Improving the sensitivity of gastrointestinal helminths detection using the Mini-FLOTAC technique in wild birds

Dante Lobos-Ovalle
Universidad Austral de Chile
Claudio Navarrete
Universidad Austral de Chile
Juan G. Navedo
Universidad Austral de Chile
Miguel Peña-Espinoza
Universidad Austral de Chile
Claudio Verdugo (claudioverdugo@uach.cl)
Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile
https://orcid.org/0000-0002-2866-260X

Short Report

Keywords: Black-necked Swan, Brown-hooded Gull, fecal egg count, Hudsonian Godwit, waterbirds

Posted Date: March 31st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-370319/v1

Version of Record: A version of this preprint was published at Parasitology Research on August 4th, 2021.
See the published version at https://doi.org/10.1007/s00436-021-07267-9.

Abstract

High-performance validated tests are essential for successful epidemiological monitoring, surveillance of parasitic infections, and comparative studies in wildlife populations. The Mini-FLOTAC is a novel flotation-based technique for the sensitive detection and quantification of gastrointestinal parasites that is recently being explored for use in wildlife. A limitation of any flotation-based copromicroscopic method is the selection of the flotation solution (FS), which might influence the performance of the test. However, no study has compared the influence of using different FS in the Mini-FLOTAC technique for parasite detection in wild birds. Here, we evaluated the diagnostic performance of the Mini-FLOTAC in three waterbird host species using two widely used FS: saturated salt (NaCl; specific gravity 1.20) and saturated zinc sulfate ($ZnSO_4$; specific gravity 1.35). One hundred fresh fecal samples were analyzed for parasite fecal egg counts (FEC). Regardless of the host species, fecal samples evaluated with the Mini-FLOTAC method using $ZnSO_4$ resulted in a significantly higher detection rate and higher FEC of strongylid, capillarid, cestode, and trematode parasites, than samples analyzed with the NaCl solution. Our concise study demonstrated the importance of using an appropriate FS for the identification of parasite eggs in wildlife species, especially in hosts with an expected aggregated distribution and low parasite load such as waterbird hosts. The higher analytical sensitivity of the Mini-FLOTAC technique achieved with $ZnSO_4$, and its applicability to fieldwork, highlights this method as a promising tool for the quantitative surveillance of parasite infections in wild bird populations.

Introduction

Precise quantification of parasite infections in wildlife represents a major methodological limitation for the identification of key epidemiological parameters and further ecological consequences of host-parasite interactions in natural populations (Thompson et al. 2010). Moreover, parasite populations are highly aggregated in a small number of hosts (Poulin 1993) limiting the performance of traditional diagnostic methods. Consequently, the use of sensitive, precise, and properly validated tests can greatly contribute to the successful wildlife population-based epidemiological monitoring and surveillance (Stallknecht 2007; Budischak et al. 2015).

In wildlife parasite identification is mostly performed by post-mortem examination of hosts (e.g., lethal sampling), which involves highly trained professionals but also ethical limitations (Budischak et al. 2015). As a non-invasive alternative, fecal egg counting techniques are used for the detection of gastrointestinal (GI) parasites, although mainly for qualitative diagnostic purposes, and more recently also for quantification of infection intensities (Coker et al. 2020). Among the quantitative methods, the Mini-FLOTAC is a novel and sensitive flotation-based technique for the detection and quantification of parasitic elements (PE) from GI helminths (i.e., eggs or larvae of nematodes, cestodes, and trematodes) and protozoa (i.e., cysts and oocysts) (Barda et al. 2013; Cringoli et al. 2017). The use of the Mini-FLOTAC for detection of GI parasites has been validated in several domestic and wild mammals, demonstrating higher diagnostic sensitivity and accuracy than commonly used copromicroscopic methods such as the McMaster method (Cringoli et al. 2017; Catalano et al. 2019). In birds, this new technique has been

modified and applied for quantitative parasite diagnosis in domestic species and wild-caught individuals (Borrelli et al. 2015; Bortoluzzi et al. 2018; Carrera-Játiva et al. 2018; Daş et al. 2020), and has only recently been explored for the diagnosis of coccidia and helminths in wild birds (Carrera-Játiva et al. 2018; Coker et al. 2020). However, there are still some methodological gaps in the use of the Mini-FLOTAC that are needed to improve the detection of parasites in wildlife species, particularly for the use in wild birds.

