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PERSPECTIVES

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An Ecologically Framed Comparison of The Potential for Zoonotic Transmission of Non-Human and Human-Infecting Species of Malaria Parasite

Nicole F. Clark[a,b] and Andrew W. Taylor-Robinson[c,d,*]

[a]*Institute for Applied Ecology, University of Canberra, Bruce, Australia;* [b]*College of Medicine and Public Health, Flinders University, Australia;* [c]*Infectious Diseases Research Group, School of Health, Medical & Applied Sciences, Central Queensland University, Brisbane, Australia;* [d]*College of Health & Human Sciences, Charles Darwin University, Casuarina, Australia*

The threats, both real and perceived, surrounding the development of new and emerging infectious diseases of humans are of critical concern to public health and well-being. Among these risks is the potential for zoonotic transmission to humans of species of the malaria parasite, *Plasmodium*, that have been considered historically to infect exclusively non-human hosts. Recently observed shifts in the mode, transmission, and presentation of malaria among several species studied are evidenced by shared vectors, atypical symptoms, and novel host-seeking behavior. Collectively, these changes indicate the presence of environmental and ecological pressures that are likely to influence the dynamics of these parasite life cycles and physiological make-up. These may be further affected and amplified by such factors as increased urban development and accelerated rate of climate change. In particular, the extended host-seeking behavior of what were once considered non-human malaria species indicates the specialist niche of human malaria parasites is not a limiting factor that drives the success of blood-borne parasites. While zoonotic transmission of non-human malaria parasites is generally considered to not be possible for the vast majority of *Plasmodium* species, failure to consider the feasibility of its occurrence may lead to the emergence of a potentially life-threatening blood-borne disease of humans. Here, we argue that recent trends in behavior among what were hitherto considered to be non-human malaria parasites to infect humans call for a cross-disciplinary, ecologically-focused approach to understanding the complexities of the vertebrate host/mosquito vector/malaria parasite triangular relationship. This highlights a pressing need to conduct a multi-species investigation for which we recommend the construction of a database to determine ecological differences among all known *Plasmodium* species, vectors, and hosts. Closing this knowledge gap may help to inform alternative means of malaria prevention and control.

*To whom all correspondence should be addressed: Prof Andrew W. Taylor-Robinson, Infectious Diseases Research Group, School of Health, Medical & Applied Sciences, Central Queensland University, 160 Ann Street, Brisbane, QLD 4000, Australia; Tel: +61 7 3295 1185; Email: a.taylor-robinson@cqu.edu.au; ORCID iD: 0000-0001-7342-8348.

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INTRODUCTION

Malaria is a disease of vertebrates that is caused by apicomplexan protozoan parasites belonging to the genus *Plasmodium*, among which five species are considered to be specialist human parasites [1], while up to 30 infect non-human primates [2]. There is a broad spectrum of specialization that exists in nature, so that not all specialists are parasites and not all parasites are specialists [3]. However, in changing conditions it might be argued that there are no true specialists and that generalism is simply a means to avoid one's own extinction [3,4]. While it may be reasoned that parasite species which depend on one host only are not likely to stray from such an arrangement, the very existence of zoonoses provides a counterpoint [5]. Like those between humans and *Plasmodium* species, the relationship between host and parasite is not mutually exclusive [6]. Hence, the ability to infect a range of hosts forms a strategic and imperfect relationship, rarely dependent on a single factor but instead a collection of unique and varied biological drivers [7]. One might consider that when placed under specific pressures, all species are destined to specialize. However, this does not guarantee success, especially in the case of parasites, as specialization is rarely conducive to either host or parasite success [3].

As evidenced by the current COVID-19 pandemic as a prime example of a disease of zoonotic origin [8], one need not understand the origin of a disease in order to take control and prevention measures. There are many other examples of epidemics of newly emerging and re-emerging infectious diseases that continue to pose a risk to public health [9]. Indeed, to date only one infectious human disease, smallpox, has been eradicated successfully [10]. Significant proportions of the global population have experienced ill-prepared management of healthcare responses to unexpected localized epidemics that have swept across South America, Asia, and Africa, taking millions of lives [11]. However, prevention is better than cure, particularly when it concerns *new* and emerging infectious zoonotic diseases [12,13]. Without identifying potential threats, we cannot hope to prevent all zoonotic disease, as it is not sufficient to simply identify established human pathogens and to treat only outbreaks of those known diseases that they cause [13-15]. The emergence of SARS-CoV-2 has demonstrated a lack of knowledge in relation to zoonoses, highlighting that what we think we know is often contrary to what we are able to observe, let alone quantify [8,12,13]. Unfortunately, such is the short-term nature of politics that governments are committed to resolve issues head on, identifying a problem only when it becomes one [16], so in effect we wait until the new disease finds us. However, there is undeniable logic in considering that this "band-aid approach" will eventually fail because for zoonotic diseases it is not a question of *if*, but instead *when*, and how soon the next outbreak will occur.

