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Original Article

Prevalence and Pathology of Endoparasitic Infections of Rock Pigeons (*Columba livia*) in Taiwan

Chih-Li Yu[1] Yen-Li Huang[2*]

Abstract

Pigeon racing is popular in Taiwan, but most lofts are located on residential roofs or in attics, which means pigeons could potentially spread parasites to humans. It is necessary to investigate the prevalence and diversity of endoparasitic infections in pigeons. This study investigated endoparasitic infections in 301 pigeons (both loft-kept racing and feral pigeons) in Taiwan using gross and histopathological examinations. The identified endoparasites included *Trichomonas* spp. (30.2%, 91/301); *Ascaridia* spp. (22.3%, 67/301); *Haemoproteus* spp. (20.6%, 62/301); *Eimeria* spp. (11.0%, 33/301); *Capillaria* spp. (7.3%, 22/301); *Hexamita* spp. (6.0%, 18/301); *Raillietina* spp. (4.7%, 14/301); *Heterakis* spp. (2%, 6/301); and *Dispharynx* sp., *Paratanaisia* sp., and *Trypanosoma* sp. (0.3% each, 1/301). Data analysis showed that the prevalence of parasitic infections was significantly higher in feral pigeons than in loft-kept racing pigeons, particularly for infections caused by *Trichomonas*, *Raillietina*, and *Ascaridia* spp. (p <0.05). However, the severity of parasitic infections observed in histopathological lesions was greater in loft-kept racing pigeons. Notably, *Ascaridia* was observed in the pancreas and pancreatic ducts of pigeons for the first time in this study. Despite intensive anthelmintic use, endoparasitic infections were still present in loft-kept racing pigeons. Developing more effective strategies to manage and control parasites in pigeon populations is essential for safeguarding the health and well-being of the birds.

Keywords: pigeon, endoparasites, blood parasites, histopathology, prevalence, Taiwan

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Introduction

Numerous studies have been conducted to examine parasites in rock pigeons (*Columba livia*) across various countries, aiming to comprehend the range of parasite diversity and the prevalence of parasitic infections, including endoparasites and ectoparasites. A brief review of previous studies on parasitic infections in pigeons indicates that common endoparasites include *Haemoproteus* spp., *Trichomonas* spp., and *Ascaridia* spp., while the most frequently encountered ectoparasite is *Columbicola columbae* (Alkharigy *et al.*, 2018; Borji *et al.*, 2013; Eljadar, 2012). Exploring diseases in pigeons, whether in racing or wild counterparts, is crucial for owners to understand the health status of their birds and identify infectious agents, pathogens, and potential zoonotic diseases (Abed *et al.*, 2014; Radfar *et al.*, 2012; Tayyub *et al.*, 2021).

In Taiwan, three distinct groups of rock pigeons are recognized: poultry, racing pigeons, and feral pigeons. Pigeon racing or related events are popular, so farmers invest substantial time and resources in training racing pigeons. However, less than 1% of racing pigeons completed seven races in a season. Pigeons' health is extremely important to increase the chance of winning the race. If pigeons have pathogenic infections such as circovirus, they could be disqualified from the race (Huang *et al.*, 2017). In Taiwan, racing pigeons that have been disqualified, lost, or wandered away often transition into feral pigeons and reproduce in urban areas.

Parasitic infections in pigeons usually cause a reduction in growth rate, weight loss, and poor health conditions (Ombugadu *et al.*, 2019). The host may even suffer serious injury or death due to the migration or highly virulent strain of parasites or because of excessive immune effector activities (Hoffmann *et al.*, 2002; Clark *et al.*, 2004; Day *et al.*, 2007). Awareness of the detection of pathogens caused by parasitic infections empowers diagnostic assessments of diseases in racing pigeons, enabling enthusiasts to take proactive measures to avert illnesses. This proactive approach helps minimize the chances of illnesses arising and decreases the risk of disqualification in competitions due to parasite-related concerns.

Racing pigeon lofts are usually located on the rooftops or attics of human residential buildings in Taiwan so that pigeons could potentially spread parasites to humans. Under the advocacy of One Health (Cunningham *et al.*, 2017), research on pigeon diseases in urban areas has become increasingly important. If some domestic and racing pigeons do not return to the loft, they may become feral pigeons. Studying the pathology and transmission methods of pigeon parasites is crucial because they are widespread and could potentially transmit parasites to wild birds (Dranzoa *et al.*, 1999; Tu *et al.*, 2019). Feral pigeons are known to harbor at least 70 different human pathogenic microorganisms, seven of which have been documented to be transmitted to humans (Haag-Wackernagel & Bircher, 2010; Haag-Wackernagel & Moch, 2004). Endoparasites such as *Toxoplasma gondii*, *Cryptosporidium* spp., and *Giardia duodenalis* can be transmitted to humans through contact with pigeon feces, feet, or feathers (De Lima *et al.*, 2011). Ectoparasites of feral pigeons, like the pigeon tick (*Argas reflexus*), can migrate into human living spaces when they lose their natural hosts. Bites from these ticks can cause severe health issues in susceptible individuals.

There are many types of parasites; examples of *Helminth* parasites are discussed, frequently observed in domestic pigeons and farmed pigeons. A very high local density of breeding pigeons could lead to heavy nematode infections (Basit *et al.*, 2006; Salem *et al.*, 2022). However, Islam *et al.* (2010) have noted that feral pigeons exhibit a higher susceptibility to helminthiasis compared to domestic birds. This increased susceptibility is likely due to their constant exposure to soil, which acts as a significant reservoir and transmission site for parasites. Consequently, the presence of potential pathogenic risks in both loft-kept racing pigeons and feral pigeons underscores the necessity of investigating infections in these populations.

