The protozoan parasite *Trichomonas gallinae* causes adult and nestling mortality in a declining population of European Turtle Doves, *Streptopelia turtur*

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SUMMARY

Studies incorporating the ecology of clinical and sub-clinical disease in wild populations of conservation concern are rare. Here we examine sub-clinical infection by *Trichomonas gallinae* in a declining population of free-living European Turtle Doves and suggest caseous lesions cause mortality in adults and nestlings through subsequent starvation and/or suffocation. We found a 100% infection rate by *T. gallinae* in adult and nestling Turtle Doves ($n = 25$) and observed clinical signs in three adults and four nestlings (28%). Adults with clinical signs displayed no differences in any skeletal measures of size but had a mean 3·7% reduction in wing length, with no overlap compared to those without clinical signs. We also identified *T. gallinae* as the suggested cause of mortality in one Red-legged Partridge although disease presentation was different. A minimum of four strains of *T. gallinae*, characterized at the ITS/5·8S/ITS2 ribosomal region, were isolated from Turtle Doves. However, all birds with clinical signs (Turtle Doves and the Red-legged Partridge) carried a single strain of *T. gallinae*, suggesting that parasite spill over between Columbidae and Galliformes is a possibility that should be further investigated. Overall, we highlight the importance of monitoring populations for sub-clinical infection rather than just clinical disease.

Key words: disease, feeding ecology, supplementary food, necropsy, PCR.

INTRODUCTION

The avian disease trichomonosis has a global distribution and widespread infection potential, and is now considered a major contributing factor to the regulation and even decline of avian populations (Stabler, 1954; Krone *et al*. 2005; Forrester and Foster, 2008; Robinson *et al*. 2010; Amin *et al*. 2014). In recent years, trichomonosis has undergone a European spread as a consequence of avian migration from the UK and has been linked to widespread declines in finch (Fringillidae) populations (Robinson *et al*. 2010; Lawson *et al*. 2011*b*, 2012; Lehikoinen *et al*. 2013; Ganas *et al*. 2014). This recent trichomonosis epizootic reported in finches is thought to have resulted from parasite spill over of one clonal strain of the *Trichomonas gallinae* parasite from Columbidae to new host species at shared communal garden feeding stations (Lawson *et al*. 2012; Ganas *et al*. 2014). Within the UK, *T. gallinae* has recently been reported within four species of Columbidae (Lennon *et al*. 2013).

Trichomonosis can result in death by suffocation and/or starvation due to caseous ulcerations/lesions (Stabler, 1954). However, host susceptibility and parasite virulence vary, and hosts often show no clinical signs unless they are nestlings or are infected with a pathogenic strain (BonDurant and Honigberg, 1994; Bunbury *et al*. 2008*b*; Sansano-Maestre *et al*. 2009; Robinson *et al*. 2010). The trichomonad life cycle has no intermediate host and transmission can occur horizontally through mutual courtship feeding, or vertically via transfer of crop milk from adults to nestlings, as well as indirectly through shared food and water sources (Stabler, 1954; Kocan, 1969).

The European Turtle Dove *Streptopelia turtur* (hereafter referred to as 'Turtle Dove') is a trans-Saharan migrant, the populations of which have undergone sustained declines in abundance and contractions in range. At a pan-European level, Turtle Doves declined by 73% between 1980 and 2010 (PECBMS, 2012). In the UK, declines of 93% were recorded between 1970 and 2010 (Eaton *et al*. 2012), with a coinciding 51% reduction in range (Balmer *et al*. 2013).

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Turtle Dove population declines on UK breeding grounds have been attributed to a reduction in breeding productivity (Browne and Aebischer, 2004), alongside a concurrent dietary switch from 'natural' arable plant seeds to anthropogenic food resources such as grain piles in farmyards (Browne and Aebischer, 2003). The dietary switch and the reduction in breeding attempts may reflect diminished availability of any food rather than quality alone. This change in feeding behaviour increases the potential for interactions between the main UK species of Columbidae and other granivorous farmland birds, including introduced game birds known to be carriers of *T. gallinae* (Pennycott, 1998; Höfle *et al*. 2004).

Limited information is available about the infection rate of the *T. gallinae* parasite in free-living Turtle Doves, though Muñoz (1995) found an infection rate of 50% in Spain. Lennon *et al*. (2013) found a high incidence of trichomonad parasite infection (86%) in Turtle Doves on breeding grounds in the UK; as high as or higher than in any resident species of Columbidae.

