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Seasonal variation of *Microsporidia MB* infection in *Anopheles gambiae* and *Anopheles coluzzii* in two different geographical localities in Benin

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Abstract

Background *Microsporidia MB*, a naturally occurring *Anopheles* symbiont was shown to strongly impair *Plasmodium* transmission without imposing deleterious fitness effects on larval development, fecundity, adult survival, and adult sex ratio, and for these reasons it is being proposed as a promising tool for malaria control. However, there is a limited knowledge about its ecology, transmission dynamics in the environments with varying abiotic conditions, and whether these could impact on the mode of host transmission. This study aimed to determine the presence and prevalence of *Microsporidia MB* in rice fields in Benin during both the dry and wet seasons.

Methods *Anopheles* larvae and adults were collected from rice fields and houses around rice fields in two locations (Koussin-Lélé and Magoumi). The collections took place during both the dry and wet seasons. The larvae and adults were molecularly identified to species level using molecular techniques and they were also screened for the presence of *Microsporidia MB* using PCR following standard protocols. Moreover, breeding sites were also analysed.

Results The species identification results revealed that *Anopheles coluzzii* was the main species in Koussin-Lélé, accounting for 100% of the 1718 samples, while *Anopheles gambiae* sensu stricto (*s.s.*) was predominant in Magoumi accounting for 98.17% of the 986 samples. In Koussin-Lélé, *Microsporidia MB* prevalence of 14% (n = 276) was observed, whereas in Magoumi, only two samples (0.3%) were tested positive out of 667 screened in the dry season. During the wet season, the prevalence of *Microsporidia MB* symbiont was low with rates of 0.7% (5) in Koussin-Lélé and 0.6% (8) in Magoumi. However, the prevalence was relatively moderate in adult field collected mosquitoes with 3.09% (n = 1554) followed by larvae samples with 2.93% (n = 682) and adults emerged from field-collected larvae with 2.67% (n = 1235). Analysis of breeding sites revealed high concentrations of nitrate, nitrite and copper in Magoumi during the dry season, which was associated with a low prevalence of *Microsporidia MB* symbiont in this area. In the wet season, high concentrations of lead and nitrate were recorded in Koussin-Lélé with high concentration of copper in Magoumi, and both localities showed low prevalence of *Microsporidia MB* infection.

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Conclusion This study revealed a high prevalence of *Microsporidia MB* symbiont in Benin during the dry season. Further investigations might be necessary, and modelling of the prevalence and characteristics of breeding sites could help predict the presence of this symbiont in other locations and countries.

Keywords *Anopheles gambiae*, *Microsporidia MB*, Dry, Wet, Season, Benin

Background

Malaria continues to be a major health concern in sub-Saharan Africa, where 97% of the global malaria deaths occur annually [1]. Efforts to control the disease largely rely on using insecticides to prevent mosquitoes from transmitting the malaria parasites to humans [2, 3]. The effectiveness of the insecticide-treated nets (ITNs) and indoor residual spray (IRS) depends factors such as mosquito susceptibility to the class of insecticide, adequate coverage rates, timely implementation, and user compliance [4, 5]. Unfortunately, the emergence of insecticide resistance in malaria vector populations has undermined control efforts, making it harder to eliminate malaria. As a result, there is a growing need for new strategies to control malaria vectors and reduce the parasite transmission.

