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Review

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Domestic reptiles as source of zoonotic bacteria: A mini review

Valentina Virginia Ebani[™]

Department of Veterinary Science, University of Pisa, I 56124 Pisa, Italy

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ABSTRACT

Captive reptiles, always more often present in domestic environment as pets, may harbor and excrete a large variety of zoonotic pathogens. Among them, *Salmonella* is the most well-known agent, whereas there are very scant data about infections by mycobacteria, chlamydiae and leptospirae in cold-blooded animals. However, the investigations that found antibody reactions and/or the bacteria in samples collected from free-ranging and captive reptiles show that herpetofauna may be involved in the epidemiology of these infections. The present review reports the updated knowledge about salmonellosis, mycobacteriosis, chlamydiosis and leptospirosis in reptiles and underlines the risk of infection to which people, mainly children, are exposed.

1. Introduction

Cold-blooded animals are always more often present in the houses of persons who choose these animals as companion pets together or instead of dogs and cats. This trend has mainly expanded in the European Union (EU) member states that currently are the largest importers of reptiles [1]. Turtles and tortoises are the most common reptiles living in domestic habitat. However, a large number of persons keep snakes and lizards in their houses, too. Sometimes these animals are kept in cages, but often they are free to move in the rooms coming into close contact with the owners and their house furnishings. Captive and free-ranging reptiles, even though they are asymptomatic, may harbor and excrete a large number of different pathogens that can determine infections in human beings.

Zoonosis is mainly caused by bacterial pathogens. Among them, salmonellae excreted by reptiles are well-known agents responsible for human infections [2], whereas information about the spread among reptiles of other zoonosis, such as

Tel: +39 0502216968 Fax: +39 050221694

Fax: +39 U3U221094

E-mail: valentina.virginia.ebani@unipi.it

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mycobacteriosis, chlamydiosis and leptospirosis that in humans may cause severe pathologies, is scant.

Infected herpetofauna is a serious threat for the public health, because it may be a source of zoonotic pathogens for many people. First of all, captive reptiles represent a risk for the owners, in particular for children that have close contact with their pets, and for professional categories, such as veterinarians, zoo and circus personnel. Moreover, free-ranging animals seem to be involved in the epidemiology of some zoonotic infections, because they contribute to spreading pathogens in the environment.

The present review reports the updated knowledge about salmonellosis, mycobacteriosis, chlamydiosis and leptospirosis in reptiles, with particular emphasis on the pathologies caused in these animals.

2. Salmonellosis

Several studies have shown that reptiles are often infected by different *Salmonella* spp. serovars [3]. Reptiles intermittently excrete salmonellae in their feces. In fact, the excretion is not continuous and this aspect may represent an obstacle to identify infected reptiles, if they are submitted to only one bacteriological exam. *Salmonella*-infected reptiles usually are a direct source of infections for other cold-blooded animals. However, poikilothermic animals often become infected through

[™]First and corresponding author: Valentina Virginia Ebani, DVM, PhD, Department of Veterinary Science, Viale delle Piagge 2, I 56124 Pisa, Italy.

the ingestion of contaminated food, both vegetables and living prey animals (mice, chicks). Pet reptiles are source of salmonellae for other household animals, mainly dogs and cats, which contribute to the spreading of the pathogens in domestic environment and increasing the occasion of infections for the human beings.

Captive reptiles may contract salmonellae in different occasions: in the country of origin before export, during traveling, or in the animal stores. In all cases, travel and new habitat determine stress for animals, favoring the fecal excretion of intestinal pathogens. Stress conditions are particularly frequent for turtles since they are often kept in overpopulated breeding ponds, where the risk of exposure to enteric pathogens is very high.

Salmonellae may contaminate chelonian eggs during the passage through the cloaca or when they are buried in moist soil or sand. These bacteria are able to quickly cross the shell within 1 h of exposure [4]. Moreover, the transovarial transmission has also been reported [5].

It is believed that *Salmonella* transmission during pregnancy can occur in ophidians; in fact it seems that salmonellae may colonize the snakes' ovaries. Moreover, salmonellae may be present inside the eggs, because some ophidian species lay eggs surrounded by a thin membrane, instead of a true shell, which may be easily overpassed by microorganisms [6].

