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Endoscopy as a novel method for assessing endoparasite burdens in free-ranging

European shags (*Phalacrocorax aristotelis*)

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Abstract

1. Parasites are a key driver of evolutionary processes in wild animal populations. However, assessing host endoparasite burdens non-destructively is problematic. Collection of faecal samples can be difficult, and faecal egg counts may not always be a reliable indicator of infection intensity.

2. Here we report on endoscopy as a method for assessing natural burdens of nematode parasites *Contracaecum rudolphii* in a wild seabird, the European shag (*Phalacrocorax aristotelis*, L.). We aimed to measure natural individual parasite burdens and repeatability of burdens over time, and verify that treatment with ivermectin removed parasites.

3. Endoscopy was rapid, averaging 6 minutes (n=159), with no obvious adverse effects on behaviour or breeding success compared to non-endoscoped birds.

4. Nematode burdens in the oesophagus and proventriculus of conscious shags were counted and classified as absent, low, medium or high using a flexible gastroscope with a camera attachment that recorded video footage.

5. Re-assessment of worm burdens was highly accurate, with 94% of randomly selected videos (n=50) giving identical categorical scores, and 70% of worm counts (n=40) giving the same total or differing by only one worm.

6. All birds were parasitized by *C. rudolphii*. Natural burdens were significantly higher in males and in late breeders.

7. Individuals had highly repeatable categorical parasite scores over time with 65% of control birds sampled more than once (n=17; mean interval between assessments= 10.8 days) showing no change in scores. However, although the rank ordering of bird’s based on categorical scores remained constant, more finely resolved quantification indicated a slight seasonal decline in worm counts within individuals.

8. Treatment with ivermectin (4mg/kg of bird weight) resulted in complete removal of parasites. There was some evidence of temporal declines in worm counts with lower doses of
ivermectin, including a dose (0.7mg/kg) previously shown to impact chick survival and
growth.

9. Endoscopy has considerable potential for investigating individual variation and temporal
changes in endoparasite burdens and drug efficacy. Applicability and limitations of this
method for other host-parasite systems are discussed.

Keywords

Nematode; gastroscope; faecal egg count; worm; gastrointestinal; macroparasite

Introduction

Parasites are ubiquitous in wild animal populations and considered to be a key driver of
evolutionary processes (Sheldon & Verhulst 1996). There is much theoretical evidence that parasites
can regulate and destabilise host population dynamics (Anderson & May 1978; Dobson & Hudson
1992) and a wealth of empirical evidence indicating that they impact the mass, body condition and
fecundity of domestic animals (e.g. Dimander et al. 2000; Nieuwhof & Bishop 2005). However,
parasites comprise an overlooked but important component of ecological communities with relatively
few studies having considered the impact of parasites on wild animals (see Tompkins et al. (2011) for
a recent review). This is despite the fact that substantial individual heterogeneity in parasite burdens
can occur (Shaw & Dobson 1995), which can lead to adaptive phenotypic evolution if related to both
phenotype and fitness (Wilson & Nussey 2010).

Host-parasite studies are severely impeded by difficulties associated with detecting and
measuring endoparasite burdens in wild hosts. Faecal egg counts are often the only available method
for quantifying individual parasite burdens without destructive sampling, and have been used in a
variety of different species (e.g. Gulland & Fox 1992; Irvine et al. 2001). However, such counts may
be an unreliable index of worm burdens, due to density dependent worm fecundity (Anderson &
Schad 1985; Tompkins & Hudson 1999), temporal variation in egg shedding rates (Shaw & Moss
1989), lack of egg shedding by larval parasite stages, or poor sensitivity at low worm burdens
Moreover, faeces may be difficult to sample in the field. Destructive sampling of the host to quantify parasite burdens cannot be undertaken if the host is of conservation importance or longitudinal data are of interest. Manipulation of parasite burdens experimentally using anti-parasite drugs has limitations since the impacts of treatment may vary depending on initial worm burdens and evaluation of drug efficacy may not be possible without destructive sampling of hosts. Development of a non-destructive method for assessing parasite burdens is therefore crucially important in advancing our understanding of host parasite systems.

