

**SURVEY OF GASTROINTESTINAL**  
**PARASITES IN TORTOISES IN THE**  
**UNITED KINGDOM**

**Submitted in part fulfilment of the requirements for the  
Royal College of Veterinary Surgeons (RCVS) Diploma  
in Zoological Medicine**

**2012**

**Word count 9683 words**

# **Contents**

<b>Introduction</b>	3-26
<b>Materials and Methods</b>	27-30
<b>Results</b>	31-45
<b>Discussion</b>	46-52
<b>References</b>	53-63
<b>Acknowledgements</b>	64
<b>Appendix 1: Examples of reference pictures for tortoise parasites</b>	65-66
<b>Appendix 2: Faecal Parasitology Protocols</b>	67-68
<b>Appendix 3: Questionnaire data</b>	69-76

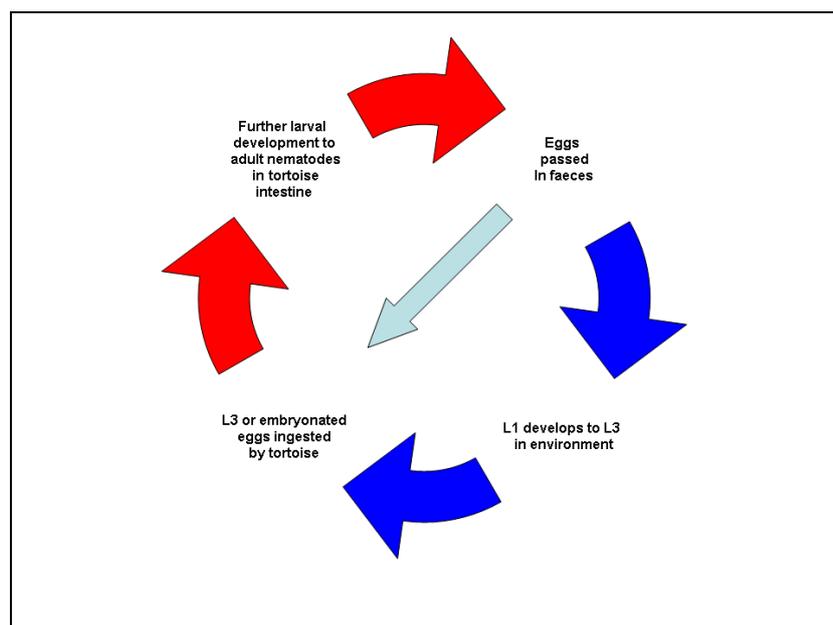
## Survey of gastrointestinal parasites in tortoises in the UK

Gastrointestinal parasites are commonly diagnosed on routine faecal screens of terrestrial pet chelonians. There are many types of gastrointestinal parasites, but broadly they may be divided into helminths and protozoa. Helminths may be further subdivided into trematodes, cestodes and nematodes. Trematodes and cestodes appear to be rarely reported, probably due to the fact that most tortoises' husbandry in the UK ensures that they are unlikely to come into contact with the intermediate hosts which these parasites require in order to complete their life cycle. Nematodes, in contrast, are commonly reported with oxyurids, ascarids, hookworms and strongyles all appearing in the literature, although their significance is uncertain.

### Gastrointestinal nematodes in tortoises

Most of the gastrointestinal nematodes reported in tortoises (with the exception of Atractidae) share the same direct life cycle (see **Figure 1**). Understanding of this is important in order to make decisions on the most appropriate methods of diagnosis and control.

**Figure 1: Basic outline of a direct nematode life cycle**



Eggs are passed in the faeces and these may either hatch in the environment or after ingestion by the host. Temperature and humidity are important factors in determining time of hatching with temperatures in the range of 18-26°C and 100% humidity being optimal for many nematodes. At lower temperatures, this process will slow and below 10°C cease altogether. Once hatching has occurred larvae develop, with the first two larval stages being dependent on bacteria as a food source. Either free living L3 or embryonated eggs (depending on nematode species) are then ingested by the host, and further larval development to mature egg-producing adults occurs within the host gastrointestinal system [Urquhart 1996, Frank 1981].

The prepatent period has not been established for many of these nematode species and may be complicated in tortoises by the effect of hibernation on the nematode life cycle. Oxyurids have been found to pass through the hibernation period, probably at an arrested larval stage, and a rise in oxyurid eggs excreted in faeces has been reported after hibernation especially in young animals [Capelli 1998].

In mammals, hypobiosis (arrested larval development) is a known feature of the life cycle in many nematodes. Depending on climate and geographical location this may occur in winter or during particularly dry periods. A periparturient rise in eggs is also common, usually in the spring time and is thought to occur due to the effect of elevated prolactin levels on temporarily reducing host immunity. This may also be augmented by the maturation of hypobiotic larvae [Urquhart 1996]. However, considering the reproductive differences in reptiles the periparturient rise is unlikely to be relevant, although hypobiosis does appear to occur [Capelli 1998].

### **Oxyurids (pinworms)**

There are a wide variety of oxyurids reported in tortoises including *Oxyuris*, *Tachygonetria*, *Alaeuris*, *Ortleppnema*, *Hemdiella* and *Thaparia* spp. [Wilkinson 2004]. In addition, the development of diagnostic techniques in recent years, especially electron microscopy, has resulted in the discovery of new species and reclassification of previously defined species [Bouamer 2001, 2006]. From a clinical viewpoint however, there

is no evidence that individual species differences are significant in terms of their pathogenicity or any beneficial effects so they will not be discussed further.

Adult worms are small-medium sized nematodes measuring 1.5-7mm in length [Mitchell 2007], with a whitish appearance [Martinez-Silvestre 2011]. They are located in the large intestine, where they feed on intestinal contents. Their life cycle is direct, as previously described, and infection of the host usually follows ingestion of an embryonated egg [Mitchell 2007].

Ova are variable in morphology with different stages of embryonation often seen (see **Figures 2-4**). In general, however, they measure ~ 130µm x 40µm and may appear either oval or asymmetrical (D-shaped). The surrounding shell may also be variable in diameter, but lacks the scalloped surface and very thick walls typical of ascarids. Opercula may be present at one or both ends [Thapar 1925].

Eggs hatch in the small intestine, where larvae develop and then migrate to the large intestine where adults may be found. The complete life cycle is thought to take approximately 40 days based on observations in other reptiles [Frank 1981]. Visceral larval migration is not reported. Oxyurids are generally considered to be commensals within the chelonian intestinal tract and have been suggested to have a beneficial effect in churning up faecal matter and so prevent constipation [Telford 1971]. However, high numbers of oxyurids have also been associated with anorexia [Martinez-Silvestre 2011] and even deaths post-hibernation, possibly due to their effect on depriving their host of nutrition [Frank 1981].

### **Ascarids (roundworms)**

*Angusticaecum holopterum* is the most common ascarid reported in tortoises [Holt 1979]. Adult worms may be large, measuring up to 10cm in length [Frank 1981], and often have a pale appearance [Schneller 2008]. They may be found attached to intestinal mucosa and feed on mucosal fluid, products of host digestion and cellular debris. Exact details of the life cycle are unknown but it appears to be direct, and infection of the host follows ingestion of an egg. Ova are typically round, thick-shelled and measure 80-100µm x 60-80µm (see **Figure 5**) [Jacobson 2007]. However, one important difference is that in

some nematode species, larvae may migrate through the viscera [Sprent 1980] resulting in pathology. In one report an adult nematode (*Angusticaecum* spp.) was removed from an aural swelling in a *Testudo graeca* [Cutler 2004].

Generally, however, they are unlikely to cause disease in low numbers, although gastrointestinal obstruction by adult nematodes has been reported [Keymer 1978]. Other pathological effects including intussusception, gastrointestinal ulceration, coelomitis, thromboembolism and avascular necrosis have also been associated with ascariasis [Frye 1991].

Ascarids from other species such as *Toxocara canis* have been experimentally inoculated into *Testudo graeca*, but do not reproduce or appear to have any pathogenic effects in tortoises at normal environmental temperatures. Interestingly, when exposed to a constant environmental temperature of 37-38°C, tortoises' natural resistance to *Toxocara* was eliminated [Merdivenci 1965], but this is unlikely to be relevant for tortoises in the UK.

### **Atractids**

*Atractis dactyluris*, a viviparous nematode, has been incidentally detected at low numbers in tortoises [Holt 1979]. Adult worms measure 4-6mm in length and are characterised by six distinct lips around the buccal opening. Unlike other more common tortoise nematodes, ova hatch in utero, releasing third stage larvae which are passed in faeces. Their lifecycle is direct with animals being infected through ingestion of larvae, but internal re-infection is also possible, leading to the potential rapid multiplication of parasite numbers [Thapar 1925].

*Proatractris* is a similar nematode, but has also been reported in *Geochelone carbonaria* and *G. pardalis*, to cause significant morbidity and mortality. Pathology included increased submucosal lymphoplasmacytic infiltration in the *lamina propria* of affected caecum and colon and in some cases extensive necrosis of the colonic mucosa. Treatment with piperazine and fenbendazole was unsuccessful although dosages were low [Rideout 1987].

## **Other gastrointestinal nematodes in tortoises**

Strongylids (hookworms) have been described in tortoises. Examples include *Chapiniella* in *Gopherus polyphemus* [Lichtenfels 1981] and *Camallanus* in *Testudo hermanni*, *T. graeca* and *T. horsfieldii* [Rataj 2011], but detailed descriptions of life cycle or pathogenicity in these species are lacking. It is thought that adult worms may attach to intestinal mucosa resulting in intestinal damage and potential anaemia [Frye 1991]. Ova are thin-walled, bluntly-rounded at the ends, with the developing embryo filling most of the shell (see **Appendix 1**) [Greiner 2006].

## **Other helminths in tortoises**

### **Acanthocephalans (thorny-headed worms)**

Acanthocephalans (thorny / spiny headed worms) have been described in *Terrapene carolina* but are usually reported in aquatic or semi-aquatic chelonians [Jacobson 2007]. Adult worms are identified by the presence of a proboscis covered in rows of hooks, which are used to attach to the host intestinal wall. Their lifecycle is generally indirect with arthropod or crustacean intermediate hosts. Ova have a characteristic 3 layered appearance and contain larvae (see **Appendix 1**) [Frye 1991].

### **Trematodes**

Trematodes (flukes) are also usually reported in aquatic or semi-aquatic chelonians, although have been identified in *Testudo graeca* and *T. marginata* [Rataj 2011]. They are suggested to be relatively non-pathogenic in tortoises, although potentially migration of flukes could result in granulomatous inflammation and death has been reported in aquatic chelonians [Johnson 1998]. Their life cycle is indirect with an intermediate host required in order to replicate, so are rarely encountered in captivity. Ova are yellow or orange in colour, thin shelled and often operculated. Diagnosis generally requires specialised faecal sedimentation techniques [Wilkinson 2004].

## **Cestodes**

Cestodes (tapeworms) are also usually reported in aquatic or semi-aquatic chelonians although have been identified in *Testudo graeca* [Rataj 2011]. They are suggested to be non-pathogenic and have an indirect life cycle, requiring an intermediate host in order to replicate. Proglottids filled with eggs are normally passed in the faeces and individual ova may be characterised by their variable number of refractile hooklets [Frye 1991].

## **Protozoa in tortoises**

The significance of protozoa in chelonian faecal samples appears even more uncertain, with some authors suggesting that all reptiles will harbour protozoa of some kind and that in a natural state these organisms are unlikely to be pathogenic [Keymer 1981]. In captivity however, there are various reports of disease in reptiles associated with high protozoal burdens [Scullion 2009] and a few examples will therefore be discussed.

## **Amoebae**

Amoebae are a group of protozoa, distinguished by the presence of their pseudopodia. Their life cycle is direct with reproduction occurring asexually [Scullion 2009]. Infection occurs by ingestion of a cyst, which develops into a motile trophozoite within the intestinal tract. These multiply by binary fission, either invading the mucosa directly or forming cysts which are passed in faeces [Lane 1996]. Various species (including *Hartmanella*, *Acanthamoeba*, *Entamoeba*, and *Endolimax*) may be present within the chelonian gastro-intestinal tract but are typically considered non-pathogenic [Wilkinson 2004].

The main exception is *Entamoeba invadens*, an important gastro-intestinal pathogen of many reptile species. Originally it was believed that this parasite was a commensal in the intestinal tract of herbivorous reptiles, with initial reports of disease described only in carnivorous species of snakes and lizards. Herbivorous chelonians were thought to be just carriers of this disease and never clinically affected. It was

suggested that they were resistant due to the plant material in their diet which provided starch necessary for the amoeba to encyst, rather than invading the intestinal mucosa [Lane 1996]. Only giant tortoises were originally thought to be susceptible [Klingenberg 1993]. More recently however, a variety of reports have described fatal amoebiasis in a variety of omnivorous and herbivorous tortoises including *G. carbonaria* [Jacobson 1983], *G. denticulata*, *G. sulcata*, *Gopherus polymerus* [Hollamby 2000], *Acinixys planicauda* [Ozaki 2000] and *Geochelone pardalis* [Philbey 2006]. Juvenile tortoises appeared more susceptible [Hollamby 2000], and both lack of food within the gastrointestinal tract [Jacobson 1983] and environmental temperature appear to play a role in parasite proliferation [Barrow 1960]. A variety of clinical signs were described, but mucoid dysentery appeared characteristic in most cases, with associated anorexia, wasting, dehydration and eventual death. On post-mortem, many animals were found to have ulceration throughout their intestinal tract, especially within the duodenum and colon. Other pathological changes include abscessation and areas of necrosis within many visceral organs, hepatitis, nephritis, and myonecrosis. Disease appeared to spread rapidly due to the parasite's direct life cycle and is difficult to eradicate fully due to the resistance of both the amoeba and cysts which can survive in the environment for over 14 days at 8°C [McConnahie 1955].

Diagnosis of amoebiasis is possible by identification of amoeba, trophozoites (9-38.6 µm) or quadrinucleated cysts (9-24 µm) in a fresh faecal smear (**see Appendix 1**). It may be difficult however, to differentiate these cysts from those of other non-pathogenic amoeba, with both false-positive and false-negative results common [Keymer 1981]. Multiple cloacal flushes and even in vitro culture are therefore recommended if disease is suspected [Cranfield 1999].

## **Flagellates**

Intestinal flagellates are generally thought to be non-pathogenic in chelonians, although it has been suggested that they are more commonly found in the faeces of sick chelonians [Wilkinson 2004]. Some authors in contrast, suggest that excessive numbers of flagellates may actually be a cause of anorexia and diarrhoea [Bone 1992]. The typical flagellate life cycle is direct with reproduction occurring asexually by binary fission.

Various species may be present within the chelonian gastro-intestinal tract, but trichomonads appear the most common in the literature [Schneller 2008].

It is important however, to differentiate these “commensal” intestinal flagellates from the pathogenic flagellate *Hexamita parva*, which can result in fatal renal disease. Disease has been described in a wide variety of tortoises including *Testudo horsfieldii* and *T. marginata* [Zwart 1975], but appears to be uncommon in the UK [Wilkinson 2004]. Infection probably occurs by ingestion of an infective cyst, which passes through the gastrointestinal tract and via the cloaca up the ureters to the kidneys where the parasite encysts. Transmission is thought to be via the urine. Clinical signs of disease include anorexia, weight loss and polydipsia. Disease may be suspected by detection of the protozoa with its characteristic six flagella within a fresh urine sample (or urine mixed with faecal sample), but this may be difficult to differentiate from trichomonads and as with other flagellates, will rapidly desiccate and die in small samples. Definitive diagnosis requires detection of the parasite on renal biopsy. Characteristic post-mortem findings include nephritis and in some cases enteritis and infection of the bile ducts [Zwart 1975].

### **Coccidia (excluding *Cryptosporidium*)**

Coccidia are small protozoal parasites, characterized by the intracellular nature of their life cycle. An infective oocyst is ingested and within the gastro-intestinal tract sporozoites are released. These invade epithelial cells, developing into trophozoites, before undergoing schizogony (asexual reproduction) and then gametogony (sexual reproduction), resulting in the production of oocysts containing 4 more infective sporozoites [Barnard 1994, Scullion 2009].

In chelonians, over 30 species of coccidia have been isolated including *Eimeria* and *Caryospora* spp. [McAllister 1989]. Types of oocysts are generally differentiated by the number of sporocysts contained within an oocyst [Barnard 1994].

Infections are generally asymptomatic but may contribute to debility in sick animals. Outbreaks of enteritis have been reported due to *Caryospora cheloniae* in both captive and free-living *Chelonia mydas* [Leibovitz 1978, Gordon 1993]. Various cases of intranuclear

coccidiosis have also been described in a variety of tortoises including *Geochelone radiata*, *G. pardalis*, *Indotestudo forstenii*, *Chersina angulata* and *Manouria impressa*

[Jacobson 1994, Garner 2006, Innis 2007, Schmidt 2008].

Clinical signs of intranuclear coccidiosis appeared variable but included anorexia, lethargy, wasting and ocular/nasal discharges. Diagnosis was made post-mortem on histopathology of multiple tissues, although the exact type of coccidia and route of infection was uncertain [Garner 2006]. No oocysts were detected within faecal samples of affected tortoises, so it is unlikely that this is a parasite likely to be seen on faecal screens [Innis 2007].

## Ciliates

*Balantidium* and *Nyctotherus* are commonly found ciliates, which have both been suggested to be commensals of the gastrointestinal tract in tortoises helping digest cellulose [Frye 1991]. An increased number may be detected at times of gastrointestinal disturbance and have been suggested to cause colitis [Bone 1992] but as with many protozoa, it is unclear if they were the inciting factor or increased in number as a consequence of intestinal disease. *Balantidium* has also been described in the liver of heavily infected tortoises, associated with abscesses [Schneller 2008].

Both protozoa share a similar direct life cycle. They reproduce by sexual reproduction with conjugation of two ciliates and exchange of micronuclei. The “new” ciliates then divide by binary fission [Lindsay 2007]. The exact details of reptile ciliate life cycles are unknown, but transmission is suggested to be via an infective cyst. This is ingested, excysts in the small intestine and produces trophozoites. These colonise the large intestine where they replicate and also form new infective cysts [Bosschere 2012]. The *Balantidium* trophozoite may be identified by its ciliate appearance and oval shape and measures 60 x 40-45µm. Cysts are round and measure ~ 55µm. *Nyctotherus* trophozoites are larger measuring 50-260 x 30-90µm. Cysts are ovoid, operculated and a similar size to the trophozoites (see **Appendix 1**) [Barnard 1994].

## **Detecting parasite infections in practice**

Clinical signs of parasite infection may vary from none to anorexia, diarrhoea, intestinal obstruction, weight loss, tenesmus, prolapses and even anaemia and death in exceptionally severe burdens [Wilkinson 2004].

Diagnosis of an endoparasite burden is usually fairly straightforward, by examination of a fresh faecal sample to detect ova, larvae, or protozoa. Fresh samples are best as protozoa may be inactive in older samples and eggs may have hatched, resulting in larvae which are difficult to identify. Alternatively faeces may be stored in a fridge to prevent eggs hatching [McArthur 2004].

Various different techniques have been described, but the most common is a direct smear. This usually involves mixing a small amount of faeces with a similar volume of warmed saline and applying a coverslip. This technique is particularly useful for identifying motile protozoa, which could otherwise be difficult to spot. It will also detect moderate to heavy nematode burdens.

For less severe nematode burdens, or to quantify egg counts, flotation methods may be used to concentrate ova. The principle is that eggs should be less dense than the flotation media so should float to the top (with the exception of trematode eggs which are heavier). A variety of flotation solutions may be used with the most common being saturated sugar solutions, saturated salt solutions and zinc sulphate.

Alternatively faecal centrifugation or sedimentation methods may be performed and may be useful for the detection of trematode ova [Wilkinson 2004]. Various stains are also available to aid in parasite identification [Klingenberg 2000]. None of these methods however, were used in this study so will not be discussed here further.

It is also important to note that, although ova may be detected by the above methods, definitive species typing usually requires examination of an adult worm, which is often difficult to obtain ante-mortem [Urquhart 1996].

## **Treatment of parasite infections in tortoises**

### **Nematode treatment**

Even if treatment is deemed necessary, there is scarce literature on the efficacy of available treatments on gastrointestinal parasites in chelonians. The only general consensus is to avoid the use of ivermectin in the treatment of chelonians, as it has been found to be toxic in some chelonian species, resulting in paresis, flaccid paralysis, hepatic lipidosis and death. This is likely to be due to its action on GABA receptors and has been suggested to be due to either increased permeability of the blood-brain barrier in chelonians allowing it to reach the CNS, or to a higher dependence on peripheral GABA neurons [Teare 1983].

