One Health 19 (2024) 100848

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Salmonella carriage by geckos detected within households in Malawi

Catherine N. Wilson [a,b,c,l,*], Patrick Musicha [b,c], Mathew A. Beale [c], Yohane Diness [b], Oscar Kanjerwa [b], Chifundo Salifu [b], Zefaniah Katuah [b], Patricia Duncan [d], John Nyangu [d], Andrew Mungu [d], Muonaouza Deleza [e], Lawrence Banda [e], Lumbani Makhaza [b], Nicola Elviss [f], Christopher P. Jewell [g], Gina Pinchbeck [a], Nicholas R. Thomson [c,k], Nicholas A. Feasey [b,i,j], Eric M. Fèvre [a,h]

[a] *Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK*
[b] *Malawi Liverpool Wellcome Programme, Blantyre, Malawi*
[c] *Wellcome Sanger Institute, Hinxton, UK*
[d] *Ministry of Agriculture and Food Security, Department of Animal Heath and Production, Malawi*
[e] *Lilongwe University of Agriculture and Natural Resources, Bunda, Lilongwe, Malawi*
[f] *UK Health Security Agency, London, UK*
[g] *Lancaster University, Lancaster, UK*
[h] *International Livestock Research Institute, Nairobi, Kenya*
[i] *Liverpool School of Tropical Medicine, Liverpool, UK*
[j] *The School of Medicine, University of Andrews, St. Andrews, UK*
[k] *The London School of Hygiene and Tropical Medicine, London, UK*
[l] *The Queen's Veterinary School Hospital, University of Cambridge, UK*

ARTICLE INFO

Keywords:
Salmonella
Gecko
Antimicrobial resistance
Malawi
Household

ABSTRACT

Salmonella was isolated from 23/79 (29.1%) pooled gecko stool samples from households in southern Malawi. Whole genome sequencing of 47 individual isolates within this collection revealed 27 *Salmonella* serovars spanning two subspecies. Our results demonstrate that geckos play an important role in the carriage of *Salmonella* within households.

1. Introduction

Salmonella is a major cause of foodborne disease globally and this zoonosis represents a concern to both human and animal health. *Salmonella* can be carried asymptomatically within the stool of humans, animals and within the environment. Serovars of *Salmonella enterica* subspecies *enterica* are consistently associated with disease in warm-bloodied animals, while non-*enterica* subspecies of *Salmonella enterica* have been associated only with opportunistic infection in humans [1,2]. Some of the *S. enterica* subsp. *enterica* serovars, including *S. enterica* subsp. *enterica* serovar Typhimurium and *S. enterica* subsp. *enterica* serovar Enteritidis, have a broad host range. Others are associated with disease in a single species but are also, less commonly, associated with disease in other animal species. Host-restricted serovars only cause disease within a single species. This means that ecologically, characterisation of the *Salmonella* spp. present within a household is important to gauge the potential zoonotic risk for other species present.

The specific pathway of direct transmission of *Salmonella* spp. between animals to humans or vice versa requires high-resolution genomic techniques and is extremely difficult to capture. Frequently, inference for the potential for transmission is made by the documentation of carriage of specific *Salmonella* isolates within the faeces of the host animal species and the presence of the identical serovar or sequence type within the faeces of the 'recipient' host [5].

There are over 1850 species of gecko worldwide [3]. It has not been thoroughly documented which species of gecko are present in Malawi, although *Chondrodactylus turnei, Hemidactylus mercatorius* and *platycephalus, Lygodactylus angularis, bonsi* and *rex* and *Pachydactylus capensis* and *kataganus* have been previously reported to be present within the country [3]. Geckos are primarily nocturnal, although some individuals

* Corresponding authors at: Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, UK.
E-mail addresses: cnw25@cam.ac.uk (C.N. Wilson), eric.fevre@liverpool.ac.uk (E.M. Fèvre).

https://doi.org/10.1016/j.onehlt.2024.100848
Received 30 June 2024; Accepted 1 July 2024
Available online 11 July 2024

may be active during the day. The diet of geckos includes arthropods such as isopods, centipedes, spiders, scorpions, cockroaches, beetles, moths, flies, mosquitoes as well as vertebrates such as other geckos and anoles [4]. Free-living and captive reptiles have been routinely identified as reservoirs of *Salmonella* spp. [6–8]. Reptiles have been previously found to carry a number of serovars, some of which are linked to invasive disease in humans [9,10]. Previously, a high prevalence (23.9%) of *Salmonella* carried within gecko faeces has been described in Asia [11]. A previous study in Nigeria published in 1985 used microbiological techniques to detect a carriage rate of (30%) amongst ninety geckos across a more limited diversity of *Salmonella* serotypes [12]. The following study reports the prevalence of carriage and genomic characterisation of *Salmonella* detected within the faeces of free-living geckos within a household environment in Africa.

