Veterinary Integrative Science 2019; 17(1): 65-73

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

A survey on blood parasites of birds in Chiang Mai province

Nithidol Buranapim[1,*], Pariya Chaiwisit[1] , Attawit Wangkawan[1] and Saruda Tiwananthagorn[2,3]

[1]Department of Companion Animal and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
[2] Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand.
[3] Center of Excellence in Veterinary Bioscience, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, 50100, Thailand

Abstract

Blood parasites are one of the most remarkable bird parasitism. Though these agents may not the main cause of death in avian species, they interfere with vital organs normal functions. This study was aimed to survey and provide the occurrence of blood parasites in birds within Chiang Mai. Blood samples were collected from 111 birds composed of red-backed sea eagles (n=2), rock doves (n=52), red junglefowls (n=14), spotted wood-owl (n=1), pheasants (n=13), peafowls (n=2), purple swamphen (n=1), spotted dove (n=1), common mynas (n=3), tree sparrows (n=6), red-whiskered bulbuls (n=13), and Asian barred owlets (n=3). Blood was drawn from brachial wing veins then stored in capillary tubes and EDTA microcentrifuge tubes. Blood samples were centrifuged to evaluate packed cell volume (PCV) and Woo's technique was performed to identify *Trypanosoma* spp. To identify other blood parasites, thin blood smears were made by dying with Giemsa's stain and observed under 40x, 100x ,400x, 1000x microscopic magnifications. From 111 samples, 54 samples gave blood parasite positive results (48.65%). Further investigation showed 18 samples were positive with more than one parasite species. Four major group of blood parasites were detected, including Microfilaria 35.14%, *Haemoproteus* spp. 17.12%, *Leucocytozoon* spp. 8.11%, and *Trypanosoma* spp. 4.50%. Bird that positive to blood parasites can show normal or low PCV values and may appear to be as healthy as normal birds while act as a vector.

Keywords: Avian, *Haemoproteus* spp., *Leucocytozoon* spp., Microfilaria, *Trypanosoma* spp.

***Corresponding author:** Nithidol Buranapim, Department of Companion Animal and Wildlife Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand. Tel: (+66) 53-948015, Fax: (+66) 53-948015, E-mail: armvet64@hotmail.com, armvet64@gmail.com

Article history; *received manuscript: 10 October 2018*
revised manuscript: 22 October 2018
accepted manuscript: 24 October 2018
published online: 7 November 2018
Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

Current number of naturally bird species surveyed in Thailand are 982 species: 567 native species, 326 migratory species, and 89 species of them appears to be both. Seasonally, migrating birds have two periods of seasonal migration which is are to escape from cold and to lay eggs. The migration periods are diverse depends on bird species and the geographic area (Chanittawong et al., 2006). Disease transmission is often caused by the vectors which affects both human and animal health (Savage et al., 2009).

The most common blood parasites found in avian species are *Haemoproteus* spp., Microfilaria, and *Trypanosoma* spp. Vectors of the disease, blood sucking insects, allow the parasite to mature and increase their number within the vectors and the bird itself. These are some examples of agents and their vector such as avian plasmodium with Culicidae, and *Haemoproteus* spp. with Ceratopogonidae and Hippoboscidae (Atkinson et al., 2008). Severity of the disease depends on parasite species, stage of infection, quantity of the parasites, environment, stress, and the age of the bird. Typical clinical signs of infection include depression, weakness, anorexia, and in some cases, a sudden death (Quillfeldt et al., 2011). Other avian species such as chickens also have important blood parasites as well such as *Leucocytozoon* spp. and *Trypanosoma* spp. Those infected by the parasites may present clinical signs or stay in subclinical state (Ritchie et al., 1994). In 2000, Leucocytozoonosis and malaria was found in laying hens in Nakornsrithammarat with 10% morbidity rate and 1.5% mortality rate. Clinical signs presented in the study including depression, anorexia, pale comb and wattle, egg production reduction, and diarrhea (Worasing et al., 2001). Another study also reported blood parasites detected from backyard chickens within Chiang Mai province (Takang et al., 2017).