The use of endoscopy to diagnose endoparasite infections is routinely used in human and veterinary medicine (e.g. Croese & Speare 2006; Sum & Ward 2009) but to our knowledge, only one published study reports utilisation of this technique in the wild. Jackson and Cooper (1988) used a gastroscope to measure prey digestion rates in sooty albatrosses (*Phoebetria fusca*), and suggested that this also had potential for assessing parasite burdens. Here we report on the first use of this technique to measure natural endoparasite burdens and drug efficacy in a wild animal host, the European shag. Shags are a useful model species for testing endoscopy because parasite prevalence is high (Abollo, Gestal & Pascual 2001; Reed *et al.* 2008) due to their fish prey being heavily parasitized (Groenewold, Berghahn & Zander 1996). In addition, adults at our study colony can be repeatedly caught during the breeding season and diet studies on conscious birds involving stomach flushing with c15mm diameter tubes have already been undertaken without any discernible adverse effects (Wanless, Harris & Russell 1993; Daunt *et al.* 2007). Finally, there is experimental evidence that parasites have a detrimental effect in this population, with late breeding adults treated with ivermectin having higher survival of male offspring (Reed *et al.* 2008) and treated last-hatched chicks having faster growth rates than controls (Reed *et al.* 2012). However, measurement of parasite burdens and treatment efficacy was not possible and hence the mechanisms behind the observed sex and phenology differences could not be elucidated.

This study had three main aims: to (i) use endoscopy to quantify individual endoparasite burdens and assess variation in burdens between hosts; (ii) measure repeatability of individual host parasite burdens over time and (iii) demonstrate that treatment with a suitable dose of ivermectin removed parasite burdens.
Materials and Methods

Study area and species

Fieldwork was undertaken in 2010 and 2011 on the Isle of May, Scotland (56°11′N, 2°33′W). As part of a long-term population study, individual shags were uniquely marked with one metal (British Trust for Ornithology) and one plastic darvic ring. Adults are sexually dimorphic, with males being 22% larger than females (Wanless & Harris 1997), and are sexed by vocalizations (Snow 1960). The breeding season is protracted, with an average of 4.2 weeks per year between first and median laying date (unpublished data; estimated from weekly checks of ca. 100 pairs at monitoring plots throughout the Isle of May between 1984 and 2008). Laying dates for birds used in the study were estimated from daily checks.

Shags are infected with the anisakid nematode *Contracaecum rudolphii* Hartwich, 1964, which attaches to the lining of the proventriculus and lower oesophagus (Abollo, Gestal & Pascual 2001; Reed et al. 2008). Post-mortem examination of archived carcasses of 7 adults and 2 chicks from the Isle of May confirmed infection with *C. rudolphii* in the proventriculus and lower oesophagus (J. Chantrey & D. Kowalek unpublished data). Shags become infected with third stage larvae via their fish diet, which is predominantly lesser sandeels (*Ammodytes marinus*) and butterfish (*Pholis gunnellus*) during the breeding season (Wanless et al. 1993; Daunt et al. 2007). Larval worms moult to become sexually mature adults in the final seabird host (Anderson 1992; Moravec 2009).

Ethical Considerations

Endoscopy is a licensed procedure and was undertaken under Home Office Project Licence PPL60/4001 and conducted by trained personnel (S. Burthe) holding a personal Licence (PIL40/6722). The work had full ethical approval from the University of Edinburgh.
and CEH’s Ethics Committees and the Home Office. Furthermore, as this was a novel technique that is usually undertaken in a clinical setting, the work was initially carried out under full independent veterinary supervision, first on anaesthetised and then on conscious birds. Anaesthesia was induced via a face mask with isofluorane and then delivered via a 3.5mm endotracheal tube. Our decision to endoscope conscious birds thereafter was made in conjunction with the veterinarian to improve bird welfare, by minimising the overall length of the procedure and significantly reducing (from several hours to <5 minutes) the time taken for the animal to return to normal behaviour, which was considered of primary importance since the birds were breeding. The veterinarian’s overall assessment of the procedure confirmed it as being ‘mild’, the lowest severity banding for animal research undertaken in the UK. Discomfort to the birds was deemed to be at an acceptable level given the short duration of the procedure and the ease of entry of the endoscope to the proventriculus due to the anatomy of the species, and the method of feeding offspring, whereby food is regurgitated whilst the chick’s head and neck are full inserted inside the parent’s throat.

