

28 **Abstract**

29 Trichomonosis, caused by the protozoan *Trichomonas gallinae*, is an emerging infectious disease in
30 finches, and is more commonly found in columbids and raptors. Infections can be sub-clinical or cause
31 morbidity and mortality, but the parasite is currently only detectable by incubation of an oral swab.
32 Here, we test whether *T. gallinae* parasites can be detected by PCR from faecal or non-cultured
33 samples from the oral cavity and gastrointestinal tract of infected Turtle Doves (*Streptopelia turtur*).
34 PCR did not detect *T. gallinae* parasites in any faecal samples screened, and in only 1 of 11 oral /
35 gastrointestinal samples (from the mouth of a nestling suspected to have died from trichomonosis). We
36 conclude that both oral swabs and parasite culture are still necessary to detect the sub-clinical presence
37 of *T. gallinae* infection in birds.

38

39 **Main article**

40 Trichomonosis is an emerging infectious disease in finches (Aves: Fringillidae) within the UK and
41 across Europe (Robinson et al. 2010; Lawson et al. 2011). The protozoan agent of trichomonosis, *T.*
42 *gallinae*, is globally distributed and more commonly found in columbids and raptors where it can have
43 sub-clinical or chronic impacts (Bunbury et al. 2008a) as well as causing both adult and nestling
44 mortality (Krone et al. 2005; Bunbury et al. 2008b; Amin et al. 2014; Stockdale et al. 2015).

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46 We recently highlighted the importance of monitoring sub-clinical infection in vulnerable populations,
47 rather than just monitoring mortality (Stockdale et al. 2015). However screening techniques for *T.*
48 *gallinae* infection are invasive and wild birds can be difficult to sample in the field, requiring the
49 location and capture of individuals, followed by swabbing of the mouth, oesophageal tract, and crop
50 and subsequent incubation of the swab (reviewed by Amin et al. 2014). *Trichomonas gallinae* is a
51 parasite of the oesophageal tract; however, there are occasional morphological reports of *T. gallinae*
52 from faecal samples (e.g. Ponce Gordo et al. 2002; Badparva et al. 2014). Here, we test whether
53 screening non-cultured faecal and seed samples from birds with known *T. gallinae* infection using
54 sensitive PCR techniques may provide an alternative, less invasive, method to screen live birds for the
55 presence of sub-clinical *T. gallinae* infection.

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57 We obtained faecal samples from Turtle Doves (*Streptopelia turtur*) handled as part of a wider
58 autecological study of Turtle Dove ecology in south-east England (UK) (e.g. Dunn et al. 2015;
59 Stockdale et al. 2015). Samples were collected either directly from birds during handling, or from the
60 inside of clean bird bags. All samples (n=78) were frozen as soon as possible after collection (1 – 8 h)
61 until subsequent analysis. We also obtained seed samples from the mouth (n=2), crop (n=4),
62 proventriculus (n=2) and gizzard (n=3) of five recently dead nestlings (recovered dead either in the nest
63 after chilling/abandonment, underneath the nest, or nearby following depredation). Trichomonosis was
64 suspected in only one nestling due to an empty crop. Seed samples were frozen 1-8 h after collection.

65
66 DNA was extracted from seed samples using a standard ‘salting out’ procedure, and from each faecal
67 sample using a QIAamp DNA Stool Mini Kit (Qiagen, Manchester, UK) following a modified
68 protocol. To maximise DNA yield, we extended the inhibitor binding step to 5 min, extended the
69 digestion step to 30 min, extended the drying step to 3 min centrifugation and finally reduced the
70 elution volume to 100µl following a 5 min incubation. DNA extraction was confirmed in all cases by
71 amplification of a 280-355 bp amplicon within the ITS-2 region using primers designed to target
72 dietary components (Dunn et al. unpubl.). We obtained two positive controls of DNA from *T. gallinae*
73 parasites collected using standard crop swabs and culture procedures (e.g. Bunbury et al. 2005; Lennon
74 et al. 2013; Thomas et al. unpubl.). All PCRs for *T. gallinae* detection were run in a 50ul reaction
75 volume with 1 X PCR Buffer, 2mM MgCl₂, 0.2mM each dNTP, 0.5 µM each primer (TFR1 and TFR2;
76 Gaspar da Silva et al. 2007) 1.25 U GoTaq Flexi (Promega, Madison, WI) and 1µl template DNA. The
77 PCR protocol consisted of an initial denaturation at 94°C for 5 min, then 35 cycles of 94°C for 45 sec,
78 63°C for 30 sec and 72°C for 45 sec, and a final extension at 72°C for 5 min and was carried out on a
79 Gene Amp ® PCR System 9700.

