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A widespread survey of avian haemosporidia in deceased wild birds of Japan: the hidden value of personally collected samples

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ABSTRACT. Widespread surveys of avian haemosporidia (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) in wild birds have substantially advanced information on the haemosporidian fauna of Japan. However, many areas and bird species remain insufficiently investigated. Bird carcasses collected for personal specimen collection seldom reach academic audience particularly in the veterinary field. The presence of avian haemosporidia was investigated in these personally collected bird carcasses, in order to better understand the avian haemosporidian fauna in Japan. Bird carcasses were donated through personal contact upon approval of the study. Tissue samples were collected from the birds and examined for haemosporidian parasites using nested-PCR targeting the cytochrome *b* gene. One hundred and forty-three birds of 85 species were donated, including 34 species and two subspecies that were molecularly or collectively investigated for the first time in Japan. Avian haemosporidian DNA was detected from 37 of the 134 tested birds (27.61%). In 8 bird species, avian haemosporidia was detected for the first time. Twenty-nine lineages were detected, including 8 novel and 9 known lineages detected in Japan for the first time. Furthermore, 16 lineages were detected from novel host species. While information that could be drawn was limited and risk management of zoonotic diseases needs re-consideration, these findings expanded information on the host range and distribution of several lineages. Collectively, this method of investigation using personally collected bird samples can provide important additions to more fully understand the avian haemosporidian fauna of Japan, as well as other areas with limited investigations.

KEYWORDS: avian haemosporidia, cyt*b*, deceased bird, Japan, personal collection

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Avian haemosporidia (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) are blood parasites that infect birds of various species throughout the world [40]. These parasites are transmitted by blood-feeding arthropods: *Plasmodium* by mosquitoes (Culicidae), *Haemoproteus* by louse flies (Hippoboscidae) or biting midges (Ceratopogonidae), *Leucocytozoon* by black flies (Simuliidae) or biting midges [35, 40]. Transmission is possible anywhere as long as competent vector and host species are present. In some naïve species, avian haemosporidia is known to cause severe symptoms, which may lead to drastic population declines or even extinction [2, 41]. For example, several cases of lethal haemosporidiosis have been reported in *ex-situ* populations of penguins (Sphenisciformes) and parrots (Psittaciformes) [10, 33]. Additionally, other effects such as reduced reproductive success and delayed migration have also been reported [6, 25]. Monitoring the haemosporidian distribution and diversity is therefore important from both the ecological and conservational perspective.

Studies on avian haemosporidia in wild birds of Japan began in the early 20th century, and since then, avian haemosporidia have been investigated in birds throughout Japan [27, 30]. Widespread investigations of the parasites reported an overall haemosporidian prevalence of 10.6–21.1% in the investigated wild birds [15, 17, 27, 30]; although some species such as *Corvus* crows of Hokkaido Prefecture (93.2–95.8%), and coal tits (*Periparus ater*; 64.3%) and willow tits (*Poecile montanus*; 81.8%) of Saitama Prefecture revealed considerably high parasite prevalence. While some studies took place in distinct areas of Japan such as Minami-Daito Island in southwest Japan and Mt. Tateyama which is part of the Northern Japan Alps [29, 36], studies tended to concentrate in certain areas such as the Kanto Region and Hokkaido Prefecture [15, 17, 28, 42]. Furthermore, previous studies mostly investigated birds captured via mist netting and other methods, and rescued birds administered to wildlife rehabilitation facilities. However, bird

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species are limited to those of certain environments in the former and to those inhabiting areas surrounding the facilities in the latter. Hence, species that can be covered in these investigations are biased towards certain species. Additionally, studies have suggested that heavily infected birds are less active compared to healthy or sub-clinically infected birds [40]. Therefore, investigations in live birds by mist netting and other methods may be biased towards sub-clinically infected individuals [40], and may be insufficient to uncover the whole haemosporidian fauna.

