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REVIEW

Diversity and role of cave-dwelling hematophagous insects in pathogen transmission in the Afrotropical region

Judicaël Obame-Nkoghe^{1,2}, Eric-Maurice Leroy^{1,2} and Christophe Paupy^{1,2}

The progressive anthropization of caves for food resources or economic purposes increases human exposure to pathogens that naturally infect cave-dwelling animals. The presence of wild or domestic animals in the immediate surroundings of caves also may contribute to increasing the risk of emergence of such pathogens. Some zoonotic pathogens are transmitted through direct contact, but many others require arthropod vectors, such as blood-feeding insects. In Africa, hematophagous insects often play a key role in the epidemiology of many pathogens; however, their ecology in cave habitats remains poorly known. During the last decades, several investigations carried out in Afrotropical caves suggested the medical and veterinary importance particularly of insect taxa of the Diptera order. Therefore, the role of some of these insects as vectors of pathogens that infect cave-dwelling vertebrates has been studied. The present review summarizes these findings, brings insights into the diversity of cave-dwelling hematophagous Diptera and their involvement in pathogen transmission, and finally discusses new challenges and future research directions.

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Keywords: Africa; bat; cave; Diptera; emergence; zoonotic pathogen

INTRODUCTION

Caves have been used by earlier human ancestors, and then also by modern humans during the Paleolithic age to face climate hazards or for various shamanic cults. Previous investigations have highlighted the importance of caves for human life, culture and survival.¹ Currently, caves are becoming more and more anthropized, mainly for resource gathering by the nearby human populations or for economic purposes, such as ecotourism or mining. Therefore, the associated health risks, particularly those related to potential zoonotic pathogens that circulate among cave-dwelling vertebrates, need to be addressed. Contacts between animal reservoirs, such as bats living inside caves, and recipient hosts, such as humans and wild or domestic animals entering or living close to caves, may favor pathogen spillover to new hosts. In specific environmental conditions, these pathogens could easily cross species barriers and cause the emergence of new zoonotic diseases.

Among such pathogens, the most threatening for humans and domestic animals are viruses that, due to their diversity, are responsible for several diseases such as hemorrhagic (for example, Filoviridae), respiratory (for example, coronaviruses), or encephalitic (for example, Henipaviruses) syndromes; bacteria causing bartonellosis (*Bartonella* spp.); and fungi, such as those causing histoplasmosis (*Histoplasma* spp.).^{2–4} It is currently unknown whether blood parasites housed by cave-dwelling animal reservoirs can develop in humans, but they remain a potential zoonotic risk, as known for viruses. The recent

discovery in humans of the monkey parasite *Plasmodium knowlesi* stands out as an example of the potential transfers of animal parasites to humans.⁵

While some zoonotic pathogens can be transferred through direct contact with the animal reservoir, others require blood-feeding arthropods. In caves and in the surrounding areas, humans and animals are almost inevitably exposed to hematophagous arthropods, especially insects. Although hematophagous insects play a key role in the epidemiology of zoonotic pathogens, very little is known about their ecology and distribution in cave ecosystems. Nevertheless, previous investigations have highlighted the great diversity of some insect groups, especially mosquitoes, sand flies, biting midges, bugs and other ectoparasites, in caves worldwide.⁶

In the Afrotropical region, most of the knowledge on cave-dwelling arthropod communities stems from studies carried out in West, Central and East Africa. Although very basic and mainly restricted to insects, these studies are still the reference for hematophagous dipterans, such as mosquitoes (Culicidae), biting midges (Ceratopogonidae), sand flies (Phlebotominae) and bat flies (Nycteribiidae and Streblidae). Members of the Diptera order have been particularly studied because of their importance in human and animal health, but other insect groups, such as bugs and fleas, have also been occasionally detected in African caves.⁷ The present review summarizes the knowledge gained during forty years of investigations on the diversity of hematophagous dipterans living in Afrotropical caves and their

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involvement in pathogen transmission. It also discusses new challenges and future research directions in this field.

