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Short communication

Genetic lineages of *Salmonella enterica* serovar Kentucky spreading in pet reptiles

Magdalena Zając [a,*], Dariusz Wasyl [a], Andrzej Hoszowski [a], Simon Le Hello [b], Krzysztof Szulowski [a]

[a] National Veterinary Research Institute, Department of Microbiology, Al. Partyzantów 57, 24-100 Puławy, Poland
[b] Institut Pasteur, Unité des Bactéries Pathogénes Entériques, Centre National de Référence des Salmonella, World Health Organization (WHO) Collaborating Centre for Reference and Research on Salmonella, Paris, France

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ABSTRACT

The purpose of the study was to define genetic diversity of reptilian *Salmonella enterica* serovar (*S.*) Kentucky isolates and their epidemiological relations to the ones from poultry, food, and environmental origin in Poland. Between 2010 and 2012 twenty-four *S.* Kentucky isolates derived from snakes ($N = 8$), geckos ($N = 7$), chameleons ($N = 4$), agamas ($N = 1$), lizard ($N = 1$), and environmental swabs taken from reptile exhibition ($N = 3$) were identified. They were characterized with antimicrobial minimal inhibitory concentration testing, *Xba*I–PFGE and MLST typing. The profiles compared to *S.* Kentucky available in BioNumerics local laboratory database ($N = 40$) showed 67.3% of relatedness among reptile isolates. Three genetic lineages were defined. The first lineage gathered 20 reptile isolates with 83.4% of similarity and wild-type MICs for all antimicrobials tested but streptomycin in single case. The remaining three reptilian and one post-exhibition environment *S.* Kentucky isolates were clustered (87.2%) with isolates originating from poultry, mainly turkey, food, and environment and presented variable non-wild type MICs to numerous antimicrobials. The third *S.* Kentucky lineage was composed of two isolates from feed (96.3%). The results suggest diverse sources and independent routes of infection. Most of the isolates belonged to reptile-associated clones spread both horizontally and vertically. Simultaneously, PFGE profiles and MLST type indistinguishable from the ones observed in poultry point out carnivore reptiles as possible vector of infection with multidrug and high-level ciprofloxacin resistant (MIC $\geq$ 8 mg/L) *S.* Kentucky. Public awareness and education are required to prevent potential reptile-associated *S.* Kentucky infections in humans.

1. Introduction

Reptiles are considered reservoir of a wide variety of *Salmonella* subspecies and serovars, being easily colonized with vertical and horizontal transfer (Chiodini, 1982; Chiodini and Sundberg, 1981). There are reports on reptile-associated *Salmonella* transmission to humans (Bertrand et al., 2008; Warwick et al., 2001), precisely in relation to the high popularity of pet reptiles kept not only in private households in terrarium but also allowed to roam freely throughout the house (Mermin et al., 2004).

Salmonella enterica subsp. *enterica* serovar (*S.*) Kentucky was rarely noted in reptiles, with a few isolates being reported in Japan, England and recently in Poland (Aiken et al., 2010; Nakadai et al., 2005; Zając et al., 2013). Recently, multidrug and high-level ciprofloxacin resistant (MIC $\geq$ 8 μg/mL) *S.* Kentucky has gained epidemiological importance in several countries causing infections in food

* Corresponding author. Tel.: +48 81 889 3043; fax: +48 889 3000.
E-mail address: magdalena.zajac@piwet.pulawy.pl (M. Zając).

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animals, and, presumably *via* contaminated food, in humans (Beutlich et al., 2012; Le Hello et al., 2011; Turki et al., 2012a). In autumn 2009 it occurred in turkey flocks and turkey meat in Poland (Wasyl and Hoszowski, 2012) with some health consequences for humans in the following years (NIH, 2012).

