DETERMINING THE PRESENCE OF *SALMONELLA* SPECIES AND ITS ANTIBIOTIC RESISTANCE IN CAPTIVE SNAKES OF MVR SNAKE PARK, KANNUR

SUMITHRA SOMANATH

(19-MSVP-09)

DISSERTATION

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

(Wildlife Studies)

2022

Faculty of Veterinary and Animal Sciences

Kerala Veterinary and Animal Sciences University

KVASU CENTRE FOR WILDLIFE STUDIES

KERALA VETERINARY AND ANIMAL SCIENCES UNIVERSITY

POOKODE, WAYANAD 673576

KERALA, INDIA

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DECLARATION

I hereby declare that this dissertation entitled **"Determining the presence of *Salmonella* species and its antibiotic resistance in captive snakes of MVR Snake Park, Kannur"** is a bonafide record of research done by me during the course of research and that the dissertation has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Date: **(19-MSVP-09)**

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CERTIFICATE

Certified that this dissertation, entitled **"Determining the presence of *Salmonella* species and its antibiotic resistance in captive snakes of MVR Snake Park, Kannur"** is a record of research work done independently by SUMITHRA SOMANATH (19-MSVP-09) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him/her.

Place: Pookode
Date:

Dr. Chintu Ravishankar
Chairman
Advisory Committee

CERTIFICATE

We, the undersigned members of the advisory committee of **SUMITHRA SOMANATH (Roll no: 19-MSVP-09),** a candidate for the degree of Master of Science in Wildlife Studies, agree that this dissertation entitled **"Determining the presence of *Salmonella* species and its antibiotic resistance in captive snakes of MVR Snake Park, Kannur"** may be submitted by **SUMITHRA SOMANATH (Roll no: 19-MSVP-09)** in partial fulfilment of the requirement for the degree.

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EXTERNAL EXAMINER

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INTRODUCTION

1.INTRODUCTION

Reptiles are one of the most diverse groups of animals that inhabit almost all continents and types of environments. They are often asymptomatic carriers of *Salmonella* species as they carry and shed these bacteria in their faeces. Besides the classical animals being known as the reservoir for *Salmonella* species, different reptiles such as turtles, iguanas, chameleons, and snakes have also been identified as possible sources of *Salmonella* infections (Seepersadsingh and Adesiyun, 2003; Schröter *et al.*, 2004). There are several reports of reptile related Salmonellosis outbreaks in humans and numerous studies have been performed to detect *Salmonella*e in both wild and captive reptiles (Kennedy, 1973).

The increased demand for reptiles as pets, has also increased the risk of infections. Infected herpetofauna poses a serious concern to public health. Cold blooded animals may harbour a wide range of pathogens of zoonotic importance. They can be asymptomatic carriers of different pathogens which they may frequently shed into the environment. The people who come in contact with reptiles, especially reptile owners, zookeepers, and veterinarians are at increased risk of infections. Better captive management and good personal hygiene are essential measures to prevent the direct transmission of infections from animals to humans. The behaviour and physiology of many wild animals are still unknown. Illegal wildlife trade and consumption of wild animal meat are common in many parts of the world. Urbanization and deforestation have permitted close interaction of many wildlife species in the proximity of human settlements. Thus, humans and domestic animals are more prone to zoonotic pathogens from wildlife. All these factors contribute to the decline of biodiversity and thereby disturb the ecological balance and ultimately end up as a major public health concern (Hilbert *et al.*, 2012). Disease outbreaks like the recent COVID-19 outbreak also raise serious concern for the threat posed by wildlife as a potential source of emerging infectious diseases.

For a sustainable ecosystem, there should be healthy interaction at the human-wildlife interface. Any anthropogenic activity disrupting healthy interaction could act as a trigger for pandemic outbreaks in the future. The health authorities, veterinarians and the general public should be aware of the potential zoonotic pathogens in important wildlife species like snakes, flying mammals and birds and the possible route of pathogen transmission. An understanding and awareness of these can reduce the potential risk of zoonosis to a considerable extent in the future.

Snakes are considered to be one of the sources of *Salmonella* infections in humans. Captive snakes become exposed to *Salmonella* when they interact with wild snakes harbouring *Salmonella* or through the diet. Captive reptiles are often fed with rodents that are either bred or shipped from outside the facility (Goupil *et al.*, 2012). When the ambient temperature is not optimal and accommodation is unsanitary, the immune system is often suppressed and thus the bacteria get an opportunity to settle within them. Some snakes may harbour bacterial pathogens from the wild, and clinical diseases become established once they are in captivity.

Salmonella is a facultative, gram-negative, non-spore producing, bacillus belonging to the Enterobacteriaceae family (Gugnani *et al.*, 1986; Gutema *et al.*, 2019). It has been identified that *Salmonella* is the most common causative agent of zoonotic diseases due to their biological characteristics such as logarithmic growth rate, tolerance to a wide variety of environments, and natural affinity with a wide group of hosts. The transmission of reptile mediated Salmonellosis is mainly through the faecal-oral route. People often get infected by direct contact via animal handling or indirect transmission through the consumption of contaminated food or water as well as contact with contaminated surroundings. Species commonly encountered in reptile mediated Salmonellosis include *S. java, S. stanely, S. marina, S. poona, S. arizonae, S. heutenae* and *S. indica* (Warwick *et al.*, 2001).

The golden age of antibiotics began with the discovery of penicillin by Alexander Flehming in 1928. The synthesis of a variety of antibiotics over the past three decades caused a dramatic increase in the usage of antibiotics in both animal and human health sectors.

In addition to controlling deadly infectious diseases such as cholera, anthrax, tuberculosis, and botulism, antibiotics helped make many advancements in modern medical procedures. However, the misuse of antibiotics triggered a rapid rise of multidrug resistant bacteria, leading to some diseases becoming untreatable with conventional antimicrobials. A considerable increase in emerging resistance coupled with poor infection control practices can easily disseminate resistant bacteria into the environment. The emergence of antibiotic resistance in different strains of bacteria has been a growing concern of public health in recent decades. The rise of antibiotic resistance involves a network of interactions in the ecosystem, among microbes, antibiotics, and resistant genes. Multidrug resistant bacterial infections are difficult to treat and even conventional combinations of antibiotics may fail to stop the infection. At present there is a shortage of effective antimicrobial treatments and this results in high morbidity and mortality (Frieri *et al.*, 2017).

Bacteria become resistant to antimicrobial agents as a result of mutations or through the exchange of genetic materials via plasmids and transposons. Large scale use of antibiotics in the society has fuelled the crisis of antimicrobial resistance. Unreasonable use of antibiotics in the health sector as well as over the counter use of the antibiotics among common people have favoured the selection of resistant bacteria. Resistant strains of bacteria are easily passed to humans through the food chain resulting in serious consequences including treatment failure and the rapid outbreak of pathogenic infections (Piddock, 2012; Mir *et al.*, 2015).

One health is a collaborative and multidisciplinary approach applied at the local and global levels. It emphasizes the better health of humans, animals and the

environment. It involves the contribution from multidisciplinary fields. Experts in the field of human health (doctors, nurses, public health practitioners, epidemiologists), animal health (veterinarians, agricultural workers), and environmental science (ecologists, wildlife experts) including policymakers, law enforcement agencies, agriculture, pet owners and communities, must collaborate, communicate and cordially act. No single individual, organization or industry can solve the issues at human-animal-environmental interface on their own.

The present study was taken up on the basic idea of one health. The prime aim of this study is to determine the presence of *Salmonella* in snakes and evaluate their antibiotic resistance. The purpose of the study is to investigate whether captive snakes are a reservoir of antimicrobial resistant bacteria, using *Salmonella* as a model organism. The study was carried out with the following objectives.