In contrast, milbemycin at 0.5-1mg/kg administered either as an oral suspension or by subcutaneous injection on day 1 and 8 did not appear to cause any deleterious reactions in one study involving *Trachemys scripta elegans* and *Terrapene carolina* [Bodri 1993]. This is surprising as milbemycin is believed to work in a similar way to ivermectin by binding GABA receptors and glutamate-gated chloride channels. However, data on efficacy is limited, and generally it is recommended that avermectins and milbemycin are avoided in chelonians [Jepson 2005]. Piperazine citrate treatments should also be avoided due to concerns of toxicity due to the citrate ions precipitating hypocalcaemia [Soifer 1978].

Levamisole is available as an oral medication on the Small Animal Exemption Scheme (Beaphar reptile wormer®) in the UK and is marketed as a “routine wormer to keep reptiles free from whipworm, hookworm and other roundworms”. The only recommended doses in the literature are anecdotal doses which generally range from 10mg/kg for intracoelomic or intramuscular injection [Jacobson 1983, Girling 2004] to 50-300mg/kg *per os* [Wilkinson 2004]. Generally the lower end of the dose range is recommended for chelonians. Levamisole works by interfering with nematode carbohydrate metabolism by blocking fumarate reductase and succinate oxidase activity. As a result, worms are paralysed and often expelled alive. Potential side effects in the host, range from gastrointestinal signs to nicotine-like effects e.g. salivation, muscle tremors, excitability and even death especially when administered

parenterally [Rees Davies 2004]. No studies have been published regarding safe or effective doses in tortoises and efficacy has been suggested to be unsatisfactory against tortoise ascarids and oxyurids [Wilkinson 2004].

The benzimidazoles are generally considered to be the group of drugs with the widest margin of safety and have been used to successfully treat a wide range of nematode infections in reptiles. They work by binding nematode  $\beta$ -tubulin, preventing the formation of microtubules and are effective against adult, immature, arrested larval and egg stages [Heggem 2008].

In the UK fenbendazole is the most commonly used treatment. Studies however have not been carried out to determine the most effective dose for treatment of parasites. This is reflected by the wide variation in suggested treatment regimes including: 50-100mg/kg given orally as a one-off treatment, repeat dosing 2-4 weeks later [Holt 1982] or divided over 3 days [Girling 2004]. Intracolonic use has even been described [Innis 2008]. Initially, fenbendazole was considered to have no significant side effects, but after an overdose caused death in Fea's vipers [Alvarado 2001], it has since been demonstrated that two courses of 50mg/kg fenbendazole, given daily for 5 days per course can cause profound leukopenia in *Testudo hermanni* [Neiffer 2005]. Repeated treatments given 2-3 weeks apart may be safer, but could be unnecessary as it can take up to 31 days for one dose of fenbendazole to have full effect at reducing egg counts. Recent work also indicates that oxfendazole appears to be faster acting in reducing egg counts than fenbendazole [Giannetto 2007]. In mammals fenbendazole is metabolised to oxfendazole but it is unknown if this conversion occurs in chelonians [Short 1987]. Oxfendazole has been suggested to have a wider safety margin than fenbendazole, but there is little evidence to support this theory. Suggested doses are 65mg/kg per os [Highfield 1996].

Alternative benzimidazole treatments have been advocated with varying success including mebendazole at 25mg/kg *per os* and thiabendazole at 55mg/kg per os [Soifer 1978], but doses are anecdotal, treatments are not readily available in practice and studies have not been carried out to assess their efficacy.

In recent years, some of the newer endoparasiticide spot-on formulations such as emodepside and praziquantel spot-on (Profender®) have also been trialled in

tortoises. Emodepside is one of a new class of anthelmintics, which acts against nematodes by stimulation of pre-synaptic receptors [Schilliger 2008]. Praziquantel works by inducing calcium ions to influx across the parasite tegument resulting in muscular spasm, changes in the metabolism and properties of surface membranes and also decreases enzyme activities in the parasite [Harnett 1988]. The spot-on combination was administered to *Testudo horsfieldii* weighing <100g at 21.5mg/kg and 85.5mg/kg respectively and appeared to effectively reduce egg counts to 14% of the initial egg count. Response was slow and a higher dose was suggested in juvenile tortoises. In tortoises weighing >100g, in contrast treatment was ineffective. This was postulated to be due to the reduced skin surface to body mass ratio and increased thickness of skin [Brames 2010].

Imidacloprid and moxidectin spot-on (Advocate®) has also been trialled in various reptiles including *Graptemys versa* at 2 – 10 times the recommended dosage for dogs. Treatment also appeared effective with no reported side effects [Melhorn 2005], but in view of the previous reports on avermectin toxicity and the alternative treatments available, caution is to be advised if considering using this in other chelonian species.

### **Trematode and cestode treatment**

Reports of treatment for trematodes and cestodes in tortoises are equally limited, but various praziquantel doses have been suggested ranging from 5-30mg/kg given either *per os* or by intramuscular injection [Wilkinson 2004].

A study in *Chelonia mydas* demonstrated the effect of 50mg/kg praziquantel, given *per os* three times over a 24 hour period against cardiovascular flukes [Adnyana 1997]. These doses however were probably excessive, with a later pharmacokinetic study demonstrating that 25mg/kg given *per os* three times, three hours apart was likely to be effective against flukes in the same species [Jacobson 2002]. Unfortunately most of the suggested treatment regimes would require hospitalisation which is not ideal for administering sequential oral treatments to a tortoise. Injectable praziquantel is no longer available commercially in the UK. In future however, the advent of more combined spot-on endoparasiticide treatments, such as emodepside-praziquantel (Profender®) may provide a more practical route for treating these infections, but further research is necessary in this area [Brames 2010].

Alternative drugs may also be effective against trematodes or cestodes in some cases, including various benzimidazoles or niclosamide. There is little evidence however, that these are useful in tortoises.

### **Protozoa treatment**

Treatment of protozoa is usually unnecessary, but may be considered if burdens are considered excessive, or clinical signs are associated with infection.

Metronidazole is the most commonly reported treatment and has been used in snakes and lizards at a variety of dosages including: 20mg/kg every 48 hours for > 2 weeks [Kolmstetter 1997], or 100mg/kg *per os* given as a single dose repeated 2 weeks later [Jacobson 1999]. The only study in chelonians used a dose of 20mg/kg administered via intracoelomic injection and several deaths were reported, but it was unclear whether this was related to adverse effects of metronidazole or coincidental [Innis 2007]. A disadvantage of metronidazole would be that although effective against amoebic trophozoites, it is not completely effective against amoebic cysts, so may need to be combined with an alternative drug in order to totally clear infection. Iodoquinol has been used both alone and in combination with metronidazole and has resulted in cessation of cyst shedding in the majority of cases at 50mg/kg once daily *per os* for 21 days [McArthur 2004].

Alternative anti-protozoal drugs used in chelonians include chloroquine (effective against amoebic trophozoites but not cysts) used at 50mg/kg weekly by intramuscular injection for 3 doses [McArthur 2004], paromomycin (effective against amoebic cysts) but with no reported doses in chelonians, or dimetridazole at 40mg/kg once daily *per os* for 5-8 days [Holt 1981].

Coccidial infections will, however, require alternative anti-protozoals, usually sulfa drugs such as trimethoprim sulfadiazine, which has been recommended at 15-30mg/kg once daily by intramuscular injection for 7 days [Lane 1996], or 25mg/kg once daily *per os* for 7 days [Girling 2004]. Anecdotally, toltrazuril has also been used for

treatment of intranuclear coccidiosis, but dosages or efficacy are not published in chelonian species [Wilkinson 2004].

### **General parasite treatment**

In addition, to specific anthelmintics for the individual tortoise, it is also important to consider other in-contact tortoises and the environment. If treatment is considered necessary for one individual, treatment should usually be initiated for all in-contacts to prevent immediate re-infection. Access to any intermediate hosts should also be prevented if the parasite has an indirect life cycle [McArthur 2004]. Good environmental hygiene is also advised; for an indoor enclosure this may involve a complete change of substrate and disinfection of enclosure and furniture. It should be noted, however, that most disinfectants have not been proven to have a direct effect on parasites, so the procedure of thorough cleaning and removal of faeces may be more important than the disinfectant chosen [Aycicek 2001].

Supportive treatment may also be necessary for the debilitated individual and any obvious problems in husbandry or diet should be corrected [Schneller 2008]. Repeat faecal samples after treatment to assess efficacy may be advised, although optimum timing has not been determined.

## *Cryptosporidium* in tortoises

*Cryptosporidium* is a small protozoal parasite, frequently encountered in a wide variety of wild and captive reptiles worldwide [Upton 1989]. Initially reported in snakes, it was found to cause hypertrophic gastritis, regurgitation, weight loss and death [Brownstein 1977], although enteritis without gastritis has also been described [Brower 2001]. In lizards, enteritis is the most common form, with associated anorexia, diarrhoea, weight loss and eventual death [Terrell 2003], although gastritis may also occur [Dillehay 1986, Oros 1998]. Less common presentations include aural polyps [Fitzgerald 1998], cloacal prolapse and cystitis [Kik 2011]. Alternatively an asymptomatic carrier state appears to be common [Deming 2008].

In chelonian species however, reports are scarce and little is known about the incidence or pathogenic effect of this parasite. *Cryptosporidium* was initially reported in *Geochelone elegans* with regurgitation [Heuschele 1986] and then a *Geochelone carbonaria* with progressive weight loss [Funk 1988], but was not confirmed to be the cause of clinical signs in either case. Infection has also been reported in *Clemmys muhlenbergi* [Graczyk 1996], *Chelonia mydas* [Graczyk 1997], *Geochelone radiata*, *G. elegans*, *Indotestudo spp.* and *Gopherus polyphemus* but again no association with clinical disease was confirmed [Raphael 1997]. More recent molecular studies have examined isolates from *Testudo graeca*, *T. hermanni* and *T. marginata*, some of which did show intestinal symptoms, such as diarrhoea, consistent with cryptosporidiosis [Traversa 2008].

Infection associated with clinical disease was first confirmed in an Egyptian tortoise (*Testudo kleinmanni*), presenting with clinical signs of enteritis, which died despite treatment. On post-mortem examination, histology confirmed heavy infection with *Cryptosporidium* affecting at least 80% of epithelial cells and although not thought to be the main cause of death, infection was considered to have been a contributory factor [Graczyk 1998]. Intestinal cryptosporidiosis has since been identified on post-mortem examination of a Russian tortoise (*Testudo horsfieldii*) and a Pancake tortoise (*Malacochersus tornieri*) and gastric cryptosporidiosis in another *Testudo horsfieldii*. The main presenting signs in each case were lethargy and anorexia, with no associated regurgitation or diarrhoea [Griffin 2010]. An outbreak of suspected gastric

cryptosporidiosis has also been described in a group of *Testudo hermanni*. Disease produced lethargy, anorexia, regurgitation of haemorrhagic fluid and mucus and resulted in death in 12% of individuals despite treatment [McArthur 2004].

Despite the fact that cryptosporidiosis is commonly described, there is little information about the exact species of *Cryptosporidium* which are found in reptiles. One reason for this is that *Cryptosporidium* oocysts often appear morphologically similar and until recently there have been few molecular studies to characterise isolates [Fall 2003]. Currently there are 19 distinct species of *Cryptosporidium* known to affect reptiles, amphibians, birds and mammals. *C. serpentis* and *C. varanii* (syn. *C. saurophilum*) appear to be the main species involved in infection in pet reptiles [Fayer 2010]. However, *C. parvum* and *C. muris* have also been identified in faecal samples [Pedraza-Diaz 2009] and have been suggested to originate from mammalian prey and pass through the reptile gastrointestinal system, but not actually to be pathogenic [Graczyk 1996]. Other genotypes have been reported in a variety of reptiles but are not currently recognised as species, although a new species of *Cryptosporidium* infecting tortoises (*C. ducismarci*) has been proposed [Traversa 2010].

Currently, reptilian strains have not been proven to cause zoonotic disease, but mammalian strains can be disseminated by tortoises, potentially causing significant problems in immuno-compromised humans, so appropriate precautions should always be taken [Traversa 2008].

Little is known about the life cycle of *Cryptosporidium*, but it is presumed to be a direct life cycle similar to that of other coccidian parasites. Two types of oocysts may be formed – the majority being thick walled in order to be passed in faeces and survive in the environment, but some being thin walled and immediately capable of re-infecting the host [Barnard 1994, Scullion 2009].

Pathological effects in the intestine occur due to hyperplasia of enterocytes and thickening of villi, leading to a loss of absorptive surface area and consequently diarrhoea, weight loss, dehydration and death [Terrell 2003]. In the stomach, pathological effects include hypertrophy of gastric mucosa and atrophy of granular cells. This may be accompanied by oedema and inflammation of the lamina propria and submucosa.

Consequently, regurgitation and anorexia occur leading to weight loss and death [Brownstein 1977]. Alternatively, the parasite may cause no significant disease [Deming 2008]. Severity of disease may depend on the immunocompetence of the host, or other concurrent disease, but the exact factors which play a role in triggering clinical disease are currently unknown.

Transmission is via the faeco-oral route, although oocysts are remarkably resilient within the environment and also spread via fomites and water [Graczyk 1997].

Diagnosis is challenging due to variable shedding of the oocysts in faeces. Repeat testing is therefore usually recommended to screen for the parasite [Deming 2008]. Alternatively samples may be obtained (depending on the species) from the mucus of regurgitated food items, by gastric lavage, or cloacal washes [Graczyk 1996]. Samples may be examined by a flotation method, or a direct smear. Sensitivity is increased with a modified acid-fast stain (*Cryptosporidium* oocysts measure 4-8µm and appear red on a green background) (see **Figure 6**). It should be noted however, that the sensitivity of these stains for the emerging strains of *Cryptosporidium* has not been established. A more sensitive test would be a direct immunofluorescence antibody test (e.g. Merifluor) which has been shown to be 16 times more sensitive in detecting the parasite than modified acid-fast stain alone. Modified acid fast stains combined with immunofluorescent antibody testing are thought to be the best non-invasive way of screening for *Cryptosporidium* [Graczyk 1995].

Commercial enzyme immunoassays are also available, but are less sensitive to non-*Cryptosporidium* parvum strains than immunofluorescent antibody tests [Graczyk 1996]. Serology has been advocated as an additional screening aid to ensure that *Cryptosporidium* infection is not incidental, but is not commercially available and it should always be used in combination with other tests as it will not identify early infection [Graczyk 1997].

Definitive diagnosis of cryptosporidiosis requires histopathology to confirm the presence of the parasite within a vacuole at the border of epithelial cells. Associated histopathological findings reported in chelonians have included either gastritis or enteritis with infiltration of the *lamina propria* with heterophils, lymphocytes and

macrophages [Jacobson 2007]. Biopsies may be obtained surgically or endoscopically, but the parasite can have a patchy distribution within the stomach, so false-negative results are not uncommon. Therefore, definitive diagnosis is often made post-mortem [Cranfield 1996].

Histopathology would rule out non-pathogenic mammalian strains of the parasite which may have been ingested incidentally. Alternatively PCR techniques may be used to characterise the exact species of *Cryptosporidium*, and are beginning to be more readily available for use in practice [Xiao 2004].

Treatment of cryptosporidiosis is another challenge with no completely successful treatment regime reported. Trimethoprim sulphamethoxazole treatment has been reported to reduce oocyst shedding, but results are inconsistent [Funk 1988]. Halofuginone treatment in snakes appeared effective in stopping oocyst shedding, but caused severe hepatotoxicity and nephrotoxicity and did not result in complete resolution of cryptosporidiosis. Spiramycin resulted in no significant change in the pattern of shedding or histological presence of cryptosporidiosis in treated animals [Graczyk 1996]. Paromomycin treatment in Gila monsters reduced clinical signs and led to a cessation of oocyst shedding but results appear inconsistent [Pare 1997]. Bovine hyperimmune colostrum has shown more promise with small studies in snakes and monitors showing a significant reduction in oocyst shedding, in addition to histopathological resolution of disease after a course of treatment [Graczyk 1998, 2000]. In geckos however, in which intestinal cryptosporidiosis is more common, colostrum treatment appeared less efficacious, possibly due to changes to the structure of colostrum immunoglobulins after passing through the stomach [Graczyk 1999].

Supportive treatments including the use of an immunomodulator consisting of inactivated virus and equine serum protein have also been advocated, although mechanism of action and efficacy are not described [Schneller 2008]. In chelonian species colostrum treatment appears ineffective at clearing parasite infection and no other successful treatments have been reported [Graczyk 1999].

In view of the lack of successful treatment regimes, cryptosporidiosis within a collection is normally best controlled by good management and hygiene. Disinfection of the environment is important to limit the spread of disease, but oocysts can be

difficult to totally eradicate. Iodophores, cresylic acid, sodium hypochlorite, benzylkonium chloride and sodium hydrochlorite have all been trialled and found to be ineffective. Ammonia (5%) and formalin (10%) appear effective but with a contact time of 18 hours at 4°C and these may not be the most practical solutions [Campbell 1982]. Currently, moist heat (45-60°C for 5-9 minutes), freezing or desiccation appear to be the most effective ways to clear the environment [Cranfield 1996].

Euthanasia should always be considered if *Cryptosporidium* is causing clinical disease within an individual, or if being shed within a collection. Although diagnosis is difficult, repeated *Cryptosporidium* screens should always be included as part of quarantine procedures within a collection. Some authors have advocated the administration of dexamethasone in order to promote immuno-suppression and therefore faecal shedding, but this carries a risk of increasing shedding of other pathogens, and allowing the reptile to be colonised by novel pathogens so is not routinely recommended [Wright 1997]. Any animals found to be shedding, should be isolated in a separate room with separate feeding and cleaning utensils, treated at the end of the day, or potentially culled. Eradication once infection is established within a collection could be attempted by identification of infected reptiles and their removal, but due to the low sensitivity of available diagnostic tests and the difficulty in eradicating environmental oocysts, this approach would be difficult. Instead, preventing infection from entering the collection is of utmost importance [McArthur 2004].

## **The significance of parasite infections in practice**

The host-parasite relationship is often balanced in the wild without any obvious clinical effects on the host. However, in captivity reptiles are under stress and may not be immunocompetent if husbandry is inappropriate. In this situation, parasites can multiply rapidly, especially those which do not require an intermediate host [Schneller 2008].

Unfortunately there is a lack of data regarding the prevalence of all these parasites in captive populations. The only published data in the UK are two from studies. One is a review of clinical and pathological findings in seventy tortoises, which identified nematodes in 30% of tortoises [Holt 1979]. These were all identified as ascarids, with a concomitant oxyurid infection in only 5% and *Balantidium* in 4%. The other is a necropsy survey of a variety of chelonians kept in a zoological collection which identified nematode infections in 43.8% cases with a mix of ascarids and oxyurids found, but there appeared little evidence of associated pathologic changes [Keymer 1978]. More recently however, some authors have described that < 75% of chelonian patients passed oxyurid eggs in their faecal samples [McArthur 2004]. Epidemiological surveys carried out in other countries including Italy, Germany and Slovenia have also shown a high prevalence of gastrointestinal parasites in tortoises, specifically oxyurids, but the relevance of these studies to the situation in the UK is currently unknown [Traversa 2005, Pasmans 2008, Papini 2011, Rataj 2011].

Routine faecal screening is often advised as part of a preventative health program for captive tortoises [Pasmans 2008], but the finding of gastrointestinal parasites on these faecals from an otherwise healthy tortoise can therefore be a conundrum for the veterinary surgeon. Without knowledge of the normal parasite burden for a species, it can be difficult to determine what level of parasites may be a problem for an individual tortoise. The decision on whether treatment is necessary is therefore normally based on individual opinion.

