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Monitoring infection load of oxyurid (nematoda) and *Isospora* (coccidia) in captive inland bearded dragons (*Pogona vitticeps*)

Caitlin Pike, Sally Hsieh, Marleen Baling

Abstract

Endoparasites, such as helminths and protozoans, have been associated with mortality and gastrointestinal disease in reptiles, with particularly high prevalence observed in pet reptiles. We conducted an observational study on six juvenile captive inland bearded dragons (*Pogona vitticeps*), to monitor the presence and estimate the abundance of intestinal endoparasite via faecal samples (egg counts using modified McMaster) over three months. We detected two types of endoparasites, coccidia *Isospora* sp. and oxyurid *Phyrngodon* sp. Oxyurid nematode ova shed almost 2.5 times higher egg count per gram of faeces in the afternoon compared to the morning, but the estimated egg counts did not significantly vary from August to October 2022. In contrast, for *Isospora*, there was no difference in oocyst counts between the two times of day sampled, but the shedding of *Isospora* oocyst increased over the three months. Often *Isospora* sp. are recorded to be highly pathogenic and can cause mortality in juvenile reptiles, whereas oxyurid are regarded as beneficial to their host unless they are found in high burdens. In this study, we did not find any significant association between the estimated quantity of egg / oocyst and the body condition of the dragons during the three months. Further studies are required to investigate pathogenicity or benefit of oxyurid to their hosts at different development stages. Finally, the storage period between collection and egg / oocyst quantification varied in this study, which may affect the estimated count of the eggs or oocysts. However, we did not find any apparent influence on time when faecal samples were processed to the number of eggs / oocysts that we counted.

Keywords

Exotic pets, captive reptiles, nematodes, egg count, oocyst count, animal welfare, animal husbandry, faecal sampling

Introduction

Reptiles are common hosts to many endoparasites, and most of them generally do not cause adverse effects on reptiles' health (Rataj et al., 2011; Baling et al., 2013; Raś-Noryńska & Sokół, 2015; Baling & Mitchell, 2021). Infection of endoparasites such as protozoan and helminth can be asymptomatic in reptiles, where the infected hosts may not exhibit disease, particularly if the infection load is low or if the species may provide benefit to the host. For example, pinworms or oxyurid nematodes (roundworm) are often found in the lower gastrointestinal tract of herbivorous reptiles such as inland bearded dragons, *Pogona vitticeps* (Reynolds & Tyrell, 2007; Schmidt-Ukaj et al., 2017). The endoparasite has a direct life cycle, where infection and reproduction occur within the main host. The presence of oxyurid is often regarded as beneficial, because it improves the absorption process during digestion in reptiles, particularly in herbivores (Šlapeta et al., 2018). So reptiles infected with oxyurid generally do not show any signs of disease.

However, endoparasites that persist in captive populations are often recorded in high prevalence because of continual reinfections of the hosts living in smaller confined spaces (cf. their natural larger home ranges) (Loukopoulos et al., 2007; Papini et al., 2011; Rataj et al., 2011; Machin, 2015; Raś-Noryńska & Sokół, 2015; Ellerd et al., 2022). Therefore, the quality of husbandry of their keepers or owners, e.g., frequency of cleaning, can have a significant effect on the reinfection rates in pet reptiles (Wilson, 2010; Papini et al., 2011; Wolf et al., 2014; Rom et al., 2018; Hallinger et al., 2020; Kehoe et

al., 2020; Mendoza-Roldan et al., 2020). Higher parasite load would generally increase incidences of disease and mortality in the hosts, even if the parasite is considered beneficial (Wilson, 2010; Šlapeta et al., 2018; Ellerd et al., 2022). Higher load also increases the likelihood of zoonoses (Pasmans et al., 2008; Ballester et al., 2010; Rataj et al., 2011; Cervone et al., 2016; Corrente et al., 2017; Kiebler et al., 2020; Mendoza-Roldan et al., 2020; Varela et al., 2022). Zoonotic infections can result in outbreaks of illness and hospitalisation mainly in young children, immune-compromised individuals and older adults (e.g., Stull et al., 2015; Varela et al., 2022). Therefore, many regard reptiles as high-risk animals for pet-associated infections (Chomel et al., 2007; McBride, 2016)

