A REVIEW OF GASTROINTESTINAL PARASITES IN TORTOISES

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Gastrointestinal parasites are commonly found in the faeces of both wild and captive tortoises (Wilkinson 2004). In low numbers they often appear to cause no obvious problems. In a confined captive situation, however, tortoises may be under stress and immunosuppressed especially if overcrowded, hygiene is poor or husbandry is inappropriate. In this situation, parasites can multiply rapidly if they do not require an intermediate host to complete their life cycle (Schneller & Pantchev 2008).

There are many types of gastrointestinal parasites, but broadly they may be divided into trematodes (flukes), cestodes (tapeworms), nematodes and protozoa. Trematodes and cestodes usually require an invertebrate intermediate host and are predominantly reported in aquatic or semi-aquatic chelonians (Wilkinson 2004). Nematodes, in contrast, are commonly seen in terrestrial chelonians with oxyurids, ascarids, hookworms and strongyles all appearing in the literature. Protozoal parasites are also commonly reported and may include ciliates, flagellates and, less commonly, coccidia and amoeba. A recent survey of UK tortoises with no known health problems indicated that 48.6% were positive for one or more parasites: 32.4% were positive for oxyurids, 13.4% for ascarids, 13.4% for protozoa excluding *Cryptosporidium* and 0.8% for *Cryptosporidium.* Female tortoises and those that had been in their owner's possession for less than five years were at higher risk of parasites, although the type of parasite found appeared to vary depending on the age and species of the tortoise amongst other factors (Hedley *et al.* 2011). Some of the more common parasites will be discussed in more detail below.

Gastrointestinal nematodes

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

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

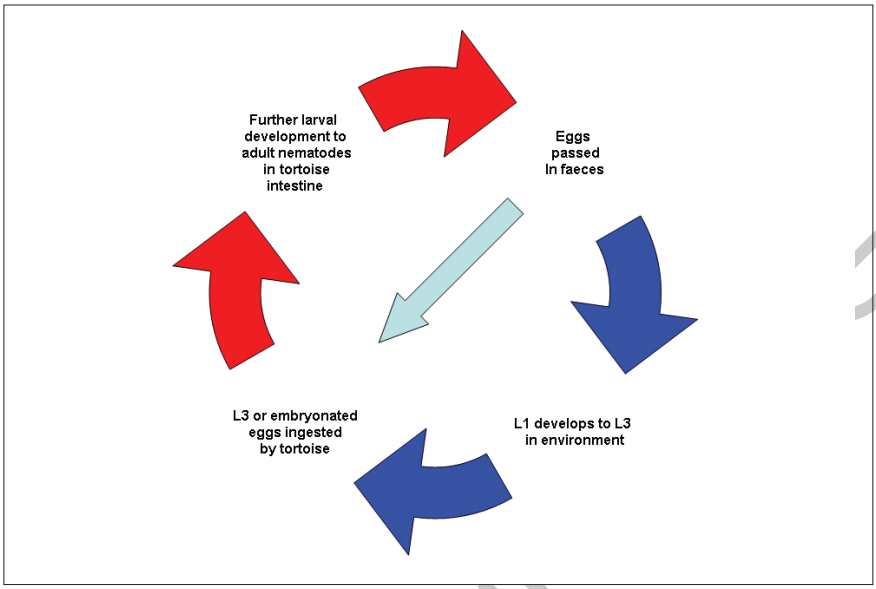

Fig. 1. The nematode life cycle.

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 & Armour 1996; Frank 1981). The prepatent period (period between infection of an individual by a parasite and detection of the parasite in that individual) 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 *et al.* 1998).