2. The study

This observational study of *Salmonella* carriage by geckos was undertaken as part of a prospective longitudinal cohort study to investigate flux of *Salmonella* between humans, animals and the environment within households in Malawi. Thirty households were sampled at three time points over the period of a year (November 2018–December 2019) in both an urban (Ndirande) and a rural (Chikwawa) study site in southern Malawi, to investigate carriage of *Salmonella* by humans, by animals and within the household environment. The two study sites lie approximately 50 km apart; Chikwawa is located 108 m above sea level, and Ndriande is much higher; 1119 m above sea level; as such the study sites have different mean ambient temperatures of 24.2 °C and 20.6 °C respectively [13]. Stool samples were collected from all humans within the household and at least one representative of each animal species present in the household, which included livestock (cattle, pigs, goats and sheep), domestic animals (cats, dogs and guinea pigs), poultry (chickens, turkeys, guinea fowl, ducks and doves) and peri-domestic wildlife (geckos, rodents and wild birds). Environmental swabs were collected from areas of high human-human, human-animal or animal-animal contact such as latrines, food preparation areas, and floors of animal pens. Between 20 and 36 samples were collected from each household, depending on the occupants of each individual household at each visit. This article presents characterisation of *Salmonella* detected within the faeces of geckos only. Assistant Veterinary Officers, who carried out the sampling, were appropriately trained by the Study Principal Investigator to recognise gecko faeces. Characteristic samples of gecko faeces (Fig. 1) were collected from the walls of households within the study. Where multiple gecko stool were collected from a single household at a single visit, these samples were pooled prior to further processing.

To detect *Salmonella,* selective microbial culture of the pooled samples of gecko stool from each household collected at a single visit was undertaken using XLD and Harlequin chromogenic agar for *Salmonella* esterase (CASE), following enrichment with buffered peptone water and Rappaport-Vassiliadis solution. Anti-sera testing against the O antigen and qPCR analysis using the *ttr* primer were used to detect and confirm the presence of *Salmonella* and a subset of the isolates (two picks from each pooled sample) were submitted for whole genome sequencing [14]. Positive (*S. Typhimurium* NCTC) and negative control samples were used at all stages of the microbiological analysis.

Ethical approval for this study was obtained from the College of Medicine Ethics Committee (Malawi) (Reference number P.02/18/2368) and the University of Liverpool Research Ethics Committee (Reference number VREC686). Informed consent from household owners was obtained for the collection of gecko faeces from each household at each visit, and for the other samples collected.

3. Results

Salmonellae were isolated from 23/79 (29.1%) of the pooled gecko stool samples. *Salmonella* positivity rate was higher in gecko samples from Chikwawa (21/47 (44.7%)), the rural study site, than Ndirande, the urban study site (2/32 (6.3%)).

Fig. 1. Characteristic gecko faeces; stool has a cylindrical, tapered shape, roughly 1.5 cm long, mainly brown coloured stool with a small white portion which contains urate excretions. Photo: C. Wilson. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Between two to three colony picks of *Salmonella* from each sample were submitted for whole genome sequencing. Forty-seven good quality *Salmonella* genomes were obtained from geckos including forty-two from Chikwawa and five from Ndirande; representing whole genome sequences from 20 discrete gecko stool samples. Of these 20 gecko samples, 19 were from Chikwawa and 1 was from Ndirande. Within sample diversity was detected in seven samples; where two different *Salmonella* serovars were detected from a single pooled sample following whole genome sequencing.

There were 27 unique genomes in the collection of 47 genomes covering 2 subspecies of *Salmonella; S. enterica* and *S. salamae* (Table 1). One whole genome sequence was obtained from isolates detected within Ndirande; *S. enterica* subspp. *Oranienburg*. The genomes isolated from Chikwawa were 11 *S. enterica* covering nine serovars, and 15 *S. salamae* covering 13 different antigenic profiles. Of these 27 different serovars, disease in humans has previously been reported to be caused by ten of these serovars (37.0%).