For fear of stating the self-evident, not all infections nor the organisms that cause disease are equal. Many infections are caused by viruses while others are caused by bacteria and some – such as malaria – are caused by protozoan parasites. Given that all species must adapt to survive or else face extinction, a number of factors are independent of infection control procedures [7]. What is now recognized as *Plasmodium* began as a simple protozoan species diverging from a single ancestor under the same pressures as the clade from which it diverged [17]. As for malaria parasites, ecological investigations have revealed that parasites first thought to be specialists infect not one but a kaleidoscope of host organisms, offering plenty of potential to diversify infectious zoonotic disease [3,18]. Therefore, parasitological research that focuses on and assumes an exclusive relationship between a single parasite and a single host species, such as that between humans and *Plasmodium* species, exposes a potential fallacy due to the contraindicatory existence of zoonoses. As history reminds us, diseases can arise in humans from animal reservoirs in just a few short decades; for instance, supposedly "non-human" malaria species are not incapable of making the switch to infect humans [19-21].

Transmitted by the infectious bite of female mosquitoes of the *Anopheles* genus, the blood-borne disease commonly referred to as human malaria has long been considered a major public health concern. The four species of malaria parasite that typically infect human beings are *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, to which may be added two relatively new discoveries of *P. knowlesi* and *P. cynomolgi* [22,23]. Malaria is responsible for the death and suffering of millions of people every year, mainly in the tropics and sub-tropics, so presenting an ongoing global health challenge to reduce its unacceptable burden [5,15,23,24]. Despite decades of development the approval of an efficacious vaccine is still a distant dream, a situation compounded by the continuing heavy reliance on increasingly less effective chemotherapeutic regimens to which parasite resistance has evolved [25]. There is a unique association between vectors, parasites, and their human host species that is complicated further by the transmission of the disease [26]. Moreover, efforts to curb the disease are confounded by the need to control the biting behavior and widespread distribution of *Anopheles* vectors that facilitate transmission of the parasite. In recent times other mosquito species have also been shown to be permissive to infection [27,28]. Due to these complex and compounding factors, how to combat malaria is regarded to be one of the greatest and most enduring problems faced by humanity [20,26].

ECOLOGICAL PERSPECTIVES OF MALARIA

Traditional disciplines such as parasitology that are founded on the observation of host-parasite interactions seek to understand epidemiological concepts [18,20,29]. However, while effective at offering ways to manage outbreaks, these approaches arguably provide little scope for identification of disease origin and hence zoonotic origin or "patient zero" [30]. Malaria is a multi-faceted, multi-dimensional host-parasite system that encompasses a complex interplay of selective pressures, including co-adaptation and speciation, all accelerated under a host-parasite relationship [4,19,31-37]. The association between host and parasite is as complex as that between parasite and vector [33]. Each stage of parasitism is critical to the process, with the end goal to reach the host [27]. By definition, a *Plasmodium* parasite requires a vector for transmission to the human host, yet this target is often confounded by virulence, resistance, and in some cases, by the death of the host [27,38]. Survival of both parasite and host may be seen as the favorable outcome of a complex arms race, where the death of a parasite signals a dead end host and over time repetition of such an event may spell local or global extinction [31,39]. Unfortunately, the success of human malaria is inordinately high [26,40,41]. Thus, finding answers to a multi-faceted, arguably 'wicked' problem requires a similarly multi-faceted approach. At a time when zoonotic diseases are on the rise, we cannot afford to ignore potentially novel solutions, particularly those that are ecologically focused [4].

