EKOLOGIJA. 2014. Vol. 60. No. 4. P. 73–83

Recent advances in vector studies of avian haemosporidian parasites

Rita Žiegytė*,

Gediminas Valkiūnas

Nature Research Centre,
Akademijos St. 2,
LT-08412 Vilnius, Lithuania

Many recent studies addressed morphological and molecular characterization, distribution, genetic diversity and evolutionary relationships of avian haemosporidian parasites (Haemosporida). However, the information about relationships between bird haemosporidians and their vectors remains fragmentary and scarce. Experimental studies on this subject are few. Recent advances in vector research of avian haemosporidians (Haemosporida) have been briefly reviewed in regard to the experimental studies, which have been carried out at the Nature Research Centre, Vilnius, during the last five years, with particular attention to widespread species of *Plasmodium* and *Haemoproteus* parasites. New information about vectors of avian malaria parasites and haemoproteids is provided and discussed. We point out high virulence of widespread *Haemoproteus* species for blood-sucking insects of the Culicidae and Ceratopogonidae, and call for additional studies on this subject. Due to widespread abortive sporogonic development in blood-sucking insects, Polymerase Chain Reaction (PCR)-based diagnostics should be carefully used in vector research of haemosporidians because it detects parasites in blood-sucking insects for several weeks after initial infection, but does not distinguish abortive parasite development. That questions vector studies, which are based solely on PCR-based tools. Demonstration of infective sporozoites in insects is essential for definitively demonstrating the insects are vectors. Because of the complicated life cycles of haemosporidians, microscopic approaches and experimental research remain essential and should be applied in parallel with PCR-based detection tools in vector studies, particularly in wildlife.

Key words: review, haemosporidians, blood-sucking dipterans, vectors, *Plasmodium, Haemoproteus*

INTRODUCTION

Haemosporidian parasites (Haemosporida) inhabit many species of land vertebrates (amphibians, reptiles, birds, and mammals) almost all over the world. They use blood-sucking dipteran insects (Insecta: Diptera) as vectors (Valkiūnas, 2005; Telford, 2009; Perkins, 2014). Haemosporidians are a relatively well studied group of parasitic protists, particularly because they include the agents of malaria, which remains one of the common human diseases in countries with warm climate. Wildlife malaria parasites of the family Plasmodiidae have been used as model objects in research of this disease for over 100 years (Garnham, 1966; Perkins, 2014). Parasites of the family Haemoproteidae have been less studied, but are certainly important because they are markedly diverse and cause severe and even lethal diseases in some bird species (Miltgen et al., 1981; Atkinson et al., 1988; Cardona et al., 2002; Olias et al., 2011; Cannell et al., 2013). Many species of *Haemoproteus* (Haemoproteidae) and *Plasmodium* (Plasmodiidae) are responsible for acute and/or chronic diseases in domestic and wild birds. These parasites are widespread in Europe and are transmitted even in

* Corresponding author. E-mail: rita.kazlauskien@gmail.com; gedvalk@ekoi.lt

parks of large cities worldwide (Valkiūnas, 2005; Atkinson, 2008), but their pathogenic influence on bird populations remains insufficiently studied (Dinhopl et al., 2015).

Haemosporidians of the genus *Plasmodium* cause malaria. Avian species of *Plasmodium* are common in birds in all continents except Antarctica (Valkiūnas, 2005; Bensch et al., 2009; Braga et al., 2011; Perkins, 2014). Species of *Haemoproteus* are also widespread in birds. Transmission of the representatives of both genera is particularly active in countries with warm and temperate climates, with overall infection prevalence exceeding 20% in many terrestrial bird populations (Greiner et al., 1975; McClure et al., 1978; Peirce, 1981; Valkiūnas et al., 2003; Pérez-Tris et al., 2007; Shurulinkov, Ilieva, 2009). Malaria parasites and other haemosporidians often negatively affect both the vectors and vertebrate hosts (Merino et al., 2000; Palinauskas et al., 2008; Knowles et al., 2010; Kazlauskienė et al., 2013; Valkiūnas et al., 2014).

