Gastrointestinal parasitic burdens in UK tortoises: survey of tortoise owners and potential risk factors

Joanna Hedley BVM&S DZooMed MRCVS [#], Kevin Eatwell BVSc (hons) DZooMed Dip ECZM MRCVS, Darren J Shaw BSc PhD,

Royal (Dick) School of Veterinary Studies and The Roslin Institute, The University of Edinburgh, Easter Bush Campus, Roslin, Midlothian, Scotland EH25 9RG

[#] Author for correspondence

Joanna Hedley

0131 650 7650

0131 650 7651 (fax)

Joanna.Hedley@ed.ac.uk

Word count 3991

Abstract

Despite gastrointestinal parasites being commonly diagnosed in captive tortoises throughout the UK, there is a lack of data regarding the prevalence. The aims of this study were to investigate the prevalence of gastrointestinal parasites in tortoises in the UK, and to investigate the factors affecting the prevalence of these parasites.

Owners were invited to submit a faecal sample from their tortoise in conjunction with a completed questionnaire covering details of signalment and husbandry. Data from the questionnaires was analysed at the end of the study. Faecal analysis was performed on samples from *Testudo hermanni*, *T. graeca*, and *T. horsfieldii*. This involved examination of direct wet preparations, a Modified McMaster technique, passive NaCl flotation and *Cryptosporidium* staining.

Of the 142 samples used, 130 were examined by the first three methods. 49% were positive for one or more parasites. Of the positive samples, 67% were positive for oxyurids, 28% were positive for ascarids, and 28% were positive for protozoa (*Balantidium*, *Nyctotherus* or flagellates). Only 1/113 (0.8%) samples was positive for *Cryptosporidium.* The most important risk factors for parasites were sex (F) and length of time (< 5 years) in owner’s possession. This survey showed that gastrointestinal parasites are frequently detected in the faecal samples of captive tortoises in the UK, but their prevalence may be influenced by various factors including sex, length of time owned, age and species.

Introduction

Gastrointestinal parasites are commonly diagnosed on routine faecal screens of captive tortoises [Wilkinson 2004]. There are a wide variety of parasites reported including oxyurids, ascarids, and various protozoa [Wilkinson 2004]. Oxyurids are generally considered to be commensals within the tortoise intestinal tract and have been suggested to have a beneficial effect in churning up faecal matter and preventing constipation [Telford 1971]. However, high numbers of oxyurids have also been associated with anorexia [Martinez-Silvestre 2011] and even death post-hibernation, possibly due to their effect on depriving their host of nutrition [Frank 1981]. Ascarids have been associated with more varied pathology due to larval migration through the viscera [Sprent 1980]. In one case report an adult nematode (*Angusticaecum* spp.) was removed from an aural swelling in a *Testudo graeca* [Cutler 2004]. Generally however, ascarids are unlikely to cause disease in low numbers, although gastrointestinal obstruction by adult nematodes has been reported [Keymer 1978]. Other pathological effects associated with ascaridiasis include intussusception, gastrointestinal ulceration, coelomitis, thromboembolism and avascular necrosis [Frye 1991].

The gastrointestinal protozoa reported in tortoises include amoeba, *Cryptosporidium*, other coccidia, flagellates, and most commonly, the ciliates [Wilkinson 2004]. Of the ciliates *Balantidium* and *Nyctotherus* are commonly seen, both of which have been suggested to be commensals of the gastrointestinal tract in tortoises helping digest cellulose [Frye 1991]. An increased number of ciliates may be detected at times of gastrointestinal disturbance and have been suggested to cause colitis [Bone 1992].

Balantidium has also been described in the liver of heavily infected tortoises, associated with abscesses [Schneller 2008].

Routine faecal screening is often advised as part of a preventative health program for captive tortoises [Pasmans 2008], but without knowledge of the normal parasite burden for a species, there is little evidence base to determine what level of parasites may be a problem for an individual tortoise. Unfortunately, despite the common reporting of infections there is a lack of data regarding the prevalence of these parasites in captive tortoise populations. The only published data in the UK are from two small studies. One is a review of clinical and pathological findings in 70 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 study was a necropsy survey of a variety of 144 tortoises kept in a zoological collection which identified nematode infections in 43.8% cases with a mix of ascarids and oxyurids found [Keymer 1978].

