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DNA Prevalence of Eukaryotic Parasites with Zoonotic Potential in Urban-Associated Birds

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Simple Summary: Zoonoses are a growing threat to human health. Therefore, their surveillance is especially important in densely populated areas such as cities. Modern cities are, in fact, urban agglomerations where the population lives in urban centers and surrounding towns interspersed with agricultural land, forest patches and parks, where it is common to come into contact with several animal species, some of which may act as vectors of zoonoses. In this work, we evaluated, using molecular techniques, the zoonotic parasites carried by urban birds (White Stork, Lesser Black-backed Gull and two species of exotic parakeets), with the aim of better understanding the zoonotic risk that their faeces may pose. We detected a total of 23 genera of eukaryotic parasites, including six that are potentially harmful to human health: three fungi, one protist, and two nematodes. Our results show that the faeces of these four bird species could pose a risk to human health associated with the zoonotic parasites present in them. This should be taken into account when developing management plans for urban populations of these bird species.

Abstract: Synanthropic birds might play an important role as reservoirs of many zoonotic endoparasites; however, little information is available on many parasites and their prevalence. Here, we use an approach based on targeted metagenomic detection through the use of DNA metabarcoding of faecal samples to screen for circulating parasites in alien parakeets (*Myiopsitta monachus* and *Psittacula krameri*) and urban landfill-feeding storks (*Ciconia ciconia*) and gulls (*Larus fuscus*). We focus especially on potentially zoonotic parasites, with the aim of better understanding the zoonotic risk that these birds' faeces may pose. We detected a total of 23 genera of eukaryotic parasites: six fungi, three protists, five nematodes, two cestodes and seven trematodes. Among them, six stood out for their relevance to human health: *Cryptococcus* spp., *Aspergillus* spp. and *Candida* spp. (fungi); *Cryptosporidium* spp. (a protist); and *Ascaris* spp. and *Halicephalobus* spp. (nematodes). In parakeets, we detected *Cryptococcus* spp. and *Ascaris* spp., the latter being detected in 10–20% of the samples. In the White Stork and the Lesser Black-backed Gull, we found a high prevalence of *Aspergillus* spp. (in 15% and 50% of the samples, respectively) and *Candida* spp. (in 63% and 82% of the samples, respectively), and the presence of *Cryptosporidium* spp. in 10% of the samples. We detected *Halicephalobus* spp. in one gull sample (2%). Our results show that synanthropic birds may act as vectors and reservoirs of zoonotic parasites and their faeces could pose a risk to human health associated with the zoonotic parasites present in them. This should be taken into account when developing management plans for urban populations of these bird species.

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Keywords: *Ciconia ciconia*; *Cryptococcus* spp.; *Cryptosporidium* spp.; *Larus fuscus*; *Myiopsitta monachus*; *Psittacula krameri*

1. Introduction

Endoparasites are of global concern for human and animal health and welfare [1–3]. Many of these are zoonotic [3,4], and for which wildlife plays an important role in their transmission to humans [3,5]. Indeed, several studies involve birds in the transmission of different zoonotic parasites, such as fungi (e.g., *Cryptococcus* spp.; Kützing, 1833), protists (e.g., *Cryptosporidium* spp.; Tyzzer, 1912) and helminths (e.g., *Capillaria* spp.; Zeder, 1800), to humans [5–8]. These zoonotic parasites may have important public health implications, especially in urban regions where human–bird interactions are frequent [9].

Some fungi (e.g., *Cryptococcus* spp. or *Histoplasma* spp.; Darling, 1906) are particularly relevant in this respect, as their transmission to humans is usually linked to birds [7] and can even cause serious health problems, such as pulmonary infections (e.g., pneumonia) and meningitis [6,7]. In addition, opportunistic fungi such as *Aspergillus* spp. (Micheli, 1729) and *Candida* spp. (Berkhout, 1923) can colonise the respiratory tract of birds [10,11] and also cause respiratory infections in humans, especially in immunocompromised individuals [5,7]. Furthermore, some work suggests that synanthropic birds may be reservoirs of drug-resistant yeasts [12,13]. Several parasitic protists of birds have also been described as zoonotic [5,6]. For example, *Cryptosporidium* spp., *Giardia* spp. (Kunstler, 1882) or *Trichomonas* spp. (Donné, 1836) are well-known causes of various pathologies in humans [6,7]. These parasites can be transmitted through the ingestion of food and water contaminated with bird faeces [7]. In addition, nematodes such as *Toxocara* spp. (Stiles, 1905) or cestodes such as *Hymenolepis* spp. (Weinland, 1858) can also cause problems for human health [14,15].

