Charles University
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BLOOD PARASITES OF BIRDS AND THEIR VECTORS

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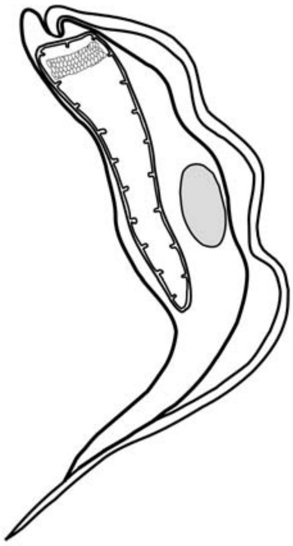

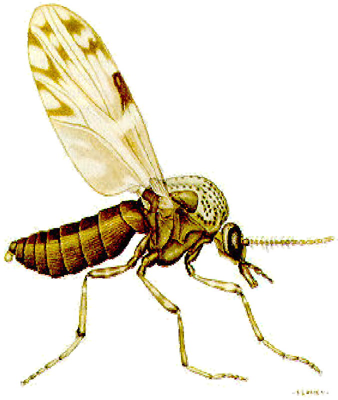

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DISSERTATION ABSTRACT

Introduction

The work is dealing with four topics: (1) identification of vectors of trypanosomes among blood-sucking insects attacking raptor nestlings, (2) mechanism of transmission of avian trypanosomes from insect vectors to vertebrate host, (3) influence of blood parasites on birds and (4) phylogenetic position of avian trypanosomes. It includes seven papers published or accepted for publication in scientific journals, one review on evolution of kinetoplast DNA network and one manuscript ready for submission.

Avian trypanosomes are widespread parasites of birds, the transmission of which remains mostly unclear, with various blood-sucking insects mentioned as possible vectors. Worldwide, about one hundred species of avian trypanosomes have been described, mostly on the basis of one host – one species paradigm, according to which a new species was assigned for every “new” bird host. Whether there is a single avian trypanosome or whether every bird species has its specific parasite has yet to be elucidated, as well as how specific the vectors of these trypanosomes are. A wide variety of blood sucking arthropods (mites, hippoboscids, biting midges, culicine mosquitoes and simuliids) has been described as vectors of avian trypanosomes. On the other hand, only few studies on experimental transmission were published and authors reported different routes of transmission of parasites to bird hosts. For most of the trypanosome species, host and vector specificities are unknown, clear-cut evidence of host-parasite relationship is still lacking, as well as evidence of the natural route of transmission.

Avian haemosporidians (family Haemoproteidae Doflein 1916) have been recorded in most of avian species and these have been found to be cosmopolitan. Hemosporidia are microscopic, intracellular parasitic protozoans found within the blood cells and tissues of their avian hosts. Three closely related genera, *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*, are commonly found in wild birds. Hemosporidia are transmitted from infected to uninfected birds by a variety of biting flies (mosquitoes, black flies, biting midges and louse flies), in which infective stages of the parasites (sporozoites) are found in their salivary glands.

Prevalence of blood parasites in European birds of prey varied considerably, depending on bird species, geographic region, habitat preferences of the bird hosts, the abundance and feeding habits of suitable insect vectors, and innate physiological differences of avian hosts.

Blood parasites are suggested to have a negative impact on their avian hosts. Infections of avian haemosporidians in highly susceptible host species and age classes may result in death, whereas pathogenicity of avian trypanosomes is ambiguous. However, the available data are mostly based on laboratory studies using domestic birds. Within natural populations of their hosts, in contrast, blood parasites are frequently considered to be organisms of low pathogenicity. Nevertheless, parasites will inevitably compete for energy and nutrients with the host, which consequently must resolve trade-offs between the amount of energy invested in reproductive effort and any immunological battle against parasites. In addition, parasites may also play an important role in sexual selection by affecting the expression of male secondary sexual traits. The Hamilton-Zuk hypothesis predicts a negative relationship between the presence and/or quantity of parasites and mail sexual traits, such as ornaments and plumage brightness, as only the healthiest individuals will be able to pay the costs of fully developed sexually selected traits.

Classification and phylogenetic position of avian trypanosomes to other members of this genus remain still unsettled. The lack of morphological features visible on blood smears to distinguish among different species shall be outbalanced by biochemical and molecular data. The 18S rRNA gene sequences are available for dozens of trypanosome species and are widely used to infer their relationship. Furthermore, we have shown that bird trypanosomes have different size of kinetoplast (k) DNA minicircles. This feature is reflected in a unique and characteristic shape of their kinetoplast and is useful in species comparison of trypanosome strain originating from different avian hosts and geographic localities.

Aims of the work

- Detect blood parasites in population of raptors (buzzard – *Buteo buteo* and sparrowhawk – *Accipiter nisus*) in the Czech Republic.
- Identify vectors of avian trypanosomes infecting birds of prey.
- Describe route of transmission and development of trypanosome infection in invertebrate vectors.
- Define influence of blood parasites on fitness and body condition of avian host in model species, Red-backed shrike (*Lanius collurio*).
- Designate phylogenetic position of avian trypanosomes on phylogenetic tree.

Summary of results

Blood parasites of raptors were investigated during the breeding seasons of 1996—2002 in Czech and Slovak Republics. Samples were collected from nestlings and adults of buzzards (*Buteo buteo*), sparrowhawks (*Accipiter nisus*), lesser-spotted eagles (*Aquila pomarina*) and kestrels (*Falco tinnunculus*). We used microscopic investigation of blood smears for determination of haemosporidian parasites and cultivation of blood for trypanosome identification. *Leucocytozoon*, *Haemoproteus* and *Trypanosoma* were found in young and adult birds and prevalence of blood protists differed according to age (higher prevalences were found in adults). The lesser-spotted eagle (*Aquila pomarina*) is reported as host of *Trypanosoma avium* and *Leucocytozoon* sp. for the first time. Neither microfilaria nor *Plasmodium* was found in any raptor species studied.