Many recent studies addressed morphological and molecular characterization, distribution, genetic diversity and evolutionary relationships of avian *Haemoproteus* and *Plasmodium* spp. However, the information about relationships between bird haemosporidians and their vectors remains fragmentary and scarce (Kimura et al., 2010). Knowledge about patterns of development of different haemosporidian species and, particularly, their genetic lineages in vectors is insufficient (Ejiri et al., 2009; Kim et al., 2009; Kimura et al., 2010; LaPointe et al., 2010; Njabo et al., 2011). A few experimental studies deal with vectors and transmission of wildlife haemosporidian parasites (Atkinson, 1991; Desser, Bennett, 1993; Valkiūnas et al., 2002; Kim et al., 2009; Levin et al., 2013). Little is known about the effects of avian haemosporidian infections on blood-sucking insects (Valkiūnas, Iezhova, 2004; Levin, Parker, 2014). These are obstacles for better understanding of the epidemiology of diseases caused by avian haemosporidian parasites and the evolutionary biology of this large and diverse group of parasites.

Due to remarkable both genetic and phenotypic diversity, the cosmopolitan distribution and relative easiness to sample and maintain under laboratory conditions, avian malaria parasites and haemoproteids are convenient objects to address important fundamental questions about pathogen evolutionary biology, ecology, emergence and many other issues. Information about genetic lineages of avian haemosporidians has been rapidly accumulating (Bensch et al., 2009; see MalAvi database http://mbio-serv2.mbioekol.lu.se/Malavi/). However, insufficient knowledge about vectors prevents understanding epidemiology of the haemosporidioses, particularly on the level of their genetic lineages. Experimental research with avian haemosporidian parasites was scarce during last 20 years (Valkiūnas, Iezhova, 2004; Kim et al., 2009). In this paper we review recent studies dealing with vector research of avian haemosporidian parasites, particularly experimental studies, which has been carried out on this issue at the Nature Research Centre, Vilnius, using models of widespread species of *Plasmodium* and *Haemoproteus* during last 5 years.

INVESTIGATION OF SPOROGONY OF THE *PLASMODIUM RELICTUM* GENETIC LINEAGES IN MOSQUITOES *CULEX PIPIENS PIPIENS*

Recent PCR-based studies provided innovative opportunities to diagnose malaria parasites and revealed huge genetic diversity of these pathogens (Perkins, Shall, 2002; Bensch et al., 2004; Ricklefs et al., 2004; Møller, Nielsen, 2007). There is several fold increase in both citations and publications on avian malaria over the past decade (Bensch et al., 2009). That indicates the rapid increase of interest on avian *Plasmodium* spp. and related haemosporidians in zoology (Iezhova et al., 2011; Zehtindjiev et al., 2012), veterinary and conservation medicine (Levin et al., 2009; Santiago-Alarcon et al., 2010; Olias et al., 2011), and particularly in evolutionary biology (Pérez-Tris, Bensch, 2005; Møller, Nielsen, 2007; Bensch et al., 2009; Santiago-Alarcon et al., 2010; Ricklefs, Outlaw, 2010; Levin et al., 2011; Marzal et al., 2011; Loiseau et al., 2012).

Numerous PCR-based and morphological studies addressed taxonomy, distribution, evolutionary biology and other issues of avian *Plasmodium* spp. biology, resulting in deposition of numerous parasite lineages in GenBank (Beadell et al., 2006; Dimitrov et al., 2010; Knowles et al., 2010; Marzal et al., 2011; Szöllősi et al., 2011; Loiseau et al., 2012). However, information

about development of different *Plasmodium* lineages in vectors remains insufficient (Ejiri et al., 2009; Kim et al., 2009; Kimura et al., 2010; LaPointe et al., 2010; Njabo et al., 2011). Comparative studies on sporogonic development of closely related lineages and different isolates of the same lineage in mosquito vectors are lacking. That is an obstacle to address epidemiology questions related to avian malaria transmission and also bias the rapidly accumulating information on *Plasmodium* spp. lineage diversity.

Plasmodium relictum lineages pSGS1 and pGRW11 have been reported in over 30 species of birds in the Old World. Both lineages seem to be rarer in the New World (Palinauskas et al., 2007; Bensch et al., 2009; Marzal et al., 2011). Remarkably little is known about sporogony of these parasite lineages (Vezilier et al., 2010).

