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Russian tortoises (*Agrionemys horsfieldi*) as a potential reservoir for *Salmonella* spp.

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ABSTRACT

A total of 80 Russian tortoises brought in Poland were examined for presence of Salmonella. Salmonella was detected in 15 out of all the animals tested (18.75%). Of the total of 56 strains, 30 (53.57%) belonged to *Salmonella enterica* subsp. enterica (I) and 26 to *Salmonella enterica* subsp. salamae (II). The predominant serotype within subspecies I was *S.* Newport, which is one of the most serotypes causing salmonellosis in humans and warm-blooded animals. *In vitro* determination of the susceptibility of Salmonella to the 10 medicinal preparations showed that all tested strains were sensitive to norfloxacin, sulfamethoxazole with trimethoprim, florfenicol, gentamicin, tetracycline and ampicillin, resistance was noted only to amoxicillin with clavulanic acid (12 strains), and intermediate sensitivity to colistin (7 strains), enrofloxacin (2 strains) and cephalexin (5 strains). These studies confirmed that Russian tortoises are a significant reservoir for Salmonella and may represent a potential source of infection for humans.

The last few decades have seen increasing interest in exotic animals, particularly reptiles and amphibians, as domestic pets. An estimated 1% of households in the USA and Europe are currently raising animals of this type, most often turtles (the term "turtle" includes tortoises and terrapins) (Harris et al., 2010). In recent years the demand for exotic pets has increased in Poland as well, which is to some extent due to the lifting of restrictions on international tourist traffic in the 1990s and the large number of reptiles and amphibians entering the country, mainly from former republics of the Soviet Union, the Czech Republic, Slovakia, Germany, and Mediterranean countries. Most frequently purchased are tortoises and semi-aquatic turtles. Nevertheless, due to their rich and diverse microbiological biota, especially the high percentage of *Salmonella* carriers, they and their environment can be a potential reservoir of these pathogens, and even are responsible for numerous human salmonellosis outbreaks (CDC, 2007; Bertrand et al., 2008; CDC, 2008).

Cases of salmonellosis in humans, caused by direct or indirect contact with exotic pets have been described in many countries in Europe and the US (Bertrand et al., 2008; Harris et al., 2010). As a result of the high percentage of infections of animal origin that had their source in reptiles, in 1975 the FDA in the United States introduced regulations prohibiting the sale and distribution of turtles less than four inches long. In the other countries the introduction of import restriction and a broad educational campaign to reduce the risk of transmission of *Salmonella* from exotic pets to humans has contributed to the protection of public health (Bertrand et al., 2008; Harris et al., 2010). In Poland, despite the regulations in effect (Act of 16 April, 2004 on protection of nature – Dz. U. 2004 No. 92 item 880 – and Council Regulation (EC) No. 338/97 of 9 December, 1996 on the protection of species of wild fauna and flora by regulating trade therein), a large percentage of many exotic animal species, including Russian tortoises, have been smuggled across the border, and their health is monitored only to a small degree (animals with clinical symptoms). In addition, there is no obligation study of *Salmonella* carrier status in exotic pets, legally distributed in pet shops, because Act of 11 March, 2004 "About health protection of animals and controlling of infection diseases of animals" in force in Poland, includes control of *Salmonella* in only farm animals and do not cover group of exotic animals.

Therefore, the information on the presence of *Salmonella* colonization of exotic pets (including Russian tortoises) on Polish territory, and potential threat they pose to humans, are very few (Wasyl and Hoszowski, 2004; Pęconek et al., 2009).

Purpose of this study was to determine the extent of carrying *Salmonella* in Russian tortoises that have been imported into the Polish territory, with particular consideration given to serotypes currently isolated from cases of salmonellosis in humans and to determine sensitivity to selected chemotherapeutic agents.

A total of 80 Russian tortoises (Horsfield's tortoise) *Agrionemys horsfieldi* were tested. The animals were confiscated by customs

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officials and placed in 30 day quarantine in the Department of Epizootiology and Clinic of Infectious Diseases of the Faculty of Veterinary Medicine, University of Life Sciences in Lublin. The material for the study consisted of swabs taken from the cloaca of each animal using sterile cotton swabs.