In order to understand *how* and *why* these infections occur, gaining an awareness of the ecology of malaria parasite systems is crucial [5,34,36,37,42,43]. However, most studies so far have not enabled accurate predictions of wide-scale changes concerning the zoonotic potential of parasite species, including *Plasmodium* [32,33,44]. By focusing narrowly on a select few model species non-human plasmodia are largely ignored. Therefore, it is useful to take a pliable perspective of these variables to predict systems flux [42,44,45]. As is frequently attempted with ecological investigations, such a view may be constructed by drawing on both new and existing knowledge that does not limit our observations to a possible specialist fallacy [3,46].

Studies that attempt to describe limiting factors among non-human malaria species are unfortunately seemingly obscured by an overwhelming body of literature that captures only a fraction of malaria-host interactions, while no research has explored the zoonotic potential and risks of non-human malaria parasites [27,47]. The application of genetics has refined potential nuances as all forms of life on earth are bound by the species concept [48], including blood-borne parasites [49]. Certainly, the success of human malarias indicates a unique and highly specialized host-parasite association [35,50,51]. Although the relative contributions of genetics versus environment are not discussed in depth here, importantly Darwinian explanations that focus on the species concept highlight the need to understand ecological perspectives which elucidate *how* specific parasite species – regardless of their phylogenetic origin – operate in their present environment, *before* they are labelled as the etiological agent of a new zoonotic disease. Thus, identifying such risk factors offers the potential to determine not only risk species but how each operates within its current ecological niche [7,14,30,52].

Several studies have employed a mixed methods approach to start to explore multi-species dimensions [35]. For instance, a few species that are etiological agents of rodent and primate malarias are being explored in place of species that cause malaria in humans [41,42,50,53]. Additionally, species that cause avian malaria have been utilized to model the transmission and overlap of multiple host and vector species by comparing risks associated with potential zoonosis [36,45,54]. In general, however, standardized methodologies limit these comparisons to a single or handful of species of either host or parasite, largely ignoring a plethora of unidentified risk factors in other potential risk species [35]. Consequently, these investigations lack insight and thus represent neither an ecological nor a multi-species approach capable of detecting critical changes that lead to zoonotic disease outbreak [31,32].

While a strong inference from these alternative studies is that multiple species can provide a platform for inquiry [6,42], there has been precious little research to address potential shifts in the various mechanisms that drive the transmission of human malaria species. Importantly, surveillance studies should consider zoonotic risk from an ecological perspective and identify factors among all *Plasmodium* species, especially those thought capable of making a switch to humans. The proceeding sections discuss potential risk factors from both biological and ecological perspectives. Such multi-disciplinary observations may not only help to identify risk factors among existing human malarias but also to determine zoonotic risk relating to a broad range of apicomplexan parasite species.

ZOONOTIC RISK

The incidence of zoonoses is on the rise [52,55]. This poses a significant public health concern that among malaria-host systems has yet to be addressed [4,35,39,43]. As discussed above, several studies have shown that parasite species respond differently to environmental and ecological pressures which accelerate changes in these

systems [4,6,36,51,56,57]. Identifying and/or evaluating the perceived risk among new and emerging infectious diseases is an important step towards the development of strategies that may be implemented to control infectious disease epidemics [39,43,51]. An equally vital step is to gain an understanding from an ecological perspective of the mechanisms that drive the development of these diseases [4]. Only through a comparative examination of the responses of multiple species experiencing the same environmental pressures can we start to comprehend those which present the greatest threat of zoonotic disease in humans [29,34,36,37,58].

It is important to ascertain whether physiological and biological limitations truly exist among all non-human blood-borne parasites. As a result, and if subject to selective pressures, might zoonotic potential exist on a sliding scale such that some species present as a greater risk than others? If so, concentrating solely on *Plasmodium* species that currently infect humans is not necessarily an accurate predictor of future human malaria risk factors since narrowing the focus may inadvertently dismiss the zoonotic potential of other blood-borne apicomplexans [27,36,37,51,59]. As a result, it may be important to consider not only how human malaria species adapt to change, such as through selective mutations for chloroquine resistance [60], but how different or how well neighboring species infecting other organisms also perform or respond to these changes [30]. This is because, despite the apparent differences between non-human and human malaria parasites to selective pressures [51], accelerated ecological and evolutionary drivers, such as rate of climate change and human encroachment [4], can drastically alter the behavior of blood-dwelling parasites, especially their biology and physiology [61].