Because each GI parasite species has PE with a particular specific gravity (s.g.) (Ballweber et al. 2014), one particular limitation of any flotation-based technique is that the flotation solution (FS) used significantly influences the diagnostic performance of the method (Cringoli et al. 2004, 2010; Barda et al. 2013). Indeed, several FS have been evaluated in mammalian hosts for the Mini-FLOTAC technique, with the most widely used being the saturated sodium chloride (NaCl; s.g. 1.20) and zinc sulfate ($ZnSO_4$; s.g. 1.35) solutions (Cringoli et al. 2017). In contrast, it is not clear which FS may perform better for the use of the Mini-FLOTAC in birds. Previous studies have detected *Macrorhabdus ornithogaster* and *Isospora* sp. in caged songbirds (O.Passeriformes) using $ZnSO_4$ solution (Borrelli et al. 2015), *Eimeria maxima* oocysts in poultry excreta using sodium nitrate (s.g. 1.20) (Bortoluzzi et al. 2018), and oocysts of coccidia (*Eimeria* spp.) in Brown Kiwi (*Apteryx mantelli*) with magnesium sulfate (s.g. 1.28) (Coker et al. 2020). Recently, Daş et al. (2020) reported that sucrose solution (s.g. 1.32) increases the accuracy of the Mini-FLOTAC technique in comparison with NaCl (s.g. 1.20) for the detection of *Ascaridia galli* and *Heterakis gallinarum* eggs in feces of poultry chickens. However, no studies have yet compared the effects of using different FS in the Mini-FLOTAC technique for the detection of PE in fecal samples from wild birds. Therefore, this study aimed to evaluate the performance of the Mini-FLOTAC technique using two different FS for the detection of helminth eggs in fecal samples from different wild bird species.

Methods

Fresh fecal samples were collected from free-living individuals from to three waterbird species with expected differences in their gastrointestinal helminth fauna: a) Brown-hooded Gull (*Chroicocephalus maculipennis*; n = 30; Fig. 1a), a trophic generalist species that is mainly infected by trophic transmission with several helminth species (Oyarzún-Ruiz and González-Acuña 2021); b) Black-necked Swan (*Cygnus melancoryphus*; n = 34; Fig. 1b), a strictly herbivorous bird infected mostly by parasites with a direct life cycle (Oyarzún-Ruiz and González-Acuña 2021); and c) Hudsonian Godwit (*Limosa haemastica*; n = 36; Fig. 1c), a long-distance migratory shorebird that host helminths species but a low infection rate (Kinsella et al. 2007). Samples were collected between November 2018 and January 2020 in different wetlands in Los Ríos (39°16' – 40°41' S; 71°35'-73°70' W) and Los Lagos (40°13'- 44°3'; 74°49' – 71°34') Regions, Chile.

Collected fecal samples from the three bird species (n = 100 independent biological replicates in total) were immediately placed in sterile 15 ml tubes and preserved with 70% ethanol at 4°C until laboratory analysis. Each sample was analyzed twice to determine parasite fecal egg counts (FEC) using the Mini-FLOTAC method (Cringoli et al. 2017) with either NaCl (s.g. 1.20) or $ZnSO_4$ (s.g. 1.35). At least 1 g of fecal sample from gulls and swans and 0.5 g from godwits were thoroughly homogenized and mixed with the respective FS at a 1:10 (sample:FS) dilution ratio in the Fill-FLOTAC device (Barda et al. 2013; Cringoli et

al. 2017). One ml of the filtered fecal suspension with each FS was transferred from the Fill-FLOTAC into one (godwits) or two (gulls and swans) chambers of a Mini-FLOTAC reading disk. After flotation, the PEs were identified under 400× in a light microscope. The FEC of each sample was expressed as eggs per gram (EPG) of feces (multiplication factor of 10 for godwits and 5 for gulls and swans).