In Japan, many bird carcasses are personally collected for taxidermy, feather, and bone specimens. Most often, the remaining parts of the bird including the organs and muscles are discarded, and these birds seldom reach academic audience. Meanwhile, in recent years, photos or information on bird carcasses are submitted to social network services (SNS) to either provide information or to offer collection of the bird for those in need of it (whether academic or personal purposes). In many cases, these birds are donated to museums and other academic institutes for both research and educational use. However, academic use tends to be biased towards particular fields of study such as taxonomy and morphology while being given relatively less attention in other fields. Veterinary scientific investigations and analyses in carcasses of wild birds have been limited to mainly mass mortality cases caused by infectious diseases such as salmonellosis and avian influenza [13]. In contrast, while there are several reports of sporadic detections, nation-wide surveys of other pathogens are scarce in Japan. While more than 600 bird species have been recorded in Japan [32], haemosporidian surveys have investigated less than 180 bird species to date. For ecological and conservational studies on haemosporidian parasites, it is essential to first make a comprehensive inventory. In this study, a variety of bird species, including species that are difficult to catch or are rarely administered to rehabilitation facilities, were collected and investigated using personally collected samples in order to accumulate data and reduce the information gap on the diversity of avian haemosporidian parasites in Japan.

MATERIALS AND METHODS

Sample collection

From 2015 to 2021, bird carcasses were collected through personal contact via e-mail and SNS. Donors include birdwatchers, researchers (including researchers outside the field of parasitology and ornithology), banders, and environmental surveyors. Birds were donated upon understanding and approval of the study. Donors were advised safe handling procedures (e.g. wearing disposable gloves and masks, placing bird carcass in two layers of plastic bags, sanitizing hands afterwards). However, note that in some cases, birds were collected prior to contact, and donors could not be advised before collection. Many samples were donated upon retrieval of certain parts (e.g. wings, feathers, intestinal organs), which were used for personal collections or for other studies. Birds collected in this study do not include "nationally endangered species of wild fauna and flora", which are designated under the Japanese law "Act on Conservation of Endangered Species of Wild Fauna and Flora" and require permission applications for transfer [26].

Carcasses were sent to Nihon University, College of Bioresource Sciences, Department of Veterinary Medicine, Laboratory of Biomedical Science, where they were kept at −20°C until further processes. Note that most of the bird specimens were frozen at −18°C or below prior to being sent to the laboratory. For most carcasses, tissue samples were collected from the liver. In three birds [one feral pigeon (*Columba livia* var. *domestica*) found in Tokyo Prefecture, one barred buttonquail (*Turnix suscitator*) found in Okinawa Prefecture, and one Japanese tit (*Parus minor minor*) found in Nagano Prefecture], samples were collected from wing muscle tissue. Note that parasite detectability by PCR has been known to differ between blood and tissue samples, but generally similar between different tissue samples [7, 37]. The effect of different tissue types in this study was therefore considered negligible. Samples were placed in microtubes with 70–99.5% ethanol and stored at 4°C until DNA isolation. When possible, the remaining bird carcasses were sent to museums or other universities to be utilized in other studies or for scientific specimens.

Molecular detection of avian haemosporidia

DNA was extracted from the tissue samples using standard phenol-chloroform method and dissolved in tris-EDTA buffer. Avian haemosporidian DNA was detected by a previously described nested-PCR protocol targeting the partial mitochondrial cytochrome *b* (cyt*b*) gene of avian haemosporidia [18]. Briefly, the primer set HaemNFI/HaemNR3 was used for 1st PCR, followed by a 2nd PCR using HaemF/HaemR2 and HaemFL/HaemR2L [11]. DNA was extracted from PCR products and directly sequenced in both directions using BigDye^{TM} terminator cycle sequence kit (ver 3.1; Applied Biosystems, Forster City, CA, USA) and an ABI 3130 Genetic Analyzer (Applied Biosystems). Obtained sequences were assembled and compared with sequences in the GenBank and MalAvi databases [3, 22]. All samples with low-quality reads or were not 100% identical to previously identified lineages were re-tested by PCR to remove possible false positives and sequence read errors. Additionally, any suspicious findings were re-tested using newly extracted DNA in order to minimize possible contaminations [4]. Results were reported to each donor, with brief explanations on the findings (e.g. distribution of detected lineages, previous investigations of the donated bird species, etc.).

