

***Salmonella enterica* subspecies *enterica* serovar Paratyphi B as a disease-causing agent in reptiles in the Czech Republic**

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ABSTRACT: The aim of this study was to describe two case studies of reptile disease, in which the primary pathogen was apparently *Salmonella enterica* subspecies *enterica* serovar Paratyphi B. Pathological examinations, sampling from different organs and cloacal swabs was performed on a dead savannah monitor (*Varanus exanthematicus*) and a sick green tree python (*Chondropython viridis*). This material was subjected to culture examination, including selective enrichment using standard methods. Typing was performed using MALDI-TOF and strains were also serologically typed. The utilisation of D-tartrate was confirmed biochemically and also using PCR. Antibiotic susceptibility was determined by the standard disc diffusion method using Mueller-Hinton agar without blood and antibiotic discs. In both cases the detected *Salmonella enterica* subspecies *enterica* serovar Paratyphi B was positive for D-tartrate and exquisitely susceptible to chloramphenicol, tetracycline, ampicillin, amoxicillin/clavulanic acid, gentamicin, ceftazidime, enrofloxacin and piperacillin.

Keywords: paratyphoid; *Varanus exanthematicus*; *Chondropython viridis*; D-tartrate; treatment

Typhoid fever remains a serious health problem in human populations in many countries and is responsible for 16 million cases of the illness and 600 thousand deaths annually. The causes of this disease are *Salmonella* Typhi and to a lower extent (rate) A, B and C serovars of *Salmonella* Paratyphi. Mortality among people treated with antibiotics differs between developed and developing countries (1% vs 10–30%; Chart et al. 2007). In the Kauffmann-White classification, *Salmonella* Paratyphi B is characterised by somatic antigens O: 1,4,5 and 12 and the flagellar antigen H: b. Already in 1941, Kauffmann isolated strains that had the ability to utilise D-tartrate from human patients in

Java using D-tartrate dehydrase. Therefore, these strains were designated as *Salmonella* Paratyphi B varietas Java. Both variants (D-tartrate⁺ and D-tartrate⁻) also have distinct epidemiological properties (Kauffmann 1941).

The variant which does not utilise D-tartrate and which forms a thin cell wall (slim wall), causes so-called extra-intestinal infection and invasion of host tissue accompanied by fever, septicaemia and in some cases, meningitis in infants and immunocompromised patients. The D-tartrate⁺ (*Salmonella* Java) is less virulent, and tends to cause gastrointestinal disease accompanied by watery diarrhoea and abdominal pain, usually with the absence of py-

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rexia (Chart 2003; Hernandez et al. 2012) Martinez-Urtaza et al. isolated both variants of *Salmonella* Paratyphi B (D-tartrate⁺ and D-tartrate⁻) not only from human patients but also from marine molluscs. Two of the isolated strains exhibited resistance to 16 antibiotic substances included in laboratory panel tests. The same authors stated that, in this connection, the marine environment and the consumption of raw molluscan shellfish should be considered dangerous for human health (Martinez-Urtaza et al. 2006).

Toboldt et al. (2012) described the findings regarding the D-tartrate⁺ strain variant of *Salmonella* Paratyphi B (varietas Java) isolated in Germany between 2002 and 2010 from human patients, poultry and reptiles. According to the same authors, this variant is the cause of gastroenteritis worldwide and since the 1990s it has been increasingly isolated especially from poultry in Germany, the Netherlands and Belgium. Other sources of this variant are tropical fish and turtles but also food and raw materials such as goat cheese and alfalfa sprouts. The same serotype and variant have also been described in equids in India (Singh et al. 2009). Other authors report the occurrence of this serotype in cattle or in calves (Evans et al. 2005). Furthermore, Hernandez et al. (2012) linked the occurrence of *S. Paratyphi B* varietas Java with aquarium fish and reptiles, especially turtles and identified farmers and their children as being at risk of infection.

Bzdil et al. (2012) examined a total of 31 624 samples of veterinary origin from commercial and hobby farms in the Czech Republic in the years 2006–2010. *Salmonella* strains were detected in 1180 cases (prevalence of 3.73%). *S. Paratyphi B* was not detected. Multidrug resistance to antibiotics in 85 strains of *S. Paratyphi B* (varietas Java) isolated in Germany in 1995–2001 was described by Miko et al. (2002). In all cases, the authors reported trimethoprim, spectinomycin and streptomycin resistance and also in 71% of cases to sulfamethoxazole, in 62% to nalidixic acid, in 51% to ampicillin, in 7% to tetracycline, in 3% to kanamycin-neomycin and in 3% to gentamicin. The differential diagnostics of disease in reptiles should include clinical signs of metabolic and nutritional deficiencies, bacterial and viral diseases and endoparasitic infestations leading to impaired nutritional status, anorexia, apathy, diarrhoea, and vomiting. The most serious condition which should be considered is inclusion body dis-

ease (caused by arenavirus), which is usually fatal (Stenglein et al. 2012).

