Higher prevalence of avian blood parasites infection in adult females compared to adult males of brown falcon (*Falco berigora*)

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Abstract:

Haemosporidians are blood parasites that infect a wide range of birds worldwide. In this study, we examined blood samples collected from brown falcons sampled from southern Victoria, Australia, and examined overall and sex differences in infection rates. 199 birds, 108 females and 91 males, were sampled via microscope assessment of stained blood smears. Female brown falcons were found to have a higher percentage of blood parasites than males (54.3%; n = 108 vs. 45.7%; n = 91, respectively). The majority of parasites found in the study were of microfilarial worms (38.7%), *Haemoproteus spp.* (11.6%), *Plasmodium spp.* (5.0%), with only 0.5% of samples containing *Leukocytozoan spp.* There was not a significant difference between males and females in intensity of blood parasite infection between the sexes for any blood parasite type (p-value < 0.05). This relatively high overall parasitism rate could indicate underlying chronic parasitism in the sampled population that may be having an impact on individuals, particularly during times of high stress. The greater prevalence of infection among females may be related to different sex roles during the breeding season and thus vulnerability to vector exposure, as well as other factors such as vector abundances and host immunological capabilities.

Key words: Brown falcon; *Plasmodium*; *Haemoproteus*; *Leukocytozoan*; Microfilarial worms.

Introduction:

Haemosporidians are blood parasites that infect a wide range of bird's worldwide (Valkiunas, 2005; Arif et al., 2016). The increasing number of emerging zoonotic outbreaks such as H5N1 avian influenza and West Nile Virus has prompted a greater focus on blood parasites in conservation studies (Brown et al., 2006; Ishak et al., 2008). While there is a range of potential avian blood parasites that can infect avian hosts, all are spread through a variety of blood-sucking insect intermediate hosts, with parasites reproducing sexually in their insect vectors before transmission to an avian host and subsequent asexual reproduction therein. Three genera of blood parasites primarily infect avian hosts, each with different vectors of infection. *Plasmodium* are spread between birds by mosquitoes of the *Culicidae* family, *Haemoproteus* species are spread by biting midgets (*Ceratopogonidae*) and louse flies (*Hippoboscidae*), while *Leukocytozoon* are spread by blackflies of the *Simuliidae* family (Atkinson and Van

Riper, 1991; Valkiunas, 2005; La Pointe et al., 2012). Furthermore, most *Haemoproteus* and *Leukocytozoan* species are restricted to a narrow range of potential host species, all of whom are typically within the same host family. Whereas *Plasmodium* species have a much broader host specificity, with single species able to infect hundreds of different avian hosts utilizing a variety of vector genera (Atkinson & Van Riper, 1991; reviewed in Bennet et al., 1993).

Microfilarial worms are a common blood parasite group found in the blood of wild bird species. Microfilariae are produced by mature filarial nematode females and released into the host body, where they enter the circulatory system and live in the blood or skin. Haematophagic arthropods like biting midges, black flies, fleas, mosquitoes, lice, mites, and ticks spread microfilariae of different nematode species. Peripheral blood can be easily used to identify microfilariae (Binkienė et al., 2021).

