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Microbial Load in the Gut of Agama Lizards and Wall Geckos

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ABSTRACT

The aim of this study was to examine the differences in the microorganisms and microbial load present in the gut of some lizards from two locations. Ten samples each of the male and female rainbow lizard (*Agama agama africana*) and wall gecko (*Hemidacty lusfrenatus*) were purchased and their morphometric parameters taken while three representative samples were tested for their microbial load and the microorganisms they carry. It was observed that the Total Viable Count (TVC) of the male (1.70Cfug^{-1}), the Total Fungal Count (TFC) of the female agama lizards (0.23Cfug^{-1}) and the Total Coliform Count (TCC) of both male and female agama lizards (0.70 Cfug^{-1} and 0.63Cfug^{-1} respectively) in Abeokuta was greater than that of Ibadan at $p \leq 0.05$ while the wall geckos showed no significant difference. This work revealed that location can be a determinant factor of the type and number of microorganisms present in the gut of lizards.

Keywords: Lizards, microbial load, microorganisms, morphometric, samples

INTRODUCTION

Reptiles are animals with back bone (vertebrates), their skin is made up of either scales or bony plates and in some of them, and it is a combination of both scales and the bony plates. Such animals include lizards, tortoises, chameleons, snakes, crocodiles, tuatara e.t.c. The tuatara is one of the known large reptiles; it looks like a lizard and commonly found on the island off the coast of New Zealand. It is the last survivor of a whole group of ancient reptiles (EMBL Reptile Database, 2003). About 6000 different kinds of reptiles are known to live in the entire world. They are of diverse sizes, shapes and habits because reptiles have gone through many changes since their ancestors lived millions of years ago. Lizards are extremely varied in form. There are long, slender snake-like racers; earthworm-like burrowers; stumpy-tailed short-bodied rock dwellers; long-tailed varieties capable of running swiftly on sand, earth and the surface of water; lumbering monsters living on land or in trees; spiny pancake-shape species; slick-skinned agile tree climbers and burrowers and still other too varied and numerous to describe (Ogundimu, 2019).

Geckos are small when compared to an average agama lizard; they are common reptiles classified to the family Gekkonidae and are mostly found in warm climates throughout the world (Keller *et al*., 2002). These small wall geckos found in houses are non-venomous and not harmful to humans, there is no record of any harmful report of geckos found on the wall of houses. There is an assumption that gecko exctreta contains bacteria that could have varied effect on the environment. When they are warm, they can be active, but when they are cold, they are sluggish or inert. They develop a horny scale cover on the surface of their skin and this helps to prevent the loss of body water. Respiration is by lung, but improved by the development of ribs into a bellows like device that expands and contracts the pleural cavity (Gordan, 1995). Geckos are

distinct among reptiles in their vocalizations, making chirping sounds in social interactions with other geckos. Many species of wall geckos have suction toe pads that enable them to climb smooth vertical and even horizontal surfaces with ease (Keller *et al*., 2002). A number of reptiles, lizards inclusive have been incriminated as the source of bacteria pathogens (Oboegbulem & Iseghohimhen, 1985). More than 1000 Salmonella serovars have been isolated from reptiles (Mitchell, 2006). Agama lizards and geckos are commonly found both in rural and urban area in Nigeria; they have been observed to have association with human in a number of ways. Agama lizards are commonly seen around farm houses and animal pens with free access around the vicinity of residential houses, animal pens, animal feeds and water sources (Ogunleye, Ajuwape, Alaka, & Adetosoye, 2013); this instigates the suspicion of this animal capable of disease transmission possibly through their faeces which may be loaded with the pathogens. The insight into the role of Agama agama lizards in disease transmission can be traced to the 1950s when Salmonella Agama was first characterized as a new serotype of Salmonella enterica from faeces of agama lizard (Agama agama) in Nigeria (Collard & Sen, 1960).