This paper describes two case studies of reptiles in which *Salmonella enterica* ssp. *enterica* serotype Paratyphi B was detected.

MATERIAL AND METHODS

The dead savannah monitor (*Varanus exanthematicus*) was received for examination in January 2016 and cloacal swabs from a green tree python (*Chondropython viridis*) were taken in March 2016. At the necropsy of the savannah monitor, samples for microbial examination were taken from the heart, lung, liver and cranial section of the intestine. Cultivation was carried out aseptically from deep tissue. Each organ culture was inoculated on blood agar (MPBA), Endo agar (EA) xylose lysine deoxycholate agar (XLD) and Rambach agar (RA; all Trios s.r.o, Praha, Czech Republic) using calibrated loops (volume 10 µl; RC Biologix, St Louis, USA).

Non-selective cultivation in buffered peptone water (BPW) in a ratio of 1 g tissue to 9 ml medium was also carried out. Incubation was carried out in a thermostat at 37 ± 1 °C for 18 ± 2 h. Selective cultivation of material was carried out in the following manner: 0.1 ml of incubated BPW were inoculated on two dishes (diameter 90 mm) with semisolid Rappaport-Vassilliadis agar (MSRV; Oxoid CZ a.s., Brno, Czech Republic), and incubation was carried out at 41.5 ± 1 °C for 24 ± 3 h. Inoculation was performed on one plate of XLD and one plate of RA (both Trios s.r.o, Prague, Czech Republic) by removing colonies from the edge of the growth zone using a loop (ca. 10 µl; R.C. Biologix, St Louis, USA). The isolated strains were identified using molecular MALDI-TOF (Bruker Daltoniks GmbH, Bremen, Germany) and *Salmonella* spp. were further serologically tested using serum O: 1,4,5,12; H: b; H: 1,2 (SIFIN GmbH, Berlin, Germany and Denka-Seiken Co., Ltd., Tokyo, Japan). Susceptibility was determined by the standard disc diffusion method using Mueller-Hinton agar (MHA; Trios s.r.o, Praha, Czech Republic) and antibiotic discs containing chloramphenicol (30 µg), tetracycline (30 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (20 + 10 µg), gentamicin (10 µg), ceftazidime (30 µg), enrofloxacin (5 µg) and piperacillin (100 µg; Oxoid CZ a.s., Brno, Czech Republic). Values were interpreted according to CLSI standards. All used discs and

media were tested with the *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) reference strains (Bzdil 2003).

A swab sample of Transbak with Amies agar and charcoal (Dispolab s.r.o, Brno, Czech Republic) from the green python (*Chondropython viridis*) was also primocultivated on MPBA, EA, XLD and RA and incubated in the thermostat at 37 ± 1 °C for 24 ± 3 h. After primocultivation, sterile swabs were wrapped in 10 ml BPW and cultivated in the same way as pathological material with selective cultivation in MSRV and inoculation on MPBA, EA, XLD and RA. Suspicious strains were isolated on MPBA, EA, XLD, RA or on nutrient agar (Trios s.r.o, Prague, Czech Republic) and the plates were incubated again at 37 ± 1 °C for 24 ± 3 h. Isolated *Salmonella* spp. strains were typed and antibiotics susceptibility was determined. Investigated material was prepared by standard methods in accordance with current legislation and using standard laboratory equipment as described previously.

Basic parasitological examinations of the dead savanna monitor were performed visually by inspection of the body surface and the digestive tract and other internal organs during autopsy. Subsequently, coprological examination by flotation technique of the content of the final section of the intestine was performed using the Breza solution.