More recently it has been suggested that <75% of pet tortoises passed oxyurid eggs in their faecal samples [McArthur 2004]. Epidemiological surveys carried out in other countries including a survey of 62 tortoises in Italy, 1251 chelonians in Germany and 563 chelonians in Slovenia have also shown a high prevalence (between 43-82%) 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].

In addition, the factors affecting parasite burden have not been investigated in captive tortoises, although it has been suggested that age and husbandry setup may play a role in influencing the prevalence of parasites [Traversa 2005]. Therefore, the aims of this study were two fold - to investigate the prevalence of gastrointestinal parasites (specifically nematodes) in tortoises in the UK, and to investigate the factors affecting the prevalence of gastrointestinal parasites.

Materials and Methods

The prevalence of gastrointestinal parasites in tortoises in the UK would ideally be obtained by assessment of a properly stratified randomised sample of the entire tortoise population. Unfortunately, there are no robust data available on this population from which an unbiased sample could be taken, so in order to try and obtain a measure of the prevalence, tortoise owners throughout the UK were invited to submit samples of their tortoise's faeces which were analysed free of charge from March – September 2010. Owners were contacted by advertising the project through tortoise/reptile interest/owner groups, in addition to contacting vets through veterinary publications. The study was limited to the Hermann's tortoise (*Testudo hermanni*), Spur-thighed tortoise (*Testudo graeca)*, Horsfield tortoise (*Testudo horsfieldii*) and Leopard tortoise (*Stigmochelys pardalis*), as these are the more common species kept in captivity in the UK [Pendry 2002]. 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 by means of a macroscopic examination and a direct wet preparation [Cooper 2009]. If >0.5g of faecal material was available, a passive flotation was also performed (using a saturated NaCl solution at a set dilution) and a modified McMaster technique in order to provide a quantitative assessment of worm egg count [Zajac 2006]. Finally, a smear of any remaining faecal material was stained for identification of *Cryptosporidium* (using Pro-Lab Diagnostics Cryptosporidium Staining Kit ®). 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. 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. origin of tortoise, time in owner's possession, individual/group housing, indoor/outdoor enclosure, substrate, previous parasite checks and worming). These questionnaires were assigned the same reference number as their associated faecal sample, but data from the questionnaires were not analysed until the end of the study. Incomplete questionnaires were included in data analysis.

Data from the questionnaires were analysed using R version 2.12.1 (The R Foundation for Statistical Computing). $p<0.05$ was taken to indicate statistical significance. Standard univariate logistic regression (hereafter '*univariate*') was carried out for all risk factors for being positive for any parasite as well as specific parasites (ascarid, oxyurids, protozoa). For analysis of substrate as a risk factor, tortoises were categorised into groups depending on whether newspaper was used or not, and whether soil was used or not. Odds ratios and associated 95% confidence intervals were calculated. Associations where $P<0.25$ [Hosmer 2000] were then entered into a multivariable univariate logistic regression model (hereafter '*multivariable'*) and variables excluded until obtaining a final minimal statistically significant model.

Results

Between March and August 2010 a total of 243 samples were returned from various locations around the UK (**Figure 1**). Of these, only 146 samples met the requirements of the survey (with the other samples being >24 hours old, from tortoises with current health concerns or from group situations). The 146 samples consisted of 63 *Testudo hermanni*, 55 *T. graeca*, 24 *T. horsfieldii*, and 4 *Stigmochelys pardalis*. Due to the low numbers of samples from *S. pardalis*, it was decided to exclude these 4 samples from further results and statistical analysis.

Of the remaining faecal samples, all 142 were tested for parasites via the direct method, 141 (99%) by the flotation method and 130 (92%) were tested via McMaster (the latter due to lack of sample material). Sixty-nine samples (49%) were positive to at least one method (**Table 1**), though there were 7 samples that were negative for the direct (D) and flotation (F) methods where McMaster's (M) were not performed. The flotation technique detected the presence of the most parasites overall (58/141 – 41.%; D:54/142 – 38%, M:38/130 – 29%) and more specifically the most oxyurids (F:32%, D:23%, M:26%). In contrast, when looking at ascarids the direct wet preparation detected the most parasites (D:11%, F:8%, M:5%), and the direct method was also more sensitive at detecting protozoa in samples (13%) compared to the flotation technique (4%). No single diagnostic method detected all apparently positive samples of any parasites, with only 28/130 (22%) samples where all 3 methods were used being positive to all 3 methods (**Figure 2**), though 37/38 positive McMaster samples were also positive for the flotation technique.

