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Full Length Research Paper

High prevalence of *Mansonella perstans* infection maintained in South Benin by *Culicoides milnei*, *Culicoides imicola*, and *Culicoides inornatipennis*

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Mansonellosis is a vector-borne infection caused by different species of filarial nematodes of the genus *Mansonella*, including *ozzardi*, *perstans*, and *streptocerca*. The infection is mainly transmitted by bloodsucking midges of the genus *Culicoides*. All *Mansonella* species are known to induce little to no symptoms in humans. Due to the asymptomatic nature of the infection, epidemiological and immunological data are almost inexistent. Here, we collected blood samples from 88 volunteers in 3 major departments of South Benin and analyzed using parasitological and molecular approaches, the presence of *Mansonella* infections in the region. Polymerase chain reaction (PCR) and entomological identification strategy were then used on 252 potential vectors collected in the same area to identify those hosting the parasite. While microscopic observations indicate a prevalence of 27.3% of *Mansonella perstans* infections, PCR analyses revealed a much higher burden (40.9%). Molecular analyses further showed that 2.27% of the tested individuals were positive for *Mansonella streptocerca*. Moreover, data from molecular identification of the parasites and morphological examination of the vectors revealed that out of 11 *Culicoides* species identified in the study region, *milnei*, *imicola*, and *inornatipennis* were positive for *M. perstans*. Our findings suggest PCR as a tool of choice to analyze the prevalence of Mansonellosis and demonstrate that *M. perstans* is the predominant *Mansonella* spp. in South Benin. Finally, the present study supports the hypothesis that a high transmission of *M. perstans* is maintained in the region of South Benin by three main *Culicoides* spp., including *milnei*, *imicola*, and *inornatipennis*.

Key words: *Mansonella*, *M. perstans*, vectors, *Culicoides.*

INTRODUCTION

Humans are the definitive hosts for several filarial nematode parasites, including mansonelliasis. *Mansonella perstans, Mansonella streptocerca,* and *Mansonella ozzardi* are the three agents that cause mansonelliasis

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(Simonsen et al., 2011). *M. perstans* is a human filarial nematode transmitted by vectors spread over sub-Saharan Africa (Raccurt, 2018), Central and South America, and the Caribbean.

It is transmitted by microscopic blood-sucking flies known as midges (Chung et al., 2020). *M. ozzardi* appears to be primarily linked with humans, but patas monkeys (Erythrocebuspatas) have been infected with them for experimental purposes (Ferreira et al., 2021). The black fly *Simulium amazonicum* and the midge *Leptoconops bequaerti* may operate as concurrent vectors in various parts of South America and Haiti, respectively (Conte et al., 2003). *M. streptocerca* is primarily a human parasite, but it has been known to infect wild chimps in rare instances. Biting midges of the genus *Culicoides*, like other *Mansonella* species vectors, are the principal vectors (Gaillard et al., 2020). Vector-borne diseases are spread by insects such as mosquitoes, ticks, and fleas. Infectious illnesses can be transmitted from one host (carrier) to another via various vectors (da Silva et al., 2017). There are now 14 vector-borne diseases in the United States of national public health concern (Bélard and Gehringer, 2021). These diseases cause many human illnesses and deaths yearly and should be reported to the Centers for Disease Control (CDC) and Prevention's National Notifiable Diseases Surveillance System. The CDC received 51,258 reports of vector-borne illness cases from state and local health officials in 2013 (Tang et al., 2010).

The irregularity, dispersion, and commonness of vector-borne diseases are heavily influenced by environmental factors, exceptionally high and low-temperature limits, and precipitation patterns (Vijayvargiya et al., 2019). Climate change can alter natural factors such as vector population size and thickness, vector endurance rates, the overall wealth of disease-carrying creatures (zoonotic) supply hosts, and microorganism proliferation rates, resulting in altered climate patterns and an increase in extreme events that can influence infection flare-ups by altering organic factors such as vector population size and thickness, vector endurance rates, and microbe multiplication rates (Confalonieri and Dutra, 2014, Raccurt, 2018). The risk of infection spreading to individuals may increase due to these changes (Jamison et al., 2015). South Africa is known for its wide range of weather conditions. The country's climate varies from tropical to subtropical high summer precipitation regions in the north to semi-arid conditions in the northwest (Gehringer et al., 2014). As we get closer to the focal point, the stature decreases, as do the temperatures during the winter months. Many parts of the country have weather conditions that allow massive numbers of adult *Culicoides* midges to stay active all year, with daytime temperatures occasionally falling below 0°C (Laidoudi et al., 2020). During the mild winter, small populations of infection-infected adult midges may thus live long enough between episodes. The vector spans should be shorter than the most severe season of viremia in the vertebrate population to ensure continued transmission of viral illnesses (Keiser et al., 2008). *Culicoides* biting midges are the most common hematophagous insects and can be found worldwide (Agbolade et al., 2006). They transmit a broad spectrum of human, domestic, and wild animal infections (Debrah et al., 2017). The seasonal abundance of *Culicoides* midges, the vector of Bluetongue and African horse sickness viruses (BTV/AHSV), the incidence of viruses in midges and black flies of the *Simulium* species family for *M. ozzardi* (Wanji et al., 2019) were determined in three geographic locations in South Africa (Shelley and Coscarón, 2001). In the current research, we will identify, discuss and highlight the different vectors of *Mansonella* species in Benin.