The pathology of birds infected with blood parasites varies, with different levels of severity. For instance, *Haemoproteus danilewaskiyi* causes an eosinophils, basophils, and heterophils proliferation which can be a result from inflammation of liver, lungs, or spleen. Elevated eosinophils and decreased PCV responses to parasitic infections. Blood protein concentration increases due to the immunoglobulins production or loss of fluids. Increasing immunoglobulin level correlates with *Plasmodium* spp. and *Leucocytozoon* spp. infection as well. Results from the study of glucose usage in pigeon cells revealed that red blood cells infected with *Haemoproteus columbae* used 100 times more glucose than normal cells. Therefore, the low glucose level of the host leads to weakness and anorexia (Donovan et al., 2008).

Previous studies in Thailand have reported types and prevalence of blood parasites in birds within the Bung Boraphet area. Several blood parasites were detected from 9 different birds, including 8 species of *Haemoproteus* spp.; *H. herodiadis*, *H. fallisi*, *H. dicruri*, *H. payevski*, *H. otocompsae*, *H. sanguinis*, *H. paseris* and *H. orizivorae*; and 4 species of *Plasmodium* spp.; *P. elongatum*, *P. lophurae*, *P. vaughani*, and *P. circumflexum*. *T. avium* was the only one species detected from *Trypanosoma* genus. From this study, 99 out of 633 birds were infected with blood parasites and the prevalence was 15.64% with a 95% confidence interval (Prompiram et al., 2015). For the study investigating blood parasites in natural birds within Chiang Mai province, rich in natural area and variety of birds, still have not been thoroughly studied. Therefore, this study aims to survey types of blood parasites in birds from different study groups within Chiang Mai. The findings will represent the current blood parasites situation of birds in Chiang Mai and a solution guideline in the future.

MATERIALS and METHODS

Sample collection

Total 111 samples were collected during 1 June – 30 November 2017 from both free ranging and captive birds. The birds in this study were either free-ranging wild or captive within Chiang Mai province. The free-ranging birds were caught from Tha Pae Gate, Nong Buak Hat Park and Huai Hong Khrai Royal Development Study Centre using nets, and were restrained for blood collection. Samples of captive birds were collected from captive birds in Huai Hong Khrai Royal Development Study Centre and birds that were brought in for treatment at the Chiang Mai University Veterinarian Teaching Hospital, which were restrained by their owners and the procedures were performed by a veterinarian. Blood was collected via brachial wing vein or the jugular vein (Sakas et al., 2002) with a 24-27 gauge hypodermic needle, then stored in capillary tubes and micro centrifuge tubes with EDTA to prevent coagulation (Samour, 2016).

All animal procedures were approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand (S15/2560) (Permission number U1-00652-2558).

Hematology and blood parasite detection

Packed cell volume (PCV) was determined by the hematocrit centrifugation method using a Hettich Zentrifugen EBA12® centrifuge. After making a thin blood film on 24 mm x 50 mm glass slide dyed with Giemsa's stain (Bennett, 1970; Campbell et al., 2007), blood parasites were identified and categorized into two groups through light microscope. The first group is blood parasites residing outside red blood cells and the other group residing within. Parasites identification was based on characteristics, shape, borders, and pathologic symptoms of the host. The hematocrit centrifugation method (Woo's technique) was applied in motile parasites. *Trypanosoma* spp. and Microfilaria would be detected between red blood cells and plasma (buffy coat) with a light microscope.

Statistical analysis

Infection rates of blood parasites in birds within Chiang Mai province in this study are displayed descriptively in number of birds positive with parasites.