Oxyurids (pinworms)

There are a wide variety of oxyurids reported in tortoises (Wilkinson 2004). 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 to 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

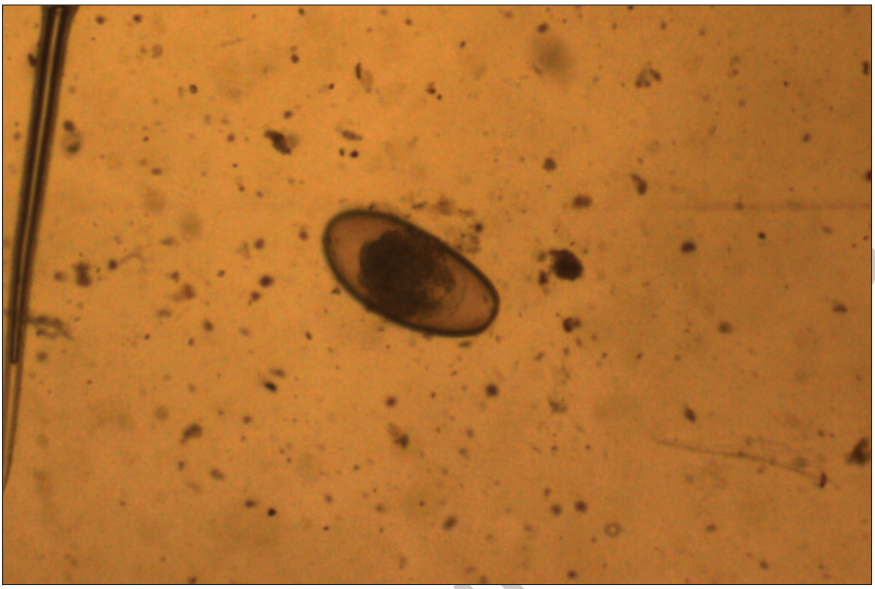

Fig. 2. Microscopic image of an oxyurid egg.

an embryonated egg (Mitchell 2007). Ova are variable in morphology, with different stages of embryonation often seen, but usually appear either oval or asymmetrical (D-shaped) (Fig. 2). The surrounding shell may also be variable in diameter, but lacks the scalloped surface and very thick walls typical of ascarids (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). Larval migration outside the gastrointestinal tract is not reported. Oxyurids are generally considered to be commensals within the tortoise's 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 *et al.* 1979). Adult worms may be large, measuring up to 10cm in length (Frank 1981) and often have a pale appearance (Schneller & Pantchev 2008) (Fig. 3). 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

Fig. 3. Adult ascarids may be seen in the faeces.

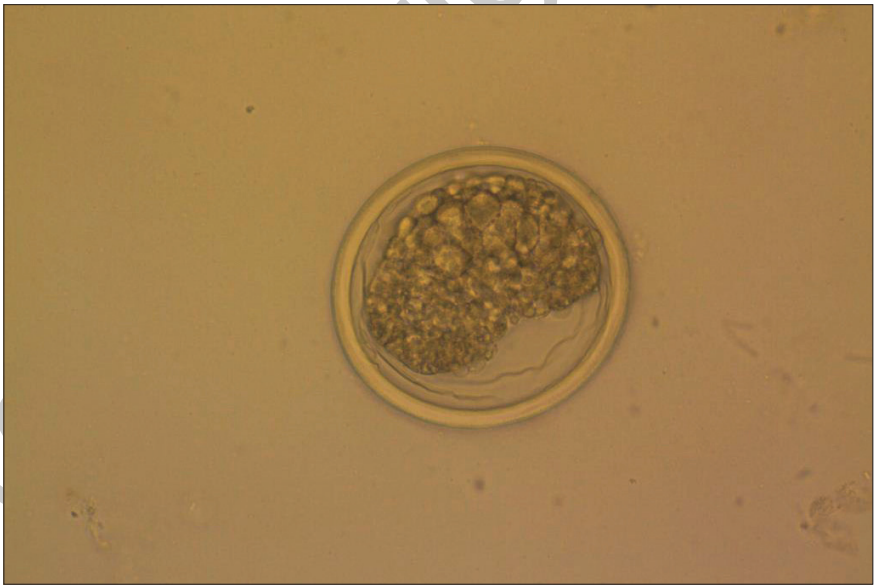

Fig. 4. Microscopic image of an ascarid egg.

host follows ingestion of an egg. Ova are typically round and thick-shelled (Fig. 4) (Jacobson 2007). However, one important difference from oxyurids is that the larvae of some ascarids may migrate through the body (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) in addition to ulceration and infection in some cases (Frye 1991). Ascarids from mammals have been experimentally inoculated into *Testudo graeca,* but do not reproduce or appear to have any pathogenic effects in tortoises at normal environmental temperatures (Merdivenci & Sezen 1965).