METHODOLOGY

Specimen collections and storage

The study areas, known to be highly endemic for *Mycobacterium ulcerans* infection from the earlier screening of school children, include three big communes: Abomey, Couffo, and Mono. The communities in these areas are located in reasonably flat bushland and extensive border swamps. The Davougon village is situated approximately 150 km from Cotonou. The neighboring villages border the river Couffo and its associated swamps on their east side. Domestic water is collected mainly from communal boreholes with hand pumps, but some people also collect water from the swamp and an unprotected spring between the villages. The populations mainly practice subsistence farming (bananas, cassava, sweet potatoes, maize, beans, and other vegetables) and keep animals (cows, goats, sheep, pigs, ducks, chickens, and pigeons) on a small scale. Some cassava is also grown as a cash crop, and a few males are engaged in charcoal burning. Most houses in these areas have brick walls and iron sheets. Few houses have mud walls and are roofed with dried grass or iron sheets (Figure 1). Blood specimens (0.5-1 ml) from patients with known *M. perstans* and characteristic *Culicoides* gnawing midges were collected and stored in EDTA tubes. Standard microscopy and PCR-based techniques were used to reach the first conclusions. *M. perstans* was identified using Knott's focus, which was identified using thin blood films. The extra living creatures were identified using laboratory-grown, clinically approved PCR tests, which were confirmed by Sanger sequencing of a segment of glpQ after PCR enhancement. Except for the *Mp* test, in which samples were frozen at -80°C for an extended period, all samples were stored at 4°C prior to further analysis, and nucleic corrosive was isolated within four days of collection to avoid the degradation of the genetic material from the sample.

Sample preparation

The DNA test was carried out in a laminar stream hood. The MolYsis Complete 5-unit (Molzym, Bremen, Germany) kit was used for DNA extraction and host DNA evacuation. Because of the small sample volume, the manufacturer recommended using the "small-size test convention," except that elution was done using 70 µL of deionized water. The REPLI-g Single Cell Kit (Qiagen, Hilden, Germany) was used to enhance the entire genome in a separate room from the test readiness. Agencourt AMPure XP dots (Beckman Coulter, Brea, CA) were used to purge enhanced DNA with a globule volume of 1.5X the sample volume. By adding sub-