Salmonella was detected according to official standards in force in Poland (PN-EN ISO 6579:2003). From each culture a maximally of five colonies characterized by macromorphology typical of *Salmonella* were selected. These were transferred to a nutrient agar for biochemical identification confirming that the bacteria belonged to the *Salmonella* genus, using the microtest Entero test 24 (Pliva-Lachema Diagnostika). Serotyping was performed according to Kauffmann–White scheme (Popoff, 2001). The susceptibility of each strain was determined using the Kirby–Bauer disk-diffusion method (Bauer et al., 1966), using disks were saturated following antibiotics: gentamicin (10 μg), colistin (10 μg), norfloxacin (5 μg), enrofloxacin (5 μg), tetracycline (30 μg), amoxicillin with clavulanic acid (30 μg), cephalexin (30 μg), ampicillin (10 μg), sulfamethoxazole with trimethoprim (25 μg) (Oxoid), and florfenicol (30 μg) (Krka). The results were interpreted based on NCCLS norms (M2-A7 and M7-A5).

In our study, *Salmonella* was isolated from 15 to 80 animals tested (18.75%). Depending on the data provided by the other authors, the percentage of positive isolations in turtles has ranged from 5–6% to 85% (Gopee et al., 2000; Nakadai et al., 2005; Hidalgo-Vila et al., 2008). Such marked differences are partly due to the lack of standardization, as to how and when material is collected for testing, different sources for the samples (direct swab, faeces) and different procedures for isolating the bacteria. Moreover, in the case of turtles, *Salmonella* are excreted periodically and a marked correlation is noted with the hibernation period and with environmental stress (Bauwens et al., 2006; Schumacher, 2006).

Due to the fact, that individual animals can be carriers of various subspecies and/or serotypes simultaneously, we distinguished more than one colony of *Salmonella* from each turtles. Of the total of 56 strains, 30 (53.57%) belonged to *Salmonella enterica* subsp. *enterica* (I) and 26 to *Salmonella enterica* subsp. *salamae* (II) (Table 1). Further serotyping determined that within subspecies I, the dominant taxon was the serotype S. Newport (10/30 strains) which is isolated from six turtles.The remaining serotypes belonged to *S. Eastbourne* (2 strains), isolated from the same turtle, and 1 each to *S.* Eko, *S. Tshiongwe* and *S.* Chincol. For the remaining 15 strains of *S. enterica* subsp *enterica* and 26 strains of *S. enterica* subsp *salamae*, the serotypes could not be determined (Table 1). Although our laboratory is able to identify most of routinely isolated serotypes of *Salmonella*, the results may indicate a limited pool of specific sera. These undefined isolates are likely to be very rarely isolated from clinical or environmental samples in Poland, and will require further identification.

The distribution and occurrence of both subspecies and serotypes in individual animals was not uniform, which was also reported by the other authors (Nakadai et al. 2005; Pedersen et al., 2009). Homogeneous *Salmonella* taxa were found only in 10 tortoises: *S. enterica* subsp. *enterica* in seven and *S. enterica* subsp. *salamae* in three. When serotype was considered alongside subspecies as a differentiating criterion, in five turtles two *Salmonella* taxa were found simultaneously, and in one specimen two subspecies and three serotypes were noted (Table 1).

This distribution of *Salmonella* subspecies differs from that reported by other authors, according to whom the tortoise, like other reptiles and amphibians, is colonized mainly by *S. enterica* subsp. *enterica* (43–80%) and *S. enterica* subsp. *diarizonae* (10–40%), and to a much lesser degree by the other subspecies of *Salmonella* (Briones et al., 2004; Bauwens et al., 2006). The presence of only *Salmonella* subspecies *enterica* and *salamae* in tested animals could be explained by the long period during which the animals are kept together in a small space (transport, quarantine), which combined with heightened stress factors probably led to increased excretion of the pathogens into the environment and to cross-contamination of individual specimens.

With regard to the spectrum of *S. enterica* subsp. *enterica* serotypes isolated from the reptiles, a high degree of variation was noted. These animals can also be colonized by serotypes most often responsible for infection, such as *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, *S.* Virchov, *S.* Infantis, and *S.* Hadar (Pasmans et al., 2005; Bauwens et al., 2006; Hidalgo-Vila et al., 2008), and those which are occasionally isolated from humans and warm-blooded animals (Baudart et al., 2000, Bertrand et al., 2008; Pedersen et al., 2009).

Most of the serotypes defined by us, have been previously reported. Our study showed that the predominant serotype was *Salmonella* Newport, and was found in 6 of 15 turtle carriers. This serotype is located on the top list of the most frequently isolated serotypes from humans, animals and foods of animal origin (Poppe et al., 2006; Foley and Lynne, 2008). There have been reported numerous cases of infection, especially in children, which often led to life-threatening conditions. In the US have also been reported outbreaks caused by this serotype (CDC, 2002; CDC, 2010)

Table 1
Salmonella strains isolated from turtles.