RESULTS

Collected samples

In total, samples were collected from 143 birds of 15 orders 85 species. The vast majority of birds belonged to the order Passeriformes (84 birds), followed by Charadriiformes (12), Anseriformes (9), Columbiformes (8), and Gaviiformes (7). Birds were found across 23 prefectures of Japan, from the northernmost Hokkaido to the southernmost Okinawa Prefectures. Bird species and subspecies with a limited distribution were also obtained. Conditions when found ranged from individuals with no apparent injuries to individuals in which only parts of the body remained. The presumed causes of death included road casualties, window collisions, predation, and

stranding. It is noteworthy that in many individuals, parasites including chewing lice (Phthiraptera), hard ticks (Ixodidae), louse flies (Hippoboscidae), and roundworms (Nematoda) were found during examination and necropsy (unpublished data).

Among the collected birds, DNA could not be extracted from the liver of 9 birds [1 Pacific golden plover (*Pluvialis fulva*), 1 rhinoceros auklet (*Cerorhinca monocerata*), 1 Japanese pygmy woodpecker (*Yungipicus kizuki nippon*), 1 pale thrush (*Turdus pallidus*), 1 Siberian rubythroat (*Calliope calliope*), 1 red-flanked bluetail (*Tarsiger cyanurus*), 2 Daurian redstarts (*Phoenicurus auroreus auroreus*), and 1 Eurasian tree sparrow (*Passer montanus*)], most likely due to excessive decomposition. These individuals were removed from further analyses.

Avian haemosporidian detection

The remaining 134 birds of 15 orders 81 species were tested by PCR for avian haemosporidia. To the best of our knowledge, 25 species and two subspecies were investigated for avian haemosporidia for the first time in Japan (Table 1). Additionally, 9 species had been previously morphologically investigated by observing blood smears, but were molecularly investigated for avian haemosporidia for the first time in Japan.

Avian haemosporidian DNA was detected from 37 of 134 birds (prevalence: 27.61%; Table 1). Among the tested bird species, avian haemosporidia was detected for the first time in 8 species, most of which were investigated in Japan for the first time. A total of 29 haemosporidian lineages were detected: 6 *Plasmodium*, 8 *Haemoproteus*, and 15 *Leucocytozoon* lineages. Eight novel lineages were detected (Table 2). These lineages were assigned unique lineage names according to MalAvi [3] and deposited to the GenBank database (NCBI website; www.ncbi.nlm.nih.gov/BLAST) under the accession numbers LC701760-LC701767. Among the 21 known lineages, 9 lineages were detected in Japan for the first time. Additionally, 16 lineages were detected from novel host species.

DISCUSSION

In this study, personally collected birds throughout Japan were investigated for avian haemosporidia. This method of sampling for a widespread survey of avian haemosporidia was used for the first time in Japan. While information that can be drawn from these samples is very limited as discussed below, this study shows that personally collected birds can supplement sampling from live birds and collectively contribute to more fully reveal the avian haemosporidian fauna of Japan.