Recent studies have shown that a naturally occurring endosymbiont, *Microsporidia MB*, has been found in *Anopheles* mosquitoes in parts of Africa [6–8]. *Microsporidia MB* was first reported in *Anopheles arabiensis* in Kenya [6] and later in *Anopheles coluzzii* and *Anopheles gambiae* sensu stricto (*s.s.*) in Ghana [7]; these mosquitoes are the main malaria vectors in many parts of sub-Saharan Africa [9, 10]. *Microsporidia MB* has been shown to strongly impair the transmission of *Plasmodium* parasite without affecting host fitness [11]. The impairment of *Plasmodium falciparum* by *Microsporidia MB* occurs as a result of priming host immunity, and have been shown not to interfere with host fitness parameters such as larval development time, adult longevity and female fecundity [6]. Besides, *Microsporidia MB* does not impose sex bias and these characteristics suggest that it is a true symbiont of the *Anopheles gambiae* complex, since similar characteristics have been observed in *An. gambiae s.s.* and *An. arabiensis* [8, 11]. However, it is not apparent whether *Plasmodium* blocking is intensity-dependent or whether there are several strains of *Microsporidia* circulating in *An. gambiae* sensu lato (*s.l.*) and if that is the case whether there exist varied blocking phenotypes among the strains. *Microsporidia MB* also has the ability to be transmitted by both horizontal and vertical modes (horizontal, from one adult individual to another of the opposite sex through insemination, and vertically, from mother to offspring through transovarial means) [6, 8, 11]. The rate of horizontal transmission ranges from 33 to 53% and occurs only between adults of opposite sexes. Females acquiring *Microsporidia MB* from the infected males were found to possess sperms in their spermathecae suggesting *Microsporidia MB* transfer via insemination [8, 11]. The presence of *Microsporidia MB* in the female ovarioles and germariums was linked to transovarial transmission [6]. In contrast to other mosquito-associated microsporidians, *Microsporidia MB* does not confer any significant negative effect on the host's fertility, fecundity, development, and longevity, and is avirulent in most species within the *An. gambiae* complex [11]. These characteristics suggests that *Microsporidia MB* could be harnessed as a promising candidate and implemented as a self-sustainable malaria control tool [12].

Symbionts can have crucial roles in the adaptation of their host insects. In the same host populations, infection dynamics can be influenced by ecological and geographic factors, such as temperature, precipitation, vegetation, longitude, latitude, and altitude [13–16]. The infection pattern of endosymbionts in spider mites is closely linked to environmental factors. Higher annual mean temperatures increase the frequency of infection by *Wolbachia* spp., while higher altitudes increase the frequency of infection by *Spiroplasma* spp. and *Candidatus cardinium hertigii* [17]. In the pea aphid *Acyrthosiphonpisum*, the geographic distribution of endosymbionts in natural populations shows a strong association with host plant species, precipitation, and temperature [13]. While these findings demonstrate a close association between the natural environment and other endosymbiont infection frequencies, it is not clear if the same effects hold true with regards to *Microsporidia MB* infection rates in *Anopheles* from diverse environments. It was shown recently that *Microsporidia* infection rates in *An. arabiensis* could not accurately be predicted because of the fluctuating infection rates [6]. However, it was apparent that the prevalence of *Microsporidia MB* increased shortly after rainfall peaks with the highest proportion of infected *An. arabiensis* recorded in January during the dry season [6]. Since these studies were undertaken in rice growing areas where agricultural pesticides and pollutants are commonly used it is highly likely that abiotic factors, such as heavy metals, could have significant impact on infection rates. The use of phosphate fertilizers by the rice growing farmers can lead to incremental accumulation of heavy metal trace elements and it would be interesting to establish whether abiotic factors, such as heavy metal pollutants can impact on symbionts infection rates.

The increased rates in environmental contamination with heavy metal pollutants such as copper have been shown to significantly impact the ecology of *Anopheles* malaria vector species, and increasing evidence shows that *Anopheles* vectors can tolerate and survive in habitats polluted with heavy metals [18]. This is despite their proclivity for clean aquatic environments. However, these associations could also result in the direct or indirect impact on endosymbionts and/or their hosts [18, 19]. Therefore, understanding the ecology and favourable conditions mediating host-endosymbionts interaction is critical especially for symbionts, such as *Microsporidia MB* that has far-reaching ramification in malaria control. To this end, the main objective of this study was to screen for the presence of *Microsporidia MB* in *An. gambiae s.l.* in rice fields from selected parts of Benin during the dry and wet seasons and establish whether pollutants in the larval habitats influenced *Microsporidia MB* infection rates.

