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

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ABSTRACT

Two species of *Salmonella* are reported from captive *Phelsuma* spp., one of which (*Salmonella 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. *Salmonella 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 *Salmonella* spp. of which over 1300 serotypes have been isolated from reptiles (Harvey & Price, 1983). According to Cooper (1981), however, the small number of *Salmonella* (< 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*), chuckwallas (*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 *Salmonella* 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 zoonoses of snakes and lizards. In one study (Onderka & Finlayson, 1985) twenty-two (48%) out of forty-six lizards were infected with various *Salmonella* 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. Oboegbulem & Iseghohimhen (1985) reviewed the potential health risk from peridomestic Wall geckos (*Gecko gecko* and *Hemidactylus* sp.) 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 *Salmonella* in the herbivorous lizard, *Uromastix hardwicki*. The occurrence of *Salmonella* 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; deHamel & 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 *Salmonella 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. laticauda* 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-

plementation (especially D_3) and Heinz "fruit salad" with "Cricket Plus". A course of Dioralyte (an electrolyte balancing fluid) was also given to prevent dehydration. A control stock of specimens (*P. abbotti* Stejneger, *P. madagascariensis grandis*, *P. flavigularis* Mertens, *P. standingi* Metheun & Hewitt and *P. barbouri* Loveridge) were maintained at the junior author's premises.

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 (Oxytetracyline, Ampicillin and Furazolidone) were given orally in water by pipette to *Phelsuma* specimens. Oxytetracyline (dosage 50 mg/kg/day^{-1}) was administered to one *P. lineata chloroscelis* 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^{-1}) was then administered for 12-14 days to four *P. laticauda*, two *P. madagascariensis grandis* and one *P. quadriocellata*. Furazolidone (dosage 0.025 mg/g/day^{-1}) was administered for nine days to one *P. quadriocellata*, 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 benzalkolium 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-desoxycholate 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 individual *Phelsuma* specimens to observe the frequency of *Salmonella* excretion. After death, samples from the liver, intestines and blood were cultured for *Salmonella* following the method described by Needham (1981, 1985) and Harvey & Price (1983).

Tissues were fixed either in Bouin's Fluid or in 70% ethanol, embedded in paraffin, sectioned at 7μm and stained with haematoxylin and eosin. Selected tissue sections were stained by the Gram method and examined using the Indirect Fluorescent Antibody Technique (IFAT).

Antibiotic senstivity tests were performed on the *Salmonella* isolates (Pat Sheehan, Dept. of Agriculture, Cork).

RESULTS

Clinical signs of illness in all cases, included apathy towards food, semi-solid faeces, raised frequency of ecdysis, dysecdysis, reduced response to external stimuli and dryness and looseness of skin, (an indication of dehydration, David Smyth, pers. comm.). Death generally occurred within three weeks of the first clinical signs, in the case of the smaller(< 140mm) species and up to six weeks in larger(> 170mm) species. Ten geckos died within eight weeks. These comprised one female *P. madagascariensis grandis*; one male and two female *P. lineata chloroscelis*; one female and four juvenile *P. laticauda* and one male *P. quadriocellata*. A further two individuals (one male *P. laticauda* and one male *P. madagascariensis grandis*) exhibited clinical signs of salmonellosis, were treated with antibiotics and after a period of 34 days and 46 days respectively, clinical signs ceased. Supportive therapy probably assisted in recovery and is strongly recommended in all cases of illness (Jackson, 1981; Lawrence, 1983).

The causative organism of the outbreak proved to be *Salmonella houten*, Subgenus IV 43: z4, z23: - (confirmed by the Dept. of Agriculture, Cork and Colindale Laboratories, London).