RESULTS

Number and types of birds

Samples were collected from 111 birds composed of red-backed sea eagles (*Haliastur indus*), rock doves (*Columba livia*), red junglefowls (*Gallus gallus*), spotted wood-owls (*Glaucidium cuculoides*), pheasants (*Lophura nycthemera*), peafowls (*Pavo cristatus*), purple swamphen (*Porphyrio porphyrio*), spotted dove (*Streptopelia chinensis*), common mynas (*Acridotheres tristis*), tree sparrows (*Passer montanus*), red-whiskered bulbuls (*Pycnonotus jocosus*), and spotted owlet (*Strix seloputo*). Birds are divided into wild birds and captive birds (Table 1).

Table 1 A list of bird species collected in the study.

Wild birds			Captive birds		
Common name	**Scientific name**	**N**	**Common name**	**Scientific name**	**N**
Red-backed sea-eagle	*Haliastur indus*	2	**Spotted dove**	*Spilopelia chinensis*	1
Rock dove	*Columba livia*	52	**Common myna**	*Acridotheres tristis*	3
Red junglefowl	*Gallus gallus*	14	**Tree sparrow**	*Passer montanus*	6
Spotted wood owl	*Strix seloputo*	1	**Red-whiskered bulbul**	*Pycnonotus jocosus*	13
Pheasant	*Lophura nycthemera*	13	**Asian barred owlet**	*Glaucidium cuculoides*	3
Purple swamphen	*Porphyrio porphyria*	1	**Peafowl**	*Pavo cristatus*	2
Total		83	**Total**		28

Packed Cell Volume

Seventy-nine samples were examined for packed cell volume (PCV). The average PCV was 34.44 (SD = 3.41), then birds were divided into two groups according to PCV results. Birds with normal PCV value (PCV ≥ 30) had 61.64% infection rate while birds with low PCV value (PCV < 30) revealed up to 75% infection rate.

Woo's technique

Examined by Woo's technique, 43 out of 81 samples were parasites positive (53.09%). Amongst these positive samples, Microfilaria and *Trypanosoma* spp. were detected in 39 samples (24 *Columba livia*, 11 *Gallus gallus*, and 4 *Lophura nycthemera*) and 5 samples (4 *Columba livia* and 1 *Pavo cristatus*), respectively (Table 2). One sample (2.33%) was co-infected by both species.

Table 2 Prevalence of blood parasites detected by Woo's technique.

Bird	Blood parasite	Positive/number	Percentage
Columba livia	Microfilaria	24/52	46.15
	Trypanosoma spp.	4/52	7.69
Gallus gallus	Microfilaria	11/14	78.57
Lophura nycthemera	Microfilaria	4/13	30.77
Pavo cristatus	*Trypanosoma* spp.	1/2	50.00

Thin blood smear

The morphological comparison was used to classify blood parasites infected in red blood cells. Microscopic findings revealed that 28 out of 111 samples were positive for the blood parasites (25.23%). Amongst these positive samples, 19 (*Columba livia*), and 9 (1 *Acridotheres tristis*, 6 *Gallus gallus*, and 2 *Lophura nycthemera*) samples were infected with *Haemoproteus* spp. and *Leucocytozoon* spp. respectively (Table 3).

Table 3 Result of blood parasite by thin blood smear.

Bird	Blood parasite	Positive/number	Percentage
Acridotheres tristis	*Leucocytozoon* spp.	1/3	33.33
Columba livia	*Haemoproteus* spp.	19/52	36.54
Gallus gallus	*Leucocytozoon* spp.	6/14	42.86
Lophura nycthemera	*Leucocytozoon* spp.	2/13	15.38

Blood parasites and bird species

Leucocytozoon spp. was distinguished in three bird species including common mynas (*Acridotheres tristis*), red junglefowls (*Gallus gallus*), and pheasants (*Lophura nycthemera*). While *Haemoproteus* spp. was only detected in rock doves (*Columba livia*), Microfilaria was found in rock doves (*Columba livia*), red junglefowls (*Gallus gallus*), and pheasents (*Lophura nycthemera*). Lastly, *Trypanosoma* spp. was positive in rock doves (*Columba livia*), pheasants (*Lophura nycthemera*), and peafowls (*Pavo cristatus*) (Table 4).

