

Culicoides' species from a *Leishmania* transmission hotspot and efficacy of the Captor® suction light trap

Espécies de *Culicoides* de um hotspot de transmissão de *Leishmania* e eficácia da armadilha de luz de sucção Captor®

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Abstract

Culicoides have medical and veterinary importance, as they play a role as vectors of viruses, protozoa, and nematodes that cause diseases. Despite the relevance to public health, greater attention has been given to other insect vectors. Therefore, the objective of this study was to evaluate the efficiency of the Captor® light trap in capturing *Culicoides* that could be examined for the presence of *Leishmania* DNA. The insects were captured in a rural area of Santa Maria, Rio Grande do Sul, Brazil, where canine and human visceral leishmaniasis have been diagnosed. Adult insects were collected weekly, from 6:00 pm to 6:00 am, for a 12 month period using a Captor® brand suction light trap. All *Culicoides* were identified at species level. Pools of *Culicoides* were tested using the Polymerase Chain Reaction (PCR) technique for the detection of *Leishmania* DNA. A total of 16,016 specimens were collected (71.54% females and 28.39% males), divided among seven species. In the DNA analysis, none of the pools showed a positive result for *Leishmania*. The data presented demonstrate that the trap is efficient and can be an alternative for use in entomological research. They also demonstrate that, despite the females having hematophagous habits, similar to other vector insects, they did not have contact with *Leishmania* in the studied locality.

Keywords: arthropods, insects, epidemiological surveillance.

Resumo

Culicoides têm importância médica e veterinária, pois desempenham um papel como vetores de vírus, protozoários e nematóides que causam doenças em animais e humanos. Apesar da relevância para a saúde pública, maior atenção tem sido dada a outros insetos vetores. Portanto, o objetivo do estudo foi avaliar a eficiência da armadilha luminosa Captor® na captura de *Culicoides* que pudesse ser verificada quanto à presença de DNA de *Leishmania*. Os insetos foram capturados em uma área rural de Santa Maria, Rio Grande do Sul, Brasil, onde foram diagnosticadas leishmaniose visceral canina e humana. Os insetos adultos foram coletados semanalmente, das 18h às 6h, com armadilha luminosa de sucção da marca Captor® por doze meses. Todos *Culicoides* foram identificados em nível de espécie. Pools de *Culicoides* foram testados pela técnica de PCR para detecção de DNA de *Leishmania*. Foram coletados 16.016 espécimes (71,54% fêmeas e 28,39% machos), divididos em sete espécies. Na análise de DNA, nenhum dos pools apresentou resultado positivo para *Leishmania*. Os dados apresentados demonstram que a armadilha é eficiente e pode ser uma alternativa para uso em pesquisas entomológicas. Demonstram também que, apesar das fêmeas apresentarem hábitos hematófagos, semelhantes a outros insetos vetores, não tiveram contato com *Leishmania* na localidade estudada.

Palavras-chave: artrópodes, insetos, vigilância epidemiológica.

Introduction

Culicoides Latreille, 1809 have medical and veterinary importance, as they play a role as vectors of viruses (e.g., Bluetongue Virus and Oropuche Virus), protozoa (e.g., *Leucocytozoon* and *Haemoproteus*) and filarial nematodes (e.g., *Mansonella* and *Onchocerca*) that cause diseases for animals and humans. In addition to the vectorial importance, they cause discomfort due to the




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painful bites that causes itching and local irritation (Borkent & Spinelli, 2007; Mellor et al., 2000). Despite the public health relevance of biting midges for public health in the Americas, including Brazil, *Culicoides* has been neglected if compared to other insect vectors (Rebêlo et al., 2016).

Leishmaniasis is an important parasitic disease transmitted by sand fly vectors resulting from infection by hemoprotozoa of the genus *Leishmania* (Monteiro, 2017). It is a public health problem worldwide and is part of the group of neglected diseases, which disable or kill millions of people representing an important medical need (Brasil, 2010). In the years 2017 to 2019, the prevalence of canine leishmaniasis diagnosed at the "Hospital Veterinário Universitário - Universidade Federal de Santa Maria" was 24.4% (Mariga et al., 2021). Between 2019 and 2020, 40 dogs were diagnosed with the disease at the same veterinary hospital (Johan, 2021) and in 2021 two human cases were confirmed, with one death (Silveira, 2022).