One of the most popular exotic pet reptile species is the inland bearded dragon. In captivity, they are commonly infected with oxyurid nematodes, coccidia and flagellates (Reynolds & Tyrell, 2007; Schmidt-Ukaj et al., 2017; Amaral et al., 2021). These endoparasites typically infect the gastrointestinal system of the host and have direct life cycles, where the host acquires them via ingestion of infected faecal particles. Oxyurid infections seen in the inland bearded dragon (and reptiles in general) are only from the family Pharyngodonidae (Rom et al., 2018). Oxyurids are considered beneficial for their hosts, but when the infection load is high, they can cause diarrhoea, impaction, anorexia and death of their hosts (Goldberg & Bursey, 1992; Loukopoulos et al., 2007; Reynolds & Tyrell, 2007; Machin, 2015; Kehoe et al., 2020). Therefore, treatment is only conducted when oxyurid load is high in the host. Coccidia, such as *Isospora* and *Eimeria*, are apicomplexan protozoa that are common and widespread in many pet reptiles. *Isospora* can be highly pathogenic to reptiles, with the largest impact on juvenile mortality (Machin, 2015; Walden & Mitchell, 2021). *Isospora amphiboluri* are commonly found in bearded dragons. However, infected hosts are often seen to be asymptomatic (Stahl, 2003; Machin, 2015) unless they occur in high loads and within young hosts (Greiner, 2003; Reynolds & Tyrell, 2007; Jorge et al., 2013).

Due to the common occurrences of infection in captivity, regular parasitological faecal examinations are often advised to improve the health standard of pet reptiles (Reynolds & Tyrell, 2007; Cervone et al., 2016; Schmidt-Ukaj et al., 2017; Ellerd et al., 2022). Veterinarians often recommend regular parasitic examinations for their pet reptiles, e.g., to be conducted every six months to every year and when obtaining new individuals (Pasmans et al., 2008; Raś-Noryńska & Sokół, 2015). However, such examinations can be expensive for the keeper, and frequency, timing and method of sampling may bias the likelihood of detection of parasites (Villanúa et al., 2006; Jorge et al., 2013). A common reason for the non-detection of infections is the shedding intermittency of the parasites, where gastrointestinal parasites can produce / shed oocytes or eggs once to several times a day at times (Jorge et al., 2013; Bahrami & Shams, 2015). These changes in shedding are hypothesised as an adaptation for parasites or pathogens to maximise their transmission by taking advantage of a short-term environment that is favourable to their spread (Pigeault et al., 2018). For example, there is strong evidence of higher shedding of coccidia oocysts in the afternoons compared to the mornings in various bird species hosts (e.g., Brawner & Hill, 1999; Hudman et al., 2000; Misof, 2004; López et al., 2007; Morin-Adeline et al., 2011; Coelho et al., 2013). This higher prevalence of shedding in the afternoon has been attributed to reduced destruction of oocysts due to desiccation and damaging UV radiation, which may increase survivorship of the oocyst in the environment (Martinaud et al., 2009; Biard et al., 2022). Many studies recommended afternoon sampling for parasite checks (López et al., 2007; Filipiak et al., 2009; Morin-Adeline et al., 2011). However, there are no records that we know of on shedding frequency patterns in gastrointestinal parasites in pet reptiles such as oxyurid nematodes. So effective sampling is critical for the detection of endoparasites.

Another issue for endoparasite examination techniques such as faecal examination is the reliability of the sample processing. Often, we need to analyse samples as soon as they are collected, preferably within 24 hours (Pasmans et al., 2008; Raś-Noryńska & Sokół, 2015; Tedjo et al., 2015; Vogtmann et al., 2017; Byrd et al., 2019), so that the gut microfauna is present and easy to identify. Depending on the target endoparasite, some fragile endoparasites, such as protozoan *Giardia* and trichomonads, may deteriorate quickly outside of the host, so processing of samples needs to be done within 30 minutes to several hours (Zajac et al., 2021). However, some parasites can still be isolated from dried faecal samples and contaminated water six months after the removal of the animal (see Rosenstein et al., 1965; Mermin et al., 2004). But there is no detailed protocol for reptiles. Some suggest that reptile coccidia may be able to resist deterioration longer (Divers, 2022).

This study aimed to monitor the infection load of

endoparasites in captive inland bearded dragons. Our study objectives were to: i) identify nematode and coccidia present; ii) compare egg or oocyst counts between different times of the day; iii) monitor parasite load over three months; iv) determine the association between parasite load to body condition; and v) determine whether there is an association between parasite load and sample processing time.