Reptiles infected by salmonellae do not usually develop disease. However, septicemia, pneumonia, coelomitis, abscesses, granuloma, hypovolemic shock, osteomyelitis and death have been observed in some cases [7–9]. In humans, salmonellosis caused by infected cold-blooded animals is a very serious public health problem. Human beings may have diarrhea, vomiting, fever, but also more severe illness due to septicemia, bone marrow and nervous system infections [2,5,10–17].

On the basis of several bacteriological investigations carried out on samples collected from reptiles, it seems that there are no host-specific serovars better adapting to a reptile species or order. In fact, several *Salmonella* serovars can be isolated from herpetofauna. Serovars belonging to *Salmonella enterica* subsp. *houtenae* and *Salmonella bongori* are usually related to reptiles. However, *Salmonella enterica* subsp. *enterica* has also frequently been isolated from cold-blooded animals. In some cases infrequent serovars have been found, but *Salmonella typhimurium* responsible for severe and well-known pathologies in humans and animals has been encountered, too [18,19].

Salmonellae excreted by captive and wild reptiles are often resistant to several antibiotics. Resistance has been observed against antibiotics commonly employed in therapy, such as cephalosporins, penicillins, sulfonamide-trimethoprim, but also against 'new' molecules. For example, recent studies have found resistance to tigecycline that is often employed against enter-obacteria [19,20]. Resistance to antibiotics is a severe and increasing concern for the treatment of veterinary and human Salmonella infections. Moreover, antibiotic-resistant salmonellae may act as a source of genes responsible for the antibiotic resistance that are transferred to other bacteria present in the gastrointestinal tract.

3. Mycobacteriosis

Infections caused by non-tuberculous mycobacteria (NTM) may occur in ophidians, saurians and chelonians that usually develop granulomas in different body sites [21]. NTM are largely

present in the environment: soil, dust, water and plants. Animals contract the mycobacterial infection through cutaneous lesions and ingestion of contaminated food and/or water. Coldblooded animals with a deficient immune system are most likely to be at risk. Stress, inadequate nutrition and concomitant diseases represent important risk factors [8]. Symptomatology due to mycobacteria in reptiles is related to the body site involved. However, many animals often show weight loss although they maintain the appetite.

All NTM can induce bacteriaemia and reach several organs, such as lung, liver, spleen, kidney, heart, bone, gonads, nervous system and joints [8,22]. In these sites, NTM cause grayish-white granulomas that on histological examinations show macrophages, multinucleated giant cells, lymphocytes, heterophils, and plasma cells. Older granulomas may be surrounded by fibrous connective tissue [23].

Among NTM, several species have been isolated from reptilian lesions. In particular, *Mycobacterium chelonae* (*M. chelonae*) has been frequently detected in turtles and tortoises with different clinical signs and lesions. The most common alterations in chelonians are plastral ulcerations, cutaneous and oral mucosa lesions, sepsis and disseminated intravascular coagulation [22,24–26]. However, *M. chelonae* has been identified as the cause of granulomatous lesions in saurian and snakes, too [27].

Mycobacterium genavense, usually considered an environmental bacterium responsible for severe infections in immunocompromised patients, has recently been associated to clinical pneumonia, with anorexia, lethargy and weight loss, in two captive snakes, one zoo-born python (Python molurus) and one exotic green snake (Philodryas olfersii). The two animals, which died of natural causes, showed whitish nodules in trachea, lungs and liver during the necropsy [28].

Typical disseminated granulomas have been observed in cold-blooded animals infected by *Mycobacterium fortuitum* (M. fortuitum), Mycobacterium kansasii, Mycobacterium haemophilum, Mycobacterium marinum, Mycobacterium kumamotonerse and Mycobacterium ulcerans [29–34].

Mycobacterium szulgai has been considered as the causative agent of lethal pneumonia in a captive adult freshwater crocodile (*Crocodylus johnstoni*); at the necropsy, the animal showed fibrinous exudate in the pleural cavity and multiple white military nodules in the right lung [35].

Despite the lesions observed in the cases reported above, cold-blooded animals are considered naturally resistant to mycobacteria, because they often harbor these microorganisms symptomlessly. A survey carried out on fecal samples collected from clinically healthy pet reptiles found about 16% of shedding animals; *M. chelonae, Mycobacterium peregrinum, M. fortuitum and M. fortuitum*-like were isolated. Moreover, a strain belonging to a not yet described *Mycobacterium* species was found [33].

