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Original Contribution

Cloacal Aerobic Bacterial Flora and Absence of Viruses in Free-Living Slow Worms (*Anguis fragilis*), Grass Snakes (*Natrix natrix*) and European Adders (*Vipera berus*) from Germany

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Abstract: Disease problems caused by viral or bacterial pathogens are common in reptiles kept in captivity. There is no information available on the incidence of viral pathogens or the physiological cloacal bacterial flora of common free-living reptiles in Germany. Therefore, 56 free-living reptiles including 23 European adders (*Vipera berus*), 12 grass snakes (*Natrix natrix*) and 21 slow worms (*Anguis fragilis*) were investigated on the island Hiddensee in northeastern Germany. Pharyngeal and cloacal swabs were taken immediately after capture. Bacteriological examination was performed from the cloacal swabs to study the aerobic cloacal flora. Molecular biological examination included amplification of DNA or RNA from adeno-, rana- and ferlaviruses as well as culturing on Russell's viper heart cells for virus isolation. *Salmonella* spp. were isolated from European adders but not from the other reptiles examined. The minimal inhibitory concentration was determined from the isolated *Salmonella* spp. However, some potentially human pathogenic bacteria, such as *Proteus vulgaris*, *Aeromonas hydrophila*, *Klebsiella pneumoniae* and *Escherichia coli* were isolated. Viruses were not detected in any of the examined reptiles. To the authors' best knowledge, the present study is the first survey of viral pathogens in free-living snakes and slow worms in Germany and the first survey of cloacal aerobic bacterial flora of slow worms.

Keywords: enterobacteriaceae, *Salmonella*, free-living reptiles, ferlavirus, hiddensee

Introduction

Reptiles can harbour pathogenic microorganisms asymptomatically and serve as potential reservoirs of infection for humans, domestic animals and other reptiles. Reports about the occurrence of viral pathogens of free-living snakes and lizards are rare (Gravendyck et al. 1998; Calle et al. 2001; Marschang et al. 2002; Allender et al. 2006, 2008; de Matos et al. 2011, 2013; Ascher et al. 2013) and still missing for free-living snakes and lizards from Germany. Ferlaviruses (Paramyxovirinae) are frequently described in captive snakes from Germany (Papp et al. 2010; Pees et al. 2010) and have been shown to be important

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primary pathogens causing respiratory diseases in captive viperid snakes (Jacobson et al. 1997). Reports of ferlavirus detection in captive and in free-living lizards are rare (Marschang et al. 2002). Ranaviruses have an increasing prevalence in captive and wild animals, although more in amphibians and fish than in snakes and lizards (Chinchar 2002; Miller et al. 2011; Stöhr et al. 2013). Ranaviruses are major pathogens of chelonian species, as shown by the epidemic in wild box turtle populations of North America (Johnson et al. 2008; Allender et al. 2011). Detection of adenovirus and reovirus is frequently described in captive snakes and lizards from Germany (Papp et al. 2009; Pees et al. 2010; Abbas et al. 2011; Schmidt et al. 2013).

