SALMONELLOSIS DUE TO SALMONELLA HOUTEN IN CAPTIVE DAY GECKOS
(GENUS: PHELSUMA GRAY)

TRICHA J. MURPHY AND ALAN A. MYERS

Department of Zoology, University College, Cork, Ireland

(Accepted 19.11.92)

ABSTRACT

Two species of Sabnonella are reported from captive Phelsuma spp., one of which (Sabnonella houten), was pathogenic. Twelve geckos developed clinical signs of anorexia, diarrhoea, dehydration and cachexia. Ten died over a period of eight weeks and deaths occurred three to six weeks from the commencement of illness. Necropsy findings included dehydration, emaciation and liver necrosis. Sabnonella houten was isolated. The response of sick geckos to antibiotics and supportive therapy is discussed.

INTRODUCTION

The normal flora of reptiles includes a wide range of Gram +VE and Gram -VE microorganisms especially of the family Enterobacteriaceae (Escherichia coli, Proteus spp., Klebsiella spp., Aeromonas spp. and Pseudomonas spp. Bacteria also play an important role in reptilian diseases (Cooper, 1981). Potentially pathogenic organisms include Sabnonella spp. of which over 1300 serotypes have been isolated from reptiles (Harvey & Price, 1983). According to Cooper (1981), however, the small number of Sabnonella (< 4%) found in commensal reptilian flora are usually non-pathogenic. Salmonellosis was first reported in reptiles by Caldwell & Ryerson (1939) in wild horned lizards (Phrynosoma solare), chuckwalla (Sauromalus ater) and Gila monsters (Heloderma suspectum) in Arizona, Texas. They named the pathogen S. arizona and found it to be most similar to a Salmonella sp. initially isolated from a case of human pyrexia in Dar-es-Salaam, East Africa. Since then, and primarily because of the problem of zoonoses, salmonellosis has attracted considerable attention amongst herpetologists and veterinarians. Comparatively little is known about the treatment of the disease in reptiles since it is generally asymptomatic, especially in the early stages of infection and thus therapy is difficult.

The majority of work on Sabnonella to date, in relation to reptiles, has been carried out with turtles and tortoises, since these come in contact with humans more frequently than do other reptiles, as a result of their popularity as pets. However, within the last 20 years an increasing number of studies have investigated zooneses of snakes and lizards. In one study (Onderka & Finlayson, 1985) twenty-two (48%) out of forty-six lizards were infected with various Sabnonella serotypes and five (11%) died from salmonellosis. The percentage of all reptiles harbouring Salmonella is estimated at 93.7% and may be as high as 77% in lizards (Chiodini & Sundberg, 1981). Because of the zoonotic implications of this carrier rate, reptiles have been the subject of extensive studies. Obegbulem & Isegbohimhen (1985) reviewed the potential health risk from peridomestic Wall geckos (Gecko gecko and Hemidactylus spp.) and suggested that they may act as a primary reservoir or natural carrier of Salmonella for humans. Similar findings are outlined by Dhiraputra & Chavalittamrong (1979) in Gecko fascicularis in Bangkok. Kaura, Sharma & Singh (1970) found a 95.5% carrier-rate of Sabnonella in the herbivorous lizard, Uromastix hardwickei. The occurrence of Sabnonella in lizards has important epidemiological implications and an increasing number of studies have considered the routes of transmission of infection (Hinshaw & MacNeil, 1947; Collard & Montefiore, 1957; Chambon, Le Minor & Martin, 1959; Kaura & Singh, 1968; Kaura et al., 1970). Refai & Rohde (1969) and Sadek (1970) suggested transmission of Salmonella from mosquitoes and other flies to geckos and from humans to reptiles (Iveson, 1979). Fears of zoonoses are not unfounded. A retrospective survey of laboratory-confirmed cases of human clinical salmonellosis in the U. S. estimated that 14% of the approximately 2 million cases each year were turtle-associated (Lamm et al., 1972). Whether a similar pattern occurs in other countries is unknown. Several workers have implicated reptiles as the source of human infections (e.g. Plows, Fretwell & Parry, 1968; delHamel & McInnes, 1971; Lamm et al., 1972; Altmann et al., 1972; Anon., 1992). Other workers have reported similarities between serotypes isolated from humans and those from reptiles in the same area (Mackey, 1955; Collard & Montefiore, 1957; Collard & Sen, 1960; Bockemühl & Moldenhauer, 1970; Kourany, Myers & Schneider, 1970; Baker, Anderson & Allard, 1972; Kaura et al., 1972; Kumar & Sharma, 1978; Helm, 1981; Minette, 1984).