Genotypic antimicrobial resistance (AMR) determinants were carried by 8/27 (29.6%, 95% CI 15.8–48.5%) of the unique genomes, seven from Chikwawa and 1 from Ndirande. Six of the eight AMR determinants were *fosA7*, two were a variant of the *fosA7* gene; *fosA7.7*. These AMR determinants were all chromosomally integrated.

4. Discussion and conclusions

Here we present for the first time a characterisation of *Salmonella* carried by household geckos in Malawi, demonstrating high carriage rates of *Salmonella* within gecko stool, similar to the 30% and 31.7% prevalence of *Salmonella* documented in gecko faeces from ninety samples collected in Nigeria [12,15]. Other studies across the world have documented a range of prevalences; 23.8% in Vietnam [11], 9% from Iraq [16]. Some of the serovars carried by geckos may have the potential for pathogenicity to both animals and humans, eg *S. Enteritidis*, *S*. Poona and *S*. Concord.

We have found a significantly higher carriage of *Salmonella* within rural households in comparison to those located in an urban area. The

Table 1
Results of whole genome sequencing of *Salmonella* isolates collected from geckos, including genotypic and phenotypic antimicrobial resistance profiles. CHH*/NHH* = individual household number prefix, ukn = unknown, AMR = antimicrobial resistance, S = sensitive, I = intermediate, R = resistant, NA = not applicable.

Study site	Household number	Visit number	Sample ID	Subspecies	Serovar	ST	Genotypic AMR	Phenotypic AMR					
								Tetracycline	Nalidixic acid	Chloramphenicol	Cefpodoxime	Streptomycin	Ampicillin
Chikwawa	CHH1	1	21	salamae	II 1,13,23:z29:e,n,x	1188	none	S	S	S	S	I	R
Chikwawa	CHH1	2	20	salamae	II 1,4,12,27:-:1,5	ukn	*fosA7*	S	S	S	S	I	S
Chikwawa	CHH1	1	21	salamae	II 35:z29:1,5,7	ukn	*fosA7*	S	S	S	S	S	S
Chikwawa	CHH1	2	20	enterica	Concord	694	none	S	S	S	S	S	S
Chikwawa	CHH2	2	5	salamae	II 1,4,12,27:-:1,5	ukn	*fosA7*	S	S	S	S	I	S
Chikwawa	CHH2	3	21	enterica	Anatum	64	none	NA	NA	NA	NA	NA	NA
Chikwawa	CHH2	3	21	enterica	Gaminara	2152	none	NA	NA	NA	NA	NA	NA
Chikwawa	CHH2	3	5	enterica	Kingabwa	546	*fosA7.7*	S	S	S	S	I	S
Chikwawa	CHH3	3	13	enterica	Jangwani	3918	none	NA	NA	NA	NA	NA	NA
Chikwawa	CHH4	2	20	salamae	II 1,13,23:z29:-	ukn	none	NA	NA	NA	NA	NA	NA
Chikwawa	CHH4	3	18	enterica	Poona	2566	none	S	S	S	S	S	S
Chikwawa	CHH5	3	6	salamae	II 41:l,[z13],[z28]:z39	ukn	none	S	S	S	S	I	S
Chikwawa	CHH5	1	22	enterica	Ahuza	2215	*fosA7.7*	R	S	S	S	S	S
Chikwawa	CHH6	1	20	salamae	II 35:g,z62:-	ukn	none	S	S	S	S	S	S
Chikwawa	CHH7	2	13	salamae	II 39:a:1,5,7	ukn	none	S	S	S	S	S	S
Chikwawa	CHH8	1	18	salamae	II 1,4,12,27:e,n,x:e,n,x	ukn	*fosA7*	S	S	S	S	I	S
Chikwawa	CHH8	1	18	enterica	Gaminara	2152	none	R	S	S	S	I	S
Chikwawa	CHH9	2	22	enterica	Enteritidis	366	none	R	S	S	S	S	S
Chikwawa	CHH11	3	14	enterica	Bukavu	5410	none	S	S	S	S	S	S
Chikwawa	CHH12	3	18	salamae	II 6,7:z:z6	ukn	none	S	S	S	S	R	S
Chikwawa	CHH12	3	18	enterica	Gaminara	2152	none	S	S	S	S	S	S
Chikwawa	CHH14	2	16	salamae	II 1,9,12,46,27:b:1,5	ukn	none	S	S	S	S	S	S
Chikwawa	CHH15	1	8	salamae	II 6,7:z:z6	ukn	none	S	S	S	S	I	S
Chikwawa	CHH15	3	6	salamae	II 35:g,z62:e,n,x	ukn	*fosA7*	S	S	S	S	S	S
Chikwawa	CHH15	2	19	salamae	II 1,13,23:e,n,x:e,n,x	ukn	none	S	S	S	S	S	S
Chikwawa	CHH15	2	19	salamae	II 1,13,23:-:e,n,x	ukn	none	S	S	I	S	I	S
Ndirande	NHH6	3	9	enterica	Oranienburg	ukn	*fosA7*	S	S	S	S	S	S

