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SHORT NOTE

Assessing parasite infections from avian faecal samples: the old methods are still the best

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There are many species of parasitic worms that use a range of bird species as definitive hosts. These parasites can have serious pathological effects on birds, inducing high sickness and mortality rates during peaks of infection (Atkinson *et al.* 2008). Unfortunately, little information exists on bird parasites in New Zealand. Notwithstanding McKenna's comprehensive checklist (McKenna 2010), even the basic question "What parasite species infect which bird species?" remains largely unanswered.

Many parasites live in the gastrointestinal tract of their bird hosts, so that acquiring information on the parasite species infecting birds usually requires sacrificing birds for dissection. The alternative, dissecting birds that are discovered dead, is a useful way of gaining information, but it is neither reliable nor a systematic method of surveying avian parasite biodiversity. For example, examining only birds discovered dead may lead to over-estimates of parasite prevalence if the parasites were associated with higher rates of mortality. Endoparasite infections can, however, to some extent be evaluated non-destructively by examining parasite eggs in bird faeces.

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To explore the diversity of intestinal parasites in New Zealand birds using a non-destructive approach, 3 methods for the detection and counts of parasite eggs in faecal samples were assessed. We tested the efficacy of a novel egg flotation device and counting method developed for agricultural use (Menixis Ltd., New Zealand: see Sowerby *et al.* 2011), and not hitherto tested on wild bird faeces. We compared this with a classical faecal egg count method, the McMaster slide (Gordon & Whitlock 1939), and also with a "total float" approach that examines in detail all, or almost all, of the faecal sample (Proudman & Edwards 1992).

We collected faeces from individual birds at 4 freshwater sites in South Island on several occasions in late summer. Lake Waihola (46° 01′ 14.1″ S, 170° 05′ 05.8″ E), Hawksbury Lagoon (45° 36′ 16.1″ S, 170° 40′ 27.4″ E), Lake Hayes (44° 58′ 59.4″ S, 168° 48′ 19.8″ E) and Lake Tuakitoto (46° 13′ 42.5″ S, 169° 49′ 29.2″ E) were sampled when the water was naturally low and birds were roosting or feeding on sand spits, beaches or grass banks. Birds were observed with binoculars, either individually or in small groups (<5 individuals) until defecation was observed. Freshly released faeces were immediately collected with a plastic spoon and transferred into a small sealable bag labeled with the bird species,

Table 1. Bird species sampled and diversity of helminth eggs found in faeces. Numbers in columns indicate number of different *types* of eggs found for each taxonomic category using the total float method. See text for scientific names.

Bird species	No. of samples	Cestode	Nematode	Trematode	Other*
Black swan	1	1	2**	0	1
Feral goose	1	0	0	0	0
Little pied shag	1	1	2	0	4
Mallard	2	3	0	4	1
Paradise shelduck	2	0	1	0	2
Pied stilt	4	2	2	0	5
Scaup	1	1	0	0	1
South Island pied oystercatcher	2	1	2	0	2
Variable oystercarcher	1	1	0	2	2

*Eggs of unknown origin or oocysts of protists; **also two small entire nematode worms

location, date and time. Samples were then stored on ice until back in the laboratory where they were stored in a fridge at 4°C. Samples were then processed within 2 to 3 days of collection. This method of sampling is very accessible, albeit time consuming, requires limited equipment and reduces the disturbance of the birds since there is no need for capture.

Nine species of bird were sampled: mallard (*Anas platyrhynchos*), scaup (*Aythya novaeseelandiae*), pied stilt (*Himantopus himantopus*), feral goose (*Anser anser*), black swan (*Cygnus atratus*), little shag (*Phalacrocorax melanoleucos*), paradise shelduck (*Tadorna variegata*), South Island pied oystercatcher (*Haematopus finschi*) and variable oystercatcher (*H. unicolor*), and a total of 15 samples collected (see Table 1). As a control, we dissected the intestines of 2 mallards (shot by licenced hunters in 2015) and compared the observed intestinal parasite load with the content of the faeces taken directly from the cloaca.

Each of the 15 faecal samples was macerated in a saturated saline solution (350 g NaCl to 1 L tap water). Four 1 mL subsamples were taken from the mixture; 2 for the egg flotation cassette and 2 for McMaster's slides. For total floats, the remaining sample was placed into two 4 ml test tubes and allowed to stand for 10 minutes to let any eggs float to the surface. The surface was then touched to a cover slip and placed on a slide. The sample on the commercial device was viewed by specialist computer program (MICRO-I: Menixis Ltd., New Zealand). The program photographs the single microscope field of view visible when buoyant particles, such as eggs, are concentrated around a cylindrical rod in the fluid meniscus. The McMaster's slide was examined under a dissection microscope and the total float fraction was examined under a compound microscope at all magnifications up to x1000.