No adult helminth parasites were identified. Of the 69 positive samples where a positive result was obtained with any method (**Figure 3**), 67% were positive for oxyurids, 28% were positive for ascarids, and 28% were positive for protozoa (*Balantidium*, *Nyctotherus* or flagellates). Only 17% of these samples were positive for more than one parasite, and only 2 samples were positive for oxyurids, ascarids and protozoa. *Cryptosporidium* testing was only able to be completed in 113 cases due to insufficient quantity of some of the samples and one sample was positive. No strongylids, trematodes or cestodes were detected.

In those 130 samples tested with McMaster, oxyurid and ascarid distributions were highly overdispersed with eggs per gramme (epg) values ranging from 0 to 44,650 for oxyurids (**Figure 4**) but with a median of 2,075 epg for the 34 McMaster positive samples, and values ranged from 0 to 4,100 for ascarids (**Figure 4**), with a median of 325 epg for the 6 McMaster positive samples.

The *univariate* analyses of the presence of any parasites revealed a significant association with the sex of the tortoise ($p=0.031$), with females (59%) more likely to be positive for parasites than males (39%, $OR=2.2$, **Table 1**). In addition, tortoises were more likely to be positive for parasites if they had been owned for ≤10 years (58%) compared to if they had been owned for >10 years (30%, $p=0.002$, $OR=3.26$). There were no statistically significant *univariate* associations between the presence of parasites and any other factors ($p>0.056$, **Table 1**).

Carrying out a *multivariable* model with all the variables in Table 1 where $p<0.25$ (Age group, sex, length of ownership, whether kept on newspaper, whether previous parasites found, whether previous treatment had occurred, and whether the tortoises hibernated) resulted in 3 variables remaining in the final minimal model. The results did not qualitatively change with respect to sex and length of ownership (sex: $p=0.034$, $OR_{female}=2.31$ (1.07-5.00); length of ownership: $p=0.001$, $OR_{\leq 10yr}=3.97$ (1.74-9.09)). However, the *multivariable* model also revealed that once sex and length of ownership had been taken into account, previous treatment for parasites was significantly associated with more tortoises being positive in the current survey ($p=0.033$, $OR_{previous\ treatment}=2.35$ (1.06-5.11)). The statistically significant risk factors for parasites are summarised in **Table 3**.

Oxyurids

A statistically significant *univariate* association was found between the presence of oxyurids and species of tortoise ($p=0.003$) with the odds of being positive for oxyurids if *T. hermanni* or *T. horsfieldii* more than if *T. graeca* (OR=3.36 and 5.11, respectively, **Table 2**). A statistically significant association was also found between the presence of oxyurids and age, with tortoises <10 years old more likely to be infected ($OR_{\leq 5yrs}$ & $OR_{>5\&<10yrs} > 6.5$) compared to older tortoises. In addition tortoises that were, non rehomed ($OR_{petshop}$ & $OR_{breeders} >2.7$), that had been owned for a shorter period of time ($OR_{\leq 10yrs}=17.4$), had no previous parasite problems or treatment ($OR_{no}>2.43$) and had not been hibernated ($OR_{no}=3.21$) were all associated with oxyurid infection (all $p<0.03$, **Table 2**). There were no statistically significant associations between the presence of oxyurids and any other factors ($p>0.078$). *Multivariable* analyses of the above variables and the 4 other variables where $P<0.25$ (outside the majority of time, newspaper or soil substrates and sex) on oxyurid presence revealed that length of time the tortoise had been owned ($OR_{\leq 10yrs}=23.9$ (4.9-

116.1)) and a history of no previous parasite problems (OR_{no}=6.20 (1.47-26.15)) remained significant risk factors. In contrast tortoises that spent the majority of time on soil were apparently protected from infection (OR_{soil}=0.21 (0.06-0.071), P<0.009).