Materials and Methods

Study species and enclosure design

We used six juvenile inland bearded dragons that were 30 weeks old (in August 2022), and all were siblings from the same egg clutch. The dragons were housed indoors in separate enclosures at the Research Room at Unitec | Te Pūkenga (Tāmaki Makaurau / Auckland, Aotearoa / New Zealand). Room temperature was maintained at 27–28 °C during the day (07:00–18:50 hrs) and 25 °C at night. Room humidity fluctuated between 35% and 50% relative humidity. Main room lights (5,000K fluorescent light) were manually turned on and off at 8:30–17:30 hrs daily. For each enclosure, we provisioned a UV light (Arcadia D3+ Reptile Tube 12% T5, UK) and, at one end, a heat lamp with a halogen bulb (Philips halogen lamp, 35W) over a terracotta refuge and tile. These lights were set with a timer to turn on and off 07:00–19:00 hrs daily. In each enclosure, dragons were provided with a hot zone (the hottest spot was 35–40 °C) and a cool zone (27–28 °C). The cool zone also has a terracotta refuge placed over sandpaper, and that zone was misted daily with dechlorinated water.

Husbandry

Dragons were fed twice a day with calcium-dusted invertebrates (mealworm *Tenebrio molitor*, soldierfly *Hermetia illucens* and black field crickets *Teleogryllus commodus* [when available]) and a mix of vegetation (white cabbage, mesclun, alfalfa sprouts, courgette, carrots, parsnips, apple). Enclosures were cleaned weekly with 10% SteriGENE® (Kahuvet Veterinary Equipment, New Zealand) and rinsed with dechlorinated water. We collected weekly morphometric measurements (mass, snout–vent length [SVL]) and digital images of each dragon. The dragons were soaked in lukewarm water (c. 37 °C) for 10–12 mins if required (e.g., if dehydration occurred).

Faecal collection and laboratory work

All faecal samples were collected twice daily (morning, 09:00–11:00 hrs, and afternoon, 13:00–15:00 hrs, New Zealand Standard Time) for two consecutive weeks in each month (August–October 2022). We noted the condition of each faecal sample (dry or wet, large or small). Samples were kept in separate clean vials and stored in a 4 °C refrigerator at the Applied Molecular Solutions Centre (Unitec | Te Pūkenga) between six and 35 days.

We followed the modified McMaster faecal egg count protocol from Zajac et al. (2021). We used Sheather's sugar solution (Zajac et al., 2021), where 454 g of granulated sugar was dissolved in 335 mL of boiling water. After cooling to room temperature, we then checked the SG (1.2–1.25) of the solution with a hydrometer. For each faecal sample, we weighed 2 g and then added 28 mL of Sheather's sugar solution for a total solution mix of 30 mL. If the full faecal sample mass was <2 g, we added distilled water to the sample to equate to 2 g. We mixed the faecal sample and sugar solution, and then we sieved the solution mix using a tea strainer. The sieved solution mix was pipetted onto a two-chambered (0.15 mL each chamber) McMaster slide (Chalex, USA) and allowed five minutes for settlement time. The prepared slide was examined under a Nikon YS100 compound microscope (Nikon Instech Co., Japan) for egg or oocyst identification and quantification (100× magnification). Any eggs or oocysts present were systematically counted along the gridded slide. If there was a high number of eggs / oocysts (>50 eggs / oocysts in most of the grids), we counted the number of eggs / oocysts in the first column in each chamber and multiplied by six (i.e., the total number of grids per chamber).

Statistical analysis

We used the following formula to standardise the estimation of the egg or oocyst counts for each sample (Zajac et al., 2021):

$$epg = \frac{No.eggs\ counted \times (\frac{T}{V})}{F},$$

where *epg* is number of eggs per gram (for coccidia: oocysts per gram, *opg*), *T* is the total volume of solution mix (i.e., 30 mL), *V* is the volume for the two-chambered McMaster slide (i.e., 0.3 mL), and *F* is mass of the faeces used.

Due to the samples being processed at different times, we looked to see if there was a correlation between duration (the number of days between when samples were collected to when the samples were processed and eggs / oocysts counted) and the estimated number of eggs per gram or oocysts per gram using Spearman's rank correlation. There was no significant correlation between duration and number of eggs / oocysts (oxyurid: p = 0.16, ρ = 0.22, N = 42; *Isospora*: p = 0.06, ρ = 0.29, N = 42) (see Appendix 1); therefore, we could continue to our comparisons.

We averaged the values for each dragon for each variable of interest (time of day, months). We calculated the body condition of each dragon per month as the cubed root of body mass divided by body length (SVL) (Labocha et al., 2013). We determined whether there was an effect of time of day by comparing the number of eggs / oocysts between morning and afternoon using a Wilcoxon rank sum test. We also determined whether there was any effect of month or body condition on the number of eggs / oocysts for the dragons using a Kruskal-Wallis test and Spearman's rank correlation. All calculations were conducted using R version 4.2.2 (R Foundation for Statistical Computing).