1. Identify the presence of *Salmonella* in the faecal samples of snakes in captivity
2. Isolate the *Salmonella* strains from the faecal sample of captive snakes
3. Assess antibiotic resistance of *Salmonella* species by Kirby-Baier disk diffusion method

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REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 ANTIBIOTICS

Antibiotics are medications that kill or inhibit the growth of bacteria. Hence, they are used in the treatment of infectious bacterial diseases. (Paterson *et al.*, 2016)

In 1942 Waksman defined antibiotics as any substance produced by a microbe that inhibits or destroys the growth of other microorganisms (Woodruff, 2014).

According to the article by Hamad (2010), only two new antibiotics were discovered during the past 30 years; oxazolidinone and linezolid which were discovered in 2000 and 2003 respectively.

2.1.1 Antibiotic Usage in Veterinary Medicine

Shortly after the development of antimicrobial drugs, they were used in veterinary medicine to treat mastitis in dairy cows. The antimicrobial drug usage varies between species and is dependent on several factors (Jhonston, 1998).

Antimicrobial drugs were also used as growth promoters and as prophylactic agents (Silbergeld *et al.*, 2008) against respiratory diseases and liver abscesses (Doster *et al.*, 2018). Moreover, it was a common practice to treat the herds of livestock with antibiotics to avoid the spread of diseases which resulted in antibiotic overuse. In this scenario, uninfected animals were also exposed to antibiotics (Economou and Gousia, 2015). Streptomycin, gentamicin, kanamycin, penicillin, cefamandole and chloramphenicol are the most commonly used antibiotic drugs in veterinary practice.

2.2 ANTIBIOTIC RESISTANCE

Antibiotic resistance in bacteria was first reported by Rammelkamp and Maxon (1942). They reported that, before the discovery of penicillin there existed antibiotic resistant bacteria as scientists identified penicillinase as an enzyme in *E. coli* that inhibits the action of penicillin.

Harrison and Lederberg (1998) defined antibiotic resistance as a defensive mechanism adopted by the microorganisms against the action of antibiotics.

Bacteria became resistant to antimicrobial agents as a result of chromosomal changes or through the exchange of genetic material via plasmids and transposons. Tenovar (2006) explained that bacteria receive resistance genes by transformation, transduction, or conjugation. Large scale use of antibiotics in the community and hospitals has fuelled the crisis of antimicrobial resistance (Piddock, 2012). These pathogenic agents may be either resistant to single antibiotics or multiple antibiotics.

2.2.1 Antibiotic Resistance in *Salmonella*

Ejo *et al.* (2016) conducted a cross-sectoral study on the prevalence of antimicrobial resistant bacteria in the food items of animal origin in Brazil. The *Salmonella* isolates were resistant to tetracycline, amoxicillin, ampicillin, nitrofurantoin and cephalothin. The study concluded that the resistant *Salmonella* present in foods of animal origin can contribute to foodborne infections in Ethiopia.

2.2.2 Emergence of Antibiotic Resistant Bacteria

The emergence of antibiotic resistance in different bacterial species has been a growing public health concern in recent decades. Antimicrobial resistance in bacterial pathogens is associated with high morbidity and mortality. The infections caused by resistant bacteria are difficult to treat and mostly untreatable

with conventional antibiotics. Currently, there is a shortage of effective therapies, lack of adequate preventive measures, and overuse and abuse of antibiotics. Along with these, inappropriate drug prescription, large scale use in agriculture, as well as lack of new antibiotics contribute to the rise of antimicrobial resistance (Ventola, 2015; Frieri *et al.*, 2017). The emergence of antibiotic resistance in bacteria affects the overall community and environmental health. This also led to an increase in the number of infections requiring costly treatment.

The resistant strains of bacteria are present in the environment and they circulate among humans, animals and the environment. According to a review by Rolain *et al.* (2012), antibiotic resistance involves interactions in an ecosystem among microbes, antibiotics, and resistant genes. The emergence of a new bacterial strain cannot be predicted. Antibiotic resistant genes naturally exist in the environment. Hence, studying different reservoirs and discovering new resistant genes in bacteria have to be carried out so that the emergence of multidrug resistance can be predicted. Also, this calls for the immediate need for developing new and more effective drugs that can combat the emerging crisis.

2.3 BACTERIAL DIVERSITY IN REPTILES

Goldstein *et al.* (1981) studied aerobic bacterial flora of Garter Snakes. An aggregate of 126 strains of aerobic and facultative bacteria was isolated from 82 Garter Snakes. Majority of these bacterial strains were potential human and snake pathogens. Among them, *Staphylococcus* spp. was the most common isolate. Three species of *Salmonella*, one *Shigella* isolate, and eight unidentified non-fermentative, gram negative rods were also recovered. It was noted that it was quite possible that Garter Snakes could act as a reservoir of potential human pathogens.

Soveri and Seuna (1986) examined samples taken from 23 captive non-venomous snakes for aerobic bacteria. The most prominent bacteria found were Gram-positive rods and Gram-positive cocci belonging to the family

Micrococcaceae. *Salmonella virchow* was found in isolates from two snakes. They suggest that most of the bacterial flora isolated from the oral cavity may be occasional environmental bacteria.

Blaylock (2001) studied normal oral bacterial flora from Southern African snakes. In this study, eighteen snakes that belonged to 11 species were selected. Isolates obtained included members of *Enterobacteriaceae*, Gram-positive cocci, and anaerobes. Most of the bacterial isolates were, *Proteus* spp., *Pseudomonas* spp., *Salmonella arizonae* and *Staphylococcus epidermidis*.

A study conducted by Jho *et al.* (2011) identified bacterial flora in snakes imported from Vietnam. In this study, oral and cloacal samples were collected from eighteen Burmese Pythons imported from Vietnam. They isolated fourteen bacterial strains including *Aeromonas hydrophila*, *Citrobacter freundii*, *Corynebacterium jeikeium*, *Enterobacter spp.*, *Enterococcus spp.*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas spp.*, *Staphylococcus spp.* from both oral and cloacal samples.

A study by Lam *et al.* (2011) determined the patterns of oral bacterial flora and their sensitivity to antibiotics in captured native snakes in Hong Kong. Their result revealed that venomous snakes harboured more pathogenic bacteria. Among the venomous snakes, the Chinese Cobra (*Naja atra*) harboured the highest number of bacterial flora.

Dipineto *et al.* (2014) determined oral bacterial flora of *Python regius* kept as pets. The oral cavity examination of snakes revealed the presence of a wide range of gram-negative bacteria predominantly comprising of *Pseudomonas spp.*, *Morganella morganii*, *Acinetobacter calcoaceticus*, and *Aeromonas hydrophilia*. Additionally, *Salmonella spp.* was recorded in 15 per cent of tests and were serotyped as *S. blukwa*, *S. diarizonae*, *S. enteritidis* and *S. oranienburg*. The antimicrobial susceptibility testing showed that bacterial isolates were highly susceptible to enrofloxacin and ciprofloxacin.

Dehghani *et al.* (2016) identified bacterial flora in the oral cavity of snakes in Kahan, Iran. Samples were obtained from venomous and non-venomous snakes. The identified bacterial pathogens included species of the genera *Bacillus*, *Enterococcus*, *Escherichia*, *Proteus*, *Pseudomonas*, *Providencia*, *Salmonella* and *Staphylococcus*. This study revealed that both the venomous and non-venomous snakes harboured a wide variety of bacterial flora in their oral cavity. Hence, when these snakes bite a person, there is a risk of transferring these bacteria to the victims thereby increasing the bite complications. Therefore, along with antivenom treatment, the probability of causing bacterial infections also should be considered.

Artavia-León *et al.* (2017) determined the diversity of aerobic bacteria isolated from oral and cloacal cavities of free-living snakes in the Costa Rica rainforest. They examined both oral and cloacal swabs collected from the families *Viperidae* and *Colubridae*. Among the different bacteria identified, the most predominant bacterial species were *Aeromonas hydrophila*, *Bacillus* sp, *Enterococcus faecalis*, *Proteus hauseri*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Salmonella enterica diarizonae*, *Staphylococcus spp.* and *Stenotrophomonas maltophilia*. The study indicated that diverse bacteria found in snakes can act as opportunistic pathogens and enhance the risk of zoonosis in humans.