To the best of the authors' knowledge, studies investigating parasites in pigeons, encompassing loft-kept racing and feral pigeons in Taiwan (Tsai & Lee, 2006) are lacking. This study aimed to investigate the prevalence of endoparasitic infection and the diversity of endoparasites in pigeons. In urban areas, parks, and suburban squares in Taiwan, there is a potential for human interaction with both racing pigeons and feral pigeons. Therefore, including loft-kept racing pigeons and feral pigeons is necessary when conducting research on parasites. Furthermore, simultaneously exploring the potential presence of parasites posing superficial risks to native wild bird species and even humans is imperative.

Materials and Methods

***Sample*:** The archives of the Laboratory of Veterinary Pathology at Asia University Veterinary Teaching Hospital and the Clinical Avian Medicine & Molecular Medicine Laboratory at National Pingtung University of Science and Technology (NPUST) were searched for rock pigeon necropsy submissions with a diagnosis of parasitic infection, covering the period from January 1, 2010, to December 31, 2023. Submissions from the Clinical Avian Medicine & Molecular Medicine Laboratory at NPUST included cases received between January 1, 2010, and June 30, 2018, when the laboratory was closed. Loft-kept racing and feral pigeons brought to the Asia University Veterinary Teaching Hospital between January 1, 2022, and December 31, 2023, were examined using a standard procedure that involved checking their crop, feces, and blood smears for pathogens. The animal hospital determined that the case was incurable and arranged for animal euthanasia, followed by a necropsy to diagnose pathogenic factors. All specimens sent to the Laboratory of Veterinary Pathology for examination were dead animals, and no live animal testing was involved. These cases were subsequently used as undergraduate teaching materials involving animal dissection. The laboratory operates at Biosafety Level 2 Plus (BSL-2+), which is capable of detecting avian influenza; however, a virus culture has not been performed. In the event of a suspected notifiable infectious disease, the case is referred to the Veterinary Research Institute, Ministry of Agriculture, New Taipei City, Taiwan.

***Data collection*:** Data collected from all samples included breed or production type (racing or feral), county of origin, year of submission, examination results from crop, blood, and fecal smears, histological methods used for pathogen diagnosis, gross and microscopic findings, and the presence of concurrent infections. In this study, a total of 301 pigeons were selected, of which 236 birds were from the loft-kept racing pigeons and 65 birds were from feral pigeons. The group of loft-kept racing pigeons was distinguished by identification bands worn on their legs. These pigeons underwent careful selection, accompanied by a comprehensive medical history provided by their owner, and they are all less than 1 year old. The group of feral pigeons comprises two parts. One consists of naturally wild pigeons and the other includes lost pigeons, which were previously used for racing but got lost during a race, subsequently turning free-range pigeons and being found by the general public.

All pigeons in urban areas, whether kept in lofts or feral pigeons, share close proximity to human activities. Only 65 feral pigeons were selected for this study, including cases of injury death and those that underwent pathological examination. The breeding locations are concentrated in southern Taiwan, including Pingtung City (24 cases), Pingtung County (141 cases), Kaohsiung (35 cases), and Tainan (2 cases). In the central region, breeding sites are found in Taichung (15 cases) and Chiayi (19 cases), while in the northern region, Taoyuan (8 cases) is notable. Firal pigeons predominantly originate from Taichung City (43 cases) and Pingtung County (20 cases). Other pigeons that did not undergo a complete examination were not included. All pigeons, including loft-kept racing pigeons and feral pigeons with complete medical records and necropsy cases with histology findings, were selected as samples for this study. Comprehensive information on the crop, blood, and fecal examination data of birds was collected.

***Microscopic examination*:** Allocate the microscopic examination into crop, blood, and fecal examinations. The inspection method follows the procedures established by the animal hospital.

For crop examination, a disposable sampling swab (High-Quality Medical Absorbent Cotton Swab, 148mm ±10mm overall length, 2.5mm ±0.5mm stick diameter) was used to collect a sample from the crop wall of the pigeons. The collected sample was smeared onto a clean glass slide. A small drop of the sample was placed on the slide and allowed to dry. The smear was then prepared on a microscopic slide using swabs obtained from the crop of all pigeons, stained with Liu's stain (Baso Company, Taiwan), and examined under light microscopy for parasite identification. Liu's staining was performed according to the following method: Add approximately 1-2ml of Liu's Solution A to the sample and allow it to stain for 30 seconds. Next, add Liu's Solution B to the sample, using an amount approximately twice that of Solution A. Gently blow air over the surface of the solution to create a rippling effect, ensuring thorough mixing of the two solutions. Allow the sample to stain for 1 minute and 30 seconds. Rinse the slide with water, let it dry, and then examine it under the microscope.

A hypodermic needle (18-20G) was used to prick the tip of the toe. The fingertip was first disinfected with alcohol, followed by a light prick to obtain a drop of blood, which was then placed onto a microscopic slide. A thin blood smear was prepared by spreading the drop of freshly collected blood on the slide, allowing it to air-dry, and subsequently staining it with Liu's stain for the identification of hemoparasites.

For fecal examination, a small amount of feces was placed on a microscopic slide and mixed with a drop of sterile normal saline solution (sodium chloride 0.9%, Taiwan Biotech). A cover glass was then applied to prepare a thin smear using the direct smear method. The slide was examined under the microscope to identify endoparasitic oocysts, eggs, or protozoa. For fresh, unpreserved fecal samples, a direct wet mount can be used to detect the presence of motile protozoan trophozoites. It is important to avoid hypotonic solutions, as they may cause lysis of the trophozoites.

***Gross and histopathological examination*:** Post-mortem examinations were conducted following previously described methods (Huang *et al*., 2017). Gross findings were recorded at necropsy. The gross pathology examination primarily describes the location, size, color, softness, hardness, and surface characteristics of the specimens. Representative tissues from various organs, including the brain, heart, liver, spleen, lung, kidney, proventriculus, gizzard, intestine, thymus, bursa, and skin of the infested birds were collected. These tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Subsequently, tissue blocks were sectioned at a thickness of 4μm and stained with hematoxylin and eosin for microscopic examination (Bancroft & Gamble, 2008).