80
81 To test the sensitivity of our analysis, we carried out a sixfold 1:10 dilution series on our positive
82 samples of cultured *Trichomonas* parasites. We treated seed samples as non-cultured controls to test
83 the necessity of culturing oral swabs following collection. All individuals from which faecal and seed
84 samples were collected tested positive for *T. gallinae* infection using standard crop swab and culture
85 techniques (Lennon et al. 2013; Stockdale et al. 2015; Thomas et al. unpubl.). Only one faecal sample,
86 collected from a nestling (nestling 23 in Stockdale et al. 2015), was from a clinically affected bird

87 (which had matted feathering around the beak, and yellow caseous lesions within the oesophageal tract
88 which were found upon gross necropsy; Stockdale et al. 2015).

89

90 We successfully amplified *T. gallinae* DNA from our positive cultured controls diluted to 1:1,000
91 (Figure 1). The same PCR protocol failed to amplify DNA from any of our faecal samples, and all but
92 one of our uncultured seed samples (Figure 1). A single seed sample, collected from the mouth of the
93 nestling suspected to have died from trichomonosis, tested positive.

94

95 Recent work has suggested that faecal diagnostics can be used to detect blood parasites in some
96 primates, although this technique failed when applied to birds (Martinsen et al. 2015). *T. gallinae* is
97 occasionally reported from microscopic analysis of avian faeces (e.g. Ponce Gordo et al. 2002;
98 Badparva et al. 2014) but these identifications are based on morphology and none of these infections
99 thus far have been confirmed by PCR. It is possible these identifications may be of other trichomonads
100 besides *T. gallinae* (e.g. Amin et al. 2014), or that faecal diagnostics may occasionally be effective for
101 *T. gallinae* infections in other species.

102

103 We failed to amplify *T. gallinae* DNA from either faecal samples or uncultured seed samples from
104 Turtle Doves testing positive for *T. gallinae* using standard sampling methods, thus confirming that
105 standard oral swab and culture techniques are both necessary for confirmation of sub-clinical *T.*
106 *gallinae* infection in wild birds.

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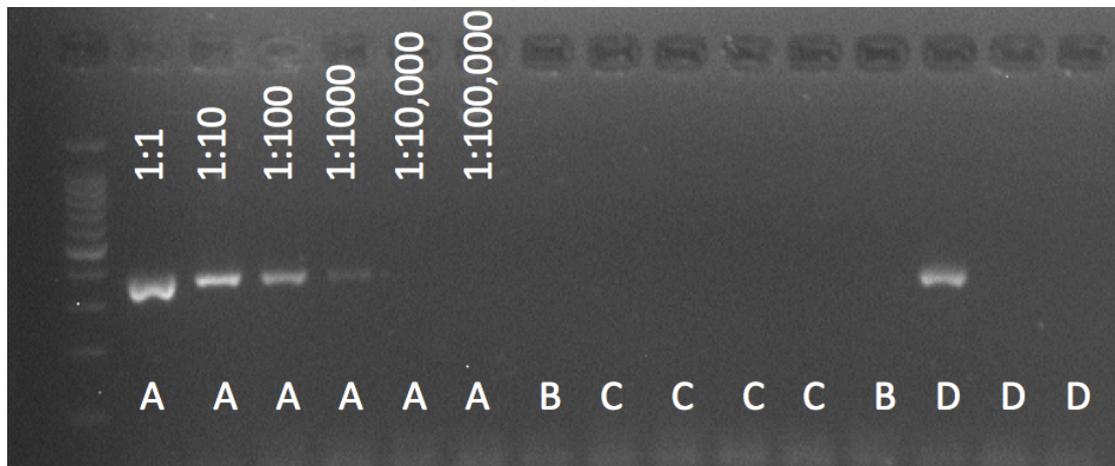
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143

144 **Fig 1** PCR products from a subset of reactions visualised on an agarose gel. Lane 1 contains a 100 bp
145 ladder



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147 A: 1 – 1:100,000 positive control dilution series

148 B: PCR negatives

149 C: Faecal samples

150 D: Seed samples from the gastrointestinal tract

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