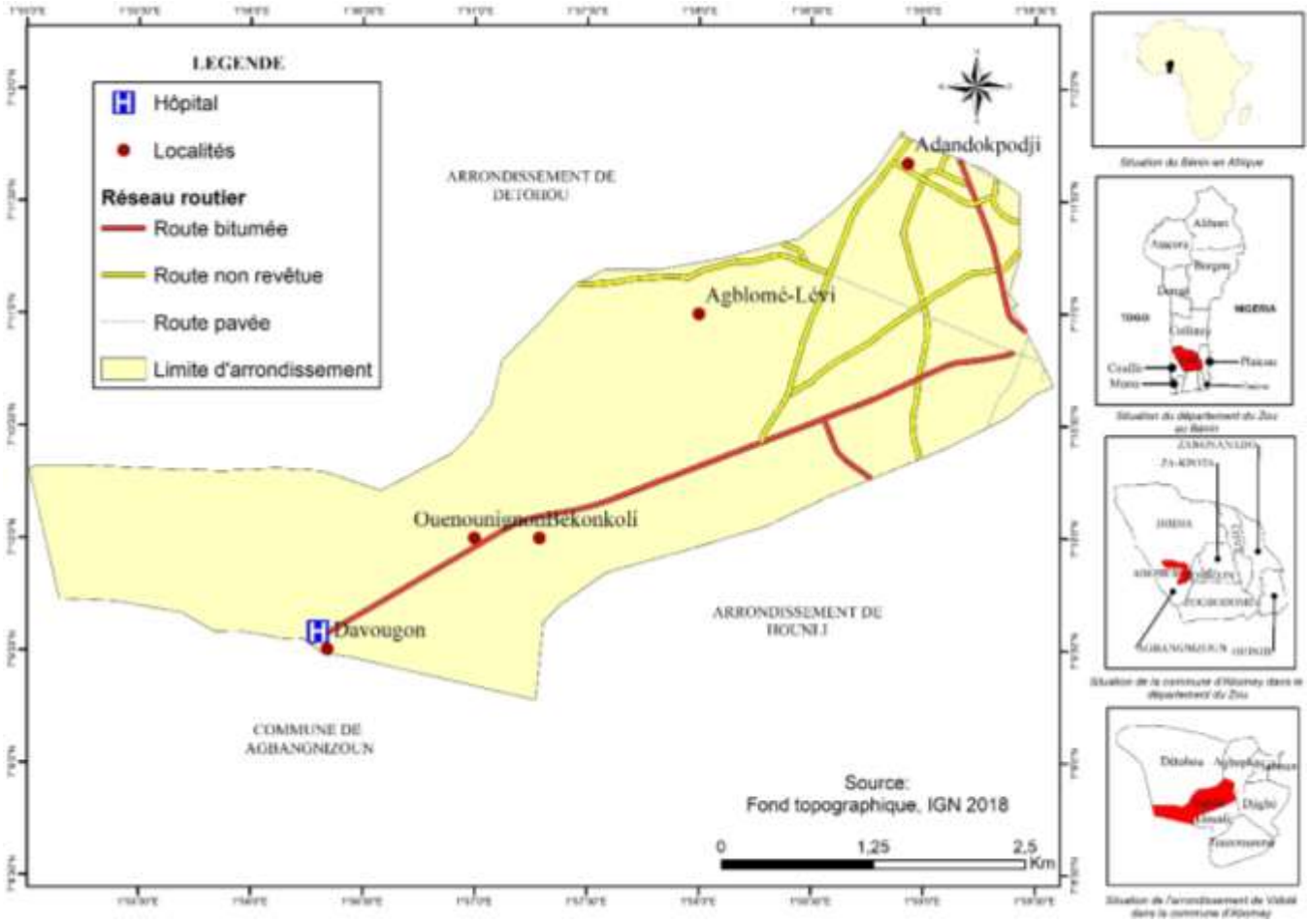

Figure 1. Map showing the study area.
Source: Authors

atomic grade water (DNase/RNase free), cleaned DNA was attenuated to a concentration of 2 ng/L. A final test volume of 50 µL was used for sequencing.

Collection and analysis of vectors

From December 2020 to July 2021, samples of *Culicoides* biting midges were collected bimonthly between 6:00 and 9:00 a.m. following the previously described human bait method (Agbolade et al., 2005). *Culicoides* samples were immediately taken to the laboratory, where they were identified, dissected, and examined for filarial larvae using a dissecting binocular Olympus® CX23 microscope.

Sequencing

Adult midges captured using light traps were stored at 20°C in 70% ethanol. Total DNA extraction, mitochondrial cytochrome c oxidase I (cox 1) PCR amplification, and sequencing analysis were carried out as previously described (Matsuda et al., 2009). The sequencing data were deposited in GenBank, and the sequences of *Culicoides* species, as determined here and in previous work of Matsuda et al. (2009), were aligned with BioEdit v7.0 for comparison.

Molecular biological methods

Isolation of plasmid DNA

According to the manufacturer's instructions, Plasmid DNA was filtered using the QIAprep® MiniprepKit (Qiagen), using a 30 µl elution volume. A QIAcube automated workstation separated genomic and plasmid DNA from various samples (Qiagen).

DNA extraction

A single adult midge was squished using a plastic pestle in a 1.5-ml microcentrifuge tube. According to the manufacturer's instructions, the DNeasy Tissue and Blood Kit extracted complete DNA from the hatchlings (Qiagen, Hiden, Germany).

Polymerase chain reaction (PCR)

PCR is a comprehensively utilized standard strategy to create high measures of any ideal DNA grouping *in vitro* (Mullis et al., 1986). The standard reaction blend and PCR cycling boundaries are displayed in Table 1.

Table 1. PCR reaction mixture and cycling parameters.

1x PCR reaction mixture		PCR cycling parameters		
Component	**Volume [µl]**	**Cycles**	**Temperature (°C)**	**Time**
5x Reaction buffer (Including $MgCl_2$)	10	1 cycle	98	5 min
dNTP-mix (10 mM)	1	-	98	10 s
Primer forward (10 µM)	2.5	30 cycles	72	30 s
Primer reverse (10 µM)	2.5	-	72	30 s/kb
gDNA (100 ng)	X	-	-	-
Phusion® HF (2 U/µl)	0.5	1 cycle	72	10 min
Ultrapure water	ad 50	-	8	∞

Source: Authors

Table 2. Primers used for quantitative real-time PCR.