A search for possible vectors of trypanosomes of raptors was performed on buzzard and sparrowhawk nests using air-sucking miniature CDC traps without a light bulb or by the sticky papers. Dissection of insects and light microscopy examination of this intestine and salivary glands for the presence of flagellates was performed. Black flies (mainly *Eusimulium securiforme* and *E. latipes*), hippoboscid flies (*Ornithomyia avicularia*), mosquitoes (*Culex pipiens pipiens*) and biting midges (mainly *Culicoides festivipennis*, *C. kibunensis* and other 12 species), were found to contain trypanosomatids in their intestine. No trypanosomes were found in salivary glands.

Trypanosomes from the raptors and blood-sucking insects were isolated, and their 18S rRNA sequences were used for species identification and for the inference of intra- and interspecific relationships. Together with the trypanosome isolated from a black fly, the bird trypanosomes formed a well-supported *Trypanosoma avium* clade. The isolates derived from hippoboscid flies and mosquitoes are most likely avian trypanosomes infecting birds other than the studied raptors. Analysis of the kinetoplast, that has features characteristic for the avian trypanosomes (minicircle size – restriction digestion of kDNA and agarose gel electrophoresis; dimensions of the kinetoplast disc – transmission electron microscopy of cells collected from culture), provided further evidence for the identification of vectors. It is suggested that all trypanosomes isolated from raptors included in our study belong to the *T. avium* complex and are transmitted by the ornithophilic simuliids such as *Eusimulium securiforme* and *E. latipes*.

Trypanosomatid parasite from biting midge was not a member of genus *Trypanosoma*. We describe the first case of a natural infection of biting midges by a kinetoplastid protozoan. Flagellates from a female of *Culicoides kibunensis* captured at a bird nest were introduced into culture and characterized by light and electron microscopy. However, since the morphological data were inconclusive, the novel endosymbiont-free trypanosomatid was assigned to *Herpetomonas* primarily on the basis of the 18S and 5S rRNA gene sequences. We named this parasite *Herpetomonas ztiplika* n.sp. The species name is derived from the Czech name of the host insect, meaning biting midge.

Trypanosomes identified as *Trypanosoma avium* were investigated in the natural vectors, ornithophilic black flies *Eusimulium latipes*. Parasites formed plugs and rosettes in the hindgut of the vector and were attached on the cuticular lining of the black fly anterior intestine (ileum) by hemidesmosome-like plaques. Hindgut stages from infected black flies were experimentally transmitted into canaries (*Serinus canaria*) by ingestion of vectors and by contamination of host conjunctiva. This is the first evidence of such transmission in avian trypanosomes. Parasites survived in peripheral blood of birds at the least ten months. In contrast to the direct inoculation of insect stages, parasites from culture failed to produce infection in experimental birds; this has consequences in laboratory studies of host susceptibility and transmission.

The regurgitation of metacyclic stages from the cardia is supposed to be the prevailing mechanism of some trypanosomatid parasite transmission (genus *Leishmania*). This regurgitation may result from the damage of the stomodeal valve of natural vectors (sand flies of genera *Phlebotomus* and *Lutzomyia*) and its mechanical block by the parasites. We found the similar phenomenon also in avian trypanosomes transmitted by *Culex* mosquitoes. Vectors with the late-stage infections were processed for the optical and transmission electron microscopy. Localization of the late infections was similar to leishmania in sand flies: massive plug of flagellates was observed in cardia region. Parasites (epimastigote stages) attached to the stomodeal valve in large numbers, the adhesion occurred by the formation of zonal hemidesmosome-like plaques. Degenerative changes of the valve included degradation of the filamentous structures on the apical end of cylindrical cells and the separation of the cuticular lining. Blocked and damaged valve of the vector may facilitate regurgitation of parasites into the vertebrate host. The phenomenon may occur generally in trypanosomatids transmitted by bite of nematoceran *Diptera*.

We investigated the occurrence of blood parasites on the Red-backed Shrike *Lanius collurio*. Selected traits of shrike body morphology, male plumage and reproduction were studied with respect to the presence and intensity of haematozoan infection in blood samples collected from 172 breeding adults. *Haemoproteus lanii* was found to be the most common parasite (72.7 %). The prevalence of other parasites (i.e., haemoproteids *Plasmodium* sp. (cf. *relictum*) and *Leucocytozoon* sp., kinetoplastid *Trypanosoma* sp. and microfilariae *Aproctella stoddardi*) was markedly lower. Females infected by *Haemoproteus lanii* initiated egg-laying later in the season than uninfected females. Among males, infected individuals had significantly larger melanin-based tail colour patterns (a secondary sexual trait) than uninfected individuals. Moreover, mating was assortative with respect to infection.

Analyses based on 18S rRNA sequences, dimensions of the kinetoplast disc and size of kinetoplast (k) DNA minicircles were used to differentiate among large trypanosomes parasitizing birds of the Old World. These trypanosomes with typical striated appearance formed two well-supported groups – the '*Trypanosoma avium*' clade and the '*Trypanosoma corvi*' clade. Interestingly, the isolate derived from the central European hippoboscid fly (*Ornithomyia avicularia*) is closely related to *T. corvi* from a raven captured in the U.K., whereas trypanosome obtained from the blood of a raven of the Central Europe origin is a typical member of the '*T. avium*' clade.

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