Knowledge about the normal gastrointestinal flora is limited for the majority of free-living reptile species. Most studies have concentrated on a small subgroup of bacteria that are known to be zoonotic or on reptile species with commercial interest as pets (Schröter et al. 2004; Ebani et al. 2005; Geue and Löschner 2002; Krautwald-Junghanns et al. 2013). Furthermore, it has been well established that many reptiles harbour Gram-negative bacteria as part of their normal flora and that these microbes are either commensal or opportunistic (Rosenthal and Mader 2006). The purpose of this study was to investigate the culturable aerobic cloacal bacterial flora of common free-living snakes and lizards and to investigate the shedding of pathogenic viruses in those free-living species. Slow worm (*Anguis fragilis*), grass snake (*Natrix natrix*) and European adder (*Vipera berus*) are common squamate species in Europe, with the latter one reaching the highest population density on the island of Hiddensee in Germany (Ortlieb et al. 2012). Slow worm is a semifossorial lizard, spending much of the time hiding underneath objects (Brown and Roberts 2008). Their habitat consists of rocks, grass and damp environment, so that their food consists of slugs and worms. Grass snakes are non-venomous colubrid snakes, which also live in a damp environment near water and feed almost exclusively on amphibians. The European adder is a venomous viper species, dwelling in different habitats including chalky downs, rocky hillsides, moors, sandy heaths, meadows, rough commons, edges of woods, sunny glades and clearings, bushy slopes and hedgerows, coastal dunes and stone quarries (Street 1979). This species also has the widest spectrum of feed, including small mammals, young birds, reptiles and amphibians. To the authors' best knowledge, the present study is the first survey of viral pathogens in free-living snakes and slow worms in Germany and the first survey of the cloacal aerobic bacterial flora of slow worms.

Materials and Methods

Animals

In total 56 reptiles, including 23 European adders, 12 grass snakes and 21 slow worms, were captured manually between June and October 2012 on the island Hiddensee (54°32′24″N 13°5′34″E) in northeastern Germany (Table 1). The landscape at the capture locations was characterised by coastal dune heathland, dry meadow and coastal meadow (Fig. 1). The length of most of the tested animals was measured with a measuring tape and the age was classified as subadult or adult by examining the size and the colour of the animals. Gender determination was done based on the body size, tail length and colour. Every animal was documented with a photo of the scale pattern to avoid recapturing. After sampling, every reptile was immediately released.

Sample Collection

Samples were taken with individually packed microbiological sterile swabs (Applimed, Châtel-St-Denis, Freiburg, Switzerland) from the pharynx (one per animal) and from the cloaca (three per animal) by rotating the swab in the mouth or cloaca in order to obtain mucosal cells. The swab from the pharynx and one swab from the cloaca were placed into MicroTest™M4® medium (Remmel, Dartford, Kent, UK) cooled with frozen gel packs and transported to the Institute of Environmental and Animal Hygiene and Veterinary Medicine, University of Hohenheim for virological examinations.

Of the other two cloacal swabs, one was subsequently inserted into a tube containing Amies transport medium (Heinz Herenz Medizinalbedarf, Hamburg, Germany) and the third was placed in Rappaport–Vassiliadis (RV) broth (Oxoid Deutschland GmbH, DE) cooled with frozen gel packs and transported to the Clinic for Birds and Reptiles, University of Leipzig, for bacteriological examinations.

Virological Examination

Samples for virological testing were treated with ultrasound as described previously (Papp et al. 2010). RNA was prepared from 300 µl of sample as described by Boom et al. (1990). DNA was prepared from 200 µl of sample using the DNeasy® Blood and Tissue Kit (Quiagen GmbH, Hilden, Germany) as described previously (Abbas et al. 2011). RT-

Table 1. Number, capture habitat, age, gender and body size of sampled free-living reptiles form the island of Hiddensee

	Slow worm (*Anguis fragilis*) (n = 21)	Grass snake (*Natrix natrix*) (n = 12)	European adder (*Vipera berus*) (n = 23)
Capture habitat			
Coastal dune heathland	10 (48%)	5 (42%)	16 (70%)
Dry meadows	1 (5%)	3 (25%)	6 (26%)
Coastal meadows	10 (48%)	4 (33%)	1 (4%)
Age			
Adult	14 (67%)	6 (50%)	14 (61%)
Subadult	7 (33%)	6 (50%)	9 (39%)
Gender			
Female	12 (57%)	5 (42%)	14 (61%)
Male	4 (19%)	4 (33%)	6 (26%)
Not determined	5 (24%)	3 (25%)	3 (13%)
Body size	24.9 ± 5.4 cm	n.m.	45.4 ± 12.4 cm

n.m. not measured.