The three most common protozoan avian blood parasite genera (*Haemoproteus, Plasmodium,* and *Leukocytozoan*) nonetheless can cause life-threatening disease in their avian hosts that can end in mortality (Hakarainen et al., 1997; Londono et al., 2007; Petra et al., 2011). Even when not fatal, blood parasite infection is generally harmful to the avian host and can have a significant impact on bird health (Marzal et al., 2004; Lay et al., 2011). For example, previous studies have determined that avian blood parasite infections can cause reduced productivity, delays in breeding, reduced speed, weight loss, and poor appetite, this may even contribute to extinction of bird species (Marzal et al., 2004; Lay et al., 2011; Arif et al., 2016). For avian haemosporidians, temperature and precipitation are significant abiotic factors that may encourage parasite diversification by fostering parasite development and vector breeding opportunities. Not all factors affect the prevalence of bird parasites, including the abundance of vectors. Another significant factor that may affect the prevalence of parasites in birds is the immunological capacity of the host. Moreover, migration strategy may have an impact on the prevalence of haemosporidian infections in birds because migratory species are subject to various parasites and vectors due to habitat changes throughout their annual cycle. (Clark, et al., 2014; Gutiérrez-López et al., 2015; Rodrigues et al., 2021). Avian blood parasites can be detected by many different methods. One of the most common methods is quantifying the prevalence of parasites within thin blood smears by microscopic examination (Lucas & Paul, 2003; Catrina, 2019; Valkinas et al., 2006). Another method is using molecular techniques such as polymerase chain reaction (PCR) (Moody A. and Chiodini P., 2000). Smears are cost-effective and easy to evaluate, even though they require more manual work.

Despite ease of access to survey methods, blood parasite prevalence and intensity in raptorial species has received comparatively little research attention (Petra et al., 2011; Brown et al., 2006), despite these species being located at the top of the food chain. More research is imperative to advance our understanding of the genetic diversity, true diversity, and host specificity of blood parasites in raptor birds, particularly among Australian species. Our study therefore examined blood parasite load in adult brown falcons (*Falco berigora*), which belong to the Falconidae family and are one of the most widely distributed species in Australia (Blakers et al., 1984; Marchant and Higgins, 1993). Brown Falcons are carnivorous raptors that feed on a wide range of prey, ranging from insects and small mammals such as mice, through to larger mammals including rabbits, birds and reptiles (Mc Donald et al., 2003). The breeding season of the Brown Falcon, occurs from June to December, where old stick nests of other species such as raptors and ravens are appropriated as a nesting site (Marchant and Higgins, 1993; McDonald et al., 2003). Females typically incubate the eggs, feed young nestlings, remove remnants of food and any dead chicks or unhatched eggs while remaining near the nest site, while males range more widely during the breeding season to hunt, providing enough food for both females and the young nestlings once hatched, and also occasionally help with the incubation of eggs (McDonald et al., 2003; McDonald, 2004). Females are slightly larger than males and the species displays marked sexual dimorphism in size and colour (McDonald 2003b). This study aimed to determine the most common types of blood parasites present in adult brown falcon blood smears and determine the prevalence and intensity of blood parasites in these samples across the population, as well as examine any sex differences based on the different sex roles during reproduction.

Methods and Materials:

A total of 199 blood samples were collected from Brown Falcons captured at the Western Treatment Plant near Melbourne, Victoria (38° 1′ 17.52″ S, 144° 34′ 25.82″ E) (from 2000 to2002). In this study, blood was taken via venipuncture of the ulnar (wing vein) and collected using capillary tubes as part of an in-depth study of the breeding ecology of this species (McDonald, 2004). The birds were also sexes molecularly (McDonald, 2003). Thin blood smears were prepared after blood collection by air-drying before being fixed in absolute methanol. The fixed smears were then stained with Quick Dip solution I (Fronine, Sydney) five times each for one second, followed by Quick Dip Solution II (Fronine, Sydney) five times each for one second. The benefit of using Quick Dip Solution is that it provides consistent and high quality blood film staining, enabling researchers to differentiate between the different types of white blood cells and their details, such as nuclear and cytoplasmic structure (Campbell and Ellis, 2007). The blood smears were then rinsed thoroughly with distilled water until clear and air-dried.

Blood parasites were assessed using a manual counting method. We aimed to view a minimum of 2000 cells in the twenty-five randomly chosen fields of view for each blood smear. The blood parasites found were identified to the genus level and photos taken were of different parasites using a digital camera (Coolpix 5400, Nikon, Korea).