Using the commercial egg flotation and counting device we were unable to discern or identify a single parasite egg in the 15 samples, not even when parasites were known to be present in the dissected mallards. The McMaster slides fared little better, with only 2 slides (from the pied stilt) showing a single 'species' of parasite egg, although at least 6 were found in the total float from the same bird. There are 2 reasons why the commercial unit and the counting slide failed to be successful. First, most of the eggs (and those items assumed to be eggs) found in wild bird faeces are considerably smaller than either technique is calibrated to detect. For example, the eggs of *Haemonchus*, *Ostertagia* and *Trichostrongylus* spp., that the unit is intended to detect, are generally 60 to 85 μm long. Most of the eggs of parasite species found within bird faeces in this assay were in the region of 10 to 25 μm long. Secondly, the only species large enough to be seen were present as only 1 or 2 specimens per sample, and the chances of these being picked up in a small subsample of the faeces are negligible. They were only detectable because the total float method examines a large fraction of a faecal sample.

The results of our study suggest that the best, and ultimately the only way to assess the faecal samples with any reliability was to examine all, or almost all, of the sample by the "total float" method (all samples revealed one or more egg type). This concentrates the parasite eggs from the entire faecal sample in the meniscus of water at the top of a test tube and enables visualisation of the eggs that float to the surface. Using this method we were able to enumerate several different types of eggs from various bird hosts, some of which were identifiable to family level (Table 1). No single reference is available for wild bird parasite egg identification, but image similarity searches on the internet form a starting point for identifying egg

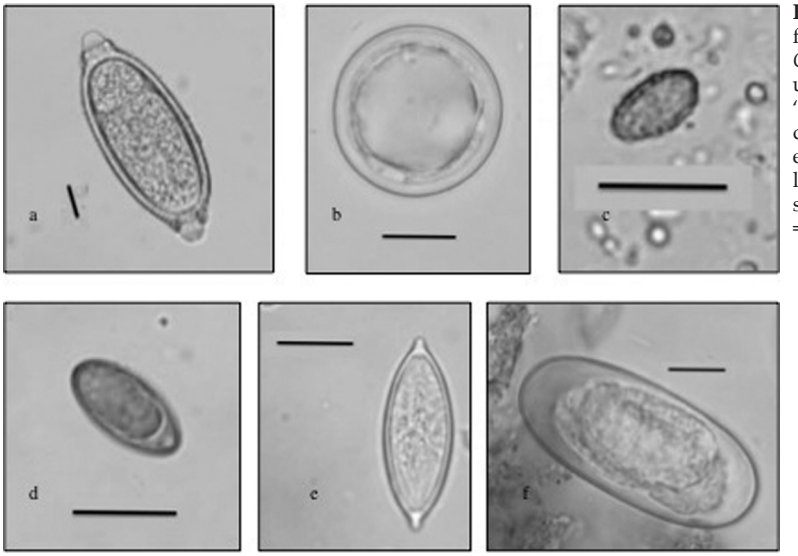

Fig. 1. A selection of parasite eggs from the faeces of a pied stilt: a) *Capillaria* sp. egg [nematode]; b) unknown round egg or cyst with thick 'shell'; c) ascarid egg [nematode]; d) unknown pigmented trematode egg; e) unknown egg (?) found in large aggregations; f) embryonated spirurid egg [nematode]. Scale bar = 10 μm.

types to family. As an example of what was found, Fig. 1 shows a selection of eggs found in the faeces of a pied stilt. Three items were recognisable as nematode eggs and could be identified to family level (Fig. 1a, c and f). The remaining 3 were unknown egg types. In terms of numbers of eggs, egg types in a, c, d and f were found as single examples, 25 were found of egg b, and egg c was found in large numbers (250+) often in aggregations of about 50. Extracting and sequencing DNA from each type of egg would be a logical step to further identify what intestinal parasites these birds are harbouring. By way of illustrating how little is known about avian parasites in New Zealand, the entire literature for pied stilt parasites in New Zealand includes mention of just 7 endoparasites (McDonald 1997), only one of which is named to species level. A single nematode, listed as *Capillaria* sp. may possibly refer to the egg in Fig. 1a, otherwise none of the eggs found is referable to a reported genus, implying at least that the pied stilt is a new host record for each parasite species, or possibly that each of the eggs belongs to a worm as yet undescribed.