PCR detection of a portion of the L gene of ferlaviruses was carried out as described previously (Ahne et al. 1999; Papp et al. 2010). Samples were examined for the presence of adenoviruses (AdV) by nested PCR targeting the DNA polymerase gene (Wellehan et al. 2004; Papp et al. 2009). Screening for the detection of ranaviruses was carried out using a PCR targeting a portion of the major capsid protein (MCP) gene (Mao et al. 1997; Marschang et al. 1999). Isolation of viruses was attempted from all samples on Russell's viper heart cells (VH-2, ATCC: CCL-140), as it was demonstrated that mixed viral infection is not unlikely (Abbas et al. 2011). Especially for detection of reovirus, cell-culture on Russell's viper heart cells is a more sensitive tool, since previous studies have shown that reoviruses isolated from reptiles can differ from one another both serologically and molecular biologically (Blahak et al. 1995; Wellehan et al. 2009; Pees et al. 2010).

Bacteriological Examination

As initial isolation media, columbia blood agar (Oxoid, Wesel, North Rhine-Westphalia, Germany), MacConkey agar (Oxoid) and brilliant-green agar (Oxoid) were streak inoculated with the samples and then incubated at 41°C for 24 h under aerobic conditions. If after this time no or scanty growth was present the incubation time was prolonged for another 24 h. Colonies demonstrating distinctive macroscopic appearance were considered separate organisms and isolated on new plates for identification. The isolated bacteria were identified using standard microbiologic techniques including Gram-staining, morphologic characteristics, catalase and oxidase reactions and growth in limiting media. Representative Gram-negative isolates of all distinct organisms were further identified using the multi-substrate-identification kit BBL Crystal™ E/NF (Becton–Dickinson, Franklin Lakes, NJ, USA). Among Gram-positive bacteria, cocci and *Bacillus* were identified to the genus based on colony morphology, catalase and oxidase reactions as well as microscopic appearance after staining.

Isolation of *Salmonella* and Determination of Serotypes

RV broth were incubated at 41°C for 24 h, and subsequently a loopful (10 µL) of RV broth culture was streak inoculated onto a xylose lysine tergitol-4 (XLT-4) agar (Oxoid Deutschland GmbH, DE) and a brilliant-green (BGA) agar plate (Oxoid Deutschland GmbH, DE), respectively. Plates were incubated for another 24 h under aerobic conditions at 41°C. All suspicious colonies were screened for *Salmonella* with a rapid plate agglutination test using Enterocolon Anti-*Salmonella* A67, omnivalent (Sifin GmbH, Berlin, DE). Up to five *Salmonella*-confirmed colonies were stored up to 4 days at −20°C in Roti®-Store cryotubes (Carl Roth GmbH + Co. AG, DE) and were sent to the German National *Salmonella* Reference Laboratory in Berlin for serotyping. The classification of *Salmonella*