Primer name	Sequence (5´→ 3´)
16S rRNA-for	TTGCTATTAGATGAGCCTATATTAG
16S rRNA-rev	GTGTGGCTGATCATCCTCT

Source: Authors

PCR and sequencing analysis

PCR items were cleansed using a NucleoSpin® Gel and PCR Clean-up Kit (MACHEREY-NAGEL, Düren, Germany) and ligated into the pCR4-TOPO vector utilizing the TOPO®TA pack (Thermo Fisher Scientific, Karlsruhe, Germany). Plasmids were changed into competent *Escherichia coli* cells (Table 2), and states containing recombinant plasmids (12 white settlements) were picked and developed for the time being utilizing standard conditions. Recombinant plasmid DNA was removed for the time being from *E. coli* societies utilizing a QIAprep Plasmid Miniprep Kit (Qiagen, Hilden, Germany). The measures of additions were dictated by province PCR of the plasmid DNA employing pTOP-seqprimers (Thermo Fisher Scientific). Plasmids with fragments of the right size were exposed to sequencing. Sanger sequencing was performed by Seqlab GmbH (Göttingen, Germany). The arrangements acquired from each gene clone were adjusted, and agreement successions characterized forward and inverted strands (n=24). BioEdit (Hall, 1999) and Blast (Altschul et al., 1990) were utilized for the arrangements investigation.

Primers

For cloning into articulation vectors and sequencing, preliminary steps were used. The right portion shows the toughening temperatures used in PCRs and the limiting compounds. The primers used in the reaction are shown in Table 2.

Molecular identification of vectors

The DNeasy Blood and Tissue Kit (Qiagen) was utilized to seclude DNA from the blood of gnawing midges. The full-blood dinner investigation was done per the distributed mosquito convention and the specialized details given by Lassen et al. (1972). The samples were first screened with an animal-type explicit groundwork. The presence of a PCR item from some random sample in gel electrophoresis was viewed as a positive outcome for that sample. Eurofins MWG|Operon sequenced the filtered PCR items on a business premise (Ebersberg, Germany). The produced FASTA documents were then used to distinguish species utilizing the GenBank DNA succession data set's nucleotide-nucleotide essential arrangement searches apparatus (BLAST)[1]. Few samples identified as positive by the species-explicit groundwork pair were then enhanced with the widespread preliminary pair, and the subsequent successions were approved in GenBank. If any sample did not give good results due to technical errors such as sequencing issues or deficient DNA extraction, the samples were repeatedly reanalyzed before being discarded or tagged irrelevant.

RESULTS

This study included 88 patients with suspected Mp attending the hospital. There were 66 (75%) females and 22 (25%) males in the present study (Figure 2). Microscopically by counting chamber technique, *M. perstans* was observed in 24 patients amongst 88 samples obtained from patients representing the prevalence of *M. perstans* (Table 3). Among 88 samples, 36 were positive on PCR based on *M. perstans* Internal Transcribed Spacer1 (MpITS1) (Table 5). 15 (22.73%) females were observed positive for *M. perstans*, while 9 (40.90%) males were observed with *M. perstans* microscopically (Table 4). Based on PCR, 25 (37.87%) females and 11 (50%) males were observed to be positive for *M. perstans* (Figure 2 and Table 4). Six locations were sampled during the *Culicoides* investigation. Out of 252 Ceratopogonidae collected, 67 were *Culicoides*. Eleven *Culicoides* spp. were identified, including *Culicoides bolitinos, Culicoides grahami,*