As mentioned above, the role of birds as reservoirs and vectors of zoonotic parasites could be especially relevant in regions where interactions between humans and birds are common. For example, in urban and peri-urban areas, where high densities of birds and humans are combined, the risk of transmission is likely to be high [7,9,16]. In urban areas, it is common to observe high densities of some birds, such as pigeons, sparrows, or parakeets (invasive alien species in Europe) [7,9]. Among these, invasive alien species deserve special attention. These may carry a greater number of pathogenic species, as they may have weaker defence mechanisms against native parasites [17,18] and, more importantly, they might move parasites that are exotic to the region [18,19]. In addition, in some European cities, populations of some previously uncommon birds, such as gulls and storks, are greatly increasing [20,21], but information on parasites in their synanthropic populations is scarce. These species usually feed on landfill sites, where the acquisition of pathogens is more likely to occur, as landfills provide the perfect conditions for the development of pathogens [7,21]. Moreover, it is known that drugs such as anti-inflammatory or antimicrobial drugs accumulate in landfills, which encourages the development of antibiotic-resistant bacterial pathogens [21,22]. However, there is no information on their potential role in the transmission of eukaryotic parasites. Therefore, there is still a significant knowledge gap regarding the presence of eukaryotic parasites in synanthropic birds. This information is particularly relevant in urban and peri-urban areas, which are becoming more and more common in cities, and where human contact with wildlife is greatly increased.

DNA metabarcoding is a powerful tool for parasite detection and systematic risk assessment [23–25]. Currently, this technique is frequently used for the study of parasites [23,26,27]. It can quickly provide information on several organisms in a sample, so that, theoretically, using one or a few genetic markers could provide information about all the eukaryotic parasites present in the sample [23,28]. In addition, this technique allows for working with faeces, a non-invasive approach, and obtaining very precise information on the presence of organisms with very low parasite loads [24].

In this work, we examined the eukaryotic parasites of four synanthropic birds (two that frequently feed in landfills and two invasive alien parakeet species) through targeted metagenomic detection, and estimated their prevalence. With this aim, we used DNA metabarcoding of faecal samples, using two 18S rRNA gene markers, a 180 bp broad-spectrum eukaryotic marker and a 490 bp nematode-specific marker. Our main objective was to provide a broad picture of the endoparasites of these species and, thereby, a better understanding of the zoonotic risk that their faeces may pose to humans.

2. Materials and Methods

2.1. Sample Collection

We collected samples of four host synanthropic bird species in Madrid, Spain (Table 1; Supplementary Material Table S1): White Stork (*Ciconia ciconia*; Linnaeus, 1758; hereafter *C. ciconia*), Lesser Black-backed Gull (*Larus fuscus*; Linnaeus, 1758; hereafter *L. fuscus*), Monk Parakeet (*Myiopsitta monachus*; Boddaert, 1783; hereafter *M. monachus*) and Roseringed Parakeet (*Psittacula krameri*; Scopoli, 1769; hereafter *P. krameri*). *C. ciconia* samples were collected in Colmenar Viejo (Madrid, Spain), close to the Northwest Madrid landfill. *L. fuscus* samples were collected in Manzanares el Real (Madrid, Spain), where they rest beside the water reservoir after feeding in Madrid landfills. Faecal samples of *C. ciconia* and *L. fuscus* were collected as fresh as possible (still wet) from the ground in roosting areas. *M. monachus* and *P. krameri* samples were provided by the Area de Gobierno de Medio Ambiente y Movilidad of the Madrid City Council, where these two species live in parks in large numbers (Table 1). Samples of these two species were collected by cloacal swabs during the capture days for the population control of invasive alien species by the technicians of the Madrid City Council. Samples were stored in individual containers in 96° pure ethanol during the field work and frozen in the laboratory at −20 °C until DNA extraction [29].

Table 1. Target host bird species and sample size. "Sample with data" refers to the number of samples with data after sequencing and filtering. More information about the samples' origin can be found in Supplementary Material Table S1.

Scientific Name	Collected Samples	Sample with Data
Ciconia ciconia	45	41
Larus fuscus	45	43
Myiopsitta monachus	80	79
Psittacula krameri	10	10

2.2. Sample Processing

First, we performed DNA extractions using the MagBind Environmental DNA 96 Kit (Omega BioTek, Norcross, GA, USA) according to the manufacturer's instructions. The quality of extracted DNA was checked through agarose gel electrophoresis and its concentration measured using Picogreen (Invitrogen, Waltham, MA, USA).

