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Salmonella Associated with Snakes (Suborder Serpentes)

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1. Introduction

In modern society, snakes (suborder Serpentes) are valued for their use in scientific research. Although being perceived with fear and repugnance by many people, snakes play an essential role in nature and influence many aspects of human life and culture. For centuries, snakes have developed significant scientific, ecologic, economic, cultural, and religious importance (Table 1).

Scientific	Laboratory animals for biomedical research
	Research on venoms for the development of new therapeutic drugs
	Venom collection for antiserum production
Ecologic	Control of rodents and other vertebrates
	Prey items for several carnivore species (mammals, birds and reptiles)
	Important components of the food web
	Ambassadors for environmental education programs and zoological parks
Economic	Exotic pets
	Skin and meat production
Cultural	Popular symbolism, including as the symbol of the medical sciences
	Mythological symbolism
	Subjects in public demonstrations and entertainment
Religious	As a symbol and representation of gods and other religious elements
	Use in various modern religious cults

Table 1. Importance of snakes

Direct contact is necessary between humans and snakes for many of the activities described above; however during such handling, one is susceptible to contamination by any microbe or pathogen that may be on the animal surface or in its secretions. Such contamination may occur not only by direct contact, but also indirectly through objects, food and inanimate surfaces that had previously been in contact with reptiles.

Among the several microorganisms inhabiting the body of snakes, Gram-negative bacteria are considered one of the most significant groups. Although they may be part of the saprophytic microbiota, oftentimes they can behave as pathogens and lead to well-defined diseases or act as opportunistic invaders of their animal or human hosts. There are many

Gram-negative bacteria of medical significance, and *Salmonella* is a particularly important genus due to its implication for public health.

2. Etiology

The current classification of *Salmonella* is complex. The genus is divided into two major species: *S. enterica* and *S. bongori* (subspecies V). *S. enterica* is further divided into six distinct subspecies based on biochemical differences: *enterica* (subspecies I), *salamae* (subspecies II), *arizonae* (subspecies IIIa), *diarizonae* (subspecies IIIb), *houtenae* (subspecies IV), and *indica* (subspecies VI). *Salmonella* strains may be further classified by serology into over 2500 serotypes (serovars) using the Kauffman and White classification scheme. This scheme defines the serogroup according to the expression of somatic lipopolysaccharide O antigens (LPS), and the serotype based on the expression of flagellar H antigens (Barrow et al., 2010; Grimont & Weill, 2007; Mitchell & Shane, 2001). Besides serving for classification purposes, the variations in LPS structure provide each serotype a different degree of virulence (Paré et al., 2006). Serotypes may be referred to by a name or an antigenic formula. In this chapter, strains will be referred to by the genus followed by the serotype or the number of the subgroup: *Salmonella enterica enterica* serotype Typhimurium will be referred to as "*S.* Typhimurium", *Salmonella enterica diarizonae* 60:r:z_{15} as "*S.* 60:r:z_{15}", *Salmonella enterica diarizonae* (IIIb) as "*S.* IIIb", and so forth.

Distinct patterns of infection (pathovars) may also be used to classify serotypes. There are three pathovars: the first includes a few serotypes that produce severe systemic disease in healthy animals; the second pathovar comprises serotypes that affect young animals and pregnant or egg-laying females; the third pathovar includes serotypes that cause infection in immunocompromised animals (Barrow et al. 2010).

The classification of strains based on their host range (host-specific, host-restricted, ubiquitous) is controversial (Barrow et al., 2010). Clinical signs involving the digestive tract are the predominant manifestations of the infections by the generalist serotypes. These infections are characterized by high morbidity but low mortality. In contrast, infections caused by serotypes adapted to specific animal species or a restrict group of hosts will tend to present low morbidity and high mortality (Hoelzer et al., 2011).

In reptiles, *Salmonella* inhabits the distal small intestine and the colon (Carvalho, 2006); however its presence seldom produces primary disease, as the bacteria seem to be part of the indigenous gut flora of these animals (Mitchell, 2004).

The first reports of *Salmonella* isolation from reptiles date back to cases involving lizards in 1939. In 1944, the bacterium was first isolated from a Bull Snake (*Pituophis catenifer*) and in 1946 from a turtle. However it was not until 1953, when a human was shown to have probably acquired salmonellosis from a chelonian, that these isolations were considered relevant (Mathewson, 1979). Hinshaw & McNeil (1944, 1945, 1946, 1947) were two researchers that greatly contributed on the first *Salmonella* isolations from reptiles.

There is a very broad diversity of serotypes amongst reptiles (Pedersen et al., 2009). From all known serotypes, approximately 40% are predominantly associated with these animals (Hoelzer et al., 2011). Even though there are no reptile-specific serotypes, subspecies III is known to be the most common in snakes (Warwick et al., 2001). Other studies have also demonstrated that *Salmonella enterica diarizonae* (IIIb) corresponds to 92.8% of the serotypes

isolated from *Bothrops jararaca* (Bastos et al., 2008) and to 100% of the isolates from *Bitis nasicornis* and *Bothriechis schlegelii* (Schröter et al., 2004), and that the subspecies *S.* IIIa and *S.* IIIb are also generally predominant in various other snake species (Sá & Solari, 2001).

In the past it was generally believed that subspecies III was a part of the reptile normal gastrointestinal microbiota (Orós et al., 1996). While serotypes of all *Salmonella* subspecies have been isolated from snakes (Bemis et al., 2007), *S.* IIIa seems to be the most pathogenic for these animals as it is most frequently isolated in cases with clinical disease (Lamberski et al., 2002; Ramsay et al., 2002). It must be taken into account, however, that due to recent changes in the nomenclature and structure of antigenic formulas used to classify *Salmonella* strains it is often difficult to determine to what extent the previous reports involved the subspecies IIIa and IIIb (Bemis et al., 2007). In Brazil, the subgroup III (formerly classified as genus *Arizona*) was first isolated in snakes in 1973, by Moreno and colleagues.

The retrieval of *Salmonella* strains from a reptile does not *per se* elucidate its role as a disease or potential zoonosis, as clinically healthy snakes may host highly virulent serotypes (Johnson-Delaney, 2006). Furthermore, the development of disease depends on a variety of factors such as animal species, individual susceptibility, stress, and inoculated dose (Carvalho, 2006).

3. Epidemiology

3.1 Prevalence

One of the first estimates of *Salmonella* prevalence among snakes was performed by Chiodini & Sundberg in 1981, who described rates of infection ranging from 16 to 92%. Another study conducted by Chiodini (1982), found that 55 out of 56 snakes (98%) were naturally infected with *Salmonella*. Several other studies determined the prevalence of these bacteria in snakes throughout the world: 92.5% and 69.2% in Australia (Iveson et al., 1969; Scheelings et al., 2011), 24% in Austria (Pfleger et al., 2003), 64.7% in Brazil (Bastos et al., 2008), 71.6% in Germany and Austria (Geue & Löschner, 2002), 69.7% in Taiwan (Chen et al., 2010), and 15% in Trinidad (Gopee et al., 2000). Prevalence is generally high in snakes; however it is important to consider these numbers may vary broadly depending on the evaluation methods (Johnson-Delaney, 2006).