Table 4 Positive blood parasite samples detected from each avian species identified by Woo's technique and thin blood smear.

Bird	Blood parasite	Positive/number	Percentage
Acridotheres tristis	*Leucocytozoon* spp.	1/3	33.33
Columba livia	*Haemoproteus* spp.	19/52	36.53
	Microfilaria	24/52	46.15
	Trypanosoma spp.	4/52	7.69
Gallus gallus	Microfilaria	11/14	78.57
	Leucocytozoon spp.	6/14	42.86
Lophura nycthemera	*Leucocytozoon* spp.	2/13	15.38
	Microfilaria	4/13	30.77
Pavo cristatus	*Trypanosoma* spp.	1/2	50.00

Blood parasite infection rate

Out of 111 birds, 54 birds (48.65%) were positive with blood parasite infection. Four major genus of blood parasites were identified including *Leucocytozoon* spp., *Haemoproteus* spp., Microfilaria, and *Trypanosoma* spp. (Figure 1). Microfilaria was the most common blood parasite detected (35.14%). *Haemoproteus* spp., *Leucocytozoon* spp., and *Trypanosoma* spp. was found in 19 (17.12%), 9 (8.11%), and 5 (4.50%) samples respectively. Eighteen samples (16.22%) had various parasites co-infection. Co-infection of *Leucocytozoon* spp. and Microfilaria was detected in 6 samples (33.33%) while *Haemoproteus*-Microfilaria co-infection was found in 11 samples (61.11%). The co-infection between 3 major agents (*Haemoproteus* spp., Microfilaria, and *Trypanosoma* spp.) presented in one sample (5.56%) from positive samples (Table 4).

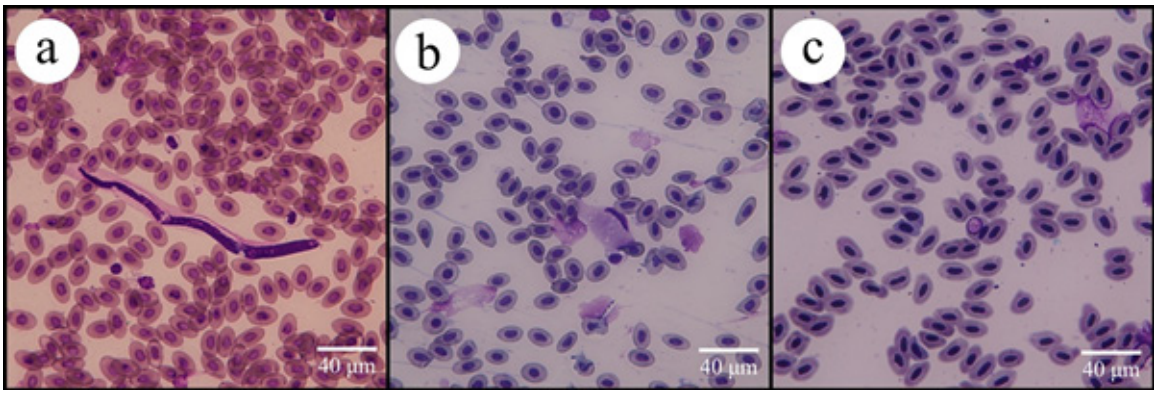

Figure 1 Light microscopic findings (1000x) of blood parasites: Microfilaria (a), *Leucocytozoon* spp. (b) and *Haemoproteus* spp. (c).

Blood parasite infection comparison according to groups of birds revealed that *Leucocytozoon* spp. were detected in both wild birds (9.64%) and captive birds (3.57%). *Trypanosoma* spp. was identified in both wild birds (5.06%) and captive birds (50%) as well. On the other hand, *Haemoproteus* spp. and Microfilaria were discovered only in wild birds with 22.89% and 49.37% infection rate respectively (Table 5).