Shaikh *et al.* (2017) evaluated the oral microbiota of venomous snakes commonly found in India and assessed their antibiotic susceptibility. Oral swabs of 27 snakes representing Indian Cobra, Russell's Viper, Saw Scaled Viper, and Common Krait were selected for the study. The most common bacteria isolated from the samples were *Morganella morganii*, *Escherichia coli*, *Aeromonas aeruginosa*, *Staphylococcus aureus*, *Bacillus spp.*, *Micrococcus spp.* and *Clostridium perfringens*. The study showed that oral bacterial flora of snakes had great diversity of Gram-positive and Gram-negative bacteria.

Panda *et al.* (2018) examined the oral cavity of the Indian Cobra (*Naja naja*) for bacterial diversity. They collected oral swabs from six healthy Indian Cobras. Their microbiological examination indicated the dominance of Gram-negative bacteria over Gram-positive bacteria. The oral bacterial flora in Indian Cobra included bacteria such as *Salmonella* spp. (*S. typhi, S. paratyphi A*), *Pseudomonas* spp. (*P. aeruginosa, P. fluorescence*), *Proteus* spp. (*P. mirabilis, P. penneri, P. vulgaris*), *E. coli, Morganella* spp., *Citrobacter* spp. (*C. diversus, C. freundii*), *Aeromonas* spp. (*A. hydrophila, A. salmonicida*), *Enterobacter* spp. (*E. aerogens*), *Acinetobacter* spp. (*A. baumannii*), *Neisseria* spp., *Serratia* spp., *Bacillus* spp. (*B. cereus, B. megaterium, B. atrophaeus* and *B. weihenstephanensis*), *Enterococcus* spp. (*E. faecalis, E. faecium*), *Staphylococcus* spp. (*S. aureus, S. epidermidis*), *Alcaligenes* spp., *Chryseobacterium* spp. and *Micrococcus* spp. All the isolates were subjected to antimicrobial susceptibility testing. This study demonstrated that proper diagnosis and treatment should be provided for snake bite patients since oral bacteria might cause infections in the victims.

2.4 SALMONELLOSIS IN ANIMALS

The potential of reptiles as carriers of *Salmonella* and *Arizona* spp. was investigated by Iveson *et al.* (1969). The study indicated that there was no difference between the serotypes isolated from captive and wild reptiles. The study showed that lizards were more prone to *Salmonella* infections when compared to snakes.

Kennedy (1973) isolated *Salmonella* spp. from snakes and other reptiles. Along with *Salmonella,* other bacteria including *Arizona hinshawii, Proteus* spp, *Citrobacter freundii,* and *Enterobacter* spp. were also isolated from the samples.

Faldae *et al.* (1976) isolated *Salmonella* from captive animals in Nigeria. In this study they could isolate six serotypes of *Salmonella*, namely *Salmonella offa, Salmonella glostrup, Salmonella wimborne, Salmonella dublin, Salmonella saint paul* and *Salmonella webridge*. For the first time, *Salmonella wimborne* and

Salmonella glostrup were reported in Nigeria. They also looked at the antibiotic susceptibility of all six serotypes. The result showed that all these strains were susceptible to nitrofurantoin and chloramphenicol but they were resistant to sulphafurazole and penicillin.

Hoff *et al.* (1977) isolated *Salmonella* from free-ranging lizards in Florida. Ten isolates were positive for *Salmonella* and six *Salmonella* serotypes namely *S. flint*, *S. florida*, *S. gaminara*, *S. meunchen*, *S. miami* and *S. oranienburg* were recovered from these isolates.

Cambre *et al.* (1980) investigated the prevalence of *Salmonella* and *Arizona* in the reptiles housed at the National Zoological Park in Washington, DC. In this study, serotypes such as *Salmonella enteritidis*, *Salmonella choleraesuis* and *Arizona hinshawii* were recovered. This study also showed that snakes showed the highest rate of infection when compared to lizards and other reptiles.

Gugnani *et al.* (1986) isolated *Salmonella* and other enteropathogenic bacteria from Wall Geckos in Nigeria. They examined bacterial flora of 150 Wall Geckos (*Hemidactylus brookei*). A variety of bacteria including *Salmonella*, *Shigella sonnei*, *Edwardsiella tarda*, *Enterobacter* spp., *Citrobaeter freundii*, *Serratia marcescens*, *Proteus* spp., *Klebsiella pneumoniae* and *Escherichia coli* were isolated. The most prevalent bacteria were *Salmonella*. Among the eight serotypes of *Salmonella*, *S. hvittingfoss* and *S. typhimurium* were the most predominant serotypes.

Kalvig *et al.* (1991) isolated *Salmonella* from laboratory housed Iguanid lizards (*Sceloporus* spp). They captured lizards from the Costa Rica dry forest and housed them in the laboratory over a study period of 3 months. They isolated *Salmonella* spp. from the lizards. The study concluded that individual animals could develop systemic infections as a result of stress in captivity.

Burnham *et al.* (1998) conducted a cohort study on the prevalence of faecal shedding of *Salmonella* among captive Green Iguanas (*Iguana iguana*). All the 12

Iguanas were positive for *Salmonella* and multiple serotypes were isolated from them.

Otokunefor *et al.* (2003) carried out a study to analyse the carriage rate of *Salmonella* in the gut and droppings of pest lizards in Nigeria. Three lizard species, namely the Agama Lizard (*Agama agama*), Wall Gecko (*Geckonidae*), and Snake Lizard (*Ameiva ameiva*) within the University of Port Harcourt campus and the University Village, were captured. Gut analysis showed a higher rate of *Salmonella* carriage in lizards that inhabited the outdoor environment namely the Agama and Snake Lizards than the Wall Geckos that dwelled indoors.

The prevalence of *Salmonella* in several reptiles was evaluated by Corrente *et al.* (2004). They isolated *Salmonella* strains and compared different culture media for isolation. Fifty percent of the samples were positive for *Salmonella* spp. and most of them belonged to *S. enterica*. The common serotypes recovered were *Salmonella newport*, *Salmonella muenster*, *Salmonella senftenberg*, *Salmonella aqua*, *Salmonella gaminara*, *Salmonella havana*, *Salmonella poona*, *Salmonella oldenburg* and *Salmonella minnesota*. The study confirmed the high prevalence of *Salmonella* strains in reptiles, especially in snakes and chameleons.

Ebani *et al.* (2005) investigated *Salmonella enterica* in the faeces of reptiles. In this study, *Salmonella* enterica was isolated particularly from Iguanas. A large number of *Salmonella* isolates were obtained from chelonians.

Palmgren *et al.* (2006) conducted a study on *Salmonella* in Black-headed Gulls (*Larus ridibundus*) in which they investigated the prevalence and *Salmonella* epidemiology. They found that 2.7 per cent of the individuals were positive for *Salmonella* and that the most predominating was *S. typhimurium*. All the *S. typhimurim* isolates were compared to the isolates from humans and domestic animals, and were found to be of the same serotype. The study showed that there existed a relationship between *S. typhimurium* isolates of gulls, humans

and domestic animals indicating that Black-headed Gulls might play a role in the spread of *S. typhimurium.*

Scheelings *et al.* (2011) determined the prevalence of *Salmonella* in captive and wild reptiles. The result of this study revealed that *Salmonella* was more prevalent in captive reptiles. The most prevalent *Salmonella* serotype was *S. enterica diarizonae*. They suggested that the *Salmonella* prevalence was linked to diet. Carnivorous reptiles shed *Salmonella* more frequently in their faeces than insectivorous reptiles. Furthermore, captive reptiles that fed with rodents were positive for *Salmonella*.