Differences in the performance of each FS for the detection of parasite eggs in the Mini-FLOTAC were evaluated as follows. The detection rate (number of samples positive to parasite eggs / total number of samples analyzed) of each FS was calculated for each bird species and per parasite egg type, then ordered in 2×2 contingency tables and compared using a McNemar's Chi-squared (χ^2) test with continuity correction tests. The agreement in the detection rate of positive samples between the two FS was evaluated using Cohen's kappa (*k*) statistic. Differences in the FEC detected by the two FS in samples from the same host species were evaluated using untransformed EPG data (per parasite egg-type) in GLM with negative binomial distributions. All analyses were performed in R and a level of $P < 0.05$ was considered significant. All procedures performed with animals in this study were approved by the Ethical Committee on Animal Research, Universidad Austral de Chile, Chile (385/2020), and the Chilean Agriculture and Livestock Service (4559/2018) in compliance with Chilean laws and regulations.

Results And Discussion

We identified several helminth PE in the three bird species, including nematode (i.e. strongylid and capillarid), trematode, and cestode eggs, although with significant differences in the detection rate and FEC between bird species and depending on the FS used (Table 1). Strongylid eggs were detected in all three host species, whereas capillarid and trematode eggs were identified in Brown-hooded Gull and Black-necked Swan. Cestode eggs were only observed in Brown-hooded Gull. No further characterization of detected helminth eggs into genus or species was performed, but several species of gastrointestinal nematodes (e.g. *Amidostomum* sp., *Capillaria* sp., *Contracecum* sp., *Viktorocara* sp.), trematodes (e.g. *Stephanoprora* sp., *Echinostoma* sp., *Notocotylus* sp.) and cestodes (e.g. *Tetrabothrius* sp., *Sobolevicanthus* sp., *Echinocotyle* sp.) have been previously identified in the wild bird species investigated (Kinsella et al. 2007; reviewed by Oyarzún-Ruiz and González-Acuña 2021).

Table 1

Number (*n*) of fecal samples positive to parasite eggs (detection rate) and arithmetic mean of eggs per gram (EPG) of feces (min–max of EPG) of gastrointestinal helminths detected in three wild bird species from Southern Chile by using two different flotation solutions (FS) in the Mini-FLOTAC technique.

	Strongylids		**Capillarids**		**Trematodes**		**Cestodes**		**All helminth eggs**	
	NaCl	**$ZnSO_4$**	**NaCl**	**$ZnSO_4$**	**NaCl**	**$ZnSO_4$**	**NaCl**	**$ZnSO_4$**	**NaCl**	**$ZnSO_4$**
Brown-hooded Gull (*n* = 30)										
n positive (%)	0	7[a] (23)	2 (7)	8 (27)	3 (10)	6 (20)	0	2[a] (7)	5 (17)	19* (63)
EPG (min-max)	0	1.33[a] (0–10)	0.33 (0–5)	1.83* (0–10)	0.5 (0–5)	1.52* (0–15)	0	0.33a (0–5)	-	-
Black-necked Swan (*n* = 34)										
n positive (%)	5 (15)	9 (26)	1 (3)	15* (44)	1 (3)	12* (35)	0	0	6 (18)	24* (71)
EPG (min-max)	1.17 (0–20)	1.32 (0–5)	0.14 (0–5)	3.52* (0–25)	0.44 (0–15)	4.26* (0–30)	0	0	-	-
Hudsonian Godwit (*n* = 36)										
n positive (%)	0	8[a] (22)	0	0	0	0	0	0	0	8[a] (22)
EPG (min-max)	0	2.5[a] (0–20)	0	0	0	0	0	0	-	-

*Significant differences in positive samples between FS analyzed by McNemar's Chi-squared (χ^2) test with continuity correction tests ($P < 0.05$) or in FEC per parasite egg type between FS following analyzes by GLM (negative binomial) of untransformed EPG data ($P < 0.01$). [a]No statistical analysis were applied (only samples analyzed with $ZnSO_4$ solution were positive to parasite eggs).