Methods

Study sites

Two rice cultivation sites were surveyed for this study Koussin-Lélé (7° 27.00; 002° 271E) in the district of Covè (southern Benin) and Magoumi (8° 001N; 002° 205) in the district of Glazoue (center of Benin). Rice farming is the main activity in both localities. Koussin-Lélé is located ~ 160 km from Cotonou in southern Benin, within a sub-equatorial climate zone. The area experiences two wet seasons, from April to July and from mid-September to late October; and two dry seasons, from November to March and from August to mid-September. Annual rainfall ranges between 1500 mm and 2000 mm. In this locality, rice cultivation has been practiced since 1972 by a cooperative of farmers on a 165 ha lowland area (Fig. 1). Rice is grown at least twice a year (from October to July) with controlled irrigation. Farmers use herbicides to manage weed and insecticides for crop protection.

The village of Magoumi (8° 001 N; 002° 205) is located in the district of Glazoué at the altitude of 185 m. Situated at 6 kms West of Glazoué, the village of Magoumi is bordered to the North by the village of Assanté, to the South by the village of Houala, to the East by Ogirin-boubou, and to the West by Ouèdèmè. It covers an area of 45 km^2, which is 3% of the total area of the district of Glazoué. The hydrographic network of the village of Magoumi consists of small rivers fed by rainwater. There are primarily two types of soil: dominant tropical ferruginous soils and hydromorphic soils that are favourable for rice cultivation, market gardening, and yam production. The municipality of Glazoué has Sudano-Guinean climate, characterized by a long-wet season from March to July and a shorter wet period from September to November. The dry season separate these two periods. The region experiences significant temperature fluctuations, with an average annual temperature of around 28 °C. While rainfall in Glazoué is generally sufficient for rice cultivation, its distribution is key to meeting the crop's water needs. The area's tropical ferruginous soils are well suited for rice farming [19, 20].

Mosquito collection

Larvae and adult mosquitoes were collected throughout the year 2022 during the dry and wet seasons. Indoor adult mosquitoes were collected using electric aspirators. After collection, the mosquitoes were morphologically identified as member of *An. gambiae* complex following the keys of Gillies and De Meillon. as well as Gillies and Coetzee [21, 22]. They were then placed in small cups with 10% sugar solution on cotton and brought back to the insectary (IITA insectary). Larvae were collected using the dipping method from 09am to 3 pm using dipping method [23]. *Culex* and other mosquito larvae were discarded and only *An. gambiae s.l.* larvae were pooled together according to their developmental stage, and reared by providing powdered Tetramin fish food. The larvae were reared to adults under insectary conditions of 25 ± 2 °C and relative humidity of 70 to 80%. Additionally, water from breeding sites in each sampling site was collected for electrochemical analysis of heavy metals and physico-chemicals parameters.

Molecular analysis

Species identification

The genomic DNA was extracted from three categories sampling mosquito: adults collected indoor (F0), larvae reared till the adult stage named F1 and from larvae (L3, L4) in each collection sites using the Livak method [24]. The members of the *An. gambiae* complex were identified by PCR [25, 26]. Prior to the extraction, larvae or adult mosquitoes were rinsed in 95% ethanol to eliminate any contaminants. DNA extraction was performed on a single larvae and adult *Anopheles*.

Microsporidia MB identification

DNA samples were screened for *Microsporidia MB* using MB18SF/ MB18SR primers (MB18SF: 5′CGC CGGCCGTGAAAAATTTA3′ and MB18SR: 5′ CCT TGGACGTGGGAGCTATC3′) designed to target the *Microsporidia MB* 18S rRNA gene 336 region. Positive DNA samples of *Microsporidia MB* were obtained from Dr. Eric Ochomo's Entomology Laboratory (KEMRI CGHR-Kisian) and were used in each set of reactions run. The PCR cyclic conditions used were; initial denaturation at 95 °C for 15 min, further denaturation at 95 °C for 1 min, followed by annealing