Statistical analysis:

All statistical analyses were calculated using the Statistical Packages for Social Sciences (SPSS) (v.26, IBM Statistics, Chicago). Unless otherwise specified, data are expressed as mean and standard deviation (SD). Data was analyzed to generate the frequency of gender in blood samples by descriptive statistics. A Spearman's coefficient correlation was performed to examine the relationship between gender of brown falcon birds and types of blood parasites. The alpha level of 0.05 was considered as the cut-off value for statistical significance.

Results:

Blood parasites were examined in 199 blood samples from brown falcons (108 females and 91 males). Female brown falcons were found to have a higher percentage of blood parasites than males (54.3%; n = 108 vs. 45.7%; n = 91, respectively). (Table 1)

Table 1: The distribution of brown falcon blood samples with at least one blood parasite detected based on sex.

Sex	Count	%
Male	91	45.7
Female	108	54.3
Total	199	100

Male and female brown falcon blood parasite infection intensity

Table 2 shows that there were no significant differences between male and female blood parasite infection intensity for either *Haemoproteus,* Microfilarial worms, *Plasmodium* and *Leukocytozoan* (p-values all > 0.05).

Table 3: The comparison between male and female in terms of each blood parasites.

Blood parasites	Sex	N	Mean	Standard Deviation	Mann Whitney	P value
Haemoproteus	Male	91	0.52	2.70	4770	0.522
	Female	108	1.67	14.20		
Microfilarial worms	Male	91	0.71	0.93	4350	0.110
	Female	108	0.56	0.94		
Plasmodium	Male	91	0.08	0.34	4874	0.794
	Female	108	0.07	0.35		
Leukocytozoan	Male	91	0.01	0.11	4860	0.276
	Female	108	0.00	0.00		

Discussion:

In this study, the blood samples of brown falcons showed a relatively high prevalence infection with at least one haemosporidian parasite species, with 51.3% of birds sampled infected. Furthermore, the majority of the study samples were counted for *Haemoproteus* and microfilarial worms. These worms are highly specialized parasites of tissues and tissue spaces of birds, mammals, amphibians and reptiles. Mature filarioid nematode females produce microfilariae, which are released into the host body, enter the circulatory system, and live in the blood or skin. Microfilariae of various nematode species are spread by hematophagous arthropods such as biting midges, black flies, fleas, mosquitoes, lice, mites, and ticks. Microfilariae are easily detected in peripheral blood (Binkienė et al., 2021; Krizanauskiene et al., 2006).

The prevalence of *Plasmodium* and *Leukocytozoan* were found in relatively small numbers in the blood samples of brown falcons sampled. In natural infections, *Plasmodium* species parasitemia is frequently mild, but chronic infections can still cause health impacts, particularly in stressed birds (Marzal et al., 2004; Lay et al., 2011). In addition, temperature and precipitation are important abiotic variables for avian haemosporidians that may promote parasite diversification by promoting parasite development and vector breeding opportunities (Clark, et al., 2014). The abundance of vectors is not the only factor that influences the prevalence of bird parasites. The host's immunological capabilities are another important factor that may influence parasite prevalence in birds, as is the habitat occupied. Brown falcons are not migratory, which has been a cause of increased infection (Gutiérrez-López et al., 2015; Rodrigues et al., 2021), however perhaps the large amount of standing water at the sampling site increased the risk of infection and encounter rates with hosts. The lack of a sex difference in infection rates was surprising, given female falcons are the main incubators and sit on nests while males move more widely throughout their territory, so we were anticipating higher infection rates among female other than male brown falcons. Male falcons under attack from biting insects can move their position to another area, whereas incubating female falcons must just sit it out.

Conclusion:

In this study, two major blood parasites were found in large numbers: *Haemoproteus* and microfilarial worms in males and females of the bird samples, as well as *Plasmodium* and *Leukocytozoan* in small numbers in falcon blood smears. Female brown falcons have a higher infection rate than males, which is related to their incubating period and nesting habits. In addition, some factors that may influence parasite prevalence in birds include climate change, vector abundances, host immunological capabilities, and migration.

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