Here we describe mortality in adult and nestling Turtle Doves caused by a single strain of the protozoan parasite *T. gallinae*, strongly suggested through gross necropsy and subsequent isolation, culture and sequencing of extracted parasites. We also cultured *T. gallinae* parasites from artificial food and water sources, suggesting likely routes of transmission.

MATERIALS AND METHODS

Birds were sampled during May–July 2012 on farms in East Anglia, UK at three sites in Essex (Tolleshunt D'Arcy: 51°77′N, 0°79′E; Marks Tey: 51°88′N, 0°79′E; and Silver End: 51°85′N, 0°62′E) and one in Norfolk (Hilgay: 52°56′N, 0°39′E). Sites were baited with either Wheat *Triticum spp*., Oil Seed Rape *Brassica napus*, or a standard wild bird seed mix (Maize *Zea mays L*, Sunflower *Helianthus annuus*, Pinhead Oatmeal *Avena sativa*, Wheat, Red Dari *Sorghum L*., Red and Yellow Millet *Panicum miliaceum*, Hempseed *Cannabis sativa* and Canary seed *Phalaris canariensis*) in areas where farmers regularly provided supplementary food or grain tailings, known to be an increasingly important constituent of Turtle Dove diet in the UK, especially in the early breeding season (Browne and Aebischer, 2003). Adults were caught at each site with either whoosh nets or mist nets (Redfern and Clark, 2001). Individuals displaying clinical symptoms of trichomonosis (feathering around the beak matted, wet and discoloured by regurgitated saliva) were caught at two of the sites in Essex (Tolleshunt D'Arcy and Marks Tey), approximately 18 km apart.

Every bird captured was ringed with a British Trust for Ornithology individually numbered leg ring, weighed with a digital balance (Satrue, Taiwan, ±0·1 g) and standard morphometrics were recorded (wing length ± 0·5 mm with a slotted rule, tarsus length ± 0·1 mm and head–beak length ±0·1 mm with Vernier callipers; Redfern and Clark, 2001). The oral cavity, throat and crop of each bird were also swabbed using an individual sterile viscose swab, which was then used to inoculate an individual InPouch culture kit (Biomed Diagnostics, Oregon, USA). Culture kits were incubated at 37 °C for 3–7 days in order to give the protozoan parasites sufficient time to culture (Bunbury *et al*. 2005) before isolating parasites using a standard procedure (further detailed in Lennon *et al*. 2013). Samples were then frozen until subsequent analysis.

In June and July 2012, we also equipped all captured adult Turtle Doves caught with tail-mounted Pip3 radio-tags (Biotrack, Dorset, UK) weighing 1·7 g (< 1·5% of body mass), to help in locating nests. Some of these birds showed clinical symptoms of trichomonosis (see above) but none appeared lethargic or had any apparent difficulty breathing, and all flew strongly upon release. Turtle Dove nests were found by monitoring the movements of radio-tagged birds and cold-searching suitable habitat known to contain territorial males. Nests were monitored every 2–3 days and when nestlings reached 7 days old, they were ringed, weighed and were swabbed using the same procedure as for adults. Where nestlings were smaller than expected ($n = 2$), swabs were taken from the oral cavity only so as not to risk damaging the oro-pharangeal lining.

When fresh carcasses of adults ($n = 2$) or nestlings ($n = 2$) were found (i.e. those displaying no or minimal signs of autolysis), a swab of the oral cavity, throat and crop was taken (as described above), and any fly eggs or maggots present were removed. The carcasses were then stored in newspaper and kept at 4 °C until gross necropsy could be performed (within 48 h of being found). A further three nestling carcasses that we could not examine post-mortem, due to significant fly damage, were swabbed for trichomonosis. A moribund Red-legged Partridge *Alectoris rufa* was also found at one site, and whilst it did not exhibit diagnostic clinical symptoms of trichomonosis (it was sat in the middle of the farmyard, unresponsive to stimuli with closed eyes and 'fluffed up' feathers), the bird was retrieved for necropsy, since it had shared a feeding site with adult Turtle Doves showing clinical signs of the disease.

All investigative gross necropsies were carried out by JES following a standard simplified protocol as previously described (van Riper and van Riper, 1980; Cooper, 2004; Bunbury *et al*. 2008*b*) involving both external and internal observations, taking samples from any lesions found for subsequent DNA analysis and the documentation of findings. Clinical signs of trichomonosis in gross necropsy can include swollen head and eyes and yellow caseous lesions predominantly found within the oral cavity, pharynx and

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upper digestive tract (Stabler, 1954; Bunbury *et al*. 2008*b*).

