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Faculdade de Medicina Veterinária

GASTROINTESTINAL PARASITE SCREENING IN PET REPTILES IN THE AREA OF PERTH, WESTERN AUSTRALIA

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In "Jesusalém"

ABSTRACT

GASTROINTESTINAL PARASITE SCREENING IN PET REPTILES IN THE AREA OF PERTH, WESTERN AUSTRALIA

Reptiles' popularity as domestic pets increased in the last decades, demanding a correspondent improvement in reptile medicine and parasitology, both for animal welfare and public health reasons, since many reptile parasitic infections are zoonotic.

A parasitological survey was carried out in a pet reptile population in the area of Perth, Australia, between April and June 2015. Faecal samples were collected from 57 reptiles, 9 lacertilian species (n=11) and 10 ophidian species (n=46). Samples were screened for the presence of gastrointestinal parasites by fresh smear and faecal flotation. After both techniques, 18% of the samples contained parasitic forms, five of which were pathogenic organisms. A total of six parasitic forms were identified, including oxyurids (52,38%), *Strongyloides* sp. (14,29%), acarids (14,29%), *Nyctotherus* spp. (9,52%), ascarids (4,76%) and pentastomids (4,76%).

Parasitic prevalence was higher in wild-caught specimens (36,6%) than in captive bred ones (25,9%), higher in reptiles kept in less than optimal conditions (62,5%), than in those kept in adequate conditions (26,54%) and also higher in animals that didn't receive adequate anti-parasitic treatment (38,10%), than in those who did (28,47%).

Results from the present study show the importance of anti-parasitic therapy and good husbandry in preventing reptile parasitic diseases and keeping a healthy reptile pet.

Key words: Reptiles, Parasites, Direct smear, Faecal flotation, Western Australia

RESUMO

RASTREIO DE PARASITAS GASTROINTESTINAIS EM RÉPTEIS DE ESTIMAÇÃO NA ÁREA DE PERTH, AUSTRÁLIA OCIDENTAL

A popularidade dos répteis enquanto animais de estimação aumentou nas últimas décadas, exigindo avanços na medicina e parasitologia de répteis, tanto por razões de bem-estar animal, como de saúde pública, sendo que várias doenças parasitárias em répteis têm carácter zoonótico.

Um rastreio parasitológico foi realizado em uma população animal de répteis de estimação na área de Perth, Austrália, entre abril e junho de 2015. Colheram-se amostras fecais de 57 répteis, 9 espécies de sáurios (n=11) e 10 de ofídios (n=46). As amostras foram analisadas para pesquisa de parasitas gastrointestinais através das técnicas de esfregaço direto e de flutuação fecal. Utilizando ambas as técnicas encontraram-se formas parasitárias em 18% das amostras, cinco das quais correspondentes a organismos patogénicos. Identificaram-se seis formas parasitárias: oxiurídeos (52,38%), *Strongyloides* sp. (14,29%), acarídeos (14,29%), *Nyctotherus* spp. (9,52%), ascarídeos (4,76%) e pentastomídeos (4,76%).

Observou-se que a prevalência de parasitas era mais elevada em animais capturados a partir da natureza (36,6%), que em répteis nascidos em cativeiro (25,9%), bem como em animais mantidos em piores condições (62,5%) do que aqueles que dispunham de condições adequadas (26,54%). Encontrou-se maior número de parasitas em animais que não estavam corretamente desparasitados (38,10%), do que naqueles que tinham a desparasitação em dia (28,47%).

Os resultados do presente estudo demonstram a importância da terapêutica antiparasitária e de maneio adequado na prevenção de doenças parasitárias em répteis, assim como na manutenção de um animal saudável.

Palavras-chave: Répteis, Parasitas, Esfregaço fecal, Flutuação direta, Austrália Ocidental

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ABREVIATION LIST

CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora

cm – centimetre

CT – computed tomography

DPaW – Department of Parks and Wildlife

FMV – UL – Faculty of Veterinary Medicine – University of Lisbon

g – gram

kg – kilogram

m – metre

mg – milligram subcutaneous

ml – millilitre

µm – micrometre

NSW – New South Wales

PECABO – partial ear canal ablation and bullae osteotomy

PO – *per os*

POTZ – preferred optimum temperature zone

PCV – packed cell volume

SC – subcutaneous route

sp. – species

spp. – more than one species

UPV – Unusual Pet Vets

UV – ultraviolet light

WA – Western Australia

WAHS –West Australian Herpetological Society

x – times

1 ACTIVITIES DEVELOPED DURING THE CURRICULAR INTERNSHIP

The curricular internship took place between the 1st of March 2015 and the 1st of July 2015 with The "Unusual Pet Vets" (UPV) in Perth, Western Australia. UPV clinic is specialized in exotic pets, and it allowed the intern to work with the state of the art equipment and facilities of Murdoch University Veterinary Hospital and Balcatta Veterinary Hospital, experience a different reality and attitude towards the role of the veterinarian physician and learn from the amazing team of the UPV clinic.

To retrieve as many faecal samples as possible, the intern attended monthly meetings of the West Australian Society of Herpetologists (WASH) and established contact with a number of private reptile keepers and breeders, as well as with the Armadale Reptile and Wildlife Centre and the Western Australian Museum.

Throughout the course of the internship, the intern had the opportunity to observe and assist all aspects of the clinic work: consultations, hospitalization, surgery, clinical analysis and diagnostic imaging.

During the consultations the intern would observe and assist, restraining animals for better examination or treatment. The intern also had the opportunity to start a number of consults, gather the patient's history and carry out the physical examination, as well as discuss with the client important aspects of husbandry and feeding. Some of the most commonly observed medical conditions where dental disease and gastrointestinal hipomotility in rabbits and guinea pigs, respiratory disease in rats, neoplasia in ferrets, liver disease in birds and fungal infections in psittacines.

Regarding the hospitalized animals, the work conducted included cleaning the patient's beds and changing the water and food bowls, as well as preparing and administering medication, force feeding critically ill patients and monitoring all cases. The hospitalized patients included the surgical cases of the day and critically ill animals that needed regular monitoring, supportive therapy and medication. The most common cases were surgical patients, "floppy bunny" cases and birds and rabbits with gastrointestinal disease.

Being a well-known exotic pet clinic, the surgery case load was quite extensive, allowing the intern to observe a large number of surgeries, including routine procedures as sterilizations and castrations in rabbits, guinea pigs and ferrets, dental corrections in rabbits and guinea pigs, leg amputations in small birds, tail amputations in reptiles and less common procedures as the partial ear canal ablation and lateral bulla osteotomy (PECABO) in rabbits and a radius and ulna fracture stabilization with pins in a rabbit.

The intern had an active role in the surgical moments, administering the pre-surgical medication, placing catheters in small mammals and monitoring patients under anaesthesia and in recovery from anaesthesia. On some occasions, the intern was given the chance to scrub in and help the assistant surgeon on more demanding procedures, as well as to do some simple procedures on her own, under supervision, such as rabbit and ferret castrations and guinea pig and rabbit dental corrections.

Regarding the clinical analysis field, the activities developed involved staining and analysing blood samples, including manual cell count and packed cell volume (PCV) assessment. Microscopic analysis of faecal material and crop washes was common practice, particularly in avian patients with gastrointestinal disease.

In the imaging department the intern assisted with positioning and monitoring patients for radiography and computed tomography (CT). The majority of rabbits with dental disease were previously submitted to CT in order to determine the affected teeth and to assess the integrity of the mandibular and maxillary bone to better quantify the risks of the following surgery in cases of dental extraction. All patients submitted to the PECABO procedure were previously submitted to CT to confirm bullae infection and assess its extension.

Other extra-curricular activities developed included a venomous snake handling and relocation course, monthly West Australian Herpetological meetings and work with Armadale reptile and wildlife centre and with the Western Australian Museum.

2 INTRODUCTION

Nowadays, when someone looks for a new pet to adopt the possibilities are wider than just the regular cat or dog. Amongst these new pets are small mammals (guinea pigs, rabbits and rats), birds, amphibians (axolotls and frogs), invertebrates (spiders and scorpions) and reptiles.

Herpetoculture, the keeping of reptiles or amphibians in captivity as a hobby or for commercial purposes, has been increasing in popularity all over the world. The most varied reasons attract hobbyists to reptile keeping, whether it is for the magnificent reptile patterns and morphs, the complex beauty of the enclosures or simply for the exoticism of having a snake or a lizard at home. An additional favourable point to keeping a pet reptile is how easy these animals are to keep once the enclosure is properly set up.

In Western Australia (WA), reptile keeping is limited to native species and requires a license according to the skill level of the desired reptile. For these reasons, WA was a bit of a late bloomer in the matter of reptile breeding and keeping. The West Australian Herpetological Society holds monthly meetings with the purpose to discuss reptile husbandry principles, to inform new owners how to keep a healthy pet and other relevant subjects.

Unfortunately, in some cases, the search for new and unusual pets does not necessarily reflect on better informed owners, which results in poor husbandry – the main cause of disease in reptiles (Griffin & Reavill, 2014). This and other subjects related with reptile welfare are discussed in the present dissertation.

Amongst the several pathologies that may affect pet reptiles are parasitic infections. Wild reptiles are believed to harbour a low amount of parasites and to establish a commensal relationship without being negatively affected. In captivity, due to a number of reasons such as bad environmental conditions, poor husbandry and increased stress levels, their immune systems' ability to sustain such infections is diminished, resulting in diseased animals. Another aggravating factor is the permanent contact with their own excrements, and therefore, with parasitic forms that may be expelled and are capable of re-infection. This results in heavier parasitic loads and in lower immune responsiveness (Jacobson, 2007; Klingenberg, 2004).

Despite the clinical and parasitological relevance of parasitic infections, a lot is yet to be researched on the subject of the prevalence of endoparasites in pet reptiles. To the best of the author's knowledge, no studies have been previously conducted on the prevalence of gastrointestinal parasites in pet reptiles in Perth, WA. There are a few studies aiming to identify parasites in wild reptiles or in reptiles kept in zoological collections, but studies specifically in pet reptiles are scarce. Rataj, Lindtnert-Knific, Vlahovic, Mavri & Dovc (2011) conducted an exhaustive study on the prevalence of endoparasites in pet reptiles in

Scandinavia and Papini, Manetti & Mancianti (2012) performed a coprological survey in pet reptiles in Italy.

The present study aims to shed some light on the prevalence of gastrointestinal parasites in pet lizards and snakes in the area of Perth, WA, and to assess the conditions in which the same reptile population is kept.

Additional research needs to be conducted in order to explore the zoonotic aspects of parasitic infections, to co-relate husbandry, stress and sanitary conditions with parasitic loads, and better characterize parasitic infections in pet reptiles.

3 BIBLIOGRAPHIC REVIEW

3.1 CHARACTERIZATION OF THE STUDIED SPECIES

Like traditional pets, reptiles have specific requirements that must be satisfied in order to maintain a healthy animal, such as matching the original habitat's conditions, providing the adequate lighting, the optimum temperature and the appropriate food items. Knowledge of these requirements has a tremendous importance both for the veterinarian clinician and for the veterinarian parasitologist.

Reptile keeping and trading in Australia is strictly regulated on a state-by-state basis. As all the samples were collected in Western Australia, the legislation details mentioned below are the ones imposed by the Government of Western Australia and controlled by the Department of Parks and Wildlife (DPaW). A license is required to keep any reptile species in WA, depending on the category the desired reptile is included. A list of the approved reptiles and all five categories is presented in appendix I. This measure and the effort that a potential reptile keeper has to put into getting a license, increase the chances that reptiles are kept by responsible owners and breeders.

The Code of Practice for the Private Keeping of Reptiles (Sate of NSW and Office of environment and heritage, 2013) states that reptile importation from overseas for inclusion in private collections is prohibited under Commonwealth law. In WA it is also prohibited to import reptiles from other states to prevent spreading and introduction of infectious diseases in the territory. An example of such disastrous effects would be the introduction in WA of the Inclusion Body Disease virus, a retrovirus that causes progressive damage to nerves, brain, spinal cord and internal organs, decimating entire collections in a short period of time.

3.1.1 SUBORDER SAURIA / LACERTILA - LIZARDS

3.1.1.1 Infraorder Autarchoglossa – Family Varanidae

3.1.1.1.1 Australian monitor (*Varanus giganteus*, Gray 1845)

Figure 1: *Varanus giganteus*. Source: Ryman, 2009.

Also known as "perentie" (Figure 1), this lizard inhabits the semidry and desert areas of central and northern Australia, from the rocky outcrops and escarpments to the open woodland, sand ridges and plains. Males have a snout-vent length between 75 and 90 cm and females are slightly smaller, between 55 and 70 cm (Ryman, 2009).

Wild perenties feed on what they can find - invertebrates, small reptiles, birds, small mammals, eggs and fish. Larger specimens can feed on wallabies as well. In captivity their diet should be equally varied, including invertebrates three to five times a week and a whole prey once a week (Ryman, 2009).

Preferred body temperature for this species is around 35ºC; it is important to provide a hotspot at 35ºC and a temperature gradient throughout the enclosure with a cooler area at 25ºC in the summer and 20ºC in the winter months (Ryman, 2009).

Varanus giganteus is included in Appendix II of the Convention on the International Trade in Endangered Species (CITES 2015). This species is not listed in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.2 Infraorder Autarchoglossa – Family Scincidae

3.1.1.2.1 Northern blue tongue skink (*Tiliqua scincoides intermedia*, Mitchell 1955)

Figure 2: *Tiliqua scincoides intermedia*. Source: Ball, 2015.

Northern blue tongue skinks (Figure 2), inhabit coastal heaths, forests, woodlands and less arid regions of the interior throughout the Northern Territory and the northwestern Western Australia. Adults reach a snout-vent length of 30 cm WASAH, 2014a).