Gastrointestinal protozoa

A wide variety of protozoa have been detected in chelonian faecal samples, 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 associated with high protozoal burdens (Scullion & Scullion 2009) and a few examples will therefore be discussed.

Ciliates

Balantidium and *Nyctotherus* are commonly found ciliates, which have both been suggested to be commensals of the tortoise's gastrointestinal tract and aid digestion of 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 associated with liver abscesses in heavily infected tortoises (Schneller & Pantchev 2008). The exact details of these ciliate life cycles are unknown, but transmission is suggested to be via an infective cyst. This is ingested, travels to the small intestine and produces trophozoites. These colonise the large intestine where they replicate and also form new infective cysts (Bosschere & Roels 2012). The *Balantidium* trophozoite may be identified by its ciliate appearance and oval shape, whereas cysts are round. *Nyctotherus* trophozoites are larger but their cysts are ovoid, operculated and a similar size to the trophozoites (Barnard & Upton 1994).

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). Various species may be present within the chelonian gastrointestinal tract, but trichomonads appear the most common in the literature (Schneller & Pantchev 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 & Truyens 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 renal changes and, in some cases, inflammation of the intestines and infection of the bile ducts (Zwart & Truyens 1975).

Detecting parasite infections in tortoises

Clinical signs of parasite infection may vary from none to anorexia, diarrhoea, intestinal obstruction, weight loss, straining to pass faeces, prolapses and even anaemia and death in exceptionally severe burdens (Wilkinson 2004). Diagnosis of a parasite infection is usually fairly straightforward. Sometimes adult worms may be seen in the faeces, or alternatively a faecal sample may be examined microscopically 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 (Fig. 5). 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 (Fig. 6). 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. It is important to

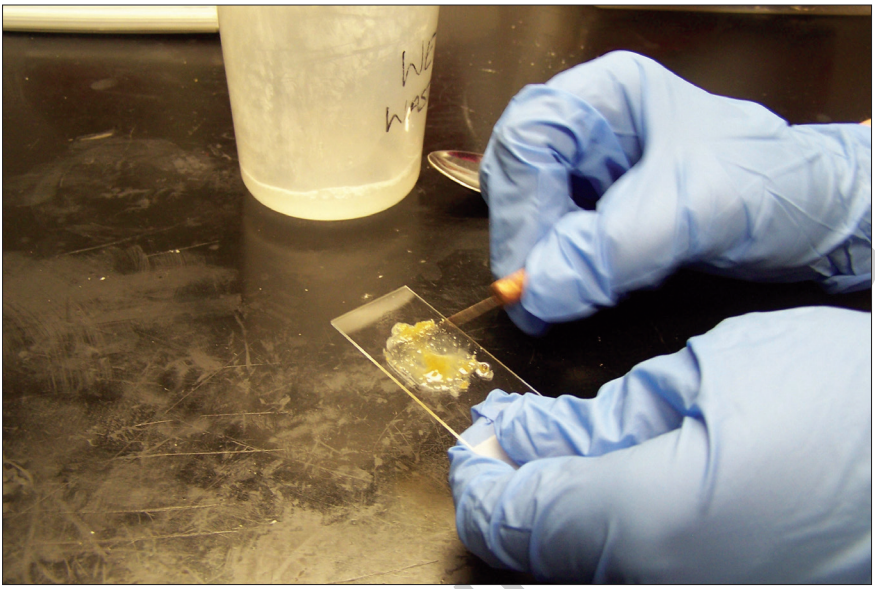

Fig. 5. Making a direct wet preparation of faeces.