Ascarids

Age was also found to have a statistically significant *univariate* association with the presence of ascarids, but in contrast with oxyurids, fewer parasites were detected in younger tortoises and no ascarids were detected in tortoises <5 years old. Tortoises that had been owned for a shorter period of time were also *less* likely to be infected ($OR_{\leq 10yrs}$=0.27, **Table 2**, P>0.063 for other variables).It was not possible to run *multivariable* models with both age and time owned included due to their confounding of each other. Therefore separate *multivarariable* analyses of these 2 variables and the 4 other variables where P<0.25 (species, outside majority of the time, previous problems and hibernation) were run. Neither final model included the 4 other variables – with just either age or time owned remaining, depending on which was entered in the multivariable model initially.

Protozoa

For protozoa the only *univariate* statistically significant associations were with whether the tortoise was kept with other tortoises (OR_{other}=3.14), or kept on soil (ORsoil=3.94, **Table 2**, P>0.052 for other variables). *Multivarariable* analyses of these 2 variables and the 6 other variables where P<0.25 (sex, origin, ownership period, newspaper substrate, previous parasites and problems) not only revealed that while the presence of other tortoises was still associated with protozoal infection (OR_{other}=3.53 (1.18-10.61), P=0.024), soil as substrate was not and in addition, as for oxyurids shorter ownership was associated with more protozoal infection

($OR_{<10yrs}$=5.01 (1.08-23.11) P=0.039).

Discussion

Gastrointestinal parasites were detected commonly in the tortoise faecal samples received in this survey, with a prevalence of 48.6%. Other authors have reported a higher prevalence (<75%), but have not specified if this includes both healthy and sick tortoises [McArthur 2004]. Previous surveys in the UK both indicated a slightly lower prevalence (30% and 44% respectively) despite being based on sick tortoises and including post-mortem data [Holt 1979, Keymer 1978].

The data presented in this paper are based only on samples which owners chose to submit from apparently healthy tortoises. We were therefore dependent on owners recognizing that their tortoises were healthy. In addition the samples we obtained may reflect particularly pro-active owners. The effect of samples being sent through the post could also not be examined in this study as the freshness of each sample at the time of postage could not be verified. We therefore cannot be certain that these data were representative of the entire UK tortoise population, but this is the largest such study carried out in the UK and therefore results do markedly add to our knowledge base.

Of the samples in this study, 32.4% samples 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 limited UK tortoise studies, 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 19% of cases, oxyurids in 16%, and protozoa in 23% of cases [Keymer 1978]. These previous studies were however performed at a time when the majority of the pet tortoise population in the UK were wild-caught, which may explain the conflicting results. Epidemiological surveys carried out on tortoises in Italy, Germany, and Slovenia revealed a varying prevalence of parasites ranging from 43 to 82% and in agreement with the present study, all found that oxyurids are the most common parasite detected in tortoises [Papini 2011, Pasmans 2008, Traversa 2005, Rataj 2011]. These studies were based mainly on captive-bred tortoises which are likely to represent a more similar population to that in this study.

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 female tortoises, but the association is not statistically significant, possibly due to the limited sample size. 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]. Male mammals and birds frequently have higher parasite burdens (proposed to be due to the immunosuppressive effects of testosterone), but there is less evidence in reptiles [Poulin 1996, Salvador 1995]. Female mammals have however, been suggested to be at increased risk at times when their oestrogen and progesterone levels are increased as both of these hormones may have immunosuppressive effects [Klein 2004]. 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].

This 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. These tortoises were younger and less likely to ever have been treated for parasites, so either or both of these factors could explain the decreased prevalence of parasites in those owned for longer periods. 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 with different parasites having a higher prevalence in different age groups [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 [Traversa 2005]. 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 [Traversa 2005]. In painted turtles it has even been suggested that parasite adaptability decreases as age of parasite increases so, the chance of a parasite surviving hibernation reduces each year, explaining reduced nematode burdens in older turtles [Esch 1967]. Alternatively changes in feeding habits of the host after sexual maturity were also suggested to play a role [Esch 1967].

Oxyurids

Those tortoises owned a shorter time were again at higher risk of being infected with oxyurids, though this is confounded by age with tortoises owned for a shorter time also being younger. 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, almost all tortoises which previously had parasites detected had been treated with anthelmintics, 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.

Other variables were associated with oxyurid infection at the *univariate* level (species of tortoise, age, origin, hibernation) but were not present in the final *multivariable* model once length of time in ownership, previous parasite problems and soil as a substrate had been taken into account. The probable main reasons for this was confounding within the data set; for example the majority of the rehomed tortoises were individuals that had been owned for longer and were therefore older, were more likely to hibernate and may have been less likely to have parasites anyway.