As a control test we dissected the guts of 2 mallards, setting aside around 3 cm of the cloaca and its contents as a representative faecal sample. One bird was particularly heavily infested as the guts contained hundreds of trematodes of at least 5 different species, and at least 3 different types of cestode including many hundreds of *Fimbriarioides* sp. Many of the trematodes and most of the cestodes were at a mature stage of their life cycle and would be expected to be producing eggs or shedding proglottides, yet none was seen on the commercial device. However, even eggs of those species in large quantities are unlikely to have been detected because of their small size (*e.g.,* microphallid trematodes present in their hundreds, but their eggs are less than 20 μm in diameter, and fimbriarioid cestode oncospheres are about 10 μm in diameter)(Fig. 2). Three trematode eggs were visualised by the McMaster method and 13 eggs of 6 different types were detected using the total float method. Fig. 2 shows a selection of eggs from parasitic helminths found in the duck's intestines.

The results of this trial have contributed some preliminary knowledge on the gastro-intestinal parasites found in some water birds, as well as confirming that the established but labour-intensive method of faecal egg examination remains the only reliable one. Further, knowledge of parasites and diseases are of obvious importance to the well being of birds in the wild, especially in New Zealand where many species are endemic and often endangered, so the research is an important complement to other past and present research projects on New Zealand birds.

Despite the apparent advantage of using faeces to survey parasite loads, this method of parasite assay has some drawbacks. Some species of birds are more difficult to sample than others. For example, some birds defecate almost exclusively in the water – we were particularly interested in getting samples of Australasian crested grebe (*Podiceps cristatus*) faeces because the species is the likely host of a newly discovered larval trematode, but they are rarely found on the shore except at breeding time. In addition, we are still lacking information on the adults of parasites found in birds, and on the link between infection intensity and egg counts. It is known that in some

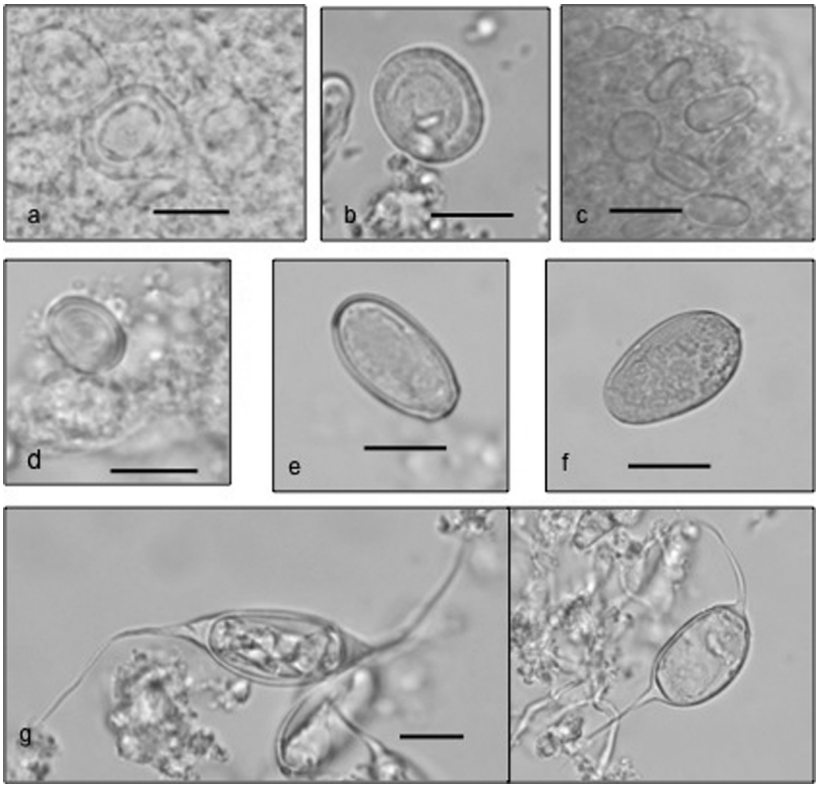

Fig. 2. A selection of eggs from parasitic worms in the intestine of a mallard duck: a) and b) egg of cyclophyllidean cestode; c) eggs within mature proglottid of cyclophyllidean cestode; d) egg of *Fimbriariella* sp. [cestode]; e) egg of *Maritrema* sp. [trematode]; f) egg of microphallid species [trematode]; g) 2 eggs of *Notocotylus* sp. [trematode]. Scale bar = 10 μm.

