S17-03: IS MOLECULAR XENOMONITORING A USEFUL TOOL FOR MONITORING VISCERAL LEISHMANIASIS TRANSMISSION IN THE PERI-ELIMINATION PHASE?

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The numbers of VL cases across the Indian Subcontinent (ISC) have decreased markedly as a result of interventions implemented during the regional kala-azar elimination programme. Consequently, active case detection is not a cost-effective surveillance approach moving forward and more sensitive surveillance approaches are required. For other vector-borne diseases targeted for elimination, such as lymphatic filariasis (LF) and onchocerciasis, molecular xenomonitoring (MX), detection of pathogen DNA/RNA, has served as a proxy of human infection to complement disease surveillance activities. The primary objective of the MX study was to investigate whether MX could be used to define endpoints of VL transmission and play a role in post-elimination surveillance of VL (similar to using MX in the LF elimination programme to monitor residual transmission post-MDA). To calculate these endpoints requires estimates of the *L. donovani* DNA infection and infectiousness rates in *Phlebotomus argentipes* females, the only know vector of VL in ISC, to be made and compared with VL infection rates in humans. The study was conducted in 12 villages (endemic, previously endemic and non endemic) located in 5 districts in Bihar. Using previous infection data, a sample size calculation was performed and a target of 3,750 *P. argentipes* females was required to detect differences in *L. donovani* DNA prevalence rates between treatment groups (levels of endemicity). Following a significant sampling effort undertaken between 2018-2021, which was interrupted by the COVID-19 pandemic and local flooding, we collected over 3,900 *P. argentipes* females for *L. donovani* screening. Each female sandfly was dissected at the upper

thorax in our attempts to quantify sandfly infection (using pools of lower thorax-abdomen sections) and infectiousness (using individual head/thorax sections) rates for *L. donovani* DNA. Prior to screening, 10 real time PCR assays, targeting different genes of the *L. donovani* genome, were tested to evaluate sensitivity and specificity. Most of these published protocols were developed specifically for detecting *L. donovani* in human patient samples. When used to detect *L. donovani* in sandflies, we found that some PCR targets cross reacted to either sandfly DNA, or to the DNA of non target pathogens, so were not fit for purpose. We optimized two protocols that were specific for *L. donovani* DNA detection in sandflies. DNA extractions were performed on 172 pools of lower thorax-abdomen sections of between 4-14 sandflies/pool (pooled by collection date and village). Pools were analysed for the presence of *L. donovani* DNA using a qPCR protocol with Taqman primers and probes. Samples were considered positive if Ct values were lower than the limit of detection by the assay (Ct<31). All samples and controls were run in duplicate. Sequencing was performed on any pools that were preliminary considered to be positive for *L. donovani*. Following molecular analyses, none of the pools were confirmed as positive for *L. donovani* DNA. The effort required to collect large numbers of female *P. argentipes* for *L. donovani* detection, during this period of low endemicity, was challenging and resource demanding. In conclusion, establishing a relationship between *Leishmania* infection rates in human and sandfly populations may not be feasible in elimination scenarios. Although RT-PCR may be considered the 'gold standard' for detecting pathogen DNA by researchers, it is an unrealistic approach for programmatic use when infection rates in vectors are so very low (0-0.03% estimates in Bihar at this time). We recommend that to optimise the use of limited programmatic resources, new field-friendly tools are required with protocols for simultaneous detection of *Leishmania* and other pathogens present in sandfly and mosquito populations. To address this need, we have developed a point-of-need multiplex tool for screening sandflies and mosquitoes for a range of pathogens, bloodmeal sources and for insecticide resistance mutations. This isothermal recombinase polymerase amplification test (RPA), is a highly sensitive and selective isothermal amplification technique, operating at 37–42°C, with minimal sample preparation and capable of amplifying as low as 1–10 DNA target copies in less than 20 min. It is

therefore more suitable than other methods for field use. The multiplex tool requires field validation but, when combined with a mobile data management system to facilitate timely data reporting, may offer a method for rapidly screening all blood-fed sandflies and mosquitoes in a collection to determine which pathogens are circulating in an area of concern. An integrated vector surveillance approach may be the most feasible path forward in vector-borne disease control and elimination efforts.

Keywords MOLECULAR XENOMONITORING; *Leishmania donovani*; ELIMINATION, MULTIPLEX TOOL; INTEGRATED VECTOR SURVEILLANCE

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