Ascarids

For ascarids, the most important risk factor appears to be length of time in owner's possession but in contrast with oxyurids, tortoises which have been owned a longer time (>10 years), 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 at higher risk from oxyurids.

Protozoa

Only a small number of samples were positive for protozoa and the fact that the samples were > 24 hours old would have made them suboptimal for protozoa detection [Wilkinson 2004]. Despite this a few variables did emerge from the analysis. 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. As with oxyurids shorter ownership was also associated with a greater prevalence of protozoa. The other risk factor which appeared in the univariate analyses was the substrate used. Tortoises kept on soil were apparently at higher risk of protozoal infection than tortoises kept on alternative substrates, though as stated above use of soil substrate is confounded with ownership. However, further research would be needed into these areas ideally with examination of fresh samples for detecting protozoa.

Other parasites

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 diagnostic test was not designed to detect reptile *Cryptosporodium* so there may have been false-negative results. This study does however show clearly that *Cryptosporidium* oocysts are not seen as a common finding in the tortoise samples screened by this method. Therefore if oocysts are found, their origin should be investigated further, ideally by molecular typing [Xiao 2004]. This is especially important in view of the zoonotic potential of some strains of *Cryptosporidium* [Traversa 2008].

Different diagnostic methods

All 142 samples were tested for parasites via the direct method, all but 1 by the flotation method and 130 were tested via McMaster (the latter due to lack of sample material).The flotation technique was found to detect the most parasites in this study, in particular for oxyurids. In contrast, for ascarids and protozoa the direct wet preparation 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 both oxyurid and ascarid ova would be detected best by flotation methods, both being similar in size [Thapar 1925, Jacobson 2007, Greiner 2006]. 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 the use of passive flotation instead of centrifugation, but further research would be necessary to determine the best method for detecting tortoise ascarids.

Conclusion

This study demonstrates that gastrointestinal parasites are frequently detected in routine faecal screens of tortoises in the UK, without any associated health concerns detected by their owners. Oxyurids were the most common parasite detected (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. In contrast, *Cryptosporidium* is not a normal finding on a routine faecal screen and should be investigated further if present.

Acknowledgements

The authors would like to thank Clare Guichard for help processing samples and all the vets and owners that submitted samples

Figure legends

Figure 1: Distribution across the UK of the samples that that contributed to the survey; the circle size indicates the number of samples submitted by each area based on postcode. Source: 2001 Census Output Area Boundaries. Crown Copyright 2003. Crown Copyright material is reproduced with the permission of the Controller of The Stationery Office.

Figure 2: Venn diagram to show parasites identified by different diagnostic methods (McMaster, Flotation, Direct), n = 130 where all 3 techniques were carried out on a sample (N=) number of samples positive for a particular technique.

Figure 3: Venn diagram to show parasites identified (excluding *Cryptosporidium*), from 69 positive samples. (N=) number of samples positive for a particular parasite

Figure 4: Histograms to show the quantity of (a) oxyurids and (b) ascarids found in tortoise faecal samples. X-axes have been modified to aid visualisation of frequencies.

Table Legends

Table 1: *Univariate* logistic regression analyses of association of putative risk factors and parasitic infection (any parasite). In all cases, the number of tortoises positive, the prevalence (95% confidence intervals), the Odds ratio (95% confidence intervals) compared to a reference level are given as well as, overall LR-test and individual level Wald's P-values as appropriate. ref - reference level.

Table 2: *Univariate* logistic regression analyses of association of putative risk factors and particular parasites ((a) oxyurids, (b) ascarids and (c) protozoa), where $P<0.05$. OR – Odds ratio, see Table 1 for further table description. NA – no odds ratio possible as no individuals were infected.