The factors affecting parasite burden have also not been investigated, although one of the Italian studies implies that age and husbandry setup may play a role in influencing

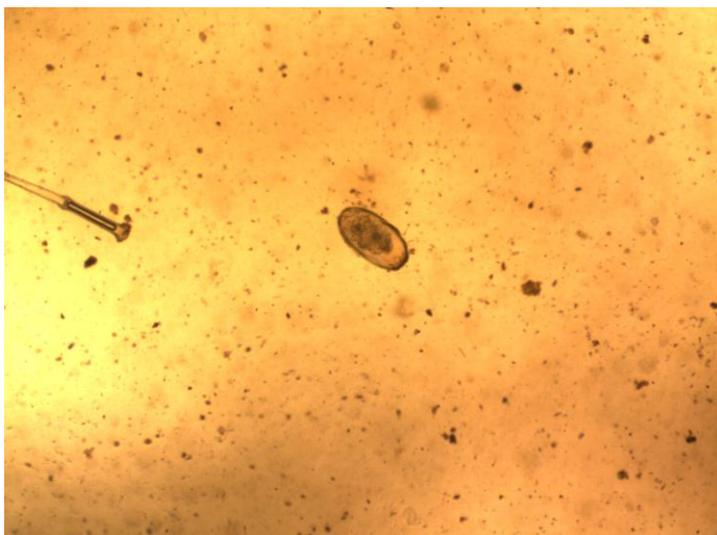
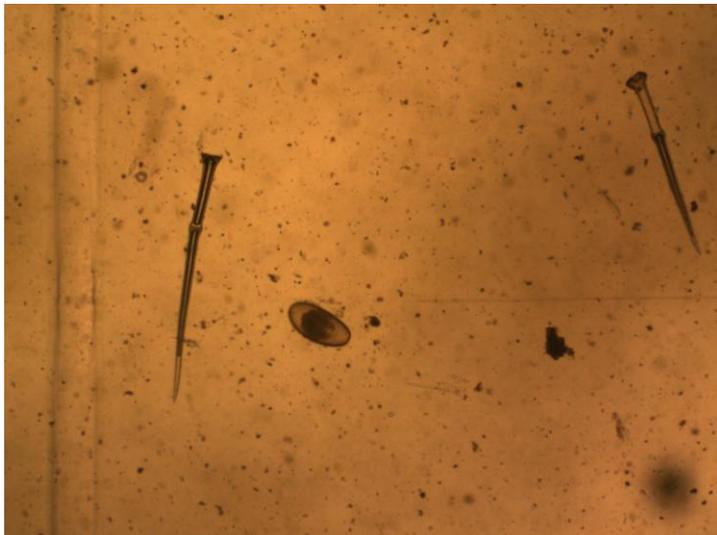
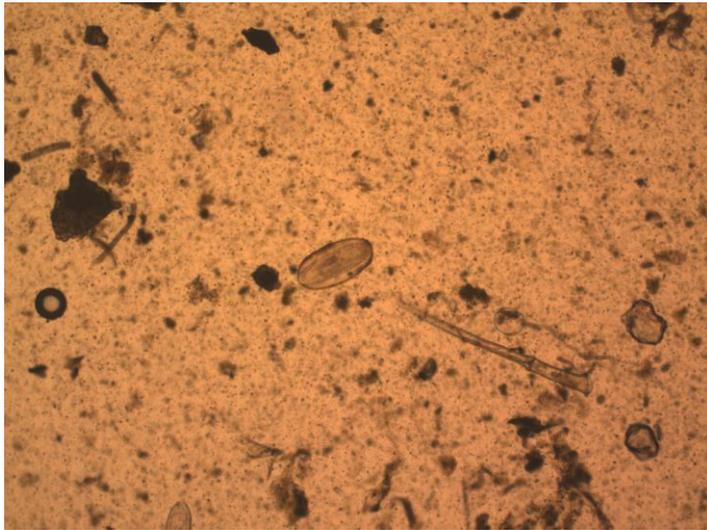
prevalence of parasites [Traversa 2005]. More information about factors affecting parasite burdens would be useful in helping to advise clients on prevention of parasite problems.

The aims of this study were therefore:

- To investigate the prevalence of gastrointestinal parasites (specifically nematodes) in tortoises in the UK
- To investigate the factors affecting the prevalence of gastrointestinal parasites

The study was limited to *Testudo hermanni*, *graeca*, *horsfieldii* and *Stigmochelys pardalis* (syn. *Geochelone pardalis*), as these are some of the more common species kept in captivity in the UK.

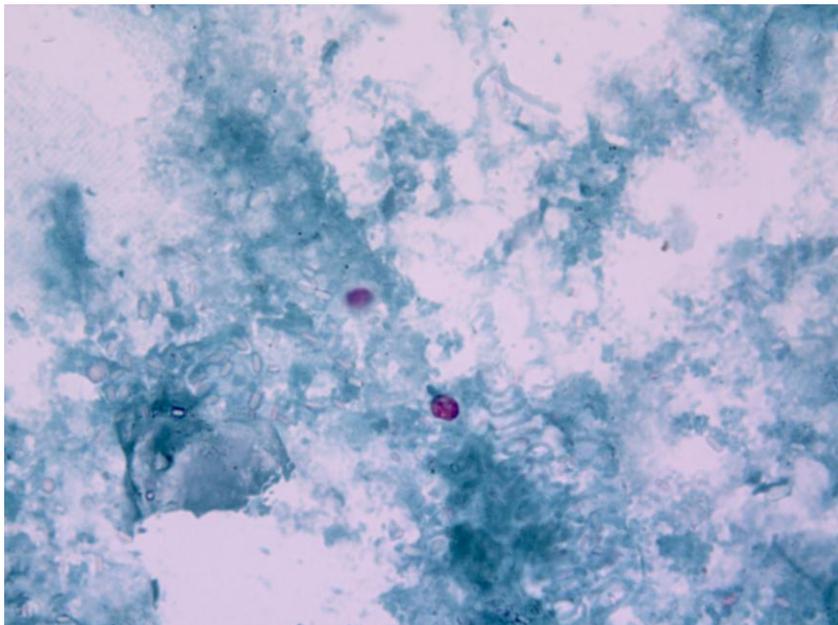
**Figures 2-4: Different appearances of oxyurid ova from *Testudo* spp.**



**Figure 5: Ascarid from *Testudo* spp**



**Figure 6: *Cryptosporidium* from a *Testudo* spp.**



## **Materials and Methods**

Tortoise owners throughout the UK were invited to submit samples of their tortoise's faeces which were analyzed free of charge from March – September 2010. Tortoise owners were contacted by advertising the project through tortoise/reptile interest/owner groups, in addition to contacting vets through veterinary publications. Owners were asked to collect a fresh faecal sample from their tortoise and send it via first class post immediately during Monday -Thursday, so that all samples could be analysed within 24 hours of being voided. On arrival samples were placed in the fridge (4°C) and given a unique reference number.

All faecal samples were examined on the day of receipt. This consisted of a macroscopic examination, a direct wet preparation [Cooper 2009] and a flotation (using a saturated NaCl solution). A modified McMaster technique was also performed in order to provide a quantitative assessment of worm egg count [Zajac 2006]. Finally, a faecal smear was stained for identification of *Cryptosporidium* (using Pro-Lab Diagnostics Cryptosporidium Staining Kit ®) (see **Appendix 2 for protocol**). Nematodes were classified as oxyurids, ascarids or strongylids based on morphological descriptions of ova [Thapar 1925, Jacobson 2007, Greiner 2006]. Protozoa were classified as *Balantidium*, *Nyctotherus*, flagellates, coccidia, or *Cryptosporidium*, also based on morphological descriptions [Barnard 1994], but grouped together (with the exception of *Cryptosporidium*) for later statistical analysis. A sample was defined as positive for a specific parasite, if it tested positive using any one of the diagnostic methods, although the efficacy of the diagnostic methods were later compared.

In return for providing this free service, owners were asked to send back a completed questionnaire (see **Figure 7**). This could be either printed from a website and submitted by post, or filled in and submitted online. The questionnaire covered details of their tortoise's signalment and husbandry (e.g. substrate, indoor/outdoor enclosure, individual/group housing). These questionnaires were assigned the same reference number as their associated faecal sample, but data from these was not analyzed until the end of the study.

Data from the questionnaires was analysed using Minitab® (Minitab Inc, Pennsylvania) and R version 2.12.1 (The R Foundation for Statistical Computing).  $p < 0.05$  was taken to indicate statistical significance.  $\chi^2$  tests, Kruskal-Wallis tests and standard logistic regression with odds ratios (OR) and 95% confidence intervals were calculated to evaluate the potential risk factors for the different parasites. Multivariable univariate analyses were then performed, where significant risk factors were evaluated using multiple logistic regression.

**Figure 17: Questionnaire detailing tortoise signalment and husbandry**



**Faecal Parasite Survey of pet tortoises in the UK**

The survey includes a questionnaire to look at the relationship between how tortoises are kept and their numbers of internal parasites. This survey is open to all tortoise owners in the UK. Owners are asked to collect a fresh faecal (poo) sample from their tortoises and send it to us on the same day. This sample will be examined free-of-charge if the questionnaire below is fully completed. Samples must be sent by first class post Monday to Thursday. Please see the separate instructions on how to package your samples. Only certain species of tortoises (see Q2) are being considered for this survey. These animals must have had **no health concerns in the past year**.

**Questions**

Q1. Where do you live? – County and postcode (e.g. EH9)

Q2. What is the species of your tortoise?

- a) Hermann's (*Testudo hermanni*)
- b) Spur-Thighed (*Testudo graeca*)
- c) Horsfield (*Testudo horsfieldii*)
- d) Leopard (*Stigmochelys pardalis*)
- e) Other – sorry, faecal samples from other species will not be accepted for this survey

Q3. How old is your tortoise?

Q4. What sex is your tortoise?

- a) Male
- b) Female
- c) Don't know

Q5. Where did you get your tortoise from?

- a) Pet shop
- b) Breeder
- c) Rescued
- d) Home-bred
- e) Other – please specify

Q6. How long have you had your tortoise?

Q7. Does this tortoise mix with any other tortoises?

- a) Yes – please list number and species of other tortoises
- b) No

Q8. Do you own any other reptiles?

- a) Yes – please list number and species

b) No

Q9. In what kind of environment do you keep your tortoise?

If more than one, please tick all below that are relevant, but indicate in which set-up your tortoise spends most time (if this varies seasonally, please describe)

- a) Garden (free-range)
- b) Garden (penned area)
- c) Vivarium
- d) Open-top pen inside
- e) Free-range in house

Q10. On what substrate (bedding) do you keep your tortoise?

If more than one please tick all below that are relevant, but indicate which substrate is used most

- a) Newspaper
- b) Sand
- c) Soil
- d) Shavings
- e) Woodchip
- f) Hemp
- g) Other – please specify

Q11. Has your tortoise had any previous parasites/worms of which you are aware?

- a) Yes – please give details
- b) No

Q12. Has your tortoise had any previous worming treatment?

- a) Yes – please give as much detail as you remember (e.g. date of last worming, drug given, frequency, used to treat infestation or as prevention)
- b) No

Q13. Has your tortoise had any other previous medical problems?

- a) Yes – please give details
- b) No

Q14. Do you hibernate your tortoise?

- a) Yes – if so, when does hibernation start and end? (e.g. Oct-Feb)
- b) No

Q15. Are you a member of a tortoise charity?

- a) Yes (please specify which one(s))
- b) No

Please continue on an extra sheet if required. Please send your samples along with the completed questionnaires to:-

**Exotic Animal and Wildlife Service, Hospital for Small Animals, Easter Bush Veterinary Centre, Roslin, Midlothian EH25 9RG.**

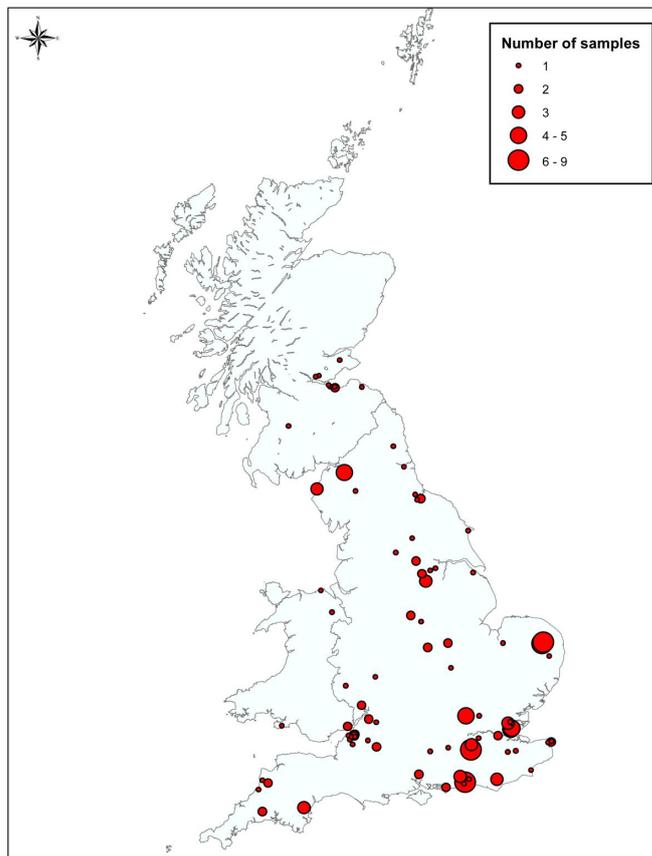
In order for us to contact you with results of our investigation **please supply your email address**. Please write clearly and legibly.

Thank you for your time in answering these questions.

## Results

A total of 243 samples were returned with completed questionnaires from various locations around the UK (see **Figure 8**). Of these, only 146 samples met the requirements of the survey (with the other samples being >24 hr old, from unhealthy tortoises or from group situations). These consisted of samples from 63 *Testudo hermanni*, 55 *T. graeca*, 24 *T. horsfieldii*, and 4 *Stigmochelys pardalis* (see **Appendix 3 for further details**). Due to the low numbers of samples from *S. pardalis*, it was decided to exclude these 4 samples from further results and statistical analysis.

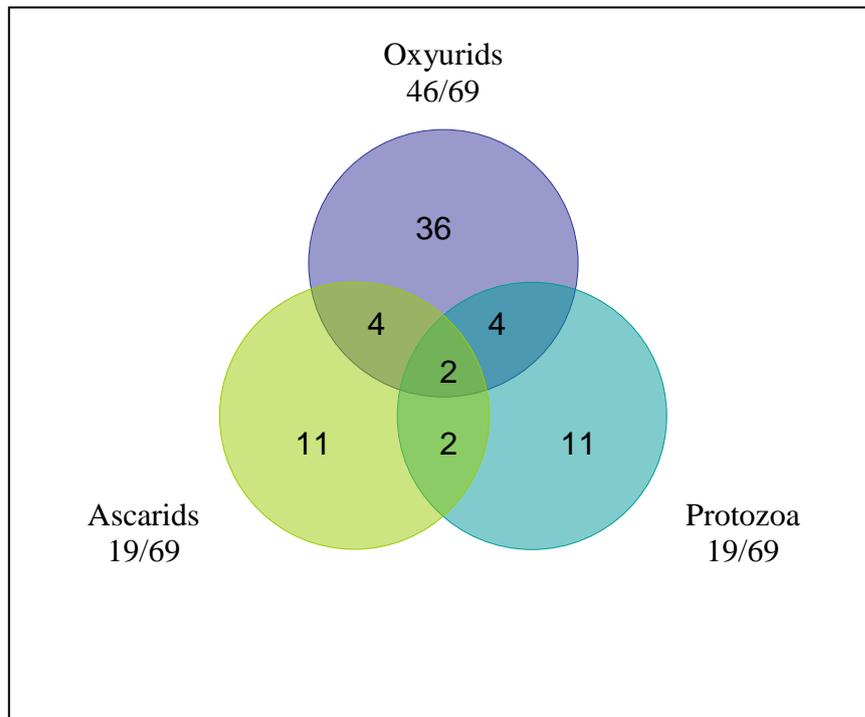
**Figure 8: Map to show distribution of samples received from around the UK**



69/142 (48.6%) samples were positive for parasite ova or protozoa. No adult parasites were identified. Of the positive samples, 46/142 were positive for oxyurids, 19/142 were positive for ascarids, 19/142 were positive for protozoa excluding *Cryptosporidium* and 1/113 was positive for *Cryptosporidium* (see **Figure 9**).

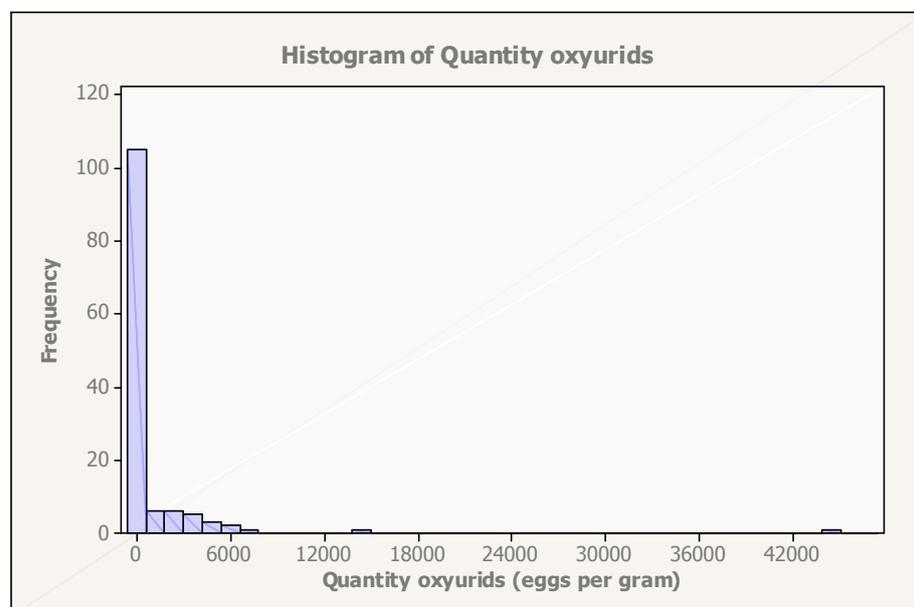
*Cryptosporidium* testing was not able to be completed in all cases due to insufficient quantity of some of the samples.

**Figure 9: Venn diagram to show parasites identified (excluding *Cryptosporidium*), n = 69 positive samples**

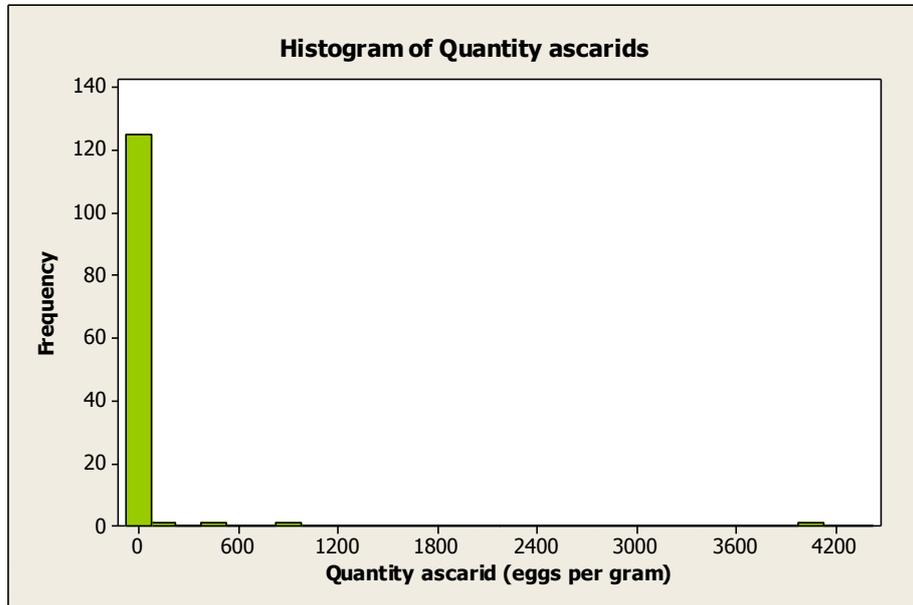


The McMaster technique revealed eggs per gram (epg) values ranging from 50 to 44650 for oxyurids (see **Figure 10**) with a median of 2075 epg. In contrast for ascarids, values ranged from 50 to 4100 (see **Figure 11**), with a median of 325 epg.