Wild blue-tongues feed on insects, arthropods and snails, as well as flowers, fruits and berries. In captivity a similar diet should be offered, occasionally including raw meat or small mice, aiming to maintain a balanced diet and, therefore, a healthy pet (West Australian Herpetological Society, 2015).

The appropriate vivarium for this species must include a 35ºC basking spot and full spectrum UV light 8-10 hours per day, otherwise they are incapable of synthetizing D-vitamin which is essential for calcium absorption, resulting in diminished bone density WASAH, 2014a).

Tiliqua scincoides intermedia is not currently listed in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.2.2 Shingle-back skink (*Tiliqua rugosa rugosa*, Gray 1825)

Figure 3: *Tiliqua rugosa rugosa*. Source: Australian wildlife secrets magazine (2016).

Commonly known as "bobtail" (Figure 3), this blue-tongued lizard is characterized for having enlarged scales and a short, round tail. This species displays a predominantly diurnal behaviour, although bobtails can also be active at night when the temperature is elevated. Adults can reach 25 cm snout to vent length (Cogger, 2014).

Slow moving, bobtail skinks are widely distributed throughout the southern half of Australia, occupying different dry habitats, as the coastal heaths, woodlands, shrublands and sandy deserts (Cogger, 2014).

Activity period, feeding habits and vivarium requirements are the same as described above for northern blue tongue skinks.

Tiliqua rugosa rugosa is included in category 2 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.2.3 Western blue tongue skink (*Tiliqua occipitalis*, Peters 1863)

Figure 4: *Tiliqua occipitalis*. Source: Cebu Zoo, 2011.

Western blue tongue skinks (Figure 4) are well adapted to arid environments, as the coastal dunes, woodlands and sand plains. They can be found all through the western and southern-western coast, southern Northern Territory, central-western New South Wales and northwestern Victoria. Adults reach a snout-vent length of 30 cm WASAH, 2014a).

Activity period, feeding habits and vivarium requirements are the same as described above for northern blue tongue skinks.

Tiliqua occipitalis is listed in category 2 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.2.4 Western Spiny-tailed skink (*Egernia stokesii badia*, Storr 1978)

Figure 5: *Egernia stokesii badia*. Source: Lee-Steere, 2008.

Western Spiny-tailed skinks, also known as Gidgee skinks (Figure 5), inhabit semi-arid and woodland areas in southwest Western Australia. They can be found hiding under piles of wood or metal, associated with rocky habitats and around dead or dying trees. Adults can reach 19,4 cm (snout to vent length), making this one of the largest species in its genus (Lee-Steere, 2008; Mcdonald, 2013).

The name "spiny-tailed" is due to the spiny scales in their tails, which they use to wedge themselves to crevices in the wood or rock. This makes them very difficult for predators to remove. These lizards frequently live in hollow branches of Gidgee Trees (*Acacia* sp.), which grants them the nickname: "Gidgee skink" (Hosking, 2010).

Gidgee skinks are omnivorous; in the wild, juveniles feed mostly on insects, whereas adults ingest a large amount of plant material. As such, their diet in captivity should consist mostly on leafy vegetation, some fruit and a smaller amount of live prey, such as crickets or mealworms (Jackson, 2014; Mcdonald, 2013).

Gidgee skinks are diurnal animals, and they like to bask in the sun. They need a 40ºC hotspot, and temperature on the rest of the enclosure should be about 30ºC during the day and 25ºC

during the night. Full spectrum UV light bulbs or tubes are necessary to keep a healthy spiny-tailed skink (Jackson, 2014).

Egernia stokesii badia is now listed as vulnerable in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.3 Infraorder Gekkota – Family Diplodactylidae

3.1.1.3.1 Marbled gecko (*Oedura marmorata*, Gray 1842)

Figure 6: *Oedura marmorata*. Source: Zozaya, 2012

Marbled geckoes (Figure 6) are adapted to many environments, hiding under fallen leaves, small stone caves or strolling through open plains at night. They can be found across most Australia, except for the arid deserts. Their maximum length is 15 cm and they are more active during the night (Leisk, 2008d).

Their diet in captivity consists predominantly on crickets and other insects. They should be fed every two days (Leisk, 2008d).

This species' preferred optimum temperature zone is around 29-30ºC. A temperature gradient can be created using a heat pad under the vivarium, providing a temperature of 31-32ºC on half the enclosure and 27ºC on the cool end (Leisk, 2008d).

Oedura marmorata is listed in category 3 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.4 Infraorder Gekkota – Family Gekkonidae

3.1.1.4.1 Leaf-tailed gecko (*Uroplatus phantasticus*, Boulenger 1888)

Figure 7: *Uroplatus phantasticus*. Source: Rationalia, 2009.

Leaf-tailed geckoes (Figure 7) are originally from Madagascar, where they inhabit the montain rainforests. This peculiar looking gecko is one of the smallest in its genus with only 12,5-15 cm. As the name suggests, its whole body mimics the colour and shape of leaves, so that it is easily mistaken for a dead leaf. They tend to remain motionless for hours during the day and hunt during the night (Gundy, 2006).

Wild leaf-tailed geckoes feed on several different insects. In captivity their diet should include crickets, mealworms, silkworms, moths and other insects, aiming to maintain a healthy balance and variety (Gundy, 2006).

Due to their natural habitat leaf-tailed geckoes are adapted to high humidity levels and cooler temperatures. Humidity levels within the enclosure should be kept between 75 and 90% and temperatures for this species should never exceed 25ºC, the ideal being 21-24ºC. As mentioned before, this species has nocturnal habits, so a day/night cycle is very important to regulate their activity period (Gundy, 2006).

UV light demands are dubious due to this gecko's nocturnal behaviour. While some authors defend that nocturnal geckoes do not rely on full spectrum UV light for a fully functional calcium metabolism, other authors argue that in their natural habitat they are exposed to UV light, therefore it should be provided in captivity as well (Dunlop, 2015).

Uroplatus phantasticus is listed as threatened on Appendix II of the Convention on the International Trade in Endangered Species (CITES 2015) and a special permit is required to keep this species in Western Australia.

3.1.1.5 Infraorder Iguania – Family Agamidae

3.1.1.5.1 Australian frilled-neck lizard (*Chlamydosaurus kingii*, Gray 1827)

Figure 8: *Chlamydosaurus kingii.* Source: Mahony 2014.

The frilled-neck lizard (Figure 8), commonly called frilly, owes its name to the characteristically large extendible frill used to frighten predators. This is an arboreal, diurnal species, which inhabits the southern New Guinea and the north coastal Australia. With males reaching up to 90 cm, its the second largest lizard in the Agamidae family (Corning, 2015).

These lizards are omnivorous, therefore their diet in captivity consists of insects, mice and chopped fruits and vegetables (Corning, 2015).

As this species is adapted to the warmest areas of Australia, temperatures in the enclosure must be quite high. Temperature on the basking spot should be 37ºC, on the warm side 29-32ºC and on the opposite end of the vivarium 23ºC, creating a temperature gradient. Frillies need full spectrum UV light and humidity levels around 70% to keep them hydrated (Corning, 2015).

Chlamydosaurus kingie is listed in category 4 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.5.2 Australian thorny dragon (*Moloch horridus*, Gray 1841)

Figure 9: *Moloch horridus*. Source: Ogwen 2004.

The Australian thorny devil (Figure 9) inhabits the sandy plains and ridges and the shrub areas throughout Western Australia, the southwestern coast, Northern Territory and western Queensland. Fully developed thorny dragons can reach 9 cm (Sherbrooke, 2015).

With the whole body, tail and limbs covered with rows of spines and elongated scales protruding in every direction, this lizard resembles a mass of dried plant material. This appearance combined with the ability to quickly change its colour, make thorny dragons experts in camouflage (Sherbrooke, 2015).

Another example of this species' great adaptability is their water absorption method. Through a capillary system in their body they absorb water from wet sand and vegetation, transporting it all the way to their mouths (Mcdonald, 2011).

These dragons feed only on a few ant species, particularly from the genus *Iridomymex* (Mcdonald, 2011).

This is a very hard species to keep in captivity due to its highly specific feeding habits.

Moloch horridus is not listed in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.1.5.3 Western bearded dragon (*Pogona minor minor*, Manthey & Schuster 1999)

Figure 10: *Pogona minor minor*. Source: Pilbara Pythons, 2014a.

Commonly known as "beardies" (Figure 10), this small species of bearded dragon measures about 41 cm. "Beardies" have a predominantly diurnal nature and can be found throughout southwestern Australia inhabiting the coastal dunes, woodlands and sand plains (WASAH, 2014b).

In the wild these dragons feed on a variety of insects and arthropods. In captivity they should be offered a selection of insects (mealworms, crickets, grasshoppers, beetles) every two days and, periodically, minced meat or pinkie mice and fruit (WASAH, 2014b).

In captivity it's important to provide a 35ºC hotspot and a temperature gradient reaching cooler temperatures on the opposite side of the vivarium. Full spectrum UV light is essential for at least 8 hours per day, otherwise they are incapable of synthetizing D-vitamin which is essential for calcium absorption, resulting in diminished bone density (WASAH, 2014b).

This species is listed on category 2 in the approved reptile keeping list in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2 SUBORDER OPHIDIA (SNAKES)

3.1.2.1 Family Boidae - Subfamily Boinae

Snakes in the Boinae Subfamily occur in many different habitats, such as high cloud forest clearings or edges, woodlands, dry tropical forest and semi-desert, as well as urban habitats due to the abundance of mice. They are non-venomous constrictors, that is, they kill their preys by coiling around them and strangling them until they suffocate. Boids are fairly arboreal and have a nocturnal or crepuscular behaviour (Kaplan, 2014; Lindemann, 2009).

In the wild, Boas feed on a wide range of mammals, birds, reptiles and amphibians. In captivity their diet is based on mice, rats, rabbits and chickens of the appropriate size (Kaplan, 2014).

The majority of snakes in this family possess very characteristic pelvic spurs. Another interesting feature is the existence of two functional lungs, which only occurs in boas and pythons (Lindemann, 2009).

3.1.2.1.1 Red-tailed boa (*B. c. constrictor*, Linnaeus 1758)

Figure 11: *B. c. constrictor*. Source: Diaz-Figueroa, 2013.

Red-tailed boas (Figure 11) are originally from Central and South America, including northern Mexico, the Andes Mountains, northern Argentina and Peru. They also inhabit several islands of the Pacific coast and the Caribbean, and a few islands off the coast of Belize and Honduras (Lindemann, 2009).

A peculiar feature of this species is the absence of heat-sensing pits, common in other boas. Boa constrictors are aglyphous, they possess rows of long recurved same sized teeth, instead of elongated fangs. These snakes are ovoviviparous and adult specimens commonly reach 3 m (Lindemann, 2009; Petco, 2012).

Recommended temperature for this species is about 35ºC on the basking spot and 25ºC on the opposite end of the vivarium (Petco, 2012).

B. c. constrictor is listed as threatened on Appendix II of the Convention on the International Trade in Endangered Species (CITES 2015) and its keeping in Western Australia requires a special permit.

3.1.2.2 Family Boidae - Subfamily Pythoninae

Snakes in the Pythoninae subfamily are commonly known as pythons. These non-venomous constrictors are mostly active during the night but it's not uncommon to find them basking in the sun during the day as a way to increase their body temperature (Cogger, 2014).

A characteristic feature of this family is the evidence of the remnants of their hind limb and pelvic structures, in the form of cloacal spurs. All Australian pythons are oviparous, the female lays the eggs and incubates them until they hatch. In captivity pythons are fed mice, rats and chickens of the appropriate size (Cogger, 2014).

All species mentioned below are listed as threatened on Appendix II of the Convention on the International Trade in Endangered Species (CITES, 2015).

3.1.2.2.1 Black headed python (*Aspidites melanocephalus*, Krefft 1864)

Figure 12 *Aspidites melanocephalus*. Source: Lawrance, 2015a.

Black headed pythons (Figure 12) are found in the northern half of Australia. They avoid extremely arid areas, opting for the humid coastal forests and dry tropical woodlands (Cogger, 2014).

This is a nocturnal species, that feeds on small mammals, birds and reptiles. Adults reach a maximum length of 2,5 m (Cogger, 2014).

Preferred body temperature is 32ºC, therefore the warm end should be around 35-36ºC whereas the cold side should be around 27-29ºC (Leisk, 2008a).

Aspidites melanocephalus is rated category 4 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2.2.2 North western carpet python (*Morelia spilota variegata*, J.E.Gray 1842)

Figure 13: *Morelia spilota variegata.* Source: Lawrance, 2015b.

Northern carpet pythons, also known as Darwin carpet pythons (Figure 13), are mainly arboreal reptiles, found throughout the wet woodlands and savannah of Northern Australia. They are mostly nocturnal, arboreal and feed on terrestrial vertebrates (Cogger, 2014).

Males can reach 1,8 m whereas females can grow up to 2 m (West Australian Herpetological Society, 2015a).

Temperature on the basking spot should be between 32ºC and 35ºC and on the cold side around 20ºC, creating a temperature gradient between the two areas (West Australian Herpetological Society, 2015a).

Morelia spilota variegata is rated category 4 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2.2.3 Olive python (*Liasis olivaceus olivaceus*, Gray 1842)

Figure 14: *Liasis olivaceus olivaceus.* Source: Davies, 2015.

Olive pythons (Figure 14) can be found on the coast and hinterland of northern Australia, from northwestern Western Australia to western Queensland and also in Pilbara, occupying different habitats, like the monsoon forest, the savannah woodland and rocky hills associated with watercourses or caves (Cogger, 2014; Pearson 2003).

In the wild, olive pythons feed on a variety of birds, reptiles and mammals (Cogger, 2014; Pearson 2003).