Relevant publications were identified by searching the NCBI PubMed and ISI Web of Knowledge databases and the database of the Office of the Assistant Secretary of Defence for Energy, Installations, and Environment (Armed Forces Pest Management Board (<http://www.afpmb.org/content/literature-retrieval-system>) using key word combinations that included 'Diptera', 'Hematophagous', 'Cave-dwelling', 'Afrotropical', 'Ethiopian Region', 'Culicidae', 'Phlebotominae', 'Ceratopogonidae', 'Nycteribiidae', 'Streblidae', 'Caves', 'Cave-dwelling bats', 'Cave-dwelling vectors' and 'Haemosporidia'. Articles in French language were also included because they are particularly numerous on this topic in this part of the world. French articles were retrieved using the Horizon database, hosted by the Institut de Recherche pour le Développement (www.horizon.ird.fr) and the same key word combinations translated in French. Most of the cited literature is available online, but some of the articles can be made available upon request.

Caves are suitable breeding places for insects

The cave physical and micro-climatic features. As in other parts of the world, Afrotropical caves are characterized by stable levels of temperature/relative humidity and the absence of light.⁸ Temperature usually ranges between 22 and 28 °C and relative humidity between 95% and 100%;⁹ however, both are modulated by air flows that generate micro-climatic variations according to the cave architecture and especially the size and shape of the internal chambers.¹⁰ Differences in the rock physical nature and in the seepage or stream water features result in a great variety of caves that are mainly classified in caves formed in non-soluble or in soluble rock.¹¹ Caves formed in non-soluble rock are generally of volcanic origin, whereas soluble rock caves are usually found in karst landscapes (that is, areas rich in limestone and characterized by sinks, ravines and underground streams)¹¹ or more rarely in areas with lateritic rocks (that is, rocks rich in iron and aluminum), as observed in Gabon.¹²

Caves provide 'board and lodging'. Availability and characteristics of breeding places. In caves, preimaginal development takes place in different breeding sites, from water pools to damp substrata, according to the dipteran group. Mosquitoes breed in residual water contained in rock pools or in shallow pools along the edge of underground rivers.¹³ Larvae of biting midges develop in muddy substrata with a semi-liquid to slightly damp powdery texture.¹⁴ Sand flies need generally dryer breeding sites and, depending on the species, immature stages can develop deep in the soil or at the surface.¹⁵ Although the different breeding sites have specific features, all contain organic matter to support preimaginal development. As the absence of light excludes the development of photosynthetic organisms, the endogenous sources of organic matter are mainly of bacterial and animal origin (decomposing products and bat droppings). Exogenous organic matter can be brought inside the cave through water runoff.

On the other hand, bat flies (Nycteribiidae and Streblidae), like all members of the superfamily Hippoboscoidea, reproduce via viviparous puparity. Thus, immature stages, from eggs to L3 larvae, develop in the uterine cavity until larviposition and progression from L3 larvae to pupae. Pupae that are not motile are found on rock walls or vaults close to their bat hosts until adult life.¹⁶

Blood source availability. Like for the immature stages, adults of hematophagous dipterans have developed physiological and behavioral adaptations to the specific environmental conditions inside caves (that

is, temperature and relative humidity) and to the vertebrate hosts used as blood sources. Among mammals, bats and rodents are the main vertebrates found in Afrotropical caves.¹⁷ Bats form very large colonies and live in high promiscuity, thus providing an unlimited blood source. More than 300 species from 70 genera have been reported in the Afrotropical region and at least 95 species from 34 genera, including Yangochiroptera and Yinpterochiroptera (for example, *Miniopterus* sp., *Rousettus* sp., *Hipposideros* sp., *Coleura* sp., *Rhinolophus* sp., *Pipistrellus* sp., *Nycteris* sp.), roost in caves.¹⁸ All these bat species can potentially serve as blood sources for cave-dwelling dipterans, although only some species have been clearly identified as such.^{19,20} The African porcupine (*Atherurus africanus* Gray) and rodents of the genus *Praomys* are the main taxa bitten by hematophagous cave-dwelling dipterans in African caves.¹⁷

