

Do Invasive Mosquito and Bird Species Alter Avian Malaria Parasite Transmission?

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Abstract: Alien mosquito and vertebrate host species may create novel epidemiological scenarios for the transmission of pathogens naturally circulating in the invaded area. The exotic Monk parakeet (*Myiopsitta monachus*) has established populations in Europe and is currently considered an invasive pest. Due to their high abundance in urban areas, Monk parakeets could be involved in the transmission of pathogens, potentially affecting wildlife and livestock. To test this hypothesis, we determined the prevalence and diversity of three vector-borne parasites, namely *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, in Monk parakeets from Barcelona. Many areas of southern Europe shelter high densities of the invasive Asian tiger mosquito *Aedes albopictus*, which in addition to native mosquito species could affect the transmission of mosquito-borne parasites, such as avian *Plasmodium*. Thus, we also sampled mosquitoes in the area to trace their blood-feeding hosts and determine the presence of *Plasmodium* parasites. Monk parakeets were neither infected by *Plasmodium* nor by *Haemoproteus* parasites, and only five individuals (3.13%; $n = 160$) were infected by *Leucocytozoon*. Monk parakeets were bitten by *Culiseta longiareolata* and represented 9.5% of *Culex pipiens* blood meals. The invasive *Ae. albopictus* showed a clear anthropophilic feeding pattern, with humans dominating its diet. Three *Plasmodium* lineages were detected in pools of *Cx pipiens*. These results suggest that *Plasmodium* circulating in the area cannot develop in the invasive Monk parakeet, in spite of the relatively high fraction of native mosquito vectors feeding on this species in its invaded distribution range.

Keywords: *Aedes albopictus*; avian malaria; *Haemoproteus*; invasive species; *Leucocytozoon*; mosquitoes; parrots; *Plasmodium*

1. Introduction

Pathogens are an important factor explaining the invasion success of alien species [1]. The Enemy Release Hypothesis proposes that invasive species proliferate in invaded areas because they lose their original parasites present in their native distribution range [2]. In addition to this possibility, the Novel Weapons Hypothesis proposes that invasive species may harbor new pathogens to the immunological naive native populations [3]. However, locally circulating pathogens may also be able

to infect alien hosts [4], although the transmission of pathogens with complex life cycles may be limited by the occurrence of competent hosts allowing its successful transmission [1]. This may be the case for vector-borne pathogens that require the coexistence of competent vertebrate hosts and insect vectors to be effectively transmitted from an infected individual to a susceptible host. Thus, different factors may explain the absence of infections by vector-borne pathogens in alien species in the invaded areas including those affecting the transmission routes of pathogens, such as 1) the absence of competent vectors for the transmission of pathogens and 2) limited contact rates between vectors and susceptible vertebrate hosts.

Avian malaria parasites of the genus *Plasmodium* are commonly found to infect birds and are transmitted by mosquitoes [5,6]. The life cycle of these parasites includes asexual reproduction stages in the vertebrate hosts and a sexual phase in the mosquito vector. To be transmitted, the parasite requires the bite of a competent mosquito on an infected bird. In addition to *Plasmodium*, birds usually suffer infection by the related *Haemoproteus* and *Leucocytozoon* parasites. These two parasite genera have a similar life cycle to *Plasmodium* but are transmitted by different vectors [5]. *Culicoides* and louse flies are the main vectors of *Haemoproteus* of the subgenera *Parahaemoproteus* and *Haemoproteus*, respectively, while *Leucocytozoon* are transmitted by blackflies [6]. Avian haemosporidians have been used as study models to assess the role of parasites on the invasive success of different alien species including house sparrows (*Passer domesticus*) [7,8] and mynas (*Acridotheres tristis*) [9]. However, the role of insect vectors to explain the epidemiology of these pathogens affecting invasive avian species has traditionally been neglected.

Mosquitoes represent an important public health concern as sources of nuisance due to their bites to humans and as vectors of pathogens [10]. As in the case of other hematophagous insects, mosquitoes show a certain degree of feeding preference, with some species feeding mainly on a particular group of vertebrates, such as birds or mammals, while other species show opportunistic behaviors [11,12]. The common house mosquito *Culex pipiens* frequently occurs in European urban areas where it may play a central role in the transmission of avian malaria parasites [13–16]. During the last decades, the invasive Asian tiger mosquito *Aedes albopictus* has dramatically increased its distribution range from the native area in Asia to other continents [16]. This species is currently present in most countries of the Mediterranean basin, reaching high abundances in densely populated areas and producing important nuisances to humans and a risk to public health [17]. As well as humans, other mammals and occasionally birds are bitten by *Ae. albopictus* which could potentially affect the transmission of mosquito-borne pathogens in the invaded area [12,18].

