Confirmation of avian trichomonosis among wild birds in Ireland

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Abstract

Trichomonas gallinae is the causative protozoan parasite of avian trichomonosis, an infectious disease of wild bird species. Since an outbreak in the UK in 2005, avian trichomonosis has resulted in mass mortality events in greenfinch (*Chloris chloris*) and chaffinch (*Fringillia coelebs*) populations. Given its proximity to the UK, it is important to monitor the emergence of *T. gallinae* in Ireland. This study proposed to sample greenfinch, goldfinch (*Carduelis carduelis*) and feral pigeon (*Columba livia domestica*) in Ireland with the aim of culturing and isolating *T. gallinae*. The oral cavity of birds was sampled with an oropharyngeal swab, which was then inoculated into Biomed InPouch™ TF test kits. *T. gallinae* infection was diagnosed in 9 of 60 sampled birds (6 greenfinch and 3 feral pigeon), giving a total infection prevalence rate of 15% and providing evidence that avian trichomonosis is present among, but may not be limited to, greenfinch and feral pigeon populations in Ireland. It is also the first laboratory-based diagnosis of *T. gallinae* from live, free-living birds in Ireland to our knowledge. It is possible that avian trichomonosis is contributing to the recent decline in greenfinch populations in Ireland. Additionally, the presence of yeast cells in some positive samples did not appear to impede trichomonad growth, which may suggest a potential symbiotic relationship between *T. gallinae* and yeast species.

Keywords Avian parasitology · Emerging infectious diseases · Finches · Protozoal disease · *Trichomonas gallinae*

Introduction

Avian trichomonosis in wild birds is caused by the protozoan trichomonad *Trichomonas gallinae* (Amin et al. 2014). Trichomonosis is characterised by caseated, necrotic lesions of the respiratory and upper digestive tracts and may cause difficulty breathing and swallowing, leading to significant loss of weight and muscle mass and, ultimately, death (Hawn 1937; Amin et al. 2014). It has been linked to past population declines of several bird species including European turtle dove (*Streptopelia turtur*) and Mauritian pink pigeon (*Nesoenas mayeri*) (Bunbury et al. 2008) and is also believed to be one of the major contributing factors related to the extinction of passenger pigeon (*Ectopistes migratorius*) in North America (Stabler et al. 1954; Bunbury et al. 2007).

Trichomonosis is endemic to columbid (pigeon and dove) species, with feral pigeon (*Columba livia domestica*) considered to be the chief reservoir host (Stabler et al. 1954; Bunbury et al. 2007; Chi et al. 2013). It is highly transmissible among individuals (Stelter and Kummerfeld 2014); direct bill-to-bill transmission is particularly common, including from parent birds to nestlings (Amin et al. 2014). In recent years, trichomonosis has become an emerging infectious disease (EID) among fringillid (finch) species in Europe, most notably greenfinch (*Chloris chloris*). A dramatic decline in greenfinch abundance followed the emergence of trichomonosis in the UK; after an initial index case in 2005, breeding populations of greenfinch declined by 35% (Robinson et al. 2010). Severe declines were observed in areas with high previously documented trichomonosis-related mortality, suggesting that a shift in *T. gallinae* host specificity to a novel species (greenfinch) had occurred.

It is likely that trichomonosis in finches may spread from the UK to neighbouring Ireland due to geographic proximity. Anecdotal evidence suggests an occurrence of trichomonosis among finches in Ireland; however, no official or definitive data exists. The Irish Garden Bird Survey (coordinated by BirdWatch Ireland; <https://birdwatchireland.ie/>) shows that

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current breeding greenfinch abundance is about half that recorded in the mid-2000s, coinciding with the emergence of trichomonosis in the UK. Thus, the objective of this study was to sample finch and pigeon populations in Ireland to test for the presence of *T. gallinae*. Our results provide the first laboratory-based diagnosis of *T. gallinae* from live, free-living birds in Ireland.