The most prevalent *Salmonella* strains in snake fecal samples may vary over time (Schröter et al., 2004), however such fluctuations have yet to be understood (Schröter et al., 2006), but it has been suggested that failures of the isolation methods and intermittent elimination of the bacteria in the feces may be involved (Chiodini & Sundberg, 1981).

3.2 Sources of infection

Fecal shedding is well documented in snakes (Ramsay et al., 2002) however other potential sources of infection must be considered (Warwick et al., 2001). The bacteria may be present in the oral cavity and there is a remote possibility of transmission through bites (see Treatment). The bacteria can also be eliminated via the cloaca in the eggs, feces, urine, and possibly other secretions.

Ramsay et al. (2002) reported that in a colony of rattlesnakes, some of which presented *Salmonella*-associated osteomyelitis, contaminated wood shaving substrate might have been a

source of infection when snakes were transferred among cages. The transmission of *Salmonella* through inanimate objects has been well described in a *Salmonella* outbreak in Komodo Dragons (*Varanus komodoensis*) held captive at a zoo (Friedman et al., 1998). Furthermore, considering the natural tongue-flicking behavior of snakes, the ingestion of substrate during prey consumption is probably not necessary to cause infection, as the briefest contact of the tongue with a contaminated surface could be sufficient to acquire *Salmonella*. Although the bacterium is frequently present in the environment, large outbreaks in humans are rarely caused solely due to environmental contamination (Friedman et al., 1998).

Some reptiles may inoculate *Salmonella* through bites and claw scratches. It is also possible that humans could be infected through ear or eye contact with feces, urine or water from the snakes' cages (Warwick et al., 2001). A snake may become infected when maintained in poor hygiene conditions, in cages or exhibits with accumulation of excrements (Fornazari & Teixeira, 2009).

3.3 Methods of transmission

Transmission occurs primarily through the fecal-oral route, but may also occur through the ingestion of contaminated food or water. Bacteria present in inanimate surfaces or in the animals may also be transmitted indirectly (Carvalho, 2006).

Snake prey, whether wild animals captured by free-ranging snakes or laboratory animals used to feed captive snakes, may present distinct species-specific characteristics that influence the acquisition of *Salmonella*. This bacterium has been frequently retrieved from wild rodents (Hoelzer et al., 2011) and other vertebrates upon which free-ranging snakes feed, however its prevalence tends to be generally lower in wild than in captive reptiles (Gray, 2011).

Although most snakes feed on vertebrates, some have specialized on invertebrate-based diets. Mollusks and crustaceans may also be consumed by snakes and it is believed that they may be a source of *Salmonella* to humans and other animals. Information is yet insufficient to confirm whether they could be potential sources of infection for snakes, but it has been suggested that they could maintain these bacteria in natural environments (Minette, 1984).

The indirect transmission from reptiles may occur because *Salmonella* can retain its virulence for several days or months in the environment. This has been shown to be particularly important for small children, as they may often become infected without ever touching the pet reptile (Bays, 2005; Hoelzer et al., 2011).

Mermin et al. (2004) reported that *Salmonella* can been isolated from the aquarium water of chelonians several weeks following contamination, and the bacteria can be recovered from the reptiles' feces even several months after defecation. It is likely that strains from semi-aquatic snakes may persist for several weeks in the water of their cages.

Salmonella has been used for several decades in an unusual manner: as a rodenticide. Even nowadays these products are still produced in some countries, despite the risk they are known to pose to human health (Painter et al., 2004). Such rodenticides are prepared using *Salmonella* Typhimurium and *S.* Enteritidis (Hoelzer et al., 2011). In addition to the unnecessary risk of transmission to humans, the strains used in these products may infect food, livestock and wildlife.

Another form of *Salmonella* infection is vertical transmission. Investigating chelonians, Kaufmann and colleagues (1972) reported that the first studies on this subject indicated trans-ovarian infection may occur, but with unknown frequency and likely with less relevance than post-laying contamination of the eggs. Those authors did not succeed in isolating *Salmonella* from the ovarian tissues of the turtles as they had done before (Kaufmann & Morrison, 1966) and suggested their previous findings could be due to rapid bacterial penetration through the egg shell after oviposition, resulting in infected newborn animals. In the 1960s, it was already known that *Salmonella* quickly penetrated turtle eggs, contaminating them as they passed through the cloaca or were laid in contaminated soil (Chiodini & Sundberg, 1981). In the following decades, the isolation of *Salmonella* from turtle ovarian tissues and egg contents as had been performed by Kaufmann and Morrison (1966) was not reproduced (Kaufmann et al., 1972) nor investigated in other reptiles (Chiodini & Sundberg, 1981).

Snake fetuses are not sterile, and the shedding of *Salmonella* by hatchlings does not necessarily occur even when the fetuses are already infected by that microorganism (Chiodini, 1982). *Salmonella* is only shed in the feces when the digestive tract of the fetus is infected, not in systemic or coelomic infections. Moreover, because *Salmonella* may be isolated in one fecal culture and not in another, it is possible that fetuses positive to *S. arizonae* may be delivered by a female that had negative fecal cultures during pregnancy (Chiodini, 1982).

In a study carried out in Germany, Schröter et al. (2006) observed 65% *Salmonella* prevalence in newborn snakes, suggesting that colonization may have occurred during pregnancy or upon birth. There seems to be no correlation between a specific serotype and its success in being transmitted to the host's progeny.

Discussions on the vertical transmission of a pathogen are generally focused on the female, whether it is an oviparous or viviparous species. The male is not often considered, either because it is not recognized as playing a significant role in vertical transmission or simply because it is forgotten. In certain snake species sperm transfer during copulation may be very prolonged, the cloacae remaining in intimate contact during this period, which may be sufficient for the exchange of bacteria between male and female. Moreover, it has been shown that some female snakes can store viable sperm from copulation for up to 6 years, a phenomenon called *amphigonia retardata* (Mader, 2006). There is research that can help us evaluating the possibility of sexual transmission of *Salmonella* from male to female, and the potential hazard for posterior vertical transmission to the progeny (Hidalgo-Vila, 2007, as cited in Pedersen et al., 2009). For instance, Chiodini (1982) did not succeed in isolating *Salmonella* from snake testes, whilst Ramsay et al. (2002) successfully isolated *S. arizonae* from the testes of a *Crotalus willardi*, and noted that active spermatogenesis occurred despite the presence of granulomatous infiltrates.

Behavioral characteristics may interfere with the acquisition and elimination of *Salmonella* by a snake. The high prevalence of *Salmonella* in terrestrial (ground-dwelling) snakes when compared to arboreal snakes may be explained by behavioral differences and by the higher risk for the terrestrial species to come in contact with contaminated excrements in the soil or substrate (Schröter et al., 2004). Semiaquatic snakes, which spend most of their time inside water, present a greater probability to acquire *Salmonella* by hydric transmission. This becomes particularly relevant for snakes in captivity, where the concentration of bacteria may become high depending on the maintenance routine and the characteristics of their

tank or artificial lake. There is a case report of fatal bacterial sepsis in Water Snakes (*Helicops modestus*) in which the lack of flowing water was considered a contributing factor for the contamination of the cages with *Proteus vulgaris* (Coutinho et al., 2001).

