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Development of a 2D Microfluidic Paper-Based Analytical Device for the Diagnosis of Canine Visceral Leishmaniasis

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RESUMO

DE CARVALHO, H. J. C. **Desenvolvimento de um Dispositivo Analítico Microfluidico 2D Baseado em Substrato de Papel para o Diagnóstico da Leishmaniose Visceral Canina**. 78 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, Brasil, 2024.

A leishmaniose visceral (VL) é uma doença tropical negligenciada causada pelo protozoário *Leishmania infantum*, acometendo humanos e cães em áreas urbanas. O diagnóstico da leishmaniose visceral canina (LVC) constitui-se como uma ferramenta para a prevenção e profilaxia da VL, uma vez que a presença de cães infectados influencia o surgimento da doença em humanos. Para tanto, é feita associação de sinais clínicos com resultados de testes rápidos e confirmatórios; que, todavia, podem apresentar sensibilidade e especificidade variadas e reação cruzada com outros patógenos, além do alto custo associado a coleta de amostras, mão de obra especializada, armazenamento e infraestrutura laboratorial. No presente estudo, desenvolvemos um dispositivo microfluidico 2D baseado em papel para o diagnóstico da LVC por meio da detecção do biomarcador canino IgG anti-*L. infantum* em imunoensaio indireto. Ao invés de usar enzimas não estáveis para transdução de sinal, acoplamos ao anticorpo de detecção sondas iônicas altamente estáveis que aumentam estabilidade e robustez ao nosso dispositivo em relação aos ensaios convencionais baseados em colorimetria. O imunoensaio é analisado por espectrometria de massas (EM) em spray de papel, detectando a presença das sondas iônicas. Os limites de detecção e quantificação obtidos indicam uma alta sensibilidade do protótipo, e o estudo clínico realizado evidencia a capacidade do dispositivo em diferenciar amostras negativas de amostras positivas para LVC. Por fim, os estudos de estabilidade realizados demonstram que o dispositivo servirá para amostragem remota estável e armazenamento em temperatura ambiente. Ao desacoplar as etapas de coleta e análise de amostras, associando espectrômetros de massa portáteis para análise do dispositivo, pretendemos futuramente tornar o diagnóstico da LVC fácil, acurado, e amplamente acessível, combinando a facilidade dos métodos rápidos com a precisão dos métodos padrão ouro.

Palavras-chave: Canino. Diagnóstico. Espectrometria de massa. Imunoensaio. Leishmaniose.

ABSTRACT

DE CARVALHO, H. J. C. **Development of a 2D Microfluidic Paper-Based Analytical Device for Diagnosis of Canine Visceral Leishmaniasis**. 78 p. Thesis (Doctoral in Sciences) – Faculty of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil, 2024.

Visceral leishmaniasis (VL) is a neglected tropical disease caused by the protozoan *Leishmania infantum* and affecting humans and dogs in urban areas. The diagnosis of canine visceral leishmaniasis (CVL) is a tool for the prevention and prophylaxis of VL, since the presence of infected dogs influences the disease in humans as well. In this way, clinical signs are associated with results from rapid and confirmatory tests, which may present varying sensitivity and specificity, and cross-reaction with other pathogens, in addition to the high cost associated with sample collection, specialized labor, storage and laboratory infrastructure. In the present study, we developed a 2D paper-based microfluidic device for the diagnosis of CVL through the detection of the canine IgG anti-*L. infantum* in an indirect immunoassay. Instead of using non-stable enzymes for signal transduction, we coupled highly stable ionic probes to the detection antibody, increasing the stability and robustness of our device when compared to conventional colorimetry-based assays. The immunoassay is analyzed by paper spray mass spectrometry (MS), detecting the presence of ionic probes. The detection and quantification limits here obtained indicate a high sensitivity of our device, and the clinical study carried out highlights its ability to differentiate LVC positive samples from negative samples. Finally, the stability studies carried out demonstrated that the device will serve for stable remote sampling and storage at room temperature. By decoupling the sample collection and analysis steps, associating portable mass spectrometers for the device analysis, we intend in the future to make the CVL diagnosis easy, accurate, and widely accessible, combining the ease of rapid methods with the precision of gold standard ones.

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Keywords: Dogs. Diagnostic. Mass Spectrometry. Immunoassay. Leishmaniasis.

1. INTRODUCTION

Visceral leishmaniasis (VL) is a parasitic disease worldwide distributed and endemic in tropical areas. Although VL cases are underreporting, approximately 50,000 – 90,000 cases occur annually (WHO, 2019). VL is caused by protozoa of the genus *Leishmania*, mainly the specie *Leishmania infantum* in the New World (MARCONDES; DAY, 2019), affecting dogs and humans in urban areas. Dogs are the main reservoir of *L. infantum* in urban areas, and the prevalence of canine visceral leishmaniasis (CVL) cases contributes for the development of human VL in determinate area (BRODSKYN 2018; MATSUMOTO, 2021). The major trouble in its prevention and prophylaxis relies on the diagnosis of the infected canine population, which is performed through clinical signs evaluation and results obtained from rapid and confirmatory tests (RIBEIRO *et al*., 2013).

Tests currently employed for canine VL (CVL) have varying sensitivity and specificity, and may cross-react with causative agents of trypanosomiasis, ehrlichiosis, babesiosis, dirofilariasis, and borreliosis. Asymptomatic dogs frequently present a lower parasite load and a lower reactivity to serological tests, requiring the implementation of diagnostic methods with higher sensitivity and specificity (MAIA; CAMPINO, 2018). Despite its high sensitivity, PCR requires expensive laboratory infrastructure and skilled labor for sample collection and manipulation (COSTA *et al*., 2010). In all CVL confirmatory methods, the animal must be present at the clinic or laboratory, or a trained technician for sample collection – which, associated with the cost, represents an inconvenience, and make CVL diagnosis difficult, especially in remote areas.

Therefore, it is still necessary to develop CVL diagnostic test meeting the ASSURED criterion (affordable, sensitive, specific, user-friendly, rapid & robust, equipment-free, and deliverable-to end-users) established by the World Health Organization, which determines an ideal of disease diagnosis (KETTLER; WHITE; HAWKES, 2022). Thus, the present work consisted of the development of a new device for diagnosing CVL by applying advanced techniques based on Paper-Spray Mass Spectrometry (PS-MS). The prototype here developed consists of a bioactive paper in which an indirect immunoassay may be performed and posteriorly analyzed by PS-MS. The association of the CVL screening method and confirmatory analysis will allow the prototype to have a high accuracy, being low-cost, user-friendly, and appliable in remote areas for CVL screening.

4. CONCLUSION

Here we developed a 2D paper-based microfluidic device for capturing CVL biomarker in an indirect immunoassay able to be analyzed by PS-MS. By applying highly stable ionic probes to our immunoassay, in association with PS-MS analysis, we provided high stability, sensitivity, and accuracy to our method. In this way, the sampling and analysis step may be decoupled, facilitating the dogs screening and the improvement of CVL diagnosis. Our device was able to successfully diagnose positive CVL samples, presenting high sensitivity, and high stability under ambient temperature storage. Upon completion, this research will provide community health centers with unique preventive and diagnostic capability for CVL diagnosis. Furthermore, implementing regular screening of infected dogs in those areas will also benefit human health by strengthening epidemiologic surveys and implementing strategies of prophylaxis for this disease in both dogs and humans.

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