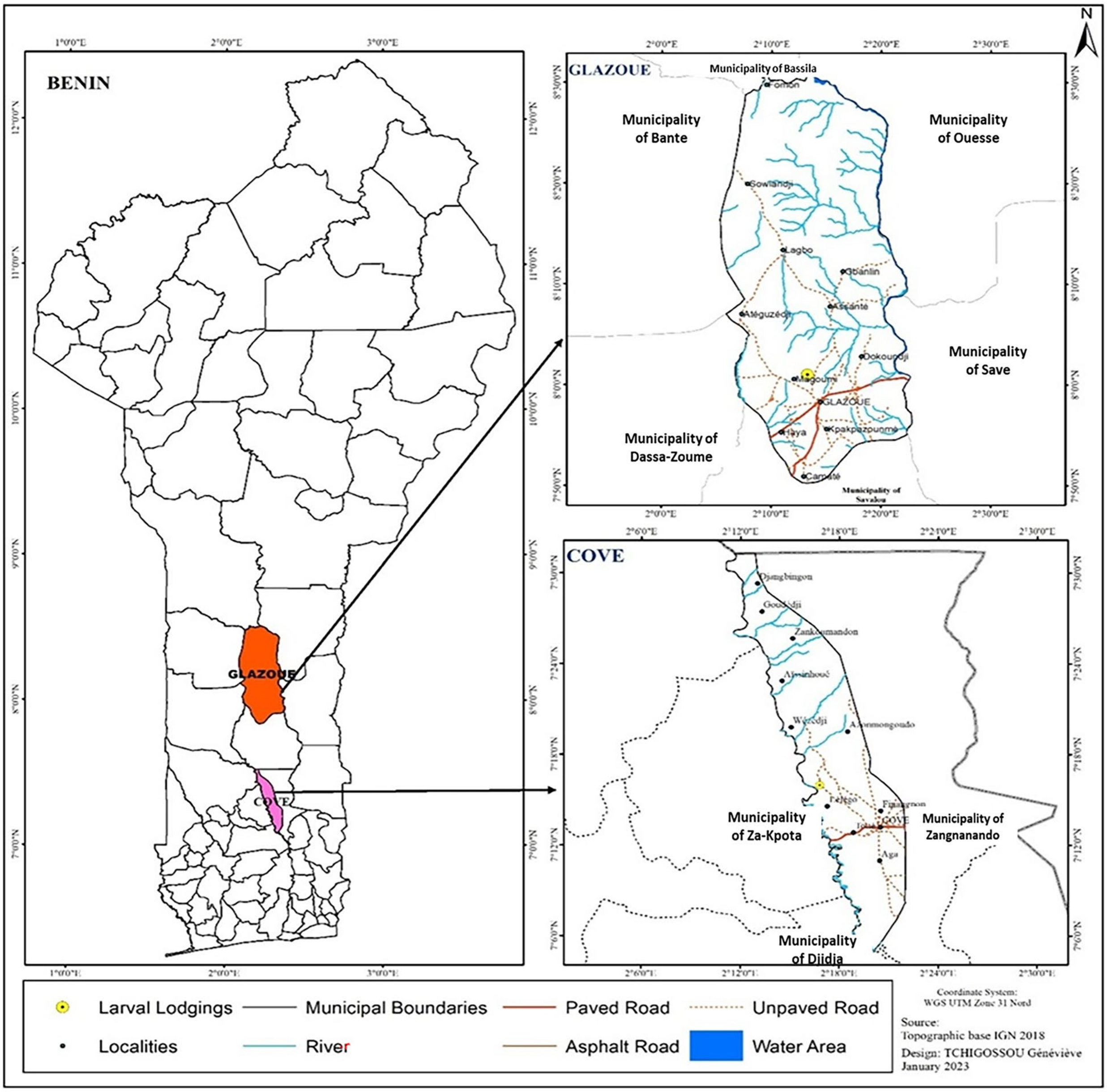

Fig. 1 Map of the study area showing the geographical location of rice sampling sites

at 62 °C for 90 s and extension at 72 °C for a further 60 s, all done for 35 cycles. Final elongation was done at 72 °C for 5 min. Products were loaded and run on a 2% agarose gel stained with Midori green (Invitrogen) and viewed under a blue-light trans illuminator. The PCR reaction was repeated for samples that showed no band for *Microsporidia MB* using an increased volume of the DNA to confirm the initial results (1 µl of the stock DNA sample was initially used and the volume is increased to 2 µl to confirm the initial results). The band size for *Microsporidia MB* detection was ~ 500 bp.