Although they often prefer to place their feces on a corner of the cage, snakes may defecate and urinate in various locations, including in watering bowls, particularly after ecdysis. Urine may be contaminated by fecal debris upon expelling through the cloaca. Some freshwater chelonians can drain water into the cloaca and release it at the nesting site to soften the soil and facilitate oviposition (Warwick et al., 2001), a behavior that may lead to the contamination of the nest and eggs.

4. Diagnostic techniques

The diagnostic method most frequently used to isolate *Salmonella* is microbiological culture (Mitchell, 2006). Isolation protocols and information on culture media are easily found in the vast scientific literature on this topic. The isolation of this bacterium generally involves: direct culture, enrichment, inoculation on culture plates, screening for suspect colonies and confirmation through biochemical and serological tests (Waltman, 2000). Most techniques and protocols used for the isolation of *Salmonella* from other sources are also used for its isolation from reptiles.

Although microbiological culture may fail to detect some asymptomatic carriers (Bradley & Angulo, 2008), it may assist investigations concerning the transmission of *Salmonella* from reptiles to humans (Gray, 2011), while biomolecular assays such as pulsed-field gel electrophoresis (PFGE) provide additional confirmatory clues.

Tetrathionate, Rappaport-Vassiliadis and selenite broths are the selective-enrichment media used to isolate *Salmonella*. To isolate this bacterium from reptiles, Mitchell (2006) recommends selenite broth. After incubation, a small aliquot of the primary enrichment broth is transferred to a selective medium. Although the direct culturing of a sample is usually unsuccessful, Bastos et al. (2008) were able to isolate multiple *Salmonella* and other enterobacteria from intestinal mucosa swabs of *Bothrops jararaca*.

Most of the broad variety of selective media used to isolate *Salmonella* from other animals has already been tested for reptiles, and the XLT4 agar has been shown to provide the best results (Bastos et al., 2008; Mitchell, 2006). In spite of the morphologic similarity of *Salmonella* colonies with those of other Enterobacteriaceae in MacConkey agar, Bastos et al. (2008) isolated 7 colonies on this agar (poorly selective media) when compared to the 13 colonies obtained from the same samples using the XLT4 agar (highly selective media); MacConkey agar is seldom used to specifically isolate *Salmonella*.

Selective screening media are used to confirm or rule out suspect colonies. The most commonly used are lysine iron agar (LIA), urea and triple sugar iron agar (TSI), besides a variety of commercial biochemical test kits (Mitchell, 2006).

After confirmation of the *Salmonella* genus, colonies can be submitted for serotyping. Subsequently, the isolates may be further identified using molecular methods such as ribotyping and PFGE (Ghilardi et al., 2006; Nair et al., 2002). Non-culture diagnostic methods can also be used for the detection of *Salmonella*, such as enzyme-linked immunosorbent assays (ELISA) and polymerase chain reactions (PCR). Both techniques can detect low numbers of bacteria in a sample (Mitchell, 2006); however are seldom applied for reptiles.

Rough phase strains are frequently isolated (Bastos et al., 2008; Geue & Löschner, 2002; Pedersen et al., 2009; Pfleger et al., 2003; Ramsay et al., 2002). These isolates agglutinate spontaneously during the agglutination serotyping tests (Difco, 2011), producing negative results that render these strains non-typable. Non-typable strains are also frequent in *Salmonella* isolated from reptiles (Bastos et al., 2008; Chen et al., 2010; Pedersen et al., 2009; Sá & Solari, 2001). Salb et al. (2002) were able to identify 42% of the isolates retrieved from Green Iguanas (*Iguana iguana*). Isolates from other vertebrates may also be similarly difficult to characterize.

Knowledge on the dynamics of this bacterium may be obtained through antigenic characterization and molecular methods (Nair et al., 2002; Sá & Solari, 2001). However, studies on the virulence of *Salmonella* in snakes still lack appropriate infection models (Bernis et al., 2007).

5. Clinical signs

In reptiles, *Salmonella* gastroenteritis leads to diarrhea (Funk, 1996), particularly in acute form. Other clinical signs include coelomitis, pneumonia, and septicemia. Hypovolemic shock and death may also occur. The feces may become green-grayish and contain mucus or blood. Small hemorrhagic spots may appear on the mucous membranes and ventral scales in animals with sepsis. Reptiles in this condition may also exhibit muscle weakness (Messonnier, 1996).

Inappetance and regurgitation with blood and mucus were observed in a Rosy Boa (*Lichanura trivirgata*) with gastrointestinal lesions (Orós et al., 1996). Extra-intestinal salmonellosis may also occur. *S. arizonae* has been associated with extensive bone lesions on the vertebrae and ribs of snakes, leading to limited mobility and prostration. Severely affected animals lose their righting reflexes and fail to capture prey (Ramsay et al., 2002). *Salmonella*-associated pneumonias in *Boa constrictor* may also lead to dyspnea (Onderka & Finlayson, 1985).

6. Pathological findings

Several reports dating back to the 1940s demonstrated that snakes may harbor *Salmonella*, often without any clinical manifestations (asymptomatic carriers) or with clinical signs and lesions typically related to the disease. Although this does not occur frequently, the first descriptions reported hepatic lesions and intestinal inflammation (Page, 1966). Inflammation and necrosis are often present in many organs. However, *Salmonella* can occur in virtually any visceral tissue of clinically healthy snakes (Chiodini, 1982) and its implication in the death of snakes and other reptiles is uncommon. In snakes, salmonellosis may manifest as necrotizing subacute severe enteritis, normally involving the distal half of the intestines (Onderka & Finlayson, 1985). The intestinal tract of two *Crotallus willardi* that died from enteritis presented heterophilic necrotizing and granulomatous lesions (Ramsay et al., 2002). A Rosy Boa (*Lichanura trivirgata*) with gastrointestinal clinical signs presented diffuse thickening of the gastric wall with necrotic and fibrinonecrotic lesions (Orós et al., 1996).

Besides the digestive tract, the urinary tract and the liver were found to be frequently infected in a survey conducted by Chiodini (1982). In snakes, *Salmonella* septicemia produced hepatitis associated to granulomas (Onderka & Finlayson, 1985).

Salmonella was speculated to be involved in a case of septic endocarditis of a Burmese Python (*Python molurus bivittatus*) (Murray, 1996), and was proven to have caused

granulomatous myocarditis in a Madagascar Dumerili's Boa (*Acrantophis dumerili*) (Schilliger et al., 2003).

Lesions associated with *S. arizonae* are frequently observed in the gastrointestinal tract, spleen, liver, oviduct and ureter. Lesions in the respiratory tract are less common. Orós et al. (1996) observed tracheal necrosis and inflammation caused by *S. arizonae* in a double-headed Honduran Milksnake (*Lampropeltis hondurensis*) which had been found dead without previous clinical signals. Onderka & Finlayson (1985) reported fibrinous alveolar exudation and interstitial inflammatory infiltration in the lungs of a *Boa constrictor* deceased from a pneumonia caused by *Salmonella*.

In snakes with osteomyelitis, the bone lesions presented heterophilic-granulomatous inflammation, osteonecrosis and sequestra, periosteal bone proliferations in the form of exostoses accompanied by heterophilic-granulomatous inflammation, and trabecular and cortical osteopenia; oophoritis and salpingitis also occured (Ramsay et al., 2002).