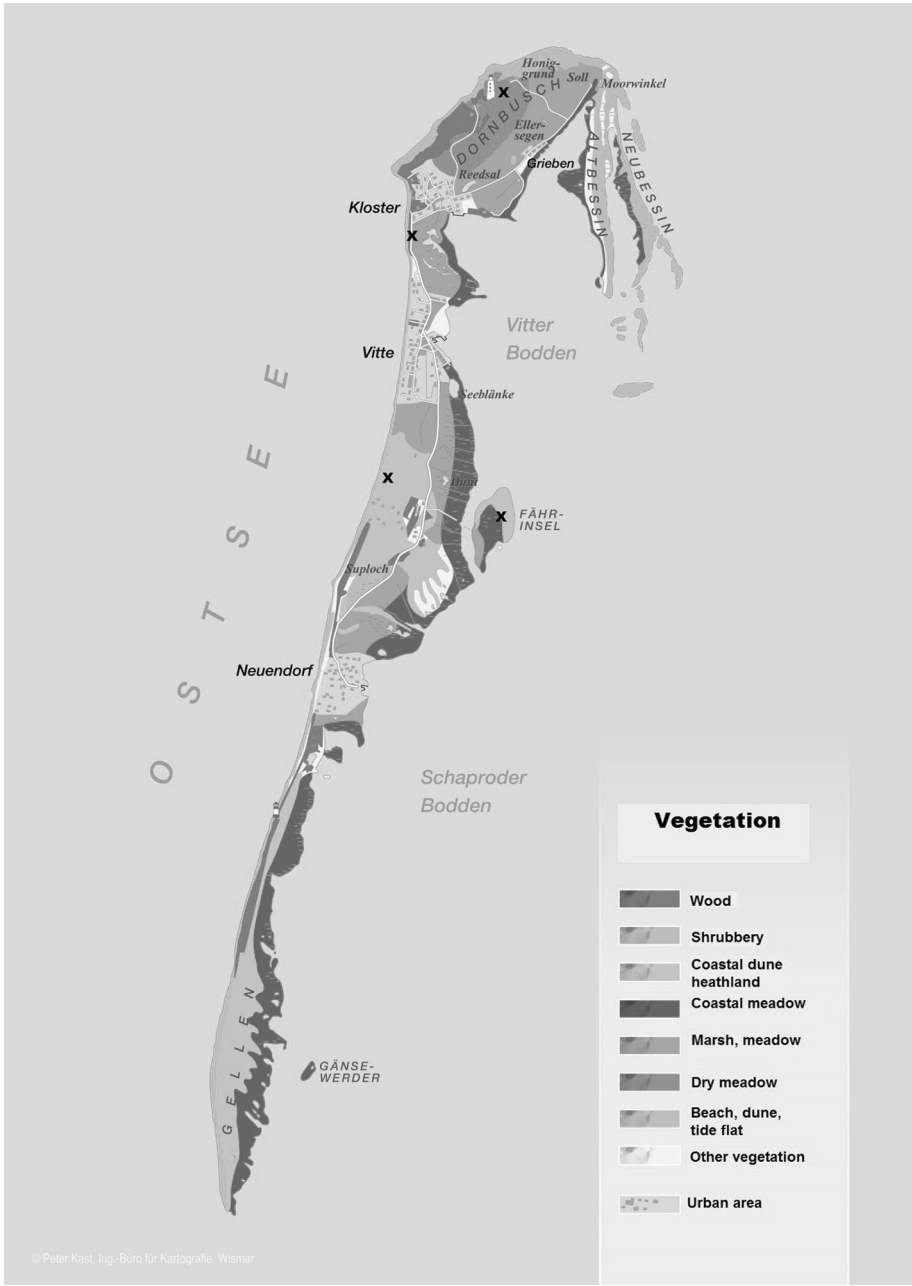

Figure 1. Vegetation map of the island of Hiddensee with *marked* (X) capture sites.

subspecies and below the subspecies level was carried out according to the White–Kauffmann–Le Minor scheme (Grimont and Weill 2007) by agglutination with O- and H-antigen-specific sera. The minimal inhibitory concentration (MIC) was determined by the agar dilution method, as recommended by the Clinical and Laboratory Standards Institute (CLSI M31-A3/2008). Used cut-off values were according to European Committee on Antimicrobial Susceptibility Testing (2012). The following antimicrobials were evaluated: ampicillin, cefotaxime, ceftacidime, chloramphenicol, colistin. ciprofloxacin, florfenicol, getamicin, kanamycin, nalidixic acid, streptomycin, sulphametoxazol, tetracycline and trimethoprim (Table 2).