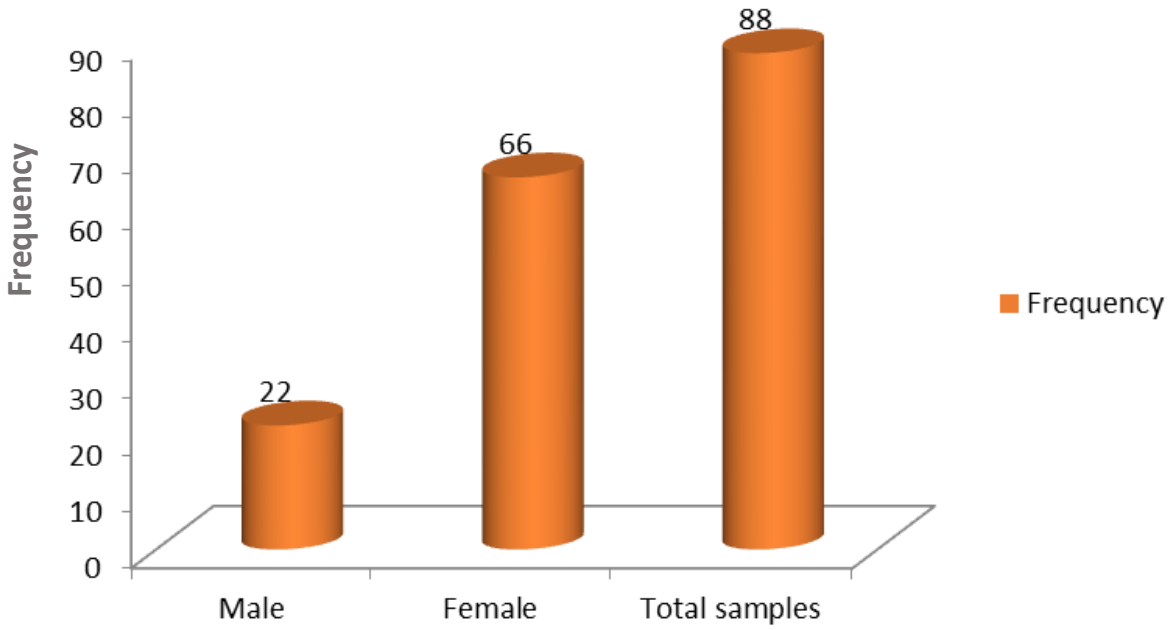

Figure 2. Gender-wise distribution of samples.
Source: Authors

Table 3. PCR-based prevalence of *M. streptocerca* in the Benin region

Total sample	Positive samples	Percentage
88	2	2.27

Source: Authors

Table 4. Gender-wise prevalence of *M. Perstans* on the microscopic and molecular basis

Positive sample on microscopy on gender basis				Positive sample on PCR on gender basis			
Male		Female		Male		Female	
No.	%	No.	%	No.	%	No.	%
9	40.90	15	22.73	11	50	25	37.87
P value		0.107				0.330	

Source: Authors

Table 5. Prevalence of *M. perstans* on the microscopic and molecular basis

Total sample	Positive sample on microscopy		Positive sample on PCR	
	No.	%	No.	%
88	24	27.27	36	40.90
P value		0.08		

Source: Authors

Culicoides milnei, Culicoides fulvithorax, Culicoides neavei, Culicoides inornatipennis, Culicoides imicola, Culicoides schultzei, Culicoides accraensis, Culicoides kibatiensis, and *Culicoides enderleini* which were

Figure 3. MpITS1. The ladder is a mixture of 50 bp size. Lane 1 to 3: PCR fragment is approximately 78 bases long.
Source: Authors