SPONTANEOUS MUTATIONS

From an anthropological perspective it is easy to overlook the fact that malaria is not exclusively a disease afflicting humanity. Hence, the evolutionary success of malaria parasites is not measured exclusively by the infection of humans [62]. Malaria parasites belong to a diverse phylum known as Apicomplexa, wherein several species belonging to the order Haemosporidia [21,34,36,37,62] are known to inflict malaria or malaria-like symptoms on a range of vertebrate host species [63]. The family Plasmodiidae comprises more than 200 species of the genus *Plasmodium* which cause malaria in animals, among them the five aforementioned species that are now recognized to infect humans [21]. Evolutionarily convergent with this, in a suggested polyphyletic group in the family Haemoproteidae, are several species of the genus *Haemoproteus,* which are also known to cause malaria among, birds, reptiles, and amphibians – collectively (if, arguably, incorrectly) referred to as "avian malaria" [34,36,39,62,63].

Most parasites that cause malaria rarely specialize; species which are responsible for avian malaria form an incredibly diverse taxon [34,36,37]. Their adaptability and potential to acquire new hosts is not an exception to the rule as most species are generalists [17,18,36,37,64-66]. However, physiological differences between avian malaria and human malaria do exist. Avian malaria parasites belonging to the Plasmodiidae (a taxon also including the Haemoproteidae) do not undergo asexual reproduction during the blood stage of the life cycle, a key difference that distinguishes *Haemoproteus* from *Plasmodium* and thus human malarias [21,35,51].

Considerable debate has long existed regarding the phylogeny of malaria parasites. Current thinking is that of the recognized human species at least *P. falciparum* is unlikely to have originated from an avian counterpart but instead is of ape origin [51,67]. An earlier proposal of haemosporidian ancestry remains unresolved as different research on limited DNA sequence data examined mitochondrial and apicoplast lineages, thereby leading to contradictory findings [5,25,34,65,68-70].

There has not been a recorded case of infection of humans by any avian malaria parasite species. An unethical study performed in the 1940s that saw two individuals of African descent inoculated with *P. relictum* demonstrated a slight rise in temperature but did not induce malaria. It was concluded that a lack of blood schizogony may explain why avian malarias do not cause disease in humans [71]. However, human disease potential has been examined for only one of several species of avian malaria. Asexual blood stage parasites are also prone to spontaneous epigenetic activation, making them physiologically capable of altering their reproductive strategies to suit prevailing conditions [72]. This suggests that if even a single limiting factor is breached, leading to just one isolated event (namely asexual reproduction by an avian malaria), hypothetically it is feasible that a single oocyte could result in a new human disease [73]. This scenario presently poses a low zoonotic risk. Yet, we should be vigilant to the future possibility of selective pressures exerted by escalating anthropogenic changes to the environment, such as climate conditions or geographical access, driving the emergence of novel parasite phenotypes [74,75].

The ability to switch between alternative reproductive strategies, such as turning on and off asexual reproduction, is firmly accepted for many unicellular organisms (prokaryotes) when subjected to rapid changes in environmental stressors such as temperature and oxygen levels [51,76-78]. Several possibilities for mutation are likely to exist as blood schizogony is not a limiting factor, extra-erythrocytic development also occurring in the bone

marrow [79]. Thus, arguably the zoonotic threat from avian malarias cannot be excluded [27,79]; several species that do not infect humans infect our close relatives – a key marker of zoonotic potential [70,80]. The phylogenetic origin of blood-borne parasites [34,36,37,81] does not compromise their ability to induce disease in higher order eukaryotes [66]. Indeed, we know that zoonoses of other pathogens can emerge even among distantly related species, as evidenced very recently by SARS-CoV-2 outbreaks reportedly originating from animal reservoirs of bats, pangolins, and mink [8,82]. In common with all disease-causing microbes, *Plasmodium* species undergo genetic mutation. Hence, while the possibility of people becoming infected by malaria parasites of avian origin and/or other haemosporidians may be slim, it should not be ruled out.