In Nigeria, particularly, just about every house has its population of geckos which are insectivorous found particularly around light sources and storage areas. This creates an opportunity for them to be vectors in disease transmission through their simple life processes of feeding and excretion as most of the food material (insects) ingested by these geckos harbour some enteric pathogens. Most often they station themselves along walls and windows sills where there is sufficient light to attract insects (Nwachukwu, Duru, Nwachukwu & Anomodu, 2014). Although they are mostlyactive at night, their presence is recognized by their faeces (Chan, Chero, Young, & Bureng, 1990). Agama lizards on the other hand are prominently seen to have close association with human especially outdoors on walls and fences and particularly around bins while wall geckos are more closely seen indoors. Lizards have been known to eliminate bacteria pathogens such as Salmonella through their digestive tract without any apparent clinical symptoms, but rather serving as potential sources of contamination as well as infections to the environment and man and animal (Shinohara *et al.,* 2008; Carvalho, Junior, Andrade & Jayme, 2013). This paper therefore aims at assessing the microbial load in the gut of the study lizards (wall gecko, male and female agama lizards) from the different locations (Ibadan and Abeokuta), occurrence of pathogens and to debunk some of the most widely held misconceptions about lizards as insignificant primary host of zoonotic diseases.

MATERIALS AND METHODS

The study was conducted in Abeokuta and Ibadan, Southwest Nigeria. Abeokuta is the capital city of Ogun State and is situated at 7°9’39”N and 3°20’54”Eon the Ogun River; 64 miles north of Lagos by railway, or 81 miles by water. Itoku market is one of the oldest markets in Abeokuta located a few distance away from the famous Olumo rock. The market is well known for the sale of adire (tie and dye) fabrics however there are other segments of the market where tradomedicinal materials are sold. Ibadan (the town at the junction of the savannah and the forest), the capital of Oyo State, is the third largest city in Nigeria by population (after Lagos and Kano), and the largest in geographical area and is situated at 7°23’47”N and 3°55’0”E. Bode market is one of the popular markets in Ibadan majorly known for the sale of both plant and animal parts for traditional medicine.

METHOD

The method of Chan *et al.,* (1990) was used. A reconnaissance survey was first made to the two markets to find out their availability but the samples were only available on request. Ten unmutilated samples of male and female rainbow lizard (*Agama agamaafricana)* and wall gecko (*Hemidactylusfrenatus*) was purchased each from Itoku market in Abeokuta and Bode market in Ibadan a day apart making 30 samples each from both locations. This number of samples was purchased for morphometric parameters and released while three representative samples each of these lizards (9 from Abeokuta and 9 from Ibadan altogether 18 samples) was taken to the laboratory at the

Institute of Agriculture, Research and Training, Moor Plantation. The samples were demobilized with chloroform, pinned down and dissected. The entire gut (from the neck region to the anal region) was severed and removed and the gut content squeezed outand mixed with some minced gut tested for their microbial load count. Smear was prepared on grease-free slides and this was then heat-fixed. The smear was stained thinly for 30secs with crystal violet and after grams iodine was flooded on the smear and this remained for 30secs. The smear was then decolourized with acetone until the purple dye no longer flows from the smear, this was washed with water and counterstained with safranin for 30secs. The smear was washed with tap water, blotted dried and then examined under oil immersion. Two results were obtained from this test and these are the Gram reaction and cell shape of each of the bacterium. The organism that retained the purple colouration was Gram positive and those that were able to take up the red colour was the Gram negative.

Plate Count Agar was used to determine total viable count, Eosin Methylene Blue Agar and MacConkey Agar was used to determine the presence of coliforms and gram negative organisms while Potato Dextrose Agar and Yeast Extract Agar was used to determine the presence of fungi and yeasts in the sample. After sterilization, the media were placed in a water bath set at 45°C to maintain the media in molten state. 1g of the sample was weighed into a test-tube containing 9mls of sterile distilled water and serially diluted to the dilution factor (10^{-6}). 1ml of the dilution factor (10^{-6}) was dispensed into labelled petri dishes. Pour plate method was used as the medium (Plate Count Agar, Eosin Methylene Blue Agar, MacConkey Agar, Potato Dextrose Agar and Yeast Extract Agar) was poured respectively into separate petri dishes as labelled and allowed to solidify. Each petri dish was duplicated. After solidifying, the plates were inverted and incubated in an incubator set at 37°C for 24 hours for the plates containing Plate Count Agar, Eosin Methylene Blue Agar and MacConkey Agar while the plates containing Potato Dextrose Agar and Yeast Extract Agar were incubated at 28°C - 30°C for 3 – 5 days. However, the yeast isolates were incubated for 24 – 48 hours. After 24 hours of incubation, the plates containing Plate Count Agar, Eosin Methylene Blue Agar, MacConkey Agar and Yeast Extract Agar were counted to estimate total viable count and total coliform count. However, plates containing 30 – 300 colonies were counted using the colony counter. The total viable count was determined using the expression:

$$\text{Total Viable Count (TVC)} = \frac{\text{Number of microbial colonies x Volume inoculated}}{\text{Dilution factor}}$$

At 72 hours of incubation, the Potato Dextrose Agar plates were also counted and recorded.