	Strongylids		Capillarids		Trematodes		Cestodes		All helminth eggs	
	NaCl	$ZnSO_4$	NaCl	$ZnSO_4$	NaCl	$ZnSO_4$	NaCl	$ZnSO_4$	NaCl	$ZnSO_4$
All samples (n = 100)										
n Positive	5	24*	3	23*	4	18*	0	2	11	51*

*Significant differences in positive samples between FS analyzed by McNemar's Chi-squared (χ^2) test with continuity correction tests ($P < 0.05$) or in FEC per parasite egg type between FS following analyzes by GLM (negative binomial) of untransformed EPG data ($P < 0.01$). [a]No statistical analysis were applied (only samples analyzed with $ZnSO_4$ solution were positive to parasite eggs).

Overall, fecal samples evaluated with $ZnSO_4$ solution resulted in a significantly higher detection rate of any type of parasite eggs (n = 51) than when analyzed with the NaCl solution (n = 11) in the three bird species (McNemar's χ^2 = 41.023, df = 1, $P < 0.001$; Table 1). The Mini-FLOTAC with $ZnSO_4$ solution detected significantly more infected individuals with strongylid, capillarid, and trematode parasites than with the NaCl solution ($P < 0.01$ for all parasites). Further, cestode eggs were only identified by $ZnSO_4$ solution (Table 1). By host species, the method with the $ZnSO_4$ solution was consistently more efficient to detect individuals with egg parasites ($P < 0.01$ for all three host species) than those analyzed with NaCl solution. A poor agreement in detection rates between FS was confirmed with low Cohen's kappa values for detection of individuals with strongylid (k = -0.02), capillarid (k = 0.11), and trematode (k = 0.12) positive samples, as well as low agreement to identify positive samples to all helminth eggs in Brown-hooded Gull (k = -0.01) and Black-necked Swan (k = 0.07). Notably, the Mini-FLOTAC analyses using NaCl were not able to detect any PE in samples from Hudsonian Godwit, whereas we were able to detect nematode (i.e. strongylid) eggs in eight individuals using the $ZnSO_4$ solution (Table 1). The only previous report in Hudsonian Godwit described the presence of the nematodes *Viktorocara limosa* and *V. capillaris* (Order Spirurida) in their breeding grounds in Alaska and Manitoba (Kinsella et al 2007). Therefore, further research is needed to confirm our findings, and fully characterize the parasite fauna hosted by Hudsonian Godwits during their non-breeding season in the Southern Hemisphere.

In terms of FEC sensitivity, the Mini-FLOTAC using $ZnSO_4$ solution was able to detect higher mean EPG of all types of helminth eggs in those infected individuals than the method with NaCl, consistently across all three bird species (Table 1). Moreover, the Mini-FLOTAC using $ZnSO_4$ solution was the only method able to detect strongylid eggs in Brown-hooded Gull and Hudsonian Godwit and cestode eggs in Brown-hooded Gull (Table 1). The method with $ZnSO_4$ solution detected significantly higher FEC of capillarid and trematode eggs in samples from Brown-hooded Gull and Black-necked Swan (GLM of untransformed EPG data; $P < 0.01$; Table 1), than those analyzed with NaCl solution, whereas FEC of strongylid eggs detected in samples from Black-necked Swan was not significantly different between both FS.

The number of samples with undetected (0 EPG) or positive (≥ 5 EPG) FEC of strongylids, capillarids, and trematodes across all bird species (total n = 100 samples) analyzed by both FS are shown in Fig. 2. Most of the samples analyzed with both FS had 0 EPG, with strongylid, capillarid, and trematode eggs being highly aggregated in few individuals in all bird species. However, while only 3–5% of the samples analyzed by NaCl were positive to these helminth eggs, 18–24% of samples analyzed by $ZnSO_4$ were positive (Fig. 2). Noticeably FEC values obtained for all parasite egg types in all three species of wild birds were very low (< 30 EPG) regardless of the FS used, therefore might be undetected by other quantitative methods (e.g. McMaster technique has a detection limit of ≥ 50 EPG). Furthermore, the low volume of individual fecal samples available from wild birds in the field (0.5-1 g in the present study) may even be insufficient for conventional quantitative flotation tests. Since the accuracy and precision of the detection of PE is highly correlated with the volume of feces examined (Torgerson et al. 2012), the microscopic examination of a larger subsample in the Mini-FLOTAC reading disk (1–2 ml subsample analyzed for FEC per fecal sample vs. 0.15–0.3 ml in the McMaster slide; Cringoli et al. 2004; Cringoli et al. 2017) partially contributed to minimize the diagnostic limitation of analyzing a small amount of fecal material. Further, results from previous studies using NaCl as a FS, especially those reporting the absence of PEs in a given bird species, might be considered with caution. These findings highlight the importance of using quantitative diagnostic techniques with higher analytical sensitivity, such as the Mini-FLOTAC and correct FS, for accurate quantification of PE from limited volumes of fecal samples that are usually obtained from wild birds.