Statistical Analysis

Statistical analysis was performed using the programme SPSS, version 17.0 (SPSS Inc., USA). Differences between number of isolated bacteria species, isolated bacterial species among reptile species, age and gender as well as location of capture, were examined with non-parametric tests for significance at a 0.05 significance level using Kruskal–

Table 2. Minimal inhibitory concentrations (μg/mL) of isolated *Salmonella* spp. determined by the agar dilution method (CLSI M31-A3/2008)

Antimicrobial	*Salmonella* sp. IIIb 14:l,v:z	*Salmonella* sp. IIIb 14:l,v:z	*Salmonella* sp. IIIb 50:z10:z	*Salmonella* sp. IIIb 50:z10:z	*Salmonella* sp. IIIb 50:z10:z	*Salmonella* sp. IIIb 50:z10:z	*Salmonella* sp. IIIb 50:z10:z
Ampicillin	≤0.5	≤0.5	1.0	≤0.5	≤0.5	≤0.5	1.0
Chloramphenicol	≤2.0	≤2.0	4.0	4.0	4.0	4.0	4.0
Cefotaxime	≤0.0625	≤0.0625	≤0.0625	≤0.0625	≤0.0625	≤0.0625	≤0.0625
Ceftacidime	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Ciprofloxacin	0.03125	0.015	0.015	0.015	0.015	≤0.008	0.015
Colistin	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0	≤2.0
Florfenicol	≤2.0	≤2.0	4.0	≤2.0	4.0	≤2.0	≤2.0
Genatmicin	≤0.25	0.5	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Kanamycin	≤4.0	≤4.0	≤4.0	≤4.0	≤4.0	≤4.0	≤4.0
Nalidixic acid	≤4.0	≤4.0	≤4.0	≤4.0	≤4.0	≤4.0	≤4.0
Streptomycin	32.0[a]	64.0[a]	16.0	16.0	16.0	8.0	8.0
Sulphamethoxazole	64.0	32.0	128.0	128.0	128.0	128.0	64.0
Tetracycline	2.0	≤1.0	2.0	≤1.0	2.0	≤1.0	2.0
Trimethoprim	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5

Used cut-off values were according to European Committee on Antimicrobial Susceptibility Testing (2012).
[a]Resistant.

Wallis test. Correlation of bacterial species, capture location and age were examined with Spearman ρ test.

Results

Most of the reptiles were captured from coastal dune heathland (31 of 56 reptiles, 55%), whereas slow worms and grass snakes frequently were captured also from coastal meadows (Table 1). Body size of European adders was between 20 and 66.5 cm and of slow worms between 18.7 and 33 cm. Measuring of the body size was not performed in grass snakes. Most of the reptiles were adult and female (Table 1). Among the examined reptiles none showed overt signs of disease. Fourteen bacterial species representing six bacterial families were obtained from the cloaca of the 56 examined free-living reptiles (Table 3). Up to five bacterial species were isolated from one individual. A mean of 2.5 ± 1.23 species were identified per reptile specimen. Slow worms harboured significantly ($P < 0.014$) more bacterial species (mean = 3.05 ± 0.8 bacterial species) than European adders (mean = 1.96 ± 1.33 bacterial species) and grass snakes (mean = 2.5 ± 1.57 bacterial species). The prevalence of the isolated species was not found to be related to age, gender or capture habitat. Molecular virological examination for ferla-, adeno- and ranaviruses as well as virus isolation in VH-2 was negative in all tested reptiles.

Enterobacteriaceae

Nine species of Enterobacteriaceae were isolated. All the examined reptile species regularly carried bacteria belonging to this family, although with dissimilar prevalence. The most common isolates among Enterobacteriaceae were *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Pantoea agglomerans*, which were present in all examined reptile species. *C. freundii* and *K. pneumonia* co-occurred significantly ($P = 0.037$) more often. *Hafnia alvei* was present in all sampled reptiles too, but at a lower prevalence. *Escherichia coli* and *Proteus vulgaris* were isolated from all reptile species except slow worms; *Enterobacter aerogenes* was isolated from one slow worm only. *P. vulgaris* were detected significantly ($P = 0.048$, $r = 0.265$) more often in grass snakes, while *E. cloacae* were detected significantly ($P = 0.017$, $r = 0.297$) more often in slow worms. *Salmonella* spp. were isolated from European adders only, being present in 34.8% (8/23) of the sampled population. All isolates belonged to *Salmonella enterica* subsp. *diarizonae* (IIIb) revealing two serotypes with the seroformula 14:l,v:z from two snakes and 50:z10:z from the remaining six snakes. Both serotypes were isolated from