reasons for this are unclear and may be related to differences in the food availability for the geckos, or climatic factors; the rural area has a higher mean average temperature (24.2 °*C*) than the urban study site (20.6 °*C*). Further work is needed to investigate this in more depth, and explore the ecological niches exploited by geckos that may lead to *Salmonella* exposure.

Some of the *Salmonella* serovars detected may have the potential to cause invasive and non-invasive disease in humans and/or animals, as documented in other published literature [17–22]. In this study, humans and animals were sampled simultaneously and there were no reports of clinical disease affecting any of the sampled hosts, therefore it was presumed all *Salmonella* carriage was asymptomatic.

Geckos play an important role in household ecology, controlling insect populations with little disturbance to human and animal inhabitants of households. At the same time, they freely move around the household environment, leaving stool on surfaces which humans may come into contact with. The finding that geckos carry serovars which have the potential to cause pathogenic disease in other species in sub-Saharan Africa has important implications for public health and adds to the known picture of movement of *Salmonella* bacteria around the household, providing a deeper understanding of the ecology of this bacterial species.

This is not a call to arms against the gecko population and any population control targeting the species at a household level is certainly not the recommendation made here. Rather, we should consider that geckos may act as a sentinel of *Salmonella* within households. It is likely that geckos are colonised by *Salmonella* following consumption of contaminated insects or environmental material around the household and so the prevalence of *Salmonella* detected within gecko stool may reflect the overall level of *Salmonella* contamination of the household environment.

It is important that hygiene precautions are taken within households where geckos are present to minimise the likelihood of transmission of *Salmonella* bacteria to other animals or humans and their environment. Important interventions to achieve this and so decrease the risk of faeco-oral bacterial transmission include good hand hygiene (particularly prior to preparation of food or following use of the bathroom), regular sweeping and cleaning of living spaces, keeping rubbish off the floor, keeping dishes and utensils clean and ideally in a sealed container between usages and cleaning the household with appropriate and effective disinfectants. These actions should help to reduce the risk of zoonotic transmission of *Salmonella* between geckos and humans.

CRediT authorship contribution statement

Catherine N. Wilson: Formal analysis, Investigation, Project administration, Writing – original draft. **Patrick Musicha:** Supervision, Writing – review & editing. **Mathew A. Beale:** Supervision, Writing – review & editing. **Yohane Diness:** Investigation. **Oscar Kanjerwa:** Investigation. **Chifundo Salifu:** Investigation. **Zefaniah Katuah:** Investigation. **Patricia Duncan:** Supervision, Writing – review & editing. **John Nyangu:** Investigation. **Andrew Mungu:** Investigation. **Muonaouza Deleza:** Investigation. **Lawrence Banda:** Investigation. **Lumbani Makhaza:** Supervision, Writing – review & editing. **Nicola Elviss:** Methodology. **Christopher P. Jewell:** Methodology. **Gina Pinchbeck:** Supervision, Writing – review & editing. **Nicholas R. Thomson:** Supervision, Writing – review & editing. **Nicholas A. Feasey:** Supervision, Writing – review & editing. **Eric M. Fèvre:** Supervision, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgement

We would like to thank the households recruited to this study for their participation in the sample collection. This work has been supported by a Wellcome Trust Clinical Fellowship award to Catherine N. Wilson, grant number 203919/Z/16/Z.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100848.

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