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Published in:
Ostrich

DOI:
[10.2989/00306525.2018.1539418](https://doi.org/10.2989/00306525.2018.1539418)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Atawal, A. F., Mgbeahurike, A. C., & Hammers, M. (2019). Microfilarial infections associated with body mass loss of Village Weavers *Ploceus cucullatus*. *Ostrich*, 90(1), 41-44.
<https://doi.org/10.2989/00306525.2018.1539418>

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Ostrich

Journal of African Ornithology

ISSN: 0030-6525 (Print) 1727-947X (Online) Journal homepage: <https://www.tandfonline.com/loi/tost20>

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To cite this article: Andong F Atawal, Anthony C Mgbeahuruike & Martijn Hammers (2019) Microfilarial infections associated with body mass loss of Village Weavers *Ploceus cucullatus*, Ostrich, 90:1, 41-44, DOI: [10.2989/00306525.2018.1539418](https://doi.org/10.2989/00306525.2018.1539418)

To link to this article: <https://doi.org/10.2989/00306525.2018.1539418>

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Microfilarial infections associated with body mass loss of Village Weavers *Ploceus cucullatus*

Andong F Atawal^{1,2*}, Anthony C Mgebeahuruike³ and Martijn Hammers^{1,4*}

¹ AP Leventis Ornithological Research Institute, University of Jos, Jos, Plateau State, Nigeria

² Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

³ Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria

⁴ Behavioural and Physiological Ecology, GELIFES, University of Groningen, Groningen, The Netherlands

* Corresponding author, email: andongfelix@gmail.com

Haemoparasitic infections in wild birds have been widely studied in order to understand parasite–host relationships, but our understanding of their impact on the host’s fitness is still limited. In this study, we quantified the associations between microfilarial infections and body mass and between microfilarial infections and haematocrit values in Village Weavers *Ploceus cucullatus*. We screened blood samples (thin smear and buffy coat) for the presence of haemoparasitic infections and measured haematocrit values. Fifty-seven percent of 91 individuals screened carried a microfilarial infection but, contrary to expectations, no other haemoparasitic infections were detected in the blood samples. We found a negative relationship between microfilarial infection status and body mass, but no relationship between infection status and haematocrit values. Our results suggest that microfilarial infections may be associated with body mass loss in wild birds.

Infections par microfilaires associées à une perte de poids chez les Tisserins gendarme *Ploceus cucullatus*

Les infections par des hémoparasites ont été largement étudiées chez les oiseaux sauvages dans le but de comprendre les relations hôte/parasite, mais notre compréhension de leurs impacts sur la santé de ces hôtes est toujours limitée. Dans cette étude, nous avons quantifié les relations entre les infections par microfilaires et le poids ainsi que le taux d’hématocrite chez les Tisserins gendarme *Ploceus cucullatus*. Nous avons analysé des échantillons de sang (frottis mince et couche leuco- plaquettaire) pour détecter la présence d’infections par des hémoparasites et déterminer le taux d’hématocrites. Cinquante sept pourcent des 91 individus dont le sang a été analysé étaient porteur d’une infection par microfilaires, mais contrairement aux attentes, aucune autre infection par hémoparasite n’a été détectée dans les échantillons de sang. Nous avons découvert une corrélation négative entre la présence de microfilaires et le poids des individus, mais pas de corrélation entre la présence d’infection et le taux d’hématocrites. Nos résultats suggèrent que les infections par microfilaires peuvent être associées à une perte de poids chez les oiseaux sauvages.

Keywords: anaemia, body condition, haematocrit, haemoparasitic infections, microfilariae, microscopy, *Onchocercidae*, *Ploceus cucullatus*, Village Weaver

Introduction

Haemoparasites, such as *Haemoproteus* and *Plasmodium* parasites, are common in many bird species, and their role as disease agents and their effects on avian ecology have received much attention (Loye and Zuk 1991; Atkinson et al. 2009). Adult nematodes from the family Onchocercidae live in tissues in vertebrate hosts, but early life stages of these nematodes may also occur as extracellular haemoparasites (microfilariae) in the hosts’ bloodstream. When insect vectors feed on blood from a vertebrate host, these microfilariae are transferred to the insect vector. Within the insect vector, the microfilariae develop into larvae after which the insect vector transmits these larvae

to a new vertebrate host. The impacts of microfilarial infections in birds may range from non-pathogenic to fatal (Campbell 1995; Simpson et al. 1996). Microfilariae have been shown to reduce survival when they co-occur with other types of haemoparasites (Davidar and Morton. 2006), but little is known about the impact of microfilarial infections on other fitness components of avian hosts, such as their body condition (Merkel et al. 2007). For example, despite microfilarial infections are usually predicted to negatively affect body mass or condition in birds (e.g. Astudillo et al. 2013), studies that have investigated this prediction are scarce and the direction of the relationship is unclear. For

example, previous studies have found no relationship (Merkel et al. 2007) or a positive relationship (Sehgal et al. 2005) between microfilarial infections and body mass.