Chiari *et al.* (2014) conducted a study in Northern Italy to isolate and identify *Salmonella* spp. from Red Foxes (*Vulpes vulpes*) and European Badgers (*Meles meles*). This study confirmed that the prevalence of *Salmonella* in foxes and badgers was low when compared to wild boars living in the same area. Different serovars of *S. enterica* were identified, among which some serovars were often associated with human illness. The result of the study demonstrated that opportunistic wild predators can indirectly infect both domestic animals and humans through the shedding of infectious pathogens into the environment.

Iovine *et al.* (2015) determined the presence of *S. enterica* and *E. coli* in free-ranging wild animals. They collected samples from two distinct regions in Brazil. The study showed that many wild animals harboured infectious pathogens and their presence might vary with respect to geographical locations.

Lukac *et al.* (2015) studied the prevalence of *Salmonella enterica* in captive reptiles. A total of 292 samples were collected from pet reptiles and the Zagreb Zoo, Croatia. The highest prevalence of *Salmonella* was observed in lizards followed by snakes and chelonians. *Salmonella enterica* serotypes such as, *Salmonella enterica arizonae, Salmonella enterica enterica* and *Salmonella enterica salamae* were identified. The study confirmed that the captive reptiles in

Croatia harboured several serotypes of *Salmonella* which might act as reservoirs of reptile mediated Salmonellosis.

Mir *et al.* (2015) studied the occurrence and serotype diversity of *Salmonella* isolates in different species of poultry (chicken, emu, and duck) and determined their resistance pattern against various antibiotics of different classes. The serotyping results showed that the majority of isolates belonged to *Salmonella enteritidis*, followed by *Salmonella typhimurium, Salmonella virchow*, *Salmonella gallinarum*, *Salmonella reading*, and *Salmonella altona*. Their observations showed that poultry could serve as an important source of transmission of these antibiotic resistant *Salmonella* serovars to humans.

Back *et al.* (2016) determined the prevalence of *Salmonella* spp. in pet turtles and their environment. They isolated *Salmonella* spp. from the faecal sample of 17 turtles. All these isolates were identified as *Salmonella enterica* through gene sequencing. This study showed that pet turtles distributed in Korea were infected with *Salmonella* spp. and their improper management would increase the risk of Salmonellosis.

Eugale *et al.* (2016) determined the prevalence and antimicrobial resistance of *Salmonella* in dairy cattle. They collected faecal samples from the peri-urban dairy farming facilities in Ethiopia where the interaction between animal and human population was very high. This study showed that the level of *Salmonella* prevalence was 2.3 per cent. Nine different *Salmonella* serotypes including *S. typhimurium*, *S. virchow* and *S. saintpaul* were identified.

Matias *et al.* (2016) isolated *Salmonella* from wild birds poached in illegal wildlife trade in Rio de Janeiro and investigated the prevalence of *Salmonella*. *Salmonella typhimurium* was isolated from Temminck's Seed Eater (*Sporophila falcirostris*) and *Salmonella panama* was isolated from two Chestnut-capped Blackbirds (*Chrysomus ruficapillus*). These birds were kept in the same cage and had no symptoms of the disease. All the serovars showed multidrug resistance.

They carried out pulse field gel electrophoresis (PFGE) analysis and the result showed 100 per cent similarity among the *Salmonella typhimurium* strain isolated from a Temminck's Seed Eater and the strains isolated from a disease outbreak in humans, in Southern Brazil. The study indicated that trafficked wild animals could be a source of salmonellosis and that it could be responsible for disease outbreaks in animals and humans. The potential for dissemination of resistant *Salmonella* through wild birds and human sources might become a problem of public health concern.

Tomastikova *et al.* (2017) conducted a study in the Czech Republic in which they determined the prevalence and characteristics of the *Salmonella* strains from captive reptiles. They identified a total of 14 *Salmonella* serotypes. The most frequently found Serotypes were *S. enterica enterica*, *S. oranienburg*, *S. fluntern*, *S. tennessee* and *S. cotham*. The result of the study showed that the prevalence of *Salmonella* spp. was more in lizards followed by snakes and chelonians. *Salmonella* was more frequently detected in carnivorous or insectivorous reptiles than in omnivorous and herbivorous reptiles.

In a study, Silva *et al.* (2018) isolated *Salmonella* spp. in Cattle Egrets (*Bubulcus ibis*). The occurrence of *Salmonella newport* and *Salmonella typhimurium* suggested that Cattle Egrets may be reservoirs of different *Salmonella* serotypes and could cause a potential risk to public health and biological diversity.

A detailed review by Gutema *et al.* (2019) showed the diversity of *Salmonella* serotypes in healthy cattle across different countries. They concluded that the *Salmonella* serotypes such as *S. newport* and *S. typhimurium* were zoonotic pathogens.

Santos *et al.* (2020) investigated the presence of *Salmonella* spp. in wild birds from the Atlantic Forest in Brazil. Only one sample from *Ceratopipra*

rubrocapilla was positive for *Salmonella enterica enterica*. According to this study, a low prevalence of *Salmonella* spp. was observed in wild birds.

2.4.1 Reptile Related Salmonellosis

As per a review by Warwick *et al.* (2001), *Salmonella* species commonly encountered in reptile mediated salmonellosis were *S. java*, *S. stanley*, *S. poona*, *S. mariana* and *S. panama*. The primary transmission route for reptile mediated salmonellosis was observed to be fecal-oral ingestion. However, terrapins, lizards and tortoises could transmit salmonellosis by claw scratches.

2.5 SALMONELLA IN HUMAN-WILDLIFE INTERFACE

In industrialized countries, non-typhoidal salmonellosis is considered as the most important foodborne infection and it can be considered as a public health problem. Humans can get salmonellosis either by direct contact (handling and exposure to infected animals) or by indirect contact via consumption of animal meat and food of animal origin or from contaminated food or water). Wild boar, wild birds and wild reptiles are the reservoirs of *Salmonella* and consumption of meat from these animals increases the incidence of salmonellosis in humans. Wild animals can act as a vector for transmitting the infections (Hilbert *et al.*, 2012).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 STUDY AREA

The present study was carried out in MVR Snake Park and Zoo, Parassinikkadavu, located at Kannur district of northern Kerala. It is one of the most popular tourist destinations in Kerala. The park houses a collection of different species of snakes including King Cobra, Indian Cobra, Rat Snake, Russel's Viper and a large collection of Pythons. The park is devoted for the preservation and conservation of snake species. They also promote various awareness programs to put an end to mythical fears and superstitions about snakes.

3.2 SAMPLE COLLECTION

Samples were obtained from different species of captive snakes. Fresh faecal samples from different species of snakes including Indian Cobra, King Cobra, Cat snake, Russel's Viper, Vine snakes and Pythons were collected from the enclosures of the snakes.

The samples were collected in sterile vials containing buffered peptone water as the pre-enrichment media. The samples were taken using the spatula provided within the vial. After collection, the samples were stored in an ice box.

3.3 IDENTIFICATION AND ISOLATION OF *SALMONELLA* SPECIES

3.3.1 Selective Enrichment

Tetrathionate broth and Rappaport-Vassiliadis (Himedia, Cat. #M-1491) broth were used as the selective enrichment media. The medium was prepared as per the manufacturer's instructions.

Using a pipette, 1ml of the inoculum (pre-enrichment broth) of each sample was transferred to 10 ml of Tetrathionate broth in the test tube and the tube was

labelled as Tube I. Using a pipette, 0.1 ml of the of inoculum (pre-enrichment broth) was then transferred to 10 ml of Rappaport-Vassiliadis broth and the test tube was labelled as Tube II. Tube I and Tube II were incubated at 36°C overnight (18-24 hours).

3.3.2 Selective Medium for Growth

Xylose lysine deoxycholate (XLD) (Himedia, Cat. #M031) agar and Brilliant green agar (BGA) (Himedia, Cat. #M016A) plates were prepared as per manufacturer's instructions.