**Endoscopy**

Endoscopy of adult shags was undertaken from late incubation to mid chick-rearing (when the chicks were approximately 25 days old) using a 103cm long, 9mm diameter Olympus GIF-PQ20 gastroscope with a 2.8mm channel for air and water, a 100º field of view and a 150 Watt halogen light source (VES) with inbuilt air and water pump, powered by a portable EU10i generator (Honda). The gastroscope tip was flexible through 210/90º vertically and 100/100º horizontally. A compact camera (Xion) connected to the endoscope was used to view and record video images on a laptop using XION DiVASMini image software (Xion Medical, Berlin).

In 2010, endoscopy was carried out on four anaesthetised adult shags and this confirmed that parasites could be quantified in the proventriculus. Due to the ease of
endoscope insertion in this species and in order to minimise the time birds were absent from their nests, endoscopy was then undertaken on 37 conscious birds in 2010. We established: i) the best time of day for the procedure; ii) that this methodology caused no adverse effects on breeding behaviour and success; and iii) the degree of observable variation in endoparasite burdens so that categorical scores could be established. As 2010 was a pilot study we only analysed data from 68 conscious shags endoscoped during the 2011 breeding season.

To ensure that birds had empty stomachs, endoscopy was undertaken between 03:30-07:30, before shags left for their first foraging trip of the day. An assistant placed a cloth over the bird’s eyes to reduce stress and held the neck stretched out on a cushion to prevent movement, with the beak open approximately 2cm. The endoscope was lubricated with KY jelly (Johnson & Johnson) and gently inserted down the oesophagus into the stomach up to a length of 50cm (the base of the proventriculus) from the tip of the beak (measured using gradations on the endoscope). Slight inflation with air helped introduce the endoscope into the stomach and facilitated effective examination. Once a clear view was obtained on the laptop, video recording was started and the scope was pulled out slowly and steadily, enabling worms to be counted, and a categorical burden to be scored as: (i) absent - no live worms seen; (ii) low- 1-10 worms; (iii) medium- 11-25 worms; or (iv) high- >25 worms (Figure 1). Exact counts of worms were only possible for burdens of <40 worms, due to worms preventing good views. Counts of worms greater than this were recorded as >40. To ensure that scores were not affected by viewing conditions we noted the presence of any food and whether the view was satisfactory (visibility scored as 1-5, with 1 being a very poor view with little confidence in the assessment, and 5 a clear view of the stomach with high confidence). The endoscope was cleaned and disinfected between birds using a high-level disinfectant TriGene wipe. The channels were cleaned with a soft brush and flushed with diluted sterilising fluid (Milton) followed by deionised water. At the end of each session
(average 6.5 birds per session) the endoscope was soaked and cleaned with MedEzyme cleaner and MedDis disinfectant (Medichem) followed by deionised water. Ideally in a clinical setting, the endoscope would be soaked for 20-45 minutes in enzymatic cleaner and disinfectant between patients. However, this approach was not practical in the field and hence we adopted a procedure based on veterinary advice that reduced the risk of disease transmission to an acceptable level given the non-sterile, challenging field conditions.

**Experimental Treatment and ivermectin efficacy**

Adult shags were weighed and injected intramuscularly into the pectoral muscle with either saline solution (controls) or an anti-parasite drug treatment (ivermectin 1% w/v, Panomec, Merial Animal Health Ltd., Harlow, UK) following endoscopy at first capture. Ivermectin is considered to be a safe drug for use in birds (Oksanen & Nikander 1989). Effective safe dose levels vary between bird species and parasites, for example doses of 4mg/kg in falcons and 50mg/kg in pheasants and chickens have been used without side effects (see review in Lierz (2001)). Shags were endoscoped repeatedly following treatment to investigate drug efficacy, with the aim being to find the minimum dose which removed worms from the proventriculus. Doses equivalent to 0.7 mg/kg of the birds weight had been used in previous experimental manipulations of adult birds (Reed et al. 2008) and found to have detectable effects on chick growth rates and survival; hence this dose was used initially. Subsequently a range of ivermectin doses were trialled (0.7; 1.0; 2.0; 3.0 and 4.0 mg/kg) with doses increased sequentially (one treatment dose per bird) to establish the dose where worm removal was complete.