Results

We collected a total of 42 faecal samples from the six bearded dragons. Two types of endoparasites were identified from this captive group. Coccidian oocysts of *Isospora* sp. were identified via their spherical to slightly subspherical shape, at approximately 20 µm in diameter. These oocysts were a mix of unsporulated and sporulated, with the sporulated oocysts containing two sporocysts (Figure 1). Large nematode (roundworms) eggs were also detected (40 × 80 µm), where they were yellow-brown ellipsoids in shape, with one long side flattened and the other side convex (Figure 1). The nematode eggs were identified as belonging to the order Oxyurida.

We found a weak significance in the estimated egg

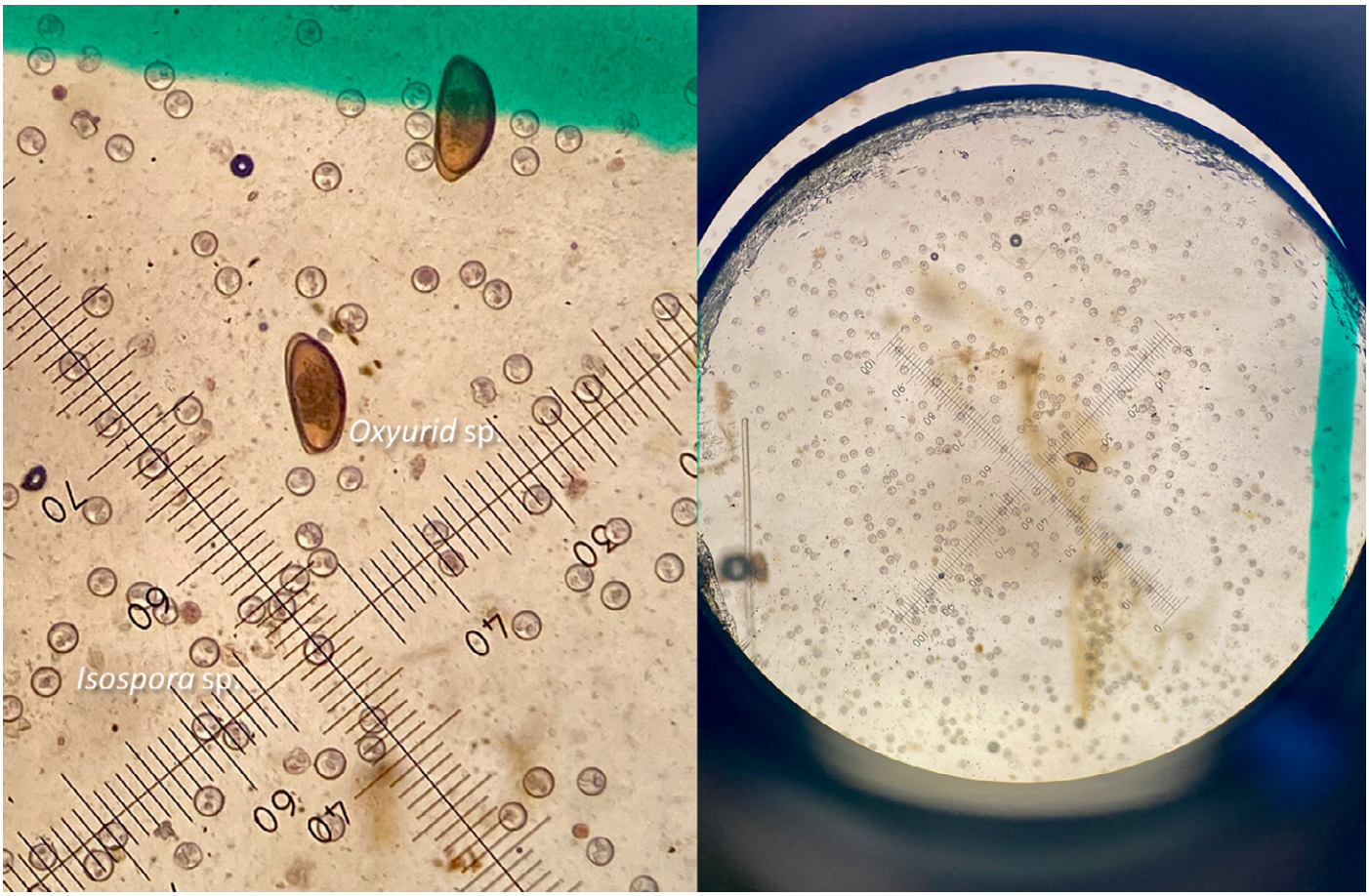

Figure 1. Two endoparasite eggs and oocysts detected in the captive inland bearded dragon. Left: Oxyurid nematode egg and *Isospora* sp. oocysts at 100× magnification (1 unit scale = 10 µm). Right: Example of high abundance of *Isospora* sp., at 40× magnification (1 unit scale = 25 µm).