All atypical mycobacteria are potentially pathogen for humans, mainly immunocompromised subjects, which may develop different lesions. In particular, lung disease, osteomyelitis, joint infections, disseminated skin and soft tissue lesions have been associated to *M. chelonae* and *M. fortuitum* in humans. *M. marinum* infection may occur in human beings after a skin wound in fresh or salt water, where this bacterium is often present, and cause a localized granuloma or lymphangitis. Persistent ulceration, draining sinuses, septic arthritis and osteomyelitis may be subsequent complications [34].

4. Chlamydiosis

Since the nineties, cases of Chlamydiosis, due to various *Chlamydia* agents, have been reported in captive and freeranging herpetofauna. The first survey detected chlamydial agents that the researchers classified, based on the microbiological tools available in those years, as *Chlamydia psittaci* (*C. psittaci*).

In 1994, Huckzermeyer *et al.* [36] isolated *C. psittaci* from liver samples of some Nile crocodiles (*Crocodylus niloticus*) with fulminant hepatitis and generalized edema. Successively, the same authors reported an outbreak of chlamydiosis in hatchling and juvenile Indopacific crocodiles (*Crodylus porosus*) with hepatitis and exudative conjunctivitis, confirming the susceptibility of crocodiles to this pathogen [37].

An avian *C. psittaci* strain was also identified in Moorish tortoises (*Testudo graeca*) with pneumonia [38].

Successively, various chlamydioses have been related to cold-blooded animals.

Microorganisms classified as *Chlamydia*-like agents have been identified in tissue samples with granulomatous inflammation of some reptiles [21,39]. However, the effective role of *Chlamydia*-like organisms in the granuloma formation is not fully understood.

Chlamydia abortus and Chlamydia pneumoniae (C. pneumoniae) have been detected in reptiles in different cases. In particular, these two pathogens, together with Neochlamydia sp., have been found in the heart with necrotizing myocarditis of a captive green sea turtle (Chelonia mydas) dead after a period with lethargy, anorexia and reluctance to dive. Moreover, the two chlamydia species have been detected in tissue samples collected from puff adders (Bitis arietans) that showed regurgitation and mild respiratory disease before their death. At necropsy the adders had severe granulomatous pericarditis, myocarditis, pneumonia, hepatitis, splenitis and enteritis [40].

Chlamydia abortus and C. pneumoniae seem to determine respiratory pathology, as suggested by their detection in a captive Burmese pythons (*Python molurus bivittatus*) with chronic intermittent respiratory disease and severe diffuse suppurative pneumonia observed at necropsy [40].

In one case, *C. pneumoniae* has been found associated to *Chlamydia felis*, a pathogen responsible for ocular and respiratory disease in cats; in detail, the two microorganisms have been detected in tissue samples collected from respiratory and gastrointestinal tracts of anorexic and lethargic iguanas (*Iguana iguana*) [40].

C. pneumoniae may be the cause of nonspecific symptomatology, as it has been observed in a flap-necked chameleon (*Chamaeleo dilepis*) that had been euthanized after a period of generally bad condition; the pathogen has been found in spleen and liver that histologically showed intra-cytoplasmic inclusions within monocytes [40].

Successively to these reports, *C. pneumoniae* has been frequently found in herpetofauna, resulting in the most wide-spread chlamydia among these animals [40–44].

C. pneumoniae and other chlamydiae have also been detected in asymptomatic captive snakes [45], suggesting that these bacteria are more widespread among cold-blooded animals than it is supposed.

The large presence of *C. pneumoniae* among reptiles is a severe threat for human health, because besides causing disease in

cold-blooded animals, this chlamydia is a well-known pathogen responsible for acute and chronic respiratory disease in humans and implicated in atherosclerosis and Alzheimer's disease [46].

C. pneumoniae DNA samples obtained from different coldblooded animals have been submitted to phylogenetic analysis and the result showed they are highly related to those of human origin.

In some cases, *C. pneumoniae* isolates found in different reptiles have been grouped in clusters that were distinct from other isolates, suggesting that these animals were infected with specific genotypes [43].