The propensity for spontaneous mutations is also established for human malaria species, exemplified by drug resistance to chloroquine and artemisinin [60]. There are several instances where spontaneous mutations have been reported among multiple *Plasmodium* species [83], suggesting current limitations of non-human malaria parasites are likely to be temporary. Risks may also come through close contact between humans and animals, providing an additional route for spontaneous mutations to occur. Additionally, recent research on the behavior of avian malarias suggests that one species may alter the behavior of its mosquito vector but the exact function of this is still not known; this knowledge gap could be masking a serious public health risk [61]. Furthermore, assessment of the genomic ancestry of six genetically distinct populations of *P. falciparum* in sub-Saharan Africa demonstrated that the phylogeography of human malaria parasites is subject to artificial pressures, selecting for resistance to uncomplicated treatments [5,24]. Genetic variance and inbreeding occurred among these isolates from the time antimalarials was introduced, where selective evolutionary constituents passed on artemisinin resistance associated with human populations [24].

While biological and physiological differences in non-human malaria parasites – in a vacuum – may *initially* act as barriers to limit zoonotic potential, given their unicellular nature and rapid replication rates, the genetic and life history traits of these organisms suggest the contrary [66,70].

MITOCHONDRIA AND PLASTIDS

Spontaneous mutations may also occur in organisms of secondary endosymbiotic origin. Plastids are highly sensitive organelles able to alter their function according to specific changes in their environment, such as light, temperature, and water loss [47]. Organisms containing a plastid can substantially alter their biology in response to epigenetic cues immediately following environmental triggers [47,84]. Unique to all apicomplexans, species of the order Haemosporidia contain a remnant chloroplast or plastid, an endosymbiotic organelle referred to as the apicoplast [85]. This ancestor of chloroplasts has lost its photosynthetic capability but has retained components of the electron transport chain for photosystem I, ie, ferredoxin and NAD^+ [85].

The apicoplast is an indispensable organelle, functioning as an important site for the metabolism of haem and type II fatty acid synthesis, making it an excellent drug target [86,87]. However, the precise role that these metabolic pathways play in the reproductive strategies and/or virulence of malaria parasites is not completely known [88-90]. Additionally, the versatility and flexibility of plastids in secondary endosymbiosis is well-documented, including accounts of complete migration of plastome transcripts and spontaneous activation of complete nuclear transcription for photosystem I and photosystem II [91,92]. Complete recovery of a photosystem in the prokaryote cyanobacterium *Anabaena* has been reported [92]. Similarly, a non-photosynthesizing apicomplexan in the gut of live corals contains the full complement of genes involved in chlorophyll biosynthesis – enabling the genetic capacity when prompted [93]. These diverse examples draw attention to the potential threat carried by advantageous and spontaneous mutations among apicomplexans.

Plastids can function with minimal genes and several photosynthetic eukaryotes are shown to adapt despite this apparent limitation, indicating possible flawed rationale in designing drugs to arrest plastids and thus, indirectly, parasite function. Plastomes of two mutant plants seemingly functioning normally showed complete arrest of photosystem II, a key component of respiration – but which apicomplexans function without – yet with survival of chloroplast function [94,95]. Similarly, plastids found in parasitic plants function comparatively to those in apicomplexans [96]. Several species of parasitic plant containing a plastid have evolved beyond a reliance, either partial or complete, for photosynthesis [96]. This marks an important parallel between parasites and the perceived competence of plastids to function efficiently while possessing minimal hardware required for energy production. This highlights the risk that spontaneous mutations to existing human malaria species as well as to new potential zoonotic species may pose to human wellbeing.

The plastid is not the only apicomplexan organelle that is subject to spontaneous mutations as the mitochondrion is also susceptible to changes. In fact, human malaria parasite species are known to alter the function of mitochondria as well as the apicoplast, evidenced by resistance to specifically targeted drugs [5,97]. It is

appropriate here to reiterate that plant parasites, which like apicomplexan parasites also share both a plastid and a mitochondrion, offer a potentially useful comparison. For the parasitic mistletoe *Viscum album* it was reported that massive gene loss had occurred due to epigenetic phosphorylation, causing altered biochemical pathways. The parasite was found to completely lack mitochondrial respiratory complex I activity [98,99], an essential function of the mitochondrial electron transport chain that until recently was considered indispensable to mistletoe. Drawing comparisons between apicomplexans and plant parasite species can provide insight into the strengths and weaknesses concerning the function of indispensable organelles in parasite species, particularly if the mitochondrial and plastid genome sequences of both are known. These examples provide support for the notion that stressors, both environmental and artificial, may trigger spontaneous mutations in secondary endosymbiotic organelles of parasite species, and thus which may be applied to the apicoplast or mitochondria in *Plasmodium* [5,24,90,96]. Monitoring such risks may confer an additional benefit by offering a deeper insight into the future management and control of existing and new human malaria species [87,100].