All carcasses except one were found at the Tolleshunt D'Arcy site in Essex. Thus swabs were taken from one feeding site and three water sources at this site (stagnant pools in artificial containers); to determine whether the associated food or water sources might be an environmental source of *T. gallinae* parasites (Kocan, 1969).

Total genomic DNA was extracted from isolated parasites and trichomonad lesions with a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA extractions were verified with a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, USA), to determine DNA concentration.

An optimized polymerase chain reaction (PCR) protocol was used with published primers (Gaspar da Silva *et al*. 2007) to amplify the ITS1/5.8S/ITS2 ribosomal region. PCR reactions were performed in 50 μL volumes containing 10 μL of extracted DNA with 0·6 μM of both primers TFR1 and TFR2, 0·8 mM dNTPs, 0·5 units GoTaq Hot Start Polymerase (Promega, Madison, USA) and 1·5 mM $MgCl_2$. The thermal profile included an initial denaturation at 94 °C for 5 min, then 36 cycles of 94 °C for 1 min, 65 °C for 30 s and 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR reactions were run on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) with three previously identified positives from Columbiformes and one negative control of molecular water. Each sample was run a total of three times to confirm the presence or absence of parasites. PCR products were electrophoresed on a 0·8% agarose gel stained with ethidium bromide in 0·5 × Tris-Borate-EDTA buffer (TBE) buffer. The presence of a 400 bp band when amplified products were observed under ultraviolet light indicated a positive sample. All positive samples were sequenced by Source BioScience (Nottingham, UK).

The ITS1/5.8S/ITS2 ribosomal region of DNA is highly conserved in *Trichomonas* spp., with a low rate of mutation (Grabensteiner *et al*. 2010), thus any sequences differing in one or more base pairs were considered to be distinct strains. We used a combination of BioEdit (Hall, 2005) and 4Peaks (Griekspoor and Groothuis, 2006) to trim, manually align, and assess forward and reverse sequences for each PCR product for sequencing. As the strain length can influence the closest matching Genbank sequence (Dunn, personal communication), all sequences from this study were initially aligned with each other in order to identify unique sequences. The longest of each unique sequence was then queried in the NCBI-BLAST database (Altschul *et al*. 1997).

To establish whether adults with clinical signs of trichomonosis differed in weight, wing length or skeletal measures of size (head–beak length and tarsus length) to apparently healthy birds, we used general linear models (GLM) in R (R Core Team, 2012). All morphometric variables were normally distributed, so we designated each in turn as the response variable in a GLM with Gaussian error distributions, and used *t* values to determine any association between clinical signs and morphometrics. All birds included in the analysis were adults (i.e. hatched the previous calendar year or before), with fully grown wings and not in active wing moult, and we also included in the analysis morphometrics from apparently healthy birds (that were all tested positive for infection by the *T. gallinae* parasite: Lennon *et al*. 2013; Dunn *et al*. unpublished data) captured during 2011 ($n = 7$) and 2013 ($n = 14$) and measured by JCD. A subset of birds was subsequently sexed by behavioural observations (through a combination of observations of purring males, and nest attendance, whereby male Columbiformes incubate during the middle of the day, and females overnight and during early morning and evening; e.g. Thorsen *et al*. 2004) but we did not include sex in the statistical model due to incomplete data.

RESULTS

Oral swabs were obtained from 18 adult and seven nestling Turtle Doves during May–July 2012 ($n = 25$; for full details of data collected from each bird see Table 1). In total 13 nests were monitored, eight of which were depredated prior to hatching. Of the five nests monitored to nestling stage (full details in Table 1), three nestlings from three nests were subsequently found dead. *T. gallinae* parasites were cultured from swabs taken from all nestlings post-mortem, although a full necropsy could only be carried out on two of these due to the state of decomposition and autolysis. One additional very small nestling (18·9 g compared to mean weight of 75·77 ± 3·82 g at 7 days ($n = 11$, including data from 2011; Dunn *et al*. unpublished data) disappeared, and was assumed to have died. A further two nests had three nestlings between them which were monitored to 7 days old: one nestling was depredated prior to fledging but the remaining two fledged successfully.