***Identification of the Parasites*:** Pigeons that tested positive for internal parasitic infections were categorized according to the type of infection identified. All collected parasites were identified following the methodology outlined by Soulsby (1986). Initial observations were conducted using low magnification (40x), followed by an increase to 100x for a more detailed examination of helminth eggs and coccidia oocysts. The 400x magnification was used after staining to observe *Trichomonas* in crop smears. Blood smears were examined microscopically at 1,000x magnification, with hemoprotozoans identified based on morphological characteristics, following the guidelines provided by Cepeda *et al.* (2019). For specific identification, especially in cases involving the *Dispharynx*, studies incorporating morphological and morphometric data were consulted, as detailed by Carreno (2008), Churria *et al.* (2011), and Severino *et al.* (2023). DNA was extracted from tissue samples that exhibited parasitic infections in atypical organs. This DNA was then subjected to PCR using specific primers to confirm the presence of the parasites (Data not shown).

***Statistical analysis*:** Data were analyzed statistically using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA). The prevalence percentage was calculated by dividing the number of positive samples by the total number of samples and multiplying the result by 100. The number of parasites in each bird was recorded and compared between feral and loft-kept racing groups using PROC GLM. Differences in parasite species between feral and loft-kept racing groups were analyzed using PROC FREQ. A p-value of less than 0.05 was considered statistically significant.

Result

Data analysis: The study investigated the prevalence of parasitic diseases infesting loft-kept racing and feral pigeons in Taiwan. The detection of endoparasite infections and the number of pigeons testing positive for each of them are reported in Table 1. This study identified six species of helminths (four nematodes, one cestode, and one trematode) and five species of protozoa (three protozoa and two hematozoa). These included *Ascaridia* spp., *Capillaria* spp., *Heterakis* spp., *Dispharynx* sp., *Raillietina* spp., *Paratanaisia* sp., *Trichomonas* spp., *Eimeria* spp., *Hexamita* spp., *Haemoproteus* spp. and *Trypanosoma* sp. of 301 pigeons examined, 122 loft-kept racing pigeons (122/236, 51.7%), and all 65 feral pigeons (65/65, 100%) infested with parasites.

Table 1 concludes that pigeons had a relatively high infection rate. The number of parasites in feral pigeons was significantly higher than that in loft-kept racing pigeons. (p <0.05) (Fig. S1). The loft-kept racing group exhibited higher infection rates of *Haemoproteus*, *Ascaridia*, and *Eimeria* than the other parasites, and the feral group showed higher infection rates of *Trichomonas*, *Ascaridia,* and *Haemoproteus*. In addition, the histopathological lesions of various organs following parasitic infection are described and shown in Table 3. The disease severity of *Trichomonas* and *Ascaridia* infections were higher in loft-kept racing pigeons. The prevalence of *Trichomonas*, *Raillietina,* and *Ascaridia* infection was significantly higher in feral pigeons than in loft-kept pigeons (p <0.05).

The detection and the prevalence of endoparasites in loft-kept racing and feral pigeons by microscopic examination: Table 2 presents the number of pigeons testing positive for various types of endoparasitic infections as identified through microscopic examination at an animal hospital. The diagnosis of *Trichomonas* infection was confirmed by crop swabs in 11.0% of loft-kept racing pigeons and in 100.0% of feral pigeons. The highest infection rates were observed for *Ascaridia* eggs, with prevalence rates of 11.0% in loft-kept racing pigeons and 24.5% in feral pigeons. Other detected parasites in loft-kept racing pigeons included *Eimeria* spp. oocysts (10.6%), *Hexamita* spp. (6.8%), *Capillaria* spp. eggs (4.2%), and *Raillietina* spp. eggs (1.7%). In feral pigeons, *Eimeria* spp. oocysts (4.6%), *Hexamita* spp. (3.1%), and *Raillietina* spp. eggs (3.1%) were identified.

The detection and the prevalence of blood parasites in loft-kept racing and feral pigeons by microscopic examination and associated clinical signs: The frequency of *Haemoproteus* sp. infection in feral pigeons (21.5%) was higher than loft-kept racing pigeons (20.3%). In this study of *Trypanosoma* infection in loft-kept racing and feral pigeons, only one feral pigeon was found to be parasitized by *Trypanosoma* sp. Additionally, this pigeon exhibited co-infection with *Haemoproteus* spp., as identified in the blood smear. Clinically, the pigeon presented with emaciation, anemia, and watery blood.

The detection and the prevalence of endoparasites in loft-kept racing by pathological examination: Most loft-kept racing pigeons infected with *Ascaridia* spp. exhibited inflammation, with the parasites filling the small intestinal lumen and compressing the intestinal mucosa (81.8%). Severe pathological lesions were observed in the liver and lungs of some pigeons heavily infected with *Ascaridia*, which exhibited severe granulomatous inflammatory cell infiltration. Adult parasites and larvae were found in the nasal passages, crop, proventriculus, gizzard, liver, and intestines. The cross-section of intestines containing *Ascaridia* spp. exhibited varying degrees of villous atrophy and fusion. The lumen of the small intestine was completely occluded with several adult and young worms (Fig. 1a). Mild-to-moderate enteritis and expansion of lamina propria with infiltration of mononuclear cells were observed. In 18 cases, granulomatous lesions were observed in the liver, and *Ascaridia* larvae were found near the lesions. The livers of infected pigeons appeared normal or yellow, with several clear white spots ranging from 0.1 mm to 1.0 mm in diameter on the liver surface. Clearly visible abscesses were slightly hardened on the cut surface of three cases of the liver, with some large larvae observed. Morphology diagnosis was moderate to severe, chronic, multifocal, granulomatous hepatitis with *Ascaridia* larvae (Fig. 1b). Granulomatous pneumonia was observed in some pigeons, but there is no evidence to suggest that the lesion was caused by ascarid nematodes. Only a few ascarid larvae were found infesting the parabronchi in one case (Fig. 1c). A large number of *Ascaridia* nematodes were found on the proventricular mucosa and gizzard surface (Fig. 1d). Infected birds exhibited an enlarged proventriculus with a thickened wall covered in whitish mucus. Although *Ascaridia* nematodes entered the crop, they did not cause emesis or remarkable trauma or lesions. In one case, focal white speckles were observed on the pancreatic surface, representing the first instance of an obstruction lesion caused by *Ascaridia* nematodes being identified prior to histopathological diagnosis (Fig. 1e). Another case showed an *Ascaridia* larvae infecting the pancreatic tissue (Fig. 1f). In a different case, an *Ascaridia* larva was observed migrating through the choanal slit.