RESULTS

Savannah monitor (*Varanus exanthematicus*) case report

In January 2016, the dead savanna monitor, weighing 2.6 kg with a history of sudden death was sent for necropsy as well as parasitological and bacteriological examinations to our laboratory. Its owner was very experienced and kept a further 20 reptiles of different species (lizards and snakes). Each reptile was provided with its own individual terrarium. The animal had been purchased five years previously from a Czech breeder. It was not possible to determine the number of generations that the species had been grown in captivity. Climatic conditions for breeding were *lege artis*. The young savanna monitor was fed with locusts and cockroaches from the owner's own stock and later with mice and young rats. Mice were bred by the owner; the rats came partially from breeding

stores. The breeder has a health certificate and the origin of the animals is verified.

Vomiting and diarrhoea, lethargy, loss of appetite and exhaustion were observed in the savannah monitor three days before death. Subsequently, the animal died. The terrarium equipment was burned, non-combustible parts were disinfected using sodium hypochlorite (Bochemie a.s., Bohumin, Czech Republic) at a concentration of 10% and after 30 min of exposure, thoroughly washed with water. No intestinal or febrile illness that could suggest paratyphoid or other salmonella infections, were observed in other reptiles, the breeder or his family members. Preventive microbiological examinations (anal swabs) of the owner and his family members were not performed.

The nutritional status of the dead animal was very good. No changes were reported on the surface of the body or on the skin. Necropsy showed a clear, yellowish exudate-filled body cavity with an admixture of fibrin fibres. Fibrin plates also covered all the body cavity organs including the gastrointestinal tract. The myocardium was flabby and a blood clot was detected in the heart chambers. The pericardial cavity was filled with clear yellowish exudate and a mass of fibrin which covered the epicardium (*cor villosum*) completely. The lungs were filled with air and drenched with oedematous reddish foamy liquid (pulmonary oedema). The peritoneum was noticeably roughened and covered focally by pseudomembranes of fibrin. The liver was slightly enlarged with strongly rounded edges, soft, elastic consistency and apparent light brown coloured liver lobes (fat dystrophy).

The gall bladder was filled with bile; the lining of bladder was without any pathological changes. The spleen was enlarged, congested and swollen with fluid (oedematous). The gastric lumen was empty of food and was filled only with a clear mucous foamy liquid. The mucosa in the fundal area was swollen and reddened. The small intestine had a noticeably thickened wall with markedly swollen mucosa. The lumen of the cranial intestinal tract was without any content.

The large intestine and cloaca were filled with a greenish-brown coloured homogeneous paste. Macroscopic inflammation of the colon mucosa was not observed. Other organs and organ systems were without pathological changes. Parasitological examinations did not reveal the presence of ectoparasites or endoparasites.

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Bacteriological culture examination of the heart, lung and liver showed the presence of *Salmonella enterica* ssp. *enterica* serovar Paratyphi B (O: 1,4,5,12; H: b; H: 1,2). The strain was sent to the National Reference Laboratory for Salmonella (State Veterinary Institute Prague) where the diagnosis was confirmed using PCR and biochemical methods (D-tartrate utilisation) and determined to be the Java type. From the cranial section of the intestine only *Escherichia coli* was isolated by primocultivation. *Salmonella enterica* ssp. *enterica* serovar Paratyphi B was detected using cultivation technique also from the intestine. The isolated strain of *Salmonella* Paratyphi B was susceptible to chloramphenicol, tetracycline, ampicillin, amoxicillin/clavulanic acid, gentamicin, ceftazidime, enrofloxacin and piperacillin. Resistance was only demonstrated to Clindamycin.

Green tree python (*Chondropython viridis*) case report

Almost two months later (March 2016), a cloacal swab from a green tree python (*Chondropython viridis*) was delivered to our laboratory. The medical history included mild apathy, disinterest in food, atypical position of the body and a slight increase in the volume of the body cavity. The animal comes from Czech breeding and at least the last two generations of his ancestors were bred in captivity. Feeding (laboratory rats) was provided by the owner's own breeding stock. Contact with other reptiles was not permitted. In this case, upon the request of a private veterinarian, we carried out only routine bacteriological examination in aerobic conditions and tested for salmonella. Primocultivation from a cloacal smear demonstrated the presence of *Klebsiella oxytoca* and *Morganella morganii* ssp. *morganii*. Using cultivation and inoculation on solid media, *Salmonella enterica* ssp. *enterica* serovar Paratyphi B (O: 1,4,5,12; H: b; H: 1,2) was detected again. The strain of salmonella was again typed using biochemical methods and PCR. We again detected a D-tartrate⁺ strain (varietas Java).