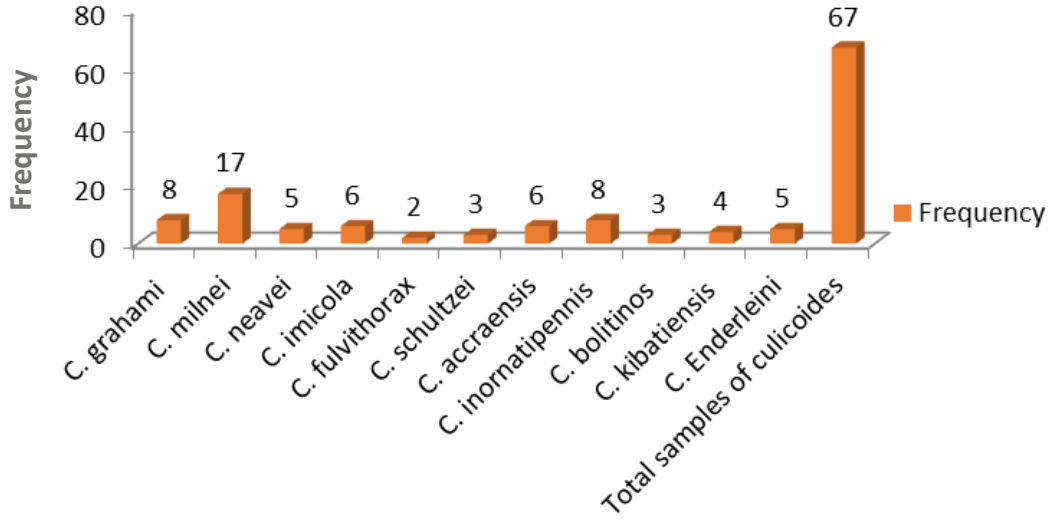

Figure 4. PCR results for vectors.
Source: Authors

depicted for the first time in West Africa, showing new local faunal species. Morphologically, the different species identified were *C. graham* (n=8), *C. milnei* (n=17), *C. neavei* (n=5), *C. imicola* (n=6), *C. fulvithorax* (n=2), *C. schultzei* (n=3), *C. accraensis* (n=6), *C. inornatipennis* (n=8), *C. bolitinos* (n=3), *C. kibatiensis* (n=4), and *C. enderleini* (n=5). Using PCR, we observed that *M. perstans* was present in *C. milnei, C. imicola,* and *C. inornatipennis*. After the conventional PCR, the bands on gel electrophoresis are as shown in Figure 5. This comparison was statistically insignificant as the p-value was higher (p=0.08) than 0.05.

M. streptocerca was reported for the first time in Benin, and out of 88 blood samples, *M. streptocerca* was observed in 2 (2.27%) samples (Table 3). *M. streptocerca* was found only in the samples positive on microscope but negative for *M. perstans* on PCR. *C. milnei* was the most abundant *Culicoides* species observed in Benin (Figure 4). BLAST analysis was done for obtained sequences, matched with the *M. perstans* and *M. streptocerca*. All the sequences were submitted in the GenBank. The accession number for the sequence of *M. perstans, M. streptocerca*, and *C. milnei* are MW644567, MW675685, and MW665129, respectively.

Microscopy, PCR, and sequencing

Microfilariae tallies controlled by microscopy ran between 50 and 1800 microfilaria/ml. All samples underwent additional examination to uncover the presence or nonappearance of *Wolbachia*. The groupings of the obtained ITS amplicons of the 7 *M. perstans* (Figure 3) mono infections from Gabon are indistinguishable from the groupings of *M. perstans* beginning from Cameroon (EU272183) and Equatorial Guinea (EU272182). The collected grouping has been kept in the GenBank data set under KJ631373.

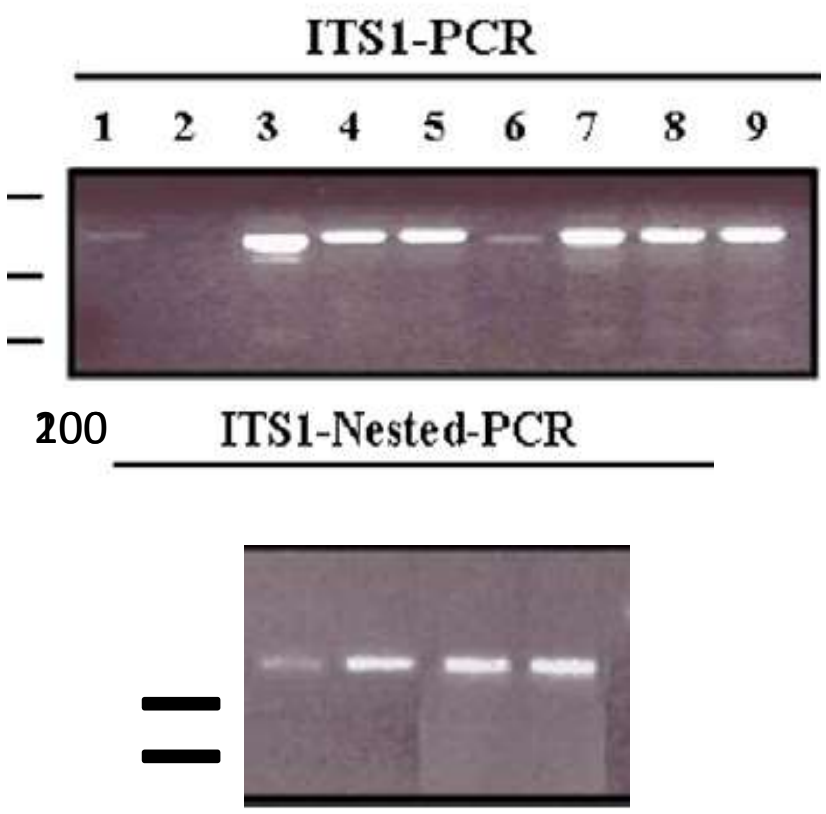

Figure 5. Prevalence of different *Culicoides* species. Differential diagnosis of filarial species. (A) ITS1–PCR products of several blood samples were included in the study. *Mp*, 484 bp. Samples 1, 4, 5, 7, 8, and 9 (B) Nested-PCRs of several samples used in the study. *Mp*, 225 bp. Samples 1, 3, and 4, *Mp*.
Source: Authors

Distinguishing proof of the species as *M. perstans* was made on morphologic measures from thick spreads of fringe blood. The shortfall of accompanying *Wuchereria bancrofti* was affirmed by midnight blood filtration and negative tests (ICT™ and TropBio™) for flowing filarial antigen. Mp_ITS1 covered 79 bp, *Culicoides* spp. ITS1 with 41 bp, *C. milnei* ITS1 with 106 bp, the detection limit was observed at C 10^{-17} g/µl, SybrGreen dye, and hybridization probe.