Table 1 Prevalence of endoparasites in loft-kept racing and feral pigeons in Taiwan between 2010 and 2023.

Parasite		Loft-kept racing pigeon (%)	Feral pigeon (%)	Total (%)	*p*-value
Helminth					
Nematodes	*Ascaridia* spp.	18.6 (44/236)	35.4 (23/65)	22.3 (67/301)	0.0041
	Capillaria spp.	6.8 (16/236)	9.2 (6/65)	7.3 (22/301)	0.5014
	Heterakis spp.	2.5 (6/236)	0	2 (6/301)	0.1941
	Dispharynx sp.	0	1.5 (1/65)	0.3 (1/301)	0.0563
Cestodes	*Raillietina* spp.	3.4 (8/236)	9.2 (6/65)	4.7 (14/301)	0.0477
Trematodes	*Paratanaisia* sp.	0	1.5 (1/65)	0.3 (1/301)	0.0563
Protozoae					
Protozoon	*Trichomonas* spp.	11.0 (26/236)	100.0 (65/65)	30.2 (91/301)	<0.001
	Eimeria spp.	12.3 (29/236)	6.2 (4/65)	11.0 (33/301)	0.1610
	Hexamita spp.	6.8 (16/236)	3.1 (2/65)	6.0 (18/301)	0.2649
Hematozoa	*Haemoproteus* spp.	20.3 (48/236)	21.5 (14/65)	20.6 (62/301)	0.8323
	Trypanosoma sp.	0	1.5 (1/65)	0.3 (1/301)	0.0563

Table 2 Prevalence of endoparasites from crop, blood, and fecal smears in loft-kept racing and feral pigeons by microscope.

	Crop smear	Blood smear	Feces smear
Loft-kept racing pigeon (%)	*Trichomonas* spp. 11.0 (26/236)	*Haemoproteus* spp. 20.3 (48/236)	*Ascaridia* egg 11.0 (26/236) *Eimeria* oocyst 10.6 (25/236) *Hexamita* spp. 6.8 (16/236) *Capillaria* egg. 4.2 (10/236) *Raillietina* egg 1.7 (4/236)
Feral pigeon (%)	*Trichomonas* spp. 100.0 (65/65)	*Haemoproteus* spp. 21.5 (14/65) *Trypanosoma* sp. 1.5 (1/65)	*Ascaridia* egg 24.5 (16/65) *Eimeria* oocyst 4.6 (3/65) *Hexamita* spp. 3.1 (2/65) *Raillietina* egg 3.1 (2/65)

Table 3 Summary table of parasites found in specified organs based on histopathology lesions in loft-kept racing and feral pigeons.

Parasite		Lesions in organ	Number of cases
Loft-kept racing pigeon	Adult *Ascaridia*	Filling the small intestinal lumen	36
		Proventriculus and gizzard whitish mucus	5
		No remarkable lesions in the crop	2
		Obstruction of pancreatic duct	1
		No remarkable lesions in the nasal passages	1
		Severe pyogranulomatous lesions in the liver	1
	Different stages of *Ascaridia* larvae	Numerous in the small intestinal lumen	36
		Multifocal granulomatous lesions in the liver	18
		Proventriculus and gizzard whitish mucus	3
		Migrate into submucous of gizzard	1
		Migrate into the lung	1
		Migrate into the pancreas	1
		No remarkable lesions in the choanal slit	1
	Capillaria Adult and larvae	Embedded in the mucosa of the intestine. Moderate to severe enteritis consists of mononuclear cells	16
	Heterakis	Few were present in the small intestinal lumen. Mild chronic enteritis consists of mononuclear cells	6
	Adult *Raillietina*	Few in number but fill the small intestinal lumen. Mild chronic enteritis consists of mononuclear cells	7
	Trichomonas	Mild multifocal pseudomembrane in the oropharynx.	17
		Moderate granulomatous lesions in the oropharynx.	5
		Moderate granulomatous lesions in the liver	4
		Moderate granulomatous lesions in the lung	4
		Moderate granulomatous lesions in the kidney	1
		Mild multifocal parasitic ingluvitis	1
	Eimeria	Mild enteritis, Macro- and microgamonts developing in the mucosal epithelium	26
	Hexamita	Mild catarrhal enteritis. *Hexamita* organisms were observed near the crypt of Lieberkuhn.	14
	Haemoproteus	Meronts in the lung	23
		Meronts in various organs	5
Feral pigeon	Adult *Ascaridia*	Few were present in the small intestinal lumen	20
		Numerous in the small intestinal lumen	2
		Proventriculus and gizzard whitish mucus	2
	Different stages of *Ascaridia* larvae	Few were present in the small intestinal lumen	8
		Multifocal granulomatous lesions in the liver	5
	Capillaria Adult and larvae	Embedded in the mucosa of the intestine	6
	Dispharynx sp.	Moderate mononuclear proventriculitis	1
	Raillietina spp.	Few in number but fill the small intestinal lumen. Mild chronic enteritis consists of mononuclear cells	6
	Paratanaisia sp.	Mild to moderate atrophy of epithelium and mild interstitial nephritis	1
	Trichomonas spp.	Mild multifocal parasitic ingluvitis	1
	Eimeria spp.	Mild enteritis with numerous in the mucosa	2
	Hexamita spp.	Mild catarrhal enteritis. *Hexamita* organisms were observed near the crypt of Lieberkuhn.	2
	Haemoproteus spp.	Meronts in the lung	1
		Meronts in various organs	
	Trypanosoma sp.	Anemia and no specific lesions	1