Materials and methods

Bird capture and sampling

Three bird species (two fringillid and one columbid) were sampled for the presence of *T. gallinae* in Ireland: greenfinch, goldfinch (*Carduelis carduelis*) and feral pigeon. Greenfinch and goldfinch were selected due to their documented association with *T. gallinae* infection in the UK and mainland Europe (Amin et al. 2014; Chi et al. 2013; Robinson et al. 2010). Feral pigeon was selected to confirm the presence of *T. gallinae*, as this species is a known reservoir host (Stabler et al. 1954; Bunbury et al. 2007; Chi et al. 2013). A total of 20 individuals from each species were sampled, following Canon and Roe (1982) and to compromise between number of samples and logistical constraints. Finches were captured in a semi-rural area (53° 20' N 7° 4' W) using mist nets on three occasions between 31 March and 15 April 2018. Feral pigeon were captured in an urban area (53° 20' N 6° 15' W) using a hand net on four occasions between 25 April and 9 May 2018. The University College Dublin Animal Research Ethics Committee granted exemption from full ethical review for capture and sampling of these animals.

Upon capture, the species, sex, age, weight and the presence or absence of exterior beak and/or oropharyngeal lesions and discharge were recorded for each individual (Table 1). A salivary sample was collected using an oropharyngeal swab. Sterile, cotton-tipped swabs were gently inserted and rotated around the bird's oral cavity. The swab was then immediately placed inside an InPouch™ TF Feline test kit (Biomed Diagnostics, White City, OR, USA) containing a growth medium to encourage trichomonad growth. Double-sampling of birds was avoided by marking finches with a metal British Trust of Ornithology issue leg ring and marking pigeons with ink on the tail feathers. After inoculation, the pouch was labelled and stored upright away from direct sunlight at an ambient temperature of approximately 20 °C until incubation.

Culture and diagnosis

On return from the field, inoculated pouches were incubated at 37 °C to allow for trichomonad growth. As the

pouches were stored in an upright position, dense materials (including epithelial cells, debris, and, possibly, protozoal organisms) settled along the bottom chamber. This area of sediment was examined by light microscopy at magnification rates of $\times 100$ and $\times 400$ at 24–48-h intervals using an Olympus System Microscope model BX40 for signs of protozoal activity, which is the gold standard method for diagnostic evaluation of protozoal infections in animals (De Waal 2012). Positive samples were removed from incubation when a diffuse amount of protozoal activity throughout the sediment was observed. Microscopic diagnosis of *T. gallinae* infection was confirmed based on the morphology of observed protozoa, i.e. four anterior flagella, an undulating membrane and axostyle (Tasca and De Carli 2003). If no protozoal activity or growth was observed after 14 days, samples were declared negative. In addition, the presence of yeast in any sample was recorded.

Impact of infection on body condition

The relationship between infection and body mass in greenfinch was tested using a linear regression model in R program (R Core Team 2021). Goldfinch and feral pigeon were excluded from this analysis because infection was not detected (goldfinch; see results) or the relevant biometrics were not recorded (feral pigeon; non-routine capture). Body mass was scaled for body size by dividing body weight by wing length, and sex and age were included as blocking variables. We also included an interaction term between infection status and the presence of yeast in the sample to test whether the presence of yeasts influences the severity of the impact of infection on body condition.

Results

Positive cases of *T. gallinae* were observed in 9 of the 60 pouches analysed (Table 2). Six greenfinch pouches displayed signs of trichomonad growth and contained a thick, opaque sediment layer, with the presence of what appeared to be yeast. Signs of trichomonad activity became evident between 24 h and 11 days incubation (Fig. 1). Three feral pigeon pouches also displayed signs of trichomonad growth and contained a thick, opaque sediment layer. The presence of yeast in this instance was clear due to the build-up of gas inside several pouches as a result of fermentation. One pouch displayed signs of trichomonad activity even before incubation commenced, while the other two pouches displayed signs between 4 and 7 days incubation. No sign of trichomonad activity was observed in any of the goldfinch pouches. A small sediment layer was observed in each pouch, most likely consisting of epithelial cells and residual debris from the oral cavity.

Table 1 For every sampled bird, the species, age (adult or juvenile), sex, biometrics (weight in grams, wing in millimetres), notes on body condition and *T. gallinae* infection result (positive or negative) was recorded. An “NA” indicates that measurements were not/could not be taken for the individual