The characterization of the isolates was done based on the morphological, physiological and biochemical characteristics of the isolates.

RESULTS

Total Coliform Count (TCC) which gives the total number of bacteria present, Total Fungal Count (TFC) gives the total number of fungi present, Total Yeast Count (TYC) gives the total number of yeast present and Total Viable Count (TVC) gives the quantitative estimate of the concentration of microorganisms in the sample organism. The count represents the number of colony forming units (cfu) per gram (g) of the sample. Table 1below shows the result of the microbial load (number and type of microorganisms present and/or contaminating the sample organism) of lizards from the two locations (Ibadan and Abeokuta) and values of TVC, TCC, TFC and TYC obtained per sample. An inoculum of approximately 10^5cfu/g in sterile wet samples over 72 hours period of observation, with viable microorganisms count between $0.1x10^5$ and $1.8x10^5$.

Table 2 and 3below shows the types of microorganisms present in the samples from the two locations. A total number of 30 representative microorganism from a total of 163 was obtained from the samples analyzed out of which 22 bacteria, 6 fungi and 2 yeast isolates. Out of the 163 microorganisms obtained from all the samples in both locations, *Micrococcus luteus* (11) was the most prevalent bacteria followed by *Staphylococcus aureus*; *Streptococcus faecium* and *Escherichia coli* (10 each); *Pseudomonas aeruginosa* and *Proteus*

vulgaris (9 each); *Serriatia marcescens* and *Aerobacter aerogenes* (7 each); *Bacillus cereus*, *Bacillus subtilis* and *Streptococcus bovis* (6 each); *Pseudomonas fluorenscens* and *Pseudomonas putida* (5 each); *Micrococcus acidophilus*, *Proteus morganii* and *Staphylococcus pneumonia* (4 each); *Bacillus macerans* and *Pseudomonas putida* (3 each); *Pseudomonas fragi* (2) and *Staphylococcus faecium*, *Klebsiella aerogenes*, *Micrococcus mecerans* and *Streptococcus mutans*(1 each). *Aspergillusterreus* (9) was the most prevalent fungi followed by *Aspergillusniger* (5); *Fusarium compactum* (4); *Aspergillus fumigatus* (3) and *Penicilliumchrysogenum* and *Fusarium oxysporum* (1 each). *Saccharomyces cerevisiae* (10) was the most prevalent yeast followed by *Saccharomyces elegans* (6). Samples from Abeokuta had the highest number of microorganisms (94) in comparison with those from Ibadan (69). The total number of bacteria, fungi and yeast from Ibadan was 50, 10 and 9 respectively while the total number of bacteria, fungi and yeast from Abeokuta was 72, 15 and 7 respectively. Four (4) bacteria (*Staphylococcusfaecium, Staphylococcus pneumonia, Klebsiella aerogenes, Micrococcus mecerans, Streptococcus mutans*) and 2 fungi (*Penicilliumchrysogenum, Fusarium oxysporum*) found in Lizards of Abeokuta was absent in those of Ibadan.