Adults usually measure about 2,5 m, but can go up to 4 m. Their considerable size deems them less recommended for unexperienced owners (Cogger, 2014).

Due to this species' tropical nature vivarium temperature should never be lower than 22ºC. The recommended temperature on the hot spot is between 35ºC and 40ºC and on the cold end around 25ºC (West Australian Herpetological Society, 2015b).

Liasis olivaceus olivaceus is rated category 5 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2.2.4 South western carpet python (*Morelia spilota imbricata*, Smith 1981)

Figure 15: *Morelia spilota variegata.* Source: Lawrance, 2015c.

The SW carpet python (Figure 15) is found in the southern Western Australia, avoiding the arid areas. Feeding habits, size and recommended vivarium temperatures are the same as mentioned for northwestern carpet pythons (West Australian Herpetological Society, 2015c).

Morelia spilota imbricata is included in category 3 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2.2.5 Spotted python (*Antaresia maculosa*, Peters 1973)

Figure 16: *Antaresia maculosa.* Source: Charlton. 2011.

Mottled or spotted pythons (Figure 16) are found in the northern Eastern Australia, from Byron Bay Hinterland in New South Wales to Cape York in Queensland, around rocky hills, open woodlands and grassy areas. These active hunters feed on small mammals and birds, reptiles, frogs and bats (Leisk, 2008b).

Preferred body temperature is 30ºC, therefore the recommended temperatures are 34-35ºC on the warm end of the vivarium and 26-27ºC on the cold side. These are small snakes, usually measuring about 1,1 m (Leisk, 2008b).

Antaresia maculosa is not listed in the West Australian Wildlife Conservation Act 1950.

3.1.2.2.6 Western stimson's python (*Antaresia stimsoni stimsoni*, Smith 1985)

Figure 17: *Antaresia stimsoni stimsoni.* Source: Pilbara Pythons, 2014b.

Commonly known as children's python, stimson's (Figure 17) are considered ideal snakes for beginners, as they are among the easiest pythons to keep. They prefer arid environments and can be found all throughout Australia, except for the far north, south and east (Cogger, 2014).

In the wild, these snakes feed on small mammals such as mice and quails. They usually grow up to 1,1 m, although they can reach 1,4 m (Leisk, 2008c).

The preferred body temperature (PBT) is 31ºC, hence the enclosure's recommended temperature is 34-35ºC on the basking spot and 27-28ºC on the cold side (Leisk, 2008c).

Antaresia stimsoni stimsoni is included in category 3 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2.2.7 Woma python (*Aspidites ramsayi*, Macleay 1882)

Figure 18: *Aspidites ramsayi.* Source: Pilbara Pythons, 2014c.

Also know as desert pythons, womas (Figure 18) prefer the arid and dry areas, such as desert and adjacent areas of the central parts of Australia. This nocturnal, terrestrial species feeds on small mammals, birds and reptiles (Cogger, 2014).

Woma pythons are great pet snakes due to their placid nature and manageable size - adults reach a maximum length of 2,7 m (Cogger, 2014).

The recommended temperature on the hot spot is 32ºC and the cold end should be between 26 ºC and 29ºC (Spinner, 2015).

Aspidites ramsayi is included in category 4 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2.3 Family Elapidae

Australia is known for having the many of most dangerous animals in the world and when it comes to snakes it certainly lives up to its reputation. Elapidae is the predominant family, which includes most Australian venomous snakes and all medically relevant species (White, 2010).

Australia's Elapidae family consists of five major groups, which are the brown snakes group, the tiger snake group, the mulga snake group, the taipans and the death adders. Included in this family are also marine sea snakes and sea kraits (White, 2010).

Elapids are characterized for having one or several pairs of relatively short, immovable fangs at the rostral region of the maxillae, used to inject venom. These elongated fangs are hollow and each connects to a duct leading to the venom gland on the caudal aspect of the upper jaw (Cogger, 2014).

Venoms of Australian terrestrial elapids are more complex than those from elapids in other continents, having different clinical presentations. The effects are predominantly neurotoxic, although some venoms can also have significant myotoxic, coagulant and anti-coagulant effects, haemolytic activity and impacts on platelet function (Cogger, 2014; White, 2010).

3.1.2.3.1 Southern Death adder (*Acanthophis antarctius*, Shaw 1794)

Figure 19: *Acanthophis antarctius*.
Source: Valentic, 2011.

The death adder (Figure 19) is considered one of the most dangerous snakes in Australia and in the world. This species can be found throughout all of southern continental Australia and all the way up to eastern and central Queensland, in open areas of low growing woody vegetation (Cogger, 2014).

Death adders have a nocturnal behaviour, preferring to bury in the sand during the day and lure their preys by twitching the tip of the tail, mimicking the movement of an insect (Cogger, 2014). This ovoviviparous species reaches a maximum length of 1 m (WASAH, 2014c).

Despite their dangerous reputation, death adders only attack if excessively provoked, being more likely to remain still when disturbed. Death adder's venoms predominant effect on humans is flaccid paralysis, due to postsynaptic neurotoxins. The venom may take effect immediately after the bite or it may be delayed for over 24 hours (Cogger, 2014; White, 2010).

Temperature should be between 30ºC and 32ºC on the basking spot and around 20ºC on the cold end of the vivarium (WASAH, 2014c).

Acanthophis antarctius is included in category 5 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.1.2.3.2 Dugite (*Pseudonaja affinis*, Günther 1872)

Figure 20: *Pseudonaja affinis*. Source: Valentic, 2013.

Dugites (Figure 20) inhabit a wide variety of areas of the southwestern Australia, including coastal dunes, heathlands, scrublands, woodlands and forests. They are also very well adapted to urban habitats, since their favourite prey is the house mouse, therefore we can find them in many towns and urbanizations (Cogger, 2014; White 2010).

These elapids usually measure approximately 1,5 m, but some specimens can reach 2 m (Beatson, 2015).

Brown snakes, such as Dugites, are active during the day and inoculate a small amount of very potent venom, which can induce defibrination coagulopathy, renal failure and microangiopathic haemolytic anaemia. Unlike death adders, dugites rarely cause placid paralysis (White, 2010).

Recommended temperatures for this species are around 34ºC on the basking spot and about 20ºC on the other end of the vivarium (WASAH, 2014c).

Pseudonaja affinis is a protected species, rated category 5 in the West Australian Wildlife Conservation Act 1950 (DPaW, 2013).

3.2 PARASITES

For this chapter the author presents a short bibliographic review on the 5 most commonly found gastrointestinal parasites in lizards and in snakes based on a paper by Rataj et al. (2011). This is the most exhaustive research study on parasites in pet reptiles to date. For this study, 949 reptiles from different species, wild caught or obtained through specialized breeders, were examined for gastrointestinal parasites through faecal analysis and necropsy, in order to better appreciate the epidemiology of reptile parasites. Parasitic prevalence on this study were 47,3% for ophidians, 76,1% for lacertilian and 88,5% for chelonians.

3.2.1 SUBORDER LACERTILA - LIZARDS

3.2.1.1 Oxyurids (Class Nematoda; Order Oxyurida; Superfamily Oxyuroidea)

Oxyurids (commonly known as pinworms) from many genera are common parasites of turtles and lizards; in fact, they are among the most common endoparasites found in reptiles. In the case of lizards, it is possible for a single animal to possess one or several species of oxyurid at a time, whereas tortoises are more commonly parasitized with several species of oxyurids at the same time (Greiner & Mader, 2006).

Pinworms inhabit the lower intestine, particularly the colon and, in most cases, establish a commensal relationship with the host. They also have a high host specificity, which is helpful in some cases when determining whether certain oxyurids are parasitic of the reptile in question or if they were parasites of the ingested prey (Diaz-Figueroa & Mitchell, 2006; Jacobson, 2007).

These nematodes have a direct life cycle, therefore elevated parasitic burdens may be attained, particularly in captive herbivorous reptiles such as iguanids and chelonians, resulting in health problems (Diaz-Figueroa & Mitchell, 2006). There are reports of heavy pinworm infections causing obstructions and impactions in iguanas and tortoises, as well as rectal prolapses in snakes and lizards (Klingenberg, 2004; Jacobson, 2007).

According to Iverson (1982), cited byJacobson (2007), oxyurids may have an important role in preventing cellulose impactions in iguanids, by mechanically breaking up the gastric contents. For this reason, and due to the parasite's commensal nature, treatment is usually avoided unless clinical signs of disease are evident.

Oxyurid eggs are commonly found in routine faecal examinations of herbivorous chelonians and lizards. Pinworms produce large eggs, measuring 130 to 140 μm, shaped in a typical cylindrical, oblong or triangular shape with a lens-shaped plug at one end (Klingenberg 2004; Greiner & Mader, 2006; Jacobson, 2007).

3.2.1.2 Strongylids (Class: Nematoda; Order: Strongylida)

The order Strongylida is a vast and complex one. A very short approach to each superfamily (Strongyloidea, Trichostrongyloidea, Ancylostomatoidea and Metastrongyloidea) is presented below, as a way to mention the important reptile parasites in this order (Durette-Desset, Beveridge & Spratt, 1994).

3.2.1.2.1 Superfamily Strongyloidea

The majority of genera in this superfamily parasitize mammals, with very few genera occurring in reptiles. For example, the monoxenous genera *Chapiniella* and *Sauricola* are parasites of tortoises (Durette-Desset et al., 1994).

3.2.1.2.2 Superfamily Ancylostomatoidea

This superfamily evolved in mammals, although there is so relationship between nematode and host groups. Members in the Diaphanocephalidae family occur in snakes and, rarely, in lizards. The most important parasite in the Diaphanocephalidae is *Kalicephalus* spp., the intestinal hookworm of snakes. This parasite has a direct life cycle; it is transmitted by ingestion of contaminated food or water or by percutaneous route (Durette-Desset et al., 1994; Klingenberg, 2007c).

3.2.1.2.3 Superfamily Tricostrongyloidea

Parasites in this superfamily are monoxenous and many occur in reptiles, as well as in mammals, monotremes and marsupials (Durette-Desset et al., 1994).

The subfamily Molineinae occurs in anurans, reptiles and mammals. *Oswaldocruzia* spp. is an example of a genera parasitic of lizards (Durette-Desset et al., 1994; Durette-Desset & Slimane, 1996).

Heligmosomoidae family includes the Herpetostrongilinae subfamily, found in Australian and Oriental reptiles. The evolution of this subfamily occurred in Australian marsupials, except for 2 genera parasitic in reptiles – one in varanids in Malasya and the other in varanids and pythons in Australia (Durette-Desset et al., 1994).

3.2.1.2.4 Superfamily Metastrongyloidea

Parasites in this superfamily occur only in mammals (Durette-Desset et al., 1994).

3.2.1.3 *Nyctotherus* sp. (Phylum Protozoa)

Several protozoans can parasitize reptiles. This Phylum consists of unicellular organisms, which can only be observed with a microscope, and includes the following groups: amoebae (e.g., *Harmannella*), coccidia (e.g., *Eimeria* and *Isospora*), flagellates (e.g., *Hexamita* and *Trichomonas*), ciliates (e.g., *Balantidium* and *Nyctotherus*), cryptosporidia and also bloodstream parasites (e.g., *Plasmodium* and *Haemosporina*) (Jacobson, 2007; Klingenberg, 2007c).

Many protozoans are commensal organisms, as is the case of *Nyctotherus* sp., commonly found in routine faecal analysis from healthy herbivorous lizards (Jacobson, 2007).

Life cycle is direct and transmission occurs through ingestion of contaminant cysts, which means that heavy parasitic burdens may occur and persist in the absence of strict sanitary and cleaning measures (Greiner & Mader, 2006; Klingenberg, 2007c).

These ciliates are relatively large, measuring approximately 60 μm in length and are uniformly covered with cilia (Greiner & Mader, 2006).

3.2.1.4 Trematodes (Class: Trematoda)

Trematodes from the three orders (Monogenea, Aspidogastrea, and Digenea) parasitize all groups of reptiles. Despite being commonly found, there is very little on the clinical course of trematode infections in reptiles (Greiner & Mader, 2006).

Monogenea trematodes have a direct life cycle. These parasites are common in fresh water turtles and are not associated with clinical signs of disease (Klingenberg, 2007c).

Digenea trematodes are the most diverse order, found in all groups of reptiles. These parasites require at least one intermediate host and can occupy almost every soft tissue organ, although the majority of digenetic parasites live in the gastrointestinal tract. Commonly found in snakes, particularly in the oral cavity and air sacs and, despite the commensal nature of the infection, they should be removed (Greiner & Mader, 2006).

Severe infections may produce anorexia, weight loss, dyspnoea and uraemia. Lesions associated with the migration process are not common with trematode infections (Klingenberg, 2007c).

Adults may be found in the mouth, cloaca or faeces and ova are sometimes found in the faeces and direct smears of lung washes. Trematode eggs are large, yellow-brownish in colour and possess an operculated end (Klingenberg, 2007c).

3.2.1.5 Ascarids (Class Nematoda, Order Ascaridoidea)

Ascarids from the Anisakidae and Ascarididae families are common parasites of reptiles, infecting snakes, lizards, chelonians and crocodiles. Specimens in the Anisakidae family parasitize marine reptiles and the Ascarididae family includes most parasites of aquatic and terrestrial reptiles. Within these families each genus has its host specificity (Klingenberg, 2004; Greiner & Mader, 2006). The most relevant members in the Ascaridoidea order are the ones belonging to genera *Ophidascaris*, *Polydelphis* and *Hexametra*, which infect snakes and lizards (Jacobson, 2007).

Most ascarid species require an intermediate host to complete their development, that is, they have an indirect life cycle. The infection is spread through ingestion of intermediate hosts, such as frogs or rodents, which carry infective larvae. These larvae migrate through several tissues in the final host and the adults finally develop in the oesophagus, stomach or small intestine of the reptile. Eggs are then passed in the faeces and develop into second-stage larvae in the soil, which are eventually ingested by the intermediate host (Klingenberg, 2004; Greiner & Mader, 2006).