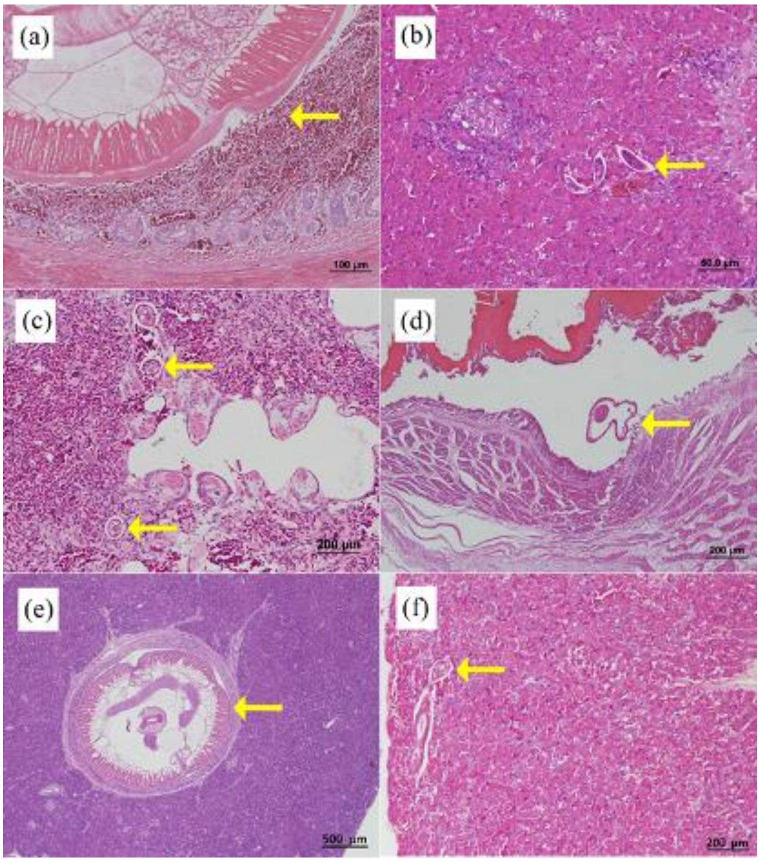


Figure 1 Histopathological changes in *Ascaridia* infections in different pigeon tissues.
(a) Segmental of jejunum in blunting of villi. There is no significant difference in the intestinal tract. There is no significant difference in lesions in the intestine between feral and loft-kept racing pigeons (H&E staining, ×200).
(b) *Ascaridia* larva-induced hepatitis during the period of liver migration (H&E staining, ×400) in loft-kept racing pigeons.
(c) *Ascaridia* larva in parabronchi of the lung (H&E staining, ×100) in loft-kept racing pigeons.
(d) *Ascaridia* larva migrate into the mucosa of the gizzard (H&E staining, ×200) in loft-kept racing pigeons.
(e) *Ascaridia* nematode migrated into the pancreatic duct (H&E staining, ×40) in loft-kept racing pigeons.
(f) *Ascaridia* larva infested and no obvious specific microscopic lesion in the pancreas (H&E staining, ×200) in loft-kept racing pigeons.

Other helminth parasite infections occurred in loft-kept racing pigeons. *Capillaria* spp. embedded in the mucosa of the duodenum. Moderate to severe expansion of lamina propria with mononuclear cell infiltrate, excess mucous layer in lumen containing desquamated duodenal epithelium, and sections of nematodes were observed in the mucosal layers. *Heterakis* infection resulted in chronic diffuse inflammation of the jejunum and ileum, with the submucosa and lamina propria being infiltrated with mononuclear cells and lymphocytes. The cross-section of the intestine infested with *Heterakis* spp. showed the nematode with the spine, surrounded by cellular debris and infiltration of inflammatory cells in the mucosa. The intestine of loft-kept racing pigeons infested with *Raillietina* spp. showed mild infiltration of lymphocytes and macrophages in the mucosa and lamina propria, with an increased number of goblet cells and enlarged columnar epithelial cells.

A gross examination of 12 loft-kept racing pigeons infested with *Trichomonas* showed severe caseous nodules in the crop, esophagus, and nasal cavity. Four pigeons showed caseous necrosis in multiple organs (Fig. 2a). Microscopically, moderate congestion was observed near the lesions, along with numerous granulomatous foci in the liver, lung, and kidney, characterized by infiltration of mononuclear inflammatory and multinucleated giant cells. *Trichomonas* spp. was also identified in liver smears stained with Liu's stain (Fig. 2b).

Other protozoal infection scenarios were observed in loft-kept racing pigeons. Gross examination of most pigeons infested with coccidian parasites showed mild enteritis, whereas histologic examination revealed only a few macro- and microgamonts developing in the mucosal epithelium. *Hexamita* was diagnosed in fresh fecal smears of racing pigeon, with only mild catarrhal enteritis observed, along with dilatation and hyperplasia of affected crypts. Large numbers of elongated *Hexamita* organisms were observed near the crypt of Lieberkuhn. Various developmental stages of *Haemoproteus*, including the branching shape of meronts, were observed mainly in the lungs, with few occurrences in the myocardium, liver, and spleen. Gametocytes were seen in the erythrocytes.

