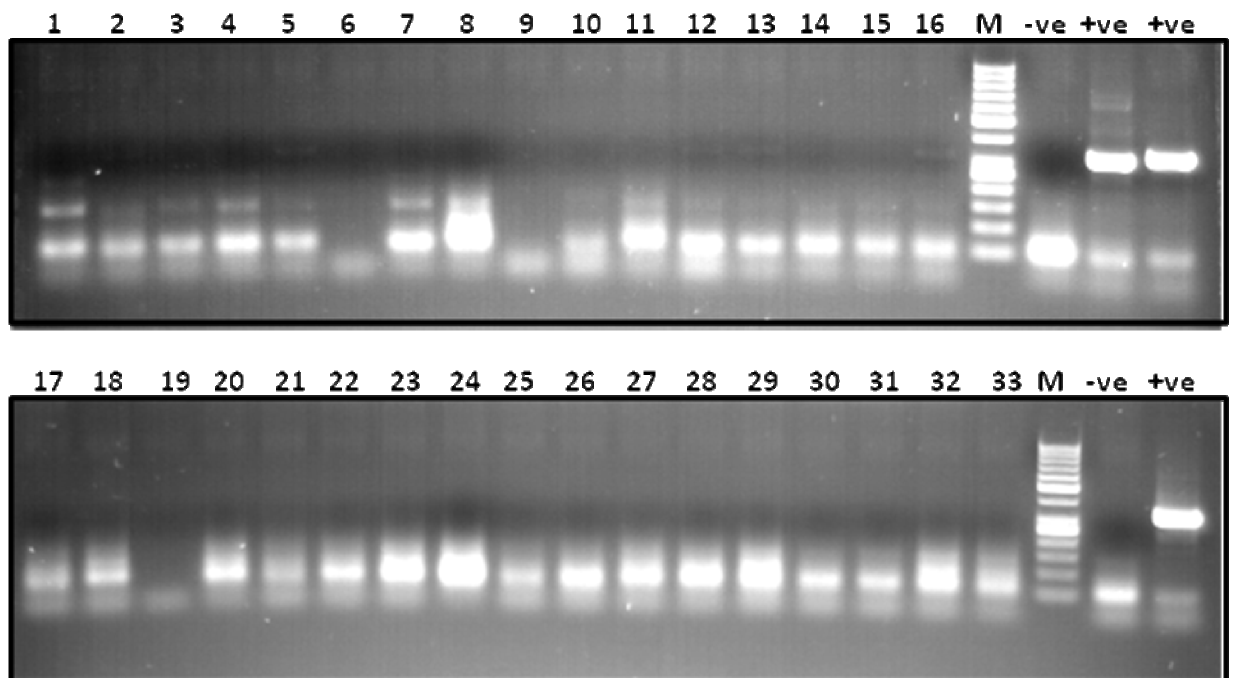

Figure 1: Agarose gel analysis of ITS1-PCR amplification

This PCR system was used as the first step in blood meal identification by reverse line blot. The used primers are biotinlyted and the same will be the amplified cytchrom b PCR product. The expected amplified band size is 350 bp. Some samples did not show any amplification products and this may due to no blood in sand fly.