Adult parasites can be found embedded deeply in the gastric submucosa, resulting in a massive sclerotic inflammatory response, or unattached within both the intestinal lumen and the bile and pancreatic ducts, causing obstruction or even perforation. Immature forms can be found adjacent to coelomic vessels and viscera (Greiner & Mader, 2006; Jacobson, 2007).

Secondary infection with opportunistic Gram-negative bacteria commonly develops following lesions in the gastrointestinal mucosa (Jacobson, 2007).

Adult ascarids may appear in faecal and regurgitated material or found in post-mortem examination within the gastrointestinal tract, measuring from a few millimetres to more than 20 cm. Migrating larvae can only be identified through histological examination of the tissues.

Ascarid eggs are easily recovered from stomach washings or by performing a faecal flotation and their appearance is quite distinctive: unembryonated, with a characteristic thick shell, measuring approximately 80 to 100 μm by 60 to 80 μm (Jacobson, 2007; Klingenberg, 2004).

3.2.2 SUBORDER OPHIDIA (SNAKES)

According to Rataj et al. (2012), the most commonly found parasites in snakes were strongylid nematodes, particularly from the *Kalicephalus* spp. but also from other unidentified species, pentastomids, ascarid eggs, *Strongyloides* spp. and *Capillaria* spp.

3.2.2.1 Strongyloid nematodes (Class Nematoda, Order Strongylida)

Strongyles belong to the superfamily *Diaphanocephaloidea* and the most important genera affecting reptiles is *Kalicephalus* spp., also known as the intestinal hookworm of snakes. According to Jacobson (2007), about 24 species presenting low host specificity have been discovered so far.

Strongyles have a direct life cycle, which causes snakes in captivity to be continuously re-infected, particularly in cases of poor husbandry and sanitary conditions. Infection can take place by faecal-oral or percutaneous route (Klingenberg, 2004).

These small nematodes can be found attached to the mucosa throughout all of the gastrointestinal tract, where they feed on the host's blood. In some cases, infections appear to be subclinical, but in severe cases the resulting lesions are associated with haemorrhagic ulceration, severe inflammation, anaemia and coelomitis. Gastrointestinal obstruction or intussusception by large masses of worms are described as associated with heavy *Kalicephalus* infection, and worms and eggs may be expelled in mucus through the mouth if the parasites are present in the oesophagus (Klingenberg, 2004; Greiner & Mader, 2006).

Other nonspecific signs, such as lethargy, debility and anorexia can also be present and secondary infection with Gram-negative bacteria is a possible complication when ulcerative lesions are present (Jacobson, 2007).

These parasites are relatively small (1 to 1,5 cm long), and can be missed on post mortem evaluation, but the thin-walled eggs are easily spotted through faecal flotation. *Kalicephalus'* eggs measure about 70 to 100 μm by 40 to 50 μm and can be embryonated. It's important to differentiate them from *Rhabdias* and *Strongyloides* eggs, which are smaller and always embryonated (Greiner & Mader, 2006; Jacobson, 2007).

3.2.2.2 Pentastomids (Class Pentastomida)

Pentastomids are divided in two orders, Cephalobaenida and Porocephalida, both represented in Australia. The study of this class of parasites is not very well developed due to risks of human transmission, to life cycle complexity and to difficulties in identifying each species. For this reason, there is little information on pentastomids' biology and pathology (Kelehear,

Spratt, O'Meally & Shine, 2013).

Veterinarians working with reptiles are more likely to find pentastomids, as nearly 90% of adult species parasitize the respiratory system of carnivorous reptiles such as large venomous and constrictor snakes, lizards, crocodilians and some piscivorous chelonians. Reptiles can also act as intermediate hosts for several of the same pentastomid species (Jacobson, 2007; Paré, 2008).

Elapids, viperids, crotalids, boids and pythonids are common definitive hosts for pentastomids, particularly since many of these snakes in private collections are wild-caught and not all owners take the care to routine de-worm their newly acquired animals (Paré, 2008).

According to Riley (1960), cited by Jacobson (2007), ophidians are infected by the genera *Armillifer, Cubirea, Gigliolella, Kiricephalus, Parasambonia, Porocephalus, Raillietiella*, and *Waddycephalus*. In a study by Kelehear et al (2013) pentastomids from the genera *Raillietiella* and *Waddycephalus* were recovered from 48 out of 81 Australian snakes belonging to 3 families: Colubridae, Elapidae and Pythonidae.

Among the species that parasitize snakes, *Armillifer* spp. deserves special attention as it is known to be involved in most cases of human nymphal pentastomiasis. Parasites are acquired when ingesting undercooked snake meat or water contaminated with snake faeces (Paré, 2008).

Two other genera deserve special mention for their role in Australia: the genus *Waddycephalus,* which comprises 10 species found in Australian snakes, particularly in the elapid family, and the genus *Parasambonia*, which occurs solely in Australian elapids. Transmission occurs through ingestion of intermediate hosts, which can be mammals, lizards or frogs (Paré, 2008).

Pentastomids are dioecious, which means there is sexual dimorphism between males and females, and most species have an indirect life cycle. Both adults and larvae are haematophagous parasites, except for *Linguatula* spp., that feeds on cells and nasal secretions from domestic mammals (Jacobson, 2007; Paré, 2008).

Eggs are ingested by the intermediate host and primary larvae hatch in the gut. The larvae then penetrate the gut wall and migrate across the coelom/abdomen until encysting in the lung and air sac or in subcutaneous tissues. There, the larvae go through several moults until they become infective nymphs (Jacobson, 2007; Paré, 2008).

The intermediate host is ingested by the definitive host, completing the cycle, and nymphs are released in the digestive tract, initiating a migration process to reach the lungs. There, they mature into young adults and male pentastomes fertilize females in an early stage of infection.

Patent females lay ova in large quantities, which are released to the exterior through coughing and sneezing or passed with faeces after ingestion. For some species autoinfection is possible, highly increasing the parasitic burden in captive reptiles (Jacobson, 2007; Paré, 2008).

Wild reptiles may carry heavy parasite loads and don't show any signs of infection. However, that's not the case with captive animals, probably due to husbandry deficiencies and increased stressed levels. Heavy parasite burdens may induce anaemia, due to the haematophagous nature of the parasites, and the extensive migration and moulting processes inflicts direct damage to the tissues (Jacobson, 2007; Paré, 2008).

According to Riley (1986) *cit in* Jacobson (2007), the moulted cuticle may be responsible for antigenic stimulation, related with increased morbidity rates in reptiles parasitized with pentastomids. Clinical signs associated with the moulting process may vanish once this process in terminated (Paré, 2008).

Embedding of adult parasites in the lung is responsible for secondary bacterial or fungal pneumonia and septicaemia, which is one of the main morbidity and mortality factors. Trachea obstruction with parasites may occur and is also involved in the death of parasitized snakes (Jacobson, 2007; Paré, 2008).

Pentastomids may be found on routine examination of faecal flotation mounts or on examination of lung washes and nasal secretions. Adult parasites can be seen in the lungs of parasitized animals through endoscopy and calcified encysted nymphs have been incidentally found on radiographic images of mammalian intermediate hosts (Paré, 2008).

Pentastomid eggs measure between 100 and 200 μm, with an ovoid, mite-like primary larva surrounded by a thin-walled external shell. In mature eggs it's possible to identify hooklets at the anterior end of the larva, as well as short, stumpy limbs (Paré, 2008).

Since most adult pentastomes are relatively large, measuring 0,5 to 12 cm, these are easily diagnosed at necropsy. The wormlike body is superficially segmented, with two compound pairs of hooks surrounding the mouth (Jacobson, 2007).

As mentioned above, pentastomes infecting reptiles carry zoonotic potential, which is demonstrated for the genera *Armillifer* and *Porocephalus*. Although *Linguatula serrata* is the most commonly pentastomid infecting humans it only parasitizes mammals and is not associated with reptiles (Paré, 2008).

More research is demanded to better know these parasites, due to the negative impact they cause on captive and wild reptiles and to the additional zoonotic potential. Additionally, it is possible that pentastomids are being introduced to Australia (Kelehear et al, 2013).

3.2.2.3 Ascarids (Class Nematoda, Order Ascaridoidea)

Ascarids' characteristics and life cycle have been described previously on this document, therefore, only details regarding ophidians will be discussed in this section.

Snakes can accommodate a moderate load of ascarids, as it is believed these parasites feed on the ingesta and not on the host's tissue. With heavy parasite loads malnutrition, gastrointestinal tract impactation and perforation may occur, causing either nonspecific signs, such as anorexia and weight loss, or specific signs such as regurgitation. Larval migration can result in abscesses or ulcerations in the lung, trachea and other sites (Klingenberg, 2004; Greiner & Mader, 2006).

As mentioned previously, Gram-negative bacteria often opportunistically invade the gastrointestinal mucosa (Jacobson, 2007).

3.2.2.4 *Strongyloides* spp. (Class Nematoda, Superfamily Rhabditoidea)

There are two important genera in the order rhabditoidea that parasitize snakes: *Rhabdias* and *Strongyloides*. These parasites share similar life cycles, consisting of a dioecious free-living phase and a parthenogenetic parasitic phase. The parasitic phase is associated with adult nematodes in the lungs in the case of *Rhabdias* or intestinal tract in the case of *Strongyloides* (Greiner & Mader, 2006).

The host can be infected directly by ingesting food or water contaminated with eggs or larvae and hatched larvae can also infect the host through percutaneous penetration. It is possible for the parasite to infect and re-infect the host without undergoing the free-living stage. After ingestion, the larvae penetrate the oral mucosa, enter the circulatory system and access the lungs. Then they ascend the trachea to the oral cavity and mature in the intestines (Jacobson, 2007; Klingenberg, 2004).

Infected snakes may display signs of respiratory distress, such as open-mouth breathing and extended glottis when larvae migrate through the lungs. Severe pneumonia and secondary bacterial pneumonia may develop, producing a mucous exudate. Intestinal infections with adult *Strongyloides* may cause diarrhoea as well as nonspecific signs of illness such as anorexia, weight loss and lethargy. Secondary bacterial infection is a possible complication as well (Jacobson, 2007).

Poor sanitary conditions, high temperatures and high humidity levels contribute to the development of the free-living stage and consequently increase the parasitic load (Greiner & Mader, 2006).

Diagnosis relies on the demonstration of embryonated eggs measuring around 60 µm by 35 µm recovered through faecal flotation or lung wash. Since both genera can be found in the

lungs and gastrointestinal system it's not possible to distinguish embryonated eggs of *Strongyloides* and *Rhabdias.* However, it is important to differentiate these small eggs from the larger embryonated eggs of *Kalicephalus* (Jacobson, 2007).

3.2.2.5 *Capillaria* spp. (Class Nematoda, Superfamily Trichinelloidea)

Capillaria spp. are included in a group of genera referred to as capillarids (Bowman, 2014). *Capillaria* are small parasites, primarily infecting the intestinal tract of lizards, snakes and crocodilians, but they can also be found in the liver and gonads. According to Jacobson (2007), *Capillaria* spp. infections in crocodilians are the most relevant.

There isn't much information on Capillaria's life cycle, but it is known that these nematodes can have a direct or indirect life cycle and that snakes can act as paratenic hosts (Klingenberg, 2004; Diaz-Figueroa & Mitchell, 2006; Greiner & Mader, 2006).

Most infections are subclinical with few reports regarding clinical signs related to *Capillaria* spp., or due to larval migration, although heavy parasitic loads may result in hepatic dysfunction (Greiner & Mader, 2006; Klingenberg, 2004).

Characteristically bi-operculated *Capillaria* eggs with a thick shell are found in the faeces or tissues of reptiles (Jacobson, 2007).

3.3 CONTROL OF ENDOPARASITIC DISEASES

While most wild reptiles suffer from endoparasitic infections, reports suggest that it's not common for these infections to lead to death or even to produce clinical signs. The fact that reptiles in the field are in constant movement searching for food and shelter minimizes infection and re-infection with parasites, resulting in a lower parasite burden (Klingenberg, 2004; Jacobson, 2007;).

Captive reptiles are normally confined to a small area, contacting with their own excretions. This way, parasites that don't need an intermediate host find the perfect conditions to reproduce and reach high levels. On the other hand, reptiles in captivity are kept isolated from other animals thus minimizing infections with parasites requiring intermediate hosts (Klingenberg, 2004; Jacobson, 2007).

Confinement, along with stress related to improper husbandry, overcrowding, poor diet and other conditions is responsible for lowering the animal's immune system and exacerbating otherwise self-limiting parasitic infections (Klingenberg, 2004). This chapter focuses on a few measures to minimize infection risk in captive reptiles.

Greiner & Mader (2006) recommend freezing the prey items before feeding as it is an effective method to inhibit parasite transmission and it also prevents injuries to the reptile due to struggle with live prey.

3.3.1 General Husbandry

The number one cause of illness in captive reptiles is poor husbandry (Griffin & Reavill, 2014). It's important to recreate in captivity the natural conditions the reptile would have in the wild; adding to the enclosure elements that resemble the species' natural landscape helps stimulate the reptile's activity (Rossi, 2006).

A very important factor in keeping a healthy reptile is the awareness of the species' preferred optimum temperature zone (POTZ). The POTZ is defined as the range of temperatures within which the animal's performance is optimized, allowing it to carry out its normal activities, such as feeding, digestion and reproduction in a more effective manner. This can be accomplished by creating a temperature gradient within the vivarium, through heating mats or heating wires and hot spot lamps, so that the reptile can move between warmer and cooler areas, thus adjusting its temperature as needed (Rossi, 2006).