This paper records the clinical and necropsy findings of a Sabnonella houten infection in a group of captive day geckos of the genus Phelsuma Gray. The efficacy of treatment is discussed.

MATERIALS AND METHODS

Twenty-four geckos (one P. quadriocellata (Peters), five P. madagascariensis grandis (Gray), ten P. laicauda Boettger, six P. lineata chloroscelis Mertens and two P. guentheri Boulenger) were maintained at the University facilities in glass or perspex vivaria within a constant temperature room (28°C). Relative humidity levels were maintained at 60-70% by daily misting. Geckos were maintained singly or in pairs, with the exception of three female and one male P. lineata chloroscelis maintained in a single vivarium. Individuals were fed twice weekly with crickets supplemented with "Cricket Plus", a mixture of puréed fruit and Heinz "fruit salad" baby food and provided with ground cuttlefish bone (calcium). All specimens were monitored on a daily basis as part of a programme of behavioural research on aspects of aggression and space utilisation. Thus detailed notes on the behaviour of each individual were available for a period of up to four months prior to the diagnosis of the disease. Emaciated individuals or individuals that refused to eat were force-fed a mixture of condensed milk, dextrose, vitamin sup-
TABLE 1. Microbial sensitivity tests conducted on Salmonella houten, isolated from Phelsuma lineata chlorostilis.

<table>
<thead>
<tr>
<th>Sensitive</th>
<th>Bactericidal</th>
<th>Bacteriostatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin/Clavulanate</td>
<td>Apramycin</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Chloramphenicol</td>
<td></td>
</tr>
<tr>
<td>Framycetin</td>
<td>Furazolidone</td>
<td></td>
</tr>
<tr>
<td>Neomycin</td>
<td>Oxytetracycline</td>
<td>Trimethoprim/ Sulphamethoxazole</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Resistant</th>
<th>Bactericidal</th>
<th>Bacteriostatic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Penicillin</td>
<td>Erythromycin</td>
</tr>
<tr>
<td></td>
<td>Streptomycin</td>
<td>Spectinomycin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sulphafurazole</td>
</tr>
</tbody>
</table>

Twelve geckos with clinical signs of salmonellosis were given antibiotics on the basis of antibiotic sensitivity tests (see results). Ten antibiotics were tested and three (Oxytetracycline, Ampicillin and Furazolidone) were given orally in water by pipette to Phelsuma specimens. Oxytetracyline (dosage 50 mg/kg/day) was administered to one P. lineata chlorostilis and three P. madagascariensis grandis for a period of three days and then discontinued on the advice of the Dept. of Agriculture, Cork. Ampicillin (dosage 3.6 mg/kg/day) was then administered for 12-14 days to four P. laticauda, two P. madagascariensis grandis and one P. quadricellata. Furazolidone (dosage 0.025 mg/g/day) was administered for nine days to one P. quadricellata, one P. laticauda and two P. madagascariensis grandis. All disposable cage furnishings and food supplies were discarded and the room and all cages were disinfected using benzalkonium chloride (as Roccal D). Subsequently hands were disinfected with "Hibiscrub" before entering the room. The remaining stocks were monitored for clinical signs of illness.

Phelsuma faecal samples less than 4-5 hours old, were pre-enriched in Rappaport-Vassiliadis Broth (Oxoid) for 12-24 hours at 37°C and then plated on to MacConkey Agar No. 3 (Oxoid) and XLD medium (xylose-lysine-deoxycholate agar) (Oxoid) and incubated at 37°C. Biochemical analysis of colonies was carried out by a variety of tests including Gram stain, oxidase test, motility, Simmons Citrate Agar, 24 hours at 37°C and then plated on to MacConkey Agar No. 3 (Oxoid) and XLD medium (xylose-lysine-deoxycholate agar) (Oxoid) and incubated at 37°C. Biochemical analysis of colonies was carried out by a variety of tests including Gram stain, oxidase test, motility, Simmons Citrate Agar, Urea Agar and API 20E strips. Serotyping was performed by the Department of Agriculture in Cork and Colindale Laboratories in London. Faecal samples were collected from the Department of Agriculture in Cork and Colindale Laboratories, London.