**Figure 10: Histogram to show the quantity of oxyurids found in tortoise faecal samples**

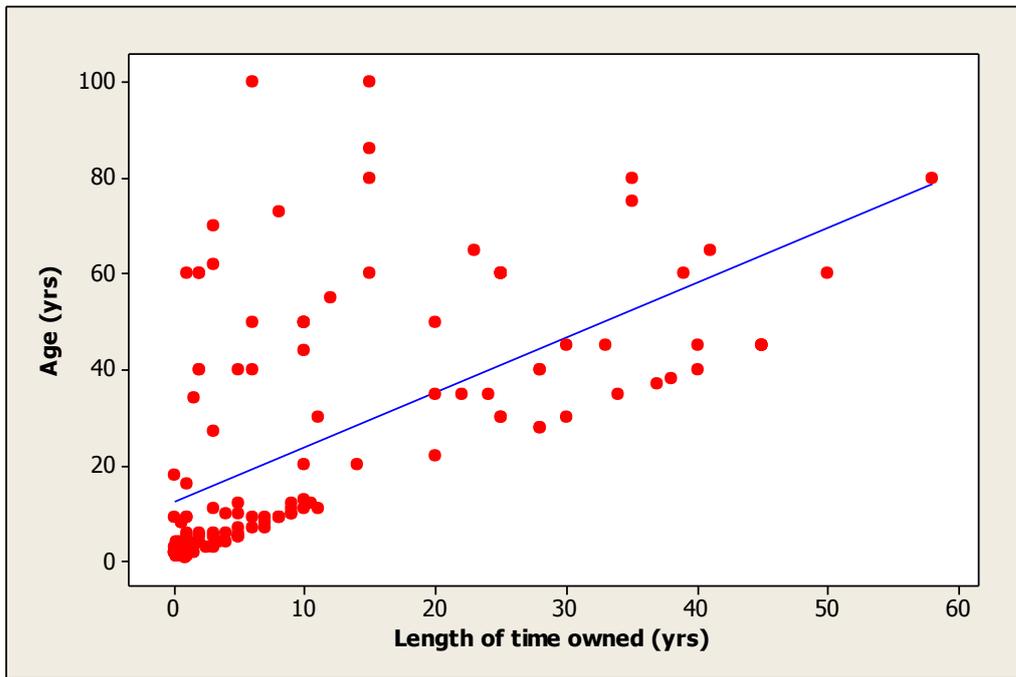


**Figure 11: Histogram to show the quantity of ascarids found in tortoise faecal samples**

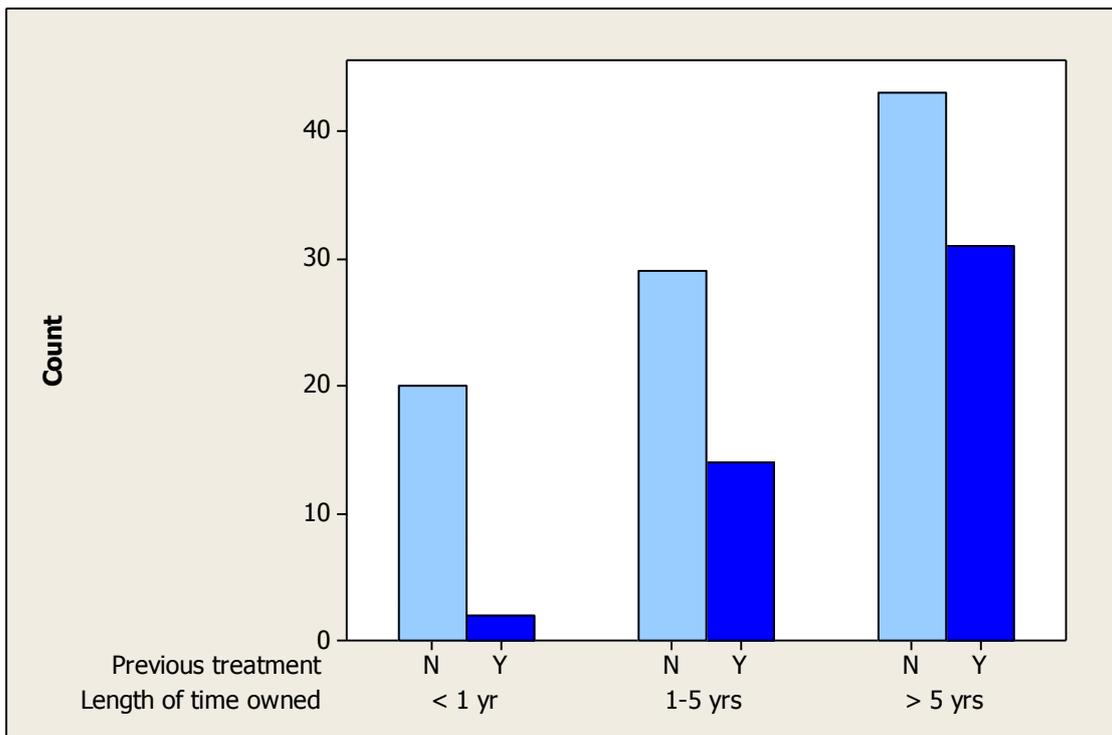


A statistically significant association was found between the presence of parasites and sex of tortoise ( $\chi^2_1=4.63$ ,  $p=0.03$ ), with the odds of being positive for parasites if female more than that if male (OR 2.2 (1.1-4.4)) (see **Table 1**). A statistically significant association was also found between the presence of parasites and length of time the tortoise had been owned ( $\chi^2_1=5.15$ ,  $p=0.02$ ), with the odds of being positive for parasites in tortoises owned >5 years less than for those owned shorter periods (OR 0.46 (0.24-0.91)). However most tortoises which had been owned for shorter periods were the younger tortoises (see **Figure 12**). Tortoises which had been owned for a longer length of time were also more likely to have been treated for parasites (see **Figure 13**).

**Figure 12 – Scatter plot to show relationship between age and length of time the tortoise was owned**



**Figure 13 – Chart to show relationship between length of time owned and previous parasite treatment**

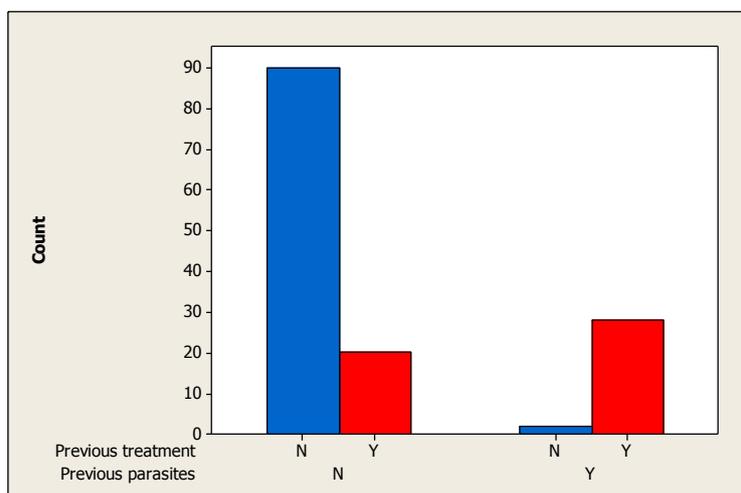


There were no statistically significant associations between presence of parasites and species, age, origin of tortoise, presence of other tortoises, presence of other reptiles, environment, substrate, previous parasites, previous treatment, previous medical problems, whether the tortoise had been hibernated or not, or whether the owner belonged to a tortoise charity ( $p>0.06$ ).

When looking more specifically at the presence of oxyurids, a statistically significant association was found between the presence of parasites and species of tortoise ( $\chi^2_2=11.9$ ,  $p=0.003$ ) with the odds of being positive for oxyurids if *T. hermanni* or *T. horsfieldii* more than if *T. graeca*, (OR 3.36 (1.4-8.06)) and (OR 5.11 (1.75-14.94)) respectively. A statistically significant association was also found between the presence of oxyurids, age (more prevalent in younger tortoises), origin of tortoise, length of time tortoise had been owned, previous parasite problems, previous parasite treatment and whether the tortoise has been hibernated or not ( $\chi^2<24.1$ ,  $p<0.03$ ) (see **Table 2**). Tortoises which had had previous parasite treatment were more likely to have had previous parasite treatment (see **Figure 14**).

There were no statistically significant associations between the presence of oxyurids and sex, presence of other tortoises, presence of other reptiles, environment, substrate, previous medical problems, or whether the owner belonged to a tortoise charity ( $p>0.051$ ).

**Figure 14 – Chart to show relationship between history of parasites and history of parasite treatment**



In contrast to oxyurids, for ascarids there was a statistically significant association between the presence of parasites and the age group of tortoise ( $\chi^2_4=14.0$ ,  $p=0.003$ ) with the odds of being positive for ascarids less if <5 years old, compared to older tortoises (OR 0.21 (0.06-0.79)) (see **Table 2**). A statistically significant association was also found between presence of ascarids, length of time the tortoise had been owned, and whether a tortoise was kept outside or inside ( $\chi^2<6.24$ ,  $p<0.01$ ).

There were no statistically significant associations between presence of ascarids and species, sex, origin, presence of other tortoises, presence of other reptiles, substrate, previous parasites, previous worming, previous medical problems, or whether the owner belonged to a tortoise charity ( $p>0.13$ ).

For protozoa there was a statistically significant association between the prevalence of parasites and whether the tortoise is kept alone or not ( $\chi^2_1=4.81$ ,  $p=0.03$ ) with the odds of being positive for protozoa if kept with others, more than if kept alone (OR 3.14 (1.06-9.25)) (see **Table 2**). A statistically significant association was also found between presence of protozoa and whether the tortoise was kept on soil or not ( $\chi^2_1=5.67$ ,  $p=0.02$ ), with the odds of being positive for protozoa if kept on soil more than if kept on alternative substrates (OR 3.94 (1.29-12.02)).

There were no statistically significant associations between presence of protozoa and species, age, sex, origin, time in owner's possession, presence of other tortoises, presence of other reptiles, environment, substrate, previous parasites, previous worming, previous medical problems, whether the tortoise had been hibernated or not, or whether the owner belonged to a tortoise charity ( $p>0.054$ ).

**Table 1: Univariate logistic regression analyses of association of putative risk factors and parasites (all oxyurids, ascarids and protozoa). In all cases, the number positive, the prevalence (95% confidence intervals), the associated  $\chi^2$  (subscript = degrees of freedom) and p-values, along with the Odds ratio (95% confidence intervals) compared to a reference level are given**

	Total	With any parasites	%	$\chi^2$ and p value	Odds Ratio
<b>Number of samples</b>	142	69	48.6(40.1-57.1)		
<b>Species</b>					
<i>Testudo hermanni</i>	63	33	52.4(39.4-65.1)	$\chi^2_2=1.68$	-
<i>Testudo graeca</i>	55	23	41.8(28.6-55.9)	p= 0.43	0.65(0.32-1.35)
<i>Testudo horsfieldii</i>	24	13	54.2(32.8-74.4)		1.07(0.42-2.76)
<b>Age</b>					
<2 yrs	10	3	30(6.7-65.2)	$\chi^2_4=7.56$	-
2-5yrs	28	18	64.3(44.1-81.4)	p=0.11	4.2(0.88-19.9)
5-10yrs	26	16	61.5(40.6-79.8)		3.73(0.78-17.9)
10-50yrs	46	19	41.3(27.0-56.8)		1.64(0.38-7.17)
50+yrs	29	12	41.4(23.5-61.1)		1.65(0.35-7.69)
<b>Sex</b>					
Male	61	24	39.3(27.1-52.7)	$\chi^2_1=4.63$	-
Female	65	38	58.5(45.6-70.6)	<b>p=0.03</b>	2.17(1.06-4.42)
<b>Origin</b>					
Pet shop	51	25	49.0(34.8-63.4)	$\chi^2_2=0.83$	-
Breeders and homebred	23	13	56.5(34.5-76.8)	p=0.66	1.35(0.5-3.64)
Rehomed	68	31	45.6(33.5-58.1)		0.87(0.42-1.8)
<b>BIOP</b>					
<5yrs	66	39	59.1(46.3-71.0)	$\chi^2_1=5.15$	
>5yrs	75	30	40.0(28.9-52.0)	<b>p=0.02</b>	0.46(0.24-0.91)
<b>Other tortoises</b>					
Yes	72	35	48.6(36.7-60.7)	$\chi^2_1<0.001$	-
No	70	34	48.6(36.4-60.8)	p=0.996	1.00(0.52-1.93)
<b>Other reptiles</b>					
Yes	34	17	50(32.4-67.6)	$\chi^2_1=0.04$	-
No	108	52	48.1(38.4-58.0)	p=0.85	1.08(0.5-2.33)
<b>Environment</b>					
Garden (free range)	22	25	47.2(33.3-61.4)	$\chi^2_1=0.06$	-
Garden (pen)	31			p=0.80	
Vivarium	6	16	44.4(27.9-61.9)		1.12(0.48-2.61)
Inside pen	25				

House (free range)	5					
<b>Substrate</b>						
Newspaper	45	19	42.2(27.7-57.8)	$\chi^2_1=3.15$	-	
Not newspaper	55	33	60.0(45.9-73.0)	p=0.08	0.49(0.22-1.08)	
<b>Previous parasites</b>						
Yes	30	10	33.3(17.3-52.8)	$\chi^2_1=3.61$	-	
No	112	59	52.7(43.0-62.2)	p=0.06	0.45(0.19-1.05)	
<b>Previous parasite treatment</b>						
Yes	48	18	37.5(24.0-52.6)	$\chi^2_1=3.61$	-	
No	92	50	54.3(43.6-64.8)	p=0.06	0.5(0.25-1.03)	
<b>Previous medical problems</b>						
Yes	32	14	43.8(26.4-62.3)	$\chi^2_1=0.39$	-	
No	110	32	29.1(20.8-38.5)	p=0.53	0.78(0.35-1.72)	
<b>Hibernated</b>						
Yes	91	40	44.0(33.6-54.8)	$\chi^2_1=2.18$	-	
No	51	29	56.9(42.2-70.7)	p=0.14	0.59(0.3-1.19)	

**Table 2: Univariate logistic regression analyses of association of putative risk factors and parasites (all oxyurids, ascarids and protozoa). In all cases, the number positive, the prevalence (95% confidence intervals), the associated  $\chi^2$  (subscript = degrees of freedom), and p-values, along with the Odds ratio (95% confidence intervals) compared to a reference level are given**

	Total	With oxyurids	%	$\chi^2$ and p value	Odds Ratio	With ascarids	%	$\chi^2$ and p value	Odds Ratio	With protozoa	%	$\chi^2$ and p value	Odds Ratio
<b>Number of samples</b>	142	46	32.4(24.8-40.8)			19	13.4(8.3-20.1)			19	13.4(8.3-20.1)		
<b>Species</b>													
<i>Testudo hermanni</i>	63	25	39.7(27.6-52.8)	$\chi^2_2=11.9$	-	6	9.5(3.6-19.6)	$\chi^2_2=3.30$	-	10	15.9(7.9-27.3)	$\chi^2_2=0.94$	-
<i>Testudo graeca</i>	55	9	16.4(7.8-28.8)	<b>p=0.003</b>	0.3(0.12-0.71)	11	20.0(10.4-33.0)	p=0.19	2.37(0.81-6.92)	7	12.7(5.3-24.5)	p=0.63	0.77(0.27-2.19)
<i>Testudo horsfieldii</i>	24	12	50.0(29.1-70.9)		1.52(0.59-3.91)	2	8.3(1.0-27.0)		0.86(0.16-4.61)	2	8.3(1.0-27.0)		0.48(0.1-2.38)
<b>Age</b>													
<2 yrs	10	20	52.6(35.8-69.0)	$\chi^2_3=24.1$	-	0	0	$\chi^2_3=14.0$		4	10.5(2.94-24.8)	$\chi^2_3=4.39$	-
2-5yrs	28			<b>p&lt;0.001</b>				<b>p=0.003</b>	0.21(0.06-0.79)			p=0.22	
5-10yrs	26	14	53.8(33.4-73.4)		1.05(0.39-2.85)	4	15.4(4.4-34.9)			4	15.4(4.4-34.9)		1.55(0.35-6.83)
10-50yrs	46	7	15.2(6.3-28.9)		0.16(0.06-0.45)	8	17.4(7.8-31.4)		0.66(0.21, 2.07)	7	15.2(6.3-28.9)		1.53(0.41-5.66)
50+yrs	29	4	13.8(3.9-31.7)		0.14(0.04-0.49)	7	24.1(10.3-43.5)			3	10.3(2.2-27.4)		0.98(0.2-4.77)
<b>Sex</b>													
Male	61	15	24.6(14.5-37.3)	$\chi^2_1=2.26$	-	9	14.8(7.0-26.2)	$\chi^2_1=0.01$	-	5	8.2(2.7-18.1)	$\chi^2_1=3.70$	-
Female	65	24	36.9(25.3-49.8)	p=0.13	1.8(0.83-3.88)	10	15.4(7.6-26.5)	p=0.92	1.05(0.4-2.79)	13	20.0(11.1-31.8)	p=0.054	2.8(0.93-8.4)
<b>Origin</b>													
Pet shop	51	20	39.2(25.8-53.9)	$\chi^2_2=12.7$	-	8	15.7(7.0-28.6)	$\chi^2_2=2.44$	-	4	7.8(2.2-18.9)	$\chi^2_2=3.78$	-
Breeders/homebred	23	13	56.5(34.5-76.8)	<b>p=0.002</b>	2.01(0.74-5.47)	1	4.3(0.1-21.9)	p=0.30	0.24(0.03-2.08)	2	8.7(1.1-28.0)	p=0.15	1.12(0.19-6.59)
Rehomed	68	13	19.1(10.6-30.5)		0.37(0.16-0.84)	10	14.7(7.3-25.4)		0.93(0.34-2.54)	13	19.1(10.6-30.5)		2.78(0.85-9.1)
<b>BIOP</b>													
<5yrs	66	31	47.0(34.6-59.6)	$\chi^2_1=11.8$		4	6.1(1.7-14.8)	$\chi^2_1=6.24$	-	12	18.2(9.8-29.6)	$\chi^2_1=2.37$	-
>5yrs	75	15	20.0(11.6-30.8)	<b>p=0.001</b>	0.28(0.13-0.59)	15	20.0(11.6-30.8)	<b>p=0.01</b>	3.87(1.22-12.34)	7	9.3(3.8-18.3)	p=0.12	0.46(0.17-1.26)

<b>Other tortoises</b>													
Yes	72	21	29.2(19.0-41.1)	$\chi^2_1=0.70$	-	9	12.5(5.9-22.4)	$\chi^2_1=0.098$	-	14	19.4(11.1-30.5)	$\chi^2_1=4.81$	-
No	70	25	35.7(24.6-48.1)	p=0.40	0.74(0.37-1.5)	10	14.3(7.1-24.7)	p=0.76	0.86(0.33-2.26)	5	7.1(2.4-15.9)	<b>p=0.03</b>	3.14(1.06-9.25)
<b>Other reptiles</b>													
Yes	34	11	32.4(17.4-50.5)	$\chi^2_1<0.001$	-	3	8.9(1.9-23.7)	$\chi^2_1=0.87$	-	3	8.8(1.9-23.7)	$\chi^2_1=0.87$	-
No	108	35	32.4(23.7-42.1)	p=0.995	0.997(0.44-2.27)	16	14.9(8.7-22.9)	p=0.35	0.56(0.15-2.04)	16	14.8(8.7-22.9)	p=0.35	0.56(0.15-2.04)
<b>Environment</b>													
Garden (free range)	22	9	17.0(8.1-29.8)	$\chi^2_1=3.13$	-	11	20.8(10.8-34.1)	$\chi^2_1=4.44$	-	9	17.0(8.1-29.8)	$\chi^2_1=0.16$	-
Garden (pen)	31			p=0.08				<b>p=0.046</b>				p=0.69	
Vivarium	6												
Inside pen	25	12	33.3(18.6-51.0)		0.41(0.15-1.11)	2	5.6(0.68-18.7)		4.45(0.92-21.47)	5	13.9(4.7-29.5)		1.27(0.39-4.15)
House (free range)	5												
<b>Substrate</b>													
Soil	25	5	20.0(6.8-40.7)	$\chi^2_1=1.97$	-	4	16.0(4.5-36.1)	$\chi^2_1<0.001$	-	8	32.0(14.9-53.5)	$\chi^2_1=5.67$	-
Not soil	75	26	34.7(24.0-46.5)	p=0.16	0.47(0.16-1.4)	12	16.0(8.6-26.3)	p=1.00		8	10.7(4.7-20.0)	<b>p=0.02</b>	3.94(1.29-12.02)
<b>Previous parasites</b>													
Yes	30	5	16.7(5.6-34.7)	$\chi^2_1=4.70$	-	4	13.3(3.7-30.7)	$\chi^2_1<0.001$	-	2	6.7(0.8-22.1)	$\chi^2_1=1.70$	-
No	112	41	36.6(27.7-46.2)	<b>p=0.03</b>	0.35(0.12-0.97)	15	13.4(7.7-21.1)	p=0.99	0.99(0.30-3.25)	17	15.2(9.1-23.2)	p=0.19	0.4(0.09-1.83)
<b>Previous parasite treatment</b>													
Yes	48	10	20.8(10.5-35.0)	$\chi^2_1=5.00$	-	5	10.4(3.5-22.7)	$\chi^2_1=0.40$	-	6	12.5(4.7-25.2)	$\chi^2_1=0.07$	-
No	92	36	39.1(29.1-49.9)	<b>p=0.03</b>	0.41(0.18-0.92)	13	14.1(7.7-23.0)	p=0.53	0.71(0.24-2.11)	13	14.1(7.7-20.8)	p=0.79	0.87(0.31-2.45)
<b>Previous medical problems</b>													
Yes	32	8	25.0(11.5-43.4)	$\chi^2_1=1.07$	-	7	21.9(9.3-40.0)	$\chi^2_1=2.33$	-	2	6.3(0.8-20.8)	$\chi^2_1=2.09$	-
No	110	38	34.5(25.7-44.2)	p=0.30	0.63(0.26-1.54)	12	10.9(5.8-18.3)	p=0.13	2.29(0.82-6.41)	17	15.5(9.2-23.6)	p=0.15	0.36(0.08-1.67)
<b>Hibernated</b>													
Yes	91	21	23.1(14.9-33.1)	$\chi^2_1=9.87$	-	15	16.5(9.5-25.7)	$\chi^2_1=2.26$	-	11	12.1(6.2-20.6)	$\chi^2_1=0.36$	-
No	51	25	49.0(34.8-63.4)	<b>p=0.002</b>	0.31(0.15-0.65)	4	7.8(2.2-18.9)	p=0.13	2.32(0.73-7.41)	8	15.7(7.0-28.6)	p=0.55	0.74(0.28-1.98)

### Multivariable univariate analyses

Multivariable univariate analyses were performed on those univariate variables for which  $p < 0.2$  (age, sex, length of time owned, substrate, previous parasites, previous treatment and whether the tortoise had been hibernated or not). However, only sex of tortoise and length of time in owner's possession remained as significant risk factors for overall presence of parasites, ( $p < 0.034$ ) with the odds of being positive for parasites in female more than if male (OR 2.21 (1.06-4.63)) and the odds of being positive if owned  $> 5$  years less than if owned shorter periods (OR 0.36(0.17-0.77)).