5. Leptospirosis

Data about *Leptospira* infection among reptiles are very scant. The first report dates back to the isolation of *Leptospira* interrogans serovar Ballum from a hog-nosed (*Heterodon* platirhinus) snake by Ferris et al. in 1961 [47]. An experimental infection of snakes (*Thamnophis sirtalis*) with the serovar Pomona was carried out by Abdulla & Karstad in 1962 and the authors found the spirochaetes in kidneys six months after inoculation and one snake was found to have an interstitial nephritis [48]. Successively, few investigations found serological positivities among reptiles [49–53].

In the last years, major attention has been focused on the study of the role of reptiles in the epidemiology of leptospirosis. Currently, this disease is considered as a re-emerging zoonosis occurring worldwide and in particular in tropical and subtropical regions where captive and free-ranging cold-blooded animals are largely present. Leptospirae are traditionally related to aquatic habitat, so it could be supposed that aquatic reptiles are more exposed to these pathogens. The detection of seropositive crocodiles that live in fresh and salt waters suggests that leptospirae can survive in both environments. Fresh water is a more suitable habitat for these spirochaetes, because salt decreases its survival rate [54]. However, some authors stated that leptospirae could survive three days in seawater [55].

It is supposed that *Leptospira*-infected reptiles excrete the spirochaetes with urine as it occurs in mammals. Consequently, people can become infected when they have contact with water, soil and food contaminated with infected urine, both in domestic and outdoor environment.

Lindtner-Knific *et al.* (2013) [56] found leptospiral antibodies in captive snakes, lizards and turtles. Among a total of 297 sera, the microscopic agglutination test (MAT) found 46 positive animals to one or more serovars. Specifically, 27.3% of snakes were positive to Grippotyphosa, Tarassovi, Copenhageni, and Pomona with antibody titers ranging from 1:50 to 1:400; 14.8% of lizards had antibodies against Grippotyphosa, Tarassovi, Canicola, Australis, Copenhageni and Hardjo with titer levels from 1:50 to 1:1000; 13.8% of turtles were seropositive to Grippotyphosa, Tarassovi, Canicola, Pomona and Copenhageni at titers from 1:50 to 1:1600.

All the animals had been imported into Slovenia and those reptiles from non-EU countries (Pakistan, Mali and Lebanon) had significantly higher prevalence (25%) than those from EU member states (10.4%).

In 2012, an investigation was carried out in Brazilian urban areas to detect *Leptospira* sp. and antibodies against this pathogen in turtles *Phrynops geoffroanus* that live in rivers, lakes and streams and are well adapted to inhabit urban rivers polluted with organic waste.

45.45% (30/66) of the turtles, tested with MAT, had specific antibodies against nine different Leptospira serovars: Copenhageni (15.15%), Pyrogenes (10.61%), Autumnalis (4.54%), Canicola (1.51%), Andamana (1.51%), Grippotyphosa (1.51%), and Castellonis (1.51%), with antibody titers ranging from 1:20 to 1:1280. Gastric and cloacal lavage samples were collected from the same animals and Leptospira sp. DNA was detected in 11 (16.67%) of the 66 turtles, 5 seropositive and 6 seronegative. The results show that the presence of specific antibodies does not always correspond to the presence of leptospirae and they could be due to a previous contact. On the other hand, the authors hypothesize that the detection of Leptospira sp. in the seronegative turtles could be related to inability of the host's immune system to respond (immunodeficiency), lack of Leptospira sp. immunogenicity, and/or too brief contact between the agent and the host to produce antibodies [57].

A recent investigation found 87.5% of the 64 tested captive Tropical Rattlesnakes (*Crotalus durissus collilineatus*) serologically positive to different *Leptospira* sp. serovars in Brazil. Most of the animals were positive to two or more serovars. Javanica resulted the most common serovar (83.9% of the samples), followed by Andamana (60.7%) and Patoc (51.8%). Antibody titers ranged from 1:25 to 1:1 600, but the most frequent were 1:25 (40.1%) and 1:50 (47.8%); titer 1:800 was found to the serovar Patoc, 1:1600 to Whitcombi and Panama

A study carried out in Mexico detected a high seroprevalence (100%) for leptospirosis among crocodilians (*Crocodylus acutus* and *Crocodylus moreletii*). Grippotyphosa resulted the serovar with the highest prevalence with antibody titers between 1:50 and 1:3 200, followed by Pomona, Wolfii and Bratislva. Even though crocodiles are not strictly in contact with humans, cases of infected people involved with handling and egg collection suggest that these cold-blooded animals could play an important role in the transmission of leptospirosis in some environments [59].