Reptile keepers should aim to mimic other wild environment conditions, such as suitable photoperiod, UV radiation and humidity levels. It's important to provide at least one hiding spot, as well as a clean water at all times and the adequate food items for each reptile species and size (Rossi, 2006).

Reptiles should be housed individually except for mating purposes to avoid injuries, dominance behaviour and competition for food, basking spots or shelters. Most important, reptiles from different species and from different areas of the world should never be housed together because they may have different commensal organisms that can be pathogenic for other species. Overcrowding and excessive handling should be avoided as these factors contribute largely to increase the animal's stress levels and potentiate cross infections (Rossi, 2006).

Failure to meet the animal's specific requirements regarding the factors mentioned above increases stress levels and contributes to lower the reptile's immune system, therefore improving the likelihood of developing a parasitic infection (Rossi, 2006).

3.3.2 Disinfection

The cleaning process plays an important role in preventing the dissemination of infectious diseases and keeping a healthy animal. Additionally, many parasites can be transmitted to humans as is the case of ascarids, protozoans and amoebas (Klingenberg, 2007b).

Cleaning and disinfection of the enclosures should be accomplished on a regular basis, using only disposable material to avoid transmitting pathogens between different animals. Hand washing and disinfecting between handling different animals also helps minimize the risk of cross infection. For the disinfection process to be more effective it's necessary to thoroughly clean and remove all organic residues. Even then, no disinfectant is 100% effective against all organisms (Rossi, 2006; Klingenberg, 2007b).

Rossi (2006) suggests using Roccal-D (a quaternary ammonium compound) diluted with water 1:200 to 1:400 or sodium hypochlorite diluted to 1 part bleach 30 parts water. Gillespie (2006) additionally recommends Chlorhexidine. To effectively eliminate cryptosporidia, Klingenberg (2007b) suggests cleaning the material with dish detergent and afterwards applying 5% ammonia for a few minutes.

Water dishes and other additional pieces that may need cleaning shouldn't be placed in the same bowl with disinfectant to avoid cross contamination (Rossi, 2006).

3.3.3 Routine faecal analysis

Faecal analysis is an efficient method to identify most gastrointestinal parasites. The first step is to differentiate the actual brown to black faeces from the white powdery urates; since the parasites are found in the faeces there's no use in analysing urates (Greiner & Mader, 2006).

Faecal samples should be as fresh as possible, but Klingenberg (2004) defends that in reptile medicine even older samples are worth processing. A possible theory is that reptilian parasites evolved to be more resilient due to the harsh environmental conditions of their hosts' habitat. For example, oxyurid eggs can resist for long periods even if subjected to temperatures between 33 and 38ºC in a desert environment, and even coccidian oocysts of *Isospora amphiboluri* have been found in samples exposed to these conditions (Klingenberg, 2004).

The clinician may have some difficulty acquiring fresh samples, since some reptiles don't defecate voluntarily at regular intervals. There are a few methods available to promptly obtain a faecal sample, for example increasing the reptile's activity, soaking the reptile in lukewarm water or physically opening the external cloacal sphincter with a lubricated cotton bud or sexing probe. As last resort a cloacal flush can be performed under light sedation (Klingenberg, 2004).

Once a faecal sample is obtained, there is a wide variety of methods available for identifying, measuring and counting different parasite stages. According to Pasmans, Blahak, Martel & Pantchev (2008) a routine parasite screening should include a direct flotation, a direct faecal smear and staining of the fresh faeces with iodine stain. Additionally, there are quantitative methods available to determine the parasitic load, such as McMaster's technique.

3.3.3.1 Faecal flotation

Faecal flotation is the most commonly used technique, as it can detect a large variety of parasites: nematode eggs and larvae, cestode eggs, several acanthocephalan eggs, pentastomide eggs, mites and their eggs, coccidian oocysts and enteric protozoan cysts (Greiner & Mader, 2006). A small amount of faeces is mixed with the flotation media, filtered into a 10mL test tube until a reverse meniscus is formed, then a coverslip is placed on top and the solution is allowed to rest for at least 10 minutes. The coverslip is then placed over a microscope slide and scanned with the 10x objective lens. The 40x objective lens is used when a higher amplification is necessary to accurately identify the parasitic forms (Zajac & Conboy, 2012).

Different concentrated salt or sugar solutions can be used. Klingenberg (2004) suggests the zinc sulphate solution with a specific gravity of 1.020 as the number one flotation media. On the other hand, Greiner & Mader (2006) uses sodium nitrate as the main flotation media, due to it's broad spectrum of activity, and sugar solution to measure coccidian oocists, as this flotation media takes more time to dry and, due to it's viscosity, allows the use of oil immersion. Appendix II contains a table (table 3) with different flotation solutions and their advantages.

Lugol's iodine stain may be used to facilitate protozoan identification, before adding the flotation media. Staining works best if performed on a direct smear since the flotation solutions, as well as the staining process, tend to promote the death of protozoans, making them harder to detect. New methylene blue or merthilolate may also be used to help visualize parasites (Klingenberg, 2004).

3.3.3.2 Direct faecal smear

Performing a direct faecal smear is the best option to identify moving ciliates, flagellates or amoeba, since the saline solution used, as well as the heat and light from the microscope, increase the movement of protozoans. This method is more efficient when performed on fresh faecal material, as its main advantage relies in the parasites' movement for easier identification (Klingenberg, 2004).

A drop of physiological saline solution is mixed with a small amount of faeces on a microscope slide and a coverslip is placed on top of the mixture. The slide is scanned with the 10x objective lens, with a high amount of contrast. A higher objective is used to better identify the parasitic forms (Klingenberg, 2004). As mentioned before, several stains may be added to the smear to help visualize and identify the parasitic stages.

Due to it's low sensitivity and great amount of debris produced this technique is best used in complement with other methods, for instance together with a faecal flotation (Zajac & Conboy, 2012).

3.3.3.3 Faecal sedimentation

A faecal sedimentation technique is performed to search for trematode eggs, and other heavy eggs. The process consists on either submit the sample to a formalin ethyl acetate centrifugation or to a manual sedimentation method, by mixing soapy water with the faecal sample. After the solution settles the supernatant is removed, leaving the sediment at the bottom of the test tube. The process is repeated until the decanted water is clear, then the sediment is transferred onto a microscope slide and scanned as described for the flotation technique (Klingenberg, 2004).

3.3.4 Quarantine protocol

Maintaining a good quarantine protocol is an essential aspect in preventing parasitic or infectious diseases from spreading to the main collection (Rossi, 2006).

Rossi (2006) suggests a quarantine period of 3 months for the majority of reptiles, and 6 months for snakes due to a number of viral infections that could prove fatal to the whole collection if given the chance to disseminate. Ideally, quarantined animals should be kept in a different room, so that no transfer of air occurs between these animals and the main collection. Quarantined animals should always be dealt with after the main collection (Rossi, 2006; Barten, 2006).

On arrival every animal should undergo a thorough physical examination, including a blood smear to rule out haemoparasites, faecal analysis to check for parasitic ova and protozoa and, in some cases, specific tests for other infectious diseases, such as inclusion body disease (Barten, 2006).

As a means to better access the animal's evolution, it is recommended to keep records of body weight, feeding schedule and appetite, general behaviour and symptoms of illness throughout the whole quarantine period. Faecal samples should be regularly retrieved and parasitic treatment administered until 3 consecutive negative samples are produced. Only then can the reptile be considered parasite free (Barten, 2006).

3.4 TREATMENT OF ENDOPARASITES

All antiparasitic agents used in reptiles were originally developed and tested for other species; the lack of research in reptiles leaves many unknown side effects. Preventive measures are always preferable, and when treatment is necessary it must be directed to previously identified agents. Nature of the parasite's life cycle and which tissues it affects are relevant in terminating a parasitic infection, as is determining whether the organism is commensal or pathological and whether it may be a vector for infectious agents. Bearing all that in mind, there is a variety of drugs that can be used in reptiles (Greiner & Mader, 2006).

Both fenbendazole and ivermectin can be used to treat nematode infections. Fenbendazole is safer and is the first choice in most cases, except in cases of drug resistance or when there is need for a fast parasite elimination, as is the case of parasitic pneumonia. In those cases, ivermectin is generally the best treatment option, as this drug leads to the parasite's death more quickly. A downside aspect of ivermectin is that killing the worms so rapidly releases antigens which can cause adverse immune reactions (Klingenberg, 2004).

The recommended agent against amoebas, ciliates and flagellates is metronidazole, although fenbendazole may also have some degree of effect. Coccidia are effectively eliminated with toltrazuril, which interferes with nucleus division among other aspects of the parasite's metabolism, or with trimethoprim/sulfamethoxazole. Praziquantel is the number one choice when dealing with cestodes and trematodes, but, once again, fenbendazole may also be used as a second choice in some cases (Klingenberg, 2004, Carpenter, 2013; Gibbons, 2014).

Table 4, found in appendix III, includes the most frequently used antiparasitic agents in reptiles, dosage and special recommendations.

Obtaining a thorough clinical history and physical examination is critical for formulating a sound diagnostic and therapeutic plan in any parasitic infestation.

4 OBJECTIVES

1. Assess the number of positive faecal samples from pet lizards and snakes in the area of Perth, WA.

2. Assess which parasites are most common in the positive faecal samples from pet lizards and snakes in the area of Perth, WA.

3. Evaluate, through a survey with an enquiry filled by all participant owners and breeders, regarding the husbandry conditions and their importance in maintaining a parasite-free reptile population.

5 MATERIAL AND METHODS

5.1 SAMPLE COLLECTION

The faecal samples for this study were collected in April, May and June from a variety of snakes and lizards kept by several breeders and keepers in the area of Perth, WA. The author contacted personally the majority of participants through meetings of the West Australian Society of Amateur Herpetologists, whilst a lesser number of samples was collected during consultation. The collected samples belonged to both wild caught and captive bred animals.

For the survey concerning epidemiological features of reptile parasitic diseases, participants in the study were asked to fill a short inquiry, found in appendix IV, regarding a few aspects of husbandry and hygiene, feeding habits, de-worming and origin of the animal.

5.2 SAMPLING

A total of 57 reptile faecal samples were collected (11 lizards belonging to nine different species and 46 snakes belonging to 10 different species) throughout April, May and June. The detailed list of species studied can be found in appendix V (tables 4 and 5).

5.3 CONSERVATION METHODS

Due to the great distance between the place where the animals were kept and the clinic, sample collection took place on a weekly basis, sometimes resulting in the analysis of week-old samples. The breeders were instructed on how to collect, identify and preserve the faecal samples, so that the samples' integrity would be maintained for as long as possible.

Samples gathered by the breeders were wrapped individually in aluminium-foil, placed inside a sealable plastic bag and identified with date of collection, animal number and species. The samples would then be kept in the fridge at 4ºC until the arranged pick up date.

Samples collected during consultation were immediately analysed.

5.4 SAMPLE ANALYSIS

Sample examination took place at The Unusual Pet Vets' laboratory, at Murdoch University. All samples were submitted to direct smear and direct flotation technique, as described previously on the chapter regarding routine faecal analysis.

5.4.1 Direct smear

To produce a direct smear a drop of saline is added to a small amount of faeces on a microscope slide and then covered with a cover slip. The faecal layer must be very thin to allow microscopic examination (Zajac & Conboy, 2012).

5.4.2 Direct flotation technique

As mentioned before, flotation technique is used to concentrate eggs from common helminths and protozoa eggs and cysts. Another positive effect is the reduction of debris, facilitating microscopic evaluation (Zajac & Conboy, 2012).

The faecal sample was thoroughly mixed with the flotation solution and then filtered into a 10ml cylindrical tube. Flotation solution was added until a reversed meniscus was formed and a cover slip was placed over the top. The solution was allowed to rest for at least 10 minutes and then the cover slip was placed over a slide for microscopic examination. The slide was then thoroughly scanned using 10x objective, switching to the 40x objective when a bigger amplification was necessary to identify the parasitic forms.

Many concentrated solutions can be used for flotation technique. Despite being less sensible than Zinc Sulfate Solution, the author chose Sheather's sugar solution for being less expensive (Zajac & Conboy, 2012). The recipe for Sheather's sugar solution can be found in appendix VI.

5.5 DATA STORAGE AND ANALYSIS

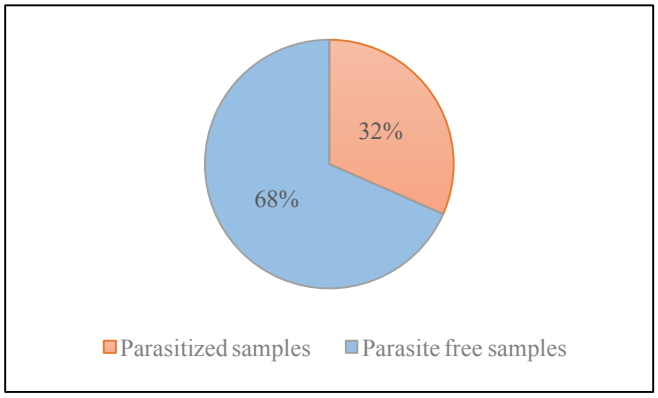
Collected data regarding parasitological and epidemiological survey performed with pet reptiles and their owners were stored in a Microsoft Excel® spreadsheet file, Version 15.16. Data was analysed using descriptive statistics parameters. Prevalence was calculated according to Bush, Lafferty, Lotz e Shostak (1997), meaning the number of individuals of the host species infected with a specific parasite species divided by the total number of hosts examined, usually expressed as a percentage.