Susceptibility was confirmed, as in the previous case, to chloramphenicol, tetracycline, ampicillin, amoxicillin/clavulanic acid, gentamicin, ceftazidime and piperacillin. *Klebsiella oxytoca* and *Morganella morganii* ssp. *morganii* exhibited the same susceptibility, except for ampicillin. The

tested salmonella strain was confirmed to be resistant only to clindamycin. Based on the observed pattern of susceptibility, therapy was initiated with intramuscular administration of Noroclav a.u.v. inj. (amoxicillin/clavulanic acid; Norbrook Laboratories Limited, Newry, Northern Ireland) at 1 ml/20 kg body weight. The preparation was administered daily for five days; the temperature in the cage was lowered to 21 °C during treatment. During the therapy, the symptoms disappeared. It could not be determined whether other actions, such as environmental sanitation and the disinfection of facilities, were carried out. Three weeks after therapy, a control microbiological examination of a cloacal swab was performed, with negative results.

DISCUSSION

This is the first diagnosis of *S. enterica* ssp. *enterica* serovar Paratyphi B isolated from veterinary material in animals in the Czech Republic (Bzdil et al. 2012). In other European countries and elsewhere in the world these detections are more common (Evans et al. 2005; Martinez-Urtaza et al. 2006; Singh et al. 2009; Hernandez et al. 2012; Toboldt et al. 2012). It remains unclear how the animals became infected, as they were kept in captivity and were not fed chickens or other animals or materials in which this salmonella serotype can occur. Therefore, it is not possible to exclude vertical transmission from parents to offspring. *S. Paratyphi B* has zoonotic potential and can cause reptile-associated salmonellosis in humans. The frequency of these diseases increases with the number of reptiles kept in households as pets. Children are most at risk due to close contacts with animals and inferior hygienic habits. Infected reptiles are often asymptomatic. It should be noted that *Salmonella* spp. shedding can be intermittent and may increase if the animal is stressed.

The initial symptoms of disease in the savannah monitor were gastrointestinal in nature. Afterwards, the autopsy showed a general sepsis, which is more commonly observed in D-tartrate⁻ variants of *Salmonella* Paratyphi B (Chart 2003; Hernandez et al. 2012). It is surprising that the disease never occurred in the breeder or his family members, which can be explained by rigorous personal hygiene and the fact that *Salmonella* Paratyphi B vari-

etas Java is less virulent than the D-tartrate⁻ variant (Chart 2003; Hernandez et al. 2012). The python had non-typical symptoms, which pointed to septic disease affecting the central nervous system; this would explain the unusual body posture. There are more frequently described these forms compared to the D-tartrate⁻ variant in the literature. The D-tartrate⁺ variant of *Salmonella* Paratyphi B was also detected in the python. In previous reports, D-tartrate-negative variants have tended to be the causative agents in reptiles (Hernandez et al. 2012; Toboldt et al. 2012).

Our isolated strains were also exquisitely susceptible to antibiotics compared with data from the literature (Miko et al. 2002; Martinez-Urtaza et al. 2006). For the python, it can be concluded that the symptoms were likely to have been caused by a bacterial infection. It is probable that the other microorganisms which were isolated, i.e., *Klebsiella oxytoca* and *Morganella morganii*, might have also participated in the disease. A similar scenario would also be observed in viral infections, such as inclusion body disease, which is accompanied by similar symptoms that are usually fatal (Stenglein et al. 2012). Amoxicillin/clavulanic acid treatment would certainly be ineffective for other diseases caused by, for example, *Mycoplasma* spp., *Chlamydia* spp., metabolic diseases or diseases resulting from nutrient deficiencies.

In conclusion, this paper describes two case reports of reptile disease, where the microbial agent was D-tartrate⁺ *Salmonella enterica* ssp. *enterica* serovar Paratyphi B. Symptoms of the disease were observed in the green tree python (*Chondropython viridis*), while specific pathological lesions were seen in the savannah monitor (*Varanus exanthematicus*). Our results suggest possible parallels and similarities with symptomatic differential diagnosis of similar diseases. We have also described therapeutic procedures leading to successful disease control. It should be emphasised that the treatment of salmonellosis in reptiles remains controversial and is accompanied by frequent relapses. Therefore, it should be applied only in pet animals and should be based on antibiotic susceptibility established in the laboratory. It should also be performed only on animals with clinical signs and the mode of drug application should be chosen according to the prevailing symptoms. If the gastrointestinal symptoms prevail, the application can be performed *per os*. However, in cases of fever

parenteral therapy is preferred. In order to assess the efficacy of the therapy, it is useful to examine control samples after treatment.

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