Twelve geckos with clinical signs of salmonellosis were given antibiotics on the basis of antibiotic sensitivity tests (see results). Ten antibiotics were tested and three (Oxytetracycline, Ampicillin and Furazolidone) were given orally in water by pipette to Phelsuma specimens. Oxytetracyline (dosage 50 mg/kg/day) was administered to one P. lineata chlorostilis and three P. madagascariensis grandis for a period of three days and then discontinued on the advice of the Dept. of Agriculture, Cork. Ampicillin (dosage 3.6 mg/kg/day) was then administered for 12-14 days to four P. laticauda, two P. madagascariensis grandis and one P. quadricellata. Furazolidone (dosage 0.025 mg/g/day) was administered for nine days to one P. quadricellata, one P. laticauda and two P. madagascariensis grandis. All disposable cage furnishings and food supplies were discarded and the room and all cages were disinfected using benzalkonium chloride (as Roccal D). Subsequently hands were disinfected with "Hibiscrub" before entering the room. The remaining stocks were monitored for clinical signs of illness.

Phelsuma faecal samples less than 4-5 hours old, were pre-enriched in Rappaport-Vassiliadis Broth (Oxoid) for 12-24 hours at 37°C and then plated on to MacConkey Agar No. 3 (Oxoid) and XLD medium (xylose-lysine-deoxycholate agar) (Oxoid) and incubated at 37°C. Biochemical analysis of colonies was carried out by a variety of tests including Gram stain, oxidase test, motility, Simmons Citrate Agar, Urea Agar and API 20E strips. Serotyping was performed by the Department of Agriculture in Cork and Colindale Laboratories in London. Faecal samples were collected from the Department of Agriculture in Cork and Colindale Laboratories, London.
Selenite Broth, which has teratogenic effects. The properties of pre-enrichment media are discussed in more detail by Harvey (1983).

Rappaport-Vassiliadis Broth was utilised in preference to preliminary studies with a wide range of growth substrates. In conjunction with the bacteriological findings, were supportive for findings of liver necrosis with heterophilic granulomas, in this study; 2, Zwart et al., 1970.

Rappaport-Vassiliadis, MacConkey Agar No.3 and XLD Medium proved effective for the isolation of Salmonella spp. and a number of other enteric organisms notably Klebsiella oxytoca, Pseudomonas spp., Citrobacter freundii, C. diversus, Enterobacter cloacae, Proteus mirabilis and P. vulgaris. Salmonella spp. appeared as small, dull colourless colonies on MacConkey Agar No. 3 and on XLD medium as distinctive orange and pink colonies with black centres resulting from hydrogen sulphide production. The Salmonella carrier-rate was 95.8% (23 out of 24 geckos) in treated stocks and no regular pattern of Salmonella excretion from the gut was detected.

Results of antibiotic sensitivity tests are given in Table 1. Ampicillin was the most effective antibiotic in the in vivo treatment of Phelsuma in this work based on the recovery of two individuals from clinical signs. Furazolidone, used on one individual, was not successful and once treatment ceased two individuals from clinical signs. Furazolidone, used on the basis of sensitivity tests and recovery from clinical signs of disease, despite being a broad spectrum antibiotic.

Furazolidone is not therefore recommended for clinical signs which recur and the individual died. Subsequent histological examination revealed necrosis of the liver and Salmonella was detected by IFAT.

Salmonella (including S. houten) were also isolated and serotyped from control specimens (P. abbotti, P. laticauda, P. standingi and P. barbouri) (Table 2).

DISCUSSION

The frequency of isolation of Salmonella from clinical pathology and necropsy specimens would indicate that this was the cause of the illness and death of the geckos. The necropsy findings of liver necrosis with heterophilic granulomas, in conjunction with the bacteriological findings, were supportive of a diagnosis of salmonellosis.

The pre-enrichment and selective enrichment procedures for Salmonella used in this study were chosen following preliminary studies with a wide range of growth substrates. Rappaport-Vassiliadis Broth was utilised in preference to Selenite Broth, which has teratogenic effects. The properties of pre-enrichment media are discussed in more detail by Harvey & Price (1983).