Table 3: Risk factors for the presence of parasites in the sample population

Table 1

Risk factor	Group	Total	With any parasites	% (95% C.I.)	Odds Ratio (95% C.I.)	P-value Overall	P-value Individual
		142	69	48.6 (40.1-57.1)			
Species	*Testudo hermanni*	63	33	52.4 (39.4-65.1)	1.53 (0.74-3.17)		0.253
	Testudo graeca	55	23	41.8 (28.6-55.9)	ref	0.432	
	Testudo horsfieldii	24	13	54.2 (32.8-74.4)	1.07 (0.42-2.76)		0.313
Age	<5yrs	38	21	55.2 (38.3-71.4)	1.65 (0.63-4.36)		0.310
	≥5-<10yrs	26	16	61.5 (40.6-79.8)	2.52 (0.84-7.58)		0.100
	≥10-<50yrs	46	19	41.3 (27.0-56.8)	0.99 (0.39-2.56)		0.995
	≥50yrs	29	12	41.4 (23.5-61.1)	ref	0.220	
Sex	Male	61	24	39.3 (27.1-52.7)	ref	0.031	
	Female	65	38	58.5 (45.6-70.6)	2.17 (1.06-4.42)		
Origin	Pet shop	51	25	49.0 (34.8-63.4)	1.15 (0.55-2.38)		0.711
	Breeders and homebred	23	13	56.5 (34.5-76.8)	1.55 (0.60-4.02)		0.366
	Rehomed	68	31	45.6 (33.5-58.1)	ref	0.661	
Been in owner's possession	≤10 yrs	97	56	57.7 (47.3-67.7)	3.26 (1.52-6.98)	0.002	
	>10 yrs	44	13	29.5 (16.7-45.2)	ref		
Other tortoises	Yes	72	35	48.6 (36.7-60.7)	1.00 (0.52-1.93)		
	No	70	34	48.6 (36.4-60.8)	ref	0.996	
Other reptiles	Yes	34	17	50.0 (32.4-67.6)	ref	0.851	
	No	108	52	48.1 (38.4-58.0)	0.93 (0.43-2.01)		
Environment	Garden (free range / pen)	53	25	47.2 (33.3-61.4)	ref	0.800	
	Vivarium / house (free range / pen)	36	16	44.4 (27.9-61.9)	0.90 (0.38-2.10)		
Substrate (majority of time)	Newspaper	45	19	42.2 (27.7-57.8)	ref	0.076	
	Not newspaper	55	33	60.0 (45.9-73.0)	2.05 (0.92-4.57)		
	Soil	25	14	56.0 (34.9-75.6)	1.24 (0.50-3.08)		
	Not soil	75	38	50.6 (38.3-62.4)	ref	0.644	
Previous parasites	Yes	30	10	33.3 (17.3-52.8)	ref	0.058	
	No	112	59	52.7 (43.0-62.2)	2.23 (0.96-5.18)		
Previous parasite treatment	Yes	48	18	37.5 (24.0-52.6)	ref	0.057	
	No	92	50	54.3 (43.6-64.8)	1.98 (0.97-4.05)		
Previous medical problems	Yes	32	14	43.8 (26.4-62.3)	ref	0.533	
	No	110	55	50.0 (40.3-59.7)	1.29 (0.58-2.84)		
Hibernated	Yes	91	40	44.0 (33.6-54.8)	ref	0.139	
	No	51	29	56.9 (42.2-70.7)	1.68 (0.84-3.36)		