7. Treatment

7.1 Antibiotics

It is not possible to eliminate *Salmonella* from the digestive tract of reptiles. Experience has shown it is impossible to raise *Salmonella*-free reptiles (Association of Reptilian and Amphibian Veterinarians [ARAV], 2009), suggesting a participation of environmental contamination in the infection, and indicating that the animals may maintain latent infections (Mermin et al., 2004).

The administration of antimicrobial agents is not recommended for enteric salmonellosis in reptiles, as some studies have demonstrated that the course of the disease is not affected by the antimicrobial therapy. On the other hand, treatment of systemic salmonellosis should include intensive care and appropriate antimicrobial therapy (Hirsh, 2004).

Whenever possible, antibiotic choice should be based on antimicrobial sensitivity testing. When this is not possible, information from the literature may be useful: *Salmonella* strains isolated from snakes are generally sensitive to aminoglycosides, quinolones and trimethoprim-sulfamethoxazole, and are often resistant to ampicillin, cefoperazone, chloramphenicol, neomycin, streptomycin and tetracycline (Bastos et al., 2008; Chen et al., 2010; Gopee et al., 2000). Multi-resistant strains are uncommon.

Bastos et al. (2008) demonstrated that *Salmonella* isolates from Jararacas were sensible to most antimicrobials tested. *Salmonella* strains carried by free-ranging snakes are also generally sensitive to antibiotics. Indiscriminate use of antibiotics can select resistant strains, however Jijón et al. (2007) pointed out that the emergence of drug-resistant bacteria is not mandatorily associated to prior antimicrobial therapy.

Several bacterial genera, including *Salmonella*, may be isolated from the mouth of snakes that are clinically healthy or with stomatitis (Mehler & Bennett, 2006). However, prophylactic antibiotic administration is not recommended for victims of ophidism if the snake species involved has venom lacking strong cytotoxic effects, such as is the case for South American Rattlesnakes (*Crotalus durissus*) (Nishioka et al., 2000). Although the incidence of abscesses on the bite wound area is approximately 12% for accidents involving South American *Bothrops* spp. (Ribeiro & Jorge, 1990, 1997), the specific involvement of *Salmonella* is clearly not a problem (Andrade et al., 1989; Jorge et al., 1990). This also applies to large constrictor snakes

and small colubrids. Among several hundred snake-bite accidents involving numerous Brazilian species, in only one case (0.4%) there was the formation of a small, easily treatable abscess (Bastos, pers. obs.). Interestingly, snake venom contains biologically active peptides with antimicrobial activity (Lima et al., 2005; Wang et al., 2008).

It is likely that the bacterial diversity found in the snake oral microbiota reflects the variation in the fecal microbiota of the live prey they ingested, particularly as the prey often defecates while it is being killed and consumed (Goldstein et al., 1979). These researchers mentioned that live rodents were used to feed the studied captive snakes, however oftentimes the studies on differences in the microbiota of wild and captive snakes do not specify which food items were offered to the animals. Another important consideration is that food and stress may alter the snake oral microbiota depending on the lifetime spent in captivity.

7.2 Probiotics and prebiotics

The capacity of the microbial flora to prevent the multiplication of a given microorganism is called competitive exclusion (Schneitz & Mead, 2000). Probiotics act in this manner: they are live microorganisms that provide beneficial effects to their host's health that go beyond their inherent nutritional value. Studies demonstrate that only a few organisms truly act as probiotics (Myers, 2007).

Studies from the 1980s and the early 1990s demonstrated that the successful colonization of the cecum of turkeys by bacteria such as *Lactobacillus reuteri* and *Bacillus subtilis* may provide protection against the establishment of *Salmonella* in the microbiota; these findings were accompanied by general improvements in viability, body mass, and feed conversion efficiency. Research on hens, however, have generally failed to reproduce those results and did not provide protection against the colonization by *Salmonella* (Hafez & Jodas, 2000). Recent studies have indicated some organisms that might be more successful in preventing *Salmonella* colonization in the ceca of hens, and Pascual and colleagues (1999) demonstrated that *Lactobacillus salivarius* strain CTC2197 may be an effective competitor against the establishment of *S*. Enteritidis in the intestinal epithelium of hens.

The administration of probiotics on the feed of Green Iguanas (*Iguana iguana*) is anecdotally said to eliminate *Salmonella* from their gastrointestinal tract. However, such effect was not demonstrated in Carpet Pythons (*Morelia spilota*), as the probiotics did not prevent the shedding of the bacterium in the feces (Holz & Middleton, 2002). Although at the moment no tests on the use of probiotics in snakes have produced successful results, efforts to characterize their intestinal microflora have been conducted aiming to identify microorganisms that may be used for the competitive exclusion of *Salmonella* (Gray, 2011).

Prebiotics are another group of substances intended to modify the intestinal environment, favoring the growth of beneficial microorganisms while preventing the proliferation of pathogens. Lactulose is a prebiotic with beneficial effects on the health of domestic animals, and has also been used to prevent the growth of *Salmonella* in their digestive tract (Holz & Middleton, 2005). This compound is used as a nutritional substrate, to promote the growth of desirable bacteria. Pathogenic bacteria, on the other hand, are unable to use this energetic substrate and their growth is suppressed by the acidification of the intestinal environment (Jankowski, 2009). Unfortunately, however, lactulose failed to prevent the elimination of *Salmonella* in the feces on studies conducted with Carpet Pythons (*Morelia spilota*) and Scrub Pythons (*Morelia amethystina*) (Holz & Middleton, 2005).

8. Prevention and control

A number of measures may be taken to interrupt the proliferation of *Salmonella* in the environment, eliminate it whenever possible and prevent it from reaching specific areas: rigorous hygiene and disinfection of facilities and equipment, isolation of sick animals and asymptomatic carriers, measures of animal welfare, and the complete removal of organic matter to facilitate disinfectant action. Sodium hypochlorite (bleach) is an effective and low-cost disinfectant that may be used for the sanitation of exhibits and cages. It should also be kept in mind that asymptomatic carriers are important sources of infection (Carvalho, 2006), as infected animals are normally isolated and monitored, thus reducing transmission (Fornazari & Teixeira, 2009).

The general public is often unaware of the risks associated to salmonellosis, failing to take basic hygiene practices while handling reptiles. Simple hygiene procedures alone can considerably decrease the probability of transmission (Hoelzer et al., 2011). Friedman et al. (1998) demonstrated that washing hands after attending a reptile exhibit or before the next meal is sufficient to prevent reptile-associated salmonellosis. This hygiene routine oftentimes is not properly followed by children under the age of five, and as a result this age group is more prone to become contaminated (Centers for Disease Control and Prevention [CDC], 2010).

The release of animals to their natural habitats, as performed by wildlife rehabilitation centers, should be thoughtful to avoid spreading *Salmonella* to free-ranging populations (Jijón et al., 2007).

Table 2 presents a list of instructions cooperatively elaborated by the Association of Reptilian and Amphibian Veterinarians (ARAV) and the Center for Disease Control and Prevention (CDC) to reduce human exposure and disease development risks involving reptiles. Table 3 provides a client educational handout developed by the ARAV.