The zoonotic potential caused by spontaneous mutations among apicomplexan parasite species should not be overlooked [27,36,59,81]. This suggests that recognized physiological barriers such as a lack of blood schizogony and organelle function do not appear to limit the potential risk factors towards zoonotic malaria. Such false securities more than likely run contrary to any risks imposed on new and emerging infectious diseases and thus must be identified [22,37,61,81,101]. Hence, regardless of any differences between non-human and human malaria species, available evidence suggests that species that do not currently infect humans could, in response to stressors, still be capable of spontaneous mutations [21,36].

SHARED VECTORS

Despite the numerosity of blood-borne parasites, records of vector species are limited. In particular, knowledge concerning vectors of avian malaria are fragmented at best and in some cases data on vectors are absent entirely, particularly for those that infect reptiles [54,64,102-104]. Although not considered to induce disease in humans, a number of vectors of non-human malaria still bite humans [105-108]. Therefore, with the exception of *P. relictum* it is unclear whether or not even a single one of these species is capable of transmitting infection to humans or whether one or more species may already cause as yet unrecognized disease in humans.

Amplifying this risk is the sharing of vectors between humans and animals, particularly those that transmit non-human malaria but also come into contact with humans to transmit other human diseases such as lymphatic filariasis [109,110]. Likewise, some vectors of avian malaria include those that transmit human malaria [64,105]. While *Haemoproteus* is not yet known to infect humans, recent findings demonstrate frequent contact between humans and this species does occur. Dissection of known dipteran vectors for species of avian malaria revealed that blood meals contained both the host and human blood, indicating a shared zoonotic risk for *Plasmodium* species, but also for transmission of other blood-borne pathogens [64,103,105].

NOVEL HOSTS AND VECTORS

Given the vast body of literature available it is difficult to discern clearly novel host trends, yet the etiology, epidemiology, and case reports of human malaria have changed over recent decades. Documented risks of the disease and the number of parasites that routinely infect humans are continuing to rise [22,57,101,111]. Moreover, the emergence of human *Plasmodium* infection have occurred via zoonoses of primate malaria parasites [53,112]. It was long thought that only four species are capable of naturally infecting humans. However, due to the discovery of zoonosis of *P. knowlesi* and now more recently *P. cynomolgi* this count has risen to six [22,113,114]. It should be noted at this point that *P. cynomolgi* has been identified only as a novel threat and is not yet officially recognized as a disease of concern [115]. Additionally, some of these species, notably *P. ovale*, contain sub-species, demonstrating plasticity among the genetic profile of human malaria parasites [116-118].

While the success of *Plasmodium* species is most likely attributed to asexual reproduction of blood stage parasites in the host [51,119], partially lowering zoonotic risk for other species, there are several plasmodia that reproduce by blood schizogony [80]. As a consequence, a number of *Plasmodium* species infecting primates have been found to be "infection compatible" with humans [53]. In fact, several cases of zoonotic infection have been recorded. Controversial studies carried out in the 1960s involved inoculations of blood containing *P. cynomolgi*, *P. simium, P. brasilianum,* and *P. inui*, all of which are known to infect non-human primates. Each of these species induced malarial infections in humans, the only difference being that they were not naturally acquired, as no natural vector for humans could be identified [113,120-122]. Alarmingly, this absence of a permissive vector to transmit non-human primate malaria parasites is all that stands in the way of preventing "infection compatible" species from causing a potentially devastating zoonotic outbreak [53,113,114,120,121]. Currently, *P. brasilianum* and *P. simium* are classified as distinct species to *P. ma-*

lariae and *P. vivax*, respectively [123,124], with which they are morphologically similar and likely genetically synonymous. As for initial reports of *P. cynomolgi*, infections of these two primate malaria parasites were very recently naturally acquired but records of the vectors responsible for disease transmission are lacking [122,125]. It is therefore unclear if existing or new vectors contributed to transmission, ie, whether or not vectors of non-human primates were responsible for transmission of these zoonotic parasites [122,125,126]. If naturally acquired infections of these species continue to occur they may induce severe clinical symptoms of malaria in humans, each leading to the potential development of a novel human disease, thus representing a series of zoonotic risks that cannot willfully be ignored [35,56,126].