Turtle N_o $\Sigma = 15$	*Salmonella enterica* subspecies						
	Salamae	*Enterica* serovar					
		Newport	Eastbourne	Eko	Tshiongwe	Chincol	Unmarked
1	5						
2	4	1					
3		1					1
4		2					
5	4	1					
6	2						3
7	2						1
8	3						
9				1			
10	5						
11							5
12							5
13	1	2			1	1	
14		3					
15			2				
Total	**26**	**10**	**2**	**1**	**1**	**1**	**15**

Table 2
Salmonella strains, in which was noted differential sensitivity profile (selected drugs).

Turtle No.[**]	Strain of *Salmonella enterica*		Chemotherapeutic drug[*]			
	Subspecies	Serovar	CT colistin	ENR enrofloxacin	AMC amoxicillin with clav. acid	CL cephalexin
1	*Salamae*	Undefined	I	S	R	I
	Salamae	Undefined	I	S	R	S
2	*Salamae*	Undefined	S	S	R	I
4	*Enterica*	Newport	I	S	R	S
7	*Enterica*	Undefined	S	I	R	S
	Salamae	Undefined	I	I	R	S
9	*Enterica*	Eko	I	S	R	I
14	*Enterica*	Newport	S	S	R	S
	Enterica	Newport	S	S	R	S
	Enterica	Newport	S	S	R	S
15	*Enterica*	Eastbourne	I	S	R	I
	Enterica	Eastbourne	I	S	R	I

S-sensitive, R-resistant, I-intermediate.
[*] Strains are sensitive to the other chemotherapeutic agents tested.
[**] The other strains are sensitive to all chemotherapeutic agents tested.

and in sensitive animals, especially cattle, has been observed that *Salmonella* Newport induced septicaemic conditions associated with high mortality (Poppe et al., 2006). The rapid spread of this serotype and such a large morbidity noted in recent years, may be connected with occurrence of multidrug resistance (MDR-AmpC, ceftriaxone and quinolone mechanisms of resistance) (Zhao et al., 2003; Poppe et al., 2006; Wasyl, 2009), which in turn, can lead to public health risk, especially when the treatment is carried out without antimicrobial sensitivity testing.

Although other serotypes are not frequently isolated from humans, it was shown that *Salmonella* Chincol, Eastbourne and Tshiongwe were responsible for cases of intestinal infections (Kaneko, 1995; Thong et al., 2004) septicaemia (Mitchel-Jones, 1984) and even outbreaks (Craven et al., 1975).

In vitro determination of the susceptibility of *Salmonella* to the 10 medicinal preparations most frequently used to treat, showed that all tested strains were susceptible to norfloxacin, sulfamethoxazole with trimethoprim, florfenicol, gentamicin, tetracycline and ampicillin. Resistance was noted only to amoxicillin with clavulanic acid (12/56 strains), usually accompanied by intermediate sensitivity to colistin (7 strains/58%), enrofloxacin (2 strains/17%) and cephalexin (5 strains/42%). The presence of the strains resistant to amoxicillin with clavulanic acid and with intermediate sensitivity to other medications was noted in the case of 7 of the 15 tortoises from which *Salmonella* was isolated, which was 47% of the infected population and 9% of all the animals tested (7/80). Surprising was, that in this group, resistance to amoxicillin with clavulanic acid occurred in all cases, accompanied in six tortoises by intermediate sensitivity to other medicines, was recorded usually for 2–3 strains within a individual turtle (Table 2). It may confirm that spreading of resistance can occur not only within the same serotype but also between different serotypes and subspecies of *Salmonella* (Poppe et al., 2006).

It is also known that the profile of *Salmonella* sensitivity can be largely conditioned by the source of the strains (frequent exposure of the microbe to medicines, use of given chemotherapeutic drugs in human and veterinary medicine, different sets of medicines approved for use in particular countries) (Pasmans et al., 2005; Vigo et al., 2010), it can be presumed that the most of Russian tortoises studied, came from a natural environment that is poorly populated and developed by man, while the animals in which showed the resistant and intermediate strains could come from other, more industrial environment. Small differences in susceptibility profiles of strains belonging to one subspecies/serotype, do not allow the exclusion that these are separate isolates. However, the prolonged, close contact of animals (travel, quarantine), enabling cross-contamination, indicates that some isolates can represent copies of the same clones.

To conclude, our study showed that Russian tortoises imported into the Polish territory are a significant reservoir for *Salmonella* (18.75% animals tested). Nevertheless, evaluating results of the study, we must also take into account the limitations due to lack of determination of the full panel of serotypes and the possibility of copies within a pool of isolated strains. But due to the fact that Russian tortoises are often kept as pets, may represent a potential source of infection for humans, especially for children who are usually in close contact with these animals. Therefore should be introduced constant monitoring of carrier rate of *Salmonella* in exotic pets and it should be enforced large-scale information campaign on the risks from reptile-related salmonellosis.

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