Urban ecosystems are considered hotspots of biological invasions where alien species could represent an important public health concern and animal health concern for pets and other animals. In particular, Monk parakeets *Myiopsitta monachus* are considered one of the most effective parrot species colonizing new territories [19]. In addition to their native range in South America, this species is currently present in areas from North America and Europe, including islands in the Atlantic Ocean (e.g., Canary Islands) [20,21]. The Monk parakeet is listed as an invasive species according to both Spanish and European regulations [22] and its abundance is quickly increasing [21,23]. In the invaded areas, Monk parakeets produce economic costs due to the damage to human infrastructures and agriculture [24,25]. Moreover, as is the case for other parrot species, Monk parakeets may be involved in the transmission of pathogens and parasites, causing diseases in humans and other animals [19,26,27].

Here, we assessed the prevalence of the avian malaria parasite *Plasmodium* and the related *Haemoproteus* and *Leucocytozoon* in Monk parakeets from the city of Barcelona, where the presence of the species was first described in the 1970s [28], and its abundance has dramatically increased during the last decades [21,29]. In addition, we screened the blood meal sources of mosquitoes and identified the *Plasmodium* parasites potentially transmitted by both native and invasive mosquitoes in the area [30,31].

2. Materials and Methods

Monk parakeets were sampled in ten sampling sites covering six of the ten districts of Barcelona, focusing on the districts with a higher density of birds (see [27]). Adult plumaged Monk parakeets were captured from 2014 to 2015 using a special Yunick Platform Trap placed at the Natural History Museum of Barcelona (Ciudadella Park) and using pre-baited clap nets at the other localities (see [32] for details of the trapping procedures). Birds were individually marked with metal rings. We obtained blood samples from a total of 160 birds to determine infection status by hemosporidian parasites. Birds were handled and blood samples taken with special permission EPI 7/2015 (01529/1498/2015) from Direcció General del Medi Natural i Biodiversitat, Generalitat de Catalunya, following Catalan regional ethical guidelines for the handling of birds. J.C.S. received special authorization (001501-0402.2009) for the handling of animals in research from Servei de Protecció de la Fauna, Flora i Animal de Companyia, according to Decree 214/1997/30.07, Generalitat de Catalunya.

Mosquitoes were collected in Barcelona city (Ciutat Vella), which included the Zoological garden. Mosquitoes were captured weekly from April to November during 2014 and 2015. Adults were captured using BG Sentinel traps (Biogents GmbH, Regensburg, Germany) supplemented with BG-Lure (BioGents GmbH, Regensburg, Germany) and CO₂ as attractants. In addition, to increase the number of blood fed mosquitoes captured, we used entomological aspirators (Improved Prokopack Aspirator, Mod. 1419, John W. Hock Company, Gainesville, FL, USA and CDC Backpack Aspirator Mod. 2846, BioQuip, Los Angeles, CA, USA), sampling mosquitoes in sinks or other resting areas. Mosquitoes were identified to species level following the procedure in [33]. The whole bodies of unfed female mosquitoes were grouped in pools containing 1–30 individuals according to the species, date of capture and sampling site. Engorged female mosquitoes were stored individually at −80 °C until further molecular analyses.

DNA was isolated from bird blood samples, the abdomen of engorged mosquitoes and from mosquito pools using a semi-automatic kit (Maxwell®16 LEV system Research; Promega, Madison, WI USA), following manufacturer specifications. Bird samples were tested for the presence of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* parasites by the amplification of a fragment of the parasite mitochondrial cytochrome b (cyt b) gene [34]. For the case of mosquitoes, we only tested samples for the presence of *Plasmodium*/*Haemoproteus* DNA, as mosquitoes transmit only *Plasmodium* parasites. We amplified a fragment of the mitochondrial cytochrome oxidase 1 (COI) gene of the vertebrate hosts from the abdomen of engorged mosquitoes [35].

Positive amplifications were sequenced using the Big Dye 1.1 technology (PE Applied Biosystems, Foster City, CA, USA) in the facilities of the Estación Biológica de Doñana (Seville, Spain) or in the Macrogen Inc. sequencing service (The Netherlands). Labelled DNA fragments of positive PCR products were resolved using the same forward and reverse primers used in the nested-PCR amplification for the case of blood parasite identifications. For blood meal identifications, amplicons were sequenced in one direction using the primer BCRV2, (Isogen Life Science, De Meern, the Netherlands) except for the case of *Ae. albopictus* blood meals that were sequenced using the primer BCVINT-RV (Isogen Life Science, De Meern, the Netherlands) (see [30]). Sequences were edited using the software Sequencher™ v 4.9 (Gene Codes Corp., © 2020–2009, Ann Arbor, MI, USA) and assigned to particular vertebrate species by blast comparison with sequences deposited in GenBank DNA sequence database (National Center for Biotechnology Information Blast) or the Barcode of Life Data Systems (BOLD). Parasite lineages were identified by comparison with those sequences deposited in GenBank database. *Plasmodium* prevalence was assessed from pool data using EpiTools [36], which estimates parasite prevalence derived from pooled samples considering the number of mosquitoes in each pool.