The detection and the prevalence of endoparasites in feral pigeons by pathological examination: *Ascaridia* infection in the feral pigeons did not show remarkable lesions. Gross examination of all feral pigeons infested with *Trichomonas* showed increased mucous secretion (Fig. 2c). The crop smear showed a large number of *Trichomonas gallinae* (Fig. 2d). The proportion of feral pigeons infected with *Trichomonas* was significantly higher than that of racing pigeons (p <0.001). All the feral pigeons examined were infected with *Trichomonas*, although only a few exhibited severe lesions. *Trichomonas* adhered to the mucosa of the upper gastrointestinal tract in these pigeons without inducing a severe inflammatory response. In contrast to the lesions observed in feral pigeons, severe multifocal caseous nodules were present in multiple organs of loft-kept racing pigeons. The severity of lesions associated with *Trichomonas* infection was notably higher in loft-kept racing pigeons compared to feral pigeons. These findings indicate that *Trichomonas* infection results in more severe pathology in loft-kept racing pigeons.

Severe coccidian infection was found in only two feral pigeons, with a large number of coccidia present in the duodenum. However, the histological cross-section of the intestine showed mild enteritis only, with no serious damage.

A gross examination revealed a heavy infection of *Dispharynx* sp. (7 -12mm long) in the mucosal lining of the proventriculus in one pigeon. The proventricular mucosal surface was burrowed by the parasites and markedly filled with a thick, gelatinous, and mucoid material. Microscopically, the parasite showed two pseudolabia, each with four characteristics of wavy cuticular cordons, and each pseudolabia had a pair of large cephalic papillae and 1 inconspicuous amphid. The cross-section of the female nematode showed numerous small (20 by 40μm), black-colored, embryonated eggs in the distended uterus of the parasite. Other findings included infiltration of mononuclear cells in the lamina propria of the proventriculus.

Paratanaisia sp. was identified in one female feral pigeon, which was presented with poor grip reflex. Histologically, the renal pelvis showed moderate to severely dilated collecting tubules, which contained numerous mature trematodes. Mild-to-moderate atrophy of epithelium and mild interstitial nephritis were observed.

Histopathological examination showed mild myocarditis, chronic-active hepatitis, lymphoid hyperplasia, and leaf-shaped protozoan parasites in the liver and blood vessels, which were similar to the findings of *Trypanosoma*. Numerous *Haemoproteus* meronts were found in the capillaries, bronchial vessels, skeletal muscle, and the endothelium of large veins. However, distinguishing between *Haemoproteus* meronts and *Trypanosoma* amastigotes by histopathological examination is difficult.

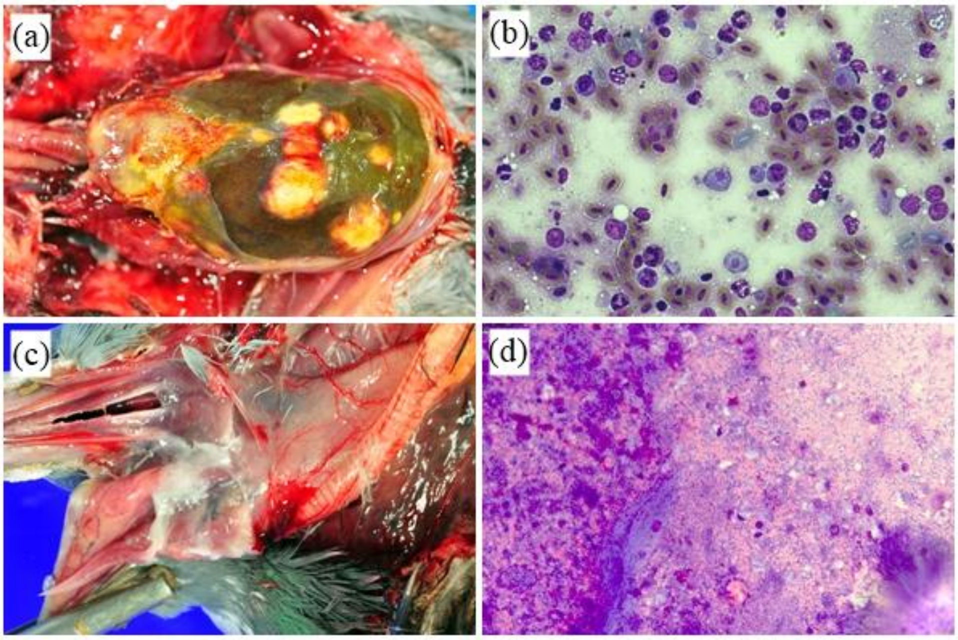


Figure 2 Trichomoniasis in pigeons.
(a) Loft-kept racing pigeon showed severe lesions with caseous necrosis in multiple organs.
(b) Smears from liver mass showed a large number of *Trichomonas gallinae*.
(c) Feral pigeon showed mild lesions with numerous mucus.
(d) Smears from crop showed a large number of *Trichomonas gallinae*.

Discussion

The comprehensive examination results revealed that pigeons, including loft-kept racing and feral pigeons, were infected with parasites, with *Trichomonas* infection being the most common. Gastrointestinal clinical signs were the most frequent findings resulting from parasitic infection in most feral pigeons, primarily affecting the upper digestive tract. Studies have shown varying prevalence rates of *Trichomonas* infections across different regions. The high prevalence rates of *Trichomonas* infections found in this study are similar to those reported in studies conducted in Spain (Sansano-Maestre *et al*., 2009), Australia (Park, 2011), Iraq (Al-Sadi & Hamodi, 2011), and Belgium (Rouffaer *et al*., 2014). In contrast, lower incidence rates, less than 10%, have been reported in Slovenia (Dovc *et al*., 2004) and Egypt (El-Khatam *et al*., 2016). Overall, there was no doubt that the prevalence of *Trichomonas* in pigeons in Taiwan is high. The infection, primarily detected in wild pigeons, can be attributed to factors such as the subtropical island climate. Severe lesions, mainly observed in racing pigeons, can be attributed to factors such as racing culture and pigeon management practices. Intensive flight training or co-infection with pigeon circovirus in racing pigeons in Taiwan may contribute to severe immunosuppression, increasing susceptibility to secondary infections by pathogens.