As discussed previously, extensive zoonosis has already occurred in a number of instances [121,127,128]. Improved DNA-based diagnostics have contributed to increased case reporting of *P. knowlesi* malaria in humans, although infection with this parasite species was probably quite prevalent historically [129]. Indeed, the identification of wide-scale infections of *P. knowlesi* in Southeast Asia was the key factor that led to the declaration of a fifth human malaria parasite [128]. This is despite human-to-human transmission being deemed unlikely [130]. Furthermore, natural transmission of *P. knowlesi* occurred through the bite of a mosquito that also transmits malaria among a species of macaque. Seemingly due to close contact of people with vectors of primate malaria, a single zoonotic event resulted in the new human disease that is evident today [22,112,121,127,131]. Of concern, the vector(s) responsible for transmission of *P. brasilianum and P. simium* are not known. In the coming decades both of these species may be declared a seventh or eighth human malaria parasite [121-123]. This dearth of information concerning both parasites and vectors of human malaria means that our knowledge on this subject is lagging behind. Investigation of zoonotic blood-borne parasites is in its infancy, yet it is critical these risks be identified so that robust public health measures can be implemented [127]. It is difficult to ascertain whether the presentation of new human malaria species is an artefact of recent change or rather it is simply the revelation of interactions that have always occurred. It may be objectively argued that several studies point to the increased intensity of these interactions in recent decades [22,106,131,132].

On a similar theme, recent research suggests the occurrence of crypsis among vectors such that there may be undescribed species of anopheline mosquito additionally transmitting malaria among humans [103]. Again, it is currently not clear whether these interactions have always occurred or whether the ecology of vectors, along with malaria parasite species, has also altered in recent decades. This adds yet another layer of complexity to zoonotic risk factors as several studies have uncovered accounts of malaria caused by *Plasmodium* species that were initially misdiagnosed, all in locations where a naturally acquired infection was reported [48,114,133]. This raises the likelihood of misidentification of both parasite and vector species [134,135], as a consequence of which identification of potential new disease transmission may be delayed. There is increasing evidence that *P. vivax*, *P. malariae*, and *P. ovale* are often misdiagnosed by conventional light microscopy examination of Giemsa-stained blood smears in regions where there is overlapping distribution of these species. Notably, *P. malariae* is frequently mistaken for *P. ovale* [136-138]. Such errors could quite feasibly extend to the misidentification of a novel human disease as an established human malaria – perhaps due in part to unconscious bias by the microscopist.

Malaria parasite-host triangulation and the strategies of these species appear more plastic and less specialized than initially defined [5,24,56]. Several *Plasmodium* species have likely adapted to exploit new and different ecological niches. Hence, hosts, parasites, and vectors of malaria are each driven to evolve under selection pressures, particularly now in response to anthropogenic factors such as increased encroachment of forested areas through urbanization and the accelerated rate of climate change [5,7,24,30,59,139]. This strongly indicates that blood-borne parasite species are already responding to such environmental flux by seeking new hosts, thus achieving novel ways to transmit malaria. As a result, it is highly probable that a number of plasmodia may be capable of switching host, as suggested by several examples [48,59,105]. However, it should be recognized that adaptation of *Plasmodium* species to their *Anopheles* mosquito vectors involves a complex molecular interplay of non-specific protein expressions [140]. Such interactions between parasite and host are not determined by a single factor. Hence, further research is required to distinguish normal risk from that of zoonotic risk, ie, drivers of disease enhancement versus apparent zoonotic risk factors.

CRYPTIC AND NON-ENDEMIC DISEASE

The means of spread of established human *Plasmodium* species is well-documented and the distribution of both parasites and vectors is closely monitored by surveillance systems in endemic countries at local, regional, and national levels. Nevertheless, it is important to identify how changing malaria parasite behavior, particularly novel vector transmission, will drive the development and geographical expansion of hitherto unrecognized disease risks. In particular, a need for epidemiological vigilance applies to existing human malaria parasites, especially in non-endemic regions. Asymptomatic cases of human

malaria are on the rise, making it challenging to detect the disease across all continents [141-143]. Incidence of asymptomatic or latent malaria infections are increasingly recognized as major contributors to the chronicity of local community disease outbreaks, especially in endemic regions where they act as reservoirs of persistent parasitemia [144]. Once thought to be benign, the long-term health of subpatent individuals is now considered to be compromised since asymptomatic malaria has been recently linked to severe presentations of malnutrition, auto-immune deficiencies, and neuropathological abnormalities [143-145].

