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Presence of leishmaniasis causing agent, *Leishmania donovani*, in biting midges (*Culicoides sp*) at a disease endemic area of Sri Lanka: Could it be a probable vector?

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Biting midges are a group of dipteran insects of the family Ceratopogonidae. Some species of biting midges are known to be vectors for filaria parasites that infect humans, and some are known to be vectors of viruses that infect livestock. Evidence suggests that biting midges may be a potential vector for Leishmania parasites in other countries. Biting midges are found in high densities in leishmaniasis endemic regions of Sri Lanka. This high density and biting nuisance suggest that these insects may have a possible role as secondary vectors (if not primary vectors) for leishmaniasis in these areas. The first criterion of leishmaniasis vector incrimination is the detection of parasites from the suspected vectors. Therefore, the present study examined the Leishmania donovani parasites circulate within biting midge populations at a leishmaniasis endemic area in Sri Lanka. The study was conducted in Medawachchiya Medical Officer of Health area in Anuradhapura District, Sri Lanka. Biting midges were collected using cattle baited net traps during December 2021. The collected specimens were identified using morphological identification keys. The specimens were surface sterilized using 70% ethanol and the DNA was extracted from the fly using MightyPrep reagent for DNA (Takara, Japan). The parasite DNA was detected using a Polymerase Chain Reaction (PCR) using Leishmania donovani specific primers that target kinetoplast minicircle gene. The amplicons were visualized under UV light after running on a 2% agarose gel stained with ethidium bromide. A total of 42 biting midges were collected and all of them were females. The collection consisted of a single species similar to Culicoides imicola in morphology. The gel electrophoresis and subsequent UV visualization indicated that two of the samples were positive for L. donovani DNA indicating a parasite circulation rate of 4.76% within the wild biting midge population. The results of the current study suggest that the L. donovani, the main causative agent of leishmaniasis in Sri Lanka, circulates within biting midge populations indicating a possibility of this species being a vector for leishmaniasis in Sri Lanka. According to the World Health Organization (WHO) criteria for leishmaniasis vector incrimination, the detection of the parasites within the insect is the first step. Further studies to assess the luxuriant growth of the parasite within midge midgut and experimental transmission using animal models are needed to confirm the vector status. Considering the medical and veterinary importance, the studies on biting midges of Sri Lanka are recommended.

Keywords: Leishmania, Parasites, Biting midges, DNA, Vector

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