Table 1: Result of Microbial load

Sample code	**Abeokuta location**				**Ibadan location**			
	TVC	TCC cfug-1	TFC	TYC	TVC	TCC cfug-1	TFC	TYC
Agama lizard 1	$1.6x10^5$	$0.8x10^5$	$0.3x10^5$	$0.6x10^5$	$1.0x10^5$	$0.4x10^5$	$0.2x10^5$	$0.5x10^5$
Female lizard 1	$1.8x10^5$	$0.5x10^5$	$0.2x10^5$	$0.5x10^5$	$1.4x10^5$	$0.3x10^5$	NIL	$0.3x10^5$
Wall gecko 1	$0.8x10^5$	$0.4x10^5$	$0.1x10^5$	$0.3x10^5$	$0.6x10^5$	$0.2x10^5$	$0.1x10^5$	$0.4x10^5$
Agama lizard 2	$1.8x10^5$	$0.6x10^5$	$0.5x10^5$	$0.4x10^5$	$1.3x10^5$	$0.4x10^5$	$0.3x10^5$	$0.4x10^5$
Female lizard 2	$1.4x10^5$	$0.7x10^5$	$0.2x10^5$	$0.5x10^5$	$1.1x10^5$	$0.5x10^5$	NIL	$0.3x10^5$
Wall gecko 2	$0.7x10^5$	$0.6x10^5$	$0.2x10^5$	$0.5x10^5$	$0.4x10^5$	$0.3x10^5$	$0.2x10^5$	$0.4x10^5$
Agama lizard 3	$1.7x10^5$	$0.7x10^5$	$0.6x10^5$	$0.6x10^5$	$1.2x10^5$	$0.3x10^5$	$0.1x10^5$	$0.3x10^5$
Female lizard 3	$1.4x10^5$	$0.7x10^5$	$0.3x10^5$	$0.5x10^5$	$1.5x10^5$	$0.3x10^5$	$0.1x10^5$	$0.5x10^5$
Wall gecko 3	$0.6x10^5$	$0.4x10^5$	$0.2x10^5$	$0.6x10^5$	$0.6x10^5$	$0.2x10^5$	$0.3x10^5$	$0.4x10^5$

KEY: TVC= Total Viable Count, TCC= Total Coliform Count, TFC= Total Fungal Count, TYC= Total Yeast Count, $cfug^{-1}$=Colony forming unit per gram

Table 4, 5 and 6 below shows the results of the comparison of the microbial load by species, sex and locations. Table 4 shows that the Total Coliform Count (TCC) was significantly higher in lizards collected from Abeokuta (0.63×10^5) than the count for female lizards collected from Ibadan (0.37×10^5). The Total Fungal Count (TFC) of 0.23×10^5 was significantly higher than that for female lizards in Ibadan. However, there was no significant difference in the Total Viable Count (TVC) and Total Yeast Count (TYC) found in female lizards collected from Ibadan and Abeokuta. Similarly, Table 5 shows that TVC and TCC was significantly

higher for Agama lizards collected in Abeokuta (1.70 x 10^5 and 0.70 x 10^5) than the respective counts in Agama lizards collected in Ibadan with counts of 1.17 x $10^5$and 0.37 x 10^5 respectively. Table 6 however shows that there was no significant difference in the TVC, TCC, TFC and TYC counts between wall gecko collected in Ibadan and Abeokuta respectively. This study further illustrates that there is no significant difference in the microbial load between wall geckos collected from Ibadan and Abeokuta; the study however established the influence of location in the TVC, TCC and TFC between the in Ibadan.

study lizards with those in Abeokuta having higher loads of the microbes than those in Ibadan. The TCC and TFC values for female lizards in Abeokuta (0.63 x 10^5 and 0.23 x 10^5) was significantly higher than their respective values of 0.37 x 10^5 and 0.03 x 10^5 for TCC and TFC respectively in female lizards in Ibadan. In the same vein TVC and TCC load for agama lizard in Abeokuta (1.70 x 10^5 and 0.70 x 10^5) was significantly higher than the TVC and TCC values of 1.17 x 10^5 and 0.37 x 10^5 respectively for agama lizards.

