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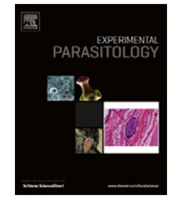

Research Brief

FLOTAC can detect parasitic and pseudoparasitic elements in reptiles

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ABSTRACT

Reptiles have increased in popularity worldwide; snakes and lizards are frequently used as pets. As a consequence of their popularity, the interest of the scientific community in these animals has increased. In order to acquire epidemiological data on the parasitic infections affecting reptiles in Italy a survey was carried out in 125 snakes and 25 lizards bred in the Campania region of southern Italy. Individual fecal samples were collected and FLOTAC was used for copromicroscopic diagnosis. Eimeriidae, oxyurids, strongylids, other gastro-intestinal nematodes and pulmonary nematodes were the most representative parasites found. Eggs of pseudoparasites (mites, oxyurids and trichurids affecting rodents) were also found. The use of FLOTAC for diagnosis of parasitic infections in reptiles has demonstrated to be a rapid and sensitive test to improve diagnosis and acquire new information on the parasitological fauna of reptiles.

1. Introduction

The use of reptiles as pets is increasing worldwide. Among these "non-conventional" animals, snakes and lizards are those most frequently kept in captivity. As a consequence of their popularity, the interest of the scientific community in these animals has increased; however, little is known about their infections, including parasites.

Captive snakes and lizards can harbor a wide variety of parasites, mostly those with homoxenous (direct) life cycle. The most commonly encountered are protozoa (i.e. *Cryptosporidium*, *Eimeria*, *Acroeimeria*, *Choleoeimeria*, *Isospora*, etc.) and nematoda (e.g. oxyurids, ascarids, strongyles, *Rhabdias* and *Strongyloides*). However, in reptiles imported as pets directly from the wild, the diversity of parasites can be much larger. As for other animal species, also in reptiles the presence of parasites is not necessarily associated with clinical signs or detectable lesions. However, the stress of captivity, improper husbandry and poor sanitation can lead to serious diseases (Modrý et al., 2001, 2004; Radhakrishnan et al., 2009; Traversa et al., 2008).

Diagnosis of parasitic infection is generally achieved by revealing parasitic elements (e.g. eggs, larvae, oocysts and cysts) in feces by routine flotation procedures and using saturated NaCl, $ZnSo_4$ or Sheather's sugar solutions (Mihalca, 2002; Modrý et al., 2001, 2004; Radhakrishnan et al., 2009; Traversa et al., 2008).

Copromicroscopic diagnosis in exotic animals can sometimes be problematic as many pseudoparasitic forms can be detected due to the extremely diverse diet (i.e. fruits, vegetables, invertebrates and small vertebrates) of reptiles. As a result, the feces of reptiles often contain artefacts such as plant and invertebrate parts, and parasite eggs of the animals used as food; this may lead to unnecessary or incorrect antiparasitic treatment (Wilson and Carpenter, 1996).

To achieve new information and to improve diagnosis of parasitic infections in reptiles, sensitive, specific and rapid techniques are required; the FLOTAC techniques have been recently demonstrated to be powerful for diagnosing a range of parasitic infections of human and veterinary importance (Cringoli et al., 2010).

The present study was aimed at investigating the occurrence of parasites and pseudoparasites in captive snakes and lizards kept as exotic pets in the Campania region of southern Italy, using for the first time in reptiles FLOTAC as copromicroscopic tool.

2. Materials and methods

From September 2009 to June 2010 individual fecal samples and cloacal swabs were collected from 150 captive reptiles (125 snakes and 25 lizards). Out of these 150 animals, 94 (62.7%) were housed in exotic animal farms, 9 (6%) in pet shops and 47 (31.3%) in individual private owners. For each animal, anamnestic data (species, sex, age, micro-environment conditions, captive-born or wild-caught origin, feeding, cohabitation, gastro-intestinal symptoms, use of anti-parasitic treatments) were recorded. All fecal samples were stored in 5% formalin and subsequently examined in the laboratory using the FLOTAC Pellet Technique (Cringoli et al., 2010). This technique is performed for samples with an

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unknown weight of fecal material. In these circumstances, the weight of the fecal material to be analyzed can be inferred by weighing the sediment in the tube (pellet) after filtration and centrifugation of the fecal sample.