Sample	Species	Age	Sex	Weight	Wing	Condition	Infection
1	Goldfinch	Juvenile	Unknown	14.3	76	No remarks	Negative
2	Goldfinch	Juvenile	Male	15.9	79	No remarks	Negative
3	Goldfinch	Juvenile	Female	27.1	85	No remarks	Negative
4	Goldfinch	Adult	Female	15.8	79	No remarks	Negative
5	Goldfinch	Juvenile	Female	15.2	77	No remarks	Negative
6	Goldfinch	Adult	Female	16.9	78	No remarks	Negative
7	Greenfinch	Juvenile	Male	22.4	86	Mucous	Positive
8	Goldfinch	Juvenile	Male	16.5	79	No remarks	Negative
9	Greenfinch	Adult	Male	26.8	88	No remarks	Negative
10	Goldfinch	Juvenile	Male	15.1	78	No remarks	Negative
11	Goldfinch	Juvenile	Male	13.9	79	No remarks	Negative
12	Goldfinch	Adult	Female	14.0	75	No remarks	Negative
13	Greenfinch	Juvenile	Male	24.9	87	Oral discharge	Positive
14	Goldfinch	Juvenile	Female	13.5	74	No remarks	Negative
15	Goldfinch	Juvenile	Female	14.3	78	No remarks	Negative
16	Goldfinch	Juvenile	Female	15.1	77	No remarks	Negative
17	Goldfinch	Unknown	Unknown	16.8	79	No remarks	Negative
18	Goldfinch	Juvenile	Male	NA	75	No remarks	Negative
19	Greenfinch	Juvenile	Male	25.8	89	No remarks	Negative
20	Goldfinch	Adult	Unknown	14.0	76	No remarks	Negative
21	Goldfinch	Juvenile	Female	13.6	73	No remarks	Negative
22	Goldfinch	Juvenile	Female	15.8	76	No remarks	Negative
23	Goldfinch	Juvenile	Male	15.0	74	No remarks	Negative
24	Goldfinch	Juvenile	Male	14.7	77	No remarks	Negative
25	Greenfinch	Juvenile	Female	26.8	84	No remarks	Negative
26	Goldfinch	Juvenile	Female	16.0	78	No remarks	Negative
27	Greenfinch	Juvenile	Female	21.7	85	Mucous	Positive
28	Greenfinch	Juvenile	Male	26.6	85	No remarks	Negative
29	Greenfinch	Juvenile	Male	28.8	85	No remarks	Negative
30	Greenfinch	Adult	Male	23.8	86	No remarks	Positive
31	Greenfinch	Juvenile	Male	25.2	86	No remarks	Negative
32	Greenfinch	Juvenile	Male	25.9	88	No remarks	Positive
33	Greenfinch	Adult	Female	26.6	82	Oral discharge	Negative
34	Greenfinch	Juvenile	Male	26.3	87	No remarks	Negative
35	Greenfinch	Juvenile	Male	28.8	90	No remarks	Negative
36	Greenfinch	Juvenile	Male	24.2	87	Oral discharge	Positive
37	Greenfinch	Juvenile	Female	25.3	88	No remarks	Negative
38	Greenfinch	Adult	Male	25.2	87	No remarks	Negative
39	Greenfinch	Adult	Male	26.1	92	No remarks	Negative
40	Greenfinch	Juvenile	Female	25.1	82	No remarks	Negative
41	Pigeon	Unknown	Unknown	350	NA	No remarks	Negative
42	Pigeon	Unknown	Unknown	330	NA	No remarks	Negative
43	Pigeon	Unknown	Male	340	NA	No remarks	Negative
44	Pigeon	Unknown	Male	410	NA	No remarks	Positive
45	Pigeon	Unknown	Unknown	NA	NA	No remarks	Negative
46	Pigeon	Unknown	Male	325	NA	No remarks	Negative
47	Pigeon	Unknown	Male	345	NA	No remarks	Negative
48	Pigeon	Unknown	Female	330	NA	No remarks	Negative
49	Pigeon	Unknown	Unknown	300	NA	No remarks	Positive
50	Pigeon	Unknown	Unknown	375	NA	No remarks	Negative
51	Pigeon	Unknown	Unknown	335	NA	No remarks	Negative

Table 1 (continued)

Sample	Species	Age	Sex	Weight	Wing	Condition	Infection
52	Pigeon	Unknown	Male	365	NA	No remarks	Negative
53	Pigeon	Unknown	Male	395	NA	No remarks	Negative
54	Pigeon	Unknown	Male	355	NA	Oral discharge	Negative
55	Pigeon	Unknown	Unknown	315	NA	No remarks	Negative
56	Pigeon	Unknown	Unknown	325	NA	No remarks	Negative
57	Pigeon	Unknown	Male	360	NA	No remarks	Negative
58	Pigeon	Unknown	Unknown	250	NA	No remarks	Positive
59	Pigeon	Unknown	Unknown	330	NA	No remarks	Negative
60	Pigeon	Unknown	Unknown	345	NA	No remarks	Negative