Ecological classification and spatial occupancy of Diptera inside caves. The current classification of the cave fauna is based on the ecological criteria proposed by earlier speleologists in which species are grouped in three categories in relation with the degree of penetration in subterranean areas: troglonec, troglophilic and troglobitic. Troglonec dipterans are accidental, occasional or regular visitors that penetrate in caves for physiological reasons, associated with seasonal climatic variations (refuge during the dry season) or with resource gathering, but that do not complete their entire life cycle inside the cave.²¹ Troglophilic species visit and usually breed inside caves, but can be found also in epigeous areas at all stages.²¹ Troglobitic dipterans are exclusively confined in subterranean areas for their entire life cycle and their presence in epigeous area is exceptional and most often fatal.²¹

In caves, dipterans colonize all spaces, including those inaccessible to humans. Adults of flying Nematocera (for example, mosquitoes, sand flies or biting midges) rest on walls, vaults or crevices in rock walls and more rarely on soil or at the bottom of rocks. Sand flies prefer places with low air flow and are frequently observed inside secondary chambers, away from high air streams.²² Blood-engorged female dipterans are regularly observed close to bats or near rodent holes, whereas males and unfed teneral females occupy the area close to the breeding site or cave walls.²² Conversely, adult bat flies live mostly on the body of bats.²³

What is known about the diversity of blood-feeding Diptera in Afrotropical caves?

Cave-dwelling Diptera remain poorly known worldwide and the very few studies dedicated to cave-dwelling hematophagous species only reported a limited number of taxa among mosquitoes, sand flies,²⁴ biting midges²⁵ and bat flies.²⁶ Most of the species of medical/veterinary interest were found in Afrotropical caves.⁶

Mosquitoes (Culicidae). Among the almost thousand mosquito species of the Afrotropical region, only twelve have been described as cavernicolous. Because of their role in bat malaria transmission, species of the *Anopheles* (*An.*) genus have been more studied in some African countries, including the Democratic Republic of Congo (DRC), the Republic of Congo and occasionally Cameroon, Central African Republic (CAR), Gabon, Guinea and Madagascar (Table 1). Besides the *Anopheles* genus, only *Uranotaenia* (*Ur.*) *cavernicola* Mattingly in DRC, *Ur. fusca* Theobald and *Mansonia uniformis* Theobald in Madagascar were detected in caves (Table 1).

Sand flies (Phlebotominae). The current knowledge on cave-dwelling sand flies in Africa is mainly based on studies carried out in Central, West and East Africa that reported the presence of several genera:

Table 1 Known cave-dwelling hematophagous Diptera in the Afrotropical region

Species	Site	Country	Vector role	References
<i>Mosquitoes</i>				
<i>An. vanhoofi</i>	Mbanza-Ngungu	DRC	Suspected vector of <i>Plasmodium</i> spp.	18,69
<i>An. rodhaini</i>	Likasi	DRC	Suspected vector of <i>Plasmodium</i> spp.	18,70
<i>An. faini</i>	Yoloha firi	DRC	Suspected vector of <i>P. roussetti</i>	18,71
<i>An. smithii rageaui</i>	Oliga	Cameroon	Vector of <i>P. atheruri</i> and <i>P. voltaicum</i>	18,36,37,72
<i>An. cavernicolus</i>	Dalaba region	Guinea	Unknown	73
<i>An. vanthieli</i>	Irangi	DRC	Vector of <i>P. atheruri</i>	18,74
<i>An. caroni</i>	Matouridi	Congo-Brazzaville	Vector of <i>P. atheruri</i>	18,75
<i>An. hamoni</i>	Meya-Nzouari	Congo-Brazzaville	Suspected vector of <i>Plasmodium</i> spp., <i>Hepaticocystis</i> spp., <i>Nycteria</i> spp.	18,76
<i>An. pauliani</i>	Aven Anjohy	Madagascar	Unknown	77
<i>Ur. cavernicola</i>	Yoloha firi	Congo-Brazzaville	Unknown	78
<i>Ur. fusca</i>	Bemaraaha	Madagascar	Unknown	77
<i>Ma. uniformis</i>	Bemaraaha	Madagascar	Unknown	77
<i>Sand flies</i>				
<i>Sl. gigas</i>	Matadi	DRC	Unknown	27
<i>Sa. emilii</i>	Loudima	Congo-Brazzaville	Suspected vector of <i>Trypanosoma vespertilionis</i>	27,51
<i>Sa. darlingi</i>	Jabel Tozi	Sudan	Unknown	27
	Sikasso	Mali	Unknown	27
<i>Sa. moucheti</i>	Koumba	Cameroon	Unknown	27
	Bébé	CAR	Unknown	27
<i>Se. mirabilis</i>	Mbanza-Ngungu	DRC	Vector of <i>Leishmania</i> spp., <i>Trypanosoma</i> spp.	10,27
<i>Se. balmicola</i>	Akok-Bekue	Cameroon	Unknown	27
<i>Se. africana</i>	Muruku	Kenya	Unknown	79
<i>Se. bedfordi</i>	Muruku	Kenya	Unknown	79
<i>Ph. somaliensis</i>	Shama-Aleh	Somalia	Unknown	27
<i>Biting midges</i>				
<i>C. brosetti</i>	Faucon	Gabon	Unknown	80
<i>C. grenieri</i>	Meya-Nzouari	Congo-Brazzaville	Unknown	80
<i>C. rageaui</i>	Meya-Nzouari	Congo-Brazzaville	Unknown	80
<i>Bat flies</i>				
<i>P. fulvida</i>	Undetermined	Congo-Brazzaville	Suspected vector of <i>P. melanipherus</i>	20,51
	Kessipoughou, Djibilong, Faucon	Gabon		20
<i>N. schmidlii scotti</i>	Undetermined	Congo-Brazzaville	Suspected vector of <i>P. melanipherus</i>	20,51
	Kessipoughou, Djibilong, Faucon	Gabon		20
<i>E. africana</i>	Mahoume	South Africa	Unknown	32
	Kessipoughou, Zadié	Gabon	Unknown	20
<i>R. simplex</i>	Undetermined	Congo-Brazzaville	Unknown	51
<i>R. seminuda</i>	Undetermined	Congo-Brazzaville	Unknown	51
<i>R. huberi</i>	Kessipoughou, Djibilong	Gabon	Unknown	20
<i>B. allaudi</i>	Kessipoughou	Gabon	Unknown	20
<i>R. leleupi</i>	Undetermined	Congo-Brazzaville	Unknown	51

In these studies, flying dipterans (mosquitoes, sand flies and biting midges) were collected manually on cave walls or using light traps. Bat flies were collected manually on captured bats.

Spelaeophlebotomus (*Sl.*), *Phlebotomus* (*Ph.*), *Sergentomyia* (*Se.*) and *Spelaomyia* (*Sa.*). *Sl. gigas* Parrot and Schwetz (Table 1) was one of the first species discovered in caves. Although for a long time this species was thought to be endemic in DRC, it was subsequently found throughout Central and West Africa.²⁷ Further investigations reported other cave-dwelling sand fly species of the genera *Phlebotomus* (*Ph.*), *Sergentomyia* (*Se.*) and *Spelaomyia* (*Sa.*) in the continent (Table 1).

Biting midges (*Ceratopogonidae*). Among biting midges, hematophagous species mainly belong to the genus *Culicoides* and, to a lesser extent, to the genera *Forcipomyia* and *Leptoconops*. These species have been largely documented in epigeous areas in South, East and West Africa, while they remain poorly studied in Central Africa.²⁸ Although

few studies have been undertaken in some caves in Central Africa, biting midges still remain under-documented in cave environments across the continent. Nevertheless, more than twenty species have been detected in caves, including fifteen new descriptions, but only three are hematophagous (Table 1), and their biology still remains unknown.