Veterinarians who treat reptiles should educate their clients who own reptiles about *Salmonella* and provide information on the recommended precautions for reducing the risk of transmission of *Salmonella* from reptiles to humans. This is especially important in households with infants and children under 5 years of age or with immunocompromised persons. The CDC recommends that households with children less than 5 years of age not own reptiles.

All veterinarians, staff and clients who handle reptiles should follow recommended precautions for reducing the risk of transmitting *Salmonella* from reptiles to humans. These precautions are also included in the ARAV client education handout and are based on good personal hygiene and not allowing reptiles to soil the owners' domicile.

All reptiles should be presumed to be carrying *Salmonella* in their intestinal tract and to be continuously or intermittently shedding it in their feces. Bacterial culture of fecal specimens from reptiles to determine *Salmonella* infection status is discouraged. If veterinarians are called upon to assist health officials in determining the cause of salmonellosis in a person, bacterial culture of combined fecal and cloacal specimens from reptiles with which that person has had direct or indirect contact are recommended. Serotyping of *Salmonella* isolates is usually needed to help discern the source of the *Salmonella* in human infections.

It is not recommended to treat healthy reptiles with antimicrobial agents with the intention of eliminating *Salmonella* from the intestinal tract. Clients who request treatment of healthy reptiles for *Salmonella* should be discouraged from such treatment and cautioned about the possibility of causing the emergence of antimicrobial-resistant *Salmonella* strains that might pose a greater health risk to humans (Bradley & Angulo, 2008).

Table 2. *Salmonella* and reptiles: veterinary guidelines

Always wash your hands with hot, soapy water after handling reptiles, reptile cages and equipment, and the feces of reptiles.

Do not allow reptiles to have access to the kitchen, dining room, or any other area in which food is prepared or eaten. Also, do not allow reptiles to have access to bathroom sinks and tubs or to any area where infants are bathed. Consider keeping your reptiles caged or limiting the parts of the house where reptiles are allowed to roam free. Always wash your hands after coming into contact with any area where reptiles are allowed to roam free.

Do not eat, drink, or smoke while handling reptiles, reptile cages, or reptile equipment. Do not kiss reptiles or share food or drink with them.

Do not use the kitchen sink, kitchen counters, bathroom sinks or bathtubs to bathe reptiles or to wash reptile cages, dishes or aquariums. Reptile owners may wish to purchase a plastic basin or tub in which to bathe or swim their reptiles. Waste water and fecal material should be disposed of in the toilet instead of the bathtub or household sink.

The CDC recommends that children less than five years of age avoid contact with reptiles and those households with children less than five years of age not own reptiles. The ARAV encourages reptile owners with young children to discuss steps to minimize risks associated with owning reptiles with their reptiles' veterinarian and their physician. Children should be supervised when they are handling reptiles to ensure that they do not place their hands or objects that a reptile has contacted in their mouths. Reptiles should not be kept in childcare centers.

Immunocompromised persons should avoid contact with reptiles.

Follow instructions from your reptile's veterinarian concerning proper diet and environment for your reptile. Healthy reptiles living in proper environments are less likely to shed *Salmonella* bacteria (ARAV, 2008).

Table 3. *Salmonella* bacteria and reptiles: client educational handout

9. Zoonotic aspects

9.1 Snake-associated human salmonellosis

Animal transmitted-salmonellosis is thought to be underreported, and the registered cases are probably biased towards uncommon or unusual characteristics. For instance, less than

1% of the reported cases of human salmonellosis are caused by reptile-associated strains (Hoelzer et al., 2011). Moreover, many patients recover rapidly without having received any treatment, further increasing underreporting (Gray, 2011).

Human salmonellosis is most frequently associated with food ingestion, however direct or indirect contact with animals in public or private environments may also be sources of infection (Hoelzer et al., 2011). Reptile-associated human infections only occur if the reptile or a feces-contaminated object is placed in the mouth. Physical contact with a reptile alone is thus not sufficient to produce infection in humans (ARAV, 2008).

Immunological, biological and behavioral characteristics are probably the reason why human salmonellosis is most frequent in children, especially the youngest (Hoelzer et al., 2011), although the disease may also occur in other age groups, including healthy adults (Warwick et al., 2001).

Many reports associate human *Salmonella* infections with isolates from snakes. Four cases were selected for discussion here; these cases will be presented in chronological order and were chosen because they are representative, interesting and illustrate the diversity of manners in which these exposures may occur. It is important to note that the development of molecular assays and their incorporation to the set of diagnostic techniques results in chronologically-increasing precision in comparing isolates retrieved from humans and reptiles in each case.

In July 1977, for the first time, *S. eingedi* was isolated from a snake in Israel and from a child that had diarrhea shortly after that. That serotype was also isolated from another child with diarrhea in the same Israeli community (Cahan, 1980).

In 1997, Paton & Mirfattahi presented the case of a 5-month-old boy with bacterial meningitis. *S. uzaramo* was isolated from the cerebrospinal fluid, blood and stool samples of the patient. The same serotype was isolated from the feces of an Indian Python (*Python molurus*) and two Ball Pythons (*P. regius*); *S. arizonae* and an unidentified isolate were also cultured from the Ball Pythons.

Jafari et al. (2002) reported *S.* Enteritidis infections resulting from a platelet donation. The apparently healthy donor had acquired an asymptomatic *S. enterica* bacteremia from his Boa Constrictor. Two patients receiving the platelet donation developed sepsis, and one of them died. The reptilian origin of the infection was confirmed through the determination of the serotype by PFGE.

A large *S.* Typhimurium outbreak occurred in the United States in 2005 and 2006. Several people had direct or indirect contact with snakes that had been fed with commercially-raised mice. The rodents had been captive raised, euthanized, vacuum-packed, frozen and sold through the internet. Cultures from three of those mice yielded pure isolates of *S.* Typhimurium that were indistinguishable by PFGE from the isolates obtained from the human cases, snakes, and environmental samples (Fuller et al., 2008).

9.2 Snake products as food items, folk remedies, and human salmonellosis

Despite the legal prohibitions and the risks of foodborne bacterial infections, many communities worldwide consume sea turtle meat and eggs. The raw or undercooked meat,

often prepared in unsanitary conditions, may result in human salmonellosis (Aguirre et al., 2006). In an outbreak of salmonellosis that occurred in Australia in 1988 it was found that 62% of the victims had ingested green turtle meat. Several people were hospitalized, and *S. chester* was isolated from undercooked sea turtle meat samples and from fecal samples of the patients (O'Grady & Krause, 1999).

Similarly to the consumption of chelonians, snakes are also used for meat production (Magnino et al., 2009). Although in the USA and in some Asian countries there are specific breeding facilities for this purpose, snakes may also often be collected from the wild. The consumption of snake meat poses a risk to the development of salmonellosis due to the high prevalence of this microorganism in these animals, and also probably because oftentimes the adequate hygiene measures are not properly carried out.