**Validation of observer repeatability**
Fifty video recordings were randomly selected and rescored six months post sampling by the same observer (S. Burthe) to evaluate whether categorical scores of parasite burdens were repeatable. Forty randomly selected videos were also rescored for worm counts. Owing to difficulties in counting high burdens, these videos were from a subset excluding those originally scored with a high burden.

**Statistical Analysis**

To evaluate whether endoscopy or treatment affected breeding success, a generalised linear model with Poisson error structure of the number of chicks fledged per nest was fitted for a subset of nests (n=147) that included the area where the endoscopy experiment was undertaken plus surrounding areas which formed part of another dosing experiment but without endoscopy. We fitted the experimental status of each individual bird in terms of whether or not endoscopy had been undertaken. Treatment status was also fitted: whether the individual had been dosed with ivermectin (low/medium (0.7-3.0mg/kg) or high (4.0mg/kg) dose), was a saline treated control or a completely unmanipulated control.

Initial categorical worm scores per individual prior to treatment were analysed using an ordinal logistic model. Previous experimental work on adult birds found variation in the effects of drug treatment between the sexes and with phenology (early or late breeders; Reed *et al.* (2008)), so we considered sex and phenology as potential explanatory variables. Phenology was quantified using a binary variable (“earlylate”) indicating whether the individual laid earlier or later than the median laying date in 2011. As parasite burdens might be expected to increase with exposure (age), and to investigate whether breeding success was correlated with parasitism, we also considered minimum age (known age if first ringed as chicks; assumed to be age 3 at ringing if first ringed as adults) and the number of chicks fledged as potential explanatory variables. Finally, we also considered Julian date of
endoscopy, in order to check that patterns were not associated with seasonal changes in parasite burdens. Phenology and breeding success are highly correlated and hence not fitted together in the same model. We considered two-way interactions between sex and either age, breeding success or phenology.

To investigate dose efficacy, worm counts were analyzed using a generalised linear mixed effects model with Poisson error structure. As it was not possible to count worm burdens in the high category that were >40 worms, these were assigned a value of 40 worms (n=14). This could potentially introduce a degree of bias, so we checked for this by fitting the same candidate model set to categorical repeated measures data (see supplementary information). Bird ID was fitted as a random effect in both cases. We included variables that were found to be important from the analysis of the natural parasite burdens, along with Julian date, treatment group and time since treatment, as potential fixed effects. The interaction between treatment group and time since treatment was also considered, and is the key variable of interest. Treatment group was either specified as a categorical variable with four groups (control, low (0.7-1mg/kg), medium (2.0-3.0mg/kg) or high (4.0mg/kg) doses), or as aggregations of adjacent dose groups (e.g. by combining the “low dose” and “medium dose” groups). Comparing the performance of different aggregations of dose groups allowed us to identify, approximately, the threshold beyond which ‘dose’ begins to have a substantial impact upon the parasite burden.

Analyses were undertaken in program R (“MASS” package (Venables & Ripley 2002); lme4 package (Bates et al. 2011); ordinal package (Christensen 2012); R Development Core Team, 2009). Models were compared using Akaike’s Information Criterion (AIC; Burnham and Anderson (2002)), calculating the difference (ΔAIC) between the AIC of alternative models relative to the “best” model with the lowest AIC. Poisson GLMMs were fitted using the Laplace approximation when performing model selection (the
glmer function in the lme4 package), but, to account for overdispersion, the final model was re-fitted using PQL (the glmmPQL function in the MASS package) to obtain parameter estimates and standard errors.