Our linear regression model detected a relationship between body mass and infection in greenfinch (Fig. 2). Scaled body mass was on average 11% lower in infected birds, after accounting for sex and age, and this relationship was significant ($P=0.002$). This model explained 39% of variation in body mass, but we acknowledge that the sample size was low ($n=20$); therefore, this result must be considered with caution. Testing body weight (as opposed to mass) revealed similar results: after accounting for sex and age, the body weight of infected greenfinch was on average 2.9 g less than uninfected greenfinch (average uninfected weight of 25.5 g), explaining 47% of variation in body weight. Our model did not detect a synergetic effect of yeast and infection on body condition, or an additive effect of yeast.

Discussion

This study confirms that the protozoal organism *T. gallinae* is present among greenfinch and feral pigeon populations in Ireland and may be considered an EID of greenfinch in an Irish setting. It is also the first laboratory-based diagnosis of *T. gallinae* from live, free-living birds in Ireland to our knowledge. Despite the small sample size (20 individuals per species) and the small geographical area covered, *T. gallinae* was readily diagnosed. Infection prevalence in

greenfinch, feral pigeon and goldfinch was 30%, 15% and 0% respectively, with a disease prevalence of the entire sample set being 15%.

It has been widely suggested that bird-feeders facilitate the spread of infection among birds, especially common garden birds such as greenfinch and goldfinch (Lawson et al. 2012). During the study, we noted that not all birds with positive infection cases exhibited visible signs of infection during capture. However, four positively tested greenfinch had foamy, slightly sanguineous salivary discharge or excessive mucoid discharge. During early trichomonad growth stages in the pouches, the motility of the active trichomonads generally appeared as small areas of movement in the sediment layer of the pouch. In later stages, movement became more diffuse throughout the sediment layer, as trichomonads proliferated. Live, motile trichomonads were observed utilising their anterior flagella under the microscope. Specimens ranged from spherical and ovoidal to piriform in shape, and the axostyle was also observed.

The presence of *T. gallinae* in Ireland may have significant consequences for finch population trends. Our results suggest that greenfinch are much more susceptible to *T. gallinae* infection than goldfinch. However, although *T. gallinae* infection was absent from our goldfinch samples, Robinson et al. (2010) confirmed that goldfinch is affected by trichomonosis in the UK, albeit to a much lesser extent than greenfinch. Avian trichomonosis is known to have negative population level effects on species abundance (e.g. Robinson et al. 2010) and may already be impacting finch abundance in Ireland. We found that infected greenfinch had, on average, an 11% lower body mass than non-infected individuals, suggesting a significant negative impact of infection on body condition (noting that the sample size in this test was 20 birds). Therefore, it may be the case that trichomonosis is negatively impacting greenfinch, especially when considered in tandem with the observed decline of breeding greenfinch evident in Irish Garden Bird Survey Data, but has not yet impacted goldfinch populations as an EID in Ireland.

This variation in susceptibility could be related to yeast; in our samples, high yeast burdens did not appear to impede

Table 2 The total number of samples, positive cases and incubation time (hours or days for each sample) are presented for the three study species

Species	Samples	Positive cases	Incubation time
Greenfinch	20	6	24 h: $n=2$ 48 h: $n=1$ 72 h: $n=2$ 11 days: $n=1$
Feral pigeon	20	3	< 24 h: $n=1$ 4 days: $n=1$ 7 days: $n=1$
Goldfinch	20	0	–

Fig. 1 Actively dividing trichomonads observed at $\times 1000$ magnification. Note the presence of 2 nuclei in each cell

trichomonad proliferation. Furthermore, co-infection of trichomonads and yeast has been observed previously (e.g. co-infection of *Trichomonas vaginalis* and *Candida* spp. in humans; Avila et al. 2017; Brown et al. 2004). Our analysis

did not detect an influence of yeasts on the severity of the impact of infection on body condition. However, the potential relevance of yeast to variation in the emergence of *T. gallinae* among species warrants further investigation. Further

Fig. 2 Relationship between body mass and *T. gallinae* infection status in greenfinch. Here, body mass was scaled for body size by dividing bird weight (g) by wing length (mm). The data show that birds with no infection tended to have a significantly higher body mass

sampling of a wider variety of species over a greater geographic range will also provide a more accurate picture of the spread and prevalence of avian trichomonosis as an EID in Ireland.

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