Inoculum from incubated Tetrathionate broth (I) and Rappaport-Vassiliadis broth (II) were spread on Xylose lysine deoxycholate (XLD) agar and Brilliant green agar (BGA) and the plates were incubated at 36°C overnight (18-24 hours).

3.3.3 Subculture of Suspected *Salmonella* Colonies

After the examination of incubated XLD agar and BGA plates, the presence of typical *Salmonella* was determined by their colony morphology. Morphologically identified *Salmonella* colonies from XLD agar and BGA were then sub cultured to nutrient agar slants. These colonies were used for carrying out biochemical confirmation and antimicrobial susceptibility testing.

3.3.4 Gram Staining

Gram staining was performed according to Cappuccino and Sherman (2002). A thin smear from the culture was prepared, air dried and heat fixed. The smear was then flooded with Crystal Violet and waited for 1 minute and gently washed with distilled water. Then the smear was flooded with Gram's Iodine and waited for 1 minute. The smear was decolorized with ethyl alcohol for 5 seconds and washed with tap water. It was then counterstained with Safranin for 45 seconds followed by washing with distilled water. After air-drying, the slides were observed at 100X magnification.

3.3.5 Biochemical Characterization

Biochemical characterization of isolated colonies was performed using HiIMViC™ Biochemical Test Kit (Himedia, Cat. #KB001). Results of the tests were interrupted as per the standard given in the results interpretation chart provided with the kit.

3.4 ANTIBIOTIC SUSCEPTIBILITY TESTING OF SALMONELLA

The antibiotic susceptibility testing was carried out for the *Salmonella* isolates using Kirby-Bauer Disk Diffusion method (Behl *et al.*, 2017).

3.4.1 Preparation of Mueller-Hinton Agar and Plating

Mueller Hinton Agar (Himedia, Cat. #M1084) was prepared as per the manufacturer's instructions. The sterility of the petri plates was checked by incubating them overnight at 36℃. Then a broth suspension of the colony was evenly spread on Mueller-Hinton (MH) agar plate.

3.4.2 Placing Antibiotic Discs

All the isolates were tested for five antibiotics; tetracycline (30 mcg), ampicillin (10 mcg), erythromycin (15 mcg), gentamicin (10 mcg) and chloramphenicol (30 mcg). Antibiotic discs were placed on inoculated agar surfaces about two or three centimetres apart with gentle pressure and incubated overnight at 36℃. The zone of inhibition diameter was measured for each antibiotic. The obtained data were compared with an interpretative chart furnished by the manufacturer to grade the test isolates as sensitive, intermediate and resistant to respective antibiotics.

RESULTS

4. RESULTS

4.1 SAMPLES COLLECTED

A total of 30 samples were collected. All the samples were collected with proper care to avoid cross contamination. *Salmonella* was successfully isolated from 20 samples. Details of the samples collected are provided in Table 1.

Table 1. Details of the samples collected

Sl. No.	**Common name**	**Scientific Name**	**Number of samples**
1	Indian Cobra	*Naja naja*	4
2	Rat Snake	*Ptyas mucosa*	8
3	Russel's Viper	*Daboia russelii*	3
4	Indian Rock Python	*Python molurus*	12
5	King Cobra	*Ophiophagus hannah*	1
6	Vine Snake	*Ahaetulla nasuta*	1
7	Cat Snake	*Telescopus fallax*	1

4.2 SALMONELLA CULTURING

A total of 20 *Salmonella* colonies were isolated from the collected samples. Suspected *Salmonella* colonies were isolated from the faecal samples of Python (*Python molurus*) and Rat snake (*Ptyas mucosa*). The details are provided in Table 2.

Table 2. Snake species positive for *Salmonella*

Species of snake	**Result**
Python molurus	*Salmonella* spp.
Ptyas mucosa	*Salmonella* spp.
Ptyas mucosa	*Salmonella* spp.
Python molurus	*Salmonella* spp.

4.3 GROWTH ON XYLOSE LYSINE DESOXYCHOLATE AGAR

Typical *Salmonella* colonies appeared as red colonies with or without a black centre.

4.4 GROWTH ON BRILLIANT GREEN AGAR

Brilliant green agar was used as another selective agar for isolating the *Salmonella* species. Typical *Salmonella* colonies appear as red colonies surrounded by brilliant red zone in the agar plate.

4.5 GRAM STAINING

The samples were Gram stained and were observed under the light microscope at 100X magnification. The samples containing Gram negative rods were isolated for further tests.

4.6 BIOCHEMICAL CHARACTERIZATION

Biochemical characterization of isolated colonies was conducted using HiIMViC™ Biochemical Test Kit by Himedia Laboratories. Colonies were inoculated into the wells using the surface inoculation method followed by 24 hours of incubation at 36°C. The isolates were confirmed as *Salmonella* according to the result interpretation chart provided with the kit.

Based on the colony morphology on selective media and biochemical tests, *Salmonella* was identified in 4 samples. The details are provided in Table 3.

Table 3. Biochemical test result of each sample (I-Indole; MR-Methyl Red; VP-Voges Proskauer; Cit- Citrate; Glu-Glucose)

Sample	I	MR	VP	Cit	Glu	Results
1	-	+	-	+	+	*Salmonella*
2	-	+	-	+	+	*Salmonella*
3	-	-	-	+	-	Not identified
4	-	+	+	+	+	Not identified

Sample	I	MR	VP	Cit	Glu	Results
5	+	+	-	+	v	Not identified
6	-	-	-	-	+	Not identified
7	-	+	-	-	+	Not identified
8	-	-	-	+	-	Not identified
9	-	+	-	-	v	Not identified
10	-	-	-	-	+	Not identified
11	-	+	-	-	+	Not identified
12	-	+	-	+	+	*Salmonella*
13	+	+	-	-	+	Not identified
14	-	-	-	+	+	Not identified
15	-	-	-	+	+	Not identified
16	-	-	-	-	+	Not identified
17	-	+	-	+	+	*Salmonella*
18	-	-	-	v	+	Not identified
19	-	+	-	-	+	Not identified
20	-	+	-	-	-	Not identified

4.7 ANTIBIOTIC SUSCEPTIBILITY TEST FOR *SALMONELLA*

All the *Salmonella* colonies were exposed to 5 antimicrobial agents. A clear hollow devoid of bacterial colonies was formed around the antibiotic discs. The diameter of the zone of inhibition was used to determine whether the isolate was resistant or susceptible to the particular antibiotic. The observations are provided in Table 4, 5, 6, 7 and 8.

Table 4. Standardized zone of inhibition to different antibiotics

	Zone of inhibition (diameter in mm)			
Antibiotics	**Disk potency**	**Resistant**	**Intermediate**	**Susceptible**
Ampicillin	10 mcg	13 or less	14 -16	17 or more
Gentamicin	10 mcg	12 or less	13-14	15 or more
Erythromycin	15 mcg	13 or less	14-17	18 or more
Tetracycline	30 mcg	11 or less	12-14	15 or more
Chloramphenicol	30 mcg	12 or less	13-17	18 or more

Table 5. Diameter of the zone of inhibition by *Salmonella* isolates. (C-Chloramphenicol; A-Ampicillin; TE-Tetracycline; GEN-Gentamicin; E-Erythromycin)

SAMPLE	ANTIBIOTICS				
	C	A	TE	GEN	E
S1	3mm	1mm	1mm	14mm	5mm
S2	4mm	0mm	4mm	15mm	1mm
S12	9mm	0mm	2mm	19mm	4mm
S17	7mm	10mm	2mm	18mm	4mm

Table 6. Diameter of the zone of inhibition by un-identified isolates. (C-Chloramphenicol; A- Ampicillin; TE-Tetracycline; GEN-Gentamicin; E-Erythromycin)

