Acta Tropica 162 (2016) 222–228

Contents lists available at ScienceDirect

Acta Tropica

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

Deforestation does not affect the prevalence of a common trypanosome in African birds

Gediminas Valkiūnas[a,*], Tatjana A. Iezhova[a], Ravinder N.M. Sehgal[b]

[a] Nature Research Centre, Akademijos 2, LT-08412 Vilnius, Lithuania
[b] Department of Biology, San Francisco State University, San Francisco, CA, USA

ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 3 July 2016
Accepted 11 July 2016
Available online 12 July 2016

Keywords:
Trypanosoma
Birds
Prevalence
Deforestation
Africa
PCR and microscopic diagnostic

ABSTRACT

In spite of numerous reports of avian *Trypanosoma* spp. in birds throughout the world, patterns of the distribution and prevalence of these blood parasites remains insufficiently understood. It is clear that spatial heterogeneity influences parameters of parasite distributions in natural populations, but data regarding avian trypanosomes are scarce. Using microscopy and molecular diagnostic methods, we analysed the variation of prevalence of avian *Trypanosoma* parasites in two widespread African bird species, the yellow-whiskered greenbul *Andropadus latirostris* and the olive sunbird *Cyanomitra olivacea*. In all, 353 birds were captured in pristine forests and agroforest sites in Cameroon and Ghana. Overall, the prevalence of avian trypanosomes was 51.3%. Five morphospecies were reported (*Trypanosoma everetti*, *T. anguiformis*, *T. avium*, *T. naviformis*, *T. ontarioensis*). *Trypanosoma everetti* predominated, representing 98% of all *Trypanosoma* spp. reports, and it was present in both avian hosts. The prevalence of *T. everetti* was significantly less in the yellow-whiskered greenbul (19%) than olive sunbird (83%), and the same pattern of prevalence was reported in these avian hosts at different study sites. We found no interaction between sites and the prevalence of *T. everetti*. For both avian hosts, the prevalence did not differ significantly between pristine forests and agroforests. This indicates the same pattern of transmission at sites with different levels of deforestation and suggests that spatial heterogeneity related to deforestation does not affect the prevalence of avian *Trypanosoma* infections. It is likely that host-related factors, but not environmental conditions favour or reduce these parasite infections in forests of sub-Saharan Africa. Microscopic and PCR-based diagnostics showed the same sensitivity in diagnostics of *T. everetti*. We discuss the implications of these findings for the epidemiology of avian trypanosomiasis in natural populations.

1. Introduction

Avian *Trypanosoma* species (Trypanosomatidae, Kinetoplastidae) are of cosmopolitan distribution. Transmission of these blood parasites occurs globally in countries with warm and cold climates, including the areas located beyond the Arctic Circle (Baker, 1976; Greiner et al., 1975; White et al., 1978; Bennett et al., 1994a; Holmstad et al., 2003; Valkiūnas, 2005; Sehgal et al., 2015). These are successful avian parasites based on their high prevalence in many bird populations worldwide. However, the biology and patterns of distribution of these protists both in avian hosts and blood-sucking insects remain insufficiently understood (Baker, 1976; Kilpatrick et al., 2006; Zídková et al., 2012; Sehgal et al., 2015). Only few studies have addressed the life cycles and specificity of these infections (Bennett, 1970; Baker, 1976; Dirie et al., 1990; Sehgal et al., 2001; Votýpka and Svobodová, 2004; Votýpka et al., 2012), and the development of trypanosomes in avian hosts and blood-sucking insects remains understudied in the great majority of described species. Available data show that avian trypanosomes develop and can be transmitted by blood-sucking dipteran insects belonging to different families of Diptera and dermanyssid mites, but the specificity of certain parasite species to certain vector groups remains insufficiently investigated (Bennett, 1970; Baker, 1976; Molyneux, 1977; Sehgal et al., 2015).

Much research has been carried out on mammalian species of *Trypanosoma*, which can cause severe disease in animals and humans (Taylor et al., 2007; Bezie et al., 2014). This is not a case with avian trypanosomes, which often are relatively benign in their vertebrate hosts (Baker, 1976). This fact may lower the interest of researchers for this group of parasites, which therefore they have

* Corresponding author.
E-mail address: gedvalk@ekoi.lt (G. Valkiūnas).

http://dx.doi.org/10.1016/j.actatropica.2016.07.003
0001-706X/

remained mainly of protistological significance. However, experimental studies suggested that these parasites may cause avian diseases when parasitemia is high; with histopathological changes and splenomegaly (Molyneux, 1973; Molyneux et al., 1983). It remains unclear how often trypanosomes cause disease in wild birds.

Due to broad specificity to avian hosts and trypanosome morphological plasticity, species diversity and taxonomy of these parasites remain debatable (Apanius, 1991; Zídková et al., 2012; Valkiūnas et al., 2011; Sehgal et al., 2015). Limited studies show that the main morphological characters of haematozoic trypomastigotes remain consistent upon repeated experimental passages of the same isolates from different avian hosts, indicating the validity of major readily distinguishable morphospecies (Bennett, 1961, 1970; Molyneux, 1973; Molyneux and Gordon, 1975; Woo and Bartlett, 1982; Sehgal et al., 2015). However, within morphospecies, cryptic speciation certainly exists and requires additional research (Votýpka et al., 2012; Zídková et al., 2012; Sehgal et al., 2015).