In a second step, amplification of the 18S rRNA eukaryotic phylogenetic marker gene was carried out. We amplified DNA samples using two pairs of primers modified to include Illumina sequencing adapters: MiniB18S_81F/MiniB18S_81R (5′-GGCCGTTCTTAGTTGG TGGA-3′; 5′-CCCGGACATCTAAGGGCATC-3′), a broad-spectrum eukaryotic primer that amplifies a 180 bp region [24], and NemF/18Sr2b (5′-GGGGAAGTATGGTTGCAAA-3′; 5′-TACAAAGGGCAGGGACGTAAT-3′), a primer specifically designed for nematode DNA amplification that amplifies a 490 bp region [30]. The former provides a more global view of the sample content, amplifying more taxa and with apparently good resolution for coccidia [24]; although, by amplifying so many different taxa, the coverage obtained for parasites is likely to be lower. The second primer set is more specific and should have a better detection rate in nematodes [30], although it is quite long and may show problems detecting degraded DNA. Amplification was performed using 4 µL of template DNA in a 44 µL reaction mixture containing 0.06 µM of each primer, 0.5 µM dNTPs and

1 U of Q5 *HighFidelity* DNA Polymerase (New England Biolabs, Ipswich, MA, USA). The thermocycler conditions consisted of 95 °C for 30 s, followed by 25 cycles of 95 °C for 10 s, 57 °C for 30 s (MiniB18S_81) or 52 °C for 30 s (NemF/18Sr2b), and 72 °C for 30 s. PCR products were then treated with 2 µL of a 1X Q5 *HighFidelity* DNA Polymerase buffer and 5 U/µL ExoI exonuclease (Thermo Scientific, Waltham, MA, USA) solution for 20 min at 37 °C and 15 min at 80 °C to remove the initial primers. The resulting products where then subjected to 10 more PCR cycles as above but with the addition of 3 µL of 10 mM primer stocks bearing (5′-3′) the required i5 and i7 Illumina adapters, 10 nt barcodes, and Illumina sequencing primers. The amplicon libraries produced were checked through agarose gel electrophoresis and their concentration measured using *Picogreen* (Invitrogen). Equimolar amounts from each library were pooled, run on an agarose gel, and the appropriate band was excised and purified using the *QIAquick Gel Extraction Kit* (Qiagen, Hilden, Germany).

Lastly, the final product was sequenced on an Illumina MiSeq NGS platform using either (i) a 300-cycle v2 reagent kit or (ii) a 600-cycle v3 reagent kit (for Mini and NemF primer pairs, respectively), following the manufacturer's instructions.

2.3. Zoonotic Parasites

We focused on the parasites that could likely pose any risk to human or bird health. On the one hand, we paid attention to those eukaryotic parasites mentioned as being zoonotic by the European Centre for Disease Prevention and Control (ECDC) and/or the US Centers for Disease Control and Prevention (CDC; Table 2). These are parasites that can be transmitted from non-human animals to humans. On the other hand, we also paid attention to any other eukaryotic parasites that could affect bird or human health. In this regard, we include *Blastocystis* spp. (Alexeieff, 1911; Protist), *Aspergillus* spp. (Fungi) and *Candida* spp. (Fungi) in the analysis, three genera of parasites that under certain circumstances, especially in immunocompromised individuals, might be important for human health and are known to be found in bird faeces [10,11,31]. Additionally, we checked all identified genera of fungi, nematodes, flatworms and coccidia (Apicomplexa) to verify whether they could be parasites of birds or could affect human health.

Table 2. Organisms reported as being zoonotic by the European Centre for Disease Prevention and Control (ECDC; source = 1) and/or the US National Public Health Agency (CDC, source = 2).