SAMPLE	ANTIBIOTICS				
	C	A	TE	GEN	E
S3	3mm	0	1mm	12mm	7mm
S4	5mm	1mm	1mm	15mm	2mm
S5	4mm	2mm	1mm	14mm	4mm
S6	3mm	1mm	1mm	13mm	3mm
S7	3mm	0	1mm	12mm	3mm
S8	2mm	0	5mm	12mm	4mm
S9	6mm	0	5mm	15mm	6mm
S10	7mm	0	4mm	14mm	5mm
S11	5mm	0	2mm	11mm	3mm
S13	10mm	0	10mm	19mm	6mm
S14	3mm	1mm	0	18mm	3mm
S15	6mm	2mm	2mm	19mm	4mm
S16	5mm	1mm	1mm	18mm	2mm
S18	4mm	2mm	2mm	19mm	5mm
S19	3mm	10mm	1mm	18mm	3mm
S20	6mm	8mm	3mm	17mm	5mm

Table 7. Antibiotic susceptibility of *Salmonella* isolates. (R-Resistance; I-Intermediate; S-Susceptible)

SAMPLE	ANTIBIOTICS				
	C	A	TE	GEN	E
S1	R	R	R	I	R
S2	R	R	R	S	R
S12	R	R	R	S	R
S17	R	R	R	S	R

Table 8. Antibiotic susceptibility of un-identified isolates

SAMPLE	ANTIBIOTICS				
	C	A	TE	GEN	E
S3	R	R	R	R	R
S4	R	R	R	S	R
S5	R	R	R	I	R
S6	R	R	R	I	R
S7	R	R	R	R	R
S8	R	R	R	R	R
S9	R	R	R	S	R
S10	R	R	R	I	R
S11	R	R	R	R	R
S13	R	R	R	S	R
S14	R	R	R	S	R
S15	R	R	R	S	R
S16	R	R	R	S	R
S18	R	R	R	S	R
S19	R	R	R	S	R
S20	R	R	R	S	R

4.8 MULTIDRUG RESISTANCE

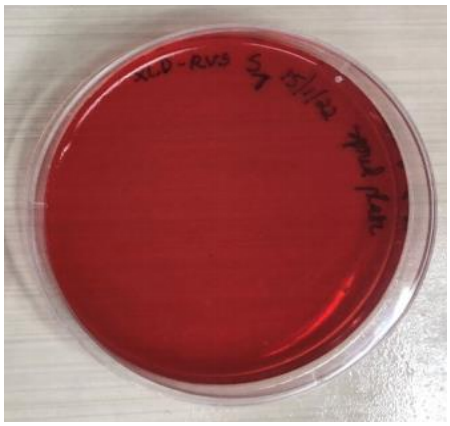
All the *Salmonella* isolates were resistant to ampicillin, tetracycline, chloramphenicol and erythromycin. However, isolates showed intermediate resistance and susceptibility to gentamicin.

Among the un-identified bacterial isolates, 75 per cent showed resistance to minimum 4 antibiotics and 25 per cent showed resistance to all 5 antibiotic drugs.

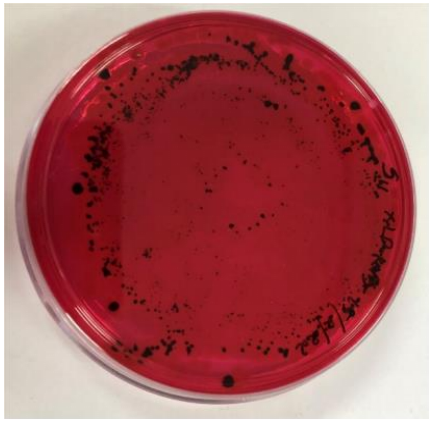
Ampicillin and tetracycline were the antibiotics to which isolates predominantly expressed resistance. However, isolates showed intermediate susceptibility to gentamicin.

Plate 1. Collection of fresh faecal sample from snake enclosure at MVR Snake Park, Kannur

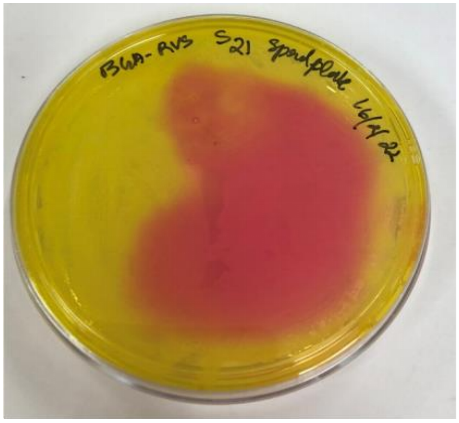
A



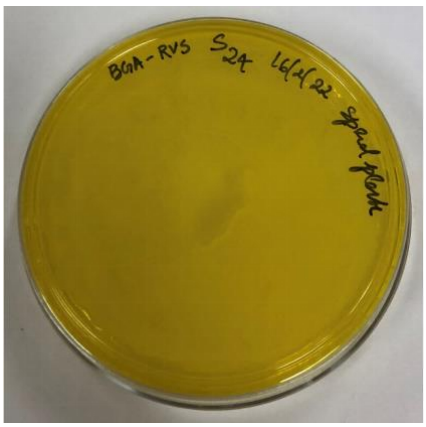
B



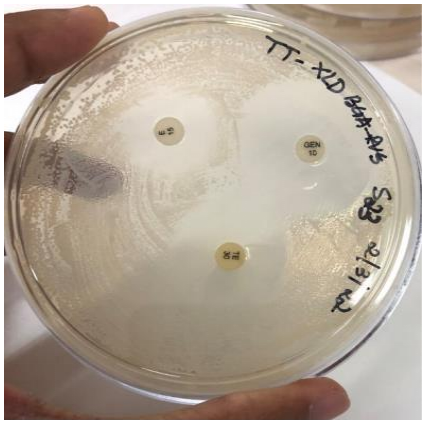
C



D

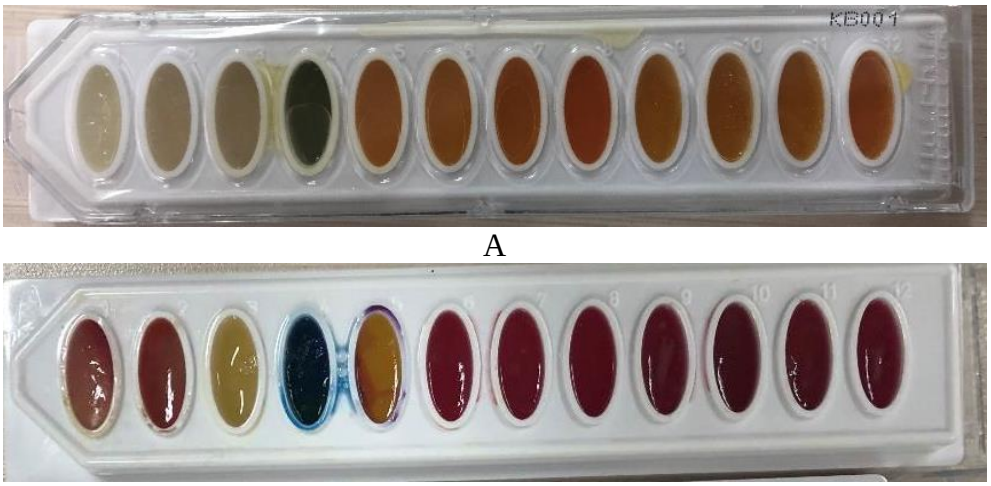


E



F

Plate 2. Bacterial growth on different culture medium. A) Uninoculated BG Agar B) Uninoculated XLD Agar C) XLD agar with black centred red colonies D) BG Agar with red colonies E) BG Agar without red colonies F) Zone of inhibition around antibiotic discs on MH Agar



A

B

Plate 3. Result of Biochemical Tests A) Reaction wells before incubation; B) Reaction wells after incubation

DISCUSSION

5. DISCUSSION

5.1 ANTIBIOTIC RESISTANCE OF BACTERIA IN THE WILD

Antibiotic resistance of bacteria due to unreasonable use of antibiotics is a major global problem. Multidrug resistance in bacteria is difficult to treat and even conventional combinations of antibiotics may fail to stop the infection. It eventually results in increased mortality and morbidity rate along with a rise in treatment expenditure (Frieri *et al.,* 2017). Studies in wild animals have revealed the presence of antibiotic resistant bacteria in a wild environment even though these animals had never been directly exposed to antibiotic therapy. Considering these studies, there is a chance for wildlife acting as a host of the antibiotic resistant bacterial gene that can spread across the globe (Radhouani *et al.*, 2014).