The gross necropsy findings were of cachexia, dehydration, pale swollen friable livers and marked congestion of the intestines. Liver samples in six cases showed abnormally large clumps of melanomacrophages (highly phagocytic tissue cells containing melanin). Large numbers of these cells had rup-

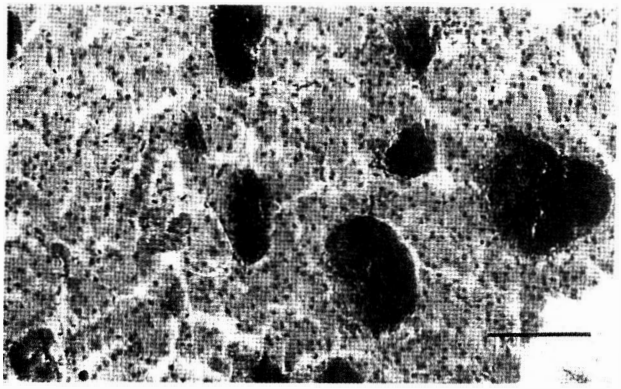

Fig. 1. Ruptured melanomacrophage cells in a liver section (stained with H & E) of a male *Phelsuma quadriocellata* (Peters), Scale bar = 50 μm

Sensitive		Resistant	
Bacteriocidal	*Bacteriostatic*	*Bacteriocidal*	*Bacteriostatic*
Amoxycillin/Clavulanate	Apramycin	Penicillin	Erythromycin
Ampicillin	Chloramphenicol	Streptomycin	Spectinomycin
Framycetin	Furazolidone		Sulphafurazole
Neomycin	Oxytetracycline		
	Trimethoprim/ Sulphamethoxalole		

TABLE 1. Microbial sensitivity tests conducted on *Salmonella houten*, isolated from *Phelsuma lineata chloroscelis*.

Phelsuma species	*Salmonella* species
P. abbotti	*S. houten*[1]
P. barbouri	*S.* 45: g, z51:-[1]
P. laticauda	*S. houten*[1]
P. lineata chloroscelis	*S. houten*[1]
P. madagascariensis	*S. enteritidis* var *chaco* and *S. soesterberg*[2]
P. standingi	*S. houten*[1]

TABLE 2. *Salmonella* species isolated from *Phelsuma* species. 1, this study; 2, Zwart *et al.*, 1970.

tured causing local dispersion of the pigment granules (Fig. 1). The livers of three individuals contained granulomas, which appeared similiar to a heterophilic granuloma i.e. an abscess induced by masses of degenerated heterophils that are elicited by bacterial organisms (Montali, 1988; Montali *et al.*, 1989).

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 freundi*, *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 the clinical signs recurred 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 *Uromastix hardwicki* 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 polymyxins) 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 recurred 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-

ried *Salmonella* in one or more internal organs such as the liver, spleen, gall bladder, ovary and testes. Gupta, Pal & Narula, (1980) isolated *Salmonella* from the liver/gall bladder of 6.5% (n = 92) *Hemidactylus flaviviridis*. In the present work post-mortem histological examination of *Salmonella* 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 toxaemic 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-radicles and in association with peroxidase, as a bacteriocidal system (Ellis, 1977). Similiar 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 *Salmonella*.

A study by Habermalz & Pietzsch (1973) on *Salmonella*, isolated from 250 species of reptiles and amphibians in Berlin Aquarium, includes *Phelsuma madagascariensis* and an unspecified *Phelsuma* sp. Two serotypes (*S. nima* 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.* 38: 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 *Salmonella* 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 midly 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 *Salmonella* 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 hardwicki*; Kourany *et al.*, 1970 - *Ameiva festiva* and Le Minor, Chambon, Bories, Marx & Charie-Marsaines, 1962 - *Leiolepis bellina guttata*).

The results of this study suggest that *Salmonella* 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 *Salmonella* which resulted in the deaths of 42% of stocks (n = 24).

PRODUCTS LISTED IN TEXT

Cricket Plus, Monkfield Nutrition, Monkfield, Bourn, Cambridgeshire CB3 7TD, England; Dioralyte, Rhone-Poulenc Rorer Ltd., Eastbourne, BN21 3YG, England; Hibiscrub, ICI Pharmaceuticals (U.K.), Kingscourt, Waterlane, Wilmslow, Cheshire, SK9 5AZ, England; Roccal D, Sanofi Winthrop Ltd., 1 Onslow Street, Guildford, Surrey, GU9 4YS, England.

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