Swabs taken from all 25 Turtle Doves tested positive for *T. gallinae*. Of these, three adults showed clinical signs of trichomonosis, with regurgitated saliva staining the feathering around the beak. A subset of 12 adults, including two of these clinically affected birds (the third was caught in May, prior to the start of radio-tagging) were radio-tagged, flew strongly upon release, and were subsequently relocated. Only the clinically affected birds are considered further here. Bird 20 (Table 1) was relocated alive on the ground ~90 m from the capture site at approximately 09:00 on the day following capture (at 19:00). The bird appeared to be gasping for breath, made no attempt to escape capture by hand

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Table 1. Summary of data collected from individual birds

ID	Outcome	Species	Age	*Trichomonas gallinae* source[a]	Post-mortem	Genbank match
1–16	Live	Turtle Dove	Adults	1	No	JN007005.1 ($n = 8$), FN433475.1 ($n = 1$), FN433473.1 ($n = 1$), AJ784785.1 ($n = 1$) and No sequence ($n = 5$)
17–18	Live	Turtle Dove	Nestling (nest 1)	1	No	JN007005.1 ($n = 1$) and FN433475.1 ($n = 1$)
19	Predated	Turtle Dove	Nestling (nest 2)	2	No	JN007005.1
20	Died	Turtle Dove	Adult	1, 4	Yes	JN007005.1
21	Predated/died	Turtle Dove	Adult	1	Yes	JN007005.1
22	Died	Turtle Dove	Nestling (nest 3)	3, 4	Yes	JN007005.1
23	Disappeared (assumed died)	Turtle Dove	Nestling (nest 3)	2	No	No sequence
24	Died	Turtle Dove	Nestling (nest 4)	3	No	JN007005.1
25	Died	Turtle Dove	Nestling (nest 5)	3	Yes	JN007005.1
26	Died	Red-legged partridge	Adult	3, 4	Yes	JN007005.1

[a] *Trichomonas gallinae* source: 1: swab collected from crop, throat and oral cavity whilst alive; 2: swab collected from oral cavity only; 3: swab collected post-mortem; 4: DNA extracted directly from lesion.

and died shortly afterwards. Bird 21 (Table 1) was relocated ~190 m from the capture site at approximately 10:00 on the morning following capture (at 16:30). We believe that this bird was predated as the carcass had been plucked, making it likely that a raptor was responsible. However, it was impossible to distinguish with certainty between predation and post-mortem scavenging. Individuals with clinical signs were lighter and had shorter wings (Table 2), showing no overlap with non-indicative individuals (Fig. 1a). There was no difference in other skeletal measures of size (Fig. 1b; Table 2).

Gross necropsies were carried out on five independent individuals as detailed in Table 1. Both Turtle Dove nestlings displayed clinical signs of trichomonosis with a swollen head and eyes and visible lesions in the buccal cavity and oropharynx (Fig. 2a and b). One adult female Turtle Dove was severely emaciated with caseous lesions found blocking the oropharynx (preventing the bird from swallowing any seed) the location and extent of which can be seen in Fig. 2c. We were unable to suggest the cause of death for the second adult turtle carcass recovered due to the paucity of remains. In contrast to the Turtle Doves examined, the Red-legged Partridge had no visible lesions within the buccal cavity or upper respiratory tract, although an oral swab taken from the dead bird tested positive for *T. gallinae* parasites. A caseous trichomonosis lesion was found to have originated within the proventriculus, grown through the wall and fused to a lobe of the liver resulting in the necrosis of the connecting tissue and discolouration (Fig. 2d).

Sequences in both directions were obtained from the 25 individuals screened; however, sequence quality from six individuals was too poor to give meaningful data (Table 1). Two identical sequences were obtained from lesions and oral swabs from three individuals (IDs 20, 22 and 26: Table 1). Overall four distinct sequences were obtained; the most common sequence (JN007005.1: 100% query coverage and 100% max identity) was isolated from 16 individuals, including all birds displaying clinical signs, all dead Turtle Doves (adults and nestlings), and the Red-legged Partridge (Table 1). Three sequences were isolated from water sources and one sequence from a feed site, which all matched Genbank sequence JN007005.1 (100% query coverage and 100% max identity; Table 1). Sequences from two individuals matched sequence FN433475.1 (100% query coverage and 100% max identity), and sequences isolated from one individual each matched Genbank sequences AJ784785.1 (99% query coverage and 98% max identity) and FN433473.1 (99% query coverage and 100% max identity; Table 1).