The higher diagnostic sensitivity of the Mini-FLOTAC method using the $ZnSO_4$ solution compared with NaCl was expected, as the increasing specific gravity of the FS results in the flotation of more PEs. However, other factors such as the fixative agent (e.g. ethanol) and the time the samples were stored before the microscopic examination (1–3 months) could have limited the detection of more PE's. Therefore, further research is needed to perform FEC analysis of fresh fecal samples from wild birds, perhaps directly in the field and taking advantage of the adaptability of the Mini-FLOTAC technique to fieldwork (Cringoli et al. 2017). Furthermore, the dynamic of gastrointestinal helminths infection in wild birds could be explored by combining a sensitive copromicroscopic method such as the Mini-FLOTAC with $ZnSO_4$, and molecular techniques for parasite identification in positive samples as previously explored in other host species (Budischak et al. 2015; Maurelli et al. 2018).

In conclusion, we were able to evaluate the performance of the Mini-FLOTAC technique with two commonly used FS with different specific gravities in the detection and quantification of gastrointestinal parasites in three waterbird species, confirming that the use of $ZnSO_4$ as FS results in a higher detection rate of infected individuals and higher FEC, compared with the NaCl solution. Our concise study demonstrates the importance of using an appropriate FS for the detection of parasite eggs in wildlife, especially in hosts with an expected low parasite load such as wild birds. A higher analytical sensitivity of the method using $ZnSO_4$ and the applicability of the Mini-FLOTAC in the field highlight this quantitative technique as a promising tool for the surveillance of parasite infections in wild birds, especially studying spatio-temporal dynamics of parasitic infections in migratory species where an improved technique is

needed to determine key epidemiological parameters that allow the understanding of further ecological consequences of parasite infections in natural populations.

Declarations

Acknowledgments

We thank Jorge Ruiz, Gabriela Biscarra, Roberto Rosas, Luis Miranda and Mario Maturana for field assistance, Juan Alarcon and Enzo Basso for the photographs. This study was supported by the Chilean Agency for Research and Development (ANID) through FONDECYT grants nr. 1191769 and nr. 3170875, and by the Centro de Humedales Río Cruces – UACh grant nr. CEHUM-2018-01-08.

Funding

This study was supported by the Chilean Agency for Research and Development (ANID) through FONDECYT grants nr. 1191769 and nr. 3170875, and Centro de Humedales Río Cruces – UACh grant nr. CEHUM-2018-01-08.

Conflicts of interest/Competing interests

The authors have no conflicts of interest to declare.

Availability of data and material

Most of the data generated or analyzed during this study are included in this published article. The dataset generated during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable

Authors' contributions

Claudio Verdugo and Miguel Peña-Espinoza contributed to the study conception and design. All resources were provided by Claudio Verdugo, Miguel Peña-Espinoza and Juan G. Navedo. Material preparation, data collection and analysis were performed by Dante Lobos-Ovalle and Claudio Navarrete. The first draft of the manuscript was written by Dante Lobos-Ovalle and Claudio Navarrete, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

Ballweber LR, Beugnet F, Marchiondo AA, Payne PA (2014). American Association of Veterinary Parasitologists' review of veterinary fecal flotation methods and factors influencing their accuracy and use