Samples weight was evaluated and 5% formalin solution was added to reach a final volume of 20 ml; each sample was homogenized and filtered. Two 15 ml conic tubes were filled with the filtered suspension up to 6 ml and were centrifuged for 3 min at 1500 rpm (about 170*g*). After centrifugation the supernatant was discarded and the two pellets (sediments) were weighted. Two different flotation solutions were used to re-suspend the two pellets: FS2 (Sodium Chloride Solution) (1200 s.g.) and FS7 (Zinc Sulfate Solution) (1350 s.g.); after homogenization, each of the two suspensions was poured into the two flotation chambers of the FLOTAC apparatus. The FLOTAC was closed and centrifuged for 5 min at 1000 rpm (about 120*g*); after centrifugation, the top parts of the flotation chambers was translated and each chamber was read under the microscope.

Parasitic elements (eggs, oocysts and larvae) were counted, photographed and measured using a light microscope at 20× and 40× magnifications (Leica DFC 490) and identified in accordance with the guidelines reported in literature (Schneller and Pantchev, 2008).

3. Results

All the 150 reptiles of this study were asymptomatic and had not received any antiparasitic treatment. Snakes diet included rodents, chickens and rabbits; lizards were fed mainly with vegetables and arthropods.

Out of the 150 samples examined, 66 (44.0%; 95% CI = 36.0–52.3%) showed the presence of parasitic elements (eggs, oocysts, larvae), specifically 16 (64%; 95% CI = 42.6–81.3%) out of the 25 lizard examined and 50 (40%; 95% CI = 31.5–49.2%) of the snakes examined (Tables 1 and 2).

Among the positive samples, multiple parasitic infections were found in 5 (31.2%; 95% CI = 12.1–58.5%) of the 16 lizards and 10 (20%; 95% CI = 10.5–34.1%) of the 50 snakes tested.

The most frequent parasites were: oxyurids, *Rhabdias*, *Kalicephalus*, *Capillaria* and Eimeriidae (Figs. 1 and 2). Pseudoparasites were also detected by FLOTAC. Specifically, eggs of *Myocoptes musculinus*, *Trichuris muris*, *Hymenolepis nana*, *Aspiculuris tetraptera* and *Syphacia obvelta* were found in 49 (39.2%; 95% CI = 30.7–48.4%) of the 125 snakes tested and eggs of mites were found in 3 (12%; 95% CI = 3.2–32.3%) of the 25 lizard examined.

Table 1
Snake genera examined and positivity to parasites and pseudoparasites.

Snake genus	N° examined	Parasites		Pseudoparasites	
		N° positive	%	N° positive	%
Boa	40	16	40.0	9	22.5
Python	30	13	43.3	10	33.3
Elaphe	25	13	52.0	16	64.0
Lampropeltis	8	1	12.5	8	100
Morelia	4	2	50.0	0	0
Aspidites	4	1	25.0	2	50.0
Acanthophis	2	0	0	0	0
Crotalus	2	2	100	2	100
Drymarcon	2	0	0	0	0
Lichanura	2	2	100	2	100
Bogertophis	1	0	0	0	0
Gonyosoma	1	0	0	0	0
Hydrodynastes	1	0	0	0	0
Lamprophis	1	0	0	0	0
Oreocryptophis	1	0	0	0	0
Rhyncophis	1	0	0	0	0
Total	125	50	40.0	49	39.2

Table 2
Lizard genera examined and positivity to parasites and pseudoparasites.

Lizard genus	N° examined	Parasites		Pseudoparasites	
		N° positive	%	N° positive	%
Pogona	6	5	83.3	0	0
Iguana	5	2	40.0	1	20.0
Eublepharis	3	2	66.7	1	33.3
Chamaleo	3	1	33.3	0	0
Clamidiosaurus	2	2	100	0	0
Basiliscus	2	2	100	0	0
Phelsuma	1	1	100	1	100
Physignatus	1	0	0	0	0
Tupinambis	1	0	0	0	0
Zoonosaurus	1	1	100	0	0
Total	25	16	64.0	3	12.0

4. Discussion

The present study represents the first survey on the parasitic fauna of lizards in Italy and of snakes in the Campania region. Data of the nematode fauna of Boidae snakes in central Italy was previously reported by Seghetti and Traversa (2007). In addition, in the