Table 3. Number of isolated bacteria species from the cloaca of free-living slow worms (*Anguis fragilis*), grass snakes (*Natrix natrix*) and European adders (*Vipera berus*)

Bacteria species	Slow worms (*Anguis fragilis*) (n = 21)	Grass snakes (*Natrix natrix*) (n = 12)	European adder (*Vipera berus*) (n = 23)
Aeromonadaceae			
A. hydrophila	0	0	1 (4.3%)
Bacillaceae			
Bacillus cereus	5 (23.8%)	1 (8.3%)	5 (21.7%)
Enterobacteriaceae			
C. freundii	7 (33.3%)	2 (16.7%)	2 (8.7%)
E. cloacae	11 (52.4%)	3 (25.0%)	3 (13.0%)
E. coli	0	1 (8.3%)	1 (4.3%)
E. aerogenes	1 (4.8%)	0	0
H. alvei	1 (4.8%)	2 (16.7%)	1 (4.3%)
K. pneumoniae	13 (61.9%)	7 (58.3%)	7 (30.4%)
P. agglomerans	4 (19.0%)	1 (8.3%)	7 (30.4%)
P. vulgaris	0	9 (75.0%)	6 (26.1%)
Salmonella spp.	0	0	8 (34.8%)
Pseudomonadaceae			
P. oryzihabitans	0	0	1 (4.3%)
Staphylococcaceae *Stapylococcus* sp.	14 (66.7%)	1 (8.3%)	3 (13.0%)
Streptococcaceae *Streptococcus* sp.	5 (23.8%)	2 (16.7%)	3 (13.0%)

snakes captured at coastal dune heathland (n = 5) and dry meadow (n = 3). The MIC was determined from seven of these eight isolates (Table 2). Both *Salmonella* sp. IIIb 14:l,v:z isolates were resistant to streptomycin. The *Salmonella* positive snakes were captured at different locations.

Pseudomonadaceae and Aeromonadaceae

Pseudomonas oryzihabitans and *Aeromonas hydrophila* were present in one European adder each.

Bacillaceae, Staphylococcaceae and Streptococcaceae

All examined reptile species carried *Bacillus cereus*, *Staphylococcus* spp. and *Streptococcus* spp.

Discussion

The island of Hiddensee is an island in the Baltic Sea with one of the highest population of European adders, so that a human contact with these snakes is common (Ortlieb et al. 2012). As reptiles can harbour potential zoonotic bacterial pathogens (Pees et al. 2013), it was consequent to investigate these free-living reptiles, to get knowledge about the cloacal aerobic bacterial flora. Several studies have isolated Enterobacteriaceae, Aeromonadaceae, Pseudomonadaceae, Streptococcoceae, Staphylococcaceae and Bacillaceae from reptilian gut contents or cloacal swabs (Roggendorf and Müller 1976; Cooper and Jackson 1981). *Salmonella*, *C. freundii*, *K. pneumoniae* and *A. hydrophila* belong to the opportunistic pathogenic bacterial species in reptiles (Cooper 2000; Rosenthal and Mader 2006; Schmidt et al. 2013). Due to the absence of clinical symptoms, these species are still likely to originate from the physiological commensal population (especially in the gut). No correlation could be shown between the habitat of capturing and the isolated bacteria, so that an environmental influence is unlikely as cause of the differences of the cloacal flora in the examined free-living squamate reptile species. Furthermore, age and gender have no effect of the cloacal flora. To the authors' best knowledge, the present survey provides the first description of the bacterial cloacal flora of slow worms. The findings revealed the same bacterial species as in the snake species examined, with the exception of *P. vulgaris*, *E. coli*, *E. aerogenes* as well as a higher prevalence of *C. freundii* and *E. cloacae* in slow worms. *Salmonella* spp., *P. oryzihabitans* and *A. hydrophila* were present in the cloaca of European adder only. Moisture of the habitat