3. Results

3.1. Blood Parasite Prevalence in Birds

Five out of the 160 (3.13%) Monk parakeets tested were infected by *Leucocytozoon* parasites corresponding to the lineage CIAE2 (100% overlap). None of the birds captured were infected by *Plasmodium* or *Haemoproteus* parasites.

3.2. Blood Feeding Patterns of Mosquitoes

The origin of blood meals was successfully identified from 295 out of 511 mosquitoes tested. Positive results were obtained for 263 *Cx. pipiens*, 20 *Ae. albopictus*, three *Culiseta longiareolata* and nine mosquitoes which were identified to the genus level (including eight *Culex* sp. and one *Aedes* sp.) (Table 1). *Culex pipiens* fed blood on both mammals (19.4%) and birds (80.6%), with 25 out of the 263 (9.5%) blood meals identified corresponding to Monk parakeets (Table 1). In addition to *Cx. pipiens*, a single *Cs. longiareolata* fed on this invasive species. In total, 18 out of 20 *Ae. albopictus* blood meals derived from humans.

Table 1. Vertebrate hosts of mosquitoes from Barcelona.

Vertebrate Hosts	Family	Host Species	<i>Ae. albopictus</i>	<i>Aedes</i> sp.	<i>Cx. pipiens</i>	<i>Culex</i> sp.	<i>Cs. longireolata</i>	Total
Mammal	Cercopithecoidae	<i>Erythrocebus patas</i>			6			6
	Equidae	<i>Equus caballus</i>			4			4
	Felidae	<i>Felis silvestris</i>	2		1			3
	Hominidae	<i>Homo sapiens</i>	18	1	37	1		57
	Rhinocerotidae	<i>Ceratotherium simum</i>			1			1
	Ursidae	<i>Ursus arctos</i>			2			2
Bird	Ardeidae	<i>Ardea cinerea</i>			7			7
		<i>Bubulcus ibis</i>			19	1		20
		<i>Egretta garzetta</i>			7			7
	Columbidae	<i>Columba livia</i>			35	2		37
		<i>Columba palumbus</i>			2			2
		<i>Streptopelia decaocto</i>			15	1		16
	Corvidae	<i>Coloeus monedula</i>			13			13
		<i>Pica pica</i>			15	2	2	19
	Paridae	<i>Cyanistes caeruleus</i>			2			2
		<i>Parus major</i>			5			5
	Passeridae	<i>Passer domesticus</i>			6			6
	Phasianidae	<i>Pavo</i> sp.			4			4
	Phoenicopteridae	<i>Phoenicopiterus</i> sp.			6			6
	Psittacidae	<i>Myiopsitta monachus</i>			25		1	26
	Spheniscidae	<i>Spheniscus humboldti</i>			47	1		48
	Sturnidae	<i>Sturnus vulgaris</i>			2			2
	Turdidae	<i>Turdus merula</i>			2			2

3.3. Blood Parasite Prevalence in Mosquitoes

The presence of blood parasites was screened in 1808 mosquitoes grouped in 308 pools (Table 2). *Plasmodium* parasites were only detected in four pools of *Cx. pipiens*. Three infected pools were trapped in 2014 (2014 prevalence for *Cx. pipiens*: 0.004; 95% interval: 0.001–0.010) corresponding to the lineages Delurb04 (two pools) and Delurb05 (one pool). During 2015, the *Plasmodium relictum* lineage SGS1 was identified from a single *Cx. pipiens* pool (2015 prevalence for *Cx. pipiens*: 0.003; 95% interval: 0.0001–0.011).

Table 2. Blood parasite prevalence found in pools of mosquitoes from Barcelona.