In contrast, for oxyuridsonly age of tortoise and previous parasite treatment remained as significant risk factors ( $p < 0.049$ ), even when including other risk factors for which  $p < 0.2$  (species, sex, origin, length of time owned, environment, substrate, previous parasites, and whether the tortoise had been hibernated or not). The odds of being positive if  $> 10$  years were less than if younger (OR 0.14(0.04-0.5)) and the odds of being positive if previously treated were less than if not treated (OR 0.41 (0.16-1.02)).

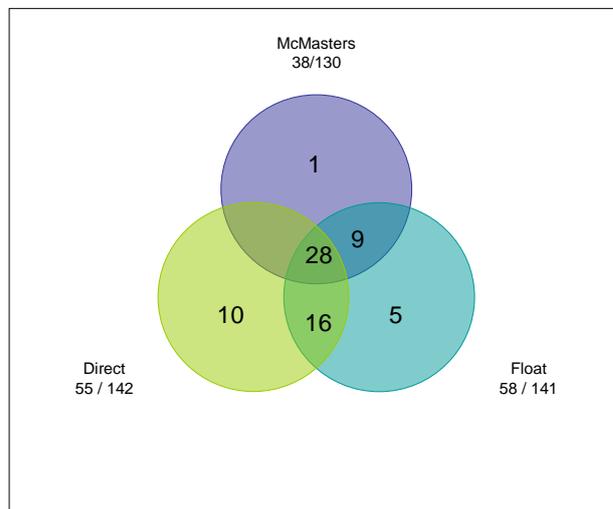
For ascarids, in agreement with the overall presence of parasites, only length of time in owner's possession remained as a significant risk factor, even when including other risk factors for which  $p < 0.2$  (species, age, environment, previous medical problems, and whether the tortoise had been hibernated or not) (**see Table 2**).

In contrast, when looking more specifically at the presence of protozoa, when including other risk factors for which  $p < 0.2$  (sex, origin, length of time owned, substrate, previous parasites, and previous medical problems), only the presence of other tortoises remained as a significant risk factor. (**see Table 2**).

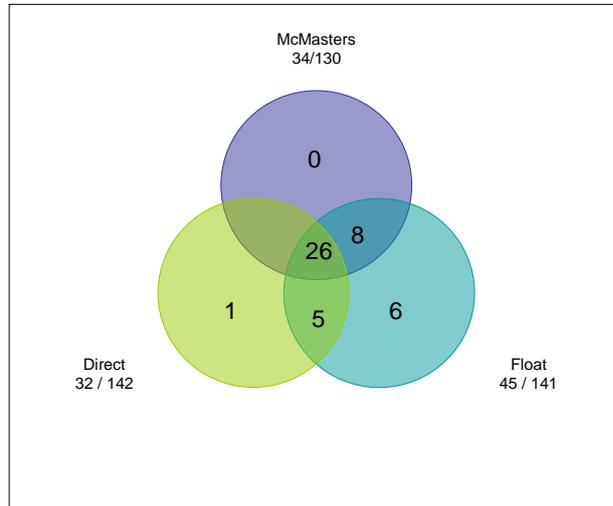
The different diagnostic methods were also assessed for their efficacy of detecting parasites. The direct wet preparation detected the most parasites (see **Figure 15**). However, no single diagnostic method detected all positive samples. In contrast, when looking more specifically at oxyurids the flotation method detected the most parasites (see **Figure 16**). For ascarids however, the direct wet preparation remained the most effective method of detecting parasites (see **Figure 17**). For protozoa the direct wet preparation also remained the most effective method with no protozoa being detected by the McMaster method (see **Figure 18**).

There were also differences noted in parasite numbers detected over the course of the year with the highest percentage of positive samples being detected in April and May (see **Figures 19-23**).

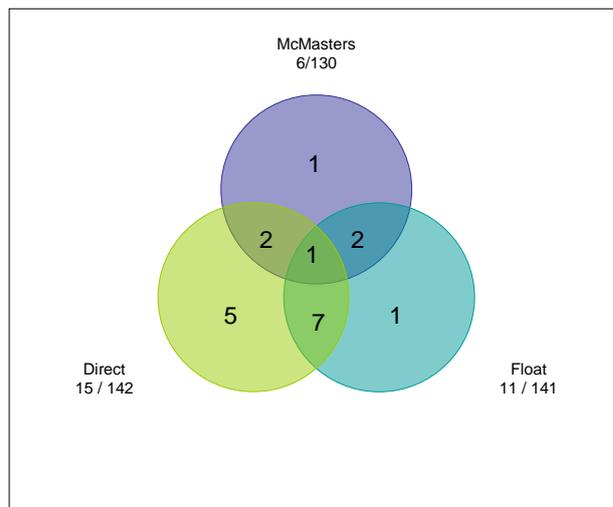
**Figure 15: Venn diagram to show parasites identified by different diagnostic methods, n =69 positive samples**



**Figure 16: Venn diagram to show oxyurids identified by different diagnostic methods, n =46 positive samples**



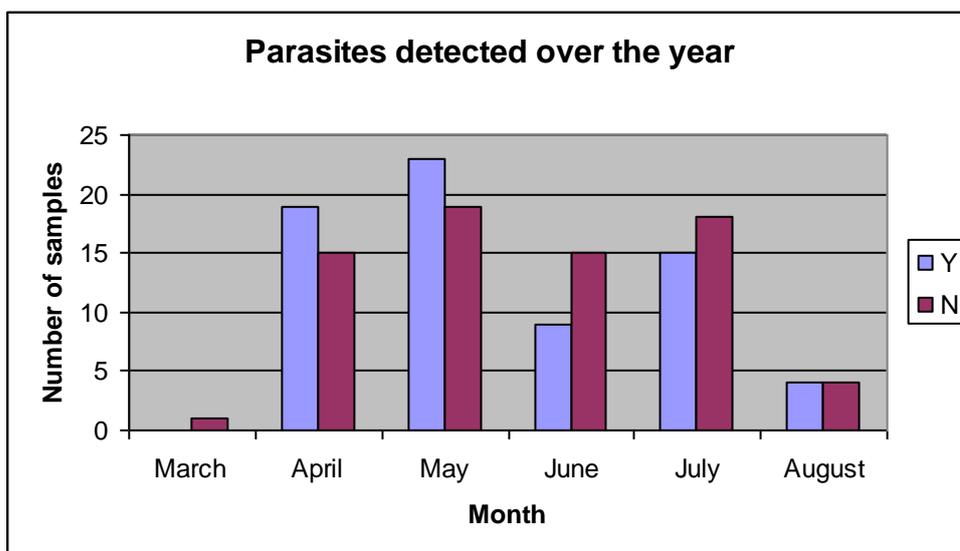
**Figure 17: Venn diagram to show ascarids identified by different diagnostic methods, n =19 positive samples**

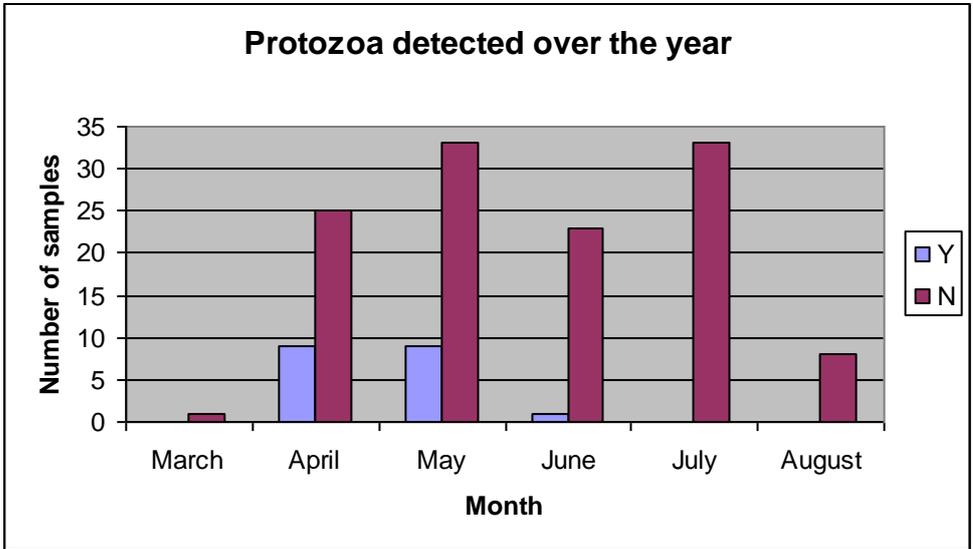
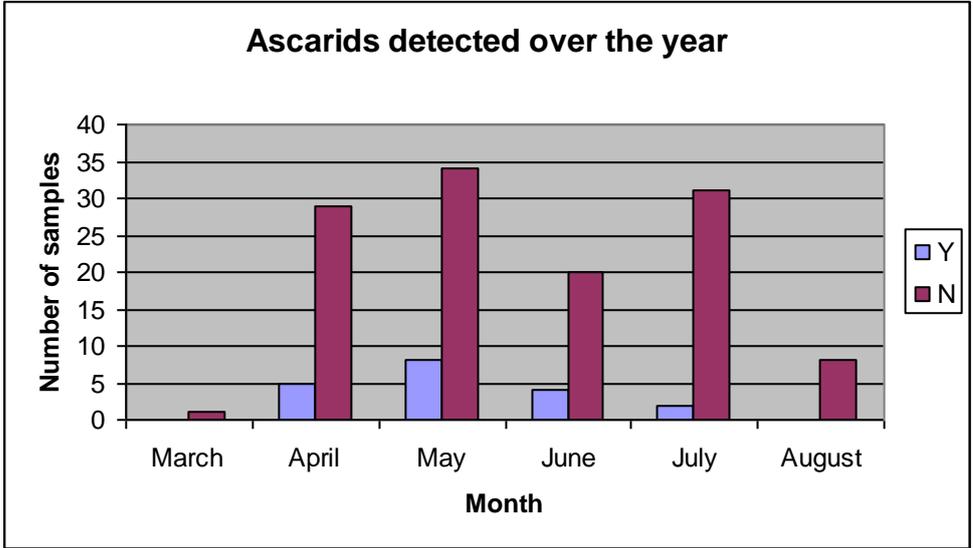
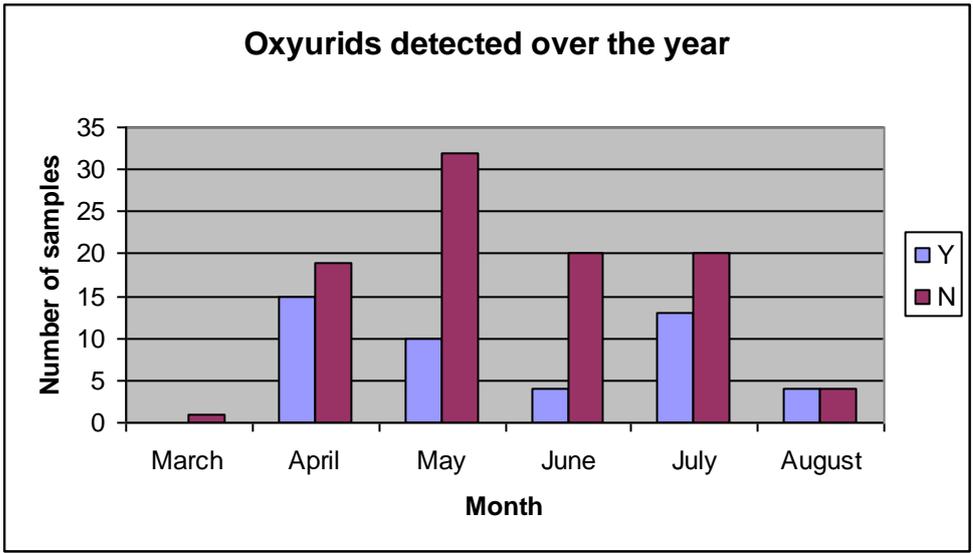


**Figure 18: Venn diagram to show protozoa identified by different diagnostic methods, n =19 positive samples**



**Figures 19-23: Bar charts to show distribution of parasites detected in each month of the study**





## **Discussion**

The results of this study show that intestinal parasites are common in pet tortoises throughout the UK, with a prevalence of 48.6% in animals without obvious clinical problems. Other authors have indicated a higher prevalence (<75%), but it is not specified if this includes both healthy and sick animals [McArthur 2004]. Previous surveys in the UK both indicated a slightly lower prevalence (30% and 43.8% respectively) despite being based on sick animals and including post-mortem data [Holt 1979, Keymer 1978].

Of the samples in this study, 32.4% were positive for oxyurids, 13.3% were positive for ascarids, and 13.3% were positive for protozoa (*Balantidium*, *Nyctotherus* and flagellates). No strongylids, trematodes, cestodes or other protozoa were detected. This contrasts with the previous UK surveys, one of which identified ascarids in all positive samples, with a concomitant oxyurid infection in only 5% and *Balantidium* in 4% [Holt 1979]; the other identified ascarids in 18.75% of cases, oxyurids in 16%, and protozoa in 22.9% of cases [Keymer 1978].

Epidemiological surveys carried out on pet tortoises in Italy, Germany, and Slovenia revealed a varying prevalence of parasites ranging from 43.1 to 81.8%. and, in agreement with the present study, all found that oxyurids are the most common parasite encountered in chelonians [Papini 2011, Pasmans 2008, Traversa 2005, Rataj 2011].

### **Risk factors for parasitism**

This study suggests that female tortoises overall are at higher risk of being positive for parasites than male tortoises. When looking more specifically at the presence of each individual parasite, more parasites are also seen in females, but the association is not statistically significant, probably due to the limited sample size. If female tortoises are at higher risk of being positive for parasites, there may be various reasons for this. A previous survey on tortoise gastrointestinal parasites found no sex differences in parasite distribution [Traversa 2005]. However, parasitology studies in other species often show differences between the sexes [Zuk 1996]. Most commonly in mammals and birds, male animals appear to have a higher parasite burden (proposed to be due to the

immunosuppressive effects of testosterone) but there is less evidence in reptiles [Poulin 1996, Salvador 1995]. Female animals may, however, be at increased risk at times when their oestrogen and progesterone levels are increased as both of these hormones may have immunosuppressive effects. Female animals may also display different behaviours to males including different patterns in feeding and time spent basking which may influence susceptibility to parasite infection [Klein 2004].

The study also suggests that tortoises which had been owned a short time (<5 yrs), are at higher risk of parasite infection than those owned for longer periods. However, most tortoises which had been owned for shorter periods were younger tortoises (see **Figure 12**). Tortoises which had been owned for a longer length of time were also more likely to have been treated for parasites (see **Figure 13**), and either or both of these factors could explain the decreased prevalence of parasites in those owned for longer periods.

### **Risk factors for oxyurids**

When looking more specifically at the prevalence of oxyurids, the study suggests various risk factors, the most important being age and previous parasite treatment.

The results suggest that younger tortoises (<10 years old) are at higher risk of oxyurid infection than older tortoises. Only one sample in this study, however, originated from a tortoise < 1 year old and this was negative. Age as a predisposing factor for parasite infections is not a novel theory in parasitology and has been described in various other species including dogs and horses [Visco 1977, Bucknell 1995]. A previous survey identified no nematodes in tortoises < 1 year old, while identifying nematodes in 100% tortoises > 1 year, but these groups were kept separately which may have influenced infection. The same survey however, identified higher egg counts in tortoises aged 1-5 years than in those > 5 years [Traversa 2005]. It was suggested that younger tortoises (1-5 years) therefore harbour more fecund species of gastrointestinal parasites and also that they are more likely to practice coprophagy, so increasing the chances of infection or reinfection.

In painted turtles it has even been suggested that parasite adaptability decreases as age of parasite increases. Therefore, the chance of a parasite surviving hibernation reduces each year, explaining reduced nematode burdens in older turtles. Alternatively changes in feeding habits of the host after sexual maturity were also suggested to play a role [Esch 1967].

The finding that previous parasite treatment reduces the risk of oxyurid infection is not surprising, as the vast majority of parasite treatments (except for one with levamisole, and one with a pyrantel and febantel combination) were with fenbendazole, which has been proven to reduce or eliminate nematodes in tortoises [Giannetto 2007].

Other risk factors which appeared to have a statistically significant association with parasite burden on univariate analyses included species, origin of tortoise, length of time owned, history of previous parasites, previous parasite treatment, and whether hibernation had occurred.

Species of tortoise has not been previously proved to be a risk factor with a previous survey showing parasites evenly distributed between *Testudo* species [Traversa 2005]. In contrast, another survey in Slovenia revealed a higher incidence of oxyurids in *T. hermanni* [Rataj 2011]. In this study, *T. hermanni* and *T. horsfieldi* tortoises were at significantly higher risk of oxyurids than *T. graeca*. This could be due to differences in feeding behaviour, time spent basking or increased species susceptibility for other unknown reasons.

Origin of tortoise also appeared to be a significant risk factor. Tortoises from pet shops, breeders and home bred were at higher risk of oxyurid infection than tortoises which had been re-homed. This could be due to space limitations and higher stocking densities in pet shops or breeding set facilities, allowing parasite burdens to build up in the environment. However, age was again a confounding factor as the majority of the rehomed tortoises were older individuals who may have been less likely to have parasites anyway.

A history of previous parasite problems also appeared to be a significant risk factor, with tortoises which had no previous history of parasites being at higher risk of oxyurid infection than those which had previously had parasites detected. However, it appears that almost all tortoises which had previously had parasites detected had been treated with anthelmintics (**see Figure 14**), in addition to some tortoises which had been treated without any evidence of parasites seen. Therefore parasite treatment is likely to have been a confounding factor.

Finally, for oxyurids, whether a tortoise was hibernated or not appeared to be a significant risk factor. Tortoises that had not been hibernated were at higher risk of oxyurid infection than those that had been hibernated. This is likely to be due to the low temperatures to which a tortoise is subjected to over hibernation, reducing although not eliminating parasite burdens [Esch 1967]. Again, age was a confounding factor, as younger tortoises were less likely to be hibernated than older tortoises.

### **Risk factors for ascarids**

When looking more specifically at the prevalence of ascarids, it was more difficult to draw meaningful conclusions from the data due to the small number of positive samples.

The most important risk factor appears to be length of time in owner's possession. In contrast with oxyurids, tortoises which have been owned a longer time (>5 yrs), were at higher risk of ascarid infection than those owned shorter periods. As previously discussed, there appeared a link between length of time in owner's possession and age and consequently older tortoises (>5yrs old) were at higher risk of ascarid infection than younger tortoises. This differs to findings in both mammals and previous findings in tortoises, where ascarids are more prevalent in younger animals [Visco 1977, Bucknell 1995, Traversa 2005]. The exact reason for this is unknown, but it is possible that ascarids are more of a historical problem (as reported in surveys 30 years ago) [Holt 1979, Keymer 1978] and that although older tortoises may still carry them, they are less of a problem in younger individuals, which appear more susceptible to oxyurids. This hypothesis is supported by the change in legislation of tortoise keeping in 1984 which banned the import, export and keeping and sale of Mediterranean species

except for captive-bred species or exempted animals sold under a licence and resulted in a change in the UK tortoise population from wild-caught *Testudo graeca* to captive bred *T. hermanni* [Pendry 2002], which appear more prone to oxyurids.

The environment in which a tortoise was kept also appeared to be a significant risk factor. Tortoises kept mainly outside were at higher risk of ascarid infection than those kept inside. The life cycle of tortoise ascarids are not fully understood, but it is possible that conditions in the outside environment would favour parasite reproduction, and would definitely be more difficult to fully clean resulting in parasite accumulation. However, older tortoises (and those which had been in the owner's possession for a longer time) were most commonly kept outside so these could be confounding factors.

### **Risk factors for protozoa**

When looking more specifically at the prevalence of protozoa, it was more difficult to draw meaningful conclusions from the data due to the small number of positive samples and the fact that the samples were > 24 hours old, making them suboptimal for protozoa detection.