Recently, Kazlauskienė et al. (2013) compared sporogonic development and morphology of two widespread lineages of *P. relictum* (pSGS1 and pGRW11) in mosquito *C. p. pipiens*. The key results of this study are that (1) the closely related lineages of *P. relictum* and (2) the different isolate of the same lineage develop synchronously and produce morphologically indistinguishable sporogonic stages and infective sporozoites in *C. p. pipiens*. It was predicted that this might be true for closely related lineages of other malaria parasite morphospecies, for instance, the genetically similar lineages of *P. relictum, P. circumflexum, P. elongatum* and some other haemosporidians. In other words, this study indicated that phylogenetic trees based on cytochrome *b* gene sequences might be helpful for predicting sporogonic development of closely related lineages in vectors. This assumption is in parallel to the Martinsen et al. (2008) study suggesting that evolution of major groups of haemosporidian parasites is closely associated with adaptation to certain groups of dipteran vectors. Data of the Kazlauskienė et al.'s (2013) study indicate that the same association also might be present in clades of genetically similar lineages on the morphospecies level of haemosporidians; that warrants further investigation. These data are helpful for better understanding of biology and diversity of avian *Plasmodium* spp., indicating possible directions for predicting sporogony patterns of numerous genetically similar lineages of *Plasmodium* spp., which sporogonic development and vectors remain unknown.

INVESTIGATION OF SPOROGONY OF THE *PLASMODIUM RELICTUM* GENETIC LINEAGES IN MOSQUITOES *CULEX PIPIENS PIPIENS* FORM *MOLESTUS*

Culex p. pipiens f. *molestus* (Forskal, 1775) is widespread in the Holarctic. This mosquito is characterized by broad ecological plasticity (Gomes et al., 2009; Vinogradova, 2000) and is particularly common in human settlements where it often breeds in sewers, but also occurs in natural sheltered habitats such as caves and other similar ecological niches. Both *C. p. pipiens* and *C. p. pipiens* f. *molestus* often occur sympatrically, particularly in countries with mild winters (Osorio et al., 2014). *Culex p. pipiens* is an important vector of avian malaria (Santiago-Alarcon et al., 2012). However, the role of *C. p. pipiens* f. *molestus* in transmission of avian malaria remains unclear. Because of peculiarities of swarming and mating behavior of *C. p. pipiens*, it is difficult to establish its new colonies and to maintain them under laboratory conditions using wild-collected insects (Vinogradova et al., 1996). That makes obstacles to use this mosquito in the experimental research aiming at better understanding of the geographical variation in susceptibility of vectors to avian malaria.

Culex p. pipiens f. *molestus* differs from *C. p. pipiens* by: 1) autogeny of females, 2) stenogamy, 3) high degree of anthropophily, and 4) absence of overwintering in diapauses (Vinogradova et al., 1996; Becker et al., 2003). These features allow *C. p. pipiens* f. *molestus* to be easily cultivated in small mosquito cages under laboratory conditions using wild-sampled insects, making this species a convenient model for experimental studies. However, it remains unclear if *C. p. pipiens* f. *molestus* is worth attention in epidemiology of avian malaria. Only one study reported complete sporogonic development of one species of malaria parasite, i. e. *Plasmodium (Giovannolaia) garnhami* in *C. p. pipiens* f. *molestus* (Garnham, 1966).

Because this mosquito form has not been reported in the list of vectors of the great majority of avian malaria parasites (Valkiūnas, 2005; Santiago-Alarcon et al., 2012), Žiegytė et al. (2014a) investigated if two widespread *P. relictum* lineages (pSGS1 and pGRW11) complete sporogony in this insect. Three key results of this experimental study should be pointed out. First, it was shown that

C. p. pipiens f. *molestus* mosquitoes readily take blood meals on birds in spite of the established opinion about high degree of anthropophily of this mosquito form (Becker et al., 2003). This mosquito should be involved in natural transmission of avian malaria, particularly in human settlements where this form is often abundant (Vinogradova et al., 1996) and transmission of avian malaria often occurs (Valkiūnas, 2005).

Second, *C. p. pipiens* f. *molestus* should be considered in epidemiology studies of avian malaria because the lineages pSGS1 and pGRW11 of *P. relictum* successfully complete sporogony in this mosquito. This finding is in accordance with former observation about successful synchronous sporogonic development of the same lineages in *C. p. pipiens* mosquitoes (Kazlauskienė et al., 2013). Both mosquitoes can act as vectors of *P. relictum* (Valkiūnas, 2005; Vezilier et al., 2010; Santiago-Alarcon et al., 2012), but epidemiological significance of *C. p. pipiens* f. *molestus* has been insufficiently addressed in avian malaria studies.