DISCUSSION

We report probably the first confirmation of mortality in free-living Turtle Doves with clinical signs

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Table 2. Summary of morphometrics for adult Turtle Doves with and without clinical signs of trichomonosis
(Values presented in bold denote statistical significance)

	Mean ± 1 SE		Statistics		
Measurement	Clinical signs ($n = 3$)	No clinical signs ($n = 31$)	t	df	P
Weight (g)	**121·40 ± 2·93**	**161·06 ± 1·92**	**−6·276**	**1**	**<0·001**
Wing length (mm)	**172·67 ± 0·83**	**179·45 ± 0·59**	**−3·493**	**1**	**0·001**
Head–beak length (mm)	46·57 ± 0·92	46·23 ± 0·15	0·623	1	0·538
Tarsus length (mm)	23·23 ± 1·17	23·52 ± 0·19	−0·416	1	0·680

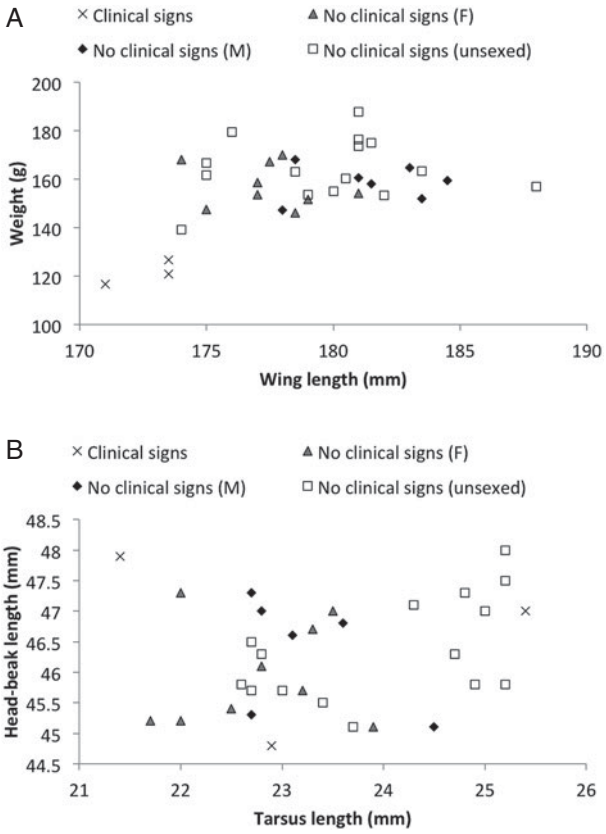

Fig 1. (a) Wing length and weight distributions and (b) head–beak and tarsus length distributions from adult turtle doves with clinical signs compared to female, male and unsexed adults with no clinical signs.

of trichomonosis. We found a 100% rate of infection by *T. gallinae* in the 25 live Turtle Doves screened during 2012. This is higher than during the previous year ($n = 14$; Lennon *et al*. 2013), and combined with previous data gives an overall infection rate of 95% ($n = 39$) across sites separated by up to 120 km. The only two individuals apparently negative for *T. gallinae* infection were two nestlings from the same nest in 2011 (Lennon *et al*. 2013). Whilst two of our nests were related to infected but non indicative adults the remaining three nests that reached the nestling stage were independent of all adults sampled. Thus, only three nestlings with clinical signs were related to any of the adults caught; and no adults with clinical signs were related to any nests.

The overall rate of *T. gallinae* infection appear unusually high when compared to other Columbidae (e.g. 19% in Spotted Dove *Streptopelia chinensis* and 59% in Zebra Dove *Geopelia striata*, Bunbury *et al*. 2007; 5·6% in Mourning Doves *Zenaida macroura*, Schulz *et al*. 2005; 34·2% in wintering Wood Pigeons *Columba palumbus*, Villanúa *et al*. 2006), with only Rock Pigeons *Columba livia* documented as having similarly high rates of infection (92%: Sansano-Maestre *et al*. 2009). Sub-clinical infection can impact on survival: for example, Pink Pigeons testing positive for *T. gallinae* infection were 13% less likely to survive for a further 2 years after screening than those testing negative (Bunbury *et al*. 2008*a*). Usually, only a very small percentage of individuals infected by *T. gallinae* display clinical signs (e.g. 0·37% of Columbidae, Sansano-Maestre *et al*. 2009; 1·9% of Pink Pigeons, Bunbury *et al*. 2008*a*). However, we report clinical signs in 28% of individuals infected by *T. gallinae* parasites (three adults and four nestlings).