	Genus	Species	Source
Fungi	*Arthroderma*	*A.* sp. (Berk, 1860)	2
	Cryptococcus	*C. neoformans* (Vuill, 1901)	2
	Enterocytozoon	*E. bieneusi* (Desportes, et al., 1985)	1
	Histoplasma	*H.* sp. (Darling, 1906)	2
	Microsporum	*M.* sp. (Gruby 1843)	2
	Sporothrix	*S.* sp. (Hektoen and Perkins, 1901)	2
Cestoda	*Dipylidium*	*D.* sp. (Leuckart, 1863)	2
	Echinococcus	*E.* sp. (Rudolphi, 1801)	1 and 2
	Taenia	*T. saginata* (Goeze, 1782)	1
	Taenia	*T. solium* (Linnaeus, 1758)	1 and 2
Nematoda	*Ancylostoma*	*A. braziliense* (De Faria, 1910)	2
	Ancylostoma	*A. caninum* (Ercolani, 1859)	2
	Ancylostoma	*A. ceylanicum* (Ercolani, 1859)	2
	Anisakis	*A.* sp. (Dujardin, 1845)	1
	Ascaris	*A.* sp. (Linnaeus, 1758)	2
	Baylisascaris	*B. procyonis* (Stefanski and Zarnowski, 1951)	2
	Toxocara	*T. canis* (Werner, 1782)	2
	Toxocara	*T. cati* (Schrank, 1788)	2
	Trichinella	*T. spiralis* (Owen, 1835)	1
	Uncinaria	*U. stenocephala* (Railliet, 1884)	2

Table 2. *Cont.*

	Genus	Species	Source
Protist	*Cryptosporidium*	*C.* sp. (Tyzzer, 1912)	1 and 2
	Giardia	*G.* sp. (Kunstler, 1882)	1 and 2
	Leishmania	*L.* sp. (Nicolle, 1908)	1 and 2
	Sarcocystis	*S.* sp. (Lankester, 1882)	1
	Toxoplasma	*T. gondii* (Nicolle and Manceaux, 1908)	1 and 2

2.4. Bioinformatic Analyses

We used Cutadapt to remove the primers from the sequences [32] and then we performed sequence processing using the R package DADA2 v1.30.0 [33–35]. First, we filtered the sequences and trimmed them based on barcode length and sequence quality. For MiniB18S_81, we trimmed the sequences to 120 pb and only allowed a maximum of 1 expected error. For NemF/18Sr2b, we trimmed the sequences to 280 pb in forward direction and to 240 pb in reverse direction and only allowed a maximum of 2 expected errors in forward and 3 expected errors in reverse. Then, we merged the forward and reverse sequences, constructed a table of amplicon sequence variants (hereafter ASVs), and removed chimeric sequences. Finally, we assigned the taxonomy using a minBoot of 50 and a Silva v132 eukaryotic 18S non-redundant database formatted for DADA2 [34,36] provided by Cabodevilla et al. [37].

Subsequently, we filtered the data by removing samples with less than 2000 reads for MiniB18S_81 and samples with less than 1000 reads for NemF/18Sr2b. We were less restrictive with NemF/18Sr2b because it is a more specific primer pair and, therefore, the coverage of parasite DNA should be higher. We also removed those ASVs with a maximum read number lower than 5 in a sample and rejected singletons and doubletons. Finally, we kept only those ASVs with taxa identification to genus level, which was 27.5% in the case of MiniB18S_81 and 55.3% in the case of NemF/18Sr2b.

From the tables of two primer sets, we selected only the ASVs identified as fungi, protist, nematodes (Metazoa) and plathelminths (Metazoa). We grouped the ASVs by genus and converted the obtained data (number of reads) into presence–absence data. Then, we combined the presence–absence data of the two primer sets into a single data table. We checked this database for the presence of the parasite genera in Table 2, as well as *Blastocystis* spp., *Aspergillus* spp. and *Candida* spp. In addition, we conducted a literature review of each of the identified genera of fungi, nematodes, flatworms and coccidia (Apicomplexa) to check whether they had been previously described as parasites, and if so, whether they could be parasites of birds or whether they could pose any risk to human health. Finally, we estimated the prevalence of each parasite genus detected.

3. Results

We detected a total of 23 genera of eukaryotic parasites (Table 3): six fungi, three protists, five nematodes, two cestodes, and seven trematodes. Among them, we detected three genera especially highlighted as zoonotic organisms by the European Centre for Disease Prevention and Control and/or the US National Public Health Agency (Table 2): one fungi (*Cryptococcus* spp.), one protist (*Cryptosporidium* spp.), and one nematode (*Ascaris* spp.). Moreover, we also detected three other genera highly relevant to human health: two fungi (*Aspergillus* spp. and *Candida* spp.) and one nematode (*Halicephalobus* spp.; Timm, 1956). Other detected genera were either of low relevance (mostly anecdotal) to human health (Table 3) or were only relevant to birds.

Table 3. Relevance of identified parasite genera to human health. Relevance is assessed qualitatively, by taking as high relevance the genera mentioned by the European Centre for Disease Prevention and Control and/or the US National Public Health Agency and ranking the rest in comparison to these based on literature information.