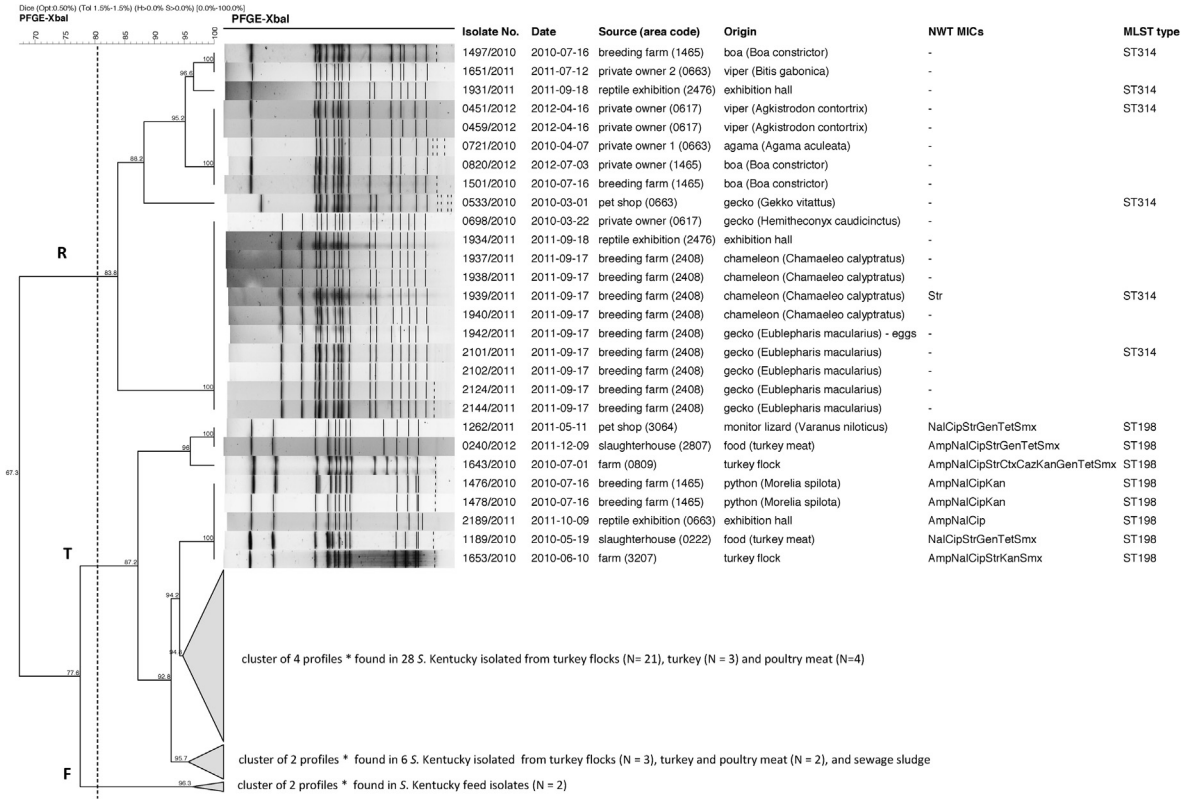
The aim of the study was to characterize *S.* Kentucky isolated from reptiles and to define their epidemiological relations to the clonal lineages currently spreading in poultry, food, and environment.

2. Materials and methods

Twenty-four *S.* Kentucky were identified according to White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007) among the isolates obtained from research project on *Salmonella* infections in reptiles in Poland. The isolates derived mostly from faecal samples collected during 2010–2012 from snakes ($N = 8$), geckos ($N = 7$, including one gecko eggs sample), chameleons ($N = 4$), agama ($N = 1$), monitor lizard ($N = 1$), and environmental surface swabs taken after reptile exhibitions ($N = 3$). Animal samples were obtained from breeding farms ($N = 13$), private pet owners ($N = 6$) and pet shops ($N = 2$). The details on sample types, sources, and isolation dates were shown in Fig. 1. Antimicrobial susceptibility testing was used to define non-wild type (NWT) minimal inhibitory concentrations (MICs) interpreted with epidemiological cut-off values (EUCAST). PFGE typing was carried out according to the PulseNet protocol following DNA digestion with *Xba*I (Ribot et al., 2006). The analysis was done with the BioNumerics (version 4.5, Applied Maths, Belgium) and *Xba*I–PFGE profiles were compared with *S.* Kentucky already present in the local laboratory database ($N = 40$). Some of those isolates originating mainly from turkey flocks, turkey farm environments, turkey meat, poultry meat, feed and sewage sludge have been described previously (Wasyl and Hoszowski, 2012). Fourteen isolates representing each of PFGE pattern were submitted to multilocus sequence typing (MLST) analysis (UCC).