Third, the susceptibility of *C. p. pipiens* f. *molestus* to the closely related lineages pSGS1 and pGRW11 of *P. relictum* was different, with different oocyst burden in the exposed insects. This finding is interesting because genetic difference in cyt *b* gene sequences between these 2 parasite strains is negligible (1 base pair), indicating that sporogony success can be markedly different even in closely related lineages of the same malaria parasite in the same mosquito species. The oocyst number is directly related to the number of transmissible sporozoites that develop in the salivary glands, and also to the mosquito longevity (Garnham, 1966; Valkiūnas, 2005). That should influence the mosquito vectorial capacity, but remains insufficiently investigated in avian malaria parasites, particularly on the level of different lineages of the same species. This study provides the first information about the patterns of sporogonic development of the lineages pSGS1 and pGRW11 in *C. p. pipiens* f. *molestus.*

HAEMOPROTEUS SPP. SPOROGONY IN THE BITING MIDGES

Numerous recent studies addressed molecular characterization, distribution and genetic diversity of haemoproteids. However, few studies deal with vectors and transmission of avian *Haemoproteus* spp. (Atkinson, 1991; Desser, Bennett, 1993; Valkiūnas et al., 2002; Martinez-de la Puente et al., 2011; Santiago-Alarcon et al., 2012; Levin et al., 2013). Biting midges of *Culicoides* (Diptera, Ceratopogonidae) and louse flies (Hippoboscidae) can transmit these parasites, but certain vector species remain unknown for the great majority of avian haemoproteids and their lineages (Atkinson, 2008; Clark et al., 2014).

The sporogony of only few *Haemoproteus* spp. has been investigated in detail in biting midges (Linley, 1985; Valkiūnas, 2005; Santiago-Alarcon et al., 2012); it is difficult to work with these insects due to their tiny size and difficulties to colonize the majority of their species (Miltgen et al., 1981; Valkiūnas, 2005; Atkinson, 2008). It was shown that *Culicoides impunctatus* (Goetghebuer, 1920) transmits several species of haemoproteids in Europe (Valkiūnas, 2005). This biting midge has been reported as a vector of *Haemoproteus belopolskyi* parasitizing blackcaps *Sylvia atricapilla* (Valkiūnas, Iezhova, 2004). However, recent molecular studies show that this blackcap parasite is actually *Haemoproteus parabelopolskyi* (Valkiūnas et al., 2007), and the vector of *H. belopolskyi*, which parasitizes icterine warblers *Hippolais icterina*, needs to be identified.

Because studies on vectors and transmission of avian *Haemoproteus* spp. are uncommon and vectors of *H. minutus* and *H. belopolskyi* are unknown, Žiegytė et al. (2014b) followed sporogony of these parasites in the biting midge *C. impunctatus*, which is widespread in Europe, willingly takes blood meal on birds and is susceptible to several haemoproteid infections (Glukhova, 1989; Blackwell, 1997; Valkiūnas, 2005). It was determined (Žiegytė et al., 2014b) that two lineages of widespread *Haemoproteus* parasites, i. e. *H. minutus* (lineage hTURDUS2) and *H. belopolskyi* (hHIICT1), complete sporogony and produce sporozoites in *C. impunctatus.* Sporogonic stages of these parasites were described and illustrated. This study indicates that *C. impunctatus* is involved in the transmission of deadly *H. minutus*, which kills captive parrots in Europe (Palinauskas et al., 2013). This biting midge is an important vector of avian haemoproteids (Valkiūnas, 2005) and worth more attention in epidemiology research of avian haemoproteosis.

DEVELOPMENT OF *HAEMOPROTEUS* PARASITES IN MOSQUITOES

Approximately 150 species of avian haemoproteids have been described (Iezhova et al., 2011). The majority of these belong to the subgenus *Parahaemoproteus* and are transmitted by biting midges belonging to *Culicoides* (Ceratopogonidae). Currently, only 10 species have been assigned to the subgenus *Haemoproteus*, all of which are transmitted by hippoboscid flies of the Hippoboscidae (Bennett et al., 1965; Garnham, 1966; Valkiūnas et al., 2010; Levin et al., 2012; Valkiūnas et al., 2013a). According to current knowledge, only biting midges and hippoboscid flies act as vectors of avian haemoproteids (Baker, 1966; Garnham, 1966; Atkinson, van Riper, 1991; Desser, Bennett, 1993; Valkiūnas, 2005). That is in accord with the molecular phylogeny studies suggesting that evolution of *H.* (*Parahaemoproteus*) and *H.* (*Haemoproteus*) parasites is closely associated with adaptation to species of the Ceratopogonidae and Hippoboscidae, respectively (Martinsen et al., 2008; Santiago-Alarcon et al., 2010; Levin et al., 2012). However, vector species have only been identified with certainty for very few *Haemoproteus* species (Valkiūnas, 2005; Santiago-Alarcon et al., 2012).