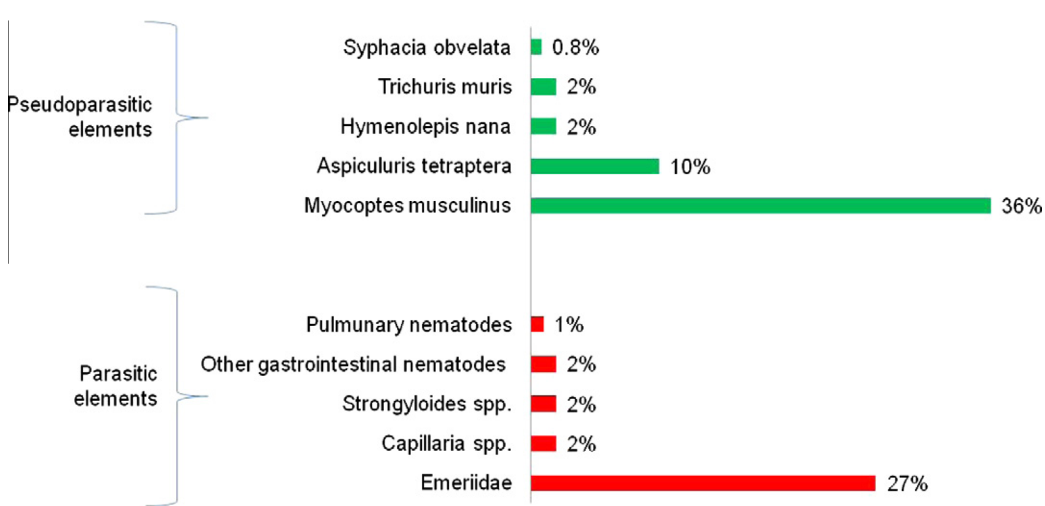

Fig. 1. Prevalence of parasitic and pseudoparasitic elements found in snakes (n = 125) bred in the Campania region (southern Italy).

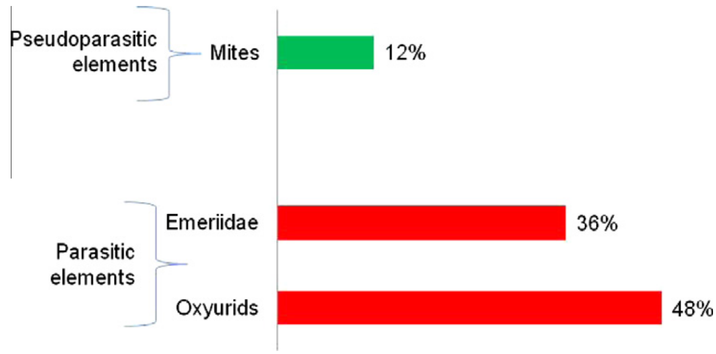

Fig. 2. Prevalence of parasitic and pseudoparasitic elements found in lizards (n = 25) bred in the Campania region (southern Italy).

present study the FLOTAC technique was successfully used for the first time for copromicroscopic diagnosis of parasites in reptiles.

Our findings show that several parasitic nematodes and protozoa infect snakes and lizards; this is in agreement with the results of other studies that report *Rhabdias*, *Strongyloides*, *Kalicephalus*, oxyurids, *Capillaria* and Coccidia including *Cryptosporidium* as the most frequent parasites in reptiles (Junker et al., 2009; Mihalca, 2002; Mihalca et al., 2010; Modrý et al., 2001; Morgan et al., 1999; Radhakrishnan et al., 2009; Xiao et al., 2004). The pathology associated with lungworm infection by *Rhabdias* is characterized by respiratory difficulties, hypoxia and severe pneumonia while lethargy, inappetence and weight of loss are cardinal symptoms of thread-worm infection by *Strongyloides* and hookworm infection by *Kalicephalus* (Junker et al., 2009; Mihalca et al., 2010; Schneller and Pantchev, 2008). Oxyurid infection can lead to lethargy, inappetence, diarrhea, prolaps of cloaca/penis, growth disturbances and reduced fertility. Coccidia are widely distributed in captive reptiles; the life cycle of these harmful protozoa is mostly direct (with the exception of *Sarcocystis* and some *Caryospora*) and they are highly pathogenic, potentially causing serious damage (Schneller and Pantchev, 2008). *Cryptosporidium* infections are also very common in reptiles (Pedraza-Diaz et al., 2009). Pseudoparasites found in lizards and snakes represent parasites affecting rodents: *M. musculinus* is a mite of which eggs are erroneously reported as reptiles oxyurids eggs; *A. tetraptera* and *S. obvelata* are oxyurids affecting rodents, missidentified with *Ophiostrongylus* (parasite of snakes) and with oxyurids affecting tortoises (*Pharyngodonidae*), respectively (Seghetti and Traversa, 2007). Eggs of *T. muris* and *H. nana* were also observed during testing of samples.

The aim of this survey was to give preliminary data on the parasitofauna of captive reptiles in Italy; the major limitation of the study was the impossibility in identifying at species level the eggs retrieved at the coprological examination; in most cases eggs and oocysts found were not strictly species-specific, representing pseudo-parasite findings that can lead to diagnostic errors.