Thirty-four bird species and two subspecies were either molecularly or collectively investigated for avian haemosporidia in Japan for the first time. This study has increased the number of Japanese bird species surveyed by approximately 20% and has reduced the information gap. Some bird species have very limited distributions even within Japan. For example, the barred buttonquail and subspecies of northern boobook (*Ninox japonica totogo*) only inhabit islands of the Kagoshima and Okinawa Prefectures [32]. Regional surveys of avian haemosporidia have been carried out in multiple areas of Japan including Hokkaido, Hyogo, Tokyo and surrounding prefectures, Tsushima Island, and Minami Daito Island [17, 27, 29, 39, 42]. Meanwhile, many areas have still not been investigated and consequently, many bird species with limited distributions have also not been investigated. Additionally, some birds such as seabirds and ducks are considered relatively difficult to catch, limiting sampling opportunities [17, 40]. In this study, seven seabird species and three duck species were investigated in Japan for the first time. By utilizing bird carcasses found throughout Japan, it was possible to investigate various birds that are otherwise difficult to investigate.

A total of 29 parasite lineages were detected, including 8 novel lineages (Table 2). Furthermore, 9 previously described lineages were detected in Japan for the first time. These results demonstrate the fact that the avian haemosporidian fauna is still understudied in Japan. Among lineages detected in Japan for the first time, *Haemoproteus* lineages EMSPO01 and EMSPO02, and *Leucocytozoon* lineage EMSPO05 detected from multiple *Emberiza* species had previously been detected in China and Russia from birds of the same host genus [14, 21, 34]. Similarly, the lineage ZOSLAT02, which had only been detected from *Zosterops* species and common myna (*Acridotheres tristis*) of Oceania [19], was detected from a Japanese white-eye (*Zosterops japonicus*). While the host range and distribution of these lineages remain unknown, detections of these lineages from closely related host species and from a neighboring area add information to further understand such characteristics of these haemosporidian lineages. Meanwhile, lineages such as PHYBOR04, PRUMOD01, and TRPIP2 which had been previously detected in distant areas such as Europe and North America [9, 24] were also detected in this study, considerably expanding the distribution range of these lineages.

Several previously described lineages, including lineages previously detected in Japan, were detected from novel host species. Molecular studies of avian haemosporidia are increasing in the Far East, but there are still only a handful of studies that have molecularly detected the parasites from wild birds [14, 16, 17, 21, 34, 36, 38, 39, 42]. Hence, bird species of the Far East have not been sufficiently investigated. In this study, many species that are only distributed in the Far East were investigated, allowing novel host species to be added to many known lineages. For some lineages with a relatively wide host range, such as BT7 and SYAT05, the findings were not surprising. Additionally, other lineages including EMSPO01, EMSPO02, HYBOR02, and TRPIP2 were detected from passerines in both previous detections and in this study. Interestingly, the lineage SYBOR02, which has been previously detected from mainly passeriform species, was detected from a long-tailed jaeger (*Stercorarius longicaudus*) of the order Charadriiformes in this study. This lineage has been described from multiple passeriform species of various countries in Asia and Europe [14, 20, 34], as well as from an anseriform species of Japan [39]. These results collectively suggest that, like many other *Plasmodium* lineages, the host range of SYBOR02 may be relatively wide.

The investigation of avian haemosporidia throughout Japan using personally collected birds also had some limitations. It is not possible to control for the number and species of birds collected, and consequently, only one to a few individuals were obtained for each species. Parasite prevalence is known to widely differ between bird species, due to several factors such as their life history,

Table 1. Species investigated in this study, with PCR detection results for avian haemosporidia