Table 5 Blood parasite comparison of wild and captive birds.

Blood parasite	Wild bird		Captive bird	
	Positive/number	Percentage	Positive/number	Percentage
***Leucocytozoon* spp.**	8/83	9.64	1/28	3.57
***Haemoproteus* spp.**	19/83	22.89	0/28	0
Microfilaria	39/79	49.37	0/2	0
***Trypanosoma* spp.**	4/79	5.06	1/2	50.00

DISCUSSION

From 111 birds in Chiang Mai, 54 of them (48.65%) were infected with blood parasites. Four major groups of blood parasites were identified including *Leucocytozoon* spp., *Haemoproteus* spp., Microfilaria, and *Trypanosoma* spp. Bird species with most infected ratios were Columbidae and Phasianidae families. The intensive infection could be in accord with their flocking nature causing disease transmission within a small population. Close contact with blood parasites vectors such as Hippoboscid flies for *Haemoproteus* spp., Microfilaria and *Trypanosoma* spp. (Pholpark et al., 2013) could also be the cause of infection as well. From other studies, Culicoides or biting midges can carry *Leucocytozoon* spp., *Haemoproteus* spp., Microfilaria, and *Trypanosoma* spp. Simulium insects can act as an intermediate host to *Leucocytozoon* spp., Microfilaria, and *Trypanosoma* spp. Mosquitoes (Culicidae) are vectors of *Plasmodium* spp., Microfilaria, and *Trypanosoma* spp. While lice can carry *Haemoproteus* spp. and Microfilaria, mites can carry only *Trypanosoma* spp. (Atkinson et al., 2008).

Bartlett (2008) reported 6 different orders of Microfilaria detected in Columbidae birds which are Aproctella, Cardiofilaria, Chandlerella, Eulimdana, Pelecitus, and Splendidofilaria. In addition, Eulimdana is a parasite which causes feather disease in rock doves. Phasinidae birds can be infected with up to 9 orders containing Aproctella, Cardiofilaria, Chandlerella, Eufilaria, Lemdana, Pelecitus, Parachoncerca, Sarconema, and Splendidofilaria.

An average value of packed cell volume in this study was 34.44% which was considered to be normal (normal PCV range = 30%-45%) (Samour, 2016). Thus, the results indicated that birds containing parasites were in subclinical or latent stage. The packed cell volume was considered higher than the study from the Bung Boraphet area which was reported with a 15.64% infection rate (Prompiram et al., 2015). However, many variable factors such as identification techniques were to be considered. For future studies, utilizing more precise identification method such as polymerase chain reaction will give more accurate results.

Sample collection in this study was divided into two groups, free ranging or wild birds, and captive birds. The results show that wild birds have a higher infection rate than captive ones, which can be explained by a higher chance of close contact with parasite vectors (blood sucking insects). The period of sample collection was between June to November, which is the breeding season of Passerine birds and when they are prone to being infected. However, surveying outside of breeding season is also significant since parasites can also transmit in non-breeding season, which environmentally supports the growth of disease vectors (Dunn et al., 2013).

CONCLUSION

From this study on blood parasite infections Chiang Mai province, 54 out of 111 birds (48.65%) were infected. Four groups of blood parasites were identified; *Leucocytozoon* spp., *Haemoproteus* spp., Microfilaria, and *Trypanosoma* spp. The results have achieved the aim of this study, which was to find preliminary information on blood parasite infections for future studies. However, a larger sample size is advised together with more location diversity to cover Chiang Mai province. Polymerase chain reaction is recommended for accurate parasite species identification in both clinically and sub-clinically infected birds (Garamszegi, 2010). If no prevention plan or disease control (vector control) is established, captive bird keepers will be directly affected by blood parasites outbreak. Therefore, bird keepers should have a good understanding of the disease to decrease the incidence of disease occurrence.

