

Salmonella in free living terrestrial and aquatic turtles

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Abstract

Detection of Salmonella in pet turtles has been the focus of extensive research, but its incidence in free living turtles is not well known. The aim of this study was to evaluate the incidence of Salmonella in terrestrial and aquatic species of chelonians inhabiting a National Park in southwestern Spain. Individuals of the terrestrial tortoise *Testudo graeca* (n = 16) and the aquatic turtles *Emys orbicularis* (n = 26) and *Mauremys leprosa* (n = 50) were investigated. Maximum incidence of Salmonella was recorded in the terrestrial species (100%). In contrast, the incidence of infected animals was low in the aquatic species, 12% in *M. leprosa* and 15.4% in *E. orbicularis*. Five serotypes of Salmonella belonging to subspecies enterica (I) and salamae (II) were identified. All serotypes were found in the terrestrial species, and three in the aquatic ones, suggesting that wild terrestrial chelonians are important reservoirs of Salmonella in our study area. Cloacal transmission during mating is the most probable mode of transmission among individuals.

Keywords: Salmonella; Salmonellosis; Free living chelonians; Transmission

1. Introduction

Salmonella commonly lives in the intestine of vertebrates, and has been frequently reported in reptiles (Chiodini and Sundberg, 1981; Millan et al., 1997; Mitchell and Shane, 2000; Abalem de Sá and

Solari, 2001; Geue and Löschner, 2002; Corrente et al., 2004; Mermin et al., 2004).

The incidence of Salmonella in turtles has been the focus of extensive research because of the common use of these animals as pets. In fact, pet turtles are frequently reported as responsible for salmonellosis in humans, especially in children (Feeley and Treger, 1969; Mermin et al., 1997; Woodward et al., 1997; Lynch et al., 1999; Torfoss and Abrahamsen, 2000; Rodgers et al., 2002). However, the incidence of Salmonella in free living reptiles is not well known (Ozsán et al., 1973; Greenberg et al., 1976; Wuthe

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et al., 1979; Bronikowski et al., 2001; Thomas et al., 2001; Briones et al., 2004).

In this study we analyse the incidence of *Salmonella* in free living individuals of three species of chelonians in southwestern Spain. The spur-thighed tortoise (*Testudo graeca*) is an herbivorous terrestrial species which is only occasionally seen in aquatic habitats where they feed on macrophytes (Andreu et al., 2000). The European pond turtle (*Emys orbicularis*) and the Mediterranean turtle (*Mauremys leprosa*) inhabit ponds, streams and other aquatic habitats, and they only move into terrestrial habitats to nest or to hibernate (Fritz, 2001). The aims of this study were to detect and quantify the presence of *Salmonella* in these free living reptiles, and to determine if the incidence is different in the terrestrial versus the aquatic species.

2. Materials and methods

During spring and early summer of 2003–2004, 16 adult tortoises (*T. graeca*) and 76 adult turtles (50 *M. leprosa* and 26 *E. orbicularis*) were captured in Doñana National Park (in southwestern Spain), a large coastal reserve characterized by sandy grounds and marshes.

After capture, every individual was housed singly in order to prevent *Salmonella* transmission among themselves. Because bacteria excretion is not continuous, for each individual three faecal samples (one

on the day of capture, and the other two samples on subsequent days) were taken from the cloaca using cotton sterile swabs. They were stored at 4 °C in Stuart transport medium for 1–4 days before cultivation and identification of *Salmonella* isolates in the National Reference Laboratory for Animal Salmonellosis in Spain (Laboratorio Central de Veterinaria, Algete, MAPA).

Cultivation of bacteria was made according to ISO 6579: 2002, with addition of some culture media that have demonstrated good performance for these samples (doc ISO/TC34SC 9 N 681). Suspect salmonellae colonies were removed for identification by biochemical tests serological identification and serotyping was performed according to Kaufmann-White scheme (Kaufmann, 1966).

3. Results

Maximum incidence of *Salmonella* was recorded in the tortoises, as positive occurrence was found in all (100%) individuals examined. In contrast, a low incidence of infected individuals was recorded in the turtles, being 12% in *M. leprosa* and 15.38% in *E. orbicularis*. The infection rate of the terrestrial turtle was significantly different from that of the aquatic turtles ($\chi^2 = 16.72$, d.f. = 1, $p < 0.0001$), while the difference between the two species of aquatic turtles was not significant ($\chi^2 = 0.172$, d.f. = 1, $p = 0.679$).

Table 1
Isolated serotypes of *Salmonella* in the three chelonian species

Subspecies	Serotype		<i>Testudo graeca</i>	<i>Mauremys leprosa</i>	<i>Emys orbicularis</i>
I	Abony	N	2	–	–
		%	12.5	–	–
I	Newport	N	2	–	–
		%	12.5	–	–
I	Potsdam	N	6	4	3
		%	37.5	66.6	75.0
II	9,12:1,v:z ₃₉	N	1	–	–
		%	36.3	–	–
II	9,12:1,v:z ₃₉ (NI)	N	3	1	1
		%	18.8	16.6	25
II	9,12:1,v:z ₂₉ :1,5	N	2	1	–
		%	12.5	16.6	–

Number and percentage of isolates. (NI) = second phase flagellar antigen not identified.