DISCUSSION

These three species are known to cause human mansonellosis. However, other *Mansonella* species, for example, the chimpanzee parasite *Mansonella rodhaini*, can occasionally infect humans, and some other species can use humans as their definitive hosts (Orihel and Eberhard, 1998, Richard-Lenoble et al., 1988), which produce patent infections with circulating microfilariae. Also, *M. ozzardi* was originally the only known parasite to cause infections in humans, and the terms “mansonellosis” or “mansonelliasis,” until the mid-1980s, were used to refer only to the infections caused by *M. ozzardi* (Connor and Neafie, 1976; Linley et al., 1983; Marinkelle and German, 1970; Nelson, 1965; Shelley et al., 1980). These terms were revised after Orihel and Eberhard included *M. perstans* and *M. streptocerca* in the genus *Mansonella* (Eberhard and Orihel, 1984; Orihel and Eberhard, 1982).

As the three species have different geographical distributions, these species also have many different vectors; specifically, *M. ozzardi* has many variating vectors that transmit these filarial infections to primates, primarily humans. Many *M. ozzardi* vectors are from the black fly family, Simuliidae, for example, *Simulium exiguum*, *Simulium amazonicum, Simulium argentiscutum, Simulium oyapockense, Simulium sanguineum, Simulium guyanensis, Simulium sanchezi*, and *Simulium minusculum*. Other vectors of *M. ozzardi* are from flies or biting midges known as Ceratopogonidae. Examples of these vectors found in the literature include *Culicoides insinuatus, Culicoides guttatus, Culicoides paraensis, Culicoides debilipalpis, Culicoides lahillei, Culicoides furens, Culicoides barbosai, Culicoides paraensis, Culicoides phlebotomus,* and

Leptoconops bequaerti (Crosskey, 1990; Lane and Crosskey, 2012; Linley et al., 1983; Shelley and Coscarón, 2001). Natural habitats and breeding sites of vectors also affect the distribution and consequent infection rates of the parasite. For example, *M. ozzardi* is transmitted by a range of *Simulium* and *Culicoides* spp. However, it appears that the parasite is most commonly transmitted by *Simulium* vectors of the *Amazonicum* species group and thus is distributed along the riverine breeding sites of these *Simulium* vectors (Shelley and Coscarón, 2001; Shelley et al., 2010). *C. phlebotomus* is the only known vector in Trinidad, which uses sandy beaches as its breeding site, and thus the parasite has a coastal distribution (Linley et al., 1983; Nathan, 1981). Such a PCR-based amplification of these filarial parasite using "species-specific target sequences" allows increased diagnostic sensitivity in comparison to traditional diagnostic methods, which include microscopy, and also allow reliable differentiation of samples taken from individuals living in co-endemic areas (Alhassan et al., 2015; Phillips et al., 2014; Ricciardi and Ndao, 2015; Shelley et al., 2001). A popular nested PCR uses a universal filariae primer to amplify a variable portion of filarial parasite ribosomal ITS1 DNA and allows for the subsequent identification of species based on the amplified fragment size (Tang et al., 2010). Nowadays, this rDNA ITS1 method has become a single-step diagnostic method by adapting it for real-time PCR (Moya et al., 2016; Thiele et al., 2016). PCR-restriction-fragment-length polymorphism (RFLP) is used to differentiate a broad range of filarial species using universal primers with a combination of the earlier techniques (Jiménez et al., 2011; Nuchprayoon et al., 2005).

This investigational research work is the first in Benin to decide upon the predominance of mansonellosis and its vectors' dispersion. Our few outcomes infer that mansonellosis is profoundly predominant in Benin. Using PCR, our investigation shows that *Mp* is present in three species of the *Culicoides* genus, namely *C. milnei, C. imicola,* and *C. inornatipennis*. In this way, our examination presumes that these three C*ulicoides* are liable for the transmission of mansonellosis in Benin. Our outcomes suggest that in Benin *M. perstans,* nematodes are exceptionally appropriated, and patients of *M. ulcerans* illness are adventitiously contaminated, so our investigation also proposes that this parasite should be considered in the Buruli ulcer patient's administration.