REFERENCES

Atkinson, C.T., Thomas, N.J., Hunter, D.B. (Eds.)., 2008. Parasitic diseases of wild birds. Wiley-Blackwell, Ames, Iowa.

Bartlett, C.M., 2008. Avian filarioid nematodes of the world, in: Wildlife disease association. Presented at the annual international conference 2008, Edmonton, Canada.

Bennett, G.F., 1970. Simple techniques for making avian blood smears. Can. J. Zool. 48, 585–586. doi:10.1139/z70-098

Campbell, T.W., Ellis, C., 2007. Avian and exotic animal hematology and cytology, 3rd ed. Blackwell Pub, Ames, Iowa.

Chanittawong, W., Chaipakdee, M., 2006. Migratory birds of Thailand. Wildlife research division, Department of national parks, wildlife and plant conservation.

Donovan, T.A., Schrenzel, M., Tucker, T.A., Pessier, A.P., Stalis, I.H., 2008. Hepatic hemorrhage, hemocoelom and sudden death due to haemoproteus infection in Passerine birds: Eleven Cases. J. Vet. Diagn. Invest. 20, 304-313.

Dunn, J.C., Goodman, S.J., Benton, T.G., Hamer, K.C., 2013. Avian blood parasite infection during the non-breeding season: an overlooked issue in declining populations?. BMC. Ecol. 13, 30. doi:10.1186/1472-6785-13-30.

Garamszegi, L.Z., 2010. The sensitivity of microscopy and PCR-based detection methods affecting estimates of prevalence of blood parasites in birds. J. Parasitol. 96, 1197–1203. doi:10.1645/GE-2531.1.

Pholpark, M., Pholpark, S., 2013. *Trypanosoma evansi* infections in Northeastern region of Thailand, Thai-NIAH eJournal, 8, 32-55.

Prompiram, P., Kaewviyudth, S., Sukthana, Y., Rattanakorn, P., 2015. Study of morphological characteristic and prevalence of haemoproteus blood parasite in Passerines in Bung Boraphet. Thai. J. Vet. Med. 45, 399-409.

Quillfeldt, P., Arriero, E., Martínez, J., Masello, J.F., Merino, S., 2011. Prevalence of blood parasites in seabirds - a review. Fron. Zool. 8, 26. doi:10.1186/1742-9994-8-26.

Ritchie, B.W., Harrison, G.J., Harrison, L.R. (Eds.), 1994. Avian medicine: principles and application. Wingers Pub, Lake Worth, Fla. pp 176-198.

Samour, J. (Ed.), 2016. Avian medicine, 3rd ed. Elsevier, St. Louis, Missouri.

Sakas, P.S., Bauck, L., 2002. Essentials of avian medicine: a guide for practitioners, 2nd ed. American animal hospital association press, Lakewood, Colo.

Savage, A.F., Robert, V., Goodman, S.M., Raharimanga, V., Raherilalao, M.J., Andrianarimisa, A., Ariey, F., Greiner, E.C., 2009. Blood parasites in birds from Madagascar. J. Wild. Dis. 45, 907–920. doi:10.7589/0090-3558-45.4.907

Takang, P., Pikulkaew, S., Awaiwanont, N., Numee, S., 2017. Prevaleance and risk factors of blood parasites infection in backyard chickens in Chiang Mai. Chiang Mai Vet. J., 157–167. doi:10.14456/cmvj.2017.15

Worasing, R., Kongkeaw, W., Tiptara, A., Anant, S., 2001. Leucocytozoonosis with avian malaria in layer chicken and treatment. The 39th Kasetsart university annual conference, Ministry of University Affairs, Bangkok (Thailand), 557-563.

How to cite this article;

Nithidol Buranapim, Pariya Chaiwisit, Attawit Wangkawan and Saruda Tiwananthagorn. A survey on blood parasites of birds in Chiang Mai province. Veterinary Integrative Sciences. 2019; 17(1): 65-73