Snake powder and dried meat, especially those prepared from rattlesnakes, are often used in folk medicine. However, several reports have associated the consumption of these products to the development of salmonellosis in humans (Babu et al., 1990), as well as infections by other Enterobacteriacea. Most of these cases occur in Mexican-American patients. Consumers often ingest such products while presenting underlying chronic and immunosuppressive conditions such as cancer, diabetes, arthritis, systemic lupus erythematosus, or acquired immunodeficiency syndrome (AIDS) (Sharma et al., 1993; Waterman et al., 1990). These conditions further complicate the cases, as *Salmonella* infections will tend to produce much more severe clinical manifestations and complications. For example, patients with AIDS may present recurrent bacteremia (Babu et al., 1990) and patients with gastric cancer may develop peritonitis (Sharma et al., 1993). Not only many *Salmonella* serotypes can be isolated from such rattlesnake capsules (Cone et al., 1990) but it also seems likely that all types of folk medicine preparations generally involve poor conditions of hygiene during their preparation, which may be yet another factor for the contamination of these products.

10. Conclusion

Numerous authors argue that *Salmonella* is a normal component of the reptilian intestinal microbiota and that 90% or more of reptiles harbor *Salmonella*, however some European researchers do not share that opinion (Hassl & Benyr, 2003), and there is evidence in other continents that some serotypes may be considerably pathogenic for the snakes (Bemis et al., 2007; Ramsay et al., 2002).

Basic hygiene practices may be sufficient to prevent most cases of human salmonellosis (Mitchell, 2004), even when it is known that snakes are very likely to be infected by *Salmonella* in both domestic and natural environments (Mermin et al., 2004).

It is vital to acknowledge *Salmonella* as an inhabitant of the digestive tract of reptiles, as this basic understanding is a solid basis to interpret the disease it may cause, to improve the life quality of these animals in captivity, and to take appropriate prevention and control measures.

11. References

Aguirre, A.A. et al. (2006). Hazards associated with the consumption of sea turtle meat and eggs: a review for health care workers and the general public. *EcoHealth*, Vol. 3, No. 3, pp. 141-153

Andrade, J.G. et al. (1989). Estudo bacteriológico de abscessos causados por picada de serpentes do gênero *Bothrops*. *Revista do Instituto de Medicina Tropical de São Paulo*, Vol. 31, No. 6, pp. 363-367

ARAV Special Publications. (2008). *Salmonella* bacteria and reptiles: client educational handout revised, In: *Association of Reptilian and Amphibian Veterinarians*, 23.04.2011, Available from:
<http://www.arav.org/ECOMARAV//timssnet/pdf/SalmonellaBacteriaandRept iles_client_final_11apr08.pdf>

Babu, K. et al. (1990). Isolation of salmonellae from dried rattlesnake preparations. *Journal of Clinical Microbiology*, Vol. 28, No. 2, pp. 361-362

Barrow, P.A.; Jones, M.A. & Thomson, N. (2010). *Salmonella*, In: *Pathogenesis of bacterial infections in animals*, 4. ed., Gyles, C.L.; Prescott, J.F.; Songer, G. & Thoen, C.O., pp. 231-265, Blackwell Publishing, Ames

Bastos, H.M.; Lopes, L.F.L.; Gattamorta, M.A. & Matushima, E.R. (2008). Prevalence of enterobacteria in *Bothrops jararaca* in São Paulo State: microbiological survey and antimicrobial resistance standards. *Acta Scientiarum. Biological Sciences*, Vol. 30, No. 3, pp. 321-326

Bays, T.A.B. (2005). Equipping the reptile practice. *Veterinary Clinics of North America Exotic Animal Practice*, Vol. 8, No. 3, pp. 437-461

Bemis, D.A., et al. (2007). Clonal relatedness of *Salmonella* isolates associated with invasive infections in captive and wild-caught rattlesnakes. *Veterinary Microbiology*, Vol. 120, No. 3-4, pp. 300-307

Bradley, T. & Angulo, F.J. (2008). *Salmonella* and reptiles: veterinary guidelines revised, In: *Association of Reptilian and Amphibian Veterinarians*, 23.04.2011, Available from: <http://www.arav.org/ECOMARAV//timssnet/pdf/SalmonellaandReptiles_vet guideline_FINAL_11apr08.pdf>

Cahan, D. et al. (1980). New *Salmonella* serovar, *Salmonella eingedi* [$6_1,6_2,7$:f,g,t:1,(2),7]. *Journal of Clinical Microbiology*, Vol. 11, No. 3, pp. 292

Carvalho, V.M. (2006). Colibacilose e salmonelose, In: *Tratado de animais selvagens: medicina veterinária*, Cubas, Z.S.; Silva J.C.R. & Catão-Dias, J.L., pp. 742-750, Roca, São Paulo

Centers for Disease Control and Prevention (CDC). (2010). Multistate outbreak of human *Salmonella* Typhimurium infections associated with aquatic frogs - United States, 2009. *Morbidity and Mortality Weekly Report (MMWR)*, Vol. 58, No. 51-52, pp. 1433-1436

Chen, C.Y. et al. (2010). Prevalence and antimicrobial susceptibility of salmonellae isolates from reptiles in Taiwan. *Journal of Veterinary Diagnostic Investigation*, Vol. 22, No. 1, pp. 44-50

Chiodini, R.J. (1982). Transovarian passage, visceral distribution, and pathogenicity of *Salmonella* in snakes. *Infection and Immunity*, Vol. 36, No. 2, pp. 710-713

Chiodini, R.J. & Sundberg, J.P. (1981). Salmonellosis in reptiles: a review. *American Journal of Epidemiology*, Vol. 113, No. 5, pp. 494-499

Cone, Lionel A.; Boughton, W.H.; Cone, Lawrence A. & Lehv, L.H. (1990). Rattlesnake capsule-induced *Salmonella arizonae* bacteremia. *The Western Journal of Medicine*, Vol. 153, No. 3, pp. 315-316

Coutinho, S.D.A. et al. (2001). Bacterial septicemia in water snakes (*Helicops modestus*) in Brazil. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Vol. 53, No. 4, pp. 1-2

Difco. *Difco Salmonella O Antisera, Difco Salmonella H Antisera, Difco Salmonella Antiserum Vi*. Product label, 23.04.2011, pp. 18

Fornazari, F. & Teixeira, C.R. (2009). Salmonelose em répteis: aspectos epidemiológicos, clínicos e zoonóticos. *Veterinária e Zootecnia*, Vol. 16, No. 1, pp. 19-25

Friedman, C.R. et al. (1998). An outbreak of salmonellosis among children attending a reptile exhibit at a zoo. *The Journal of Pediatrics*, Vol. 132, No. 5, pp. 802–807

Fuller, C.C. et al. (2008). A multi-state *Salmonella* Typhimurium outbreak associated with frozen vacuum-packed rodents used to feed snakes. *Zoonoses and Public Health*, Vol. 55, No. 8-10, pp. 481-487

Funk, R.S. (1996). Diarrhea, In: *Reptile medicine and surgery*, Mader, D.R., pp. 364-365, W.B. Saunders, Philadelphia

Geue, L. & Löschner, U. (2002). *Salmonella enterica* in reptiles of German and Austrian origin. *Veterinary Microbiology*, Vol. 84, No. 1-2, pp. 79-91

Ghilardi, A.C.R.; Tavechio, A.T. & Fernandes, S.A. (2006). Antimicrobial susceptibility, phage types, and pulsetypes of *Salmonella* Typhimurium, in São Paulo, Brazil. *Memórias do Instituto Oswaldo Cruz*, Vol. 101, No. 3, pp. 281-286