influences the bacterial flora of soil (Pohlon et al. 2013). This may be reflected in the significant differences of the total number of isolated bacteria from the cloaca of the different species, as both species living in a damper environment harbour a larger number of different bacteria species than the European adder, which has a wider spectrum of habitats. On the other hand, more hydrophilic microbes like *P. oryzihabitans* and *A. hydrophila* were isolated from European adders only. It is unlikely that dietary differences cause the divergences of the bacterial flora in those three squamate species, as all the isolated bacterial species are common in soil and/or water as well as in the gut or skin of the prey (Ducklow et al. 1979; Hird et al. 1983; Stenkat et al. 2013).

Both of the *Salmonella* serotypes isolated in the present survey have been isolated in a previous survey from European adders 1 year before (Krautwald-Junghanns et al. 2013). The prevalence of *Salmonella* (34.8%) was much higher in the present study than 1 year before, in which only one *Salmonella* was isolated from 22 examined snakes captured on the island Hiddensee. The reason for differences of the prevalence between two consecutive years is not clear, as age, capture habitat, time of sampling, animal catcher as well as sampling and cultivation methods were identical. However, it is in agreement with a previous study regarding the prevalence of *Salmonella* in European adders in Germany conducted 34 years ago (Wuthe et al. 1979). Surprisingly, no *Salmonella* were isolated from grass snakes and slow worms. The high population density of European adders on the island of Hiddensee could be effected by individual stress in those species, resulting in a significant shedding of *Salmonella* compared to the other two examined free-living squamate species. In other surveys *Salmonella,* belonging to different serotypes, were isolated with prevalence up to 73.3% from inner organs of captured free-living species of the Colubridae family (Kuroki et al. 2013). It is often stated that the prevalence of *Salmonella* in free-living snakes is typically lower. For example, among different wild reptiles admitted to the Wildlife Center of Virginia, including two snake species, no cloacal shedding of *Salmonella* was detected (Richards et al. 2004). The lower isolation success of *Salmonella* in living snakes may be due to intermittent excretion of *Salmonella* in clinically inapparent carriers. In a study carried out on green iguanas (*Iguana iguana*), 100% of the animals were found to be shedding *Salmonella* at least once during a 10-week evaluation period, but the likelihood of detecting *Salmonella* in a single faecal sample was only 76%. Therefore, the authors recommended culture of at least three faecal samples collected 1 week apart for reliable detection of *Salmonella* in these animals (Burnham et al. 1998). Since snakes feed and defecate with much lower frequencies than other reptiles, it is difficult to sample fresh faeces under field conditions. In conclusion, it can be assumed that the prevalence of *Salmonella* among free-living grass snakes and also in slow worms might be higher than our detection rate suggests. A high percentage of clinically asymptomatic reptiles carry *Salmonella* in their intestinal tracts, since they are natural reservoirs for this infectious agent (Schröter et al. 2004; Pasmans et al. 2005). Although food products like poultry, meat, eggs, dairy products or sprouts are most commonly identified as alimentary sources responsible for outbreaks of human salmonellosis, it has recently been proved that reptiles causing the Reptile-Exotic-Pet-Associated-Salmonellosis in humans (Pees et al. 2013). Transmission can occur either by direct contact with the reptile or indirectly via the environment, since *Salmonella* are extremely undemanding, and once excreted with faeces the bacterium remains viable in a wide variety of substrates like soil, water or vegetable matter (Waterman et al. 1990; Mitchell and Shane 2000). The antibiotic resistance pattern revealed that both *Salmonella* sp. IIIb 14:l,v:z isolates were resistant to streptomycin. This may caused by aminoglycoside-modifying genes in these isolates (Miró et al. 2013).

