Experimental Parasitology 165 (2016) 71–80

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

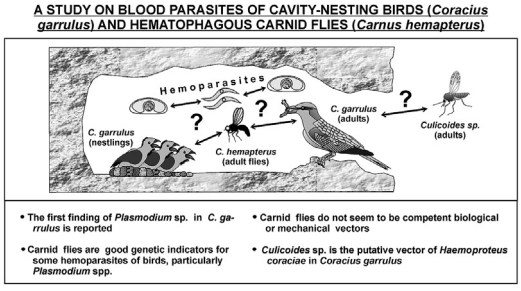
Full length article

Nest ecology of blood parasites in the European roller and its ectoparasitic carnid fly

Radovan Václav [a, *], Tatiana Betáková [b], Petra Švančarová [b], Jorge Pérez-Serrano [c], Ángel Criado-Fornelio [c], Lucia Škorvanová [b], Francisco Valera [d]

[a] Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84506 Bratislava, Slovakia
[b] Institute of Virology, Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84505 Bratislava, Slovakia
[c] Parasitology Laboratory, Departamento de Biomedicina y Biotecnología, Facultad de Farmacia, Universidad de Alcalá, ES-28871 Alcalá de Henares, Madrid, Spain
[d] Estación Experimental de Zonas Áridas (EEZA-CSIC) Ctra. Sacramento s/n, La Cañada de San Urbano, ES-04120, Almería, Spain

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form
8 March 2016
Accepted 13 March 2016
Available online 16 March 2016

Keywords:
Avian malaria
Transmission ecology
Nidicolous ectoparasites
Coracias garrulus
Carnus hemapterus
Trypanosoma

ABSTRACT

Haemosporidian parasites are considered the most important vector-borne parasites. However, vector identity and ecology is unknown for most such host–vector–parasite systems. In this study, we employ microscopic and molecular analyses to examine haemosporidian prevalence in a migratory, cavity-nesting bird, European roller *Coracias garrulus*, and its nidicolous blood-feeding ectoparasite *Carnus hemapterus*. This system is unique in that the ectoparasite is confined to a near-closed environment, in contrast to the free-wandering system of haematophagous dipterans such as mosquitoes. Blood film analysis confirms previous works in that *Haemoproteus* parasites are widely prevalent in adult rollers and belong to a single species, *Haemoproteus coraciae*. *Leucocytozoon* sp. and *Trypanosoma* sp. also are detected in adult rollers at low intensities with this technique. By means of molecular analysis, we report for the first time *Plasmodium* sp. presence in *C. garrulus*. Based on PCR results, *Plasmodium* parasites are relatively less prevalent than *Haemoproteus* parasites (20% vs. 31%) in rollers. In contrast, haemosporidian prevalences show the opposite trend for *Carnus* flies: *Plasmodium* sp. occurrence (62%) clearly predominates over that of *Haemoproteus* sp. (5%). A comparison between roller and *Carnus* samples reveals a significantly higher prevalence of *Plasmodium* sp. in *Carnus* samples. Insect survey and phylogenetic analysis suggest *Culicoides* flies as *Haemoproteus* sp. vectors, which appear to readily transmit the parasite in southern Spain. This study does not find support for *Carnus* flies to serve as biological or mechanical vectors of haemosporidians. In spite of this, nidicolous blood-feeding ectoparasites, such as

* Corresponding author.
E-mail address: radovan.vaclav@savba.sk (R. Václav).

http://dx.doi.org/10.1016/j.exppara.2016.03.014

carnid flies, appear as a suitable model for studies on the occurrence and temporal dynamics of avian haemosporidians such as *Plasmodium* sp. present at low intensities.

1. Introduction

Haemosporidian parasites are considered, due to their cosmopolitan distribution and associations with a wide range of vertebrate hosts, the most important vector-borne parasites (Garnham, 1966; Valkiūnas, 2005). In addition to direct effects of haemosporidian infection (e.g. Van Riper et al., 1986; Atkinson et al., 1995; Merino et al., 2000), long-term effects of the infection on life span and lifetime reproductive success has recently been demonstrated in birds (Ashgar et al., 2015). While concerns for human and wildlife health rise due to expanding or shifting distributions of vector-borne parasites (see Lafferty, 2009), the knowledge of vector distribution and ecology remains incomplete (e.g. Kraemer et al., 2015).

Despite the popularity of molecular techniques in detecting haemosporidians in hosts and vectors, vectors for most haemosporidians are unidentified (Cleaveland et al., 2001; Valkiūnas, 2005), and parasite–vector associations remain an enigmatic aspect of haemosporidian parasite ecology (Atkinson et al., 2008; Kimura et al., 2010; Njabo et al., 2011). For example, a long-term research conducted at a coastal region in northeast Europe suggests that vector competence can be low for some haemosporidians, even if the parasites are ingested by available haematophagous insects (Valkiūnas, 2005). It is unclear whether this result is due to the lack of competent vectors or a temporal unavailability of vectors during the breeding period of their avian hosts (Valkiūnas, 1984, 2005).