Electrochemical analysis of selected heavy metals in mosquito breeding sites

Target metals were extracted from water samples and then quantified. Extraction was done using a metal extraction kit following the manufacturer's guidelines (Trace$_2$O, United Kingdom). After extraction, 3.5 ml of the extract was added to 66.5 ml of deionized water, along with the corresponding buffer salts in a beaker. The mixture was then analysed using Metalyser HM3000 (Trace$_2$O, United Kingdom). Analysis was conducted using the standard addition method, where the sample was first analysed and then known concentrations of standards were added to the same. After conducting 3 or 4 additions, the resulting current intensities were used to plot the relationship Ip = f ([M]) and then determined the unknown concentration of the metal in the sample by projection it on to the x-axis. Copper, lead, cadmium, mercury, arsenic, nitrate and nitrite were analysed. Conductivity was measured using a Wagtech conductivity meter and TDS was recorded with a photometer.

Data analysis

The counts of symbiont *Microsporidia MB* in mosquitoes were expressed as percentages. The Chi-square test was utilized to analyse *Microsporidia MB* symbiont rates among the different study locations. Data are reported as mean ± SEM, with a significance level set at $p \leq 0.05$.

Results

Species identification

In total, 2736 *An. gambiae s.l.* were analysed for species identification from the two localities. It was observed that *An. coluzzii* was the main species found in Koussin-Lélé (Covè) (100%, n = 986), while *An. gambiae s.s.* was predominant in Magoumi (Glazoue). Out of 1750 samples analysed, 1718 were *An. gambiae s.s.* and 32 *An. coluzzii.* (Table 1).

Distribution of *Microsporidia MB* infection in *Anopheles* mosquito in the dry and wet seasons in surveyed sites

In Koussin-Lélé (Covè), 39 (14%) samples were positives for *Microsporidia MB* symbionts out of 276 analysed while in Magoumi two (2) (0.3%) were positive out of 667 screened in the dry season. A Chi-square analysis suggests a likelihood of mosquito in Koussin-Lélé being infected with *Microsporidia MB* during the dry season than *Anopheles* from Magoumi (Chi-square test; $p < 0.00001$). In contrast, collection from the wet season presented with very low infection rates ranging from 0.7% (n = 5) to 0.6%(n = 8) analysed from a total sample of 710 and 1283 from Koussin-Lélé and Magoumi, respectively (Fig. 2), which did not differ significantly in their *Microsporidia MB* infection rates (Chi-square test; $p = 0.830324$).

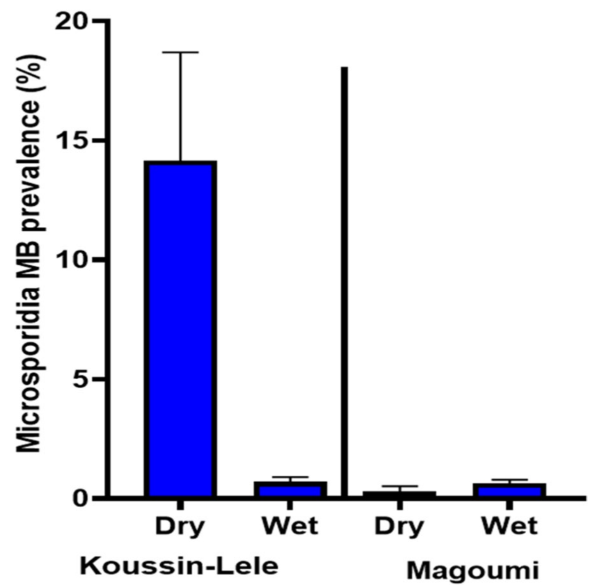

Fig. 2 *Microsporidia MB* prevalence in *An. gambiae* s.l. according to the seasons. Infection rates of *Microsporidia MB* (MB) in *An. gambiae s.l.* were evaluated during the wet and dry seasons at the Koussin-Lélé and Magoumi study locations in Benin. The bar plots display the prevalence of *Microsporidia MB*, with error bars denoting the standard error of the mean (SEM) (*$P < 0.05$)