All fatal cases of trichomonosis were linked to the same strain of *T. gallinae* found at our study sites in both Turtle Doves and Woodpigeons (Lennon *et al*. 2013), which was also isolated from the only Turtle Dove showing clinical signs during 2011 (a nestling that was predated prior to fledging; Lennon *et al*. 2013). This strain falls within the same clade as *T. gallinae* strain A (Lawson *et al*. 2011*a*; Chi *et al*. 2013; Lennon *et al*. 2013) and is identical at the ITS/5.8S/ITS2 region to the causative agent of the finch trichomonosis epizootic (Robinson *et al*. 2010; Ganas *et al*. 2014). As we only sequenced the ITS/5.8S/ITS2 region, we acknowledge that we may be observing more than one strain that is genetically different at other functional genes. The clade contains strains found in Columbidae worldwide, raptors in Spain, and finches in the USA and UK, suggesting inter- and intra-specific transmission. Further PCR work is required to determine whether or not this strain is identical to the epizootic strain reported in finches (Robinson *et al*. 2010; Lawson *et al*. 2011*a*), by examining other functional genes such as the iron hydrogenase gene (Lawson *et al*. 2011*a*; Lennon *et al*. 2013).

Necropsies carried out on intact Turtle Dove carcasses (one adult, two nestlings) strongly

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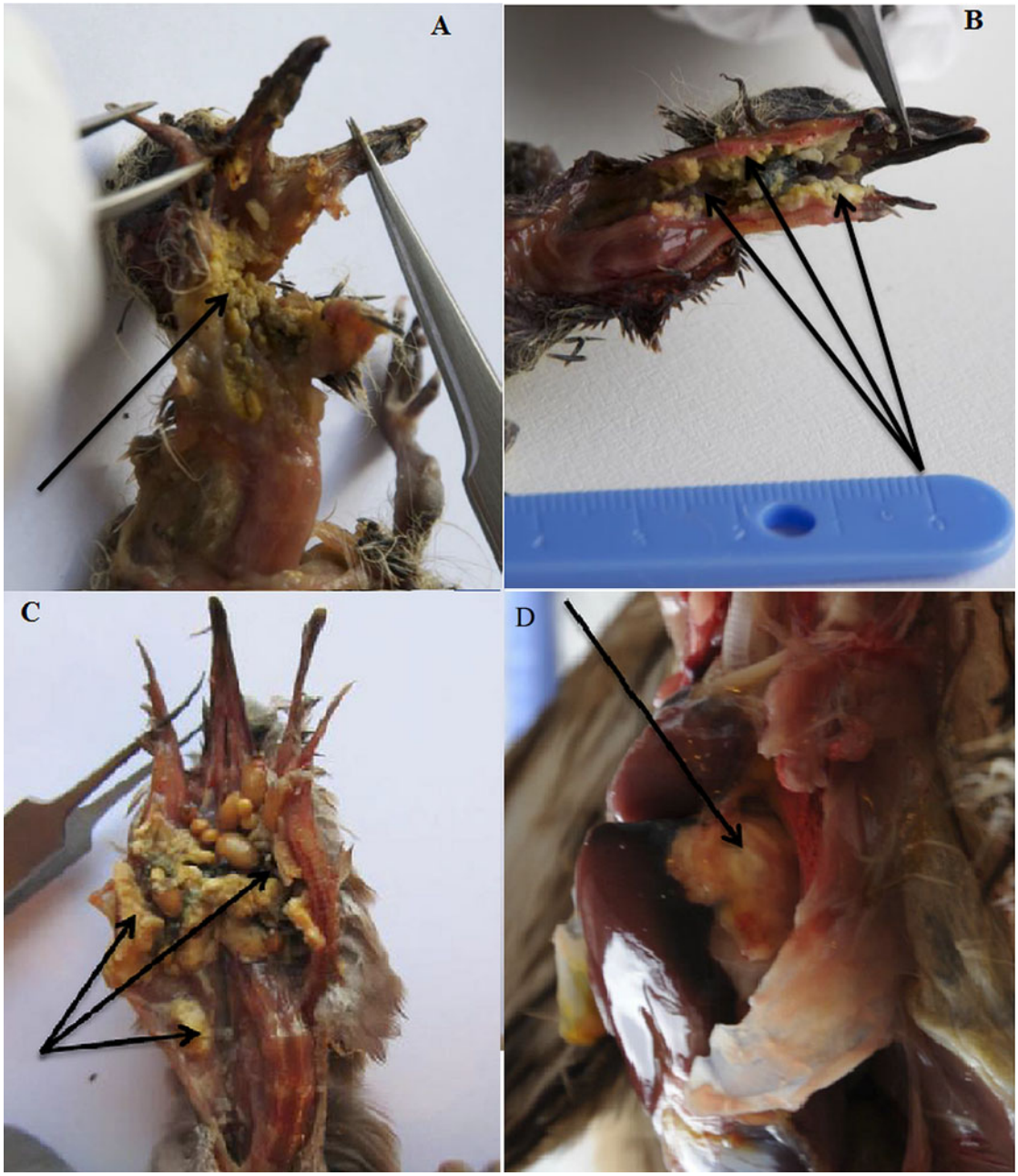