6 RESULTS

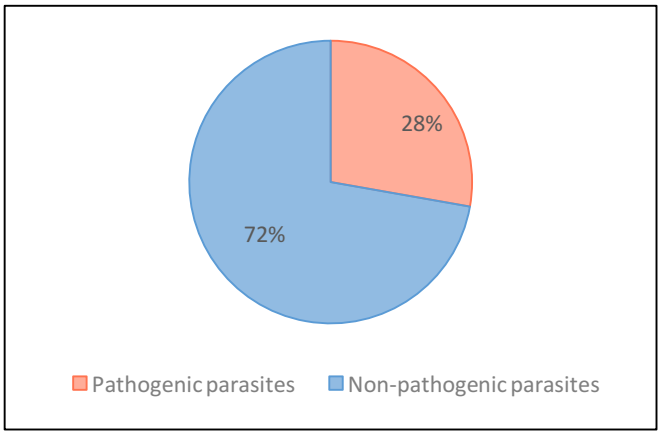
6.1 SAMPLE CHARACTERIZATION

From the 57 samples retrieved and analysed for this study, 18 (32%) contained one or more parasitic forms within either the faecal direct smear or within the faecal flotation (graphic 1). Out of these 18, five were pathogenic organisms and the remaining 13 were considered either commensal organisms or pseudo-parasites. This results in a total of 27,8% pathogenic parasites (graphic 2).

Graphic 1: Overall parasitic prevalence



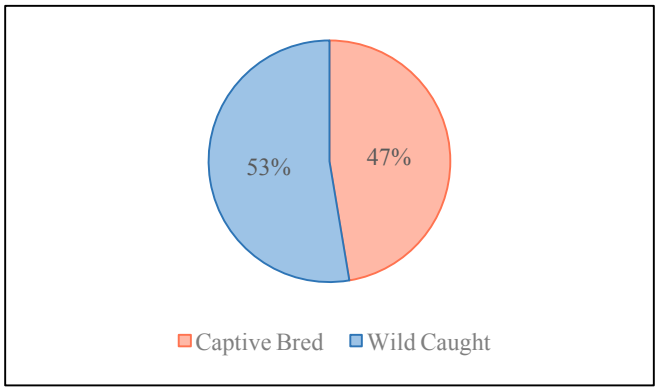
Graphic 2: Overall prevalence of pathogenic parasites



According to the questionnaire filled by all participants in this study, 30 (53%) animals were captured from the wild, whereas the remaining specimens were bred in captivity either by the actual owner or by a different breeder (graphic 3).

A total of 11 (40%) wild caught reptiles and a total of seven (26%) captive bred reptiles had parasites in their faecal samples. Regarding wild caught animals, 45,45% of the parasitized specimens had pathogenic organisms whereas only 14,29% of the parasitized captive bred specimens possessed pathogenic organisms.

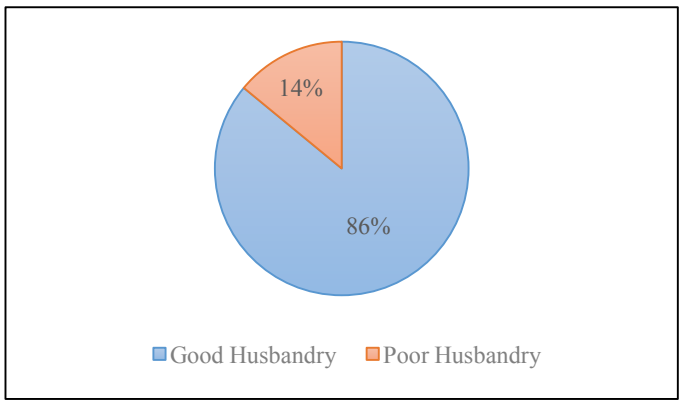
Graphic 3: Animal origin



The survey conducted showed that most participants in this study employed correct husbandry and sanitary measures and only in 8 cases (14%) these factors were unsatisfactory. Handling stress was a husbandry reprobation factor in seven of these eight cases. Husbandry quality is characterized on graphic 4.

Pathogenic parasites were found in three samples belonging to specimens provided with good husbandry and in two samples belonging to specimens provided with poor husbandry.

Graphic 4: Husbandry quality

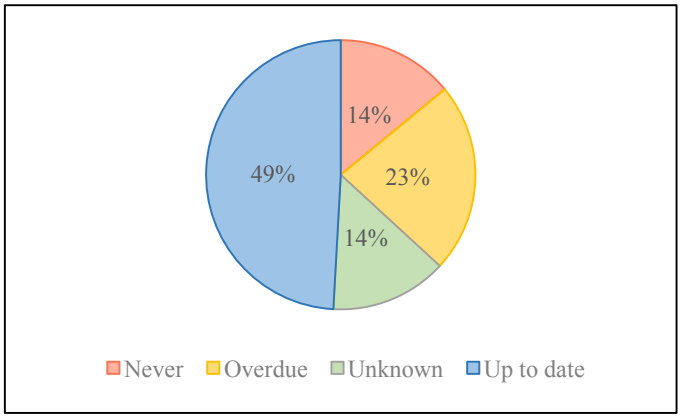


Regarding antiparasitic measures, the survey showed a total of 28 (49%) cases with up to date antiparasitic treatment and 13 (23%) cases in which treatment was overdue. Out of the remaining cases, eight (14%) never received antiparasitic treatment and in eight (14%) situations the owner didn't know whether such treatment had been employed by the previous owner (graphic 5).

A total of 8 (29%) specimens out of 28 with antiparasitic treatment up to date showed parasitic forms in the faecal analysis, although none of the referred parasitic forms were considered pathogenic.

A total of 21 (37%) specimens were not correctly de-wormed. This number is comprised of the cases in which treatment was overdue (23%) and the animals that never received any treatment (14%). Out of these 21 specimens, five (23,8%) provided samples containing only non-pathogenic organisms, such as commensal parasites and pseudo-parasites, two (9,52%) provided samples containing only pathogenic parasites and two (9,52%) contained both commensal and pathogenic organisms.

Graphic 5: Antiparasitic treatment record



6.2 PARASITIC PREVALENCE

6.2.1 Lacertilia

Out of 11 lizard samples analysed, six (54,5%) were infected with parasites. This represents 10,5% of the total analysed samples. Eggs from four different genera were found isolated or combined in these samples: acarid (Figure 21), ascarid (Figure 22), oxyurid (Figure 23) and *Nyctotherus* spp. (Figure 24).

Oxyurid eggs were the most commonly found in this study, occurring in samples from 5 lizards from five different species (*Egernia stokesii badia, Moloch horridus, Oedura marmorata, Tiliqua occipitalis, Tiliqua scincoides intermedia*). Two samples possessed two different parasitic forms: *Nyctotherus* spp. cysts occurred in one northern blue tongue skink (*Tiliqua scincoides intermedia)*, along with oxyurid eggs; ascarid eggs of unidentified species occurred in a western blue tongue skink (*Tiliqua occipitalis)*, along with oxyurid eggs as well. Another western blue tongue skink's sample contained eggs resembling those of mites.

Table 1 displays the parasitic forms found in lizards and the corresponding species from which they came from.

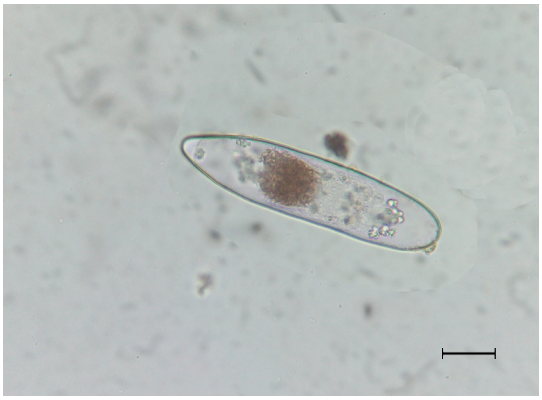


Figure 21: Unidentified acarid egg within a flotation from a western blue tongue skink (*Tiliqua occipitalis)*. (400x). Bar = 30 µm. Original.

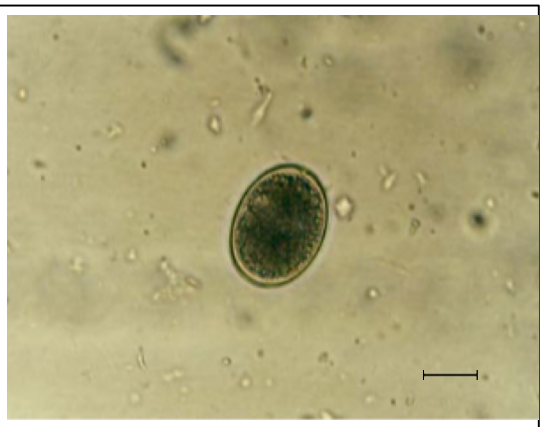


Figure 22: Ascarid egg in Leopard Gecko (*Eublepharis macularius*). Source: Rataj et al, 2011.

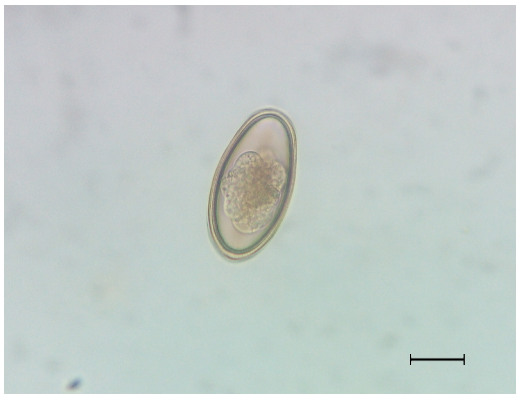


Figure 23: Unidentified oxyurid egg within a flotation from a western blue tongue skink (*Tiliqua occipitalis)*. (400x). Bar = 30 µm. Original.

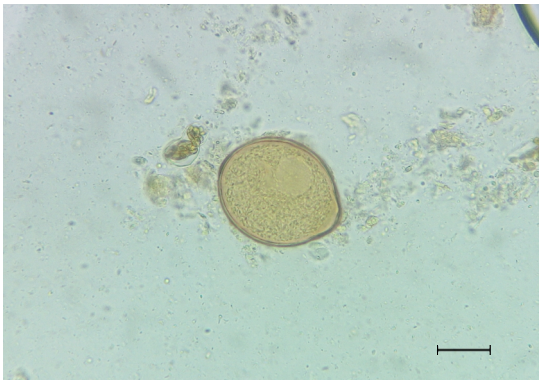


Figure 24: *Nyctotherus* spp. cyst within a direct smear from a northern blue tongue skink (*Tiliqua scincoides intermedia)*. (400x). Bar = 30 µm. Original.

Table 1: Number of positive lizards infected with different endoparasites.

Scientific name (Number of lizards)	Number (%) of Positive Animals	Endoparasite
Egernia stokesii badia (1) *Moloch horridus* (1) *Oedura marmorata* (1) *Tiliqua occipitalis* (1) *Tiliqua scincoides intermedia* (1)	5 (45,45%)	Oxyurid eggs
Tiliqua occipitalis (1)	1 (9,09%)	Acarid eggs
Tiliqua occipitalis (1)	1 (9,09%)	Ascarid eggs
Tiliqua scincoides intermedia (1)	1 (9,09%)	*Nyctotherus* spp. cysts

6.2.2 Ophidia

Out of the 46 snakes involved in this study, 12 (26%) provided samples with parasitic stages, four of which were pathogenic organisms. These numbers amount to 21% of the total samples analysed. The parasitic forms found were acarid eggs (Figure 25), *Nyctotherus* spp. cysts (Figure 26), oxyurid eggs (Figure 27), pentastomida embryonated eggs (Figure 28) and *Strongyloides*-like eggs (Figure 29).

Oxyurid eggs were found in six snakes from four different species: black headed python (*Aspidites melanocephalus*), dugite (*Pseudonaja affinis*), red-tailed boa (*B. c. constrictor*) and woma python (*Aspidites ramsayi*). This was the most commonly found genera in snakes in

this study. *Strongyloides*-like eggs were found in three black headed pythons. Acarid eggs were found in two snakes: a death adder (*Acanthophis antarctius*) and a south western carpet python (*Morelia spilota imbricata*).

Embryonated pentastomida eggs occurred in samples from a woma python (*Morelia spilota imbricata*) and *Nyctotherus* spp. cysts occurred in a stimson's python (*Antaresia stimsoni*).

One black headed python provided samples with both *Strongyloides*-like eggs and oxyurid eggs.

Table 2 displays the parasitic forms found in snakes and the corresponding species from which they came from.

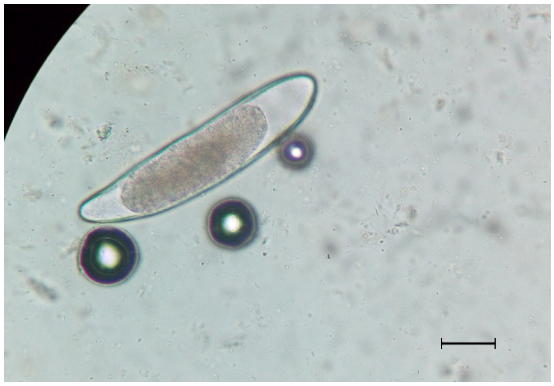


Figure 25: Acarid egg within a flotation from a death adder (*Acanthophis antarctius*). (400x). Bar = 30 µm. Original.

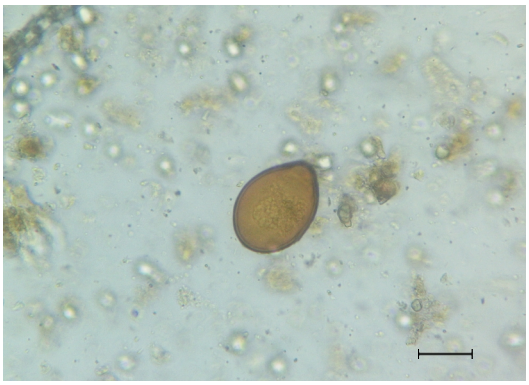


Figure 26: *Nyctotherus* sp. cyst within a flotation from a stimson's python (*Antaresia stimsoni*). (400x). Bar = 30 µm. Original.