The most important risk factor appears to be the presence of other tortoises. Tortoises that are kept with others are at higher risk of protozoal infection than those kept alone. Another risk factor which appeared to have a statistically significant association with parasite burden on univariate analyses was the substrate used. Tortoises kept on soil were at higher risk of protozoal infection than tortoises kept on alternative substrates. However, further research would be needed into these areas with specific methods for detecting protozoa.

## ***Cryptosporidium***

Of 113 samples examined, only one faecal sample was found to be positive for *Cryptosporidium*. This was from a 2 year female *Testudo hermanni* kept in a vivarium on newspaper, with one companion tortoise which tested negative. With the techniques used in this study, it was not possible to say if this was a mammalian strain which had been ingested incidentally or a true reptile pathogen. This study does however show clearly that *Cryptosporidium* oocysts are not seen as a common finding in tortoise faecal screens. Therefore if oocysts are found, their origin should be investigated further, ideally by molecular typing. This is especially important in view of the zoonotic potential of some strains of *Cryptosporidium*.

## **Comparing diagnostic tests**

The direct wet preparation was found to detect the most parasites in this study, in particular for ascarids and protozoa. In contrast, for oxyurids the flotation method detected the most parasites. However, no single diagnostic method detected all positive samples. Other authors agree that direct wet preparations are best for protozoa and flotation methods best for nematode ova [Urqhart 1996, Klingenberg 2000]. However, it would be expected that ascarid ova would be detected best by flotation methods, being similar in size to oxyurid ova. It is possible that the varying results were due to human error (e.g. insufficient sample mixing when making a suspension for the McMaster slide or flotation) or a flaw in study design, but further research would be necessary to determine the best method for detecting tortoise ascarids.

## **Seasonal variations in parasitism**

There were differences noted in parasite numbers detected over the course of the year with the highest percentage of positive samples being detected in April and May. This is in agreement with a previous study which describes a rise in parasite numbers after hibernation thought to be due to the maturation of hypobiotic larvae and a change in climatic conditions [Capelli 1998].

## **Conclusions**

This study demonstrates that gastrointestinal parasites are frequently detected in routine faecal screens of captive tortoises in the UK, without any known associated health concerns. Oxyurids are the most common parasite (especially in younger *T. hermanni* and *T. horsfieldi*), with ascarids also being identified (especially in older *T. graeca* kept outside). Ciliates and flagellates may also be present. Female tortoises, and those owned for shorter periods have a higher risk of parasitism. *Cryptosporidium* is not a normal finding on a routine faecal screen and should be investigated further if present.

## References

1. Urquhart GM, Armour J et al (1996) Veterinary Helminthology. In: Veterinary Parasitology. 2<sup>nd</sup> Edition. Blackwell Science, Oxford, 4-11
2. Frank W (1981) Endoparasites. In: Diseases of the Reptilia, Volume 1. Cooper JE, Jackson OF (eds). Academic Press, London. 291-358
3. Capelli G, Vorsato E et al (1998) Epidemiology of gastrointestinal parasites of tortoises (*Testudo hermanni boettgeri*) in captivity. *Parassitologia* 40:29
4. Wilkinson R (2004) Clinical Pathology. In: Medicine and Surgery of Tortoises and Turtles. McArthur S, Wilkinson R, Meyer J (eds). Oxford, Blackwell. 141-186
5. Bouamer S, Morand S, Bourgat R (2001) Oxyuroids of palearctic Testudinidae – new definition for *Alaeuris* (Nematoda: Pharyngodonidae) and redescription of *Alaeuris numidica*. *Journal of Parasitology*. 87(1): 128-133
6. Bouamer S, Morand S (2006) Nematodes Parasites of Testudinidae (Chelonia): List of species and biogeographical distribution. *Annales Zoologici*. 56(2): 225-240
7. Martinez-Silvestre A (2011) Massive *Tachygonetria* (Oxyuridae) infection in a Herman's tortoise (*Testudo hermanni*). *Consult Journal Special Edn* 2011: 409-412
8. Mitchell MA (2007) Parasites of Reptiles. In: Flynn's Parasites of Laboratory Animals, 2<sup>nd</sup> edn. Baker D G (ed), Blackwell Publishing, Iowa. 177-216
9. Thapar GS (1925) Studies on the oxyurid parasites of reptiles. *Journal of Helminthology*. 3(3-4): 83-150
10. Telford SR (1971) Parasitic Diseases of Reptiles. *Journal of the American Veterinary Medical Association*. 159: 1644-1652
11. Holt, PE, Cooper JE, Needham J R (1979). Diseases of tortoises: a review of seventy cases. *Journal of Small Animal Practice*. 20:5
12. Schneller P, Pantchev N (2008) Parasitology in snakes, lizard and chelonians. Edition Chimaira, Frankfurt am Main. 105-172

13. Jacobson E (2007) Parasites and Parasitic diseases of reptiles. In: Infectious Diseases and Pathology of Reptiles: Color Atlas and Text. CRC Press, Boca Raton. 571-665
14. Sprent JFA (1980) Ascaridoid nematodes of amphibians and reptiles: *Anguistacaecum* and *Krefftascaaris* n.g. Journal of Helminthology. 54:55-73
15. Cutler SL (2004) Nematode-associated aural abscess in a Mediterranean tortoise, *Testudo graeca*. Journal of Herpetological Medicine and Surgery. 14(3): 4-5
16. Keymer IF (1978) Diseases of chelonians: (1) Necropsy survey of tortoises. Veterinary Record. 103:548-552
17. Frye FL (1991) Applied clinical nonhemic parasitology of reptiles. In: Biomedical and Surgical Aspects of Captive Reptile Husbandry. Vol. I, 2nd edition, Malabar, Krieger. 281-325
18. Merdivenci A, Sezen Y (1965) The resistance of tortoises, *Testudo graeca* against *Toxocara canis* infection. Z. f. Parasitenkunde. 25: 387-392
19. Rideout B, Montali B et al (1987) Mortality of captive tortoises due to viviparous nematodes of the genus *Protractis* (Family Atractidae). Journal of Wildlife Diseases. 23(1): 103-8
20. Lichtenfels JR, Stewart TB (1981) Three new species of *Chapiniella* Yamaguti, 1961 (Nematoda: Strongyloidea) from tortoises. Proceedings of the Helminthology Society Washington. 48(2): 137-147
21. Rataj AV et al (2011) Parasites in pet reptiles. Acta Veterinaria Scandinavica. 53: 33
22. Greiner EC, Mader DR (2006) Parasitology. In: Reptile Medicine and Surgery. 2<sup>nd</sup> edn. Mader DR (ed). St. Louis, WB Saunders. 343-364
23. Johnson CA et al (1998) Fatal trematodiasis in research turtles. Laboratory Animal Science. 48(4): 340-343
24. Keymer IF (1981) Protozoa. In: Diseases of the Reptilia, Volume 1. Cooper J E, Jackson O F (Eds): Academic Press, London. 233-287
25. Scullion F, Scullion M (2009) Gastrointestinal protozoal diseases in reptiles. Journal of Exotic Pet Medicine. 18(4): 266-278
26. Lane TJ, Mader DR (1996) Parasitology. In: Reptile Medicine and Surgery. Mader DR (ed). WB Saunders, Philadelphia, 185-203

27. Klingenberg RJ (1993) Understanding Reptile Parasites: A Basic Manual for Herpetoloculturists and Veterinarians. Advanced Vivarium Systems, Lakeside, California. 59-61
28. Jacobson ER, Clubb S, Greiner E (1983) Amoebiasis in red-footed tortoises. Journal of the American Veterinary Medical Association. 187: 1192-1194
29. Hollamby S, Murphy D, Schiller C (2000) An epizootic of amoebiasis in a mixed species collection of juvenile tortoises. Journal of Herpetological Medicine and Surgery. 10: 9-15
30. Ozaki K, Matsuo K, Tanaka O, Narama I (2000) Amoebosis in the flat-shelled spider tortoise (*Acinixys planicauda*). Journal of Comparative Pathology. 123(4): 299-301
31. Philbey AW (2006) Amoebic enterocolitis and acute myonecrosis in leopard tortoises (*Geochelone pardalis*). Veterinary Record.158: 567-569
32. Barrow J, Stockton JJ (1960) The influences of temperature on the host parasite relationships of several species of snakes infected with *Entamoeba invadens*. Journal of Protozoology. 7: 377-383
33. McConnahie EW (1955) Studies of *Entamoeba invadens* Rodhain, 1934 *in vitro*, and its relationship to some other species of *Entamoeba*. Parasitology. 45: 452-481
34. Cranfield M, Graczyk T (1999) New approaches for diagnosis of *Entamoeba* infections in captive reptiles. Proceedings of the Association of Reptile and Amphibian Veterinarians. 17-18
35. Bone RD (1992) Gastrointestinal system. In: Manual of Reptiles. 1st edn. Beynon PH, Cooper JE, Lawton MPC (eds). British Small Animal Veterinary Association, Quedgeley. 107-108
36. Zwart P, Truyens EHA (1975) Hexamitiasis in tortoises. Veterinary Parasitology. 1: 175-183
37. Barnard SM, Upton SJ (1994) A Veterinary Guide to the Parasites of Reptiles. Vol. I Protozoa. Malabar, Krieger. 35-63
38. McAllister C T, Upton S J (1989) The Coccidia (Apicomplexa: Eimeriidae) of Testudines, with descriptions of three new species. Canadian Journal of Zoology. 67: 2459-2467

39. Leibowitz L, Rebell G, Boucher GC (1978) *Caryospora cheloniae* sp.n.: a coccidial pathogen of mariculture-reared green sea turtles *Chelonia mydas mydas*. Journal of Wildlife Diseases. 14: 269-275
40. Gordon AN, Kelly WR, Lester RJG (1993) Epizootic mortality of free-living green turtles *Chelonia mydas*, due to coccidiosis. Journal of Wildlife Diseases. 29(3): 490-494
41. Jacobson ER et al (1994) Intranuclear coccidiosis in radiated tortoises (*Geochelone radiata*) Journal of Zoo and Wildlife Medicine. 25: 95-102
42. Garner MM, Gardiner CH, et al (2006) Intranuclear coccidiosis in tortoises: nine cases. Veterinary Pathology. 43(3): 311-20
43. Innis CJ, Garner MM et al (2007) Antemortem diagnosis and characterization of nasal intranuclear coccidiosis in Sulawesi tortoises (*Indotestudo forsteni*). Journal of Veterinary Diagnostic Investigations. 19: 660-667
44. Schmidt V, Dyachenko V, et al (2008) Case report of systemic coccidiosis in a radiated tortoise (*Geochelone radiata*). Parasitology Research.102(3): 431-436
45. Lindsay DS, Sundermann CA (2007) Biology of Protozoa. In: Flynn's Parasites of Laboratory Animals, 2<sup>nd</sup> edn. Baker D G (ed). Blackwell Publishing, Iowa. 15-26
46. Bosschere, HD, Roels S. Balantidium sp. And Nycotherus sp.: Two common members of the digestive-tract flora in Mediterranean tortoises. Tortoise Trust website: <http://www.tortoisetrust.org/articles/balantidium.htm>. Retrieved January 13<sup>th</sup> 2012.
47. McArthur S (2004) Problem-solving approach to common diseases of terrestrial and semi-aquatic chelonians. In: Medicine and Surgery of Tortoises and Turtles. McArthur S, Wilkinson R, Meyer J (eds). Oxford, Blackwell. 347-349
48. Urquhart GM, Armour J et al (1996) The laboratory diagnosis of parasitism. In: Veterinary Parasitology. 2<sup>nd</sup> edn. Blackwell Science, Oxford. 4-11
49. Klingenberg RJ (2000) Reptilian parasite testing. In: Laboratory Medicine: Avian and Exotic Pets. Ed. Fudge AM. Philadelphia, WB Saunders. 258-264

50. Teare JA, Bush M (1983) Toxicity and efficacy of ivermectin in chelonians. *Journal of the American Veterinary Medical Association*. 183 (11): 1195-119
51. Bodri MS, Nolan TJ, Skeebe SJ (1993) Safety of Milbemycin (A3-A4 Oxime) in Chelonians. *Journal of Zoo and Wildlife Medicine*. 24(2): 171-174
52. Jepson L (2005) Prescribing for reptiles. In: *Veterinary formulary*. 6<sup>th</sup> edn. Bishop, Y M (ed) Pharmaceutical Press. 111
53. Soifer F (1978) In: *Zoo and Wild Animal Medicine*. Fowler M E (ed) WB Saunders. 138-143
54. Jacobson EJ (1983) Parasitic Diseases of Reptiles. In: *Current Veterinary Therapy VIII Small Animal Practice*, WB Saunders. 603
55. Girling S J (2004) Formulary. In: *Manual of Reptiles*. 2nd edn. Girling SJ, Raiti P (eds). British Small Animal Veterinary Association, Quedgeley. 352-356
56. Wilkinson R (2004) Therapeutics. In: *Medicine and Surgery of Tortoises and Turtles*. McArthur S, Wilkinson R, Meyer J (eds): Oxford, Blackwell, 465-485
57. Rees Davies R, Klingenberg RJ (2004) Therapeutics and medication. In: *Manual of Reptiles*. 2nd edition. Girling SJ, Raiti P (eds). British Small Animal Veterinary Association, Quedgeley. 115-130
58. Heggem B (2008). Fenbendazole. *Journal of Exotic Pet Medicine*. 17(4): 307–310
59. Holt P E (1982) Efficacy of fenbendazole against the nematodes of reptiles. *Veterinary Record*. 110: 302-304
60. Innis C (2008) Clinical parasitology of the chelonian. *Proceedings of the North American Veterinary Conference*, Orlando. 1783-5
61. Alvarado TP, Garner MM et al (2001). Fenbendazole overdose in four Fea's vipers (*Azemiops feae*). *Proceedings of the Annual conference of the Association of Reptilian and Amphibian Veterinarians*, Orlando. 35-36
62. Neiffer D, Lydick R, Burks K et al (2005). Hematologic and plasma biochemical changes associated with fenbendazole administration in Hermann's tortoises (*Testudo hermanni*). *Journal of Zoo and Wildlife Medicine*. 36(4): 661-672

63. Giannetto S, Brianti E et al (2007) Efficacy of oxfendazole and fenbendazole against tortoise (*Testudo hermanni*) oxyurids. *Parasitology Research*. 100: 1069–1073
64. Short CR, Barker SA et al (1987) Disposition of fenbendazole in the goat. *American Journal of Veterinary Research*. 48: 811-815
65. Highfield AC (1996) Parasitic Diseases. In: *Practical Encyclopaedia of Keeping and Breeding Tortoises and Freshwater Turtles*. Carapace Press, London. 109-117
66. Brames H (2010) Risks, benefits and limitations of spot-on endo and ectoparasite treatment in reptiles. *Proceedings of the 1<sup>st</sup> International Conference on Reptile and Amphibian Medicine*, Munich. 39-40
67. Schilliger L (2008) Use of the combination emodepside-praziquantel in reptiles: A new drug for endoparasite management? *Proceedings of the Annual conference of the Association of Reptilian and Amphibian Veterinarians*, LA. 11-16
68. Harnett W (1988) The Anthelmintic Action of Praziquantel. *Parasitology Today*. 4(5): 144-146
69. Melhorn H, Schmahl G, Mevissen I (2005) Efficacy of a combination of imidacloprid and moxidectin against parasites of reptiles and rodents: case reports. *Parasitology Research*. 97; S97-S101
70. Adnyana W, Ladds PW, Blair D (1997) Efficacy of praziquantel in the treatment of green sea turtles with spontaneous infection of cardiovascular flukes. *Australian Veterinary Journal*. 75(6): 405-407
71. Jacobson ER et al (2003) Plasma concentrations of praziquantel after oral administration of single and multiple doses in loggerhead sea turtles (*Caretta caretta*). *American Journal of Veterinary Research*. 64(3): 304-309
72. Kolmstetter CM, Frazier D et al (1997) Metronidazole pharmacokinetics in yellow ratsnakes (*Elaphe obsoleta quadrivittata*). *Proceedings of the American Association of Zoo Veterinarians Conference*. 26
73. Jacobson E R (1999) Use of antimicrobial drugs in reptiles. In: *Zoo and wild animal medicine - Current therapy*. 4<sup>th</sup> edn. Fowler E , Miller RE (eds). WB Saunders, Philadelphia. 190-200
74. Innis C, Papich M, Young D (2007) Pharmacokinetics of metronidazole in the red-eared slider turtle (*Trachemys scripta elegans*) after single

- intracoelomic injection. *Journal of Veterinary Pharmacological Therapy*. 30: 168-171
75. Holt PE (1981) Drugs & dosages. In: *Diseases of the Reptilia*, Vol. 2. Cooper JE, Jackson OF (eds). Academic Press, London. 551-584
76. Aycicek H et al (2001) Efficacy of some disinfectants on embryonated eggs of *Toxocara canis*. *Turkish Journal of Medical Science*. 31: 35-39
77. Upton SJ, McAllister CT, Freed PS, Barnard SM (1989) *Cryptosporidium* spp. in wild and captive reptiles. *Journal of Wildlife Disease*. 25(1): 20-30
78. Brownstein DG, Strandberg JD et al (1977) *Cryptosporidium* in snakes with hypertrophic gastritis. *Veterinary Pathology*. 14: 606-617
79. Brower A I, Cranfield M R (2001) *Cryptosporidium* sp. – associated enteritis without gastritis in Rough green snakes (*Opheodrys aestrivus*) and a Common garter snake (*Thamnophis sirtalis*). *Journal of Zoo and Wildlife Medicine*. 32(1): 101-105
80. Terrell SP, Uhl EW, Funk RS (2003) Proliferative enteritis in Leopard geckos (*Eublepharis macularius*) associated with *Cryptosporidium* sp. infection. *Journal of Zoo and Wildlife Medicine*. 34(1): 69-75
81. Dillehay DL, Boosinger TR, Mackenzie S (1986) Gastric cryptosporidiosis in a chameleon. *Journal of the American Veterinary Medical Association*. 189(9): 1139-1140
82. Oros J, Rodriguez JL, Patterson-Kane J (1998) Gastric cryptosporidiosis in a wild Frilled lizard from Australia. *Journal of Wildlife Disease*. 34(4): 807-810
83. Fitzgerald SD, Moisan PG, Bennett R (1998) Aural polyp associated with cryptosporidiosis in an iguana (*Iguana iguana*). *Journal of Veterinary Diagnostic Investigation*. 10: 179-180
84. Kik M J, Van Asten A, Lenstra J A, Kirpensteijn J (2011) Cloaca prolapse and cystitis in green iguana (*Iguana iguana*) caused by a novel *Cryptosporidium* species. *Veterinary Parasitology*. 175: 165-167
85. Deming C, Greiner E et al (2008) Prevalence of *Cryptosporidium* infection and characteristics of oocyst shedding in a breeding colony of leopard geckos (*Eublepharis macularius*). *Journal of Zoo and Wildlife Medicine*. 39 (4): 600-607

86. Heuschele WP, Oosterhuis J et al (1996) Cryptosporidial infections in captive wild animals. *Journal of Wildlife Diseases*. 22(4): 493-496
87. Funk RS (1988) Implications of cryptosporidiosis in emerald tree boas, *Corallus caninus*. In: Proceedings of the 10th and 11th International Herpetological Symposia on Captive Propagation and Husbandry. Rosenburg MJ (ed). Zoological Consortium, Chicago. 139-143
88. Graczyk TK, Cranfield MR, Fayer R (1996) Evaluation of commercial enzyme immunoassay (EIA) and immunofluorescent antibody (IFA) test kits for detection of *Cryptosporidium* oocysts of species other than *Cryptosporidium parvum*. *American Journal of Tropical Medicine and Hygiene*. 54(3): 274-279
89. Graczyk TK, Balzas GH et al (1997) *Cryptosporidium* sp. infections in Green turtles, *Chelonia mydas*, as a potential source of marine waterborne oocysts in the Hawaiian islands. *Applied and Environmental Microbiology*.. 63(7): 2925-2927
90. Raphael BL, Calle PP et al (1997) Clinical significance of Cryptosporidia in captive and free-ranging chelonians. Proceedings of the Annual meeting of the American Association of Zoo Veterinarians. 19-20
91. Traversa D, Iorio R et al (2008) *Cryptosporidium* from tortoises: Genetic characterisation, phylogeny and zoonotic implications. *Molecular and Cellular Probes* 22: 122–128
92. Graczyk T, Cranfield M et al (1998) Intestinal *Cryptosporidium* species infection in the Egyptian tortoise, *Testudo kleinmanni*. *International Journal for Parasitology* 28: 1885-8
93. Griffin C, Reavill D et al (2010) Cryptosporidiosis caused by two distinct species in Russian tortoises and a pancake tortoise. *Veterinary Parasitology*. 170: 14-19
94. McArthur S, McLellan L, Brown S (2004) Gastrointestinal system. In: *Manual of Reptiles*. 2nd edn. Girling SJ, Raiti P (eds). British Small Animal Veterinary Association, Quedgeley. 210-229
95. Fall A, Thompson RC, Morgan-Ryan U (2003) Morphology is not a reliable tool for delineating species within *Cryptosporidium*. *Journal of Parasitology*. 89: 399-402