/ oocyst counts between morning and afternoon for oxyurid nematodes (Wilcoxon rank sum test, p = 0.03, N = 6) but not for *Isospora* sp. (Wilcoxon rank sum test, p = 0.31, N = 6). There were higher estimated egg counts for oxyurid in the afternoon (median = 14,409.25 epg) compared to the morning (median = 5,968.55 epg) (Figure 2).

There was also a significant difference in the number of oocysts in *Isospora* sp. across months (Kruskal Wallis test, H (2) = 7.83, p = 0.02), where there was a marked increase in the number of oocysts between the start (August median = 1,150.15 epg) and the end of the study (October median = 28,9541.5 epg) (Wilcoxon rank sum test: August vs October, p = 0.01, N = 6; August vs September, p = 0.30; September vs October, p = 0.06, shown in Figure 3. In contrast, there were no significant changes in the number of oxyurid eggs across months (Kruskal Wallis test, H (2) = 3.26, p = 0.20).

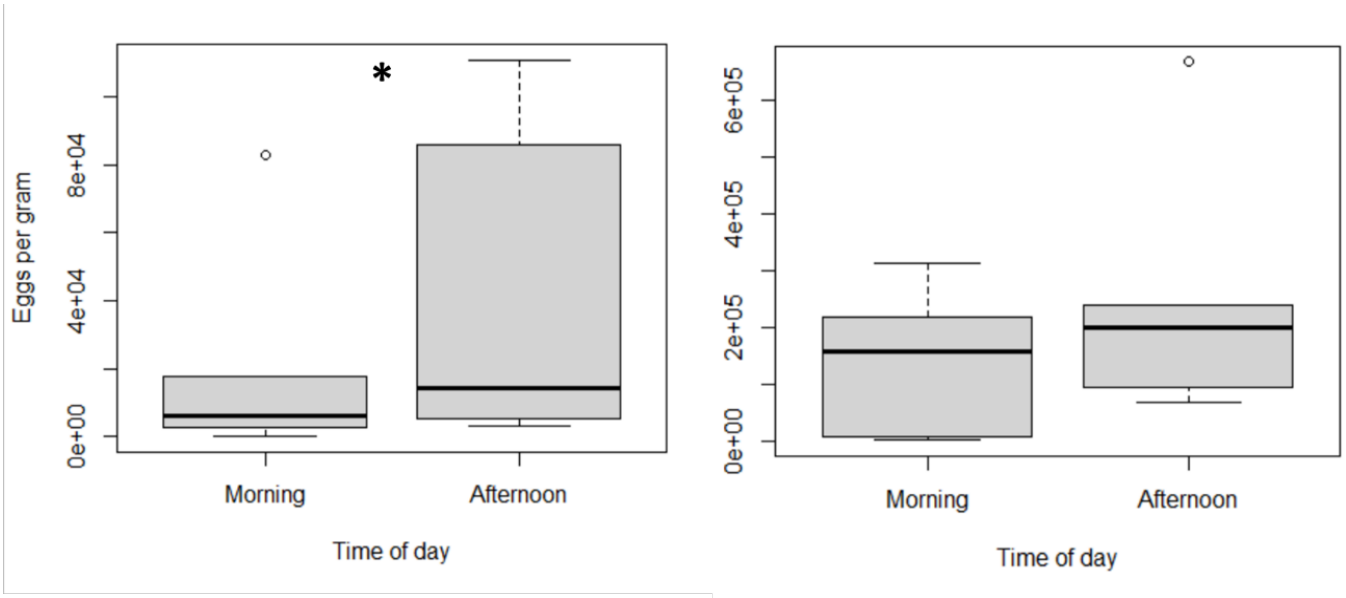

Figure 2. Estimated number of eggs / oocysts per gram for oxyurid (left) and *Isospora* sp. (right) between mornings and afternoons. Asterisk indicates a significant difference between number of eggs / oocysts in the morning and afternoon (p = 0.05*, p = 0.01**, p = 0.001***).