The disease is considered a complex of diseases with varied symptoms, transmitted by the bite of sandflies and which has shown important changes in the pattern of transmission (Alvar et al., 2012). Some authors admit the hypothesis of transmission between the canine population through ingestion of infected ticks and even copulation (Brasil, 2006). In the 1990s, a clear process of urbanization of the disease began, in addition to its geographic expansion to the southernmost states of the country, increasing its area of dissemination (Alves & Bevilacqua, 2004). In an attempt to identify alternative vectors, during studies on entomological surveys of sandflies in areas with active transmission of leishmaniasis, new species of *Culicoides* have been described (Rebêlo et al., 2016).

The identification of possible disease vectors is of significant epidemiological importance. Therefore, the objective of the study was to evaluate the efficiency of the Captor® light trap in the capture of *Culicoides* and to verify the presence of *Leishmania* DNA in the species collected in a rural area of Santa Maria, Rio Grande do Sul, Brazil, place of occurrence of canine and human visceral leishmaniasis.

Materials and methods

Adults *Culicoides* were collected weekly (three nights a week) with a Captor® brand suction light trap for twelve months (March 2020 to February 2021). The trap has a range of 120m² and a 32W ultraviolet lamp (voltage: 220 V / 60 Hz) that attracts insects and propellers that rotate at reduced speed, sucking the insects into an internal drawer inside the trap, protecting them from constant ultraviolet exposure. The trap was installed at -1.5 m above ground level in a rural property (29° 39' 37.2" S; 53° 42' 32.2" W) located in Santa Maria, Rio Grande do Sul, South of Brazil (29° 41' 03" S; 53° 48' 25" W).

The property has pets (e.g. dogs) and production animals (e.g. turkeys and chickens) in its peridomicile, as well as routinely humans. And the type of vegetation in the area is native vegetation (Atlantic Forest and country vegetation) with predominance of the Atlantic Forest, with the presence of streams and lakes in the area.

The collections were carried out between 6:00 pm to 6:00 am, the captured insects were placed inside a plastic container containing 70% ethanol and transported to the Veterinary Parasitology Laboratory of the Federal University of Santa Maria for identification. *Culicoides* were separated from other insects under a stereoscopic microscope based on morphology and grouped into morphospecies according to the wing spotting pattern.

Specimens of each morphological group were mounted on microscope slides according to Wirth & Marston (1968) with adaptations, where they were taxonomically identified at the species level with the aid of the identification keys of Wirth & Blanton (1959), Wirth et al. (1988), Spinelli et al. (2005) and Felipe-Bauer et al. (2008). The other samples were stored and preserved in a -20°C freezer in 2 mL Eppendorf® microtubes.

The capture, collection and transport of samples of the dipterans were authorized by the System of Authorization and Information on Biodiversity (SISBIO) of the Ministry of the Environment under registration n°. 76188 based on Normative Instruction n°. 03/2014.

Genomic DNA was extracted from pools with 15 specimens (using whole individuals) from each morphological group using the phenol/chloroform method similar to that described by Rebêlo et al. (2016). DNA purity and concentrations were determined using a spectrophotometer. The Polymerase Chain Reaction (PCR) for the detection of *Leishmania* DNA was performed

using specific primers for the amplification of *Leishmania* spp. known and verified in GenBank using BLAST: LITSR (5' CTGGATCATTTTCGGATG 3') and L5.8S (5' TGATACCACTTATCGCACTT 3') (Rebêlo et al., 2016) of 453pb. The reaction had a total volume of 25µl, using 2.5µl of 10X reaction buffer (100 mM Tris-HCl, pH 8.5 and 500 mM KCl), 1.25µl of MgCl₂ (50mM), 1µl of dNTPs (2,5mM) and 0.25µl of recombinant Taq DNA Polymerase (5U/µL), 1µl of each primer (10mM), 2µl of DNA (50 µL) and 16µl of nuclease-free water. Amplification was performed in a Bio-Rad® CFX96 thermocycler by: one cycle of 94°C for two minutes, 35 cycles of three steps including denaturation (94°C for 20 seconds), annealing (50°C for 30 seconds) and extension (72°C for 1 minute) and final extension of a 72°C cycle for seven minutes. The amplified product (8µl) was analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide (0.5µg/mL) and visualized under ultraviolet light. A molecular weight standard of 100bp was used. As a positive control, DNA from a strain of *Leishmania braziliensis* provided by the Protozoology Laboratory of the Federal University of Santa Catarina was used, whose DNA extraction was performed by the phenol/chloroform method. For the negative control, ultrapure water was used.