96. Fayer R (2010) Taxonomy and species delimitation in *Cryptosporidium*. *Experimental Parasitology*. 124: 90-97
97. Pedraza-Diaz S, Ortega-Mora L et al (2009) Molecular characterization of *Cryptosporidium* isolates from pet reptiles. *Veterinary Parasitology*. 23(160): 3-4
98. Graczyk TK, Fayer R, Cranfield MR (1996) *Cryptosporidium parvum* is not transmissible to fish, amphibians or reptiles. *The Journal of Parasitology*. 82(5): 748-751
99. Traversa D (2010) Evidence for a new species of *Cryptosporidium* infecting tortoises: *Cryptosporidium ducismarci*. *Parasites & Vectors*. 3:21
100. Graczyk TK, Fayer R, Cranfield MR (1997) Zoonotic transmission of *Cryptosporidium parvum*: Implications of water-borne cryptosporidiosis. *Parasitology Today*. 13(9) 348-351
101. Graczyk TK, Owens R, Cranfield MR (1996) Diagnosis of subclinical cryptosporidiosis in captive snakes based on stomach lavage and cloacal sampling. *Veterinary Parasitology*. 67:143-151
102. Graczyk TL, Cranfield MR, Fayer R (1995) A comparative assessment of direct fluorescence antibody, modified acid fast stain, and sucrose flotation techniques for detection for *Cryptosporidium serpentis* oocysts in snake fecal specimens. *Journal of Zoo and Wildlife Medicine*. 26(3): 396-402
103. Graczyk TK, Cranfield MR (1997) Detection of *Cryptosporidium*-specific immunoglobulins in captive snakes by a polyclonal antibody in the indirect ELISA. *Veterinary Research*. 28(2): 131-142
104. Cranfield MR, Graczyk T K (1996) Cryptosporidiosis. In: *Reptile Medicine and Surgery*. Mader DR (ed). WB Saunders, Philadelphia. 359-363
105. Xaio L et al (2004) Genetic diversity of *Cryptosporidium* spp in captive reptiles. *Applied and Environmental Microbiology*. 70(2): 891-899
106. Graczyk TK, Cranfield MR, Hill SL (1996) Therapeutic efficacy of halofuginone and spiramycin treatment against *Cryptosporidium serpentis* (Apicomplexa: Cryptosporidiidae) infections in captive snakes. *Parasitology Research*. 82: 143-148

107. Pare JA (1997) Treatment of cryptosporidiosis in Gila monsters (*Heloderma suspectum*) with paromomycin. Proceedings of the Annual meeting of the Association of Reptile and Amphibian Veterinarians. 23
108. Graczyk TK, Cranfield MR et al (1998) Therapeutic efficacy of hyperimmune bovine colostrums treatment against clinical and subclinical *Cryptosporidium serpentis* infections in captive snakes. *Veterinary Parasitology* 74: 123-132
109. Graczyk TK, Cranfield MR, Bostwick EF (2000) Successful hyperimmune Bovine Colostrum Treatment of Savanna Monitors (*Varanus exanthematicus*) infected with *Cryptosporidium* sp. *Journal of Parasitology*. 86(3): 631-632
110. Graczyk TK, Cranfield MR, Bostwick EF (1999) Hyperimmune bovine colostrum treatment of moribund Leopard geckos (*Eublepharis macularius*) infected with *Cryptosporidium* sp. *Veterinary Research*. 30(4): 377-382
111. Graczyk TK, Cranfield MR, Bostwick EF (1999) Therapeutic efficacy of hyperimmune bovine colostrums treatment against *Cryptosporidium* infections in reptiles. Proceedings of the Annual meeting of the American Association of Zoo Veterinarians. 6-10
112. Campbell I, Tzipori S (1982) Effect of disinfectants on survival of *Cryptosporidium* oocysts. *Veterinary Record*. 111: 414-415
113. Wright K (1997) *Cryptosporidium* controversy: When do you consider a reptile Crypto-free? Proceedings of the Annual meeting of the Association of Reptile and Amphibian Veterinarians. 169-172
114. Traversa D, Capelli G, et al (2005). Epidemiology and biology of nematofauna affecting *Testudo hermanni*, *Testudo graeca* and *Testudo marginata* in Italy. *Parasitology Research*. 98: 14–20
115. Pasmans F et al (2008) Introducing reptiles into a captive collection: The role of the veterinarian. *The Veterinary Journal*. 175: 53-68
116. Papini R, Menetti C, Mancianti F (2011) Coprological survey in pet reptiles in Italy. *Veterinary Record*. 169: 207
117. Cooper JE (2009) In-practice and field techniques of the investigation of parasitic infections. *Journal of Exotic Pet Medicine*. 18(4): 289-298

118. Zajak AM, Conboy GA (2006) *Veterinary Clinical Parasitology*, 7<sup>th</sup> edn. Blackwell Publishing, Iowa. 8-10
119. Zuk M, McKean KA (1996) Sex Differences in Parasite Infections: Patterns and Processes. *International Journal for Parasitology*. 26(10): 1009-1024
120. Poulin R (1996) Sexual Inequalities I Helminth Infections: A Cost of Being a Male? *The American Naturalist*. 147(2): 287-295
121. Salvador A et al (1995) The cost of producing a sexual signal; testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behavioural Ecology*. 7(2): 145-150
122. Klein SL (2004) Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology*. 26: 247-264
123. Visco RJ, Corwin RM, Selby LA (1977) Effect of age and sex on the prevalence of intestinal parasitism in dogs. *Journal of the American Veterinary Medical Association*. 170(8): 835-837
124. Bucknell DG, Gasser RB, Beveridge I (1995) The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia. *International Journal for Parasitology*. 25(6): 711-724
125. Esch GW, Whitfield Gibbons J (1967) Seasonal incidence of parasitism in the Painted Turtle, *Chrysemys picta marginata* Agassiz. *The Journal of Parasitology*. 53(4): 818-821
126. Pendry S and Allan C (2002) *Selling like Hot Cakes: An investigation into the trade in tortoises in Great Britain*. TRAFFIC International and Defra, UK

## **Acknowledgements**

Thank you to the following individuals:

Kevin Eatwell for being my RCVS advisor and reviewing the manuscript

Darren Shaw for his guidance with the statistics

Clare Guichard for help processing samples

All the vets and owners that submitted samples

## Appendix 1

### EXAMPLES OF REFERENCE PICTURES FOR TORTOISE PARASITES

Figure 1: *Angusticaecum holopterum* (B) and oxyurids (A) [Traversa 2005]

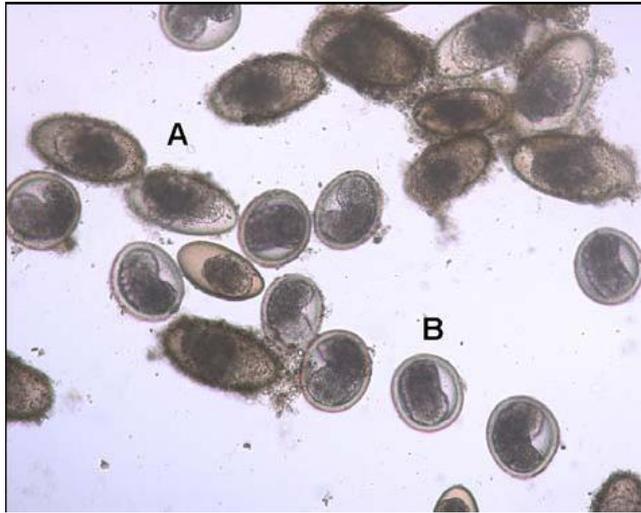


Figure 2: *Chapiniella* [Greiner 2006]

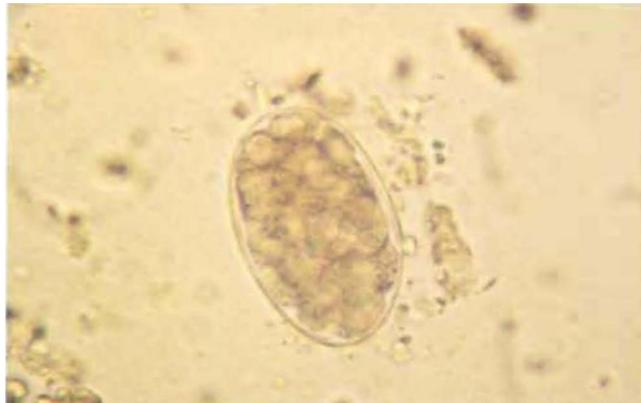


Figure 3: *Acanthocephalans* [Frye 1991]

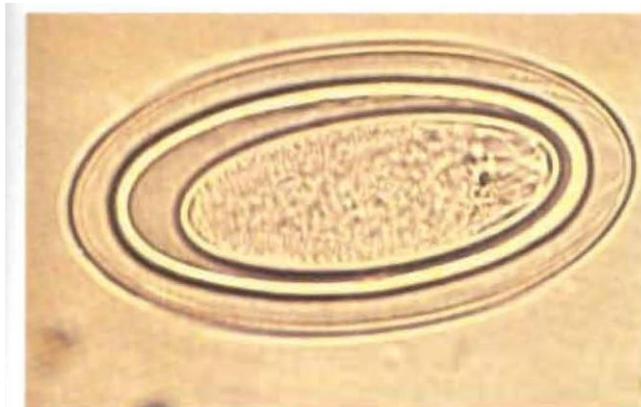


Figure 4: *Entamoeba* [Greiner 2006]

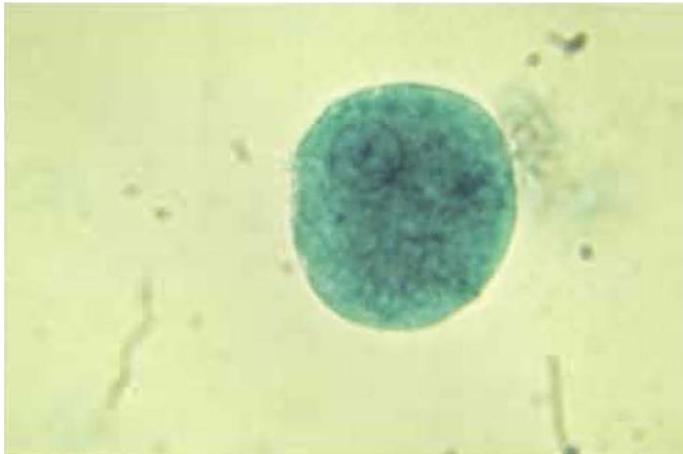
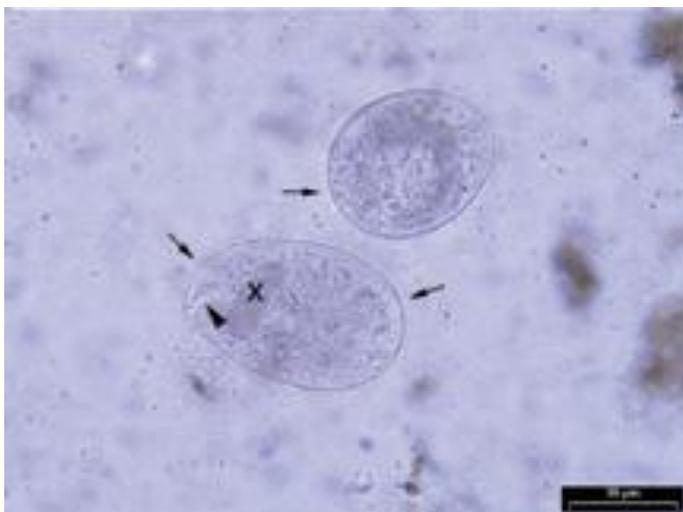


Figure 5: *Balantidium* [Bosschere 2012]



Figure 6: *Nyctotherus* [Bosschere 2012]



## Appendix 2

### **FAECAL PARASITOLOGY PROTOCOLS**

Sample must be collected one day, sent appropriately packed first class post (see Post Office Regulations) and examined the following day – to be placed in the fridge on arrival

#### DIRECT SMEAR

1. Mix equal volume faeces with equal volume normal saline (0.85%) on a slide
2. Mix thoroughly to form homogenous suspension
3. Remove any large pieces of faecal material
4. Ensure smear is thin enough to read print through it
5. Place cover slip
6. Examine whole slide starting at **x10** objective and then at **x 40** and record parasites seen.

#### SATURATED SALT FLOTATION (Modified McMaster method and direct flotation)

1. Weigh out 1g faeces
2. Mix with 14ml saturated sodium chloride solution and pour through a fine mesh sieve
3. Fill up McMasters slide
4. Leave to stand for 5 minutes
5. Examine both grids, counting number of worm eggs present at both **x10** objective and if necessary **x40**
6. Add the number of eggs in both grids, then  $\times 50 = \text{eggs/gram}$

If insufficient faeces, use 0.5g with 7ml saturated sodium chloride. If  $<0.5\text{g}$ , only direct flotation should be carried out

7. Mix remainder of faeces through a tea strainer with sufficient sodium chloride to fill a test tube
8. Place cover slip on top

9. Leave to stand for 15 minutes
10. Examine under **x10** objective and if necessary **x40**

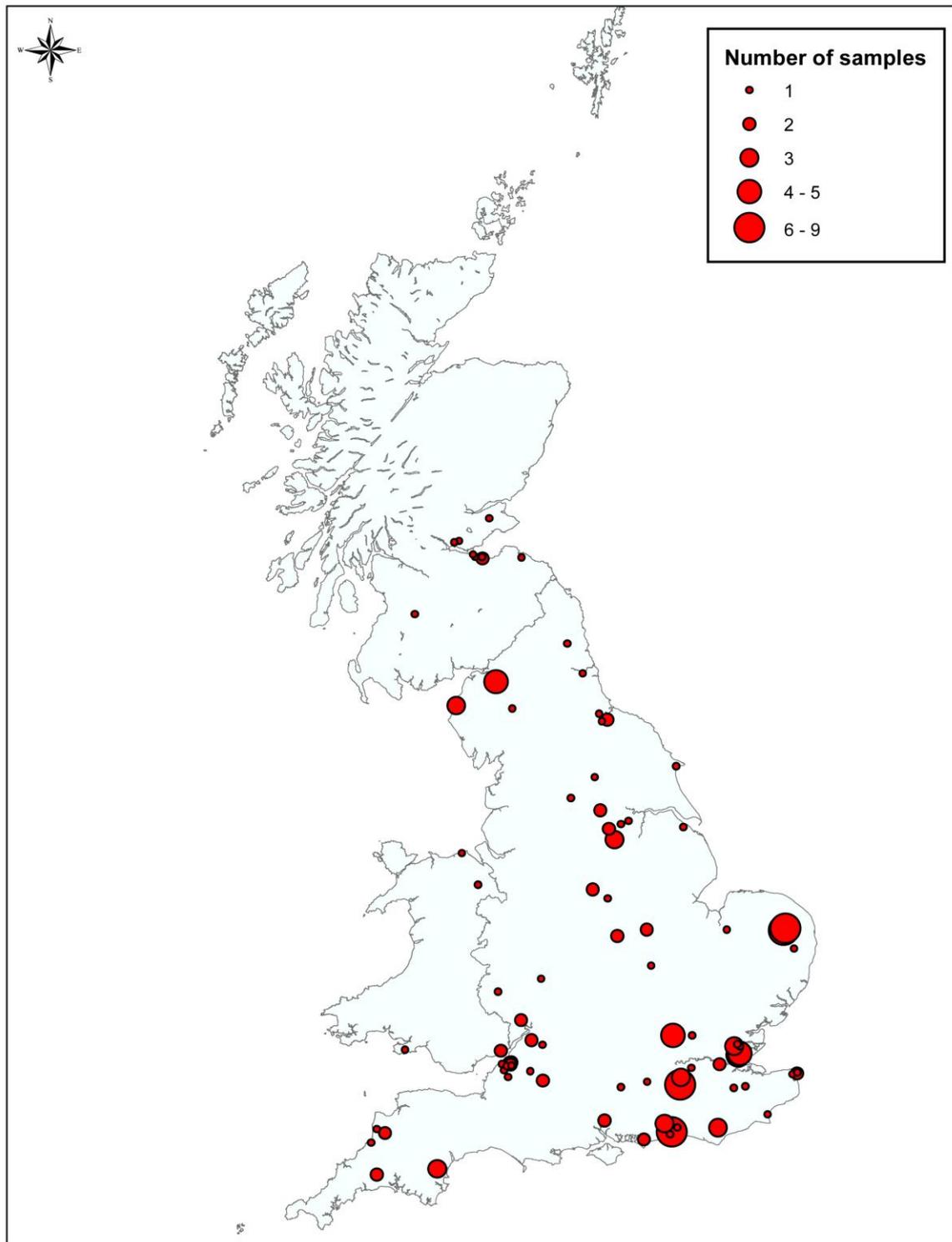
CRYPTOSPORIDIUM STAIN (Pro-Lab Diagnostics Guidelines)

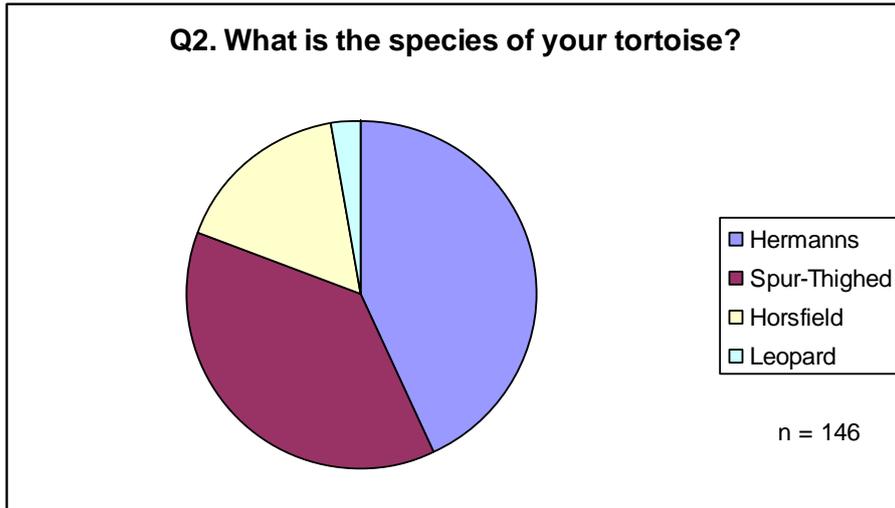
1. Prepare smear of material to be examined by emulsifying in saline on a glass slide and allow to air dry
2. Place slide on a staining rack and fix in Cryptosporidium Fixative for 1 minute. Allow to air dry
3. Apply Cryptosporidium Stain (ZN Carbol Fuchsin) to the slide and stain for 5 minutes
4. Pour off excess stain and wash with Differentiator 1. Rinse slide in water, and shake off any excess
5. Apply Differentiator 2 for 2 minutes or until no more stain washes out of the smear
6. Wash off Differentiator 2 by rinsing in water. Shake off excess water
7. Apply Cryptosporidium Counterstain (Malachite Green) and stain for 1 minute
8. Remove excess stain by rinsing slide in water. Shake off excess water
9. Blot slide gently on clean blotting paper and dry using gentle heat
10. Examine stained slide under the light microscope using oil immersion objective x 100 under oil

## Appendix 3

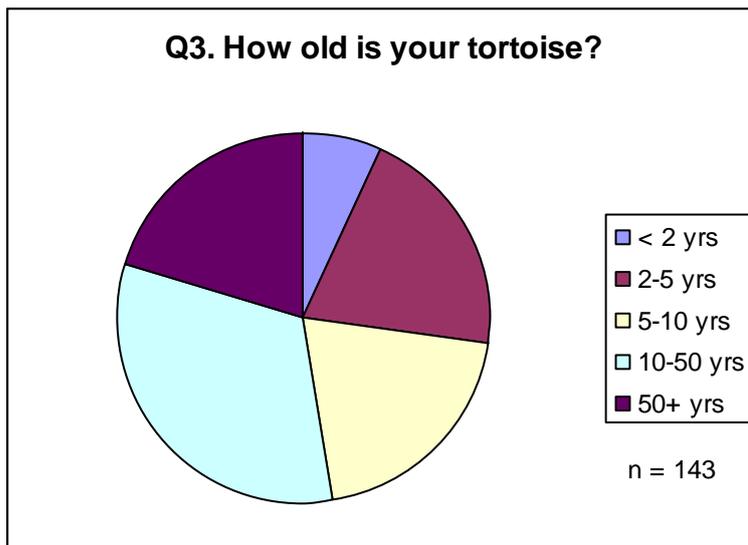
### QUESTIONNAIRE DATA

Q1. Where do you live? – County and postcode (e.g. EH9)