As part of a larger study, numerous blood samples were collected at pristine forest and agroforest sites in Cameroon and Ghana, and information about the distributions of avian *Plasmodium*, *Haemoproteus* and *Leucocytozoon* species at the same study sites was collected and analysed (Bonneaud et al., 2009; Chasar et al., 2009; Loiseau et al., 2010; Iezhova et al., 2010). Several readily distinguishable new morphospecies of avian trypanosomes were found and described (Valkiūnas et al., 2011; Sehgal et al., 2015). Here, we provide prevalence data of avian trypanosomes in two widespread African songbirds, the yellow-whiskered greenbul *Andropadus latirostris* (Pycnonotidae) and the olive sunbird *Cyanomitra olivacea* (Nectariniidae). These passeriform birds are widespread, abundant and relatively easy to sample both in pristine and agroforest sites (Thomas, 1995; Cheke et al., 2001; Bonneaud et al., 2009; Chasar et al., 2009; Smith et al., 2011), providing opportunities for comparative ecological research on their parasites. The aim of the study was to examine whether habitat differences were associated with differences in the prevalence of the predominant avian trypanosome, *Trypanosoma everetti* in common African bird species.

2. Materials and methods

2.1. Study sites and bird sampling

Birds were caught using mist-nets between 27 June and 27 July 2005 in Cameroon and between 7 and 19 June 2007 in Ghana. The methodology of catching birds (with mist nets) was similar at all study sites (see Bonneaud et al., 2009; Loiseau et al., 2010). In Cameroon, birds were caught at four sites. Two sites located in pristine forests (Zoebefame, 02°39.517′ N, 13°23.817′ E and Bobo Camp, 02°39.283′ N, 13°28′.267′ E) and two sites in agroforests (Ndibi, 03°46.00′ N, 12°13.00′ E and Nkwouak, 03°52.017′ N, 13°18.967′ E). All sites were between 600 and 700 m above sea level (asl). Mature forest sites were characterized by a layered closed canopy with tall emergent trees; the sites were located approximately 30 km from the nearest human settlement. Agroforest sites were adjacent to human settlements and had significant disturbance associated with cacao plantations, wood harvesting, burning and various other forms of rainforest habitat degradation. Detailed description of these sites, including habitat characterization using remote-sensing data is presented elsewhere (Bonneaud et al., 2009).

In Ghana, birds were caught at three study sites. Two sites were located in secondary forest, in which between 27% and 41% of tree cover remained (Agumatsa, 07°01.758′ N, 00°33.490′ E; 269 m asl and Abrafo (05°21.171′ N, 01°23.406′ E, 170 m asl). One site (Nkwanta, 05°16.912′ N, 02°38.495′ E, 85 m asl), although a secondary forest, had much lower levels of forest disturbance, with a 64% tree cover present. Detailed description of these sites, including characterization of climate and habitat variables was presented elsewhere (Loiseau et al., 2010).

In all, we examined 353 birds. Among them were 173 olive sunbirds and 180 yellow-whiskered greenbuls. The number of sampled birds was <14 individuals at two study sites in Cameroon (data are not shown). Because of insufficient numbers for reliable statistical analysis of prevalence data, we combined data from different study sites and presented them by nature of their habitat (pristine and agroforests) in Cameroon and Ghana (Table 1).

2.2. Collection of blood samples and their microscopic examination

The blood was taken by puncturing the brachial vein, and two or three blood films were prepared on ready-to-use microscopic glass slides. Blood films were air-dried within 5–15 s after preparation using a battery-operated fan, and fixed in absolute methanol. The blood films were stained in a 10% working solution of a commercially purchased stock solution of Giemsa's stain. Details of preparation and staining of blood films were described by Valkiūnas et al. (2008). For molecular analysis, approximately 50 μl of whole blood was drawn from each bird. The samples were fixed in lysis buffer (Sehgal et al., 2001); they were held at ambient temperature in the field and later at −20 °C in the laboratory.

Because samples were collected in remote field locations, culturing of trypanosomes was impractical for diagnostic purposes (Sehgal et al., 2015). All blood samples were examined microscopically. An Olympus BX61 light microscope equipped with Olympus DP70 digital camera and imaging software AnalySIS FIVE was used to examine blood films and to prepare illustrations. Approximately 150 fields were examined at low magnification (400×), and then at least 100 fields were studied at high magnification (1000×). Parasites were identified according to Baker (1956), Valkiūnas et al. (2011) and Sehgal et al. (2015). Intensity of parasitemia was calculated by actual counting of the number of trypomastigotes per 100 microscopic fields of view at 1000×. The statistical analysis was carried out using the 'Statistica 7' package. Prevalences of infections were compared by Yates corrected Chi-square (χ^2) test. A P value of ≤0.05 was considered significant.

2.3. DNA extraction, PCR amplification and sequencing

Samples collected in Ghana were screened by PCR. This was done with the aim to determine parasite lineages and compare the sensitivity of microscopy and PCR detection methods for trypanosomes (Table 2). To obtain total genomic DNA, blood was extracted following a DNeasy kit protocol (Qiagen®, Valencia, California), or the animal tissue protocol provided with the Wizard SV Genomic DNA Purification Kit (Promega Corporation, Madison, WI). The purified DNA was then used in a nested PCR protocol to amplify SSU rRNA DNA (Valkiūnas et al., 2011). PCR reactions and thermal cycling profiles were performed according to Sehgal et al. (2015). We used primers Tryp763 (5′-CATATGCTTGTTCAAGGAC-3′) and Tryp 1016 (5′-CCCCATAATCTCCAATGGAC-3′) for DNA amplification in first PCR. The second set of primers was Tryp99 (5′-TCAATCAGACGTAATCTGCC-3′) and Tryp957 (5′-CTGCTCCTTTGTTATCCCAT-3′). The fragment length was 770 bp. Products positive for infection were visualized on 1% agarose gels. The representative bidirectional sequence of *T. everetti* from yellow-whiskered greenbul sampled in Cameroon was deposited in Genbank (accession AF361430).