

A non-lethal method to sample gastrointestinal parasites from terrestrial salamanders

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The usual method to obtain gastrointestinal parasites from amphibians is dissecting sacrificed animals (e.g. Pritchard and Kruse, 1982; Goater and Goater, 2001 and references therein). However, the recent worldwide decline of amphibian populations (e.g. Daszak et al., 2003; Stuart et al., 2004) and the raising conservation concern about endangered and rare species strongly demand the finding of alternative and less disruptive methods that may reduce the overcollection of amphibians from their natural habitats. This is especially the case of endangered species that are in need of protection, such as European cave salamanders belonging to the genus *Speleomantes* Dubois, 1984 (Amphibia, Urodela, Plethodontidae). These fully terrestrial salamanders, found in SW France and Italy including Sardinia (Lanza et al., 1995), are totally protected by the European Union 92/43 directive for the conservation of biodiversity, known as "Habitat and Species". Little is known about gastrointestinal parasites of *Speleomantes* (Ricci, 1988; Ben Slimane and Durette-Desset, 1995) and, recently, a new species of *Distoichometra* (Cestoda, Nematotaeniidae) was described from preserved specimens of *S. strinatii* studied for the first time by Pastorino (1974) (Buriola et al., in press). Thus, the examination

of fresh specimens of this new parasite species was opportune. In this paper we describe the successful application of a non-lethal method to obtain gastrointestinal parasites from free-living terrestrial salamanders.

In this study, Yomesan® (Bayer Ltd, Germany), a commercial human anthelmintic prepared from niclosamide (2',5-dichloro-4'-nitrosalicylamide) was used. Niclosamide is a molluscicide used to control schistosomiasis, since it kills the fresh-water snails that act as intermediate hosts. It is very toxic to aquatic animals, but it is quickly metabolised in water and no carcinogenic or teratogenic effects were proved (World Health Organisation, 2002). In humans, this compound is used to treat infections from *Taenia saginata* (beef tapeworm), *Taenia solium* (pork tapeworm), *Diphyllobothrium latum* (fish tapeworm) and *Hymenolepis nana* (dwarf tapeworm). Since *Speleomantes* are small-sized and Yomesan® is not readily soluble in water, a diluted suspension (5 mg/ml) had to be prepared (table 1). The procedure was as follows: first MIRJ 52-S was added to 40-50 ml of deionised water heated at 40°C, then one finely crushed Yomesan tablet, sugar (used to minimise other preservatives) and AVICEL RC-591 were added. The preservative E219 was mixed last to avoid interference with MIRJ 52-S and the final volume of the suspension was set at 100 ml. *Speleomantes strinatii* salamanders were collected in three artificial cavities near Savignone (Liguria, NW Italy: 44°32'30"N, 08°58'53"E). Adult salamanders with a mean body mass assessed by a Pesola dynamometer of 3.5 g (range 2.0-4.1 g) were caged singly in plastic boxes (24 × 17 × 3.5 cm in size) in the Biospeleological Station of San Bartolomeo, an artificial underground cavity naturally colonised by a *S. strinatii* population (Salvidio et al., 1994). Two experimental trials were conducted. In a preliminary test performed in June 2003, 28 salamanders were treated with 0.25 mg/g of the Yomesan® suspension (volume range 0.1-0.2 ml), introduced directly into the stomach from the mouth, with an insulin syringe equipped with a flexible plastic tube (diameter 1.1 mm). This dosage represented an intermediate value of the one used in veterinary medicine that ranges from 0.08 to 0.5 mg/g (World Health Organisation, 1988). Caged animals were inspected three days after treatment and parasites fixed in 10% formaldehyde. In the second experiment, 20 salamanders were collected in September 2004 and treated as in the first case,

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Table 1. Chemical components of the anthelmintic suspension used to obtain gastrointestinal parasites from free-living *Speleomantes strinatii*.

Component	Quantity	Function
Yomesan® (Niclosamide)	0.5 g	anthelmintic
Methyl 4-hydroxybenzoate (E219)	0.1 g	preservative
Polyoxyethylene stearate (Mirj 52-S)	0.3 g	surfactant
Microcrystalline cellulose and carboxymethylcellulose (AVICEL RC-591)	2.5 g	excipient
Sugar	40.0 g	preservative
Deionised water	100 ml	suspension medium

but they were inspected hourly up to six hours, and then again after 24 and 48 hours from treatment. In this experiment, Cestoda were fixed in alcohol-formalin-acetic acid solution (AFA) and Nematoda in 70% ethyl alcohol. After both experiments all salamanders appeared in good health conditions and were released at their capture sites.

During the first experiment, four tapeworms were found in four different cages. However, these specimens, fixed several hours after excretion, were partially decomposed (E. Buriola, pers. obs.). In the second experiment, 10 out of 20 salamanders excreted gastrointestinal parasites, some of them still moving. Overall, 14 parasites were obtained and their microscopic examination revealed 13 tapeworms and one nematode, all suitable for taxonomic and anatomical investigations. Excretion started one hour (one parasite excreted) and lasted up to five hours after treatment: four, eight, and 13 parasites were excreted after two, three and four hours respectively (median excretion time: three hours). Two salamanders excreted two parasites each, while three tapeworms were obtained from a single individual.

This is, to our knowledge, the first successful application of an alternative method that avoids necropsy of free-living amphibian hosts to obtain gastrointestinal parasites for parasitic studies. The anthelmintic used is easily obtained in Europe, and it is prepared from a low cost chemical. We did not sacrifice experimental animals to check if all the gastrointestinal parasites were excreted. However, even if necropsy remains the

best method to sample quantitatively helminths from amphibians, the use of anthelmintic suspensions on living animals may reduce the over-collection of protected and rare species. Moreover, epidemiological studies based upon large sample sizes of common species are affordable without the dissection of the host animals. For these reasons, our results seem encouraging for both parasitologists and herpetologists, who are invited to test our method on other amphibian taxa, and even on reptiles for which this method could be easily adapted.

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