The chi-square statistical method (significance level $p < 0.05$) was used to compare the absolute frequencies of all *Culicoides* species captured in this study. This comparison was performed according to the total number of captures and the analyzes were performed using the SAS statistical program, version 9.2 (SAS Institute, 2001).

Results

During the sampling period, 16,016 specimens of *Culicoides* were collected, being 71.54% females and 28.39% males (Table 1). Seven species were identified: *Culicoides impusilloides* Spinelli & Wirth, 1984, *C. insignis* Lutz, 1913, *C. leopoldoi* Ortíz, 1951, *C. limai* Barretto, 1944, *C. pseudodiabolicus* Fox, 1946, *C. pusillus* Lutz, 1913 and *C. venezuelensis* Ortíz & Mirsa, 1950. The most statistically frequent species were *C. insignis* and *C. venezuelensis*.

After sorting and identifying the biting midges, 2,400 female specimens of the five most abundant species of *Culicoides* were selected, which were divided into 160 pools of 15 individuals, similar to the methodology carried out by Rebêlo et al. (2016) as described in Table 2. In DNA analysis, none of the pools tested positive for *Leishmania* spp.

Table 1. Species of *Culicoides* captured in Santa Maria, Rio Grande do Sul, Brazil, from March 2020 to February 2021.

Species	Female number	Male number	Absolute frequency	Relative frequency (%)
<i>Culicoides impusilloides</i>	27	4	31 ^b	0.19
<i>Culicoides insignis</i>	5,764	2,344	8,108 ^a	50.62
<i>Culicoides leopoldoi</i>	289	132	421 ^b	2.62
<i>Culicoides limai</i>	34	12	46 ^b	0.28
<i>Culicoides pseudodiabolicus</i>	3	0	3 ^b	0.01
<i>Culicoides pusillus</i>	13	9	22 ^b	0.13
<i>Culicoides venezuelensis</i>	5,329	2,056	7,385 ^a	46.11
Total	11,459	4,548	16,016	100

Using the chi-square statistical method, values followed by a different letter in the same column differ ($p < 0.05$).

Table 2. Relation between the number of pools and the total number of specimens examined according to the species of *Culicoides* collected in greater abundance for carrying out the Polymerase Chain Reaction.

Species	Pools number/ Specimens number	Total specimens examined
<i>Culicoides impusilloides</i>	1 / 15	15
<i>Culicoides insignis</i>	71 / 15	1,065
<i>Culicoides leopoldoi</i>	15 / 15	225
<i>Culicoides limai</i>	2 / 15	30
<i>Culicoides venezuelensis</i>	71 / 15	1,065

Discussion

Despite the knowledge about the vectorial importance of *Culicoides*, studies with these Diptera worldwide are still considered limited. In Brazil, studies are even more incipient. Species surveys have been carried out in different Brazilian states, however, in Rio Grande do Sul the works are scarce. Some authors contributed by identifying allergic dermatitis caused by *C. insignis* in a flock of sheep (Corrêa et al., 2007). Carrasco et al. (2014) related the interactions between the population fluctuation of *Culicoides* species such as *C. insignis*, *C. venezuelensis* and *C. caridei* and the environmental variables in the marshes of the Lagoa dos Patos estuary. Furthermore, efforts have been made by Santarém & Felipe-Bauer (2021) annually to update species lists to facilitate the study of the Ceratopogonidae family.