Such studies are difficult to design using traditional parasitology methods, particularly in wildlife because haemoproteids do not multiply in the circulation, so parasite strains cannot be maintained in birds by means of infected blood inoculation, which is easy to do in the case of avian malaria. Experimental infection of birds with haemoproteids needs injection of infective sporozoites into susceptible avian hosts. In practice, determination of susceptible vectors of these parasites requires experimental infection of blood-sucking insects by feeding them on naturally infected birds, many species of which are difficult to maintain in captivity (Baker, 1957; Fallis, Bennett, 1960; Atkinson, van Riper, 1991; Desser, Bennett, 1993; Valkiūnas et al., 2002). Such experiments are particularly difficult to do with haemoproteids parasitizing rare bird species and in remote areas.

Molecular markers provide new opportunities to detect haemosporidian lineages during light infections both in avian hosts and vectors. Mitochondrial cytochrome *b* gene markers have been successfully applied for detecting and distinguishing haemosporidian species and are easy to use (Martinsen et al., 2006; Sehgal et al., 2006; Palinauskas et al., 2007; Santiago-Alarcon et al., 2010; Iezhova et al., 2011; Martinez-de la Puente et al., 2011; Križanauskienė et al., 2012). It is attractive to apply the PCR-based methods in vector research (Ejiri et al., 2009; Njabo et al., 2009; Kimura et al., 2010; Ejiri et al., 2011; Martinez-de la Puente et al., 2011; Foley et al., 2012; Glaizot et al., 2012; Kim, Tsuda, 2012; Ventim et al., 2012; Kazlauskienė et al., 2013). Several recent studies reported the presence of *Haemoproteus* spp. lineages in mosquitoes and speculated about possible involvement of these insects in transmission (Ishtiaq et al., 2008; Njabo et al., 2011). However, sporogonic development of *Haemoproteus* spp. lineages has not been documented in mosquitoes.

Valkiūnas et al. (2013b) studied experimentally development of *Haemoproteus* (*Parahaemoproteus*) *tartakovskyi* and *Haemoproteus* (*Parahaemoproteus*) *balmorali* in *Ochlerotatus cantans* (Meigen, 1818) (Diptera, Culicidae), a widespread Eurasian mosquito. These haemosporidian parasites are common and prevalent in siskin *Carduelis spinus* and thrush nightingale *Luscinia luscinia* in Europe, respectively. It was aimed to investigate sporogonic development and survival time of these haemoproteids in mosquito *O. cantans* using microscopic and PCR-based examination methods, which were applied in parallel. This mosquito is a member of the tribe *Aedini*, many species of which transmit closely related avian *Plasmodium* parasites (Valkiūnas, 2005; Santiago-Alarcon et al., 2012).

It was showed (Valkiūnas et al., 2013b) that these *Haemoproteus* (*Parahaemoproteus*) species (1) underwent sexual processes and produced ookinetes throughout the entire digestive tract of engorged mosquitoes, including the head, thorax and abdomen; 2) ookinetes found no barrier in their way from gut contents to the gut wall and haemocoele, resulting in the presence of parasites throughout the body of the mosquitoes; 3) developed oocysts in the head, thorax and midgut wall of mosquitoes; and 4) aborted sporogonic development at the oocyst stage without formation of sporozoites. Importantly, *Haemoproteus* spp. DNA is detectable in the head, thorax and abdomen of infected mosquitoes for several weeks, indicating relatively long-time persistence of *Haemoproteus* parasites in resistant insects following an infected blood meal. In spite

of abortive sporogonic development, mosquitoes have been scored as infected by PCR when, in fact, they are not competent vectors of the parasites. This makes obstacles in direct application of PCR-based detection methods in vector research, particularly in wildlife, and questions conclusions of evolutionary studies (Medeiros et al., 2013) determining vectors of haemosporidians based solely on detection of lineages of the parasites in blood-sucking insects.