Investigated species[a]				PCR results[e]						
Species	Scientific name[b]	Finding location[c]	First investigation in Japan[d]	No. tested	No. positive	Plas	Haem	Leuc	Co-infec	First detection from species
Stejneger's scoter	*Melanitta stejnegeri*	CB* (4)	●	4	3			3		●
Black scoter	*Melanitta americana*	CB* (4)	●	4	3			3		●
Red-breasted merganser	*Mergus serrator*	CB (1)	●	1	0					
Common pheasant	*Phasianus colchicus*	OK (1)		1	0					
Green pheasant	*Phasianus versicolor*	CB (1)		1	0					
Feral pigeon	*Columba livia* var. *domestica*	KN (1), MG (1), TY (1)		3	0					
Japanese wood pigeon	*Columba janthina janthina*	SM (1)		1	0					
Oriental turtle dove	*Streptopelia orientalis orientalis*	CB* (1), TT* (1), TY* (1)		3	3		1	2		
Red collared dove	*Streptopelia tranquebarica*	OK (1)	●	1	0					
Brown-cheeked rail	*Rallus indicus*	TY (1)		1	0					
Eurasian coot	*Fulica atra*	CB (1)		1	0					
White-breasted waterhen	*Amaurornis phoenicurus*	OK (1)	○	1	0					
Barred buttonquail	*Turnix suscitator*	OK (2)	●	2	0					
Black-winged stilt	*Himantopus himantopus*	TY (1)	●	1	0					
Long-billed plover	*Charadrius placidus*	KN (1)	●	1	0					
Solitary snipe	*Gallinago solitaria*	HK (1)	●	1	0					
Pin-tailed snipe	*Gallinago stenura*	OK (1)		1	0					
Black-legged kittiwake	*Rissa tridactyla*	IB (2)	○	2	0					
Slaty-backed gull	*Larus schistisagus*	HK (1)	●	1	0					
Common tern	*Sterna hirundo longipennis*	CB (1)	●	1	0					
Long-tailed jaeger	*Stercorarius longicaudus*	IB* (1)	●	1	1	1				●
Ancient murrelet	*Synthliboramphus antiquus*	CB (1)		1	0					
Red-throated loon	*Gavia stellata*	YG (1)	●	1	0					
Pacific loon	*Gavia pacifica*	YG (6)	●	6	0					
Fork-tailed storm petrel	*Hydrobates furcatus*	IB (1)		1	0					
Sooty shearwater	*Ardenna grisea*	HK (1)	●	1	0					
Japanese cormorant	*Phalacrocorax capillatus*	CB (1)	●	1	0					
Black-crowned night-heron	*Nycticorax nycticorax*	OK (1)		1	0					
Black kite	*Milvus migrans*	NA (1)		1	0					
Eastern buzzard	*Buteo japonicus*	CB (1)		1	0					
Northern boobook	*Ninox japonica totogo*	OK (2)	● (ssp.)	2	0					
Ruddy kingfisher	*Halcyon coromanda*	TY (1), GI (1)	○	2	0					
Japanese pygmy woodpecker	*Yungipicus kizuki shikokuensis*	HG (1)	● (ssp.)	1	0					
Great spotted woodpecker	*Dendrocopos major japonicus*	NA (1)	○	1	0					
Japanese green woodpecker	*Picus awokera awokera*	MG (1)		1	0					
Grey-headed woodpecker	*Picus canus*	HK (1)	●	1	0					
Common kestrel	*Falco tinnunculus*	TC (1)		1	0					
Bull-headed shrike	*Lanius bucephalus*	NI (1), IS (1)		2	0					
Azure-winged magpie	*Cyanopica cyanus*	TY (1)		1	0					
Large-billed crow	*Corvus macrorhynchos japonensis*	KN* (1), TY* (1)		2	2		2	1	1	
Bohemian waxwing	*Bombycilla garrulus*	HK (1)	●	1	0					
Coal tit	*Periparus ater*	nd (1)		1	1			1		
Varied tit	*Sittiparus varius varius*	MG (1), YA* (1)		2	1			1		●
Japanese tit	*Parus minor minor*	KN (1), NA* (1), TY* (2)		4	2	1		2	1	
Eurasian skylark	*Alauda arvensis*	NA (1)		1	0					
Brown-eared bulbul	*Hypsipetes amaurotis*	TY (1)		1	0					
Barn swallow	*Hirundo rustica gutturalis*	YN (1)		1	0					
Asian house martin	*Delichon dasypus*	na (1)	●	1	0					
Red-rumped swallow	*Cecropis daurica*	TT (1)	○	1	0					
Japanese bush warbler	*Horornis diphone*	TT (1)		1	0					
Long-tailed tit	*Aegithalos caudatus caudatus*	NR (1)		1	0					
Kamchatka leaf warbler	*Phylloscopus examinandus*	ME (1), TY (1)	●	2	0					
Gray's grasshopper warbler	*Helopsaltes fasciolatus*	OK (1)	●	1	1		1			●
Warbling white-eye	*Zosterops japonicus japonicus*	TT (2), TY* (1), YA (1), na (1)		5	2	1	1			
Red-billed leiothrix	*Leiothrix lutea*	AI (1)		1	1			1		
White-cheeked starling	*Spodiopsar cineraceus*	TY (1)		1	0					
Chestnut-cheeked starling	*Agropsar philippensis*	HK (1)	●	1	0					
White's thrush	*Zoothera aurea*	HK (1), nd (1)		2	0					