Fig 2. Photographs from post-mortems of (a) nestling Turtle Dove 25, (b) nestling Turtle Dove 22, (c) adult Turtle Dove 20 and (d) Red-legged Partridge 26. Arrows show oropharyngeal lesions in Turtle Doves and a lesion originating in the proventriculus in the Red-legged Partridge.

suggested trichomonosis as the cause of death and identified large oropharyngeal lesions. Molecular testing of DNA extracted from the lesions confirmed the gross necropsy diagnoses. Adult 20 was severely emaciated, but in contrast adult 21 had substantial muscle reserves over the sternum suggesting that this bird might have been at an earlier stage of infection, although the paucity of remains did not allow us to establish this with any certainty. The observation of clinical trichomonosis in adult and nestling Turtle Doves is, to our knowledge, the first suggestion of mortality associated with trichomonosis caseous lesions in this species. Whilst we did not screen for other pathogens and cannot rule out the possibility of co-infection increasing susceptibility to *T. gallinae*, the final cause of death was believed to be due to *T. gallinae* lesions. Controlled experimental infections in the absence of co-infecting pathogens would be necessary to confirm trichomonosis as causing mortality.

That individuals showing clinical signs of disease were considerably lighter than those without is not unexpected: *T. gallinae* lesions constrict the oesophagus and prevent affected birds from ingesting food, resulting in decreased weight. However, the difference in wing lengths is marked, with no overlap between the wing lengths of individuals with and without clinical signs, and a mean 3·47% reduction

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in the wing length of individuals with clinical signs compared to those without. Our sample size of birds showing clinical signs is small, and thus our results should be treated with some caution. There were no differences in any skeletal measures of size, suggesting that infection may impact upon wing length during moult on wintering grounds in Africa through competition for energetic resources, rather than smaller birds simply being more susceptible to infection. Such a mechanism has been proposed previously in other host-parasite systems, with *Haemoproteus* and *Plasmodium* spp. (Marzal *et al*. 2013), *Haemoproteus* spp. (Dunn *et al*. 2013), *Leucocytozoon* spp. (Hatchwell *et al*. 2001) and *Trypanosoma* spp. (Rätti *et al*. 1993) posited to reduce feather length through competition for host resources during moult. Turtle Doves are Europe's only trans-Saharan migrant Columbid and undergo a partial post-breeding moult prior to migration, completing their moult on the African wintering grounds (Baker, 1993). Thus, individuals with clinical signs during summer 2012 may have acquired infections on, or en route to/from, their wintering grounds, or even during the previous breeding season, highlighting the need to further understand the dynamics of *T. gallinae* infection throughout the annual cycle of migratory species.

The finding of a moribund Red-legged Partridge, and subsequent suggestion of the same strain of *T. gallinae* causing markedly different pathology (through isolation of the parasite from the lesion) is interesting. Previous work had discounted the possibility of parasite spill over between Columbidae and introduced Galliformes such as Red-legged Partridges and common Pheasants *Phasianus colchicus* (Lennon *et al*. 2013), as Galliformes tend to be infected by *Trichomonas gallinarum*, which is genetically distinct from *T. gallinae* (e.g. Pennycott, 1998). However, our findings suggest that such a parasite spill over may potentially occur. This suggests that screening of Galliformes may be worthwhile in order to establish whether parasite spill over between Columbidae and Galliformes – and potentially Passerines – is a possible occurrence at shared food resources such as game bird feeders or grain spills in farmyards. Such parasite transfer may occur potentially through a similar mechanism to that suggested by Lawson *et al*. (2012) for the putative parasite spill over from Columbidae to Passerines.

