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Differences in helminth infections between captive and wild spur-thighed tortoises *Testudo graeca* in southern Spain: A potential risk of reintroductions of this species

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ABSTRACT

Although the spur-thighed tortoise, *Testudo graeca*, is one of the most widely distributed species of tortoises, its natural populations are threatened through its whole range. Particularly at south-eastern Spain, the species is mainly threatened by habitat destruction and over-collection, given that this chelonian has been traditionally considered an appreciate pet. As south-eastern Spanish wildlife recovery centers shelter hundreds of captive animals mainly coming from illegal trade or captive-bred, there is a strong debate about what to do with these animals: maintaining them in captivity all along their lives or reintroducing them to wildlife. It is well known that the reintroduction of captive animals supposes a risk for the wild population due to the uncertainty of their genetic origin and to the possible spread of infectious diseases. However, despite the increasing evidence that infectious agents are a potential health hazard for wildlife, little is known about the risk that introduced parasites could suppose for the wild populations of spur-thighed tortoise.

The present study investigates for the first time the presence of helminth eggs and worms in faeces from 107 wild and captive individuals collected from mid-March to mid-June 2010, and relates the findings to different environmental and host variables. Sixteen oxyurid species and the ascarid *Angusticaecum holopterum* were identified. This last nematode and the oxyurid species *Tachygonetria palearticus* and *T. seurati* had not been reported in Spanish wild *T. graeca* previously. The prevalence of oxyurid eggs and worms were 94% and 70%, respectively; while, ascarid eggs and worms were found in 26% and 5% of tortoises, respectively. Ascarid infections affected mostly captive animals and were associated to caparace deformities and symptoms of upper respiratory tract disease ($p < 0.05$). Oxyurid infections were not associated to negative health traits and prevalence increased with age. In free-living tortoises, the distribution of pharingodonid genera also varied according to habitat; moreover, *T. longicollis*, *T. pusilla*, *T. conica*, *T. robusta* and *Mehdiella stylosa* where significantly more frequent in wild compared to captive tortoises ($p < 0.05$). Study results highlight important differences in the nematode fauna of captive and free-living tortoises and questions one more time if the reintroductions of captive animals suppose a risk for the wild population since the former ones can harbor and distribute among free populations pathogens like ascarid nematodes.

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1. Introduction

Habitat loss, trade, human consumption or the spread of diseases are some of the threats that many species of tortoises have to face (IUCN, 1989). Although the range of the spur-thighed tortoise (*Testudo graeca*) covers part of three continents (Europe, Africa and Asia) and represents the most widely distributed species of tortoises in the western Palearctic, its global population is declining through its whole range, being classified as vulnerable species at the Red List category (IUCN, 2010). At the Mediterranean Basin, spur-thighed tortoise presents most of its range in North Africa, and only occurs in a few small and isolated European populations in the Iberian Peninsula (in Doñana National Park and in south-eastern Spain) and in some islands (Mallorca, Sardinia and Sicily). Special characteristics like the small size of these animals or their tolerance to captive conditions promote its consideration of manageable and valuable pet, which is the main reason for the continuous removal of tortoises from their natural habitat and the illegal trade. Particularly, each summer hundreds of seized North African tortoises arrive to south-eastern Spanish wildlife recovery centers, admitted as confiscated specimens. Given their proximity to the wild population, these recovery centers as well receive wild damaged animals, captive animals collected from the field and maintained for a period, or captive-bred tortoises donated by its owners (Ferrández, 2011). There is a strong debate about how to manage and what conservation strategy must be implemented with captive population. Although south-eastern Spanish wild population is threatened by the destruction of its natural habitat due to urbanization and agricultural activity and by past over-collection, most of the recovery centers avoid reintroducing captive animals into the wild population, which was a traditional practice in the past but is still carried out by particular people. For scientists, administration and naturalists it is well known that these captive tortoises are not valid for reintroductions due to the uncertainty of their genetic origin and to the possible sanitary risk of introducing pathogen agents in its natural habitat that, subsequently, could provoke a disease outbreak (Pérez et al., 2010). However, little is known about the risk that introduced parasites could suppose for the wild population.

The role of parasites as limiting factor of tortoise populations is not well understood. With few exceptions, nematodes are the only helminths infecting terrestrial chelonians, and most of them belong to the orders Oxyurida and Ascaridida, which are considered to be transmitted by the oro-fecal route. There are isolated reports of mortality associated to large ascarid infestations (Rideout et al., 1987); in contrast, oxyurids can be very prevalent and are considered to have an almost commensal relationship with its host (Roca, 1999; Gagno, 2005). Most studies of helminths infections in tortoises have been carried out in captive populations kept in zoological parks and, on the best of our knowledge, there are not published epidemiological studies of helminths infections in *T. graeca* in its natural environment, where nematode transmission may differ significantly when compared to animals maintained in captivity. Moreover, nematode distribution and impact on the host may also vary depending on human intervention in the tortoise's natural habitat.

Therefore, the aims of this study are to describe and to compare the gastrointestinal helminth fauna in wild and captive spur-thighed tortoises. Additionally, we will evaluate the correlation between the prevalence and the infection intensity with host variables as age and sex, with indicators of tortoise's health conditions and, with the free-living or captive origin of tortoises. Finally, we will discuss the obtained pattern of parasitic infection and the potential risk of spreading parasites with tortoise's reintroductions.

2. Material and methods

2.1. Study area and population

Wild south-eastern Spanish population of *T. graeca* covers approximately 2600 km^2 of semiarid coastal mountains between Almeria and Murcia provinces (Fig. 1). We sampled 66 wild tortoises from areas with low, medium and high habitat fragmentation, and 41 from enclosures of the Wildlife Recovery center (WRC) of Santa Faz, in Alicante province (southeast of Spain). Samples were collected between mid march to mid June 2010 in both groups of tortoises (wild and captive animals). Study region lies between longitude N38° 23′ to N36° 20′ and latitude W0° 30′ to W2° 20′, and has a semi-arid climate with mild winters, hot summers and limited rain (200–350 mm/year) mostly during the cooler months of the year.

Tortoises were localized by sight during standardized transects. After sampling, specimens were marked and released in the capture sites.

The forty-one captive tortoises (that correspond to 20% of the individuals held in the WRC) were animals kept

Fig. 1. Distribution of *Testudo graeca* in the Mediterranean region (modified from Fritz et al., 2009); South-eastern Spanish population is included in the red grey square. Star represents the location of the Wildlife Recovery center of Santa Faz (Alicante, Spain). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)