Moreover, identifying parasites and pseudo-parasites (either parasitic elements from other animal species or non animal structures, i.e. pollen and crystals) would be of importance, both in symptomatic and in asymptomatic animals, to understand the real pathogenic effect of some parasites and to verify the occurrence of zoonotic agent in reptiles.

The FLOTAC technique, used for this survey, has demonstrated to be highly sensitive also when we tested samples of little or even unknown weight.

This work underlines the need to collect data and to study in depth the epidemiology and the prevalence of parasitic infections in reptiles; in addition new techniques should be performed in order to improve the coprological diagnosis and overcome the difficulty in identifying parasitic species.

Conflict of interest

The FLOTAC apparatus has been developed and is patented by G. Cringoli, but it is planned that the patent will be handed over to the University of Naples "Federico II". At present, the FLOTAC technique is under detailed validation by several research groups focusing on human and veterinary parasitology. Should these validations continue to be successful, the FLOTAC technique will be provided free of charge to public research centers, including the World Health Organization and universities. The fact that one of the authors is the current patent holder of the FLOTAC apparatus played no role in the present study. All other authors have no competing financial interests.

References

Cringoli, G., Rinaldi, L., Maurelli, M.P., Utzinger, J., 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nature Protocols 5, 503–510.

Junker, K., Lane, E.P., Dlamini, B., Kotze, A., Boomker, J., 2009. Post mortem identification of *Kalicephalus colubri colubri* (Nematoda: Diaphanocephalidae) in a captive mole snake (*Pseudapis cana*) in South Africa. Journal of the South African Veterinary Association 80, 54–56.

Mihalca, A.D., 2002. Un studiu coproparazitologic la reptile captive. Scientia Parasitologica 3, 159–163.

Mihalca, A.D., Miclăuş, V., Lefkaditis, M., 2010. Pulmonary lesions caused by the nematode *Rhabdias fuscovenosa* in a grass snake, *Natrix natrix*. Journal of Wildlife Diseases 46, 678–681.

Modrý, D., Daszak, P., Volf, J., Veselý, M., Ball, S.J., Koudela, B., 2001. Five new species of coccidia (Apicomplexa: Eimeriidae) from Madagascan chamaleons (Sauria: Chamaeleonidae). Systematic Parasitology 48, 117–123.

Modrý, D., Necas, P., Mazuch, T., Kamler, M., 2004. *Eimeria atheridis* n. sp. (Apicomplexa: Eimeriidae), a new coccidium from the western bush viper *Atheris chlorechis* (Pel, 1851) from tropical Africa. Systematic Parasitology 59, 71–74.

Morgan, U.M., Xiao, L., Fayer, R., Graczyk, T.K., Lal, A.A., Deplazes, P., Thompson, R.C., 1999. Phylogenetic analysis of *Cryptosporidium* isolates from captive reptiles using 18S rDNA sequence data and random amplified polymorphic DNA analysis. Journal of Parasitology 85, 525–530.

Pedraza-Diaz, S., Ortega-Mora, L.M., Carrion, B.A., Navarro, V., Gomez-Bautista, M., 2009. Molecular characterisation of *Cryptosporidium* isolates from pet reptiles. Veterinary Parasitology 160, 204–210.

Radhakrishnan, S., Kurup, S.P., Banerjee, P.S., 2009. Endoparasitism in captive wild-caught snakes indigenous to Kerala, India. Zoo Biology 28, 253–258.

Schneller, P., Pantchev, N., 2008. Parasitology in Snakes, Lizards and Chelonians. A Husbandry Guide, Edition Chimaira, Frankfurt am Main, Germany.

Seghetti, M., Traversa, D., 2007. Indagine parassitologica in boidae allevati in cattività in Italia centrale. Large Animal Reviews 13, 107–112.

Traversa, D., Iorio, R., Otranto, D., Modrý, D., Slapeta, J., 2008. *Cryptosporidium* from tortoises. Genetic characterization, phylogeny and zoonotic implications. Molecular and Cellular Probes 22, 122–128.

Wilson, S.C., Carpenter, J.W., 1996. Endoparasitic Disease of Reptiles. Saunders Company.

Xiao, L., Ryan, M., Graczyk, K., Limor, J., Li, L., Kombert, M., Junge, R., Sulaiman, I.M., Arrowood, M.J., Koudela, B., Modrý, D., Lal, A.A., 2004. Genetic diversity of *Cryptosporidium* sp. in captive reptiles. Applied and Environmental Microbiology 70, 891–898.