	Genus	Relevance to Human Health	Pathogenicity	Literature
Fungi	***Aspergillus***	High	Opportunistic fungi responsible for high prevalence of infections in humans	[38,39]
	Candida	High	Opportunistic fungi responsible for high prevalence of infections in humans	[39]
	Stachybotrys (Timm, 1956)	Low-Medium	Toxic black mould. Pose a danger when it grows massively on cellulose-rich plant debris. Should not pose a risk in faeces.	[40]
	Malassezia (Baillon, 1889)	Low	Opportunistic fungi. Very common organism of the skin and digestive tract, usually harmless	[41,42]
	Rhodotorula (Harrison, 1927)	Low	Opportunistic fungi. A common environmental yeast, usually harmless	[43]
	Cryptococcus	High	Opportunistic fungi responsible for high prevalence of infections in humans'	[39]
Protist	***Cryptosporidium***	High	Patients with impaired immune system may develop profuse, life-threatening, watery diarrhoea that is very difficult to treat with currently available drugs	[44,45]
	Eimeria (Schneider, 1875)	Apparently no		
	Blastocystis	Low-Medium	The causes of its pathogenicity are not clear, but it seems that it can generate a dysbiosis and affect the epithelia of the digestive tract	[46,47]
Nematoda	***Ascaris***	High	Globally important parasite, especially in underdeveloped areas. Ascariasis is considered one of the most important neglected tropical diseases.	[48]
	Halicephalobus (Timm, 1956)	High	Rare parasite of humans, but can cause fatal Meningoencephalomyelitis	[49,50]
	Travassostrongylus (Orloff, 1933)	Apparently no		
	Aonchotheca (López-Neyra, 1947)	Apparently no		
	Eucoleus (Dujardin, 1845)	Low	Very rarely cause respiratory infections in humans	[51]
Cestoda	***Parorchites*** (Fuhrmann, 1932)	Apparently no	Rarely causes intestinal infection in humans	[52]
	Raillietina (Fuhrmann, 1920)	Low		
Trematoda	***Cardiocephaloides*** (Sudarikov, 1959)	Apparently no	Cercarial Dermatitis	[53]
	Collyriclum (Bremser, 1831)	Apparently no		
	Cryptocotyle (Luhe, 1899)	Apparently no		
	Diplostomum (von Nordmann, 1832)	Apparently no		
	Galactosomum (Looss, 1899)	Apparently no		
	Gigantobilharzia (Odhner, 1910)	Low-Medium		
	Posthodiplostomum (Dubois, 1936)	Apparently no		

Regarding the prevalence, among the fungi, we found the highest values in *C. ciconia* and *L. fuscus*, with an especially high prevalence of *Candida* spp. (0.63 and 0.82, respectively),

Rhodotorula spp. (0.80 and 0.91, respectively), and also *Aspergillus* spp. (0.5) in *L. fuscus* (Figure 1). However, *Cryptococcus* spp., the only genus particularly highlighted as a zoonotic organism, was only detected in *M. monachus* (Figure 1). *Stachybotrys* spp. was detected in *C. ciconia*, *L. fuscus* and *P. krameri*. In the case of the protists, we detected *Eimeria* spp. in all the host species, *Blastocystis* spp. in *C. ciconia* and *M. monachus*, and *Cryptosporidium* spp. in *C. ciconia* and *L. fuscus* (Figure 2). *Blastocystis* spp. could be identified to the subtype level, detecting subtypes 3 and 4 in *C. ciconia* (in one sample each) and subtype 4 in *M. monachus* (in two samples). We found the highest prevalence of *Cryptosporidium* spp. in *L. fuscus* (0.09). *C. ciconia* was the only species in which all three protists were detected, and it was also the only species in which both *Cryptosporidium* spp. and *Blastocystis* spp., the two genera with zoonotic potential, were detected (Figure 2).

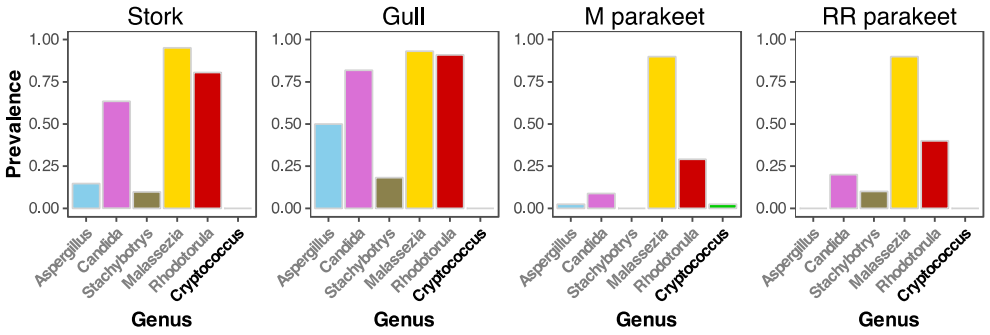


Figure 1. Prevalence of zoonotic fungi genera detected in bird faeces. All genera could parasitise humans; in black are those described as zoonotic in Table 2 and in grey are those that, according to the literature, may have some effect on human health.