– is there really one best technique? Vet. Parasitol. 204:73–80. https://doi.org/10.1016/j.vetpar.2014.05.009

Barda BD, Rinaldi L, Ianniello D, Zepherine H, Salvo F, Sadutshang T, Cringoli G, Clementi M, Albonico M (2013) Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: Experience from the field. PLoS Negl Trop Dis 7:e2344. https://doi.org/10.1371/journal.pntd.0002344

Borrelli L, Dipineto L, Rinaldi L, Romano V, Noviello E, Menna LF, Cringoli G, Fioretti A (2015) New diagnostic insights for *Macrorhabdus ornithogaster* infection. J Clin Microbiol 53:3448-3450. https://doi.org/10.1128/JCM.01564-15

Bortoluzzi C, Paras KL, Applegate TJ, Verocai GG (2018) Comparison between McMaster and Mini-FLOTAC methods for the enumeration of *Eimeria maxima* oocysts in poultry excreta. Vet Parasitol 254:21-25. https://doi.org/10.1016/j.vetpar.2018.02.039

Budischak SA, Hoberg EP, Abrams A, Jolles AE, Ezenwa VO (2015) A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. Mol Ecol Res 15:1112-1119. https://doi.org/10.1111/1755-0998.12382

Carrera-Játiva, P.D., Morgan, E.R., Barrows, M., Wronski, T., 2018. Gastrointestinal parasites in captive and free-ranging birds and potential cross-transmission in a zoo environment. J. Zoo Anim. Med. 49, 116–128.

Catalano S, Symeou A, Marsh KJ, Borlase A, Léger E, Fall CB, Sène M, Diouf ND, Ianiello D, Cringoli G, Rinaldi L, Bâ K, Webster JP (2019) Mini-FLOTAC as an alternative, non-invasive diagnostic tool for *Schistosoma mansoni* and other trematode infections in wildlife reservoirs. Parasites Vectors 12:439. https://doi.org/10.1186/s13071-019-3613-6

Coker SM, Pomroy WE, Howe L, McInnes K, Vallee E, Morgan KJ (2020) Comparing the Mini-FLOTAC and centrifugal faecal flotation for the detection of coccidian (*Eimeria* spp.) in kiwi (*Apteryx mantelli*). Parasitol Res 119:4287-4290. https://doi.org/10.1007/s00436-020-06912-z

Cringoli G, Rinaldi L, Veneziano V, Capelli G, Scala A (2004) The influence of flotation solution, simple dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. Vet Parasitol 123:121-131. https://doi.org/10.1016/j.vetpar.2004.05.021

Cringoli G, Rinaldi L, Maurelli MP, Utzinger J (2010) FLOTAC: New multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 5:503-515. https://doi.org/10.1038/nprot.2009.235

Cringoli G, Maurelli MP, Levecke B, Bosco A, Vercruysse J, Utzinger J, Rinaldi L (2017) The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat Protoc 12:1723-1732. https://doi.org/10.1038/nprot.2017.067

Daş G, Klauser S, Stehr M, Tuchscherer A, Metges CC (2020) Accuracy and precision of McMaster and Mini-FLOTAC egg counting techniques using egg-spiked faeces of chickens and two different flotation fluids. Vet Parasitol 283:109158. https://doi.org/10.1016/j.vetpar.2020.109158

Guberti V, Stancampino L, Ferrari N (2014) Surveillance, monitoring and surveys of wildlife diseases: A public health and conservation approach. Hystrix It J Mamm 25:3-8. https://doi.org/10.4404/hystrix-25.1-10114

Kinsella JM, Didyk AS, Canaris AG (2007) Helminths of Hudsonian Godwits, *Limosa haemastica*, from Alaska and Manitoba. J Parasitol 93:716-717. https://doi.org/10.1645/GE-1133R.1

Maurelli MP, Bosco A, Pepe P, Ianniello D, Amadesi A, Cringoli G, Rinaldi L (2018) Innovative tools for the diagnosis of *Echinococcus granulosus* in definitive hosts. Parasitol Res 117:2607-2612. https://doi.org/10.1007/s00436-018-5952-1

Oyarzún-Ruiz P, González-Acuña D (2021) Checklist and state of knowledge of helminths in wild birds from Chile: An update. Austral J Vet Sci 53:63-72.