The European roller *Coracias garrulus* (hereafter roller) is a near-threatened secondary hole-nesting bird, breeding in Southern Spain in burrows excavated by other birds in sandstone cliffs as well as in cavities in bridges and nest-boxes installed on cliffs and trees (Václav et al., 2011). To date, *Haemoproteus* (*Parahaemoproteus*) *coraciae*, *Leucocytozoon eurystomi*, *Leucocytozoon bennetti*, and *Trypanosoma avium* have been detected in adult rollers during the spring migration period using blood film analysis (Danilewsky, 1889; Valkiūnas and Iezhova, 1990; Valkiūnas, 1993; Shurulinkov and Golemansky, 2002, 2003).

The nest-based ectoparasite *Carnus hemapterus* is a 2 mm long blood-sucking fly that parasitizes bird nestlings of more than 50 species of 23 families and 10 orders (Grimaldi, 1997; Brake, 2011). After winter diapause, winged adult flies usually emerge in the nests when nestling hosts hatch and emergence continues throughout the whole nestling period (Valera et al., 2003). Adult flies lose their wings as soon as they locate a suitable host (Roulin, 1998). Wingless females of this ectoparasite repeatedly take blood meals from avian nestling hosts in the same cavity and lay egg batches in the nest substrate between feeding bouts (Valera and Zídková, 2012). Incubating and brooding adult birds can serve as alternative hosts for *C. hemapterus* before more suitable nestling hosts hatch in the same cavity (López-Rull et al., 2007; Calero-Torralbo et al., 2013). *Carnus* flies have never been found, neither as adults nor larvae, on the body of adult birds outside the nest, suggesting that host location occurs exclusively during the winged phase.

Although carnid flies are not usually considered potential vectors for haemosporidians (but see Fitzner and Woodley, 1985; Soler et al., 1999; Martín-Vivaldi et al., 2006), their role as possible biological or mechanical vectors has never been studied. Previously, we reported high infestation rates by *C. hemapterus* in nestlings of *C. garrulus* breeding in a semiarid region in southeast Spain (Václav et al., 2008). The system formed by the cavity-nesting roller and carnid flies is peculiar in that the ectoparasite is confined to a near-closed environment, in contrast to the free-wandering system of mosquitoes and biting midges. Such a spatially constrained feeding habitat suggests intriguing potentials for *C. hemapterus* co-evolution with both avian hosts and blood parasites.

In this contribution we focus on the nest ecology of blood parasites. Haemosporidian prevalence is scrutinized in this unusual host–ectoparasite system at the spatio-temporal scale of host nest cavities and the host reproductive period. The approach in this study is to examine first the prevalence of haemosporidians for a multi-year sample of adult and nestling rollers. Then, partial gene sequences of mitochondrial and nuclear DNA are analysed for haemosporidians found in adult and nestling rollers and in carnid flies recovered from these hosts. Phylogenetic trees are constructed in order to infer taxonomic relations of haemosporidians detected and their possible dipteran vectors. Finally, within-cavity consistency in haemosporidian prevalence in birds and flies is examined to reveal a potential temporal pattern in haemosporidian prevalence for adult and nestling rollers. We hypothesise that it should be possible to diagnose haemosporidian infection in juvenile birds by PCR-based molecular analysis of their ectoparasitic *Carnus* flies. If supported, such a diagnostic procedure would be advantageous as it may be a non-invasive indirect method for monitoring haemosporidian infections in birds.

2. Materials and methods

2.1. Site characteristics

The study area (~50 km^2) is located within sparsely populated extensive farmland in the Tabernas Desert (Almería, SE Spain, 37°05′N, 2°21′W). The climate is semiarid with long hot summers and high annual and seasonal variability in rainfall (Lázaro, 2004).

2.2. Taxonomic survey of diptera

A qualitative survey of arthropods, aimed at the identification of putative dipteran vectors, was performed in the nest-boxes of three active roller pairs using sticky cards. About 170 flies were captured during the sampling period of one week in May 2010. Sticky cards were attached to the underside of nest-box lids during the incubation/hatching period of rollers.

2.3. Protocol for blood analysis in adult and nestling rollers – samples examined by microscopic methods

We sampled adult birds by capturing them at the nests during the incubation and hatching periods in the years 2005, 2006 and 2010 (n = 98 for the three years). Blood samples from nestling rollers were obtained only in 2006 (n = 26). Blood films were fixed in absolute ethanol immediately after blood collection and stained within a month with Giemsa for 45 min. For samples collected in 2005 and 2006, infection by intraerythrocytic haemosporidians