Microsporidia MB infection according to the different developmental stages' of *Anopheles* mosquito

To investigate if mosquito samples collected at different developmental stages harbored differing *Microsporidia MB* infection rates, adults previously collected as larvae and individual adults aspirated from the same locations were used for genomic extraction and molecular quantification of *Microsporidia MB* infection rates. The rationale for this approach was to tease out whether *Microsporidia MB* infection rates was influenced by abiotic factors found within larval habitats, which provides a relatively constant development parameter as opposed

Table 1 Species characterization of *Anopheles* collected from the surveyed locations

Site	Species	Percent abundance % (n)
Koussin-lélé	*An. coluzzii*	100 (986)
Magoumi	*An. coluzzii*	1.83(32)
	An. gambiae ss	98.17(1718)

to terrestrial environments that are characterized by fluctuating temperatures and humidity, which could likely impact complex physiological state and *Microsporidia MB* infection rates.

The rate of *Microsporidia MB* was found to be greater in samples collected as larvae and subsequently developed into adult mosquitoes (F1). Among the 654 adult mosquitoes (F0) aspirated from Cove, 31 tested positive for *Microsporidia MB* representing an average rate of 4.74%. In contrast, the larvae that matured into adults (F1) exhibited an average symbiont rate of 32.35% (n = 102). Additionally, of the 98 larvae samples analysed from this area, only one was found to be *Microsporidia MB* positive. In Magoumi, the infection rate was very low. *Microsporidia MB* was detected only in field-caught mosquitoes (F0). Out of 886 samples analysed, 9 were positive (1.24%) (Fig. 3).

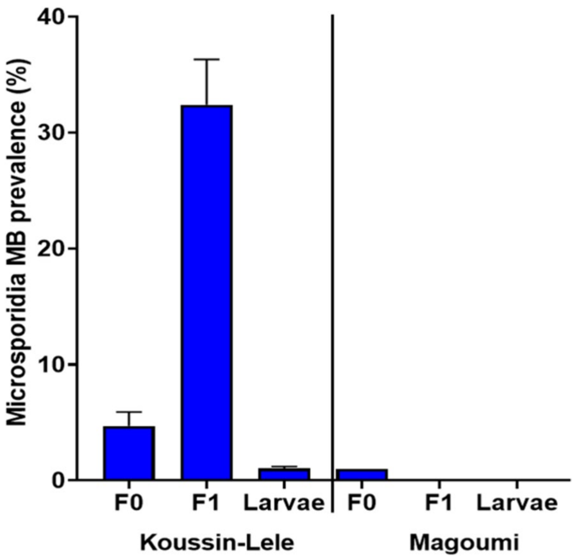

Fig. 3 *Microsporidia MB* infection according to mosquito developmental stage. Infection rates of *Microsporidia MB* (MB) in different developmental stages of *An. gambiae s.l.*from the two study sites were evaluated. The bar plots display the prevalence of *Microsporidia MB*, with error bars indicating the standard error of the mean (SEM) (**P* < 0.05)

Concentration of heavy metals in mosquito breeding waters in surveyed sites

Analysis of larval habitats revealed the presence of heavy metals at variable concentrations. High concentrations of nitrate, nitrite and copper were found in Magoumi during the dry season, which correspond with a low prevalence of *Microsporidia MB* symbiont rate. In the wet season, Koussin-Lélé exhibited high levels of lead and nitrate, while Magoumi had elevated copper concentrations, both associated with a low occurrence of *Microsporidia MB* in these areas. The results obtained were summarized in Table 2.

Discussion

Microsporidia MB, is an endosymbiont shown to completely impair the transmission of *Plasmodium* without affecting mosquito survival and therefore constitute a promising tool for malaria control. This work reports the presence of *Microsporidia MB* in *An. coluzzii* and *An. gambiae s.s.* collected from rice fields in Benin.