In all these studies, even though reptiles had high antibody titers, they did not show clinical signs. Only Perez-Flores *et al.* observed that crocodiles with the highest titers presented a generally bad condition and a lower weight than crocodiles of the same size. Septicemia due to leptospirae in crocodiles has been supposed when the animals are under continuous stress conditions [59]. However, it can be stated that reptiles may be involved in the epidemiology of leptospirosis, without developing disease.

6. Discussion

Asymptomatic and diseased reptiles may harbor and excrete a wide variety of bacterial pathogens.

Besides salmonellae, mycobacteria, leptospirae and chlamydiae, reptiles may excrete in their feces enteric bacteria (Escherichia coli, Klebsiella sp. Campylobacter spp, Yersinia spp.) that cause infection and disease in human beings. Bacteria belonging to the genus Clostridium, Staphylococcus, Streptococcus and Pasteurella may affect herpetofauna, too [8]. Aeromonas and Pseudomonas spp. are frequently isolated from different reptile species, both from clinically healthy and symptomatic individuals. These bacteria can cause dermatitis, stomatitis, cloacitis, abscesses, ear and respiratory infections and septicemia with consequent death [60].

Persons with pathologies, children, elderly and pregnant women are immunodeficient individuals and thus are at greater risk of infection than other subjects. However, any person directly or indirectly coming into contact with reptiles may contract zoonotic pathogens. Traditionally, zoo and circus personnel, veterinarians and herpetologists are the job positions with the highest risk of infection. The increased popularity of reptiles has extended this risk to a relevant number of persons that own these animals as pets. Moreover, other household animals may contract the infections from reptiles and amplify the possibility of transmission to humans.

Usually, people relates reptiles such as snakes and saurians to the risk of injuries, whereas considers turtles and tortoises as harmless animals safe for children. Small turtles, that are inexpensive animals, can be easily purchased not only from pet stores, but also from vendors at fairs and online merchants. In all cases, turtles are under high stress condition and thus they excrete harbored pathogens. These pets are often housed in a small pool of water in plastic cage where high concentration of pathogens can be reached. Children, that more frequently handle the turtles, and adult owners that change the water and clean the cages can easily become infected [16].

Owners must be informed about the risk to contract infections from reptiles. First of all, they should know that they have to wear gloves when cleaning the reptile cages or changing the water pool. Also, despite the use of gloves, it is important to wash the hands always after handling the reptiles and particularly the wounds due to bites or scratch. Owners should keep reptiles away from the kitchen and other areas where food is prepared or consumed. Moreover, owners must pay attention to the general good care (feeding, hygienic condition of habitat) of their reptiles, being aware that stressed animals have higher possibility to contract and excrete pathogens than those in good conditions.

Finally, microbiological exams should be carried out on the new animals some days after they have been introduced into the domestic environment. The test should be periodically repeated, because of the intermittent excretion of pathogens and because reptiles could become infected after their introduction in the new habitat. When a pathogen is detected, it is important to define an appropriate treatment. The antimicrobial sensitivity test, when it is possible, is of great importance to choose the most effective antibiotic and to avoid the development of antimicrobialresistant bacterial strains. If infections by leptospirae, chlamydiae or mycobacteria are diagnosed, the treatment is very difficult. Although marbofloxacin has not demonstrated fully efficacy against C. pneumonaie, Rüegg et al. suggested a treatment with this drug for 35 days [44]. In other cases, treatment with tetracycline has been successfully employed [37]. No data are available about the antibiotic therapy against leptospirosis; it can be supposed that penicillins and streptomycin are effective as in mammals. The treatment of mycobacteriosis in reptiles is not always recommended, because usually the infection is diagnosed in an advanced stage and the potential therapy is very expensive and longlasting [31].

In conclusion, several zoonotic bacteria may be excreted by captive and wild reptiles. Some of them are well known as causative agents of infection and disease in mammals and/or birds, but few studies have been carried out to determine their epidemiology and pathogenicity in cold-blooded animals. Regular monitoring of reptiles, in particular those kept in captivity, is necessary to know the spreading of the pathogens and the consequent risk of infection for human beings.

Conflict of interest statement

We declare that we have no conflict of interest.

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