Results

Endoscopy

Parasite burdens were assessed in 68 shags (25 control and 43 ivermectin treated), 43 of which were repeat sampled on 2-6 occasions. Shags responded calmly to insertion of the endoscope, remained calm throughout the procedure with no evidence of rapid breathing, and returned to the nest within five minutes after release, unless their mate had assumed nest duties. The time taken to endoscope each bird averaged 6 minutes (n=159). Occasionally the endoscope became blocked during the procedure and had to be removed and cleaned before re-insertion, resulting in processing time increasing up to a maximum of 12 minutes (n=8 assessments >8 minutes). Views of the stomach were generally excellent (83% of assessments scored 4 or 5 for visibility, only 4% scored <3). There was no evidence of an adverse effect on breeding success of endoscopy (mean number of chicks fledged: no endoscopy 1.58 (n=80); endoscopy 1.95 (n=40)) or dosing (Table 1).

Validation of observer repeatability

The same categorical score was assigned to 47 (94%) of the videos that were re-assessed. Of the three that were scored differently (originally scored as medium, medium and high and rescored as high, low and medium respectively), the category only differed by one. Twenty-eight (70%) videos that were rescoring for worm counts were within 1 of the original count (mean difference = 1.43; max difference= 8 worms).
Patterns of worm prevalence

All birds were infected with worms (100% natural prevalence). Twenty-seven individuals (40%) had low scores, 18 medium (26%) and 23 high (34%), with counts ranging from 2 to >40 worms. The best model of natural parasite burdens included sex and phenology (early or late), such that late males had the highest parasite burdens and early females the lowest (Tables 2, 3, Figure 2). Models which included the parameters Julian date, age or breeding success were less well supported (see supplementary information).

Of the 25 control birds, 17 were endoscoped more than once (total of 65 assessments). The mean interval between repeat assessments was 10.8 days (range 3-33 days, n=40). Categorical scores for individual birds were highly repeatable over time (Figure 3). However, although the overall rank order of birds remained the same over the breeding season based on categorical data, for the more finely resolved count data there was some evidence of a slight decline in worm counts within individuals over time (see Figure 5).

Assessment of ivermectin efficacy

Forty-three shags were dosed with ivermectin, with 27 birds endoscoped more than once (total of 94 endoscopy assessments). Repeat scoping occurred 1-18 days post dosing (mean 6.3 days, n=50). Complete absence of worms was only observed after dosing with the high (4mg/kg) dose (Figure 4) but there was evidence of a consistent decline in worm counts for lower doses. Birds treated with ivermectin remained worm free for at least 18 days. However, assessment of efficacy beyond this was not possible because shags at this colony became uncatchable late in the season.
The best supported models of the repeated count data included a significant interaction between treatment group and time from dose, with additive sex and phenology ("earlylate"; Tables 4 & 5; Figure 5; see supplementary information for categorical model results). Models that allowed the interaction with time from dose for the high dose group to differ from that of the other treatment groups had strong support ($\Delta$AIC < 8; see Table S3c supplementary information), whilst models where the high dose group was amalgamated with other treatment groups had very poor support ($\Delta$AIC >50). Therefore there is strong evidence that the high dose treatment group is significantly different from the other treatment groups.

There was modest support for the suggestion that low and medium dose treatments were significantly different from controls (difference in AIC of 5.8 between a model with low and medium identical to controls, relative to a model with low and medium separate from controls).

Discussion

Endoscopy proved to be a rapid and reliable method for quantifying endoparasite burdens in shags, and to our knowledge this is the first time the technique has been used for this purpose in the wild. This represents a significant methodological advance in systems where destructive sampling of the host is not possible, faecal samples are difficult to collect or faecal egg counts are unreliable indicators of parasite burdens.

Endoscopy enabled repeated quantification of natural individual parasite burdens. In contrast, destructive sampling can only provide single estimates, while in the faecal egg count method there may be intermittent or seasonal changes in egg shedding through the sexual life-cycle of the parasites. However, adopting endoscopy requires full consideration of ethical implications. Endoscopy on conscious birds significantly reduced the time taken for birds to return to normal breeding behaviour in this study. Other studies have also endoscovered
conscious animals, for example the upper respiratory tract and oesophagus in small ruminants (Stierschneider, Franz & Baumgartner 2007), suggesting that adaptation of this technique to wild mammals may be possible. As in our study, Jackson and Cooper (1988) successfully endoscoped sooty albatross and rock-hopper penguins (*Eudyptes chrysocome*), in the field without anaesthetic.