Wild as well as captive animals, can act as a reservoir of a diverse number of bacteria in their intestine which are shed into the environment. *Salmonella* species have been isolated from herpetofauna including snakes, lizards, turtles, *etc*. *S. houtenae* and *S. bongori* were usually isolated from reptiles. Wild animals, especially herpetofauna, harbour a variety of zoonotic potential bacteria (Ebani *et al.*, 2005).

S. typhimurium isolated from migratory gull population showed multidrug resistance to tetracycline, ceftriaxone and ceftiofur. However, it was susceptible to gentamycin (Palmgren *et al.*, 2006).

In this study, the antimicrobial susceptibility test was carried out for all the *Salmonella* positive isolates using the Kirby-Bauer Disk Diffusion method. A high percentage of multidrug resistant isolates, resistant to tetracycline, ampicillin, erythromycin and chloramphenicol were detected in this study.

5.2 MULTIDRUG RESISTANCE OF *SALMONELLA*

A study by Ogunleye *et al.* (2017) reported multidrug resistance of *Salmonella enterica* isolated from lizards. The isolates were resistant to chloramphenicol, ampicillin, streptomycin and kanamycin.

In this study, multidrug resistance was significantly high in *Salmonella* isolates. All four *Salmonella* isolates were multidrug resistant with resistance against at least 4 antibiotics. All the positive *Salmonella* positive isolates showed resistance to ampicillin, tetracycline, chloramphenicol and erythromycin. Among the unidentified bacterial isolates, 75 per cent showed resistance to minimum 4 antibiotics and 25 per cent showed resistance to all 5 antibiotic drugs.

Isolates were highly resistant to tetracycline and ampicillin, followed by erythromycin and chloramphenicol. However, isolates were susceptible to gentamycin.

The result of this study exhibits a high prevalence of multidrug resistance of *Salmonella* isolated from the faecal sample of snakes. The study follows the general trend of exhibiting a high rate of multidrug resistance in *Salmonella* isolated from reptiles and other animals.

5.3 ZOONOSIS AT THE HUMAN-WILDLIFE INTERFACE

Zoonotic diseases are one of the public health threats associated with close and frequent contact with wild animals. Both domesticated animals and wildlife can be a reservoir of many potential zoonotic pathogens. The influence of anthropogenic activity increases the risk of zoonotic disease emergence at the human-wildlife interface. Increased urbanization, habitat encroachment, land use changes, illegal wildlife trade, lack of proper management, and use of antimicrobial drugs aid the transmission of zoonotic diseases between wildlife and the human population (Hassell *et al.*, 2017). We have already experienced the effects of zoonotic diseases like Nipah, COVID-19, Ebola *etc*. To combat this

situation, there is an urgent requirement of more studies on bacteria and their antibiotic resistance in wild animals to guide us in regulating anthropogenic activities that disturb the equilibrium at the human-wildlife interface.

5.4 FUTURE PROSPECTS

WHO in 2011 declared antibiotic resistance as a global threat to health and raised the theme "Antimicrobial resistance: no action today and no cure tomorrow".

There is an urgent need for evaluating human-animal interactions including domestic animals and wildlife along with reduction in mass medications for animal herds in addition to including a global policy that regulates the use of antibiotics (Palma *et al.*, 2020)

Rather than careful use of antibiotics, it is recommended to adopt suitable prophylactic measures like immunization programs. Another suitable measure is to take up the usage of probiotics that directly act on the gut microbiota. On the other hand, predatory microbes are also now considered an alternative to antibiotics (Reid, 2006). Hoclzer *et al.* (2018) articulated that the administration of vaccines in the field of animal husbandry can aid in minimizing antimicrobial drug usage by preventing diseases in animals. Usage of enzymes, vaccines, innovative drugs, and immune related products derived from microbes can also substitute antibiotics.

Morrison and Saksida (2013) introduced vaccine administration against *Aeromonas salmonicida* in the farm salmon industry. This significantly reduced the usage of antibiotics.

5.5 ONE HEALTH CONCEPT

Antimicrobial resistant bacteria are found in the microbiomes of humans, animals and the environment. Adopting the One Health approach to any global

issue entails considering the interactions of domestic animals, humans, wildlife and the environment. Several governments and international organizations have adopted the One Health concept as a major attempt to combat antimicrobial resistance. AVMA (2018) defined One Health as a collaborative and multidisciplinary approach-working at the local and global levels. Their goal emphasizes the better health of humans, animals and the environment.

SUMMARY

6. SUMMARY

Antibiotics are drugs used against bacterial and viral infections. The synthesis of a variety of antibiotics over the past three decades has caused a dramatic increase in the usage of antibiotics in both animal and human health sectors. Gradually, the inappropriate use of antibiotics along with the decrease in the development of new antibiotic drugs triggered the rapid rise of antibiotic resistance in bacteria.

The emergence of antibiotic resistant bacteria is a global issue that must be addressed in a One Health approach because some diseases can be untreatable with conventional antibiotic drugs and they can affect any person or animal despite their age and country.

Salmonella is a facultative, gram-negative, non-spore producing, bacillus belonging to the *Enterobacteriaceae* family, which is considered a potential zoonotic pathogen. Reptiles are often asymptomatic carriers of *Salmonella* as they carry and shed *Salmonellae* in their faeces. Snakes are considered to be one of the sources of *Salmonella* infections in humans. Captive snakes become exposed to *Salmonella* when they interact with wild snakes harbouring *Salmonella* or through the diet. Wild snakes are never exposed to antibiotic treatment. However, they may acquire antibiotic resistant bacteria from the environment contaminated with the faeces of captive snakes which are released back to the wild or by consuming animals carrying resistant bacteria.

People often get infected by direct contact via animal handling or indirect transmission through the consumption of contaminated food or water as well as contact with contaminated surroundings. *Salmonella* species commonly encountered in reptile related Salmonellosis include *S. java, S. stanely, S. marina, S. poona, S. arizonae, S. heutenae* and *S. indica*.

Investigating the presence of antibiotic resistant *Salmonella* in captive snakes could aid in understanding their role in reptile related Salmonellosis. Hence, the present study was conducted to determine the presence of *Salmonella* species present in the captive snakes of MVR Snake Park, Kannur.

The faecal samples collected were initially cultured for *Salmonella* spp. on Xylose Lysine Deoxycholate and Brilliant Green Agar. Out of the 30 samples, pure colonies were confirmed by biochemical tests. A total of 20 isolates were obtained successfully. Biochemical tests like Indole, Methyl Red, Voges Proskauer's, citrate, and glucose were used for identifying the bacteria. In our study, isolates from 4 samples were confirmed as *Salmonella* spp. Antibiotic susceptibility testing was carried out for the identified isolates. All the isolates were resistant to more than 4 antibiotics. All the *Salmonella* isolates exhibited resistance to multiple antibiotics.

The result of this study exhibited a high prevalence of multidrug resistance in *Salmonella* isolated from the faecal sample of snakes and support the idea that the snakes can act as reservoirs of antibiotic resistant *Salmonella*.