Goldstein, E.J.C. et al. (1979). Bacteriology of rattlesnake venom and implications for therapy. *The Journal of Infectious Diseases*, Vol. 140, No. 5, pp. 818-821

Gopee, N.V.; Adesiyun, A.A. & Caesar, K. (2000). Retrospective and longitudinal study of salmonellosis in captive wildlife in Trinidad. *Journal of Wildlife Diseases*, Vol. 36, No. 2, pp. 284-293

Gray, T.Z. (2011). Update: reptiles and *Salmonella*. *Journal of Exotic Pet Medicine*, Vol. 20, No. 1, pp. 14-17

Grimont, P.A.D. & Weill, F.X. (2007). Antigenic formulas of the *Salmonella* serovars, 9. ed., revision, *WHO Collaborating Center for Reference and Research on Salmonella*, Institut Pasteur, Paris

Hafez, H.M. & Jodas, S. (2000). *Salmonella* infections in turkeys, In: Salmonella *in domestic animals*, Wray, C. & Wray, A., pp. 133-135, CABI Publishing, Oxon

Hassl, A. & Benyr, G. (2003). Hygienic evaluation of terraria inhabited by amphibians and reptiles: cryptosporidia, free-living amebas, salmonella. *Wien Klin Wochenschr*, Vol. 115, Suppl. 3, pp. 68-71

Hidalgo-Vila, J. et al. (2008). *Salmonella* in free-living exotic and native turtles and in pet exotic turtles from SW Spain. *Research in Veterinary Science*, Vol. 85, No. 3, pp. 449-452

Hinshaw, W.R. & McNeil, E. (1944). Gopher snakes as carriers of salmonellosis and paracolon infections. *The Cornell Veterinarian*, Vol. 34, pp. 248-254

Hinshaw, W.R. & McNeil, E. (1945). *Salmonella* types isolated from snakes. American Journal of Veterinary Research, Vol. 6, pp. 264-266

Hinshaw, W.R. & McNeil, E. (1946). Paracolon type 10 from captive rattlesnakes. *Journal of Bacteriology*, Vol. 51, No. 3, pp. 397-398

Hinshaw, W.R. & McNeil, E. (1947). Lizards as carriers of *Salmonella* and paracolon bacteria. *Journal of Bacteriology*, Vol. 53, No. 6, pp. 715-718

Hirsh, D.C. (2004). Enterobacteriacea: *Salmonella*, In: *Veterinary microbiology*, 2. ed., Hirsh, D.C.; MacLachlan, N.J. & Walker, R.L., pp. 69-74, Blackwell Publishing, Ames

Hoelzer, K.; Switt, A.I.M. & Wiedmann, M. (2011). Animal contact as a source of human non-typhoidal salmonellosis. *Veterinary Research*, Vol. 42, No. 34, pp. 1-27

Holz, P.H. & Middleton, D.R. (2002). The effect of probiotic feeding on *Salmonella* excretion in carpet pythons, *Morelia spilota. Journal of Herpetological Medicine and Surgery*, Vol. 12, No. 3, pp. 5-7

Holz, P.H. & Middleton, D.R. (2005). The effect of feeding a prebiotic on *Salmonella* excretion in carpet pythons, *Morelia spilota*, and scrub pythons, *Morelia amethystine. Journal of Herpetological Medicine and Surgery*, Vol. 15, No. 1, pp. 4-6

Iveson, J.B.; Mackay-Scollay, E.M. & Bamford, V. (1969). *Salmonella* and *Arizona* in reptiles and man in Western Australia. *The Journal of Hygiene (London)*, Vol. 67, No. 1, pp. 135-145

Jafari, M. et al. (2002). *Salmonella* sepsis caused by a platelet transfusion from a donor with a pet snake. *The New England Journal of Medicine*, Vol. 347, No. 14, pp. 1075-1078

Jankowski, G. (2009). Lactulose. *Journal of Exotic Pet Medicine*, Vol. 18, No. 2, pp. 156-159

Jijón, S.; Wetzel, A. & LeJeune, J. (2007). *Salmonella enterica* isolated from wildlife at two Ohio rehabilitation centers. *Journal of Zoo and Wildlife Medicine*, Vol. 38, No. 3, pp. 409-413

Johnson-Delaney, C.A. (2006). Reptile zoonoses and threats to public health, In: *Reptile medicine and surgery*, 2. ed., Mader, D.R., pp. 1017-1030, Saunders Elsevier, St. Louis

Jorge, M.T. et al. (1990). Flora bacteriana da cavidade oral, presas e veneno de *Bothrops jararaca*: possível fonte de infecção no local da picada. *Revista do Instituto de Medicina Tropical de São Paulo*, Vol. 32, No. 1, pp. 6-10

Kaufmann, A.F. & Morrison, Z.L. (1966). An epidemiologic study of salmonellosis in turtles. *American Journal of Epidemiology*, Vol. 84, No. 2, pp. 364-370

Kaufmann, A.F. et al. (1972). Turtle-associated salmonellosis: III. The effects of environmental salmonellae in commercial turtle breeding ponds. *American Journal of Epidemiology,* Vol. 95, No. 6, pp. 521-528

Lamberski, N. et al. (2002). Recovery of a pathogenic *Salmonella arizona* from four snakes at the Riverbanks Zoo. *Journal of Herpetological Medicine and Surgery*, Vol. 12, No. 4, pp. 17-22

Lima, D.C. et al. (2005). Snake venom: any clue for antibiotics and CAM? *Evidence-Based Complementary and Alternative Medicine*, Vol. 2, No. 1, pp. 39-47

Mader, D.M. (2006). Perinatology, In: *Reptile medicine and surgery*, 2. ed., Mader, D.R., pp. 365-375, Saunders Elsevier, St. Louis

Magnino, S. et al. (2009). Biological risks associated with consumption of reptile products. *International Journal of Food Microbiology*, Vol. 134, No. 3, pp. 163-175

Mathewson, J.J. (1979). Enterobacteriaceae isolated from iguanid lizards of west-central Texas. *Applied and Environmental Microbiology*, Vol. 38, No. 3, pp. 402-405

Mehler, S.J. & Bennett, R.A. (2006). Upper alimentary tract disease, In: *Reptile medicine and surgery*, 2. ed., Mader, D.R., pp. 924-930, Saunders Elsevier, St. Louis

Mermin, J. et al. (2004). Reptiles, amphibians, and human *Salmonella* infection: a population-based, case-control study. *Clinical Infectious Diseases*, Vol. 38, Suppl. 3, pp. 253-261

Messonnier, S.P. (1996). *Common reptile diseases and treatment*, Blackwell Science, Cambridge

Minette, H.P. (1984). Epidemiologic aspects of salmonellosis in reptiles, amphibians, mollusks and crustaceans – a review. *International Journal of Zoonoses*, Vol. 11, No. 1, pp. 95-104

Mitchell, M.A. (2004). Snake care and husbandry. *Veterinary Clinics of North America Exotic Animal Practice*, Vol. 7, No. 2, pp. 421-446

Mitchell, M.A. (2006). *Salmonella*: diagnostic methods for reptiles, In: *Reptile medicine and surgery*, 2. ed., Mader, D.R., pp. 900-905, Saunders Elsevier, St. Louis