Twenty-six *Salmonella* isolates belonging to the species *Salmonella enterica* were obtained. No more than one serotype was isolated from individual animals. Seventeen isolates were included in subspecies *enterica* (I), in which we identified three serotypes. The remaining nine isolates belonged to subspecies *salamae* (II), with two serotypes identified and five isolates which could not be totally identified (Table 1).

T. graeca exhibited all serotypes described (identified and not identified), with Potsdam (I) being the most frequent (in 37.5% of isolates), and with lower frequencies of six isolates of subspecies *salamae* (II), two Abony (I) and two Newport (I) (Table 1).

The aquatic turtles showed lower diversity of serotypes, with Potsdam being the most frequent, as it was isolated in 66% of infected *M. leprosa* and in 75% *E. orbicularis*. Only two or one additional serotypes belonging to the subspecies *salamae*, were isolated in these species.

4. Discussion

Among reptiles, a high incidence of serotypes of *Salmonella* has been described in snakes and lizards (Greenberg et al., 1976; Wuthe et al., 1979; Mermin et al., 1997; Burnham et al., 1998; Abalem de Sá and Solari, 2001; Geue and Löschner, 2002; Corrente et al., 2004; Pasmans et al., 2005) while some chelonians have been considered with low or no prevalence (Ozsan et al., 1973; Abalem de Sá and Solari, 2001; Geue and Löschner, 2002; Pasmans et al., 2002; Corrente et al., 2004; Richards et al., 2004). In contrast, in European tortoises from captive centres, the incidence of *Salmonella* was found to be over 70% of individuals (Pasmans et al., 2000). A recent study reporting the incidence of *Salmonella* in wild and captive reptiles in Spain (Briones et al., 2004) revealed similar proportions in chelonians, saurians and ophidians. *M. leprosa* and captive individuals of *T. graeca* were included in this study and had infection rates of 0% and 31%, respectively. A notably higher incidence of *Salmonella* was found in our study, as all tortoises were infected, and many of the turtles were *Salmonella* positive. The absence of infections in *M. leprosa* from central Spain (reported in Briones et al., 2004) may be explained by the low number of

individuals analysed in that study, as we found a low frequency of *Salmonella* in our population from Doñana National Park.

Tortoises may be considered as a very important reservoir for *Salmonella* in the study area. The most commonly reported serotypes responsible for human salmonellosis, Enteritidis or Typhimurium, were not isolated in our study. Nevertheless, the serotypes isolated have been previously reported in human salmonellosis (Chiodini and Sundberg, 1981; Woodward et al., 1997; Torfoss and Abrahamsen, 2000; Unicomb et al., 2003; Briones et al., 2004). Potsdam, the most frequent serotype isolated in this study, is not commonly found but it has been previously reported in reptiles (Burnham et al., 1998; Briones et al., 2004). Newport is widely distributed, and frequently reported in reptiles (Greenberg et al., 1976; Abalem de Sá and Solari, 2001; Geue and Löschner, 2002; Corrente et al., 2004) and in aquatic environment (Polo et al., 1999; Baudart et al., 2000). Abony has been previously isolated in tortoises (Pasmans et al., 2000) and rivers (Usera et al., 1999). Serotypes belonging to subspecies *salamae* (II) are common in ectothermic animals and in the environment (Abalem de Sá and Solari, 2001).

Salmonella survives well in the environment (Roszak et al., 1984; Polo et al., 1999; Baudart et al., 2000). In the wild, the ingestion of faeces or contaminated water is considered a probable way of colonization of *Salmonella* (Polo et al., 1999; Baudart et al., 2000; Mermin et al., 2004). Of the three species included in this study, *T. graeca* is a herbivore in which ingestion of faeces is considered unusual (Andreu et al., 2000), while the two turtles mainly feed in water, where the risk of *Salmonella* infection should be high. Thus, we consider that the high incidence of *Salmonella* that we found in tortoises might not be originated only via oral ingestion. Cloacal transmission might be a mechanism of transmission among individuals, especially as individual females are frequently mounted for copulation by different males during the breeding season (Roques et al., 2004). Frequent mounting has also been reported for aquatic European turtles, *E. orbicularis*, although the dominance strategy reported for some males suggests that this species (Rovero et al., 1999) would have lower frequency of cloacal contact among individuals than *T. graeca*. The lower frequency of sexual encounters in aquatic than

in terrestrial turtles might explain their corresponding lower incidence of *Salmonella* infections.

In general, the aquatic medium is considered a favourable environment for transmission of *Salmonella* (Polo et al., 1999; Baudart et al., 2000). However, some terrestrial reptiles have high infection rates of *Salmonella* (Greenberg et al., 1976; Burnham et al., 1998), and our results demonstrate that, within the same study area, aquatic turtles are less prone to *Salmonella* infection than the terrestrial species. It may be explained by the lower persistence of the bacteria in organisms living in aquatic medium where they are washed from skin and cloaca, while in terrestrial habitats they persist longer and are directly transmitted among individuals.

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