Table 2 Physico-chemical parameters of breeding sites collected in surveyed sites and *Microsporidia MB* prevalence recorded

	Koussin-Lele				Magoumi		
	Dry		Wet		Dry		Wet
	Chemical Concentration (%)	MB Prevalence (%)	Chemical Concentration (%)	MB Prevalence (%)	Chemical Concentration (%)	MB Prevalence (%)	Chemical Concentration (%)
Cadmium (Cd) ug/l	-		10.65(100)		-		0(0)
Lead (Pb) ug/l	16 (100)		0 (0)		31.41 (100)		0 (0)
Mercure (Hg) ug/l	1.59 (4.38)		34.74 (95.62)		29.75 (75.43)		9.69 (24.57)
Arsenic (As) ug/l	0 (0)		0 (0)		0 (0)		0 (0)
Copper (Cu) ug/l	16 (10.80)	14	132.19 (89.20)	0.7	82.5 (20.99)	0.3	310.61(79.01)
Nitrates (NO3) mg/l	137.01 (98.70)		1.8 (0.02)		3.46 (24.26)		10.8 (75.74)
Nitrite (NO2) mg/l	1150 (99.99)		0.1 (0.01)		9300 (99.99)		0.16 (0.01)
Turbidity (NTU)	708 (88.35)		93.33 (11.65)		531 (95.33)		26 (4.67)
conductivity (µs)	123.2 (7.65)		1488 (92.35)		487 (55.60)		389 (44.40)

Values in bracket on the chemical concentration column indicate changes in chemicals between dry and wet seasons from the same location.

Species identification revealed the predominance of *An. coluzzii* in Koussin-Lélé whereas in Magoumi, *An. gambiae s.s.* was the main species found in both larvae and adults. The variation of these two species in the two localities could be explained by the ecological characteristics of each site and their relative propensity, and the presence of breeding sites favouring their reproduction. Djogbenou et al. [27] reported a significant decline in the frequency of *An. coluzzii* with increasing latitudes. Koukpo et al. [28] also reported that *An. coluzzii* was the predominant species in the southern regions of Benin, while *An. gambiae* was the major species in the northern and central regions suggesting that climatic conditions could be playing an important role in the distribution of the different species [29]. *Anopheles coluzzii* is associated to permanent breeding sites and those resulting from human activities, while *An. gambiae s.s.* is more frequent in rain-dependent temporary breeding sites. The same authors also concluded that *An. coluzzii* prefers urban water collections and adapts quickly to pollution.

This study showed differences between the prevalence of *Microsporidia MB* in the dry and wet seasons, such observation was reported in *An. arabiensis* from Mwea in Kenya, where the prevalence of *Microsporidia MB* increased after rainfall peaked with the highest proportion in *An. arabiensis* recorded in January [6]. These results suggest that *Microsporidia MB* prevalence could fluctuate according to climatic conditions. Moreover, the difference in species distribution and breeding sites composition could explain the variation of *Microsporidia MB* prevalence.

Increased rates in environmental contamination with heavy metal pollutants such as copper can significantly impact the ecology of *Anopheles* malaria vector species, and increasing evidence shows that *Anopheles* vectors can tolerate and survive in habitats polluted with heavy metals [18]. This is despite their proclivity for clean aquatic environments. To assess whether natural habitats polluted with heavy metals could impose significant biological costs on host symbionts *Microsporidia MB*, larval and adult infection rates were determined across wet and dry seasons and waters collected from larval habitats used to estimate the concentration of heavy metals. Heavy metals could exert selective pressure to adversely affect biological fitness, and that this was likely to be manifested in individuals harboring *Microsporidia MB*.