Bat flies (*Nycteribiidae* and *Streblidae*). About 40 species and sub-species of *Nycteribiidae* and 32 species and sub-species of *Streblidae* are known in Africa.^{29,30} Most of them parasitize epigeous bats in the Congo Basin (Central Africa) and southern Africa. Bat flies have been recorded also in Nigeria (West Africa), Cameroon (Central Africa), Kenya, Sudan, Tanzania and Uganda (East Africa).²⁹ Specific studies

on bat flies that parasitize cave-dwelling bats remain rare across the African continent and only six species have been described so far (Table 1). Two Nycteribiidae species (*Penicilidia fulvida* Bigot and *Nycteribia schmidlii scotti* Falcoz) parasitize mainly cave-dwelling bats of the genera *Miniopterus* (Republic of Congo, Gabon) and *Rhinolophus* (Republic of Congo).^{20,31} *Eucampsipoda africana* Theodor infests the cave-dwelling *Rousettus aegyptiacus* Geoffroy in South Africa and Gabon.^{20,32} Three Streblidae species (*Raymondia simplex* Jobling, *R. seminuda* Jobling and *Raymondioides leleupi* Jobling) have been found on *Miniopterus* and *Rhinolophus* bats that live in caves of the Republic of Congo.³¹ In Gabon, *Raymondia huberi* Frauenfeld and *Brachytarsina allaudi* Falcoz infest the bat species *Hipposideros caffer* Sundevall and *Hipposideros gigas* Wagner, respectively.²⁰

What is known about the vector role of blood-feeding Diptera in Afrotropical caves?

In tropical caves, several thousand bats often aggregate in very large roosts, thus favouring the circulation of pathogens, including many viruses, such as Lyssaviruses, Filoviruses, Coronaviruses, Paramyxoviruses or Flaviviruses.³³ The presence of bat-biting dipterans in cave ecosystems increases the risk of arthropod-borne pathogen transmission because these insects are known vectors of blood parasites (for example, mosquitoes, sand flies and biting midges), viruses (for example, mosquitoes, sand flies and biting midges), bacteria (for example, mosquitoes and sand flies) and filarial parasites (for example, mosquitoes and biting midges).

Vector-borne parasites

Haemosporidia. Several arthropod-borne haemosporidian parasites have been identified in African cave-dwelling hosts, including rodents and bats. Among Plasmodiidae, *Plasmodium atheruri* Van Den Berghe, Lambrecht and Zoghi, which infects African porcupines, has been found in the salivary glands of *An. smithii* complex members in caves of Cameroon³⁴ and Ghana.³⁵ *An. vanthieli* Laarman and *An. caroni* Adam were also identified as vectors of this parasite in Republic of Congo.¹⁸ However *An. hamoni* Adam is a suspected vector of *P. atheruri* and other blood parasites of the genera *Hepatocystis* and *Nycteria*.¹⁸ Members of the *An. smithii* complex are vectors of *Plasmodium voltaicum* Van der Kaay, a parasite that infects the bats *Lyssonycteris smithii* Thomas in Ghana and *Lyssonycteris angolensis* Bocage in the Republic of Congo.^{36,37} Although their role has never been formally demonstrated, cave-dwelling *Anopheles* are also suspected to transmit *Plasmodium roussetti* Van Riel that infects the fruit bat *Rousettus aegyptiacus*.¹⁷

In caves, sand flies are mostly known for their role in the epidemiology of saurian Haemosporidia.³⁸ In Africa, about twenty *Plasmodium* species have been detected in reptiles.³⁹ Therefore the presence of various reptiles in caves could suggest a possible role of sand flies in saurian Haemosporidia circulation in these environments.

In the Afrotropical region, biting midges are considered the main vectors of bat *Hepatocystis* parasites in epigeous environments.⁴⁰ However, their role in the transmission of *Hepatocystis* and other parasites, such as *Nycteria*, inside caves is only suspected, but not formally demonstrated.¹⁷

After *Anopheles* mosquitoes, bat flies are the most documented vectors of bat malaria parasites in caves. *Polychromophilus* parasites mainly infect bats of several genera, including *Miniopterus*, *Rhinolophus*, *Myotis*, *Pipistrellus*, *Hipposideros*, *Antrozous* and *Glosophaga*.¹⁷ Most of these bats are found in Afrotropical caves, especially the species *Miniopterus minor minor* Peters, *Rhinolophus sylvestris* group Aellen and *Hipposideros caffer* that are infected by *Polychromophilus*