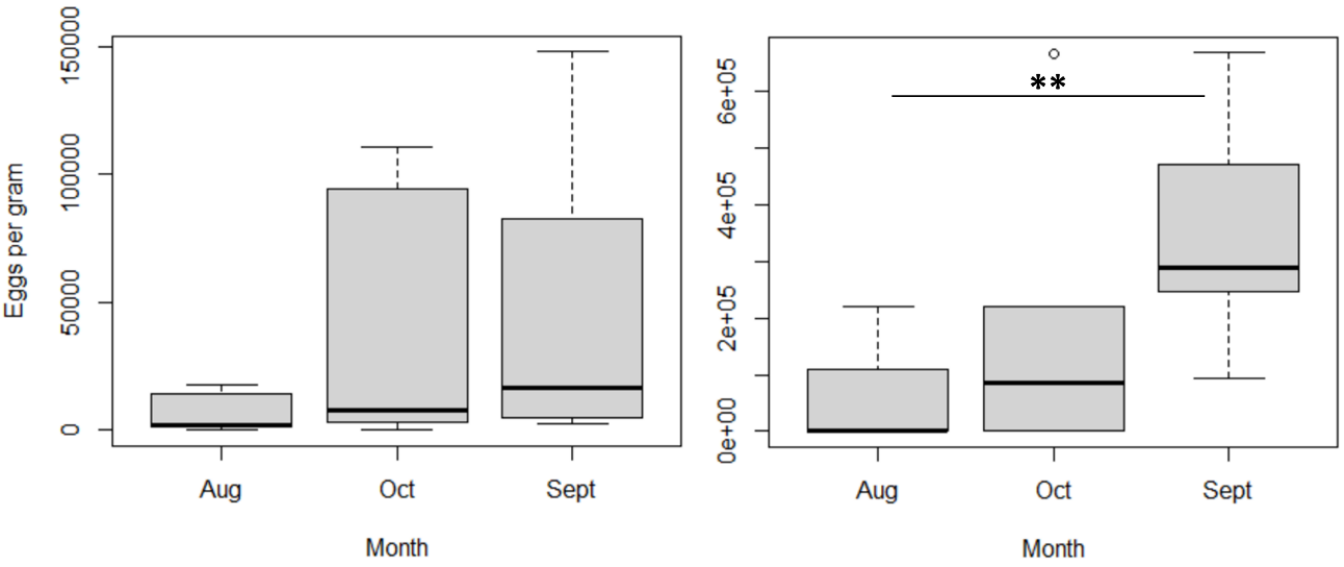

Figure 3. Estimated number of eggs per gram for oxyurid (left) and oocysts per gram for *Isospora* sp. (right) across three months (August–October 2022). Asterisk indicates a significant difference between number of eggs / oocysts in the morning and afternoon (p = 0.05*, p = 0.01**, p = 0.001***).

Overall, we did not find any significant association between the estimated egg / oocyst count of either oxyurid (Spearman's rank correlation, $p = 0.63$, $\rho = -0.12$, $N = 6$) or *Isospora* sp. (Spearman's rank correlation, $p = 0.70$, $\rho = -0.01$, $N = 6$) to the body condition of the dragons.

Discussion

In this study, we identified two endoparasites in six captive inland bearded dragons that were housed separately from the age of one week. Based on the physical attributes and shape of the eggs / oocysts and host species, we suspect these parasites were *Pharyngodon* sp. (oxyurid nematodes, pinworm) and *Isospora amphiboluri* (coccidia) (McAllister et al., 1995; Greiner, 2003; Rom et al., 2018; Walden & Mitchell, 2021). We monitored the shedding of eggs / oocysts of oxyurid nematodes and *Isospora* in all inland bearded dragons from August to October 2022. We found that oxyurid nematodes had almost 2.5 times higher egg count per gram of faeces in the afternoon compared to the morning. In contrast, there was no difference in oocysts between morning and afternoon in *Isospora*. We noted that the shedding of *Isospora* oocysts increased over time, whereas there was no change in estimated egg counts for oxyurid. There was no association between the estimated quantity of eggs / oocysts to the body condition of the dragons. Finally, we did not find any apparent influence on time when faecal samples were processed to the number of eggs / oocysts that we counted.