Table 2: Micro-organisms identified in lizards for Abeokuta location

Sample code	**Micro-organisms isolated**
Agama lizard 1	*Staphylococcus aureus, Micrococcus luteus, Serriatia marcescens, Proteus vulgaris, Bacillus cereus, Pseudomonas fluorenscens, Pseudomonas aeruginosa, Staphylococcus faecium, Escherichia coli, Aspergillus fumigates, Fusarium compactum.*
Female lizard 1	*Micrococcus luteus, Proteus morganii, Staphylococcus aureus, Pseudomonas putida, Bacillus subtilis, Staphylococcus pneumonia, Klebsiella aerogenes, Saccharomyces cerevisiae, Proteus vulgaris, Serriatia marcescens, Escherichia coli.*
Wall gecko 1	*Staphylococcus pneumonia, Staphylococcus aureus, Escherichia coli, Micrococcus mecerans, Pseudomonas putida, Micrococcus acidophilus, Bacillus subtilis, Pseudomonas aeruginosa, Aerobacter aerogenes, Saccharomyces elegans, Aspergillusterreus, Penicilliumchrysogenum.*
Agama lizard 2	*Staphylococcus aureus, Micrococcus luteus, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Streptococcus bovis, Streptococcus faecium, Escherichia coli, Bacillus cereus, Aspergillusniger, Fusarium oxysporum.*
Female lizard 2	*Micrococcus acidophilus, Proteus morganii, Bacillus macerans, Streptococcus faecium, Escherichia coli, Saccharomyces cerevisiae, Aspergillusniger, Fusarium oxysporum.*
Wall gecko 2	*Streptococcus bovis, Pseudomonas fluorenscens, Bacillus cereus, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Aerobacter aerogenes, Streptococcus faecium, Pseudomonas putida, Serriatia marcescens, Saccharomyces elegans, Aspergillusterreus.*
Agama lizard 3	*Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa, Streptococcus faecium, Aspergillusniger, Saccharomyces cerevisiae, Fusarium compactum.*
Female lizard 3	*Pseudomonas fluorenscens, Micrococcus luteus, Proteus vulgaris, Bacillus mecerans, Aerobacter aerogenes, Staphylococcus pneumonia, Streptococcus faecium, Aspergillusniger, Serriatia marcescens, Saccharomyces cerevisiae, Aspergillusterreus.*
Wall gecko 3	*Staphylococcus pneumonia, Streptococcus bovis, Staphylococcus aureus, Pseudomonas fragi, Serriatia marcescens, Micrococcus luteus, Aerobacter aerogenes, Escherichia coli, Saccharomyces elegans, Aspergillusterreus, Penicilliumchrysogenum.*

Table 3: Micro-organisms Identified in Lizards for Ibadan location

Sample code	**Micro-organisms isolated**
Agama lizard 1	*Bacillus cereus, Micrococcus acidophilus, Serriatia marcescens, Pseudomonas putida, Pseudomonas aeruginosa, Aerobacter aerogenes, Aspergillus fumigatus, Saccharomyces cerevisiae.*
Female lizard 1	*Pseudomonas fluorenscens, Staphylococcus aureus, Proteus vulgaris, Bacillus subtilis, Streptococcus faecium, Saccharomyces cerevisiae.*
Wall gecko 1	*Bacillus subtilis, Micrococcus luteus, Proteus vulgaris, Escherichia coli, Serriatia marcescens, Streptococcus bovis, Aspergillusterreus, Saccharomyces elegans.*
Agama lizard 2	*Bacillus cereus, Micrococcus acidophilus, Pseudomonas aeruginosa, Proteus morganii, Streptococcus faecium, Saccharomyces cerevisiae, Aspergillusniger, Aspergillus fumigatus.*
Female lizard 2	*Pseudomonas aeruginosa, Pseudomonas fluorenscens, Proteus vulgaris, Bacillus subtilis, Streptococcus faecium, Saccharomyces cerevisiae.*
Wall gecko 2	*Bacillus macerans, Micrococcus luteus, Proteus vulgaris, Escherichia coli, Pseudomonas fragi, Streptococcus bovis, Aspergillusterreus, Fusarium compactum, Saccharomyces elegans.*
Agama lizard 3	*Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecium, Aspergillusterreus, Saccharomyces cerevisiae.*
Female lizard 3	*Pseudomonas aeruginosa, Micrococcus luteus, Proteus vulgaris, Bacillus subtilis, Aerobacter aerogenes, Streptococcus faecium, Aspergillusterreus, Saccharomyces cerevisiae.*
Wall gecko 3	*Micrococcus luteus, Staphylococcus aureus, Proteus morganii, Escherichia coli, Streptococcus bovis, Aerobacter aerogenes, Aspergillusterreus, Fusarium compactum, Saccharomyces elegans.*