almost permanently.⁴¹ Recent investigations in Gabon caves revealed that the bat fly species *P. fulvida* and *Nycteribia schmidlii scotti* are heavily infected by *Polychromophilus melanipherus*. Moreover, the finding that both bat fly species parasitize *Miniopterus inflatus* Thomas, a natural host of this parasite,^{20,42} suggests that these bat fly species might transmit this parasite. Infections by *P. melanipherus* have also been observed in *Eucampsipoda africana*, a Nycteribiidae species that infests cave-dwelling bats.²⁰ To date, flies from the Streblidae family have never been incriminated in the transmission of haemosporidian parasites. Nevertheless, their vector role should be reinvestigated because *P. melanipherus* was molecularly detected in the streblid species *Brachytarsina allaudi* and *Raymondia huberi*.²⁰ Moreover, infecting stages of a bat malaria parasite (*Vetufefebus ovatus* Poinar) were previously observed in a streblid specimen.⁴³

Filarial, leishmanial and trypanosomatid parasites. Bats are important vertebrate hosts of filarial nematodes. Thirty-four genera of nematodes have been described⁴⁴ and *Litomosa* and *Litomosoides* (Onchocercidae) are the best known.⁴⁵ In Africa, bats, including cave-dwelling taxa,⁴⁶ are infected mainly by parasites of the *Litomosa* genus, but few cases of infections by *Litomosoides* have also been reported.⁴⁵ Infections by *Litomosa* spp. have been detected in several bat families.⁴⁷ The vectors remain unknown, although mites (Macronyssidae)⁴⁸ are suspected, as well as other dipterans, including Culicidae, Phlebotominae and Ceratopogonidae.

Sand flies are well-recognized vectors of *Leishmania* parasites in the tropics. Recent studies in caves of the Neotropical region (also called South American region) revealed that bats are infected by *Leishmania* sp.⁴⁹ In Africa, cave-dwelling bats are important natural hosts of *Leishmania* parasites;⁵⁰ however, the role of sand flies as vectors remains to be confirmed, despite their frequent co-occurrence with bats in caves.

Trypanosomatid parasites circulate among cave-dwelling reservoirs, including bats and rodents, in the Afrotropical region.⁵¹ The transmission modalities remain under-investigated. In the Neotropical region, sand flies are known vectors of trypanosomatid parasites.⁵² Conversely, in the Afrotropical region only the genus *Spelaomyia emilii* Parrot and Wanson is suspected to be involved in the transmission of *Trypanosoma vespertilionis* Battaglia that infects cave-dwelling bats in the Republic of Congo.⁵¹ Besides sand flies, mosquitoes, particularly within the genus *Culex*, are suspected to be vectors of avian,⁵³ amphibian⁵⁴ and reptilian⁵⁵ trypanosomatid parasites.

Viruses. Currently, it is not clear how hematophagous arthropods are involved in the circulation of viruses in cave ecosystems due to the limited number of studies on this question. Cave-dwelling vertebrates, including bats, are recognized hosts of a large diversity of zoonotic viruses, such as Paramyxoviruses, Lyssaviruses, Coronaviruses, Filoviruses, Hantaviruses and particularly arboviruses (for instance, the Flaviviridae, Bunyaviridae and Reoviridae families).³³ Some of them cause diseases in humans or in domestic animals.^{56,57} Most of the arboviruses or potential arboviruses found in African bats have been reported or isolated from the bat genera *Nycteria*, *Miniopterus* or *Hipposideros*.³³ Considering the major role of dipterans, such as mosquitoes, sand flies, bat flies or even biting midges, in the transmission of bat arboviruses,⁵⁸ it would be of great interest to thoroughly investigate the epidemiology of arboviruses in cave ecosystems, by carrying out arbovirus screening in cave-dwelling bats and hematophagous dipterans.