In this study, we investigated the association between microfilarial infections and body condition of Village Weavers *Ploceus cucullatus*. We expected that infected individuals would have lower body mass (assuming that higher body mass reflects better body condition) than uninfected individuals and that anaemia is more likely to occur in infected individuals.

Materials and methods

Village Weavers are residents and locally nomadic intra-African migrants. These passerines are widespread across sub-Saharan Africa – except in the south-west and north-east – and occur in almost any type of open habitat. They are polygynous colonial nesters and breed during the rainy season (Fry et al. 2000). Village Weavers were trapped in Sabon-Gari (09°89' N, 08°97' E; Plateau State, Nigeria) between 12 May and 13 June 2014. Trapping was conducted from 06:30 to 10:00 daily using mist nets. Each captured bird was fitted with a unique numbered SAFRING aluminum ring to avoid resampling of the same individuals. For each individual, we recorded body mass (to the nearest 0.1 g) and tarsus length (to the nearest 0.1 mm). Individuals were sexed based on plumage characteristics (Borrow and Demey 2014). We did not examine the captured individuals for possible lesions. In total, 91 individuals were captured and blood sampled using brachial vein puncture. From each individual captured, ~80 µL of blood was collected in a heparinised capillary tube and a thin blood smear was made.

The capillaries were centrifuged using a micro haematocrit centrifuge for 5 min. We then checked the buffy coat of the sample in the capillary tube for motile haemoflagellate infections (i.e. extracellular haemoparasites, such as *Trypanosoma* and microfilariae) and the thin smears were also screened for other haemoparasitic infections (e.g. *Haemoproteus*, *Plasmodium* and *Leucocytozoon*) using a Nikon microscope with 100× magnification under oil of immersion. Encountered infections were identified based on their morphology (Valkiūnas 2005). We also recorded the haematocrit level (i.e. the proportion of red blood cells in the blood). Normal avian haematocrit levels range from 35% to 55% and values below this range indicate anaemia (Campbell 2004).

Statistical analysis

We used a general linear model with Gaussian errors and an identity link function to investigate whether body mass and haematocrit value were related to microfilarial infections. The significance level was set at $P < 0.05$ and R version 3.2.5 (R Development Core Team 2018) was used for all analyses. Body mass and haematocrit value were the dependent variables, whereas sex, tarsus length and microfilarial infection (Yes/No) were explanatory variables. Individuals with higher body mass relative to tarsus length (which was included as a covariate in the models to account for the fact that larger individuals are likely to be heavier) were assumed to have a better body condition. We also

assumed that individuals with haematocrit values between 35% and 55% were in better body condition than birds with higher or lower values (Campbell 2004). We included the interaction between sex and microfilarial infection to test whether males and females respond differently to an infection. We also included an interaction between tarsus length and microfilarial infection to investigate the possibility that larger individuals suffer less from infections than smaller individuals. We first fitted the full models including all main effects and the interactions described above. Then, in order of least significance, we removed any non-significant interactions. Main effects were always retained in the model, regardless of their significance (see Whittingham et al. 2006).

Results

Despite screening both the buffy coat region and the thin smear for the presence of a suite of haemoparasite infections, microfilarial infections were the only type of infection detected in our sampled individuals. Microfilariae were detected in 57% (52 out of 91) of samples (Figure 1). For males, 55% of samples (18 out of 33) were positive, and for females, 59% (34 out of 58) were positive.

While statistically controlling for sex and tarsus length, body mass of infected individuals was significantly lower than that of non-infected individuals (Table 1, Figure 2a). The interaction between sex and infection was close to significance (0.06; Table 1a), suggesting that the association between microfilarial infection and body mass was stronger for females than for males (Figure 2a). On average, infected females and males were, respectively, 9% and 2% lighter than non-infected same-sex individuals. Haematocrit values were similar for infected and non-infected individuals (Table 1, Figure 2b).

Discussion

We found a negative relationship between microfilarial infection status and body mass, suggesting that microfilarial

Figure 1: Microfilaria in the buffy coat layer (Giemsa stained, 100× magnification)

infections are costly to Village Weavers. Contrary to expectation, we found no relationship between infection status and haematocrit value.

Although microfilarial infections are rarely associated with diseases, lower body mass of infected individuals may indicate that infected individuals are in poorer body condition (e.g. Atkinson et al. 2009; Astudillo et al. 2013). A potential explanation for a negative impact of nematode infections on body condition could be that the microfilariae are capable of feeding on blood serum, which contains nutrients necessary for growth and development, hereby competing or obstructing the nutrients necessary for the host's body functioning (Combes 2001; Graham 2008). Apart from affecting body condition, microfilarial infections have been incriminated for inflammation of birds' pulmonary artery, which may negatively affect tissue oxygenation and lead to a low level of general metabolism (Atkinson et al. 2009). Although our results suggest that microfilarial infections are associated with lower body condition in Village Weavers, we cannot exclude that other, unmeasured, variables (e.g. other diseases, food availability, predation risk and breeding status) have caused the lower body mass in infected individuals. Future studies might test the impact of microfilarial infection on body condition by experimentally infecting uninfected individuals.