Previous studies have reported that the vectors of *M. perstans* are biting midges (*Culicoides*) in most of the other endemic countries (Ta-Tang et al., 2018). For vector incrimination, the vector should be attracted to and must bite humans. It should be able also to carry the parasite. In determining the importance of a particular vector species in disease transmission, it is crucial to describe the disease transmission dynamics like mansonellosis. *Culicoides* midges are involved in the transmission of infective *M. perstans* larvae. This research work was thus conducted to determine the prevalence of various *Mansonella* spp. in Benin and the related vectors usually involved in the parasite transmission. Our research work is indeed aligned with the existing evidence in research that *Mansonella* spp., specifically *M. perstans* are present in various *Culicoides* spp., as after assessing the presence of *M. perstans* in C*ulicoides* spp. samples obtained from patients in Benin using PCR, we inferred that *M. perstans* in Benin is carried and transmitted by *Culicoides* vectors of which three species have been highlighted.

Our microscopic observation of the 88 samples found that 15 (22.73%) females were positive for *M. perstans,* while 9 (40.90%) males were also positive for *M. perstans*. Based on PCR, 25 (37.87%) females and 11 (16.63%) males were observed positive for *M. perstans.* Higher prevalence was observed in males as compared to females. This might be due to more exposure of males to infection than females. These findings follow the previous studies in which male prevalence was reported more than females (Debrah et al., 2017). The vector abundance could clarify the high microfilaria distribution. Though, in Benin, the microfilaria vector has not been identified. We presume it might be due to missing microfilaria or misinterpretation during observation. The limited entomologic investigations conducted in mansonellosis-endemic areas show that the prevalence of *Mansonella*-infected wild-caught *Culicoides* is either meagre (0.8%) or nonexistent. These results contradict the high incidence of *Mansonella* microfilaremia in humans. Several studies have incriminated the vector species for *M. perstans* in endemic areas.

Additionally, this problem is complicated because, for tropical *Culicoides* spp. taxonomy, no work has been done in detail (Simonsen et al., 2011). Most intensive studies have been carried out in Cameroon and Nigeria. Recent studies in Cameroon have described *C. milnei* as the vector that transmits *M. perstans* (Debrah et al., 2017). Numerous previous studies have been carried out in various countries to determine the vector for *M. perstans* in endemic areas like Nigeria and Congo, but none has been carried out in Benin. Biting midges have had a dominant presence in rural areas because rural areas provide suitable conditions for vector-breeding, such as wet mud and leaf litter (Agbolade et al., 2006; Ta-Tang et al., 2018). Various landmarks such as bushes or the margin of the pond or the head of an animal, the abundance of cassava tubercules and banana plants, underbrush, and decayed matter of plants have been reported to support *Culicoides'* breeding (Bakhoum et al., 2016). Previous studies have reported higher sensitivity of molecular methods than microscopy in detecting parasitemia and bacteremia (Parola et al., 2011; Andrews et al., 2005). In addition, real-time PCR assays for a few *Culicoides* spp. have been developed, as has a DNA microarray for identifying *Culicoides* species. The bites of

Culicoides midges transfer *M. perstans* nematodes, but it is unclear if *M. perstans*-infected midges may also carry *M. ulcerans*. For example, skin penetration is necessary to establish the presence of *M. ulcerans*, as explained in the guinea pig experiment (Marsollier et al., 2007). However, such a test was not performed in our work, so we cannot establish a strong argument related to *M. perstans* and *M. ulcerans* co-infection. Persistent filarial diseases can cause immunological adjustment and influence the host's reaction to intracellular microbes. Therefore, drives must be embraced to find minimal expense, touchy ways to deal with assistance, distinguish and group tainted people, survey treatment adequacy, and explain immunological connections between various filarial contaminations and different infections.

Conclusion

M. perstans has a high genetic diversity in different areas. The molecular identification method that we are developing is helping in epidemiology to provide a more unambiguous indication of the geographical distribution of the area of risk points where there is a high chance of having contact with the vector. Due to persistent filarial diseases, the host's reaction to intracellular microbes and immunological reactions can be compromised. Efforts should be made to explore low-cost-efficient methods to address the issue, identify the infected persons, explore the available remedies, and investigate immunological connectedness between various filarial contaminations and different infections. The current study demonstrates that mansonellosis is profoundly predominant in Benin. PCR examination confirms the presence of *M. perstans* in *C. milnei, C. imicola*, and *C. inornatipennis,* suggesting that the three *Culicoides* are liable for the transmission of mansonellosis in Benin. The nucleotide sequence-based molecular identification technique developed here successfully identified midges and will be valuable for a better knowledge of *Culicoides* biting midge environments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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