Table 2

Risk factor	Group	Positive	% (95% C.I.)	OR (95% C.I.)	Overall P	Individual P
(a) Oxyurids		46	32.4 (24.8-40.8)			
Species	*Testudo hermanni*	25	39.7 (27.6-52.8)	3.36 (1.40-8.06)		0.007
	Testudo graeca	9	16.4 (7.8-28.8)	ref	0.003	
	Testudo horsfieldii	12	50.0 (29.1-70.9)	5.11 (1.75-14.94)		0.003
Age	<5 yrs	20	52.6 (35.8-69.0)	6.58 (1.93-22.5)		0.003
	≥5-<10yrs	14	53.8 (33.4-73.4)	7.95 (2.13-29.7)		0.002
	≥10-<50yrs	7	15.2 (6.3-28.9)	1.12 (0.30-4.23)		0.865
	≥50yrs	4	13.8 (3.9-31.7)	ref	<0.001	
Origin	Pet shop	20	39.2 (25.8-53.9)	2.73 (1.20-6.23)		0.017
	Breeders/homebred	13	56.5 (34.5-76.8)	5.50 (1.98-15.29)		0.001
	Rehomed	13	19.1(10.6-30.5)	ref	0.002	
Been in owner's possession	≤10yrs	44	45.3 (35.3-55.8)	17.4 (3.99-76.1)		
	>10yrs	2	4.5 (0.5-15.5)	ref	0.001	
Previous parasites	Yes	5	16.7 (5.6-34.7)	ref		
	No	41	36.6 (27.7-46.2)	2.89 (1.03-8.12)	0.030	
Previous parasite treatment	Yes	10	20.8 (10.5-35.0)	ref		
	No	36	39.1 (29.1-49.9)	2.44 (1.08-5.51)	0.025	
Hibernated	Yes	21	23.1 (14.9-33.1)	ref		
	No	25	49.0 (34.8-63.4)	3.21 (1.54-6.68)	0.002	
(b) Ascarids		19	13.4 (8.3-20.1)			
Age	<5 yrs	0	0 (0-9.0)	NA		NA
	5-10yrs	4	16.0 (4.5-36.1)	0.60 (0.15-2.35)		0.462
	10-50yrs	8	17.4 (7.8-31.4)	0.66 (0.21-2.07)		0.479
	50+yrs	7	24.1 (10.3-43.5)	ref	0.002	
Been in owner's possession	≤10yrs	8	8.2 (3.6-15.6)	0.27 (0.10-0.73)		
	>10yrs	11	25.0 (13.2-40.3)	ref	0.009	
(c) Protozoa		19	13.4 (8.3-20.1)			
Other tortoises	Y	5	19.4 (11.1-30.5)	3.14 (1.06-9.25)		
	N	14	7.1 (2.4-15.9)	ref	0.028	
Substrate	Soil	8	32.0 (14.9-53.5)	3.94 (1.29-12.02)		
	Not soil	8	10.7 (4.7-19.9)	ref	0.017	

Table 3

Risk factors for presence of any parasites	**Risk factors for presence of oxyurids**	**Risk factors for presence of ascarids**	**Risk factors for presence of protozoa**
Female tortoises	*Testudo hermanni* or *Testudo horsfieldii*	Those >5 years old	Being kept with other tortoises
Those owned ≤10 years	Those <10 years old	Those owned for a longer period of time	Those kept on soil
Those previously treated for parasites	Those owned ≤10 years		Those owned for a shorter period of time
	Originating from a pet shop or breeder		
	A history of no previous parasite problems		
	Never having been hibernated		
	Those not kept on soil		

References

BARNARD, S. M. & UPTON, S. J. (1994) A Veterinary Guide to the Parasites of Reptiles. Vol. I Protozoa. Malabar, Krieger. 35-63

BONE, R.D. (1992) Gastrointestinal system. In: Manual of Reptiles. 1st edn. Beynon PH, Cooper JE, Lawton MPC (eds). British Small Animal Veterinary Association, Quedgeley. 107-108

BUCKNELL, D. G., GASSER, R. B. & BEVERIDGE, I. (1995) The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia. *International Journal for Parasitology* **25(6)**, 711-724

COOPER, J. E. (2009) In-practice and field techniques of the investigation of parasitic infections. *Journal of Exotic Pet Medicine* **18(4)**, 289-298

CUTLER, S.L. (2004) Nematode-associated aural abscess in a Mediterranean tortoise, *Testudo graeca*. *Journal of Herpetological Medicine and Surgery*. **14(3)**, 4-5

ESCH, G. W. & WHITFIELD GIBBONS, J. (1967) Seasonal incidence of parasitism in the Painted Turtle, *Chrysemys picta marginata Agassiz*. *The Journal of Parasitology* **53(4)**, 818-821

FRANK, W. (1981) Endoparasites. In: Diseases of the Reptilia, Volume 1. Cooper JE, Jackson OF (eds). Academic Press, London. 291-358

FRYE, F.L. (1991) Applied clinical nonhemic parasitology of reptiles. In: Biomedical and Surgical Aspects of Captive Reptile Husbandry. Vol. I, 2nd edition, Malabar, Krieger. 281-325

GIANNETTO, S., BRIANTI, E., POGLAYEN, G., SORGI, S., CAPELLI, G., PENNISI, M. G. & COCI, G. (2007) Efficacy of oxfendazole and fenbendazole against tortoise (*Testudo hermanni*) oxyurids. *Parasitology Research* **100**, 1069–1073