3. Results

Reptile *S.* Kentucky isolates have been clustered into seven *Xba*I–PFGE profiles with the 67.3% relatedness to those found in isolates from poultry, food, feed, and sewage sludge (Fig. 1). Assuming 80% cut-off similarity value, the three lineages comprising isolates from different sources were identified. The first lineage (R) gathered twenty reptile isolates representing 5 profiles with 83.8% of



Isolate No.	Date	Source (area code)	Origin	NWT MICs	MLST type
1497/2010	2010-07-16	breeding farm (1465)	boa (Boa constrictor)	-	ST314
1651/2011	2011-07-12	private owner 2 (0663)	viper (Bitis gabonica)	-	
1931/2011	2011-09-18	reptile exhibition (2476)	exhibition hall	-	ST314
0451/2012	2012-04-16	private owner (0617)	viper (Agkistrodon contortrix)	-	ST314
0459/2012	2012-04-16	private owner (0617)	viper (Agkistrodon contortrix)	-	
0721/2010	2010-04-07	private owner 1 (0663)	agama (Agama aculeata)	-	
0820/2012	2012-07-03	private owner (1465)	boa (Boa constrictor)	-	
1501/2010	2010-07-16	breeding farm (1465)	boa (Boa constrictor)	-	
0533/2010	2010-03-01	pet shop (0663)	gecko (Gekko vitattus)	-	ST314
0698/2010	2010-03-22	private owner (0617)	gecko (Hemitheconyx caudicinctus)	-	
1934/2011	2011-09-18	reptile exhibition (2476)	exhibition hall	-	
1937/2011	2011-09-17	breeding farm (2408)	chameleon (Chamaeleo calyptratus)	-	
1938/2011	2011-09-17	breeding farm (2408)	chameleon (Chamaeleo calyptratus)	-	
1939/2011	2011-09-17	breeding farm (2408)	chameleon (Chamaeleo calyptratus)	Str	ST314
1940/2011	2011-09-17	breeding farm (2408)	chameleon (Chamaeleo calyptratus)	-	
1942/2011	2011-09-17	breeding farm (2408)	gecko (Eublepharis macularius) - eggs	-	
2101/2011	2011-09-17	breeding farm (2408)	gecko (Eublepharis macularius)	-	ST314
2102/2011	2011-09-17	breeding farm (2408)	gecko (Eublepharis macularius)	-	
2124/2011	2011-09-17	breeding farm (2408)	gecko (Eublepharis macularius)	-	
2144/2011	2011-09-17	breeding farm (2408)	gecko (Eublepharis macularius)	-	
1262/2011	2011-05-11	pet shop (3064)	monitor lizard (Varanus niloticus)	NalCipStrGenTetSmx	ST198
0240/2012	2011-12-09	slaughterhouse (2807)	food (turkey meat)	AmpNalCipStrGenTetSmx	ST198
1643/2010	2010-07-01	farm (0809)	turkey flock	AmpNalCipStrCtxCazKanGenTetSmx	ST198
1476/2010	2010-07-16	breeding farm (1465)	python (Morelia spilota)	AmpNalCipKan	ST198
1478/2010	2010-07-16	breeding farm (1465)	python (Morelia spilota)	AmpNalCipKan	ST198
2189/2011	2011-10-09	reptile exhibition (0663)	exhibition hall	AmpNalCip	ST198
1189/2010	2010-05-19	slaughterhouse (0222)	food (turkey meat)	NalCipStrGenTetSmx	ST198
1653/2010	2010-06-10	farm (3207)	turkey flock	AmpNalCipStrKanSmx	ST198

Fig. 1. Phylogenetic similarity of *S.* Kentucky isolated from reptiles in comparison to *S.* Kentucky isolated from other non-human origins. Genetic lineage defined at >80% of profile similarity, a cluster of profiles at ≥95%; antimicrobial abbreviations and minimal inhibitory concentration epidemiological cut-off values (EUCAST; non-wild type > mg/L): Amp–ampicillin (8 mg/L), Ctx–cefotaxime (0.5 mg/L), Caz–ceftazidime (2 mg/L), Cip–ciprofloxacin (NWT > 0.064 mg/L; clinical breakpoint $R > 1$ mg/L), Gen–gentamicin (2 mg/L), Kan–kanamycin (4 mg/L), Nal–nalidixic acid (16 mg/L), Str–streptomycin (16 mg/L), Smx–sulfametoxazole (256 mg/L), Tet–tetracycline (8 mg/L). Vertical line at 80% similarity designates treshhold used to identify reptile-associated (R) clonal lineage, turkey-related lineage (T), and feed-related lineage (F); * profiles described by Wasyl and Hoszowski (2012).