Further studies have to be carried out to check for *Salmonella* species in wild animals like snakes, flying mammals and birds which are the victims of anthropogenic activities. For a sustainable healthy ecosystem, there should be healthy interaction at the human-wildlife interface. Health authorities, veterinarians and the general public should be aware of the potential zoonotic pathogens in wildlife and the possible route of pathogen transmission. Adoption of One Health approach and maintaining healthy interactions at the human-wildlife interface can ensure better human and animal health in the future

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DETERMINING THE PRESENCE OF SALMONELLA SPECIES AND ITS ANTIBIOTIC RESISTANCE IN CAPTIVE SNAKES OF MVR SNAKE PARK, KANNUR

SUMITHRA SOMANATH

(19-MSVP-09)

DISSERTATION

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

(Wildlife Studies)

2022

Faculty of Veterinary and Animal Sciences

Kerala Veterinary and Animal Sciences University

KVASU CENTRE FOR WILDLIFE STUDIES

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KERALA, INDIA

8. ABSTRACT

Antibiotic resistance is an emerging global issue that should be addressed by adopting One Health approach, for a healthy future. Antibiotic resistance in wild is difficult to solve due to lack of knowledge on the bacterial flora of wild animals. Reptiles, especially snakes, are considered as the carriers of *Salmonella* and transmitters of reptile mediated Salmonellosis in many parts of the world. Faecal samples from different snake species including Indian Cobra, Rat snake, Russel's Viper, Indian Rock Python, Cat snake, and Vine snakes were examined for *Salmonella*. In our study, we assessed the multidrug resistance of *Salmonella* isolates to 5 antibiotics - gentamicin, chloramphenicol, erythromycin, tetracycline and ampicillin. All the isolates exhibited resistance to all four antibiotics except gentamycin. The isolates showed high susceptibility to gentamycin. This finding supports the idea of snakes acting as potential reservoirs of multidrug resistant *Salmonella*.

Acad. Form No.19a

KERALA VETERINARY AND ANIMAL SCIENCE UNIVERSITY

Faculty of Veterinary and Animal Sciences

PROGRAMME OF RESEARCH WORK FOR DISSERTATION FOR MASTER OF SCIENCE DEGREE

1. Title of dissertation:

   Determining the presence of *Salmonella* species and its antibiotic resistance in captive snakes of MVR Snake Park, Kannur

2. a) Title of the department /KVASU research:

      Nil

   b) Project of which this forms a part:

      Nil

   c) Code No. if any, and order by which the departmental/KVASU research project is approved:

      Nil

3. a) Name of student: Sumithra Somanath

   b) Admission No: 19-MSVP-09

   c) Name of the programme: Master of Science (Wildlife Studies)

4. a) Name of Guide: Dr. Chintu Ravishankar

   b) Address: Professor
      Department of Veterinary Microbiology,
      College of Veterinary and Animal Sciences,
      Pookode, Wayanad- 673576

5. Objectives of the study:

   1. Identify the presence of Salmonella in the faecal samples of snakes in captivity
   2. Isolate the Salmonella strains from the faecal sample of captive snakes
   3. Assess antibiotic resistance of Salmonella species by Kirby-Baier disk diffusion method

6. Practical /Scientific utility:

Global trade in wildlife has been cited as an important disease transmission mechanism of growing concern in recent decades. The increased popularity of keeping exotic pets at home has also enhanced the risk of zoonotic infections.

Captive reptiles are known to be reservoirs of some important opportunistic pathogens. The oral and faecal flora of snakes are opportunistic pathogens to humans. Non-venomous snakes such as pythons and boas are kept as pets and in captivity. Poor captive management conditions expose these reptiles to a wide variety of pathogens. When the surrounding temperature is not optimal, accommodation is unsanitary, and when animals suffer from stress, the immune system in reptiles is suppressed and thus bacteria get an opportunity to settle in their host. Some snakes may harbour bacterial pathogens from the wild and clinical diseases become established once they are in captivity.

Captive reptiles also acquire bacteria from the environment or terrarium in which they are held or from other infected reptiles. Diseases such as ulcerative stomatitis, and other opportunistic infections are common in captive snakes (Bull. Wildlife Disease Association, 1966).

Salmonella is one of the most common enterobacteria. It is a ubiquitous Gram-negative rod bacterium. Over 2500 different serotypes have been identified to date for *Salmonella bongori* and *Salmonella enterica*. *Salmonella* is widely distributed in domestic animals and wildlife. One of the main characteristics of *Salmonella enterica* is that it can cause a variety of diseases of varying degrees in different hosts such as humans, swine, cows and mice. In humans, *Salmonella enterica* can cause a variety of food borne diseases such as gastroenteritis as well as systemic and persistent diseases (WHO, 2018).

Salmonella is naturally found in the gastrointestinal tract of reptiles (eg. snakes, turtles) and amphibians. *Salmonella* is carried by snakes and can be transmitted to humans through the food chain, causing harm to humans.

7. Important publications on which the study is based:

Cambre *et al*. (1980) studied salmonellosis and arizonosis in the reptile collections of Zoological Park.

Goldstein *et al*. (1981) conducted a study on aerobic bacterial oral flora of Garter Snakes regarding the development of normal flora and pathogenic potential for snakes and humans.

Gugnani *et al*. (1986) identified *Salmonella* and other enteropathogenic bacteria in the intestines of wall geckos in Nigeria.

Blaylock (2001) studied normal oral bacterial flora from some southern African snakes.

Burnham *et al*. (1998) studied the faecal shedding of *Salmonella* organisms among captive Green Iguanas and its potential public health implications.

Ebani *et al*. (2005) isolated *Salmonella enterica* from the faeces of domestic reptiles and studied their antimicrobial in vitro sensitivity.

Mir *et al*. (2015) studied isolation, serotype diversity and antibiogram of *Salmonella enterica* isolated from different species of poultry.

Eugale *et al.* (2016) determined the faecal prevalence, serotype distribution and antimicrobial resistance of *Salmonella* in dairy cattle.

Tomasticiva *et.al.* (2017) studied the prevalence and characteristics of *Salmonella* spp. isolated from captive reptiles in the Czech Republic.

Gutema *et al*. (2019) studied the prevalence and serotype diversity of *Salmonella* in healthy cattle and did a systemic review and meta-analysis of published studies.

8. Outline of the technical programme:

Faecal samples of captive snakes will be collected from their enclosures at MVR Snake Park, Parassinikkadavu, Kannur. A sufficient number of samples will be collected in sterile vials containing buffered peptone water. The samples will be sealed and transported to the laboratory on ice. Isolation and identification of *Salmonella* spp. will be based on their colony morphology and biochemical characteristics.

All isolated colonies will be subjected to different biochemical tests. All confirmed isolates will be evaluated for their antibiotic susceptibility using antibiotics of different classes. Disk Diffusion Method of Kirby and Bauer will be used for antibiotic susceptibility testing.

9. Main items of observations to be made:
   1) Cultural and morphological characteristics of bacterial isolates
   2) Biochemical characteristics of isolates
   3) Resistance of Salmonella isolates.
10. Duration of research work:
    One Semester

Signature of the Student

Project coordination group proposed: NIL

Place:

Date:

Signature of Guide

Name, address and signature of members of the Advisory committee

1. Dr. Abdul Azeez C. P.
   Associate Professor
   Department of Animal Reproduction,
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   College of Veterinary and Animal
   Sciences, Pookode

2. Dr. George Chandy
   Course Director and Special Officer
   KVASU Centre for Wildlife Studies,
   Pookode

11. References:

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WHO (Global Foodborne Infections Network). 2010. Laboratory protocol: Isolation of Salmonella spp. (5th Ed.).

CERTIFICATE

Certified that the research project has been formulated observing the stipulations laid down under the Prevention of Cruelty to Animals Act (Amendment, 1998).

Place: Pookode
Date:

Dr. Chintu Ravishankar

CURRICULUM VITAE

1.	Name of the candidate	: Sumithra Somanath
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9.	Publications Made	: Nil
10.	Membership in Professional Societies	: Nil