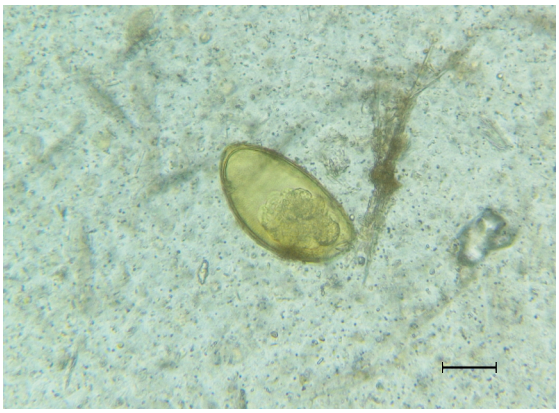


Figure 27: Unidentified oxyurid egg within a direct smear from a black headed python (*Aspidites melanocaphalus*. (400x). Bar = 30 µm. Original.

Figure 28: Pentastomida embryonated egg within a flotation from a woma python (*Aspidites ramsayi*). (400x). Bar = 30 µm. Original.

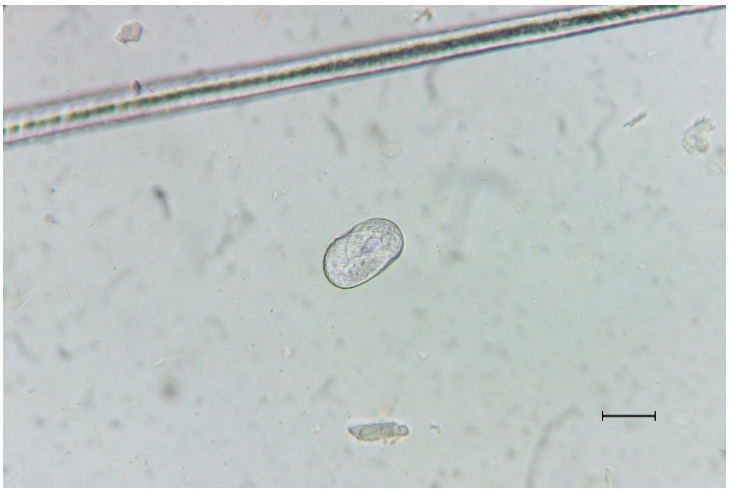


Figure 29: *Strongyloides*-like embryonated egg within a flotation from a black headed python (*Aspidites melanocephalus*). (400x). Bar = 30 µm. Original.

Table 2: Number of positive snakes infected with different endoparasites.

Scientific name (Number of snakes)	Number (%) of Positive Animals	Endoparasite
Aspidites melanocephalus (3) *Pseudonaja affinis* (1) *B. c. constrictor* (1) *Aspidites ramsayi* (1)	6 (13,04%)	Oxyurid eggs
Aspidites melanocephalus (3)	3 (6,52%)	*Strongyloides*-like eggs
Acanthophis antarctius (1) *Morelia spilota imbricata* (1)	2 (4,35%)	Acarid eggs
Antaresia stimsoni (1)	1 (0,02%)	*Nyctotherus* spp. cysts
Aspidites ramsayi (1)	1 (0,02%)	Pentastomida eggs

7 DISCUSSION

7.1 SURVEY ANALYSIS

Information on each specimen's origin, husbandry related details (feeding habits, temperature within the vivarium and handling routines), cleaning and disinfection protocols and antiparasitic treatment was acquired through the survey completed by all the owners and breeders participant in the present study.

7.1.1 Specimens' origin

Survey analysis shows that a considerable number of specimens (30, corresponding to 53%) were previously captured from the wild, which is a common method for breeders and sellers to acquire new reptiles in Australia. As not all reptile keepers de-worm their animals prior to selling, capturing reptiles from the wild may increase the parasitic prevalence in those specimens, particularly if adequate quarantine measures and anti-parasitic treatment are not employed. These infections may even be transmitted to other animals in the collection if de-contamination procedures are not executed when dealing with different animals (Rossi, 2006). All remaining specimens (27, corresponding to 47%) were bred in captivity and acquired from specialized breeders. There were no specimens acquired from pet shops, which would be a probable source for reptiles in poor health and welfare condition due to poor quality enclosures and transportation methods (failure to provide an overall decent general husbandry is the main cause of disease in captive reptiles (Griffinn & Reavill, 2014)).

The expected results were that pathogenic parasites would be more common in wild caught specimens than in captive bred ones, despite the parasitic burden being higher in captive reptiles in cases of improper sanity measures (Klingenberg, 2004; Jacobson, 2007). A total of 11 wild caught specimens provided samples containing parasitic forms, five of those pathogenic. A total of seven captive bred specimens provided samples containing parasitic forms, only one of those being pathogenic. The obtained parasitic prevalence in relation with the specimen's origin was as expected.

7.1.2 Husbandry and sanitary protocols

According to the questionnaire, the majority of participants appear to have good knowledge of their reptile's requirements and to always provide them with the adequate conditions. A small number of participants, corresponding to 14% of the total samples analysed, didn't employ ideal husbandry and vivarium conditions. Provided that most specimens belonged to

experienced owners, it was expected and verified that a large number of animals were kept in optimum conditions.

The number one cause of poor husbandry in cases analysed for this study was handling stress, in seven of the eight cases. In these cases, the animals were used for exhibition and demonstrations. Despite all the process being handled with complete humanity and with the best care for the reptile's welfare, excessive handling is a relevant stress factor that can depress the immune system and allow for commensal parasite levels to flare up or for opportunistic infections to settle in (Klingenberg, 2004; Rossi, 2006).

The remaining participant failed to provide adequate temperatures and lighting, which decreases the physiological metabolism, including the digestive system's activity. A direct cause of a reduced metabolism is gastrointestinal stasis, which creates an optimum environment for pathological microorganisms to grow and multiply. In the same case, cleaning and disinfecting protocols were inadequate, facilitating multiplication and propagation of pathogenic organisms (Klingenberg, 2004; Rossi, 2006).

Pathogenic parasites were present in samples belonging to three animals with good husbandry and sanitary conditions, although only one of these cases presented clinical signs. The absence of clinical signs is expected in these cases, as the adequate cleaning and hygiene processes help eliminate parasitic forms from the environment, thus preventing re-infections, and good husbandry and vivarium set ups allow the reptile's immune system to be more active and responding, limiting infections before heavy parasitic loads are achieved (Klingenberg, 2004; Rossi, 2006).

Pathogenic parasites were present in the faecal analysis of two specimens with a history of poor husbandry, which is an expected result. Commensal parasites were found in the faecal analysis from three specimens with a history of increased stress levels, which has also been described in the literature (Klingenberg, 2004; Rossi, 2006). Methods to evaluate the parasitic load, such as McMaster's egg count procedure, where not employed in the present study, although it could have been pertinent to evaluate whether these samples contained an excessive number of commensal parasites.

7.1.3 Anti-parasitic therapy

On the subject of anti-parasitic treatment and its frequency, the survey showed that in 49% (28) of the samples an adequate treatment was instituted and up to date. In the absence of clinical signs of disease, it was expected to find no pathogenic parasites in samples belonging to these specimens. This hypothesis was verified, as the parasitic forms found in eight of these specimens consisted of commensal or pseudo-parasitic organisms from prey origin.

A relatively large number of specimens (37%) were not correctly de-wormed. There are many possible reasons why not all animals received adequate anti-parasitic treatment. A highly probable cause would be that many reptile keepers underestimate or are not aware of the role of parasitic infections in captive animals. In some cases, unexperienced owners neglect to provide veterinary care to their pet reptiles, which explains the lack of knowledge regarding their pet's health and welfare.

7.2 OVERALL PARASITIC PREVALENCE

Parasitic forms were found in a total of 18 (32%) samples, six of these cases in lizards and 12 in snakes. Only five of the 18 parasitized samples represent pathologic threat, which is a remarkable result considering the amount of cases that didn't receive anti-parasitic treatment (37%). The remaining 39 samples were parasite-free at both faecal smear and faecal flotation analysis.

7.2.1 Lacertilia

Regarding lizards in this study, 54,5% of the analysed samples contained one or more parasitic forms.

The most common parasitic forms encountered in the present study were oxyurid eggs, in 45,45% of the analysed samples. A high prevalence of oxyurids was expected by comparison with the work done by Rataj et al. (2011), where oxyurid eggs were found in 57.1% of all lizard samples. In a different study by Bernardino (2014), faecal samples belonging to a population of *Eublepharis macularius* (leopard gecko) were screened for gastrointestinal parasites, and the results indicate an estimated prevalence of oxyurids between 61,1 and 85,9%.

According to the bibliography, oxyurids are indeed the most common parasites of reptiles. They are considered commensal organisms, but in cases of heavy infestations they are known to cause intestinal obstructions or rectal prolapses. Considering the parasite's direct life cycle, it is easy for this organism to continuously re-infect the host in the limited space to which reptiles are confined in a captive environment (Klingenberg, 2004; Greiner & Mader, 2006, Jacobson 2007).

The number of oxyurids per sample in this study didn't appear to be excessive, even in stressed specimens, although quantitative methods were not employed in this study and only through that sort of methods would be possible to accurately quantify the parasitic load in each animal.

Acarid eggs were found in one western blue tongue skink (9,09%), possibly corresponding to *Ophionyssus natricis* species, which is a common mite of blue tongues (Jacobson, 2007). This specimen was wild caught and its anti-parasitic therapy history was unknown, which places it in a good position for harbouring this sort of parasites.

Ascarid eggs occurred in a sample from a captive bred western blue tongue skink, corresponding to 9,09% of the studied lizard population. This is not an unexpected result, as Rataj et al. (2011) registered a prevalence of 6,9% in their study. This lizard was frequently used for demonstrations and handled by a great number of people, which increases stress levels and affects the immune systems' ability to terminate infections (Klingenberg, 2004; Rossi, 2006). Ascarids are usually transmitted through ingestion of parasitized intermediate hosts (Klingenberg, 2004) and this blue tongue skink was often fed snails captured from a school's backyard, which, combined with the absence of anti-parasitic therapy, may explain how a captive bred lizard became infected with ascarids.

Finally, *Nyctotherus* sp. cysts occurred in a northern blue tongue skink, which represents 9,09% of the analysed lizard samples. The results from the present study are closer to the 10,0% reported by Rataj et al. (2011) than to the 22,4% reported by Bernardino (2014). In both referred studies and in the present work, the prevalence of *Nyctotherus* sp. was lower than the prevalence of oxyurids, despite both having a direct life cycle. The results may be related with the greater fragility of protozoan in comparison with the oxyurid nematode (Zajac & Conboy, 2012).

As *Nyctotherus* sp. is a common commensal protozoan of herbivorous lizards, it is generally not associated with clinical signs of disease, although heavy loads may occur in the absence of adequate sanitary conditions and cleaning processes (Jacobson, 2007). This lizard showed no signs of disease, displaying natural behaviour at all times.

7.2.2 Ophidia

Concerning the snakes involved in the present study, 26% produced samples containing one or more parasitic forms. Only in four cases were the referred parasites considered to have a pathogenic nature.

Oxyurid eggs were the most common parasitic form encountered in snakes in this study, present in 13,04% of the analysed samples. Oxyurids found in snakes are more often rodent oxyurids, acquired through ingestion of infested prey, therefore being considered pseudo-parasites. In either case, oxyurids develop a commensal relationship with their host and only in cases where heavy infections develop, may they be responsible for causing intestinal

obstructions or rectal prolapses (Okulewicz, A., Kaźmierczak, M. & Zdrzalik, K., 2014; Klingenberg, 2007b).

Strongyloides-like eggs were found in samples from three black headed pythons, showing a prevalence of 6,52%. This parasitic prevalence was not surprising, not only because it is in concordance with the results reported by Rataj et al. (2011), but also due to the suggestive clinical signs two of the affected ophidians displayed at the moment of sample retrieval.

In one case, the wild caught python was donated to the breeder in very poor body condition, showing anorexia, muscle loss and severe cachexia, coming from a background of poor husbandry and inadequately low temperatures within the vivarium. The faecal analysis contained several *strongyloides*-like eggs and a great number of pinworms. *Strongyloides* spp. is known to cause anorexia and other unspecific clinical signs and its direct life cycle combined with poor husbandry and cleaning methods allows for an exponential parasitic multiplication and re-infection. The pinworm infestation was most likely from prey origin, since the previous owner had been force-feeding fresh-killed rodents as a mean to stimulate the snake's appetite (Greiner & Mader, 2006; Jacobson, 2007).

A different wild caught black headed python presented with anorexia and weight loss and it was verified that the faecal samples contained *strongyloides*-like eggs as well. The third python was also captured from the wild and showed no clinical signs of disease at the time of this trial.

Acarid eggs were found in two snakes (4,35%) from different species, a python and an elapid. It is common and described in the literature, that when snakes are being fed rodent prey, rodent parasites may pass in the snake's faeces, which has probably been the case with some these specimens (Klingenberg, 2007b).

Nyctotherus spp. cysts occurred in samples from a stimson's python (0,02%); as mentioned before, these are commensal organisms and in most cases do not cause disease in snakes (Jacobson, 2007).

Pentastomida embryonated eggs were present in samples from a wild caught woma python (0,02%). This python had never been de-wormed, nevertheless, it showed no clinical signs of disease at the moment of the analysis. It is possible for a snake to withstand the infection when parasitic load is low and husbandry and sanitary protocols are optimum (Jacobson, 2007; Paré, 2008).

The reported cases draw attention to the importance of knowing each animal's specific requirements and providing the adequate environment conditions to allow for physiological processes to develop naturally, as well as avoiding stress, immunological depression and disease. Occurrences like these also corroborate the fact that wild caught specimens are

capable of maintaining a healthy condition despite the parasitic infestation, whereas captive reptiles' health can be readily compromised for all the reasons described above (Klingenberg, 2004; Jacobson, 2007).