Individual members of *An. gambiae* complex exposed to heavy metal pollution have been found to show considerable modulation in their detoxification enzymes, which subsequently affects their tolerance to insecticides [30]. The implications of this phenomenon can be profound for vector management strategies, since mosquitoes that are usually susceptible to frontline insecticides are likely to exhibit resistance. To tease out if natural infection with *Microsporidia MB* differs between different developmental stages, the symbiont rates on field-caught female (F0), larvae (L) and field larvae collected and reared to adult (F1) was analysed. *Microsporidia MB* prevalence was higher in F1 mosquito than those collected as adults. Similar results were observed by Akorli et al. [7] and could more likely be due to efficient vertical transmission which depends on the *Microsporidia MB* intensity in the ovaries of the female parent, which has been shown to influence transmission to offspring. In addition, several studies reported that the microbiota composition is dynamic, varying with and among individuals of the same species [31–34]. Hence, it is important to identify the effect of environmental variables on the micro-biome especially on *Microsporidia MB* for developing and deploying symbiotic control strategies. The difference in the prevalence observed in the two localities could be mainly due to composition of breeding sites as shown by phyco-chemicals parameters analysed in this study. The mosquito gut microbiota is mainly acquired from the environment [35, 36] and its composition is largely influenced by its aquatic breeding environment [37, 38], stage of mosquito development, and geographical areas [39–42]. Significant differences of the prevalence of symbiont *Microsporidia MB* were noted in *An. coluzzii* during the dry and wet seasons in the same locality Koussin-Lélé indicating that the highest prevalence was recorded in the dry season. In addition, comparison between localities showed a significant difference between *An. gambiae s.s.* collected at Magoumi and *An. coluzzii* collected at Koussin-Lélé.

These differences in the prevalence of symbiont *Microsporidia MB* between seasons and *Anopheles* species collected from different localities could depends on several factors such as the acquisition of environmental microbes (localities) and seasonality. This corroborates previous studies showing that seasonality impacts the adult mosquito microbiota composition [43]. Moreover, the variability of breeding sites can contribute to variable concentration or presence of symbionts. In addition, it was proposed that the diet could influence the microbial composition of mosquitoes during their larval stage. It has also been found that the diversity of microbes in adult mosquitoes is reduced mainly due to the proliferation of certain bacterial species after they feed on sugar and/or blood [44, 45]. Therefore, variations in *Microsporidia MB* prevalence observed in this study could be due to the differences in the feeding histories of mosquito at their different developmental stage. Several environmental and physiological statuses could potentially affect the intensity of *MB* observed in adults and variations in infection prevalence could be governed by factors that are yet to be investigated. Future studies should undertake experimental evaluation to establish whether the concentration or a specific pollutants impact on host harbouring *Microsporidia MB*. Such studies will reveal whether these pollutants affect infection rates, transmission rates, and host fitness. This is critical because should individuals infected with *Microsporidia MB* lack inherited resistance to heavy metal contaminants, the greater survival rates of non-infected individuals with compensatory mechanisms may lead to a reduction in *Microsporidia MB* infection rates. The overall effect could manifest as a decrease in the number of *Microsporidia MB* infected individuals in areas heavily contaminated with certain heavy metals. On the other hand, if symbiont *Microsporidia MB* provides some level of tolerance to specific heavy metals, the naïve populations that are not infected may experience reduced reproductive and survival rates, potentially resulting in an increase in *Microsporidia MB* prevalence. These data will help inform strategies that aim to develop a target product profile for releasing symbionts *Microsporidia* in mosquitoes to control malaria.

Microsporidia MB possesses significant characteristics, including both vertical and horizontal transmission, suggesting its potential to maintain field infections at levels capable of effectively reducing malaria incidences. Furthermore, the transmission from infected male mosquitoes to females indicates that releasing males into the wild could eliminate the necessity of releasing biting females, a strategy likely to gain acceptance among communities in malaria-endemic areas.

Conclusion

This study revealed the presence of *Microsporidia MB* in Benin with high prevalence in the dry season. However, variations in *Microsporidia MB* prevalence observed in this study could be due to the differences in the feeding histories of mosquito at their different stage of development and environment factors.

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Author contributions
GT and RD designed the study. GT coordinated laboratory work. GT, ML, ET, PMS, RA conducted field sampling and participated in laboratory analysis of samples. GT, CD, MG, DA, MK, AO conducted the molecular analysis. GT, ML, wrote the manuscript. RA, GN advised on the study design and helped to finalize the manuscript. All authors read, reviewed and approved the final version of the manuscript.

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Availability of data and materials
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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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