Mitchell, M.A. & Shane, S.M. (2001). *Salmonella* in reptiles. *Seminars in Avian and Exotic Pet Medicine*, Vol. 10, No. 1, pp. 25-35

Moreno, G. et al. (1973). Enterobactérias isoladas de anfíbios e répteis. *Revista do Instituto de Medicina Tropical de São Paulo*, Vol. 15, No. 3, pp. 122-126

Murray, M.J. (1996). Cardiology and circulation, In: *Reptile medicine and surgery*, Mader, D.R., pp. 95-104, W.B. Saunders, Philadelphia

Myers, D. (2007). Probiotics. *Journal of Exotic Pet Medicine*, Vol. 16, No. 3, pp. 195-197

Nair, S.; Lin, T.K.; Pang, T. & Altwegg, M. (2002). Characterization of *Salmonella* serovars by PCR–single-strand conformation polymorphism analysis. *Journal of Clinical Microbiology*, Vol. 40, No. 7, pp. 2346–2351

Nishioka, S.A.; Jorge, M.T.; Silveira, P.V.P. & Ribeiro, L.A. (2000). South American rattlesnake bite and soft-tissue infection: report of a case. *Revista da Sociedade Brasileira de Medicina Tropical*, Vol. 33, No. 4, pp. 401-402

O'Grady, K.A. & Krause, V. (1999). An outbreak of salmonellosis linked to a marine turtle. *Southeast Asian Journal of Tropical Medicine and Public Health*, Vol. 30, No. 2, pp. 324-327

Onderka, D.K. & Finlayson, M.C. (1985). Salmonellae and salmonellosis in captive reptiles. *Canadian Journal of Comparative Medicine*, Vol. 49, No. 3, pp. 268-270

Orós, J. et al. (1996). Respiratory and digestive lesions caused by *Salmonella arizonae* in two snakes. *Journal of Comparative Pathology*, Vol. 115, No. 2, pp. 185-189

Page, L.A. (1966). Diseases and infections of snakes: a review. *Bulletin of the Wildlife Disease Association*, Vol. 2, No. 4, pp. 111-126

Painter, J.A. et al. (2004). *Salmonella*-based rodenticides and public health. *Emerging Infectious Diseases*, Vol. 10, No. 6, pp. 985-987

Paré, J.A.; Sigler, L.; Rosenthal, K.L. & Mader, D.R. (2006). Microbiology: fungal and bacterial diseases of reptiles, In: *Reptile medicine and surgery*, 2. ed., Mader, D.R., pp. 217-238, Saunders Elsevier, St. Louis

Pascual, M. et al. (1999). *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. *Applied and Environmental Microbiology*, Vol. 65, No. 11, pp. 4981-4986

Paton, J.H. & Mirfattahi, M.B. (1997). *Salmonella* meningitis acquired from pet snakes. *Archives of Disease in Childhood*, Vol. 77, No. 1, pp. 91

Pedersen, K.; Lassen-Nielsen, A.M.; Nordentoft, S. & Hammer, A.S. (2009). Serovars of *Salmonella* from captive reptiles. *Zoonoses and Public Health*, Vol. 56, No. 5, pp. 238-242

Pfleger, S.; Benyr, G.; Sommer, R.; Hassl, A. (2003). Pattern of *Salmonella* excretion in amphibians and reptiles in a vivarium. *International Journal of Hygiene and Environmental Health*, Vol. 206, No. 1, pp. 53-59

Ramsay, E.C. et al. (2002). Osteomyelitis associated with *Salmonella enterica* ss *arizonae* in a colony of ridgenose rattlesnakes (*Crotalus willardi*). *Journal of Zoo and Wildlife Medicine*, Vol. 33, No. 4, pp. 301-310

Ribeiro, L.A. & Jorge, M.T. (1990). Epidemiologia e quadro clínico dos acidentes por serpentes *Bothrops jararaca* adultas e filhotes. *Revista do Instituto de Medicina Tropical de São Paulo*, Vol. 32, No. 6, pp. 436-442

Ribeiro, L.A. & Jorge, M.T. (1997). Acidente por serpentes do gênero *Bothrops*: série de 3.139 casos. *Revista da Sociedade Brasileira de Medicina Tropical*, Vol. 30, No. 6, pp. 475-480

Sá, I.V.A. & Solari, C.A. (2001). *Salmonella* in Brazilian and imported pet reptiles. *Brazilian Journal of Microbiology*, Vol. 32, No. 4, pp. 293-297

Salb, A.; Mitchell, M.A.; Riggs, S. & Diaz-Figueroa, O. (2002). Characterization of the aerobic and anaerobic intestinal flora of the green iguana (*Iguana iguana*), *Proceedings of ARAV Conference*, Reno, NV

Scheelings, T.F.; Lightfoot, D. & Holz, P. (2011). Prevalence of *Salmonella* in Australian reptiles. *Journal of Wildlife Diseases*, Vol. 47, No. 1, pp. 1-11

Schilliger, L.; Vanderstylen, D.; Piétrain, J. & Chetboul, V. (2003). Granulomatous myocarditis and coelomic effusion due to *Salmonella enterica arizonae* in a Madagascar Dumerili's boa (*Acrantophis dumerili*, Jan. 1860). *Journal of Veterinary Cardiology*, Vol. 5, No. 1, pp. 43-45

Schneitz, C. & Mead, G. (2000). Competitive exclusion, In: Salmonella *in domestic animals*, Wray, C. & Wray, A., pp. 301-322, CABI Publishing, Oxon

Schröter, M. et al. (2004). Pet snakes as a reservoir for *Salmonella enterica* subsp. *diarizonae* (serogroup IIIb): a prospective study. *Applied and Environmental Microbiology*, Vol. 70, No. 1, pp. 613-615

Schröter, M.; Speicher, A.; Hofmann, J. & Roggentin, P. (2006). Analysis of the transmission of *Salmonella* spp. through generations of pet snakes. *Environmental Microbiology*, Vol. 8, No. 3, pp. 556–559

Sharma, J.; Von Hoff, D.D. & Weiss, G.R. (1993). *Salmonella arizonae* peritonitis secondary to ingestion of rattlesnake capsules for gastric cancer. *Journal of Clinical Oncology*, Vol. 11, No. 11, pp. 2288-2289

Waltman, W.D. (2000). Methods for the cultural isolation of *Salmonella*, In: Salmonella *in domestic animals*, Wray, C. & Wray, A., pp. 355-372, CABI Publishing, Oxon

Wang, Y. et al. (2008). Snake cathelicidin from *Bungarus fasciatus* is a potent peptide antibiotics. *PLoS ONE*, Vol. 3, No. 9, pp. 1-9

Warwick, C.; Lambiris, A.J.L.; Westwood, D. & Steedman, C. (2001). Reptile-related salmonellosis. *Journal of the Royal Society of Medicine*, Vol. 94, No. 3, pp. 124-126

Waterman, S.H. Juarez, G.; Carr, S.J. & Kilman, L. (1990). *Salmonella arizona* infections in Latinos associated with rattlesnake folk medicine. *American Journal of Public Health*, Vol. 80, No. 3, pp. 286-289