Kaura, Sharma & Chandiramani (1981) determined the immunological response of five Uromastyx hardwickii to Salmonella which they harboured and found an absence of haemagglutinins in the lizard sera (dilution 1:40). They concluded that this indicated a good host-parasite relationship.

Before treatment of a Salmonella infection is attempted, it is generally recommended that antibiotic sensitivity tests are performed and information on the properties of effective agents obtained (Watson, 1977; Cooper, 1981; Holt, 1981; Jackson, 1981). In general, narrow spectrum drugs are preferable since they conserve bacterial flora. Prophylactic drugs e.g. chloramphenicol should be avoided (Hamilton-Miller, 1975) and bacteriocidal drugs (penicillins, cephalosporins, aminoglycosides and polyoxymyxins) are preferable to bacteriostatic ones (Watson, 1977). Antibiotics which are essentially bacteriostatic include tetracyclines, chloramphenicol, macrolides, lincomycin, sulphonamides and nitrofurans (Watson, 1977). The use of drug "cocktails" is sometimes considered inadvisable (Jawetz, 1975; Watson, 1977) as they are generally of little benefit and can be toxic. However, ampicillin and chloramphenicol used in combination eliminated Salmonella in turtles and tortoises (Koopman & Kennis, 1976) and neomycin and oxytetracycline suppressed but did not eliminate an infection in terrapins (Pseudemys sp.) (Siebeling, Neal & Granberry, 1975). The clinical condition of the animal and the appropriate dosage to administer are other parameters to consider, especially if utilising drugs with contra-indications. Tetracycline, erythromycin or chloramphenicol are not recommended for mammals with hepatic dysfunction (Watson, 1977), although these are often the antibiotics prescribed for reptiles (Murphy, 1975; Lawrence, 1983). The efficacy of the drug at different temperatures can also vary. Studies on gentamicin, for example, have shown it to be nephrotoxic at high temperatures (e.g. above 24°C in Natrix fasciata confluens) (Hodge, 1978). Lawrence (1983) stressed that all dose regimes for reptiles should be accompanied by recommended environmental temperatures, especially if utilising drugs with contra-indications. In the present work, therapy was not fully effective and did not prevent the progression of the condition in most cases. Siebeling et al., (1975), found that antibiotics suppressed but did not completely eliminate the excretion of Salmonella from turtles. It is probable that antimicrobial treatment is most effective at the very early stages of infection when diagnosis is most difficult. For the treatment of Salmonella in reptiles, trimethoprim is a frequently recommended drug (Chris Marshall, pers. comm.). In the present work, ampicillin was used on the basis of sensitivity tests and recovery from clinical signs of disease, despite being a broad spectrum antibiotic. Lawrence et al., (1983) found that of thirty-two Salmonella isolates from reptiles tested, only four were ampicillin resistant. Furazolidone has been used successfully in fish and poultry but no report of its administration to reptiles could be located in the literature. This antibiotic is a nitrofuran, bacteriostatic drug and in this study resulted in a cessation of clinical signs which occurred once treatment ended. Furazolidone is not therefore recommended for Phelsuma. According to Bullock, Conroy & Snieszko (1971) furazolidone leaves tissue residues in fish which can build up to toxic concentrations after prolonged treatment, especially in animals with renal failure.

In apparently clinically healthy reptiles, Kaura et al., (1970), found that 16.4% (n = 134) of lizards examined car-

<table>
<thead>
<tr>
<th>Phelsuma species</th>
<th>Salmonella species</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. abbotti</td>
<td>S. houten¹</td>
</tr>
<tr>
<td>P. barbouri</td>
<td>S. 45: g, z51: -¹</td>
</tr>
<tr>
<td>P. laticauda</td>
<td>S. houten¹</td>
</tr>
<tr>
<td>P. lineata chloroscelis</td>
<td>S. houten¹</td>
</tr>
<tr>
<td>P. madagascariensis</td>
<td>S. enteritidis var chaco and S. soesterberg²</td>
</tr>
<tr>
<td>P. standingi</td>
<td>S. houten¹</td>
</tr>
</tbody>
</table>