Table 1. Continued.

Investigated species[a]				PCR results[e]						
Species	Scientific name[b]	Finding location[c]	First investigation in Japan[d]	No. tested	No. positive	Plas	Haem	Leuc	Co-infec	First detection from species
Japanese thrush	*Turdus cardis*	IS (2), NA (1)		3	0					
Pale thrush	*Turdus pallidus*	MG* (1), NR* (1), OK *(1), TT* (1), TY* (2)		6	5	3		3	1	
Brown-headed thrush	*Turdus chrysolaus*	TY (2)	○	2	0					
Dusky thrush	*Turdus eunomus*	AI* (1), HK (1), NI* (1),TT (1)		4	2			2		
Siberian rubythroat	*Calliope calliope*	HK (1), MG (2)		3	0					
Narcissus flycatcher	*Ficedula narcissina*	YG* (1)		1	1		1			
Blue rock thrush	*Monticola solitarius philippensis*	TT (1)	●	1	0					
Amur stonechat	*Saxicola stejnegeri*	HK (1), NA (1)	●	2	0					
Eurasian tree sparrow	*Passer montanus*	MG (1), TY (1)		2	0					
Japanese accentor	*Prunella rubida*	SZ* (1)	●	1	1			1		●
White wagtail	*Motacilla alba lugens*	YN (1)		1	0					
Japanese wagtail	*Motacilla grandis*	HG (1)	●	1	0					
Buff-bellied pipit	*Anthus rubescens*	NA* (1)	○	1	1		1	1	1	●
Brambling	*Fringilla montifringilla*	HG (1), nd (1)		2	1	1				
Hawfinch	*Coccothraustes coccothraustes*	GM (1), TY* (1)	○	2	1			1		
Japanese grosbeak	*Eophona personata*	nd (1)		1	1			1		
Grey-capped greenfinch	*Chloris sinica*	nd (1)		1	0					
Meadow bunting	*Emberiza cioides*	NA (2), TT* (1)		3	1		1			
Rustic bunting	*Emberiza rustica*	KN* (1)	○	1	1			1		●
Yellow-throated bunting	*Emberiza elegans*	NA (1)		1	0					
Black-faced bunting	*Emberiza spodocephala*	HK* (1), NA (1), nd (1)		3	1		1			
Grey bunting	*Emberiza variabilis*	YA* (1)		1	1			1		
Common reed bunting	*Emberiza schoeniclus*	HK (1), nd (1)		2	0					
Total				134	37	7	9	25	4	8