THE VIRULENCE OF *HAEMOPROTEUS* INFECTIONS TO BLOOD-SUCKING INSECTS

Some haemoproteids cause severe diseases in avian hosts and affect their fitness (Atkinson et al., 1988; Merino et al., 2000; Marzal et al., 2005; Cannell et al., 2013). However, little is known about the effects of *Haemoproteus* infections on blood-sucking insects. Valkiūnas and Iezhova (2004) reported mortality of the biting midge *Culicoides impunctatus* (Diptera, Ceratopogonidae) associated with experimental infections of *Haemoproteus belopolskyi*, *H. fringillae* and *H. lanii*. This biting midge transmits several species of *Haemoproteus* in Europe (Valkiūnas, 2005; Žiegytė et al., 2014b). However, effects of haemoproteids on mosquitoes and other bird-biting insects are insufficiently investigated.

Mosquitoes do not transmit *Haemoproteus* parasites and should be not adapted to this infection (Garnham, 1966; Ejiri et al., 2009; Kim et al., 2009; Santiago-Alarcon et al., 2012). A recent experimental study (Valkiūnas et al., 2013b) showed that several species of *Haemoproteus* developed numerous ookinetes and early oocysts throughout the entire body of mosquitoes *Ochlerotatus cantans*; these parasites do not complete sporogony, which is abortive at the oocyst stage. It was speculated that *Haemoproteus* infections might be virulent to bird-biting mosquitoes. To our knowledge, there are no other records about effects of haemoproteids on longevity of blood-sucking mosquitoes. However, these insects have been reported to be PCR positive for *Haemoproteus* spp. lineages across the world (Ishtiaq et al., 2008; Njabo et al., 2011; Glaizot et al., 2012; Synek et al., 2013; Valkiūnas et al., 2013b). Experimental observations are needed for a better understanding of the damage caused by *Haemoproteus* parasites in mosquitoes and other bird-biting insects. Because such studies are uncommon, Valkiūnas et al. (2014) investigated the survival rate of the mosquito *O. cantans* following experimental infection with three widespread species of avian haemoproteids, i. e. *H.* (*Parahaemoproteus*) *balmorali*, *H.* (*Parahaemoproteus*) *lanii* and *H.* (*Parahaemoproteus*) *tartakovskyi*. This mosquito is widespread in Eurasia and readily bites birds (Bernotienė, 2012). Wild-caught females were allowed to feed on naturally infected and uninfected birds, and fate of the exposed insects was followed at controlled laboratory conditions. It was determined that widespread *Haemoproteus* (*Parahaemoproteus*) species are markedly virulent and rapidly kill the majority of infected *O. cantans* mosquitoes. It is likely that the simple physical damage of mosquito tissues by ookinetes in the head, thorax and abdomen was the main reason of the sharply reduced survival rate between 0.5 and 2 days post infection. During this period of time, numerous ookinetes develop and migrate throughout the body of infected insects, but oocysts are still absent (Valkiūnas et al., 2013b). Physical damage due to perforation of tissues should be directly related to the ookinete numbers, which depend on the intensity of gametocytaemia in donor birds. This explains why the mosquito survival rate markedly depends on the intensity of infection. Mosquito mortality is high at gametocytemia exceeding 1%, but is much less at parasitemia <0.5%. In nature, blood-sucking insects are exposed to numerous environmental factors, thus mosquito mortality due to *Haemoproteus* parasites may be even more pronounced than was recorded in the laboratory. Reduced survival of blood-sucking insects implies a possible reduced involvement in transmission of other infections by decreasing the number of survived biting females. This might be significant epidemiologically, but remains insufficiently investigated. Further studies are needed to understand fully consequences of pathogenesis of *Haemoproteus* infections on mosquitoes and other blood-sucking insects, particularly during abortive sporogonic development, which remain insufficiently investigated.