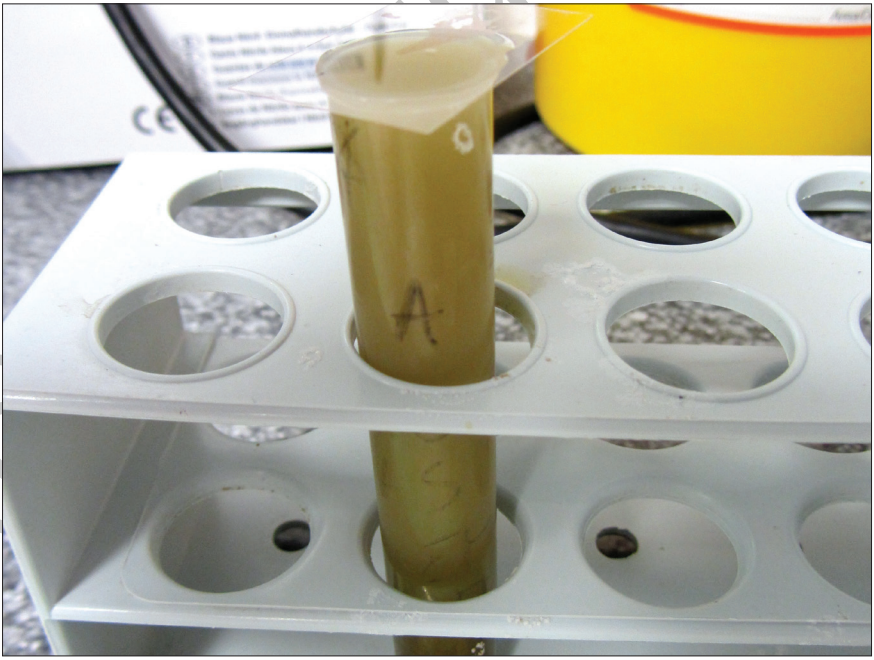

Fig 6. Performing a faecal flotation.

note that, although ova may be detected by the above methods, definitive species typing usually requires examination of an adult worm (Urqhart 1996).

Treatment of parasite infections in tortoises

Regular faecal screens are recommended to check the type and level of parasites present and determine if parasite treatment is necessary. 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 species resulting in paresis, flaccid paralysis, hepatic lipidosis and death (Teare & Bush 1983). Treatment with other wormers including milbemycin and levamisole has been suggested but data on efficacy and safety is limited and potential side effects may be severe.

In the UK fenbendazole is the most commonly used treatment for nematode infections. However, studies 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 (Holt 1982; Girling 2004; Innis 2007). Initially, fenbendazole was considered to have no significant side effects, but profound immunosuppression has been demonstrated in six *Testudo hermanni* following two five-day courses of fenbendazole treatment two weeks apart (Neiffer *et al.* 2005). Repeated treatments given two to three weeks apart may be safer, but may be unnecessary as it can take up to 31 days for one dose of fenbendazole to have full effect at reducing egg counts (Giannetto *et al.* 2007). Alternatively, other related drugs such as oxfendazole have been suggested to have a wider safety margin than fenbendazole, but there is little evidence to support this theory. In recent years, some of the newer endoparasiticide spot-on formulations such as emodepside and praziquantel spot-on (Profender®) have also been trialled in small tortoises and appear to work to some degree, although response was slow and likely to be less effective in larger tortoises with thicker scutes (Brames 2010).

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 at a variety of dosages (Innis 2007). Coccidial infections will require alternative anti-protozoals, usually sulfa drugs such as trimethoprim sulfadiazine (Lane & Mader 1996; Girling 2004).

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 *et al.* 2001). Supportive treatment may also be necessary for the debilitated individual and any obvious problems in husbandry or diet should be corrected (Schneller & Pantchev 2008). Repeat faecal samples after treatment to assess efficacy may be advised, although optimum timing for these has not been determined.

In summary, gastrointestinal parasites are commonly seen in tortoises and may not be associated with any health concerns. Regular faecal checks are recommended to ensure that numbers of parasites are not excessive and treatment is advised if numbers are high or associated with any clinical problems.

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