Table 4: Comparison of microbial load in Female agama lizard by location

Parameter	N	**Abeokuta**	**Ibadan**	T-Value	Sig P	Remark
TVC	3	1.53	1.33	1.11	0.33	N.S
TCC	3	0.63	0.37	2.83	0.05**	S
TFC	3	0.23	0.03	4.24	0.01*	S
TYC	3	0.50	0.37	2.00	0.18	N.S

* Significant at (P≤0.01), ** Significant at (P≤0.05)
NS = Not Significant, S = Significant

Table 5: Comparison of microbial load in Male agama lizard by location

Parameter	N	**Abeokuta**	**Ibadan**	T-Value	Sig P	Remark
TVC	3	1.70	1.17	5.06	0.01*	S
TCC	3	0.70	0.37	5.00	0.01*	S
TFC	3	0.47	0.20	2.53	0.08	N.S
TYC	3	0.53	0.33	1.45	0.24	N.S

* Significant at (P≤0.01), ** Significant at (P≤0.05)
NS = Not Significant, S = Significant

Table 6: Comparison of microbial load in Wall gecko by location

Parameter	N	**Abeokuta**	**Ibadan**	T-Value	Sig P	Remark
TVC	3	0.70	0.53	1.89	0.13	N.S
TCC	3	0.47	0.23	3.13	0.53	N.S
TFC	3	0.17	0.17	0.00	1.00	N.S
TYC	3	0.47	0.04	0.76	0.53	N.S

* Significant at (P≤0.01), ** Significant at (P≤0.05)
NS = Not Significant, S = Significant

DISCUSSION

The focus of this study is to identify the role lizards play as possible carriers of zoonotic diseases. Studies on the microbial load and microorganisms present in the gut of lizards has shown that they are reservoirs of harmful pathogenic bacteria which mostly depend on their environment and/or location as well as their feeding habit. From the result of this study, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa, Proteus vulgaris*, *Serriatia marcescens* and *Aerobacter aerogenes*were the most prevalent bacteria found in their gut which are capable of causing serious infections in humans depending on the level of exposure. However, transmission of these bacteria is most likely to occur through contact with water and foodstuffs as these lizards are known to frequent barns and storage areas in a bid to get their own food. Abeokuta had the highest number of bacteria and fungi isolates while Ibadan had higher yeast isolates. This result is in line with the findings of Singh *et al. (*2013) which stated that the environment (location) of Lizards is a determinant factor of the type of microorganism present. The isolation of the bacteria organisms from the study by Ajayi, Ogunleye, Happi and Okunlade (2015) confirmed the epidemiologic importance of possible disease transmission from lizards to poultry and possibly to humans, especially due to free access of these Agama agama lizards to the poultry houses, as well as their sources of food and water. Singh *et al.* (2013) also reported that it is widely accepted that potentially enteropathogenic and zoonotically important bacteria may be present in the intestine of geckos (common house lizards), and thus the gecko has been seen as potential threat in the spread of enteric diseases. He further stated that not all intestinal bacteria present in these lizards are excreted in their faeces. The bacterial population in gecko droppings may vary significantly under different environments as droppings collected from geckos living at different places had significant difference in bacterial population of droppings. However, it is important to note that most of the organisms obtained from this study are of the family Enterobacteriaceae. This group of organisms consists majorly of gram negative organisms that produce endotoxins. These toxins reside in the cell wall of the organisms. When ingested and the toxins released into the bloodstream, it causes serious health risk if proper treatment is not done.

CONCLUSION

The microorganisms and microbial load found in the gut of the study lizards varied depending on their location even though the differences were not significant. This may be due to the fact that lizards were not readily available at both markets as at when needed. The lizards were collected upon completion of the adequate number required for the study. The ecological role of these lizards in insect and pest control cannot be overemphasized, as it can be described as a symbiotic relationship; on one hand beneficial and on the other harmful. However, these relationships are essential to many organisms and the ecosystem at large, providing balance that can only be achieved by such interactions. Proper storage of food items should be done to avoid these lizards coming in contact with them, especially in the process of

drying, when going about their normal feeding habit. Further research is required to expound bacteria of public health concern that are present on the tongue and in the faeces of lizards which are the major food/water contaminants and precautionary measures to take so as not to hinder them from performing their ecological roles and creating imbalance in the ecosystem.

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