Consistent with the findings of an earlier study (Astudillo et al. 2013), we found no difference in haematocrit value between infected and uninfected individuals. The absence of a relationship between microfilarial infection and haematocrit value may be explained by the fact that microfilarial infections are extracellular haemoparasites and may not necessarily feed on or destroy red blood cells, unlike intracellular blood parasites (e.g. *Haemoproteus* or *Plasmodium* blood parasites). None of the captured birds had haematocrit values below 35%, suggesting that the captured birds did not suffer from anaemia. In fact, haematocrit values in this study ranged from 50% to 69%, which is somewhat higher than the level usually observed in birds. Some studies suggest that an increase of haematocrit value may occur during the breeding season (as in

the present study), for example because of an increase in energy demands for egg laying, incubation and parental care. Such activities increase oxygen consumption and the need for more red blood cells (Kilgas et al. 2006).

That we did not detect other haemoparasitic infections in the blood smears does not rule out that parasites were not present. For example, the number of parasites in the bloodstream may have been too low to be detected by the microscopy method we used compared to PCR methods (Jarvi et al. 2002). However, other studies have successfully used microscopy methods to investigate haemoparasitic infections in birds (Valkiūnas et al. 2008). An additional explanation for the lack of malaria-like (e.g. *Plasmodium* or *Haemoproteus*) infections in the present samples may be that infected individuals showing a sickness response from such infections are less active and therefore less likely to be caught using mist nets (Valkiūnas 2005). If this is the case, our samples will be biased towards non-infected individuals or individuals carrying a chronic infection with few or no other haemoparasites in their bloodstream.

The prevalence of microfilariae infections in the present study (57% of individuals carried an infection) was high compared with what was found in other studies on birds. Prevalence of microfilariae is generally low in birds (usually <7% across many bird species; reviewed in Sehgal et al. 2005). However, in a small number of species, a much larger proportion of individuals are infected (30% in Willow Ptarmigan *Lagopus lagopus*: Holmstad et al. 2003; 62% in Fire-crested Alethe *Alethe diademata*: Sehgal et al. 2005; 14% in Galápagos Penguins *Spheniscus mendiculus* and 42% in Flightless Cormorants *Phalacrocorax harrisi*: Merkel et al. 2007), suggesting that infection probabilities may vary greatly between hosts. Future studies should investigate whether the high prevalence of microfilarial infections is specific to Village Weavers in our study area, or whether also other bird species in our study area show high prevalence and how this relates to the abundance of insect vectors and vertebrate hosts. Future studies may use molecular methods for parasite identification and use larger sample sizes. In addition, future studies should also

Table 1: Body mass and haematocrit value in Village Weavers in relation to microfilarial infection status, sex and tarsus length. Main effects are given for a model excluding the interaction terms. Significant terms are in bold

Variable	Estimate	SE	t	P
Body mass				
Intercept	28.47	5.06	5.62	<0.001
Sex (male)	6.45	1.00	6.42	<0.001
Tarsus	0.32	0.19	1.71	0.09
Infection (Yes/No)	-2.15	0.71	-3.03	0.01
Sex × Infection	2.66	1.40	1.90	0.06
Tarsus × Infection	0.08	0.37	0.21	0.84
Haematocrit value				
Intercept	69.60	6.66	10.46	<0.001
Sex (male)	1.56	1.32	1.18	0.24
Tarsus	-0.46	0.25	-1.85	0.07
Infection (Yes/No)	-0.27	0.94	-0.29	0.77
Sex × Infection	2.87	2.65	1.08	0.28
Tarsus × Infection	-0.69	0.50	-1.39	0.17

Figure 2: Microfilarial infections and (a) body mass and (b) haematocrit value in male and female Village Weavers. Values and error bars are the mean ± SE of raw data ($n = 58$ females, 33 males)

check for adult parasites and possible lesions, especially in dead birds for which the health status can be assessed more accurately.

Acknowledgements — The study was supported by the AP Leventis Ornithological Research Institute (APLORI), Leventis Conservation Foundation, University of Jos and the National Veterinary Research Institute, Vom, Plateau State. We also thank the following people for their help with various aspects of this project: Joseph Effiong Eyo, Ikele Bright Chika, Ike Nelson Ossai, Will Cresswell, Ulf Ottosson, Sola Iwajimo, Matthew Grainger, Agbo Boniface, Konvong Shedrack Arin Izam, Ezenwaji Ngozi Evelin, Atama Chinedu Ifeanyi, Melefa Temitope Dadewura, Hinmikaye Funmilayo Faith, Ebido Chike Chukwuenyem and Elijah Sunday Okwuonu.

ORCID

Martijn Hammers <https://orcid.org/0000-0002-6638-820X>

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