In general, the shedding of eggs or oocysts has been found to vary depending on the time of day, seasons and behavioural activity in some species (e.g., Hudman et al., 2000; Filipiak et al., 2009; Dolnik et al., 2010; Morin-Adeline et al., 2011; Taylor et al., 2018; Biard et al., 2022). In particular, some studies show 4– >4,000 times higher prevalence of parasites (coccidia) in the afternoons compared to the mornings in bird hosts (e.g., Brown et al., 2001; Morin-Adeline et al., 2011; Coelho et al., 2013). These patterns seemed to occur in birds living in temperate forests, which had higher oocyst shedding in the afternoons where UV is lower and humidity is higher during that period of the day (Biard et al., 2022). However, bird hosts living in tropical rainforests were observed to have consistent shedding, where UV radiation levels and humidity are more stable throughout the day (Biard et al., 2022). The environment that the dragons were kept in for our study (i.e., constant UV and heat exposure during the day, overall low humidity levels of 35–50%) may encourage consistent shedding of *Isospora*. However, there was still significantly higher (c. 2.5 times) shedding of oxyurid eggs in the afternoon. This observation study is limited to small sample size and we suggest future experimental investigations into comparing dragons in different environments with higher sample sizes to determine factors that influence oocyst / egg shedding in oxyurid and *Isospora*. Our current study results concur with other studies' suggestion that faecal sampling is best to be conducted during the afternoons to increase the chances of parasite detection in the host (López et al., 2007; Filipiak et al., 2009; Morin-Adeline et al., 2011; Coelho et al., 2013). We also suggest further study to monitor the changes or variation in shedding over a longer period of time (e.g., comparing between seasons) with a larger sample size.

The increase of *Isospora* over time potentially suggests temporal accumulation of the parasite within the dragons. It is a typical expectation that parasite prevalence will increase over time if no treatment is applied (Walden & Mitchell, 2021). In contrast, oxyurid did not significantly change in numbers over the three months. Two likely explanations are that the symbiotic relationship results in stability of oxyurid numbers over time in healthy hosts (Šlapeta et al., 2018), or that the weekly deep cleaning conducted during this study was sufficient in maintaining the infection of oxyurid but not *Isospora*. The cleaning agent, SteriGENE, and similarly TriGENE (Ethical Agent, New Zealand) and F10 (Health and Hygiene Pty Ltd, South Africa), generally contain quaternary ammonium compounds (didecyldimethylammonium chloride), benzalkonium chloride and polihexanide (Ethical Agent, 2015; Health and Hygiene Pty Ltd, 2022). Both SteriGENE and alcohol are bactericide, virucide and fungicide; therefore, both are unlikely to remove coccidia effectively (Australasian Infectious Diseases Advisory Panel, 2008; Kines et al., 2021; Varela et al., 2022). All dragons were treated for *Isospora* at the end of the experiment (i.e., before they were returned to the private breeder).

Although there was the presence of endoparasite and accumulation of *Isospora* over time, they did not seem to have a significant effect on the apparent body condition of the dragons. All dragons were asymptomatic, except for one individual that produced watery faeces. This individual was the largest of all the dragons, and showed typical behavioural responses to handling and movement tests (M. Baling, unpublished

data). This observation mirrors previous studies that regularly showed apparently healthy pet reptiles carrying gastrointestinal parasites (Raś-Noryńska & Sokół, 2015; Ellerd et al., 2022). Grenier (2003) suggests that infected individuals that seem healthy still undergo intracellular damage due to the coccidia. But if the rate of regeneration of these cells is quick enough, the host can remain healthy in the presence of coccidia. Suboptimal conditions often elicit physical and physiological stresses in ectotherms that result in disease (Bower et al., 2019). Therefore, by minimising potential physiological and physical stressors such as suboptimal environmental conditions and hygiene, reptiles may be able to be more resilient and tolerant to infection load (Bower et al., 2019). It is likely that the environmental conditions for the dragons in our study were sufficient to prevent multiple clinical symptoms. But to confirm this, we suggest more investigation to compare the effect of optimal husbandry vs suboptimal husbandry on infection and the likelihood of disease in captive reptiles.

The typical recommendation for faecal analysis, especially faecal floatation, is to process the samples as soon as possible, i.e., within 24 hours (Raś-Noryńska & Sokół, 2015; Tedjo et al., 2015; Vogtmann et al., 2017; Byrd et al., 2019). Nematodes, such as oxyurid, can hatch if left in warm temperatures, resulting in the bias of non-presence of parasites or inaccurate egg / oocyst counts (Broussard, 2003). However, there are various circumstances that can prevent the immediate processing of faecal samples (e.g., sampling of wild animals from isolated locations). So the typical protocol is to store fresh faeces between 2–8 °C for 24-hour analysis or to preserve the samples using a formalin fixation for longer periods (Zajac et al., 2021). We did not use formalin fixation, but all our samples were stored in a 4 °C fridge (Zajac et al., 2021). If we assumed that there would be an increasing deterioration of eggs or oocysts over time, we expected a decreasing trend between estimated egg counts to an increasing duration between when the samples were collected and samples processed. But our study showed no apparent association between the final estimated egg count and the number of weeks between collection and processing of samples, which suggests that the oocysts and eggs from reptile hosts, or at least in these bearded dragons, were able to withstand up to 30 days when stored at 4 °C. Further experiments should be conducted to test the shedding of both *Isospora* and oxyurid over time (e.g., within 24 hrs vs 48 hours vs one week).