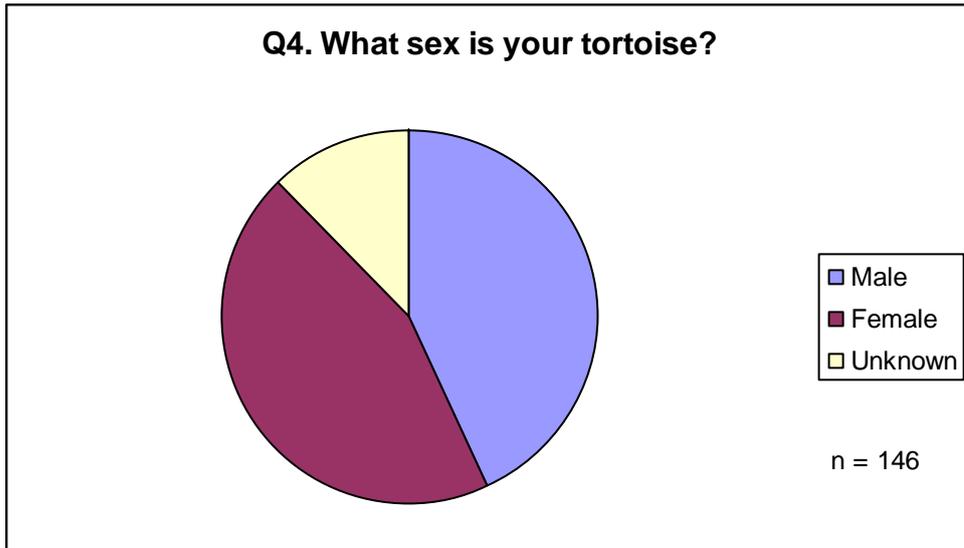
63 Hermann's, 55 Spur-Thighed, 24 Horsfield, 4 Leopard  
 n= 146



Ages were split into 5 different categories:

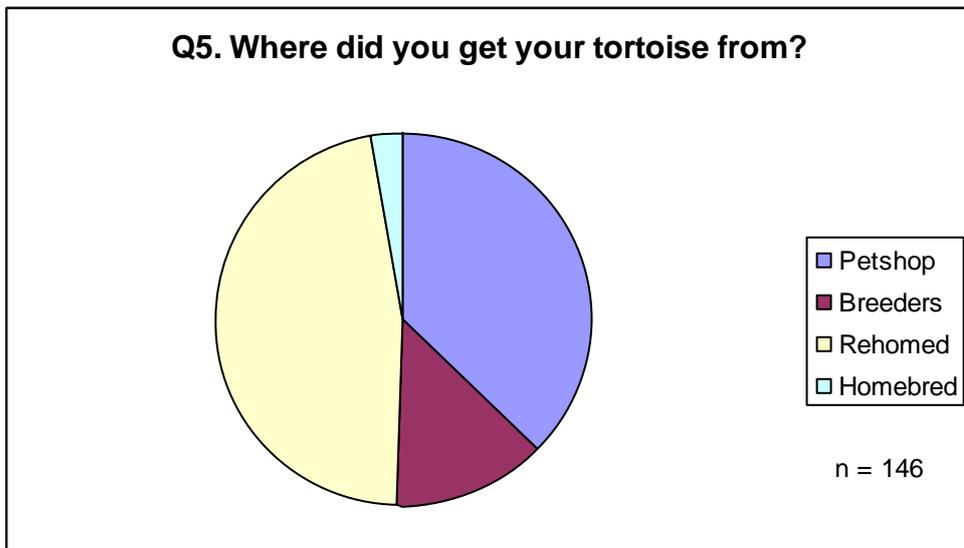
- <2 years
- 2-5 years
- 5-10 years
- 10-50 years
- 50+ years

10 (< 2 yrs), 29 (2-5 yrs), 29 (5-10 yrs), 46 (10-50 yrs), 29 (50+ yrs)  
 n = 143 (as 3 owners did not answer this question)



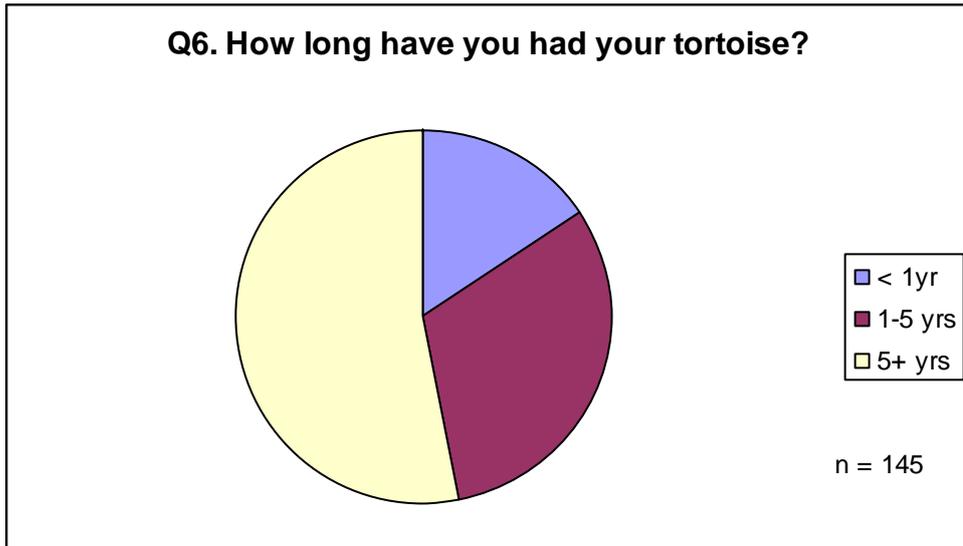
63 male, 65 female, 18 unknown

n = 146



54 pet shop, 19 breeders, 69 rehomed, 4 home bred

n = 146

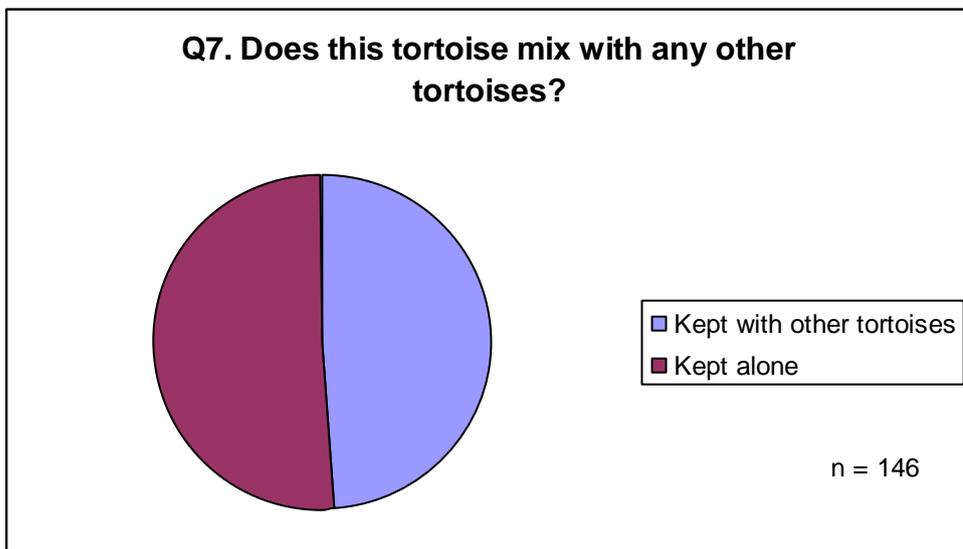


Time periods that tortoise had BIOP were split into 5 different categories:

- <1 year
- 1-5 years
- 5+ years

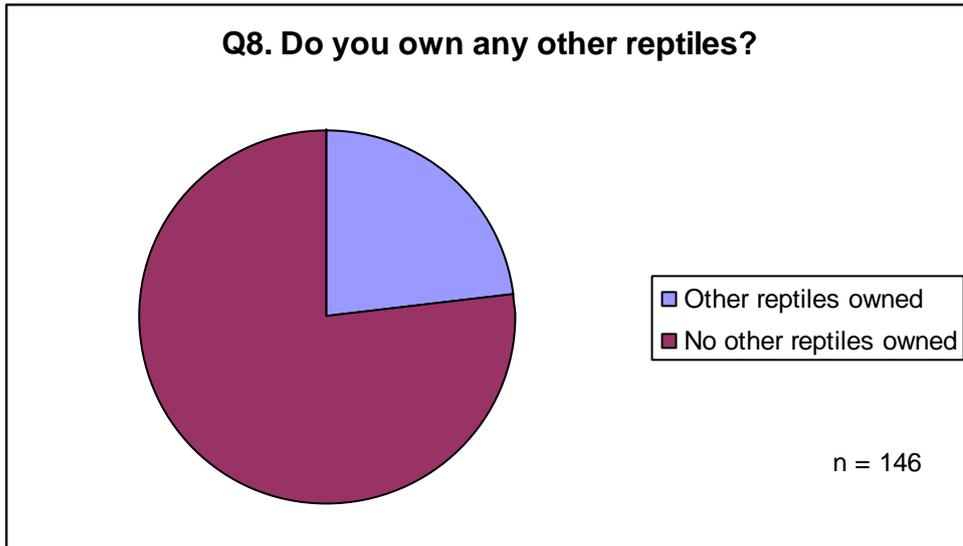
23 (< 1yr), 45 (1-5 yrs), 77 (5+ yrs)

n = 145 (as 1 owner did not answer the question)

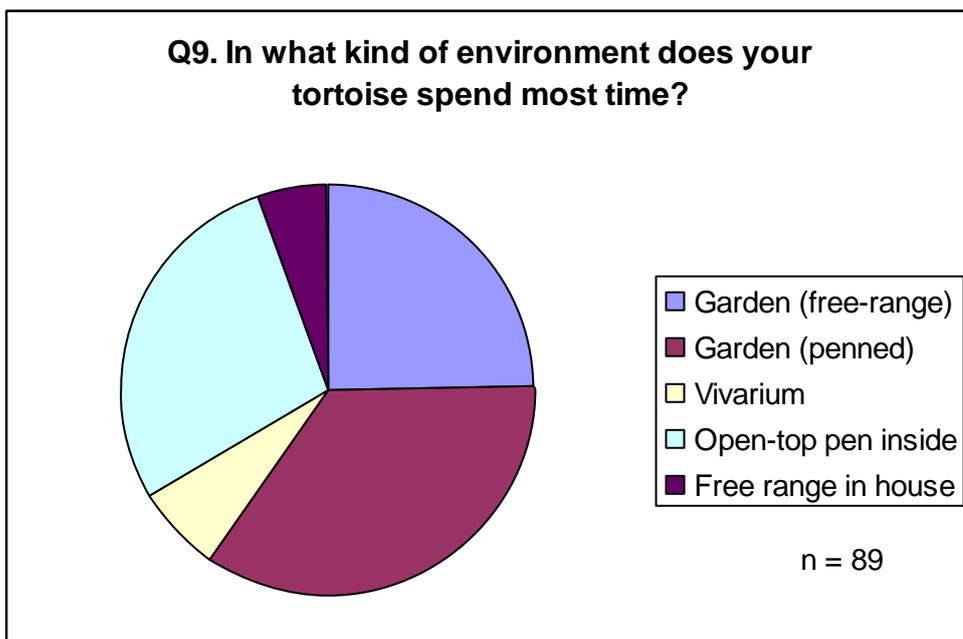


71 mix with other tortoises, 75 are kept alone

n = 146

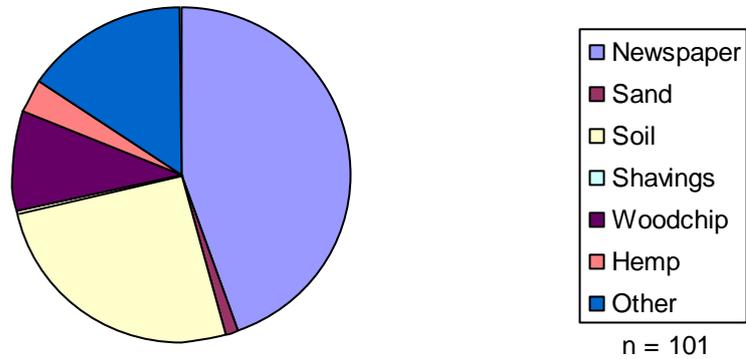


34 have other reptiles, 112 have no other reptiles  
n = 146



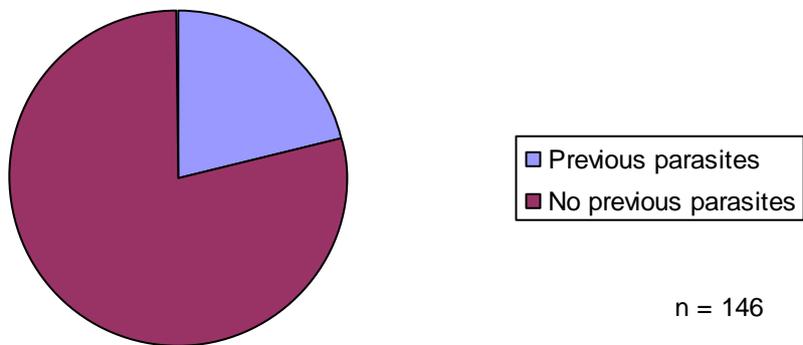
22 garden (free-range), 31 garden (penned), 6 vivarium, 25 open-top pen inside, 5 free range in house  
n = 89 (as 57 owners did not answer the question)

**Q10. On what substrate (bedding) do you mostly keep your tortoise?**

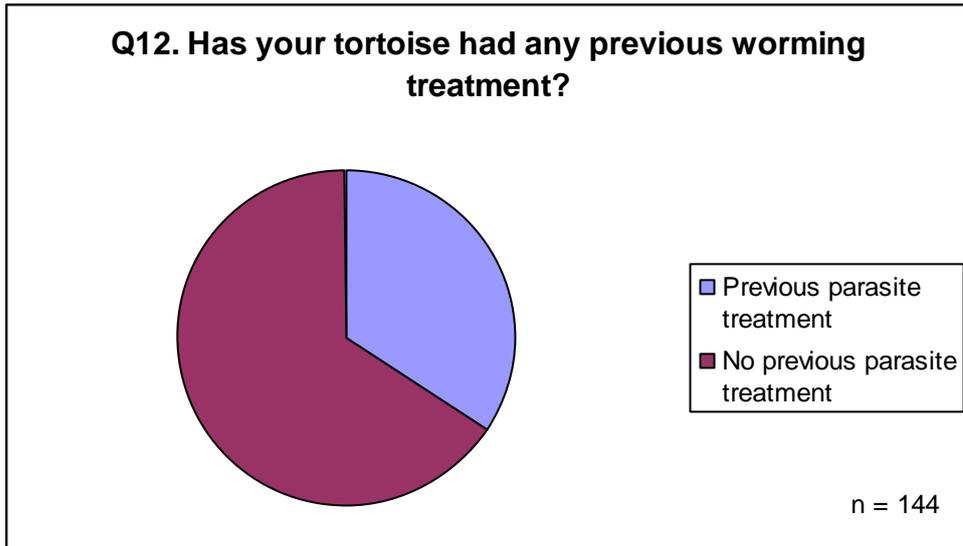


45 newspaper, 1 sand, 26 soil, 0 shavings, 10 woodchip, 3 hemp, 16 other  
n = 101 (as 45 owners did not answer the question)

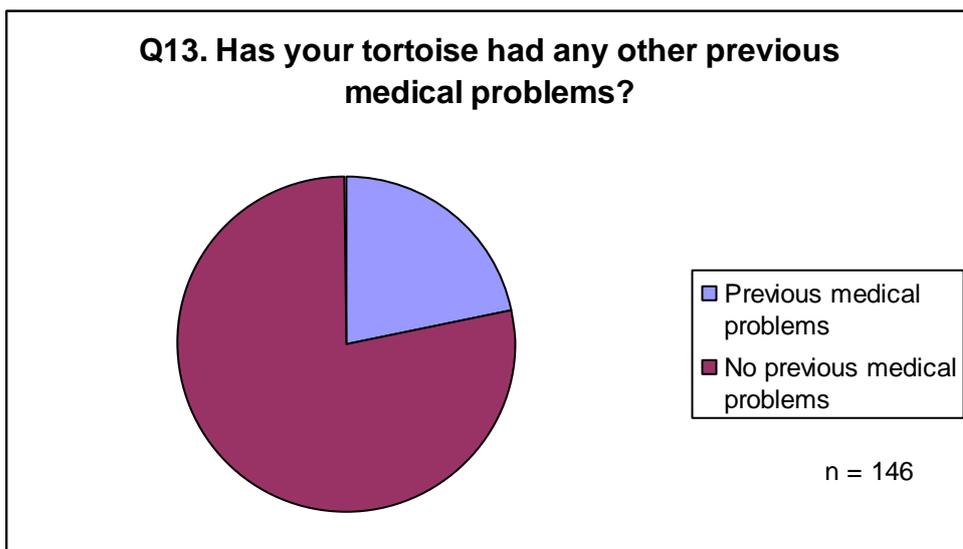
**Q11. Has your tortoise had any previous parasites/worms of which you are aware?**



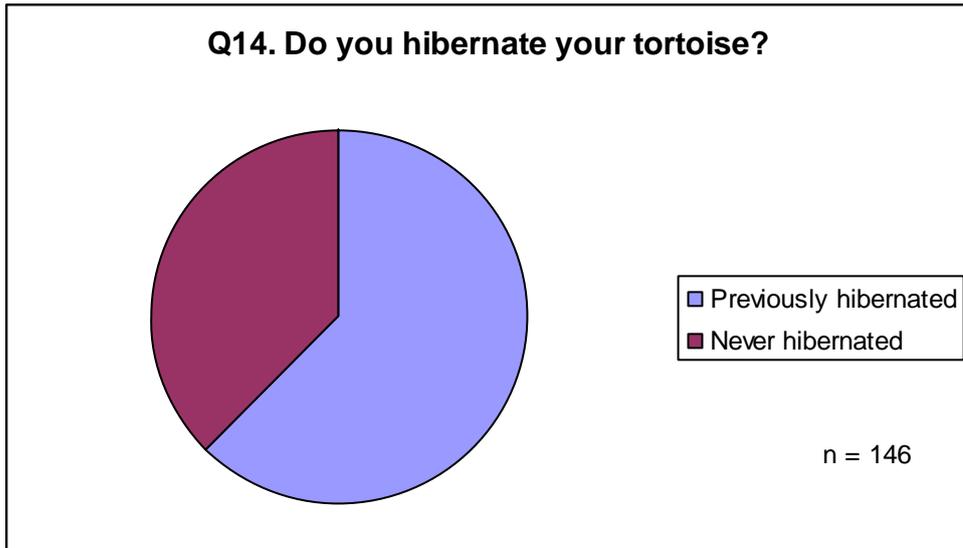
31 had previous parasites, 115 had no previous parasites (to the owner's knowledge)  
n = 146



49 had previous parasite treatment, 95 had no previous parasite treatment  
n = 144 (2 owners did not answer this question)

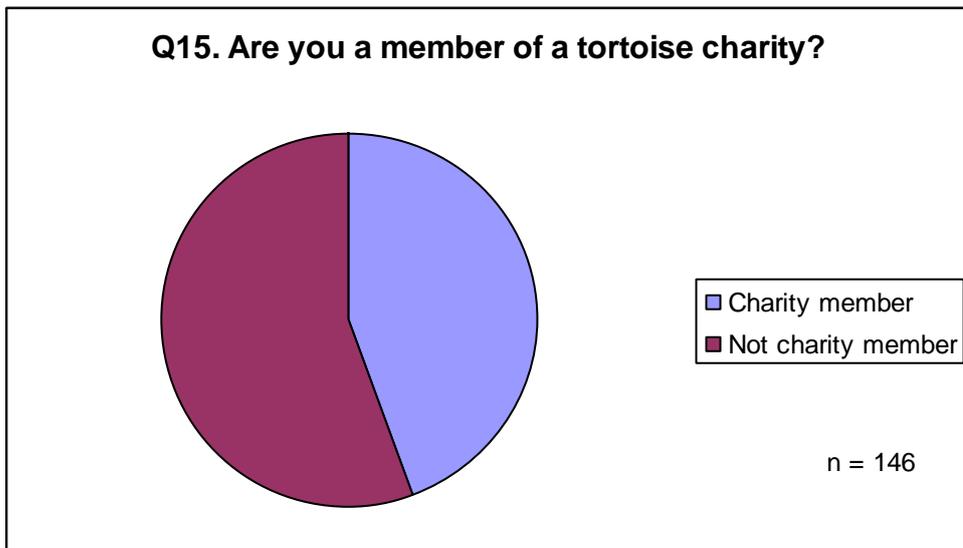


32 had previous problems, 114 had no previous problems  
n = 146



91 hibernate, 55 do not hibernate

n = 146



81 are not tortoise charity members, 65 are tortoise charity members

n = 146