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.

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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, Acrooimeria, Choleoeimeria, Isospora, etc.) and nematoda (e.g. oxyurids, ascarids, strongylids, 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 (Modry 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, ZnSO₄ or Sheather’s sugar solutions (Mihalca, 2002; Modry 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
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 170g). 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 120g); 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 obvelata* 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.

### 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
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 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 Panchtev, 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 Sarcozystis and some Caryosporida) and they are highly pathogenic, potentially causing serious damage (Schneller and Panchtev, 2008). Cryptosporidium infections are also very common in reptiles (Pedraza-Díaz et al., 2009). Pseudoparasites found in lizards and snakes represent parasites affecting rodents: M. musculus is a mite of which eggs are erroneously reported as reptiles oxyurids eggs; A. tetraptera and S. obvelata are oxyurids affecting rodents, misidentified with Ophiostriongulus (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**


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