The most common parasite in loft-kept racing pigeons was *Haemoproteus* spp., which is primarily found in blood smears. Despite the absence of distinctive clinical signs (subclinical), regular blood smears could effectively detect blood parasitic infections. The most common parasite identified in loft-kept racing pigeons was *Haemoproteus* sp., primarily detected through blood smears. Despite the absence of overt clinical signs (subclinical infections), regular blood smears proved to be an effective method for detecting blood parasitic infections. A study by Rosyadi *et al.* (2021) demonstrated a significant negative relationship between the level of *Haemoproteus columbae* parasitemia and body mass in feral domestic pigeons; specifically, a 1% increase in the number of infected erythrocytes correlated with a 5.42g reduction in body mass (Nebel *et al.*, 2019). This finding underscores the profound impact that long-lasting subclinical pigeon haemoproteosis caused by *H. columbae* can have on the overall health of farmed domestic pigeons. Pigeon flies were often observed in large numbers on the surface of wild pigeons during examination, and the prevalence of blood parasite infections in wild pigeons was high. Although no significant difference was found when compared with racing pigeons (P =0.8323), the common occurrence of blood parasite infections in Taiwan indicated that this pathogen deserved further research and control measures. A notable detail that only one case described in co-infection of feral pigeons with *Haemoproteus* sp. and *Trypanosoma* sp. showed clear clinical signs of anemia. The clinical signs of anemia were speculated to be mainly caused by *Typanosoma* because *Haemoproteus* infection was usually subclinical and can cause mild clinical signs. *Trypanosoma avium* and *T. hannai* are the most common species found among pigeons. These species are nonpathogenic, pleomorphic, and transmitted by mosquitoes, simuliids, and hippoboscids (Mandal *et al*., 2008). In Taiwan, a documented case of *Trypanosoma avium* infection in a collared scops owl (*Otus bakkamoena*) did not identify the transmission vector. Similarly, prior to our investigation, there has been no effective identification of the transmission vectors for *Trypanosoma* infections in pigeons. Understanding the pathogenicity, distribution, and significance of blood parasites in wild birds requires comprehensive epidemiological and demographic studies of the parasites, their vectors, and hosts, all supported by experimental research. Despite the inherent challenges, ongoing investigation into the transmission vectors of avian diseases remains essential, as highlighted by Valkiunas *et al.* (2004).

Domestic and free-range pigeons are often infected with helminth parasites at higher rates. The data showed that pigeons infected with *Ascaridia* spp., *Raillietina* spp., and *Capillaria* spp. were consistent with previous reports (Khezerpour & Naem, 2013; Salem *et al*., 2022), which indicated that these birds harbor various pathogens and serve as reservoirs for many parasitic infections. Helminth parasites were found not only in the gastrointestinal tracts but also in many organs. The health of loft-kept racing pigeons could be seriously damaged by parasites. Moreover, the infection rate in feral pigeons was significantly higher than that in racing pigeons (p <0.05), increasing the risk of parasitic infection spreading between pigeons and different avian species. Helminth parasites are significant in various bird species, including poultry. Species such as *Ascaridia galli* and *Heterakis gallinarum* are common helminths that affect a wide range of avian species (Ola-Fadunsin *et al*., 2019). Additionally, species like *Torameres* spp., *Dispharynx nasuta*, *Amidostomum anseris*, *Raillietina* spp., and *Davainea proglottina* are also important. These helminths can cause clinical and pathological challenges in birds, potentially leading to the transmission of infectious diseases to poultry (Permin & Hansen, 1998). This issue is particularly concerning in free-range systems, where parasitic control measures are often less stringent compared to commercial systems.

In cases of heavy infection, the *Ascaridia* spp. adversely affect the health of birds with weight loss, emaciation, slowed growth, unthriftiness, damage to the gut epithelium, fertility disturbances, catarrhal enteritis, occlusion, intestine rupture, peritonitis, and death, particularly among young birds (Mehmood *et al*., 2019). This parasite can cause hemorrhagic lesions in the liver, lungs, and intestines of domestic pigeons (Adang *et al*., 2010). *Ascaridia* spp. was the most common helminth infesting racing pigeons. This study demonstrated that the loft-kept racing pigeon infested with *Ascaridia* spp. showed severe lesions in the digestive system. However, most feral pigeons are infected with *Ascaridia* spp. did not show any significant lesions. This result could indicate that the loft-kept racing pigeons have more severe signs than the feral pigeons after *Ascaridia* spp. infection. Similar to Adang's study (2010), the histopathological effects, particularly severe lesions, were observed in many organs. In the present study, *Ascaridia* was found in the choanal slit, liver, pancreas and pancreatic duct, lung, gizzard, duodenum, jejunum and ileum of the infected domestic pigeons. Based on prior research, this finding is the first report of *Ascaridia* observed in the pancreas and pancreatic ducts of pigeons. In one case involving a loft-kept racing pigeon, an adult *Ascaridia* nematode was found obstructing the pancreatic duct, resulting in a slight thickening of the duct wall. Although no direct evidence was available, it is highly likely that this obstruction was due to an excessive number of worms migrating into the pancreatic duct, which triggered a mild inflammatory response. If the parasite migration occurred after the host's death, inflammation or thickening reactions are often absent or difficult to observe. In a separate case involving a feral pigeon, the larval stage of the *Ascaridia* nematode was detected within the pancreatic tissue. These larvae undergo visceral migration during their developmental process and eventually mature into adults. However, it remains uncertain whether the larvae migrating through the pancreas fully mature, and further investigation is needed to clarify this aspect of their lifecycle.