This study is limited to a small sample size and the restricted time for data collection (as this study is student research); however, the information that we can provide is baseline data that can be built up over time. Our study showed that oxyurid sheds 2.5 times more during the afternoon compared to the morning, whereas no difference was observed in *Isospora*. Despite the increased infection load of *Isospora* over three months, there did not seem to be an apparent negative effect on the body condition of the captive juvenile dragons.

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Conflicts of Interest

There are no conflicts of interest.

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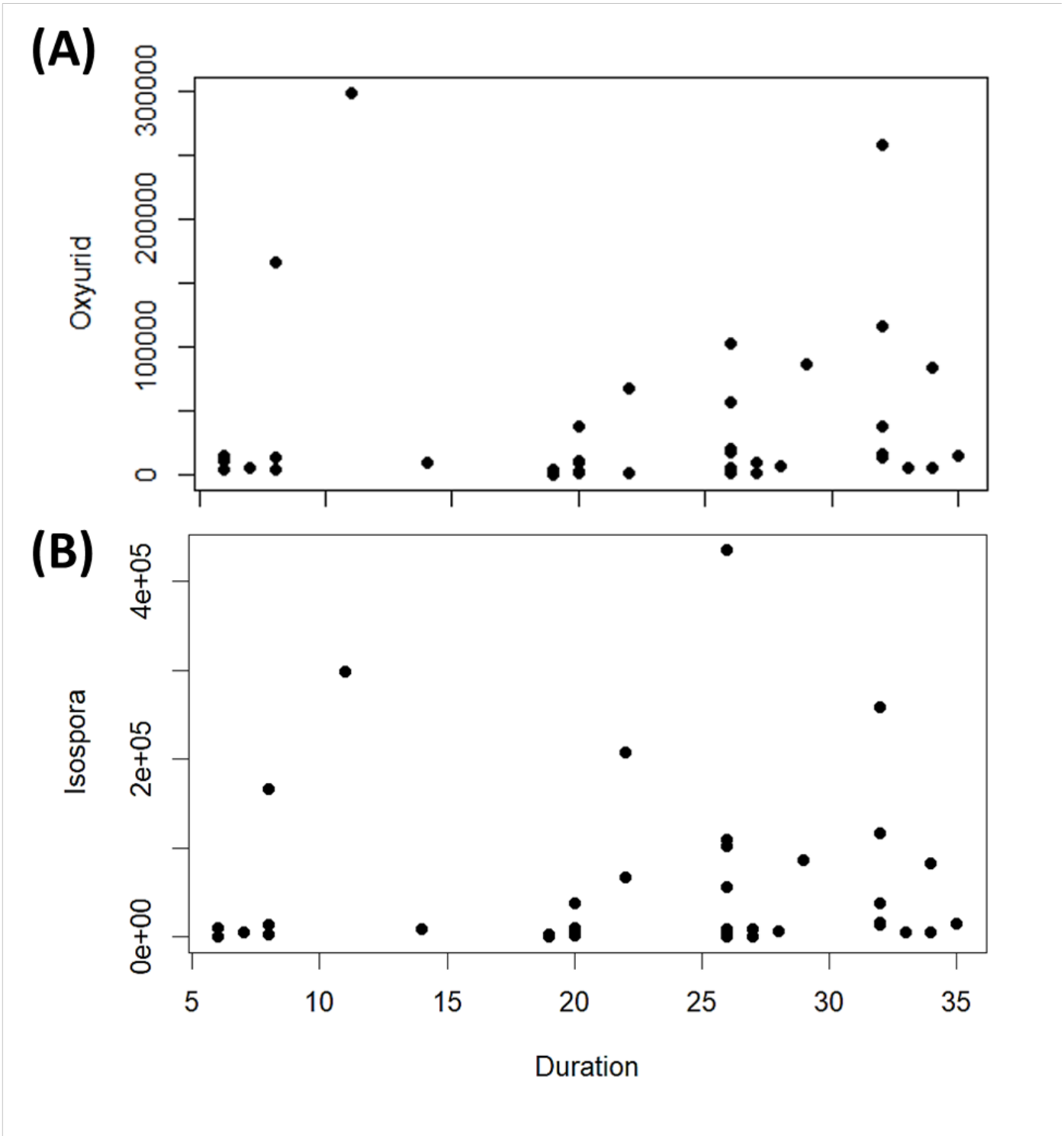
Appendix 1

Estimated number of eggs / oocysts per gram (top: oxyurid [N = 42]; bottom: *Isospora* sp. [N = 42]) against number of days between when samples were collected and quantified (Duration).



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