Bacteria. Little is known about the role of hematophagous insects in the epidemiology of bacteria that infect wild mammals living in

African caves. Among the known arthropod-borne bacteria, *Bartonella* spp. is one of the most widely transmitted among vertebrates, including bats.⁵⁹ In cave environments, bats are the main hosts of bacteria and bacteria are mainly transmitted by bat ectoparasites, such as ticks (Arachnida) and bat flies.⁶⁰ In Africa, recent studies described *Bartonella* spp. infections in the cave-dwelling bats *Eidolon helvum* Kerr and *E. dupreanum* Schlegel and Pollen, and showed the potential role of the Nycteribiidae *Cyclopodia greefi greefi* Karsch and *Cyclopodia dubia* Westwood in their transmission.^{61,62} Although the *Bartonella* species were not identified, previous phylogenetic investigations highlighted the strong similarity between the *Bartonella* spp. found in bat flies and in bats, suggesting that bat flies might be the involved vectors.⁶³

Like bat flies, sand flies also are *Bartonella* spp. vectors.⁶⁴ Although *Bartonella* spp. circulate in Afrotropical cave ecosystems,⁵⁹ sand fly role in their transmission has not been investigated yet.

Borrelia bacteria infect different bat species worldwide,⁶⁰ but little is known about African bats. Ticks are the primary vectors of *Borrelia* bacteria⁶⁵ and several mosquito genera, such as *Anopheles*, *Culex* and *Aedes*, are suspected to ensure their transmission.⁶⁶

Challenges and future research directions

The circulation of a wide range of pathogens hosted by vertebrate reservoirs in cave environments constitutes a potential major hazard for the health of humans and domestic animals because of the cave progressive anthropization. Some previous studies have shown that some species of sand flies (for example, *Sl. gigas*) and mosquitoes (for example, *An. hamoni* and *An. caroni*) are opportunistic and can feed on bats, rodents, and occasionally humans.^{10,67} Some caves that host large communities of hematophagous dipterans are located in villages, towns, and even in farming concessions.¹⁹ This can increase the contact between cavernicolous hematophagous dipterans and humans, thus favouring the accidental transmission of pathogens to humans or livestock. Such contacts and transfers could occur when people or domestic animals enter inside caves, but also via troglonec dipteran species that could bring pathogens from inside the cave to the cave surroundings, which may include areas inhabited by human populations. Depending on the pathogen and on the host susceptibility, a new pathogen could emerge in humans and cause local outbreaks, if it can find a suitable vector to ensure its epidemic transmission among humans. Due to their ability to transmit some pathogens, cave-dwelling hematophagous dipterans are of important concern for researchers and health authorities. Among the Diptera-related factors that contribute to the zoonotic hazard of cave environments, the most important are: (i) the ability to support natural enzootic and intra-specific pathogen cycles that involve a natural primary reservoir. This could increase the risk of opportunistic or accidental inter-specific transmission to humans or domestic animals; and (ii) the ability of cave-dwelling hematophagous dipterans, particularly troglonec insects, to bite humans or domestic animals, even accidentally. However, contacts between cave fauna and humans are not yet widespread and general, are still restricted to the surrounding populations and only occasionally to far-away victims.

To date, little is known about Diptera ecology and their vector role in Afrotropical caves. Indeed, basic data were collected several decades ago by few passionate researchers who made substantial efforts to overcome the inaccessibility and hostile environmental conditions of African caves. This pioneering research was not further pursued, although many questions remain unanswered about the transmission cycles involving arthropod vectors. Recent technical advances (that is, molecular biology and high-throughput sequencing tools for vector taxonomy and

pathogen screening) could certainly help filling in these gaps. The renewed interest on cave-dwelling Diptera and associated pathogens, as attested by recent studies in Gabon, provided new insights into the diversity of cave-dwelling insects of medical importance.²⁰ The discovery of several pathogens in cave-dwelling reservoirs using advanced molecular tools⁶⁸ should help assessing the risk of transfer to humans or domestic animals that live in the vicinity of caves. Indeed pathogen surveillance of wild and domestic animals in cave-associated areas would improve our knowledge on the transmission risk as well as on arthropod-borne pathogens and their associated vectors. Studies on parasite ecology, such as their life cycle and the gonotrophic cycle of vectors, could bring crucial information on how these bioecological features influence pathogen epidemiology in cave-associated areas. Extending research to caves of new areas and to other arthropod groups of medical and veterinary importance, such as bugs, fleas and ticks, will provide integrated data on the diversity, spatial distribution and role in pathogen epidemiology of cave-dwelling hematophagous arthropods.

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