Although this survey of *Culicoides* species in Santa Maria, RS, was carried out in a single environment (rural area), a high diversity of species was observed, including new records (*Culicoides impusilloides*, *C. leopoldoi*, *C. limai*, *C. pseudodiabolicus* and *C. pusillus*), which highlights the scarcity of available information, suggesting that the real diversity of *Culicoides* in the state may be even greater. In fact, a high number of *C. insignis* and *C. pusillus* were collected, which are vectors of the Bluetongue disease virus, an infectious, non-contagious, notifiable disease that affects domestic and wild ruminants, according to the World Organization for Animal Health (2022), which creates restrictions on the international movement of animals and their products. The disease infected sheep in Santa Maria in 2018 (Martins, 2018), so this information can be useful in epidemiological surveillance strategies to determine the risk of bluetongue outbreak and spread in Rio Grande do Sul.

Although only one trap was used, an expressive number of *Culicoides* was collected (16,016 specimens) in one year of research if compared to other studies found in the literature. As an example, Barros et al. (2007) obtained 2,874 specimens in one year of collections using a trap proposed by the Center for Disease Control (CDC). The Captor® light trap can be considered an efficient alternative, easy to handle, install and transport, and at a lower cost when compared to the CDC light trap (Sudia & Chamberlain, 1962), normally used for sampling blood-sucking insects (Barros et al., 2007; Laender et al., 2004; Santarém et al., 2010). Both traps attract the insect to a light source, however, the CDC trap differs in that it attracts them by a small source of incandescent light or LED light, while the Captor® attracts them by an ultraviolet light source. Both suck the insects inside through a small fan. A recent study compared the capture efficiency of two suction light traps: the ultraviolet light Onderstepoort and the incandescent light CDC type trap for collecting *Culicoides* species associated with livestock in South Africa, and the This result confirmed the superiority of the Onderstepoort trap (Venter et al., 2022). This data corroborates the methodology used, since the type of lamp is the same.

It is described that, in areas of humid and sub-humid climate, *Lutzomyia* spp. and *Culicoides* spp. can be captured in the same traps, thus *Leishmania* infection of ceratopogonids is feasible due to the obvious overlap of habitat and likely blood sources. Both are commonly collected in peridomestic environments, and studies show that they use similar hosts, which consist mainly of domestic and synanthropic animals (Costa et al., 2013; Gusmão et al., 2014; Rebêlo et al., 2010). Seblova et al. (2012) observed the development of *Leishmania* parasites in *Culicoides nubeculosus*. In Brazil, the presence of *Leishmania* DNA in *Culicoides* spp. was first reported by Rebêlo et al. (2016). The results showed the detection of *Leishmania braziliensis* DNA in *C. foxi*, *C. ignacioi* and *C. insignis*, while the DNA of *L. amazonensis* was detected in *C. filariferus* and *C. flavivenula*. However, despite the presence of natural infection suggesting a possible role as potential vectors of leishmaniasis, their ability to transmit *Leishmania* has not been confirmed and must be tested experimentally. Unlike Rebêlo et al. (2016), in our research, it was demonstrated that, despite the females having hematophagous habits, similar to sand flies, they did not have contact with trypanosomatids in the studied locality.

Considering that there are autochthonous cases of Leishmaniasis in the studied locality since 2011 (Silva et al., 2011), it is necessary to know all the possible vectors of this disease in the region, which determines the need for further studies on the subject.

Conclusion

The data presented demonstrate that the trap used is efficient and can be an alternative for use in entomological research. *C. insignis* and *C. venezuelensis* were the most frequent species in the study. They also demonstrate that, despite the females having hematophagous habits, similar to other vector insects, they did not have contact with protozoa of the genus *Leishmania* in the studied locality.

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Ethics statement

The study does not involve the use of animals and/or humans (vertebrates), therefore, the Ethics Committee is not necessary, according to the norms of the Federal University of Santa Maria.

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Conflicts of interest

JSL, DMP and SGM - The authors declare that they have no conflict of interest.

Authors' contributions

All authors made substantial contributions to this study. J.S.L and S.G.M. collected the specimens and processed the material for species identification. Data analysis and interpretation was performed by J.S.L., D.M.P and S.G.M. The first draft of the manuscript was written by J.S.L. and all authors commented on previous versions of the manuscript. The final version of the manuscript was read, critically reviewed and approved by all authors.

Availability of complementary results

With the authors upon request.

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