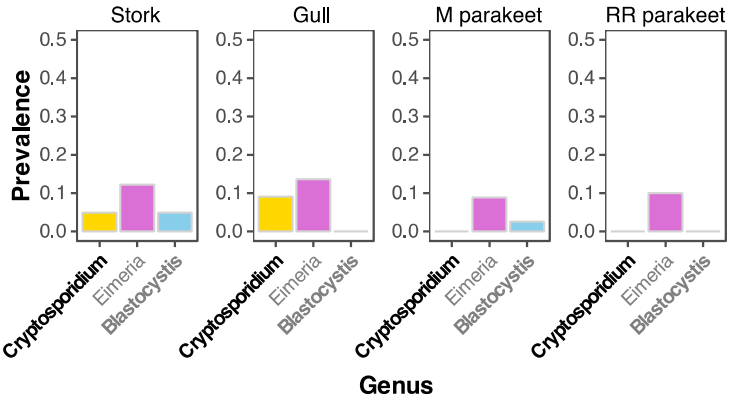


Figure 2. Prevalence of zoonotic protist genera. Genera that could parasitise humans are highlighted in bold; in black are those described as zoonotic in Table 2, and in grey are those that, according to the literature, may have some effect on human health. The genera that are not in bold are bird parasites.

On the other hand, the detected metazoan parasites varied greatly among the host species, with prevalence values between 0 and 0.2 (Figure 3). Among the five nematode genera detected, we detected *Ascaris* spp. in *M. monachus* and *P. krameri*, while the others were observed in only one species: *Aonchotheca* spp. in *C. ciconia*, *Halicephalobus* spp. and *Eucoleus* spp. in *L. fuscus*, and *Travassostrongylus* spp. in *M. monachus* (Figure 3). We detected *Ascaris* spp. in *M. monachus* and *P. krameri* with a prevalence of 0.09 for *M. monachus* and 0.2 for *P. krameri*, implying the presence of this zoonotic parasite in 1 out of 10 *M. monachus* and 2 out of 10 *P. krameri*. We detected cestodes with a prevalence higher than 0.1 in *C. ciconia* (0.12; *Raillietina* spp.) and *L. fuscus* (0.14; *Parorchites* spp.; Figure 3). We also detected *Raillietina* spp. in *M. monachus* but with a very low prevalence (0.01). The prevalence of trematodes was lower than 0.1, being detected almost exclusively in *L. fuscus* (only *Galactosomum* spp. was detected in *C. ciconia*; Figure 3). However, the combined prevalence of all the trematodes in *L. fuscus* was 0.28 (approximately in 1 in 3 *L. fuscus*). We observed

the presence of the zoonotic trematode *Gigantobilharzia* spp. in *L. fuscus*, with a prevalence of 0.02.