Poulin R (1993) The disparity between observed and uniform distributions: A new look at parasite aggregation. Int J Parasitol 23:937-944. https://doi.org/10.1016/0020-7519(93)90060-C

Rinaldi L, Levecke B, Bosco A, Ianniello D, Pepe P, Charlier J, Cringoli G, Vercruysse J (2014) Comparison of individual and pooled faecal samples in sheep for the assessment of gastrointestinal strongyle infection intensity and anthelmintic drug efficacy using McMaster and Mini-FLOTAC. Vet Parasitol 205:216-223. https://doi.org/10.1016/j.vetpar.2014.06.011

Stallknecht DE (2007) Impediments to wildlife disease surveillance, research, and diagnostics. In: Childs JE, Mackenzie JS, Richt JA (eds) Wildlife and emerging zoonotic diseases: The biology, circumstances and consequences of cross-species transmission. 1st edn. Springer, Berlin, Heidelberg, pp 445-461

Thompson RCA, Lymbery AJ, Smith A (2010) Parasites, emerging disease and wildlife conservation. Int J Parasitol 40:1163-1170. https://doi.org/10.1016/j.ijpara.2010.04.009

Torgerson PR, Paul M, Lewis FI (2012) The contribution of simple random sampling to observed variations in faecal egg counts. Vet Parasitol 188:397-401. https://doi.org/10.1016/j.vetpar.2012.03.043

Figures

Figure 1

Wild bird species studied for detection of gastrointestinal helminths in fecal samples by the Mini-FLOTAC technique: a. Brown-hooded Gull (Chroicocephalus maculipennis), b. Black-necked Swan (Cygnus melancoryphus) and c. Hudsonian Godwit (Limosa haemastica)

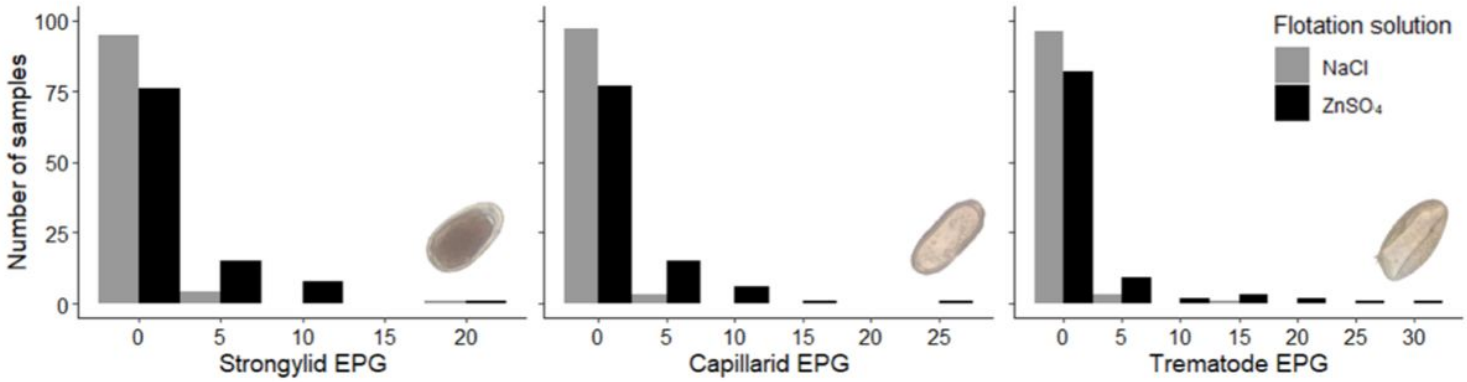

Figure 2

Number of samples with undetected (0 EPG) or positive (≥ 5 EPG) faecal egg counts of strongylid, capillarid and trematode eggs across all bird species (total n = 100 samples) analyzed by the Mini-FLOTAC technique using two flotation solutions (NaCl and ZnSO4). Helminth eggs presented in the figure correspond to representative eggs observed during the investigations