The same predominant single strain of *T. gallinae* isolated from the moribund Turtle Doves and Red-legged Partridge was also isolated from both a farmyard grain pile and three artificial water sources at one of our sites. Food and water sources have previously been postulated as potential vectors for transfer of *T. gallinae* parasites (Kocan, 1969), although Bunbury *et al*. (2007) found no positive grain samples, and only 2 out of 15 water samples to be positive for trichomonads. Whilst speculative, the unusually wet summer of 2012 may have allowed parasites to survive for longer on damp grain piles (Kocan, 1969; Erwin *et al*. 2000) meaning that individual birds may have been subjected to high and repeated doses of *T. gallinae* parasites from repeat visits to infected food and water sources. Further work should examine the survival of parasites in food and water sources in these settings to gauge natural infection rates in relation to the density of potential hosts, and weather-related factors.

Turtle Dove populations in NW Europe have been declining for decades and continue to do so. Whilst a previous intensive study of this species on UK breeding grounds found no evidence of disease-related issues (S. Browne, personal communication), no historic data on infection rates are available. The species has also undergone a dietary switch in the UK, from the seeds of arable plants (Murton *et al*. 1964) to anthropogenic seed resources such as grain piles in farmyards (Browne and Aebischer, 2003). Food stress can decrease immune function (Lindström *et al*. 2005) and induce chronic stress in birds (Clinchy *et al*. 2004), potentially increasing susceptibility to infection and the likelihood of clinical signs and this possibility cannot be negated within this system. More likely, however, is that the dietary switch undergone by this species has led to an increased risk of intra- and inter-species transference of directly and indirectly-transmitted parasites and pathogens, such as *T. gallinae*, at a restricted number of food resources shared by birds feeding at high densities (e.g. Höfle *et al*. 2004; Lawson *et al*. 2012).

Historically, the anti-protozoal dimetridazole, or Emtryl, was widely used as a prophylactic feed additive for game birds reared for sporting purposes; however, since its withdrawal in 2002 concerns have been raised about the potential impacts of motile protozoans on a wide range of species, mostly captive-reared birds (Dernburg *et al*. 2005; Callait-Cardinal *et al*. 2007). To the best of our knowledge, no literature is available examining any trends in infection rates of trichomonads in captive-reared game birds during the period since Emtryl withdrawal, although Lennon *et al*. (2013) found higher rates of trichomonad infection in Columbidae on farms with game bird feeding than on farms without, and Höfle *et al*. (2004) suggest that the supplementary feeding of game birds constitutes a risk factor for the appearance of trichomonosis outbreaks in wild birds. We suggest that the potential for parasite transfer from non-native game birds to rapidly declining native species is worthy of further investigation. Supplementary feeding of game and wild birds, especially during the late winter period when seed food is scarce, is widespread. Although Turtle Doves are summer migrants and therefore not present in Europe during the winter, given the results presented here, and the recent finch trichomonosis epizootic (Robinson *et al*. 2010), we suggest stringent

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hygiene precautions when deploying supplementary food are needed throughout the year to reduce the risk of disease transmission. These include strict adherence to guidelines to only distribute enough food to match consumption, ensure a fresh supply of food is maintained without leaving seed unconsumed and rotating feeding sites. (e.g. Natural England, 2012).

Our work highlights the importance of continued monitoring of *T. gallinae* infection in Turtle Doves and of monitoring sub-clinical infection in free-living populations rather than relying on morbidity and mortality reports alone, particularly for species where the population status gives cause for conservation concern. Further work should address the epidemiology of *T. gallinae* infection, as well as establishing any sub-clinical impacts of infection that may impact on ecological parameters such as reproductive success. *T. gallinae* is thought to be a population-limiting factor in the Pink Pigeon, despite observed pathogenicity being low (Bunbury *et al*. 2008*a*). Unless Turtle Dove feeding ecology changes to allow a reduction in infection rates, parasite infection may potentially amplify the existing reduction in reproductive output and either hasten the ongoing population decline or prevent population recovery. Greater uptake of measures that provide abundant and accessible food (e.g. fallows, seed mixes or cultivated, uncropped margins), which are available in many European agri-environment schemes, would provide birds with more dispersed feeding opportunities and thus potentially reduce disease transmission.

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