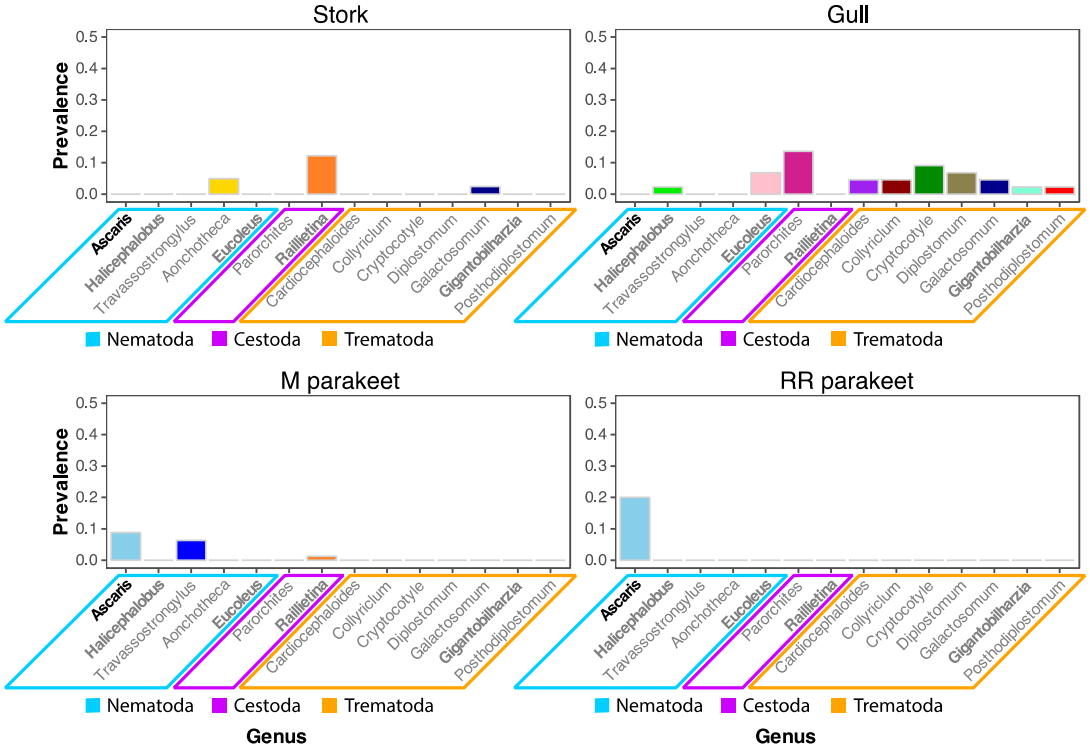


Figure 3. Prevalence of zoonotic metazoan genera. Genera that could parasitise humans are highlighted in bold; in black are those described as zoonotic in Table 2, and in grey are those that, according to the literature, may have some effect on human health. The genera that are not in bold are bird parasites; most of them are harmless to humans or have been very rarely reported in humans.

4. Discussion

Our results provide relevant information on the parasites (fungi, protists and helminths) carried by alien parakeets (*M. monachus* and *P. krameri*) and urban landfill-feeding *L. fuscus* and *C. ciconia*. During winter, these *C. ciconia* sleep on roofs within the village and spend the day eating at the landfill and its surroundings. And *L. fuscus* roost at the Santillana reservoir, which supplies water to Madrid, and feed mostly on Madrid's large landfills (Northwest Madrid and Valdemingómez). Among the parasite genera detected, six stand out for their relevance to human health; *Cryptococcus* spp., *Aspergillus* spp. and *Candida* spp. (fungi); *Cryptosporidium* spp. (protist); and *Ascaris* spp. and *Halicephalobus* spp. (nematodes). We detected *Cryptococcus* spp. and *Ascaris* spp. in *M. monachus* and *P. krameri*, the latter with a significant prevalence, being detected in 10–20% of their faeces. On the other hand, we found a high prevalence of *Aspergillus* spp. and *Candida* spp. in *C. ciconia* and *L. fuscus*, with a higher prevalence of *Aspergillus* spp. in *L. fuscus*. We also detected the presence of *Cryptosporidium* spp. in up to 1 in 10 *C. ciconia* and *L. fuscus* faeces. We only detected *Halicephalobus* spp. in one *L. fuscus* sample. Therefore, our work provides a broad picture of the parasites present in these species' faeces.

Some fungi are frequent parasites of birds, which can even impact severely on their health [5,54]. Moreover, certain of these fungi have zoonotic potential, i.e., they can also infect humans [7,55]. In this study, the six fungal genera detected have species with the potential to infect humans in some circumstances, although only three of them were particularly relevant to human health: *Cryptococcus* spp., *Aspergillus* spp., and *Candida* spp. The most common route of transmission for *Cryptococcus* spp. and *Aspergillus* spp. is airborne, via the inhalation of spores [56,57], and bird faeces can spread these spores [57]. *Cryptococcus* spp., especially *C. neoformans*, is closely associated with bird faeces [57,58],

and the spread of it in such faeces is widely documented [5,57,59,60]. In our case, we only detected it in a single sample of alien *M. monachus*. Previously, the feral pigeon had been considered a reservoir and disperser of *C. neoformans* [58,60], but this shows that alien parakeets (*M. monachus* and *P. krameri*) may also play this role, although in this case the observed prevalence was very low. *Aspergillus* spp. has also been frequently detected in bird faeces [55,56,61], and it has been suggested that these faeces may act as a vector for the spread of their spores and therefore pose a zoonotic risk [61]. We detected *Aspergillus* spp. in three of the four host species, but especially in *L. fuscus*, with a prevalence of 0.5, and in *C. ciconia* (0.15). This suggests that *L. fuscus* faeces in particular could pose a significant risk of aspergillosis, followed by *C. ciconia* faeces. Finally, *Candida* spp. has also been documented in bird faeces [12,55,61] and birds have been suggested as possible parasite dispersers [13,62]. Among the four host species, we detected *Candida* spp. in all of them. Its prevalence in *C. ciconia* and *L. fuscus* was extremely high, being detected in more than 60% and 80% of the samples.