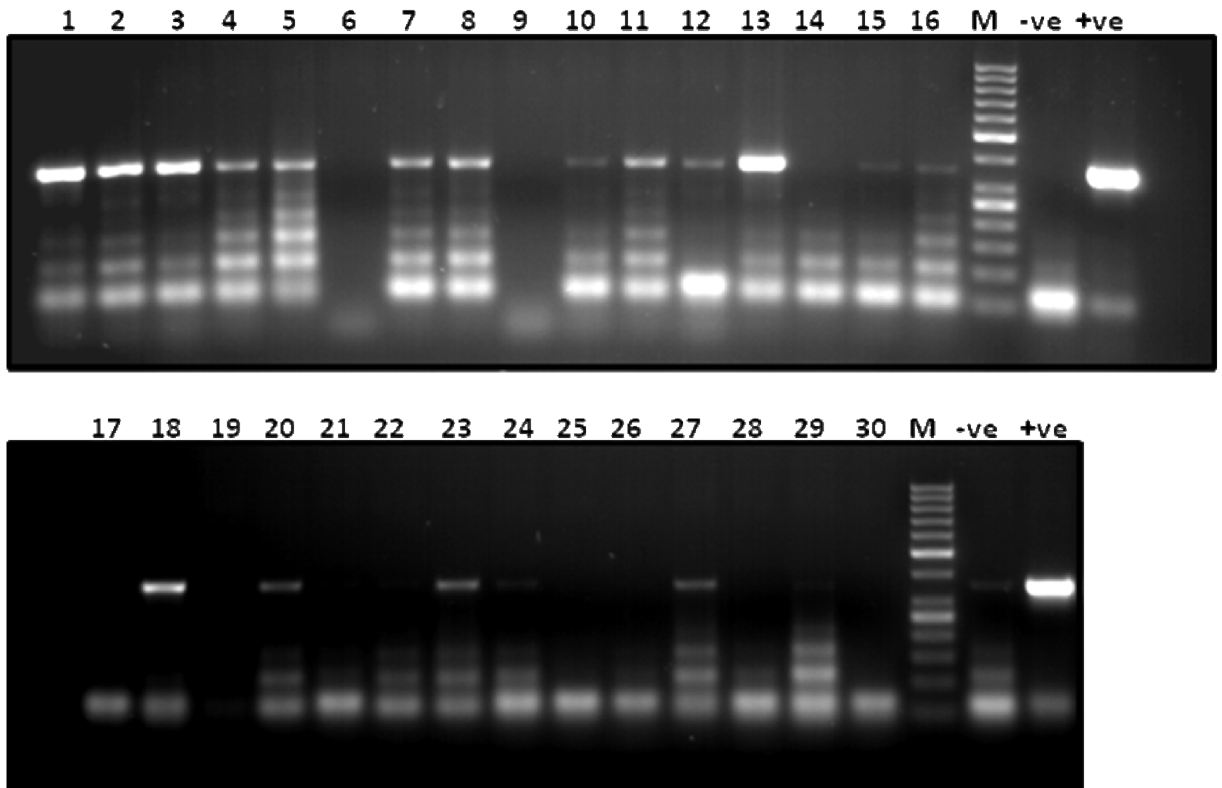

Figure 2: Agarose gel analysis of cytcochrom b-PCR

Selected strips obtained after applying Reverse Line Blot (RLB) analysis for blood meal identification. The amplified cytochrome b PCR product was hybridized with different nine probes that were bound to BiodyneC membrane, the arrangement was as indicated above

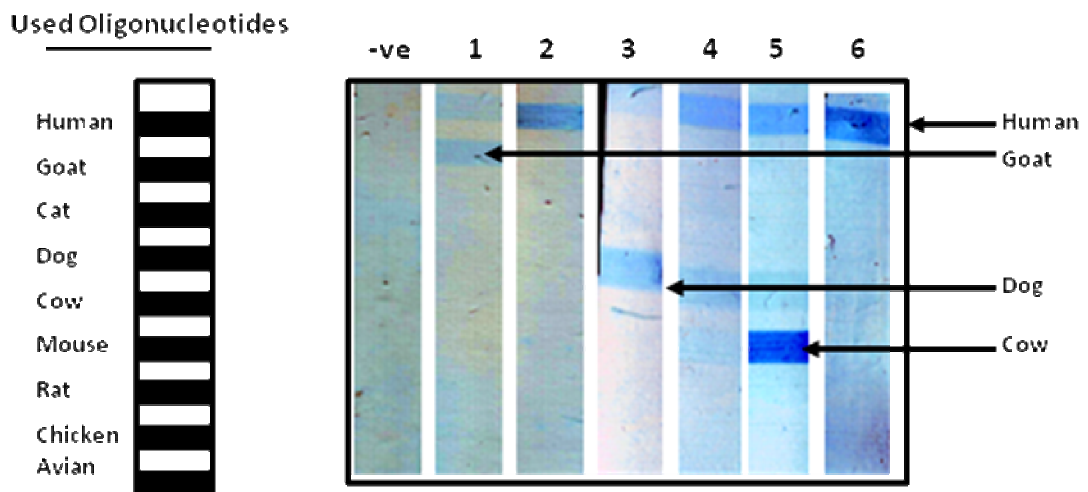

Figure 3: Selected strips obtained after applying Reverse Line Blot (RLB) analysis for blood meal identification.

Real time PCR analysis showed the fluorescence intensity of kDNA-qPCR amplification from different parasite numbers that reflects different DNA concentrations. The pink colored signals is the negative control, so any signals appears before this line will be considered a positive.

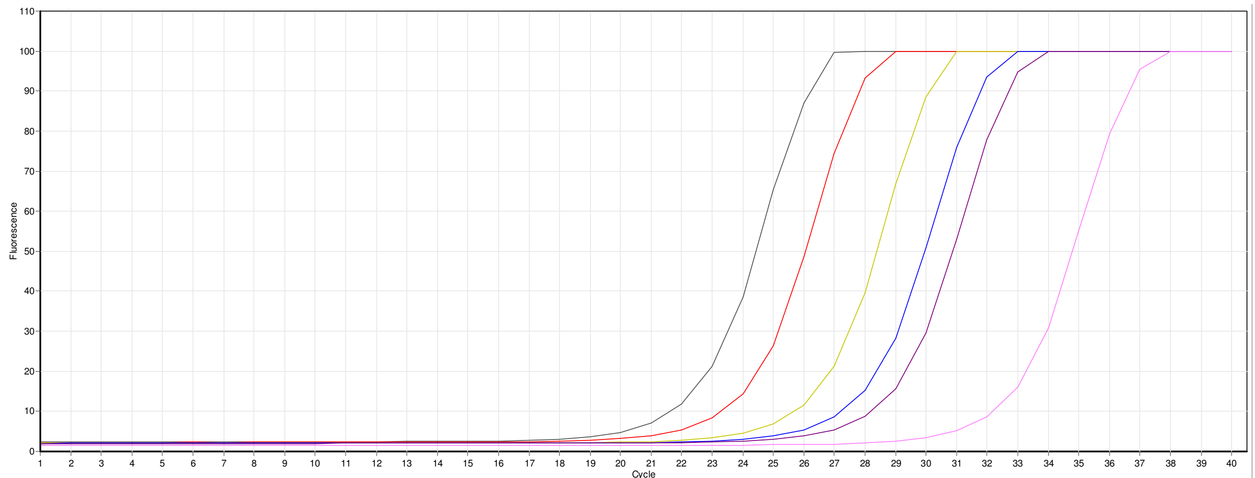

Figure 4: Real time PCR analysis

The manuscript is being prepared for publication as short communication based on the result very shortly.

iii. Proposed utilization of the experience in India:

- Determining habitat of sandflies entering into the house by food dye marking method
- Detection of natural infection of parasite using ITS-PCR and kDNA quantitative real time PCR
- Determining blood meal sources using cytochrome *b* PCR followed by Reverse-Line Blotting (RLB)
- Culturing *Leishmania* parasite microscopically isolated from sandflies as no parasite from sandflies is available in India.

Signature of ICMR-IF

ICMR Sanction No.: INDO/FRC/452/S-37/2015-16-IHD dated 12 Oct, 2015