Mosquito Species	Mosquitoes	Pools	Positive pools
<i>Aedes albopictus</i>	473	84	0
<i>Aedes</i> sp.	14	7	0
<i>Anopheles claviger</i>	1	1	0
<i>Culiseta longiareolata</i>	109	36	0
<i>Culex</i> sp.	16	9	0
<i>Cx. modestus</i>	3	2	0
<i>Cx. pipiens</i>	1190	167	4
<i>Ochlerotatus berlandi</i>	1	1	0
<i>Oc. geniculatus</i>	1	1	0

4. Discussion

We tested the role of the invasive Monk parakeet in the transmission of avian hemosporidians in an area with the presence of both native and invasive potential vectors. Monk parakeets showed an apparent absence of blood parasites of the genera *Plasmodium* and *Haemoproteus* and a very low (3.13%) prevalence of infection by *Leucocytozoon*. Parasites may be an important factor affecting the success of establishment of alien species in new areas, with those species escaping from the attack of their parasites from their native distribution range being favored [37]. Avian hemosporidians produce deleterious effects on birds, compromising the life expectancy and reproductive success of infected individuals [38–40], including parrots maintained in captivity [41]. In addition, immunological responses to parasites are costly for birds [42]; thus, invasive species may benefit from avoiding the interactions with parasites in the invaded area [4].

Among the different hypotheses proposed to explain the infection pattern of alien species in the invaded distribution range, the Enemy Release Hypothesis has received strong support [7,43]. Our results agree with this hypothesis, since the invasive population of Monk parakeets showed a very low prevalence of infection by a single parasite lineage. Similarly, invasive house sparrow populations had a lower prevalence and parasite diversity than native populations [7]. The host range may differ between parasite genera [44,45], but also clear differences between parasite lineages within each parasite genera may occur, with some lineages only developing in a restricted host range while others are able to develop in a huge number of species [46]. The phylogenetic relatedness of the avian community in the invaded area with the introduced species may determine parasite pressure on the alien species [47]. This fact may benefit parrots introduced in Europe, due to the absence of related species in the native bird community. The only parasite lineage identified from Monk parakeets was the generalist *Leucocytozoon* lineage CIAE2, which circulates between bird species of different orders in Europe, Africa and Asia (see records in Malawi database [48]). Three *Larus* species are hosts of this parasite lineage. Gulls could play a role in the transmission of this parasite in the metropolitan area of Barcelona due to their relative high density (2 ind/km²; see [49,50]). In addition, different species of the order Falconiformes are hosts of CIAE2, including records in Spain, potentially playing a role in the transmission of the parasites to Monk parakeets. Further studies are necessary to identify the main reservoirs of this parasite lineage in the area and to assess the ability of hemosporidians circulating in the area to develop in Monk parakeets.

Alternatively, it is also possible that Monk parakeets are refractory to infections by *Plasmodium* and *Haemoproteus* parasites in both native and introduced areas. Although the ability of *Leucocytozoon* parasites to infect Monk parakeets was previously reported [51] (see also [52] for records in other parakeet species from South America), to our knowledge, there are no previous records of infections by *Plasmodium* and *Haemoproteus* parasites in this species. Some bird groups show a very low prevalence or total absence of hemosporidian infections [53], with parrots representing one of these groups [52,54]. In fact, a very low prevalence of blood parasites was found in species of American Psittacidae with only 36 individuals infected out of the 1199 tested [55]. Furthermore, a single *Haemoproteus* species has been described in South American parrots [56]. However, parrots maintained in captivity are susceptible to infection by European parasite lineages, which produce high mortality rates in the infected individuals [41]. This suggests the possibility that the absence of *Haemoproteus* and *Plasmodium* infections in Monk parakeets could also be due to an extremely high mortality rate of infected individuals (see [56]). Nevertheless, this possibility remains unlikely due to the absence of reports of massive mortalities of Monk parakeets in the study area.

A priori, the absence of competent insect vectors in the area or their inability to bite Monk parakeets could also explain the absence of *Plasmodium* infections. However, invasive populations of Monk parakeets are affected by different arthropod ectoparasites in the invaded areas [27,57,58], including blood-sucking mosquitoes [30]. Monk parakeets are hosts of mosquitoes including *Cs. longierolata* and *Cx. pipiens*, representing 9.5% of the *Cx. pipiens* blood meals in this study. Both mosquito species are potential vectors of avian malaria parasites, with the last species considered a well-known competent mosquito for the transmission of avian *Plasmodium* [6,14].

5. Conclusions

Monk parakeets from Barcelona show a low prevalence of infection by blood parasites. The inability of locally circulating *Plasmodium* parasites to develop in Monk parakeets represents the most plausible reason for the absence of infections in this invasive species. This was not the case of *Leucocytozoon* parasites, which infected 3.13% of the Monk parakeets tested. Therefore, differences in the specificity/generalist characteristics of each host–parasite assemblage may determine the success of parasite development in alien species in the area. The invasion success of a species is likely affected by different factors including the suitability of urban habitats and the lack of predators in urban areas. Our results suggest that the low prevalence of infection by blood parasites found in Monk parakeets may add to all these factors, facilitating the spread of this species worldwide.

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