[a] The taxonomic order and nomenclature of bird species are listed according to the International Ornithological Congress (IOC) World Bird List version 12.1. [b] For species with multiple subspecies recorded in Japan, subspecies were determined by morphological features and geographic areas of sampling. Not all individuals could be identified to subspecies level. [c] Sampling locations are given by abbreviations of prefecture names. AI: Aichi, CB: Chiba, GI: Gifu, GM: Gunma, HG: Hyogo, HK: Hokkaido, IB: Ibaraki, IS: Ishikawa, KN: Kanagawa, MG: Miyagi, ME: Mie, NA: Nagano, NI: Niigata, NR: Nara, OK: Okinawa, SM: Shimane, SZ: Shizuoka, TC: Tochigi, TT: Tottori, TY: Tokyo, YA: Yamaguchi, YG: Yamagata, YN: Yamanashi, nd: no data. Prefectures in which positive individuals were detected are marked with an asterisk (*). Parentheses show sample numbers per location. [d] Closed circles: bird species that were investigated for haemosporidia for the first time in Japan. Open circles: bird species that were molecularly investigated for haemosporidia for the first time in Japan. (ssp.): bird subspecies that were investigated for haemosporidia for the first time in Japan. [e] Plas: *Plasmodium*, Haem: *Haemoproteus*, Leuc: *Leucocytozoon*, Co-infec: co-infection by multiple parasite genera.

habitat, and behaviors [1, 17, 18, 23]. Furthermore, body conditions and vital status are also known to affect the parasite prevalence [5, 8]. Due to these factors, it was not possible to compare the total parasite prevalence and prevalence by bird species with other studies. The largest limitation was that most birds were frozen prior to sampling. Although PCR and other molecular methods are powerful tools, morphological observations of haemosporidian parasites are essential for species identification and confirmation of infection stages and other important characteristics. However, freezing is generally known to distort the tissue and may create artifacts, and therefore, frozen samples are not suitable for histopathological observations and preparations of impression smears. In a recent study, bird carcasses were collected in Austria through a citizen science project [12]. The study aimed to assess avian mortality and its relationship to avian haemosporidian infection through histopathological examinations, and hence, citizen scientists were advised to store the birds in a cool place until the birds are picked up. In the present study, many of the donated birds had been collected for other purposes (e.g. preparing taxidermies and specimens for personal specimen collection, other studies) and were generally frozen immediately after collection. Additionally, due to limitations in transportation measures, it was not possible to obtain and sample birds within a short period of time, and birds were therefore frozen in order to avoid decomposition.

Another important factor that must be considered is the handling of bird carcasses by donors. While the collection of animal carcasses is legal in Japan (with exceptions such as protected species and areas), risks and safety procedures regarding infectious diseases must also be considered. While avian haemosporidia do not infect humans, zoonoses such as SFTS and avian influenza are increasingly becoming a problem. Consequently, the need for preventative measures through the “One Health” approach, which recognizes that the health of people, animals, and the environment are all connected with one another, are increasing [13, 31]. In this study, some donors were advised safe handling procedures prior to collection of the carcass. However, other donors had collected the birds prior to contact. In the latter case, donors may bear the risk of zoonosis infection unless the donor had prior knowledge regarding the handling of animal carcasses. In order to prevent such risks, along with proper advice during studies like this, there is a strong need to raise

Table 2. Haemosporidian lineages detected in this study, with host information from previous studies