As asymptomatic cases of malaria often pass unnoticed in non-endemic regions any suspicions of a patient are likely to be dismissed by an unsuspecting medical practitioner, so chronic illness, and even in extreme cases, death may ensue [143]. Situations have arisen in which malaria was contracted via blood transfusion as, due to a lack of screening, asymptomatic donors unknowingly transmitted the disease to immunocompromised patients [142]. Moreover, mixed infections with multiple parasite species are reported with increasing frequency [146-148]. Similarly, some of these infections, dual or otherwise, are increasingly shown to resurge after cessation of initial treatment, as well as to last very long times – three decades or more, with one case reported to be of 50 years' duration [117,149].

Asymptomatic cases of malaria have also made their way into non-endemic regions, where a phenomenon known as "baggage malaria" occurs [150]. This term describes how an asymptomatic or infectious but untreated person arriving from an endemic region passes on malaria to one or more individuals who are close contacts living in a non-endemic region [150]. Hence, by virtue of close proximity – such as living in the same household – and despite themselves not entering an endemic region, the third party contracts the disease via the bite of a novel yet compatible non-endemic mosquito species belonging to the genus *Anopheles* [133]. Asymptomatic baggage malaria likely results from a failure of continual prevention strategies and/or initial adequate treatment of the disease [151]. Since few cases of baggage malaria are symptomatic, authorities are not on high alert to the threat so that asymptomatic infections tend to go unrecognized, thereby creating an opportunity for the spread of imported malaria [141]. Temperature may also play a role, wherein the risk of baggage malaria may increase when infected individuals in non-endemic regions experience a similar climate to endemic regions [152]. This highlights the emerging threat posed by climate change due to the effect on newly permissive vectors and novel presentations of malaria in both endemic and non-endemic regions. Rising temperatures may lead to malaria gaining a foothold in places where it has not occurred in the recent past and where awareness of its dangers would be slow to be recognized.

CONCLUSION

The cumulative anthropogenic pressures imposed by escalating human development, urbanization, and accelerated rate of climate change are already shown to significantly alter malaria parasite behavior. Changes in the disease itself over recent decades are strong indicators of zoonotic risk. Exposure of *Plasmodium* species to fluctuating environmental conditions have altered clinical presentations, leading to greater intensity of symptoms, ie, drug-resistant strains causing the death of symptomatic individuals and the detrimental long-term health of asymptomatic individuals. Exacerbating these elevated public health risks is a lack of awareness of disease characteristics, potential to contract disease, and zoonotic threat.

Only a thin veil exists between non-human malaria parasites, zoonotic transmission, and the development of new, potentially deadly, human disease. As it stands, that veil is probably gossamer-thin among parasites that infect primates. Serious risks are likely to surface as a result of failure to track and identify species for which human disease (naturally acquired or otherwise) is already documented. It should be stressed that a local absence of a permissive vector is all that stands between an identifiable infectious disease and an emerging disease outbreak. This potentially deadly threat, and others such as novel species, host sharing, and spontaneous mutations at the micro- and macro-scale, should not be viewed as limiting factors that constrain zoonotic transmission.

Complacency in each of the areas described will most likely lead to a mounting threat of disease. While some risks are clearly greater than others (for example, absence of blood stage schizogony and long-term virulence in parasite physiology), in order to avoid catastrophic scenarios as emphasized by infectious diseases of pandemic resurgence, each potential constituent of zoonotic malaria should be considered among all haemosporidians. Any observable differences between *Plasmodium* and other apicomplexans are rudimentary at best. As the history of the scourge of malaria on humankind repeatedly warns, the biological threat from these prokaryotic parasitic organisms is inordinately high. Given such compounding factors as human encroachment and climate change, the risks from malaria look set to rise in coming decades. These risks create novel scenarios that, if undiscovered and/or uncontrolled, may facilitate the emergence of a zoonotic malaria, causing a new human disease that carries the threat of an epidemic or even pandemic outcome.

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