Neither pathogenic protozoans, nor flagellates were found in this study, possibly due to the techniques employed or to the quality of the samples analysed. In fact, a number of factors determined that most samples retrieved were not as fresh as recommended. The distance between the clinic and the sample retrieving places, as well as the fact that reptiles don't defecate at a regular rate were the main factors implied. The low defecation rate was aggravated by the decreasing temperature and, therefore, decreased reptile activity during the collection period. As mentioned before, it is important to analyse fresh samples in order to evaluate the presence of protozoan and flagellate parasites, as these microorganisms lose the ability to move (which aids in their identification) and degenerate quickly over time (Klingenberg, 2004; Greiner & Mader, 2006).

Limited timespan and resources didn't allow for the specific *Cryptosporidium* and *Amoeba* tests to be performed, as recommended by Pasmans et al. (2008) for quarantined animals in large collections.

8 CONCLUSION

An overall reduced parasitic prevalence of 32% was registered in the present study. Regarding pathogenic organisms, parasitic prevalence corresponded to 27,8% of the parasitized samples. Ophidians were better represented in this study, with only 26% of the examined snakes being parasitized, whereas the number of parasitized lizards corresponds do 54,5% of the participant lizard population. Oxyurid eggs were the parasitic form most commonly encountered, both in ophidians and lacertilians.

This low parasitic prevalence may be justified by the large number of specimens that were kept in the adequate conditions and received anti-parasitic therapy, but it would be interesting to perform extended analysis to increase the chance of finding intermittently shed parasites. The reduced parasitic prevalence doesn't necessarily mean that parasites were completely absent from the studied specimens, but that these parasites were not present in the analysed samples.

Regarding the participant's knowledge on their pet's basic requirements, the survey showed that only in a small number of cases (14%) did the owner fail to provide the adequate conditions, which was expected, since most specimens belonged to experienced reptile keepers. On the other hand, anti-parasitic therapy appears to be largely underestimated, as a reasonable amount of reptiles (51%) weren't adequately de-wormed. These results show that the majority of the participants are responsible and informed owners, but still manifest some reticence in providing veterinary care to their pets.

9 FINAL CONSIDERATIONS AND FUTURE RESEARCH

During the bibliographic search for this project, the author found a number of research papers regarding parasitological findings in wild reptiles throughout Australia and surveys for parasites in some reptile species, but no information was found on parasites in pet reptiles. To the author's best knowledge this was the first attempt on characterizing the parasitic prevalence in pet reptiles in the area of Perth, Western Australia.

Considering the increasing popularity of reptiles as pet animals and their growing contact with humans, a lot of work needs yet to be conducted regarding parasites in pet lizards and snakes. From a public health perspective, it would be important to explore aspects concerning zoonotic parasitic infections, how to prevent these infections, how to diagnose them on a routine basis and how they can affect the reptile's owner.

Further research needs to be performed on the matter of co-relating husbandry principles, vivarium conditions and stress levels with the occurrence of infectious diseases, as well as other non-infectious pathological processes.

Despite the favourable results in the present study, the importance of knowing each reptile's particular habitat, temperature, food and health requirements is still overly underestimated by many reptile owners, as is the practice of routinely taking the pet reptile to a veterinary consult - this results in a vast number of uninformed reptile owners who fail to provide the adequate conditions to their pet. The vast majority of diseases in pet reptiles can be prevented if the animal is kept in the adequate conditions. Furthermore, the affluence of terminally ill reptilian patients would be drastically reduced if the owners were well informed and aware of the subtle signs of disease manifested by reptiles.

Additional research needs to be conducted in order to identify and characterize parasitic infections of pet reptiles, establish the most common parasites in each genus and determine which parasites represent pathological threat. In order to get accurate results, more applied and fundamental research is demanded, with better resources and a more representative group of reptiles.

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Appendix I – **Approved Reptile Keeping List (Department of Parks and Wildlife)**

— PART 1 —

ENVIRONMENT

EV301*

Wildlife Conservation Act 1950

Wildlife Conservation (Reptiles and Amphibians) (Pet Herpetofauna) Notice 2013

Made by the Minister under the *Wildlife Conservation (Reptiles and Amphibians) Regulations 2002* regulation 4.

1. Citation

This notice is the *Wildlife Conservation (Reptiles and Amphibians) (Pet Herpetofauna) Notice 2013*.

2. Commencement

This notice comes into operation as follows —

(a) clauses 1 and 2 — on the day on which this notice is published in the *Gazette*;

(b) the rest of the notice — on the day after that day.

3. Pet herpetofauna declared

For the purposes of regulation 4 the fauna of the class *Reptilia* or *Amphibia* listed in Schedule 1 are declared to be pet herpetofauna.

4. Notice revoked

The *Wildlife Conservation (Reptiles and Amphibians) (Pet Herpetofauna) Notice 2003* is revoked.

Schedule 1 — Fauna declared to be pet herpetofauna

[cl. 3]

1. Category 1

No fauna.

2. Category 2

Scientific name	Common name
Egernia napoleonis	South-Western Crevice Egernia
Gehyra variegata	Tree Dtella
Heteronotia binoei	Bynoe's Gecko
Litoria caerulea	Northern Green Tree Frog
Litoria moorei	Western Green Tree Frog or Motorbike Frog
Pogona minor minor	Western Bearded Dragon or Dwarf Bearded Dragon
Strophurus ciliaris	Northern Spiny-tailed Gecko
Strophurus spinigerus	Southwest Spiny-tailed Gecko
Tiliqua multifasciata	Centralian Bluetongue
Tiliqua occipitalis	Western Bluetongue
Tiliqua rugosa rugosa	Bobtail

3. Category 3

Scientific name	Common name
Antaresia stimsoni	Stimson's Python
Chelodina colliei	Oblong Tortoise
Ctenophorus reticulatus	Western Netted Dragon
Egernia kingii	King Skink
Litoria splendida	Magnificent Tree Frog
Morelia spilota imbricata	Southwest Carpet Python
Nephrurus levis	Three-lined Knob-tailed Gecko
Oedura marmorata	Marbled Velvet Gecko
Underwoodisaurus milii	Thick-tailed Gecko
Varanus caudolineatus	Stripe-tailed Monitor

4. Category 4

Scientific name	Common name
Antaresia perthensis	Pygmy Python

Scientific name	Common name
Aspidites melanocephalus	Black-headed Python
Aspidites ramsayi	Woma Python
Chelodina steindachneri	Flat-shelled Turtle
Chlamydosaurus kingii	Frilled Lizard
Heleioporus albopunctatus	Western Spotted Frog
Amphibolurus longirostris	Long-nosed Ta-ta Dragon
Liasis mackloti/Liasis fuscus	Water Python
Morelia spilota variegata	Northwest Carpet Python
Varanus acanthurus	Ridge-tailed Monitor
Varanus brevicauda	Short-tailed Pygmy Monitor
Varanus tristis tristis	Black-tailed Tree Monitor

5. Category *5*

Scientific name	Common name
Ancanthophis antarcticus	Southern Death Adder
Ancanthophis pyrrhus	Desert Death Adder
Ancanthophis wellsi	Pilbara Death Adder
Boiga irregularis	Brown Tree Snake
Liasis olivaceus	Olive Python
Morelia carinata	Rough-scaled Python
Notechis scutatus	Tiger Snake
Pseudechis australis	Mulga Snake
Pseudechis butleri	Spotted Mulga Snake
Pseudonaja affinis affinis	Dugite
Pseudonaja nuchalis	Gwardar
Varanus gouldii	Gould's Monitor
Varanus rosenbergi	Southern Heath Monitor

A. P. JACOB, Minister for Environment.

Appendix II – **Flotation Solutions**

Table 3: Flotation Solutions (adapted from Zajac & Conboy, 2012)

Flotation Solution	Specific Gravity	Advantages	Disadvantages
Sodium nitrate ($NaNO_3$) **Fecasol Saturated $NaNO_3$**	1.2 1.33	Floats common helminth and protozoa eggs and cysts	Distorts *Giardia* cysts rapidly; does not float most fluke and some unusual tapeworm and nematode eggs
33% Zinc sulfate ($ZnSO_4$)	1.18	Floats common helminth and protozoa eggs and cysts; preferred for *Giardia* and some lungworm larvae.	Less effective for flotation of common tapeworm eggs than others; does not float most fluke and some unusual tapeworm and nematode eggs
Saturated sodium chloride (NaCl)	1.2	Floats common helminth and protozoa eggs and cysts	Distorts *Giardia* cysts rapidly; does not float most fluke and some unusual tapeworm and nematode eggs
Saturated magnesium sulfate (Epsom salts)	1.32	Floats common helminth and protozoa eggs and cysts	Distorts *Giardia* cysts rapidly; does not float most fluke and some unusual tapeworm and nematode eggs
Sheather's sugar solution	1.25	Floats common helminth and protozoa eggs and cysts; preferred for *Cryptosporidium* oocysts; in general, causes less damage to parasite eggs and cysts than salt solutions	Does not float most fluke and some unusual tapeworm and nematode eggs; less sensitive than $ZnSO_4$ for *Giardia*; creates sticky surfaces

Appendix III – **Recommended antiparasitic agents used in reptile medicine (adapted from Klingenberg, 2007; Carpenter, 2013)**

Table 4: Recommended antiparasitic agents used in reptile medicine (adapted from Klingenberg, 2007; Carpenter, 2013)

Parasite	Drug	Dose	Comments
Amoebas, Ciliates, Flagellates	Metronidazole	20-50mg/kg PO q24-48h , 3 doses	Drug of choice. Avoid using in pregnant females and in cases of hepatic disease.
	Fenbendazole	50mg/kg/day PO for 3-5 days	Efficient against *Giardia* sp, flagellates and ciliates
Cestodes	Praziquantel	8mg/kg PO, SC ou IM; repeat in 14days	Potential for treating pentastomids
Coccidea	Trimetropim/sulfa	30mg/kg PO q48h until negative fecal analysis	Cover against concurrent bacterial infections. Avoid using in dehydration or renal dysfunction.
	Sulfadimetoxine	50mg/kg PO q24h for 3-5days, then every 48h until negative fecal analysis	Non efficient in amoebas. Efficient in *Giardia* sp. Avoid in dehydration or renal dysfunction
Nematodes	Fenbendazole	20-50mg/kg PO SID for 3-5days, repeat in 10days if necessary	Drug of choice
	Albendazole	50mg/kg PO	Efficient in treating ascarids. Avoid using in pregnant females and in renal or hepatic disease.
	Levamisole	5-10mg/kg SC or ICe. Repeat in 14days	Narrow range of safety. Avoid using in debilitated animals, in renal and hepatic disease.
	Ivermectin	0,2mg/kg SC or IM. Repeat in 14days.	Do not use in chelonians, crocodilians, indigo snakes or skinks.
	Pyrantel pamoate	5mg/kg PO. Repeat in 14 days. 25mg/kg PO q24h for 3 days	Indicated for ascarids, hookworms and pinworms.
Trematodes	Praziquantel	8mg/kg PO, SC ou IM; repeat in 14 days	Potential for treating pentastomids

Appendix IV – **Survey requested from all breeders and owners as a mean to assess husbandry measures and patient's history**

GASTROINTESTINAL PARASITES SCREENING IN PET REPTILES

SAMPLE ID

Owner:

Species:

Sample nº:

HISTORY

Origin:

(Breeder, petshop, wild-caught)

Deworming:

- <12m
- >12m
- Never

Antiparasitic agent used:

HUSBANDRY

Temperature:

- Hot zone:
- Cold zone:

Uv Light:

- Used: Type:
- Not used

Substract:

Cleaning Frequency:

Appendix V – **Number and species of lizards and snakes studied**

Table 5: Scientific name, common name and number of examined lizards.

Scientific Name	Common Name	Number of Studied Animals
Clamydosaurus kingii	Frill-necked Lizard	1
Egernia stokesii badia	Western Spiny-tailed Skink	1
Moloch horridus	Australian Thorny Dragon	1
Oedura marmorata	Marbled Gecko	1
Tiliqua occipitalis	Western Blue Tongue	3
Tiliqua rugosa rugosa	Shingle-back skink	1
Tiliqua scincoides intermedia	Northern Blue Tongue	1
Uroplatus phantasticus	Leaf-tailed Gecko	1
Varanus giganteus	Australian Monitor	1
Total		11
Number of examined species of lizards		9

Table 6: Scientific name, common name and number of examined snakes.

Scientific Name	Common Name	Number of Studied Animals
Acanthophis antarctius	Southern Death Adder	1
Antaresia maculosa	Spotted Python	1
Antaresia stimsoni stimsoni	Western Stimson's Python	10
Aspidites melanocephalus	Black Headed Python	10
Aspidites ramsayi	Woma Python	7
B. c. constrictor	Red-tailed boa	1
Liasis olivaceus olivaceus	Olive Python	1
Morelia spilota imbricata	Southwest Carpet Python	12
Morelia spilota variegata	Northern Carpet Python	2
Pseudonaja affinis	Dugite	1
Total		46
Number of examined species of snakes		10

Appendix VI – **Sheather's sugar solution**

Sheather's sugar solution (spg 1.2–1.25) (Adapted from Zajac & Conboy, 2012)

1. Combine 355 ml of water and 454 g of granulated sugar (sucrose).
2. Dissolve the sugar in the water by stirring over low or indirect heat (e.g., the top half of a double boiler).
3. After the sugar is dissolved and the solution has cooled to room temperature, add 6 ml formaldehyde USP to prevent microbial growth (30 ml of 10% formalin can also be used, with the volume of water reduced to 330 ml).
4. Check the SPG with a hydrometer.