Lineage[a]	Host species in this study[b]	Host species in previous studies[c]
Plasmodium		
BT7	*Turdus pallidus* (1)	Anseriformes (NA), Charadriiformes (NA), Falconiformes (EU, NA), Passeriformes (AS, EU, NA, SA)
NYCNYC02	*Fringilla montifringilla* (1)	*Nycticorax nycticorax* (JP), *Tarsiger cyanurus* (JP)
PARMIN02*	*Parus minor* (1)	
SYAT05	*Turdus pallidus* (2)	Galliformes (SA), Columbiformes (OC), Passeriformes (JA, AS, EU, AF, NA, OC)
SYBOR02	*Stercorarius longicaudus* (1)	*Anas platychynchos* (JP), Passeriformes (AS, EU, AF)
ZOSLAT02	*Zosterops japonicus* (1)	*Zosterops lateralis* (OC), *Z. flavifrons* (OC), *Z. xanthochroa* (OC), *Acridotheres tristis* (OC)
Haemoproteus		
COCOR14	*Corvus macrorhynchos* (1)	*Corvus corone* (JP)
COCOR15	*Corvus macrorhynchos* (1)	*Streptopelia orientalis* (JP), *Corvus corone* (JP), *C. macrorhynchos* (JP)
EMSPO01	*Emberiza cioides* (1)	*Emberiza spodocephala* (AS), *E. godlewskii* (AS)
EMSPO02	*Anthus rubescens* (1), *Emberiza spodocephala* (1)	*Emberiza spodocephala* (AS)
FICNAR01	*Ficedula narcissina* (1)	*Ficedula narcissina* (JP)
PHYBOR04	*Helopsaltes fasciolatus* (1)	*Phylloscopus borealis* (NA), *Catharus minimus* (NA), *Turdus pilaris* (EU)
STRORI01	*Streptopelia orientalis* (1)	*Streptopelia orientalis* (JP)
STRURA02	*Zosterops japonicus* (1)	*Strix uralensis* (JP), *Hirundo rustica* (JP)
Leucocytozoon		
ANSFAB01	*Melanitta stejnegeri* (1), *M. americana* (3)	*Anser fabalis* (JP), *Cygnus cygnus* (JP)
ANTRUB02*	*Anthus rubescens* (1)	
COCCOC01	*Coccothraustes coccothraustes* (1)	
EMSPO05	*Emberiza rustica* (1)	*Carpodacus erythrinus* (EU), *Emberiza spodocephala* (AS), *E. godlewskii* (AS)
EOPER02*	*Eophona personata* (1)	
FICNAR02	*Parus minor* (1)	*Poecile montanus* (JP), *Troglodytes troglodytes* (JP), *Tarsiger cyanurus* (JP), *Ficedula narcissina* (JP)
HYBOR02	*Parus minor* (2)	*Poecile montanus* (JP), *Hypsipetes borbonicus* (AF), *Sitta europaea* (AS), *Tarsiger cyanurus* (JP)
LEILUT03*	*Leiothrix lutea* (1)	
PERATE09	*Periparus ater* (1)	*Periparus ater* (JP)
PRUMOD01	*Prunella rubida* (1)	*Prunella modularis* (EU)
SITVAR01*	*Sittiparus varius* (1)	
STRORI06*	*Streptopelia orientalis* (2)	
TRPIP2	*Emberiza variabilis* (1)	*Anthus trivialis* (AF), *Poecile hudsonicus* (NA), *Acanthis flammea* (NA), *Junco hyemalis* (NA)
TUREUN01*	*Turdus pallidus* (1), *T. eunomus* (2)	
TURPAL02*	*Turdus pallidus* (1)	

[a] Lineage names are given according to MalAvi. Novel lineages are marked with an asterisk (*). Lineages detected in Japan for the first time are shown in bold. [b] The number of individuals the lineage was detected from is shown in parentheses. Host species which the lineage was detected for the first time are underlined. [c] Previously detected host species are based on MalAvi. The host order are shown instead of host species for lineages with wide host ranges. Parentheses show the area in which the lineage was detected per host species/order. JP: Japan, AS: Asia (excluding Japan), OC: Oceania, EU: Europe, AF: Africa, NA: North America, SA: South America.

awareness of risk management to the general public.

Despite these limitations and problems to consider, sampling through personally collected bird carcasses can help to investigate avian haemosporidian diversity especially in areas that are understudies. Furthermore, many other parasites were detected from the examined carcasses, suggesting that this method of sample collection is effective for investigating other parasites as well. While comprehensive surveys of museum specimens would provide a more thorough investigation, this study demonstrates that personally collected bird samples that would otherwise remain uninvestigated can provide important additions to more fully understand the avian haemosporidian fauna of Japan. Through the increase of public awareness and education, which are both important parts of the "One Health" approach, the possibilities of citizen science projects in the veterinary field may expand.

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