GREINER, E. C. & MADER, D. R. (2006) Parasitology. In: Reptile Medicine and Surgery. 2nd edition. Mader DR (ed). WB Saunders, St. Louis. 343-364

HOLT, P. E., COOPER, J. E. & NEEDHAM, J. R. (1979) Diseases of tortoises: a review of seventy cases. *Journal of Small Animal Practice* **20**, 5

HOSMER, D.W. & LEMESHOW, S. (2000) Applied Logistic Regression: Second Edition, John Wiley & Sons. Inc, New York

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

KEYMER, I. F. (1978) Diseases of chelonians: (1) Necropsy survey of tortoises. *Veterinary Record* **103**, 548-552

KLEIN, S. L. (2004) Hormonal and immunological mechanisms mediating sex differences in parasite infection. *Parasite Immunology* **26**, 247-264

KLINGENBERG, R. J. (2000) Reptilian parasite testing. In: Laboratory Medicine: Avian and Exotic Pets. Fudge, A.M. (ed). WB Saunders, Philadelphia. 258-264

MARTINEZ-SILVESTRE, A. (2011) Massive *Tachygonetria* (Oxyuridae) infection in a Herman's tortoise (*Testudo hermanni*). *Consulta Journal Special Edition* 409-412

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). Blackwell Publishing, Oxford. 347-349

PASMANS, F., BLAHAK, S., MARTEL, A. & PANTCHEV, N. (2008) Introducing reptiles into a captive collection: The role of the veterinarian. *The Veterinary Journal* **175**, 53-68

PAPINI, R., MENETTII, C. & MANCIANTI, F. (2011) Coprological survey in pet reptiles in Italy. *Veterinary Record* **169**, 207

PENDRY, S. & ALLAN, C. (2002) Selling like Hot Cakes: An investigation into the trade in tortoises in Great Britain. TRAFFIC International and DEFRA, UK

POULIN, R. (1996) Sexual Inequalities I Helminth Infections: A Cost of Being a Male? *The American Naturalist* **147**(2), 287-295

RATAJ, A. V., LINDTNER-KNIFIC, R., VLAHOVIC, K., MAVRI, U. & DOVC, A. (2011) Parasites in pet reptiles. *Acta Veterinaria Scandinavica* **53**, 33

SALVADOR, A., VEIGA, J. P., MARTIN, J., LOPEZ, P., ABELENDA, M. & PUERTA, M. (1995) The cost of producing a sexual signal; testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behavioural Ecology* **7**(2), 145-150

SCHNELLER, P. & PANTCHEV, N. (2008) Parasitology in snakes, lizard and chelonians. Edition Chimaira, Frankfurt am Main. 105-172

SPRENT, J.F.A. (1980) Ascaridoid nematodes of amphibians and reptiles: *Anguistacaecum* and *Krefftascaris* n.g. *Journal of Helminthology*. **54**, 55-73

TELFORD, S.R. (1971) Parasitic Diseases of Reptiles. *Journal of the American Veterinary Medical Association.* **159**, 1644-1652

THAPAR, G.S. (1925) Studies on the oxyurid parasites of reptiles. *Journal of Helminthology* **3**, 83-150

TRAVERSA, D., CAPELLI, G., IORIO, R., BOUAMER, S., CAMELI, A. & GIANGASPERO, A. (2005) Epidemiology and biology of nematofauna affecting

Testudo hermanni, *Testudo graeca* and *Testudo marginata* in Italy. *Parasitology Research* **98**, 14–20

URQUHART, G. M., ARMOUR, J., DUNCAN, J. L., DUNN, A. M. & JENNINGS, F. W. (1996) Veterinary Helminthology. In: Veterinary Parasitology. 2nd edition. Blackwell Science, Oxford. 4-11

VISCO, R. J., CORWIN, R. M. & SELBY, L. A. (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

WILKINSON, R. (2004) Clinical Pathology. In: Medicine and Surgery of Tortoises and Turtles. McArthur S, Wilkinson R, Meyer J (eds). Blackwell Publishing, Oxford. 141-186

ZAJAK, A. M. & CONBOY, G. A. (2006) Veterinary Clinical Parasitology, 7th edition. Blackwell Publishing, Iowa. 8-10

ZUK, M. & MCKEAN, K. A. (1996) Sex Differences in Parasite Infections: Patterns and Processes. *International Journal for Parasitology* **26**(10), 1009-1024