However, hosts may require anaesthesia if endoscopy is overtly stressful, causes significant discomfort, or if effective restraint is not possible. Crucially, a full cost-benefit analysis should be undertaken before endoscoping conscious birds, based on the species’ biology and likely responses to endoscopy with and without anaesthesia. We recommend that anaesthesia should be undertaken initially to ensure that the endoscope is appropriate for the species in question, and that parasites can be viewed in the proventriculus. Endoscopy may be of more limited use if the parasites of interest are too small in size to be effectively viewed or are located lower down in the gastrointestinal tract. Although not possible in our study, post-mortem analysis of host burdens would provide unequivocal validation of the endoscope method for quantifying parasites and would be possible in some systems.

Natural parasite categorical scores were highly consistent within individuals, suggesting that a single observation using the endoscope, at least in this species, is sufficient to classify individual parasite burdens into broad categories. More detailed assessment of worm counts indicated temporal declines in burdens over the chick rearing period. This general consistency in scores between endoscope sessions, coupled with the re-analysis of video records, provides confidence in the ability of the endoscope to accurately determine parasite burdens in the host. The ability to record and archive videos is clearly of great benefit. Although not undertaken here, potentially the endoscope can be used to collect parasite samples from the gut using retrieval baskets or biopsy forceps (see Jones (1990) for
Endoscopy was also used for evaluating drug efficacy, with the complete removal of worms in shags post-treatment with a 4.0mg/kg ivermectin dose. There was also some suggestion that birds treated with lower doses showed steeper declines in seasonal worm burden counts compared to controls. Evaluating drug efficacy is crucial if impacts of parasites on the host are to be fully understood. Previous experimental work on shags at this colony found that adults treated with equivalent low doses of ivermectin were significantly more successful at rearing sons than controls, but this difference was only found in late breeders (Reed et al. 2008). The impact of parasites in shags may therefore be even greater than suggested by this experiment, as lower doses would not have completely removed worms. Moreover, endoscopy in 2011 indicated that late breeding birds had significantly higher parasite burdens than early breeders, thus elucidating potential mechanisms for these observed differences in treatment effects.

Given our experiences we propose that endoscopy would be applicable in the field to many host endoparasite systems, including reptile, mammal and bird hosts. Endoscopy is a standard tool in veterinary medicine, routinely used on animals including: tortoise (Pizzi et al. 2005); cats (Kubiak et al. 2002); dogs (Le Sueur, Bour & Schaper 2010); rabbits (Johnson, Drazenovich & Hawkins 2007); rats (Silverman et al. 1980); sheep and goats (Stierschneider, Franz & Baumgartner 2007); cattle (Franz 2011); horses (Raphel 1982); and seals (reported in Jackson and Cooper (1988)). Endoscopy has been particularly well utilised as a technique in avian medicine (Hernandez-Divers (2005); Gancz (2006)), including species such as cockatoo (Oglesbee & Steinohrt 2001); falcons (Jekl et al. 2006); and seabirds (Jackson & Cooper 1988; Quesada et al. 2011). Dietary studies involving stomach flushing of conscious birds using tubes inserted via the oesophagus into the stomach have been
successfully undertaken in birds ranging in size from small shore-birds (<50g in mass; Tsipoura and Burger (1999) through to albatross (Cooper, Henley & Klages 1992).\footnote{451}\footnote{452} Endoscope tubes that are capable of being flushed with air/water are available as small as 4mm in diameter (Taylor & Murray 1999), opening up opportunities for a number of species.\footnote{452}

Endoscopy potentially opens up many avenues of research into the impact of parasites on host life-history strategies and fitness. There is a wealth of long-term demographic studies across a wide range of species many of which focus on the extrinsic and intrinsic drivers of variation in individual survival and breeding success. However, the role of parasites has been largely ignored. Being able to monitor temporal changes and quantify individual heterogeneity in parasite burdens represents a major step forward for ecological research in this field. In conclusion, once a full assessment of the ethical considerations has been undertaken, endoscopy can potentially provide a rapid, reliable and repeatable method for assessing natural individual variation in hosts.