SPOROGONIC DEVELOPMENT OF *PLASMODIUM HOMOCIRCUMFLEXUM* (LINEAGE COLL4) IN MOSQUITOES

Analysis of cytochrome *b* gene sequences revealed huge diversity of lineages of avian haemosporidian

parasites (Ishtiaq et al., 2007; Beadell et al., 2009; Bensch et al., 2009; Chasar et al., 2009; Dimitrov et al., 2010; Marzal et al., 2011; Levin et al., 2013). However, sporogonic development of the majority of species and their lineages of avian *Plasmodium* spp. remains insufficiently studied (Kim et al., 2009; LaPointe et al., 2010; Njabo et al., 2011; Palinauskas et al., 2007). Palinauskas et al. (2015) isolated a new malaria parasite, *Plasmodium homocircumflexum* (pCOLL4) from a naturally infected red-backed shrike *Lanius collurio*. Because sporogony of *P. homocircumflexum* is unknown, we investigated development of this parasite in several common European mosquito species, i. e. *Culex pipiens pipiens*, *Culex pipiens pipiens* form *molestus* and *Aedes vexans* (Meigen, 1830). It was shown that none of these mosquitoes are competent vectors of *P. homocircumflexum* (Palinauskas et al., 2015). The sporogonic development of *P. homocircumflexum* (lineage pCOLL4) occurs to the oocyst stage in the mosquitoes *Culex pipiens pipiens, Culex pipiens pipiens* form *molestus* and *Aedes vexans,* but sporozoites do not develop, indicating the abortive parasite sporogony. Further studies are needed for determining vectors of *P. homocircumflexum* and many other species of avian haemosporidian parasites. This study also shows that detection of lineages of haemosporidian parasites and ookinetes and oocysts in mosquitoes does not necessarily indicate vectors (Santiago-Alarcon et al., 2012). Demonstration of infective sporozoites in insects is essential for definitively demonstrating the insects are vectors. Because of the complicated life cycles of haemosporidians, microscopic approaches and experimental research remain essential and should be applied in parallel with PCR-based detection tools in vector studies, particularly in wildlife.

Received 14 November 2014
Accepted 12 December 2014

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Rita Žiegytė, Gediminas Valkiūnas

NAUJAUSI PASIEKIMAI TIRIANT PAUKŠČIŲ HEMOSPORIDINIŲ PARAZITŲ PERNEŠĖJUS

S a n t r a u k a

Šiuo metu daug mokslinių darbų skirta paukščių hemosporidinių parazitų morfologiniams ir molekuliniams tyrimams bei šių patogenų paplitimui, genetinei įvairovei ir filogenetiniams ryšiams nustatyti, tačiau informacijos apie jų vystymąsi pernešėjuose yra labai mažai. Eksperimentinių tyrimų šia tema irgi yra nepakankamai. Mes glaustai apžvelgėme naujausius mokslinius pasiekimus tiriant paukščių hemosporidijų pernešėjus, taip pat eksperimentinius darbus, atliktus Gamtos tyrimų centre per paskutiniuosius penkerius metus. Ypatingą dėmesį skyrėme labiausiai paplitusiems *Plasmodium* ir *Haemoproteus* genčių parazitams. Pateikėme ir aptarėme naujausią informaciją apie paukščių maliarinių parazitų ir hemoproteidų pernešėjus. Pabrėžėme, kad plačiai paplitę *Haemoproteus* genties parazitai yra labai virulentiški kraujasiurbiams dvisparniams Culicidae ir Ceratopogonidae šeimų vabzdžiams ir raginame atlikti papildomus tyrimus šioje srityje. Dėl dažno hemoproteidų abortatyvaus vystymosi galimybės kraujasiurbiuose vabzdžiuose PGR paremti metodai šiems parazitams pernešėjuose diagnozuoti turėtų būti naudojami atsargiai, nes infekcijos diagnozuojamos kelios savaitės po užkrėtimo, neatskiriančios abortatyvaus parazitų vystymosi, kuris yra transmisijos aklavietė. Tai sukelia abejonių nustatant pernešėjus, kai naudojami tik PGR paremti tyrimo metodai. Gyvybingų sporozoitų nustatymas vabzdžiuose naudojant klasikinius parazitologijos metodus yra būtinas galutinai įrodyti, kad tam tikri vabzdžiai yra pernešėjai. Dėl sudėtingų hemosporidinių parazitų gyvenimo ciklų mikroskopiniai metodai ir eksperimentiniai tyrimai yra labai svarbūs ir turi būti naudojami lygiagrečiai su PGR paremtais tyrimo metodais, ypač nustatant pernešėjus gamtoje.

Raktažodžiai: apžvalga, hemosporidijos, kraujasiurbiai dvisparniai, pernešėjai, *Plasmodium*, *Haemoproteus*