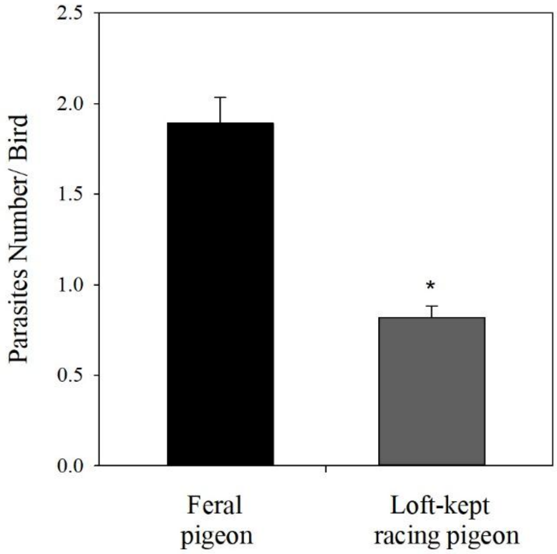
Dispharynx sp. (*Nematoda*: *Acuarioidea*: *Acuariidae)* is a widespread parasite that primarily affects the proventriculus and ventriculus of a wide range of birds, both free-living and in captivity. It can be found in various organs, including the esophagus, proventriculus, gizzard, and small intestine. The presence of this parasite is typically associated with ulcers in the proventriculus and hemorrhagic content, often observed alongside roundworms. *Dispharynx* sp. has been reported in domestic chickens, wild chickens, raptors, and small wild birds in Taiwan (Schmidt & Kuntz, 1970; Su & Fei, 2004), but it has not been documented in feral pigeons until now. The necropsy findings in feral pigeons infected with nematodes were similar to those observed in *Dispharynx nasuta* infections, as previously reported by Carreno (2008), Churria *et al.* (2011), and Severino *et al.* (2023). This study represents the first report of *D. nasuta* infection in Taiwan.

In Taiwan, renal infections by trematodes in feral pigeons have not been previously documented. The lesions associated with *Paratanaisia bragai* infection observed in this study are consistent with findings from earlier research (Menezes *et al.*, 2001; Olson *et al.*, 2003; Pinto *et al.*, 2004), which reported mild gross and microscopic lesions in the affected kidneys. Our study also confirmed that *P. bragai* infection primarily causes lesions in the kidney medulla. All members of the *Eucotylidae* family share a similar life cycle involving two hosts, with metacercariae developing in sporocysts within freshwater or terrestrial snails. These snails serve as intermediate hosts for trematodes, and snails from both wild and urban environments could potentially act as intermediate hosts for this parasite (Unwin *et al.*, 2013). Birds become infected by ingesting these snails, after which the metacercariae excyst and mature in the kidneys of the definitive host. The infection in pigeons occurs via ingestion of snails. Snails such as *Allopeas clavulinum* and *Subulina octona* have been identified as hosts for *P. bragai* in wild environments. These snails are primarily litter-dwelling species, inhabiting areas among stones, leaves, grass, and soil at altitudes ranging from 300 to 2,000 meters (Keller *et al.*, 1992). Feral pigeons are more likely to prey on snails found in soil contaminated with parasites. In contrast, loft-kept racing pigeons are less exposed to contaminated soil and secondary hosts, making them less susceptible to parasitic infections. Consequently, the prevalence of parasitic infections was found to be higher in feral pigeons than in loft-kept racing pigeons.

Despite the medical histories provided by racing pigeon owners, some have self-administered medications for their birds, with infected pigeons often experiencing more severe illnesses. The use of toltrazuril is favored due to the increasing resistance against other drugs, such as sulfonamides and amprolium, its extended effectiveness compared to substances like clazuril, and its ability to allow pigeons to develop immunity during treatment (Krautwald-Junghanns *et al.*, 2009). Additionally, numerous drug preparations are available for the treatment of nematodes in poultry, including piperazine citrate, albendazole, and fenbendazole (Salem *et al.*, 2022; Tarbiata *et al.*, 2016). While antiparasitic agents can be effective, it is crucial to follow veterinary guidelines when administering them. Additionally, extensive research has been directed towards identifying natural and safe alternatives to chemical drugs to combat the resistance of helminths and other pathogens. Many of these alternatives have proven successful in treating worm infestations and other infections. *Eimeria* infections in pigeon lofts primarily occur through the aerosolization of oocysts from dried feces, nest debris, or contaminated footwear. Contaminated food and water are also potential sources of infection (Saikia *et al.*, 2017). *Ascaridia* eggs, deposited in host feces, can complete their development in the environment, with a small proportion surviving for up to two years, leading to the accumulation of infectious eggs in the surroundings. Consequently, further research is needed to investigate factors contributing to the persistence of parasites, such as loft conditions, including litter type and stocking density, which may facilitate parasite proliferation and re-infection from the floor (Mohammed *et al.*, 2017).

Including feral pigeons in parasite research is essential, as it facilitates the simultaneous investigation of parasites that may pose risks to native wild bird species and even humans (Attia *et al.*, 2022). Feral pigeons, which often feed on insects or snails from soil contaminated with parasites, are more susceptible to parasitic infections. This study found that the prevalence of parasitic infections was higher in feral pigeons compared to loft-kept racing pigeons. Although loft-kept racing pigeons have less exposure to contaminated soil and secondary hosts, they can still come into contact with wild pigeons. The findings underscore the importance of identifying common parasites to develop more effective strategies for managing and controlling parasites within pigeon populations, thereby reducing disease control costs and ultimately safeguarding human health and well-being.

In conclusion, the study found a significantly higher prevalence of parasitic infections in feral pigeons compared to loft-kept racing pigeons. However, the histopathological analysis revealed more severe parasitic lesions in loft-kept racing pigeons. While no zoonotic parasites were detected in this study, it is recommended that pigeon management programs and public education initiatives be implemented to mitigate the risk of pathogen and parasite transmission from feral pigeons to livestock. This research provides a foundation for further in-depth studies, which are essential for developing effective preventive and control strategies.



Supplementary Figure 1 The number of parasites in each bird between the groups of loft-kept racing and feral pigeons. Bar plots showed the mean number of parasites per bird (mean ± SE). * means the p-value was below 0.05.

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