\section*{Acknowledgements}

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Supplementary Information

S1: Video explaining the rationale for developing endoscopy as a method for assessing parasite burdens of wild shags.

S2: Four video clips showing the variation in parasite burdens encountered in the wild shags, ranging from an individual that has no worms present (“absent”) following treatment with a dose of 4mg/kg of ivermectin; through low, medium and high burdens.

S3: Tables of full model results with AIC values and parameter estimates for the categorical repeated measures modelling.

References


Bates, D., Maechler, M., Bolker, B. & (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. http://CRAN.R-project.org/package=lme4.


Table 1: Parameter estimates for the analysis of number of chicks fledged per nest fitting treatment group (unmanipulated, control, low or high ivermectin dose) and whether endoscopy was undertaken.

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Table 2: The 12 best supported models of natural categorical parasite scores based on endoscopy at initial capture (n=69; see supplementary online information for full tables). “Earlylate” refers to whether a bird laid before or after the median laying date in 2011.

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</tr>
<tr>
<td>earlylate + sex * age + Julian date</td>
<td>7</td>
<td>4.099</td>
</tr>
<tr>
<td>No. chicks fledged * sex</td>
<td>5</td>
<td>4.225</td>
</tr>
</tbody>
</table>

Table 3: Parameter estimates for the analysis of natural categorical parasite scores based on the best supported model (by AIC) which included sex and “earlylate” (whether a bird laid before or after the median laying date in 2011).

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>1.726</td>
<td>0.521</td>
<td>3.312</td>
</tr>
<tr>
<td>Earlylate (late)</td>
<td>1.173</td>
<td>0.501</td>
<td>2.343</td>
</tr>
</tbody>
</table>
Table 4: The twelve best supported models for the analysis of repeated worm counts (n=159; see supplementary information for full tables). Bird ID was fitted as a random effect in all cases. Sex and “Earlylate” (whether a bird laid before or after the median laying date in 2011) were found to be important from modelling of control data and included in all models. “Time” indicates time from dose. Models were fitted with different classifications of the treatment groups: “C” is the control group; “L” is low dose (0.7-1.0mg/kg); “M” is medium dose (2.0-3.0mg/kg) and “H” is high dose (4.0mg/kg). Separation of groups is shown by a fullstop (e.g. Group (CL.M.H) would be grouped control and low, with separate medium and high dose groups). Main effects with interactions are denoted by an asterisk and interactions without main effects by a colon.

<table>
<thead>
<tr>
<th>Model parameters</th>
<th>Main treatment effect</th>
<th>Interaction</th>
<th>df</th>
<th>ΔAIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(C.L.M.H)</td>
<td>low, medium and high doses</td>
<td>High has separate effect from low &amp; medium</td>
<td>10</td>
<td>0.000</td>
</tr>
<tr>
<td>Sex + Earlylate + Time*Group(C.L.M.H)</td>
<td>low, medium and high doses</td>
<td>High has separate effect from low &amp; medium</td>
<td>9</td>
<td>0.731</td>
</tr>
<tr>
<td>Sex + Earlylate + Time*Group(C.L.M.H)</td>
<td>low, medium and high doses</td>
<td>All three doses have separate effects</td>
<td>11</td>
<td>1.323</td>
</tr>
<tr>
<td>Sex + Earlylate + Julian date + Time + Group(C.L.M.H) + Time:Group(C.L.M.H)</td>
<td>low, medium and high doses</td>
<td>High has separate effect from low &amp; medium</td>
<td>11</td>
<td>1.740</td>
</tr>
<tr>
<td>Sex + Earlylate + Julian date + Time*Group(C.L.M.H)</td>
<td>low, medium and high doses</td>
<td>High has separate effect from low &amp; medium</td>
<td>10</td>
<td>2.593</td>
</tr>
<tr>
<td>Sex + Earlylate + Julian date + Time*Group(C.L.M.H)</td>
<td>low, medium and high doses</td>
<td>All three doses have separate effects</td>
<td>12</td>
<td>3.028</td>
</tr>
<tr>
<td>Sex + Earlylate + Time*Group(C.L.M.H)</td>
<td>medium and high doses</td>
<td>Separate effects for medium and high doses</td>
<td>9</td>
<td>3.347</td>
</tr>
<tr>
<td>Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(C.L.M.H)</td>
<td>Only at high dose</td>
<td></td>
<td>8</td>
<td>3.835</td>
</tr>
<tr>
<td>Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(C.L.M.H)</td>
<td>medium and high doses</td>
<td>Separate effects for medium and high doses</td>
<td>10</td>
<td>4.695</td>
</tr>
<tr>
<td>Sex + Earlylate + Julian date + Time*Group(C.L.M.H)</td>
<td>medium and high doses</td>
<td>Separate effects for medium and high doses</td>
<td>10</td>
<td>4.925</td>
</tr>
<tr>
<td>Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(C.L.M.H)</td>
<td>Only at high dose</td>
<td></td>
<td>9</td>
<td>5.194</td>
</tr>
<tr>
<td>Sex + Earlylate + Time + Group(C.L.M.H) + Time:Group(C.L.M.H)</td>
<td>Only at high dose</td>
<td></td>
<td>8</td>
<td>5.528</td>
</tr>
</tbody>
</table>