TABLE 2. Salmonella species isolated from Phelsuma species. 1, this study; 2, Zwart et al., 1970.
ried *Sabronella* in one or more internal organs such as the liver, spleen, gall bladder, ovary and testes. Gupta, Pal & Narula, (1980) isolated *Sabronella* from the liver/gall bladder of 6.5% (n = 92) *Hemidactylus flaviviridis*. In the present work post-mortem histological examination of *Sabronella* infected specimens revealed changes in the liver, characterised by necrotic foci and large numbers of phagocytic melanomacrophages. These cells had ruptured causing local dispersion of the pigment granules which, according to Roberts (1978), is indicative of toxaeonic conditions in fish. Although little is known of these cells in reptiles, in fish they are thought to have a defensive function, acting as a source of quinone free-radicals and in association with peroxidase, as a bacteriocidal system (Ellis, 1977). Similar histological changes have been reported in the experimental infection of *Python molurus* with *S. arizona* (von Schröder & Ippen, 1970). Gram staining histologically prepared sections of liver and intestinal tissue of *Phelsuma* proved particularly effective in the detection of bacteria and IFAT proved useful in verifying their identification as *Sabronella*.

A study by Habermalz & Pietzsch (1973) on *Sabronella*, isolated from 250 species of reptiles and amphibians in Berlin Aquarium, includes *Phelsuma madagascariensis* and an unspecified *Phelsuma* sp. Two serotypes (*S. nina* and *S. mosselbay*) were isolated in cages holding *Phelsuma* mixed with other gecko species (*Oedura tryoni*, *O. monilis* and *Gecko smithi*) so the exact origin of these serotypes is unknown. Mayer & Frank (1974) isolated *S. arizona* (S. a: k: z35) from a gecko species and provide a plate of a paracolon infection in the liver of a *Phelsuma* sp. It is not clear however whether the gecko referred to in the text is the same as that illustrated. The *Sabronella* serotypes isolated from *Phelsuma* species in this study and two serotypes isolated by Zwart, Poelma & Strik (1970) from *P. madagascariensis* (subgenus I and subgenus IV strain), are given in Table 2. Subgenus II and subgenus IV salmonellae were isolated from *Phelsuma* in the present work. These are generally considered non-pathogenic or only mildly pathogenic in humans.

In Denmark, Nielson & Clausen (1975), found that imported reptiles posed a potential risk to owners and handlers. Considering the high levels of *Sabronella* occurring as commensal bacterial flora in reptiles and the increasing popularity of these animals as pets, they may constitute a public health problem. The carrier rate, however, appears to be low in wild gekkonids e.g. from 2% in *Hoplodactylus pacificus* (deHamel & McInnes, 1971) to 33% in *Peropus mutilatus* (Bockemühl & Moldenhauer, 1970) compared with other lizards which may exceed 95% (*Kaura et al., 1970 - Uromastix hardwickei; Kourany et al., 1970 - Ameiva festiva and Le Minor, Chamblon, Bories, Marx & Charie-Mansaines, 1962 - Leiolepis bellina guttata*).

The results of this study suggest that *Sabronella* bacteria are probably part of the normal commensal intestinal flora of *Phelsuma* species. However, stressed individuals (used for behavioural research), readily succumbed to an opportunistic invasion of *Sabronella* which resulted in the deaths of 42% of stocks (n = 24).

**PRODUCTS LISTED IN TEXT**


**ACKNOWLEDGEMENTS**

We are grateful to Mr. Ger Ryan, Mr. Eugene Power and Mr. Pat Sheehan, Dept. of Agriculture, Cork and Mr. David Smyth, Dunmanway, for veterinary advice; to Mrs. Therese McAuliffe, Mrs. Mary Cross, Mr. Paddy O'Reilly and Dr. Bridget Foley for assistance with bacteriology; to Dr. Chutima Hanjanvanit and Dr. Eamonn Twomey for help with histology; and to Mrs. Sandy O'Driscoll and Mr. Mark O'Shea for technical support. Thanks are extended to Mr. Eugene Power, Dept. of Agriculture, Cork; Dr. Jim Claire and Mr. Tony Fitzgerald, Microbiology Dept., Mercy Hospital, Cork; Dr. Kevin Collins and Dr. Maurice O'Donoghue, Microbiology Dept., U.C.C. for helpful comments on the manuscript.

**REFERENCES**


Kumar, A. & Sharma, V. K. (1978). Enterobacteria of emerging pathogenic significance from clinical cases in man and animals and detection of toads and wall lizards as their reservoirs. Antiope van Leeuwenhoek 44, 219-228.


SALMONELLA IN DAY GECKOS