helminth species egg release is highly subject to variation due to factors such as weather (Rickard & Zimmerman 1992; Vicente *et al.* 2005), season (Shaw & Moss 1989; Theodoropoulos *et al.* 1998; Kumba *et al.* 2003), phase of the parasitic infection (Giver *et al.* 2000) or hour of sampling (Villanúa *et al.* 2006). Thus, the conditions, date and time of collection is an important factor in presence, absence or number of eggs in the faeces. For this reason, we have not analysed counts of any species of eggs, because, without data on the variability of egg release, such counts are not meaningful. In addition, even where eggs are identifiable to major taxonomic group, identification to species level is impossible without genetic sequencing or access to the adult worms.

Our findings lead to the recommendation that, if faecal egg counts are used for assaying parasite loads of wild birds, a thorough approach must be taken. Collecting faecal samples and performing a "total float" assay is a simple procedure that could be utilised by any interested ornithologist who has access to a microscope. The work has produced new information on the parasites of a number of bird species from specific South Island populations that may prove valuable for conservation and management purposes, and for bird welfare. It shows that faecal egg assays would be useful for checking for parasite pathogens in cases of mass illness or when a population has experienced elevated rates of mortality.

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LITERATURE CITED

Atkinson, C.T.; Thomas, N.J.; Hunter, D.B. 2008. *Parasitic diseases of wild birds.* New York: Wiley-Blackwell. 592 pp.

Giver, H.; de Vlas, S.; Johansen, M.V.; Christensen, N.; Nansen, P. 2000. *Schistosoma japonicum*: day to day variation in excretion and hatchability of parasite eggs in the domestic pig, *Suis suis*. *Experimental parasitology 95*: 8-18.

Gordon, H.; Whitlock H.V. 1939. A new technique for counting nematode eggs in sheep faeces. *Journal of the Council for Scientific and Industrial Research 12:* 50-52

Kumba, F.; Katjivena, H.; Kauta, G. ; Lutaaya, E. 2003. Seasonal evolution of faecal egg output by gastrointestinal worms in goats on communal farms in eastern Namibia. *Onderstepoort Journal of Veterinary Research 70*: 265-271.

McDonald, S. 1997. The parasitology of the black stilt (*Himantopus novaeazelandiae*). *A report submitted to the Department of Conservation Threatened Species Unit.* 62 pp.

McKenna, P.B. 2010. An updated checklist of helminth and protozoan parasites of birds in New Zealand. *Web Med Central Parasitology 1*: WMC00705.

Proudman, C.J.; Edwards, G.B. 1992. Validation of a centrifugation/flotation technique for the diagnosis of equine cestodiasis. *Veterinary Record 131:* 71-72

Rickard, L.G.; Zimmerman, G.L. 1992. The epizootiology of gastrointestinal nematodes of cattle in selected areas of Oregon. *Veterinary Parasitology 43*: 271-291.

Shaw, J.; Moss, R. 1989. The role of parasite fecundity and longevity in the success of *Trichostrongylus tenuis* in low density red grouse populations. *Parasitology 99*: 253-258.

Sowerby, S.J.; Mirams, G.J.; Hill, P.C.; Paulin, M.G. 2011. An axisymmetric meniscus converges particles for microscopy. *Journal of Microscopy 244*: 230-234.

Theodoropoulos, G.; Koutsotolis, K.; Nikolaou, E.; Kalogiannis, D.; Petrakos, G. 1998. Seasonal variation of gastrointestinal nematodes of sheep in the region of Joannina, Greece. *International Journal of Parasitology 28*: 1287-1292.

Vicente, J.; Fierro, Y.; Gortazar, C. 2005. Seasonal dynamics of the fecal excretion of *Elaphostrongylus cervi* (Nematoda, Metastrongyloidea) first-stage larvae in Iberian red deer (*Cervus elaphus hispanicus*) from southern Spain. *Parasitology Research 95*: 60-64.

Villanúa, D.; Pérez-Rodríguez, L.; Gortázar, C.; Höfle, U.; Viñuela, J. 2006. Avoiding bias in parasite excretion estimates: the effect of sampling time and type of faeces. *Parasitology 133*: 251-259.

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