Table 5: Parameter estimates for analysis of worm counts based on the best supported (by AIC) model that included sex, “earlylate” (whether a bird laid before or after the median laying date in 2011), additive terms for treatment group and time from dose, and an interaction between treatment group and time from dose (high dose group separate from the other treatment groups). Model estimates were obtained using the “glmmPQL” function in R in order to account for overdispersion.
|                                | Estimate | Std. Error | df  | t value | Pr(>|t|) |
|--------------------------------|----------|------------|-----|---------|---------|
| Time from dose                 | -0.007   | 0.004      | 86  | -1.668  | 0.099   |
| Treatment group (low dose)     | -0.099   | 0.200      | 62  | -0.495  | 0.622   |
| Treatment group (medium dose)  | -0.574   | 0.281      | 62  | -2.043  | 0.045   |
| Treatment group (high dose)    | 0.009    | 0.265      | 62  | 0.033   | 0.973   |
| time from dose: treatment group (low/medium dose) | -0.024 | 0.014 | 86 | -1.647  | 0.103   |
| time from dose: treatment group (high dose) | -0.316 | 0.058 | 86 | -5.411  | 0.000   |
Figure 1: Images of the proventriculus of adult shags obtained from videos of birds exhibiting a range of *C. rudolphi* burdens: a) absent; b) low c) medium and d) high.

Figure 2: Predicted probabilities (with 95% confidence limits) of control individuals of different sex and phenology (early or late denoting whether a bird laid before or after the median laying date in 2011) having low, medium or high parasite burdens based on the best supported model by AIC which included additive sex and phenology terms.

Figure 3: Categorical scores of natural parasite burdens in adult shags assessed with an endoscope. Parasite scores have been offset slightly to facilitate identification of individuals.

Figure 4: Changes in parasite scores (categorical variable from absent to high) following initial treatment with ivermectin (day 0) for individual birds. Scores have been offset slightly to assist identification of individuals.

Figure 5: Predicted worm counts following treatment with ivermectin or controls for the best supported model by AIC that included sex, “earlylate” and an interaction between treatment group and time from dose. Points indicate raw worm counts. Controls are shown in pale grey; low dose (0.7-3.0 mg/kg) in dark grey; high dose (4.0 mg/kg) in black. Predictions are for late breeding males.
Figure 1:
Figure 2

Predicted probability

Parasite Score

low  medium  high

Early female

Early male

Late female

Late male
Figure 3

Parasite Score
Absent
Low
Medium
High

Time since initial Capture (days)

0 10 20 30 40 50 60
Figure 4

0.7-1.0 mg Dose

Parasite Score

0.7-1.0 mg Dose

High

Medium

Low

Absent

Time since Dose (days)

0 5 10 15 20 25

2.0-3.0 mg Dose

Parasite Score

2.0-3.0 mg Dose

High

Medium

Low

Absent

Time since Dose (days)

0 5 10 15 20 25

4.0 mg Dose

Parasite Score

4.0 mg Dose

High

Medium

Low

Absent

Time since Dose (days)

0 5 10 15 20 25
Figure 5

![Graph showing worm count over time since treatment](image-url)