We also detected three genera of protist parasites in the faeces of these birds, of which only *Cryptosporidium* spp. may be of actual relevance to human health [7]. It is known that birds can also carry some zoonotic protist parasites and *Cryptosporidium* spp. is one of the most important [7,63]. In this study, we only detected it in species that feed on landfills. This result is in line with what has been previously described in the faeces of synanthropic *C. ciconia* and *L. fuscus* [63,64]. We detect no *Cryptosporidium* spp. in *M. monachus*; however, this is in contrast to what has been described in other regions of the world, such as in Chile, where it is also an alien species [65]. Therefore, even if we have not detected *Cryptosporidium* spp., *M. monachus* should be considered a potential carrier of this parasite. We also detected *Blastocystis* spp. in *C. ciconia* and M parrakeet. In this case, we were able to identify the subtype of *Blastocystis* spp., identifying the sequences as subtypes 3 and 4, which are considered zoonotic [66]. However, the pathogenicity of this organism is not yet clear [67] and, therefore, we considered it not very relevant for human health. We also detected *Eimeria* spp. in around 10% of the faeces of all the species. This genus does not infect humans but can be particularly harmful to bird health [68].

Regarding helminths, we detected several genera of nematodes, cestodes and trematodes. The most relevant nematode genera detected were *Ascaris* spp. and *Halicephalobus* spp. [48,50]. *Ascaris* spp., in particular, is of great relevance to human health [48]. We detected this genus only in invasive parakeets (*M. monachus* and *P. krameri*). As we have detected *Ascaris* spp. in around 10% of the *M. monachus* samples and 20% of the *P. krameri* samples, it would be advisable to consider their faeces as a potential source for ascariasis. On the other hand, *Halicephalobus* spp. was only detected in one *L. fuscus* sample. The importance of this parasite lies in the severity of the pathology it can cause. Although infections by this parasite in humans are rare, it can lead to fatal meningoencephalomyelitis [49,50]. *Eucoleus* spp. can also rarely infect humans [51], but this genus and the other two detected (*Travassostrongylus* spp. and *Aonchotheca* spp.) could be relevant for bird health [10]. We also detected two genera of cestodes (*Raillietina* spp. and *Parorchites* spp.), of which *Raillietina* spp. can cause intestinal infection in humans [52], although this is rare. All the trematodes we detected were parasites of fish and birds without zoonotic capacity, with the exception of *Gigantobilharzia* spp., which can cause cercarial dermatitis [53]. Trematodes were almost exclusively detected in *L. fuscus*, a species for which fish are an important part of its diet [69]. It is possible that these parasites were acquired at the landfill by feeding on fish waste (if accessible), although they could also be acquired in the coastal areas of the country where they may spend part of the year. If so, *L. fuscus* could act as a vector of these parasites, spreading them in nearby bodies of water, such as the Santillana reservoir, where they usually roost. This may be particularly relevant, as in this study, trematodes were detected in 28% of the *L. fuscus* samples.

5. Conclusions

In conclusion, the faeces of all the synanthropic birds studied could pose a risk to human health associated with the zoonotic parasites present in them. This risk is probably higher in areas of urban parks or other green spaces in urban or peri-urban areas, where it is more likely to come into contact with bird faeces. In the case of invasive parakeets (*M. monachus* and *P. krameri*), this risk is mainly due to *Cryptococcus* spp. and *Ascaris* spp., while in *C. ciconia* and *L. fuscus*, it is due to *Candida* spp., *Aspergillus* spp., and *Cryptosporidium* spp., and in particular, *L. fuscus* faeces pose a risk of aspergillosis. This should be considered when developing management plans for urban populations of these species. Large populations of these species could pose a significant risk of spreading parasites, both within cities, mainly in parks, and through the contamination of water bodies such as reservoirs. Moreover, as green areas within cities are becoming more and more common, it would be advisable to establish plans for the active surveillance of these pathogens and to have good estimates of the population sizes of the vector species.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/birds5030025/s1, Table S1. Samples' origin.

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