The fact, that a large percentage of the isolated bacteria, esp. *E. coli*, *K. pneumoniae*, *P. vulgaris*, *A. hydrophila* and *B. cereus*, are known as potential foodborne pathogens for humans, is of particular interest (Cooper 2000; Pund and Theegarten 2008).

No viruses were detected in any of the free-living European adders, grass snakes or slow worms from the island Hiddensee tested. However, the small sample size does not allow final conclusions on the presence of those viral pathogens in the free-living population of reptiles of the island of Hiddensee. To the best knowledge of the authors, ferla-, adeno- or ranavirus have so far not been described in European adders, grass snakes or slow worms. A combined pharyngeal and cloacal swab proved successful to detect shedding of ferlavirus from captive and free-living squamata (Marschang et al. 2002; Papp et al. 2010). In captive boid snakes prevalence of ferlavirus was determined as 9% including snakes with remarkable clinical findings and 3.5–7.5% in clinically healthy boid snakes (Pees et al. 2010). Taking these prevalences into account, detection of ferlavirus-RNA should therefore be expected in at least one of the tested squamate reptile with 95% confidence,

assuming the test will detect 100% of infections, and that 100% of infected reptiles will be shedding the virus through the pharynx/cloaca at the time of testing in the population in case that ferlavirus is present. However, lack of ferlavirus detection in the present study could be caused by sampling locations. Ferlavirus can be amplified mainly in the lung tissue (Papp et al. 2010), and it has been assumed that a tracheal lavage improves the detection rate of ferlavirus in snakes (Pees et al. 2010). However, given to the small body size of the examined reptiles, tracheal lavage was not possible in the field, so that this could have influenced the results of the virological examinations. Cloacal shedding of adenovirus has been described in bearded dragons (*Pogona vitticeps*) (Stöhr et al. 2013; Doneley et al. 2014). Few studies exist in free-living squamata, which demonstrate antibodies against ferla- and adenovirus without shedding of the virus (Gravendyck et al. 1998; Marschang et al. 2003). Phylogenetic analyses of ferla- as well as AdV support the classification of both viruses into distinct genogroups each, with potential serodiagnostic implications (Allender et al. 2008; Marschang et al. 2009; Papp et al. 2009). As neither ferla- nor AdV have been described in the examined species to date, a serological examination was not performed. Ranavirus infection has recently been documented associated with skin lesions in different lizard species (Stöhr et al. 2013). Skin lesions were not detectable in the present free-living reptiles. In tortoises, ranavirus causes necrotizing stomatitis, so that oral swabs are recommended for detection of shedding of this virus (Johnson et al. 2008; Allender et al. 2011).

Conclusion

The present survey revealed that common viral pathogens of captive snakes and lizards were not detected in common free-living snakes and slow worms from the island Hiddensee. The prevalence of bacteria in healthy free-living snakes and lizards included in this survey is not influenced by age, gender or habitat. The presence of *A. hydrophila, B. cereus, C. freundii, E. cloacae, E. coli, E. aerogenes, H. alvei, K. pneumonia, P. agglomerans, P. vulgaris, Salmonella* sp., *P. oryzihabitans, Stapylococcus* sp. and *Streptococcus* sp. in apparently healthy individuals indicates that free-living snakes and lizards harbour potentially human pathogenic microorganisms. Accordingly, it can be concluded that Gram-negative as well as Gram-positive bacteria are common in healthy reptiles and can be part of the physiological cloacal bacterial flora of these free-living reptiles. Therefore, free-living snakes and lizards of the examined species seem to harbour potentially pathogenic bacteria in low numbers without immediate clinical consequences. This should be considered by persons who come in close contact with free-living snakes and lizards, like landscape ecologists or tourists, esp. children.

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