UNIVERSITI PUTRA MALAYSIA

THE OCCURRENCE OF SALMONELLA SP. AMONG CAPTIVE SNAKES IN KLANG VALLEY, MALAYSIA

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FPV 2022 79

THE OCCURRENCE OF SALMONELLA SP. AMONG CAPTIVE SNAKES IN KLANG VALLEY, MALAYSIA

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A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

December 2022

CERTIFICATIONS

It is hereby certified that we have read this project paper entitled "The Occurrence of Salmonella sp. Among Captive Snakes In Klang Valley, Malaysia" by Nurul Faqhira binti Abd Ghani and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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ACKNOWLEDGEMENTS

As this paper project marks the third quarter of my Doctor of Veterinary Medicine degree, I would like to express my gratitude to everyone that has helped me throughout my five years journey in vet school.

I am grateful to God for giving me strength and patience to pursue my dream and endure all obstacles that came into my way.

Dr. Rinalfi, Dr. Sharina and Dr. Azlan who has guided me through this rocky road journey.

My parents and family who have always believed in me and pushed me to follow my dreams and never be afraid to aim for the stars.

My cats; Ponyo, Panda, Zorro and Oreo for their never-ending supply of serotonin and dopamine boost.

Raihanah and Eizzah for being my best friends and support system through all years of vet school

BTS

myself.

LIST OF FIGURES

LIST OF TABLES

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan VPD 4999 – Projek Ilmiah Tahun Akhir.

KEJADIAN *SALMONELLA* DALAM KALANGAN ULAR PELIHARAAN DI LEMBAH KLANG, MALAYSIA

Oleh

NURUL FAQHIRA BINTI ABD GHANI

2022

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Salmonellosis adalah satu penyakit zoonosis yang disebabkan oleh *Salmonella* dan boleh berjangkit kepada manusia melalui laluan fecal-oral secara sentuhan langsung atau tidak langsung dengan haiwan yang dijangkiti. Ini membawa kepada kebimbangan kesihatan awam kerana salmonellosis adalah penyakit yang mengancam nyawa. *Salmonella* adalah bakteria gram-negatif daripada keluarga Enterobacteriaceae. Bakteria ini dicirikan oleh kehadiran flagella, berbentuk rod dan merupakan penghuni normal dalam saluran gastrousus reptilia. Genus

Salmonella terdiri daripada beberapa serovar iaitu S. typhimurium, S. enteritidis dan S. albany. Salmonellosis pada manusia ditunjukkan oleh gejala seperti cirit-birit, demam, sepsis, kejutan, dan juga kematian. Oleh itu, kajian ini bertujuan untuk mengetahui kejadian *Salmonella* dalam kalangan ular peliharaan di Lembah Klang, Malaysia. Sebanyak 11 sampel swab kloaka dan najis segar daripada ular peliharaan termasuk sepuluh ular sawa dan seekor ular California kingsnake dikumpul daripada beberapa sumber yang berpangkalan di Selangor. Sampel-sampel tersebut kemudiannya tertakluk kepada pengasingan dan pengecaman bakteria menggunakan Brilliant Green Agar (BGA), Xylose Lysine Deoxycholate (XLD) agar dan polyvalent 'O' antisera. Keputusan menunjukkan bahawa *Salmonella* sp. telah dikesan dalam 1 daripada 11 (9.09%) sampel ular. Kesimpulannya, walaupun kajian ini menunjukkan bahawa kehadiran *Salmonella* adalah rendah; disebabkan sampel yang tidak mencukupi, kajian yang lebih teliti dicadangkan untuk dilakukan pada masa hadapan bagi menentukan prevalens *Salmonella* dalam ular dalam kurungan.

Kata kunci: *ular, Salmonella, salmonellosis, Malaysia, peliharaan*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD4999 - Final Year Project.

THE OCCURRENCE OF *SALMONELLA* SP. AMONG CAPTIVE SNAKES IN KLANG VALLEY, MALAYSIA

By

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2022

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Salmonellosis is a zoonotic disease caused by *Salmonella* and can be transmitted to humans through faecal-oral route via direct or indirect contact with the infected animals. This leads to a public health concern as salmonellosis is a life-threatening disease. *Salmonella* is a Gram-negative bacterium from the Enterobacteriaceae family. The bacterium is characterised by the presence of flagella, is rod in shape and is a normal inhabitant in gastrointestinal tract of reptiles. The genus *Salmonella* consists of several serovars namely *S. typhimurium*, *S. enteritidis* and *S.*

albany. Salmonellosis in humans is manifested by symptoms such as diarrhoea, pyrexia, sepsis, shock, and even death. Hence, this study aimed to determine the occurrence of *Salmonella* among captive snakes in Klang Valley, Malaysia. A total of 11 cloacal swab and fresh faecal samples from pet snakes including ten ball pythons and one California kingsnake were collected from several sources based in Selangor. The samples were then subjected to bacterial isolation and identification using Brilliant Green Agar (BGA), Xylose Lysine Deoxycholate (XLD) agar and polyvalent 'O' antisera. Results showed that *Salmonella* sp. was detected in 1 out of 11 (9.09%) snake samples. In conclusion, although this study indicated that the presence of *Salmonella* is low; due to the insufficient samples, a more thorough study is suggested to be done in the future to determine the prevalence of *Salmonella* in captive snakes.

Keywords: *snake, Salmonella, salmonellosis, Malaysia, captive*

1.0 INTRODUCTION

Salmonella is a significant organism that causes substantial morbidity and mortality for both humans and animals worldwide. *Salmonella* infections or salmonellosis in humans can cause both enteric or typhoid fever and gastroenteritis or non-typhoidal disease (Ohl & Miller, 2001).

Snakes have become residents to an extensive number of houses and exhibition centres as pets in recent years. Furthermore, snakes are also kept in captivity such as zoos for exhibition and research purposes. In addition to bacteria, snakes can also carry a number of viruses and parasites including protozoa, helminths, cestodes and nematodes (Mendoza-Roldan *et al*., 2020). This has raised a concern to public health issues throughout the world. It is likely that more than 90% of reptiles such as snakes, lizards and chelonians carry *Salmonella* in their gastrointestinal tracts though they do not manifest symptoms of the disease. This happens as they are the natural reservoirs and asymptomatic carriers of *Salmonella*. Other companion animals such as dogs and cats may also contract *Salmonella* from pet snakes and may be presented with poor prognosis. Humans who

have been exposed or come in contact with carrier snakes may exhibit serious illness and potentially could be fatal (Clancy *et al*., 2016).

As *Salmonella* is shed intermittently in the digestive tract of the snakes, it is difficult to rule out that the animal is truly free from *Salmonella*. If the faecal samples or cloacal swabs are acquired during the non-shedding time of the organism, this condition might not be properly diagnosed (Saleha *et al*., 2015).

Unlike other countries, there were few to none reptile-associated salmonellosis cases that were reported in Malaysia. As a consequence, there are scarcely any published studies that concentrate on *Salmonella* in reptiles in Malaysia. This study was conducted to contribute to understanding the prevalence of this organism among reptiles hence the objective of this study is to detect the presence of *Salmonella* sp. among pet snakes in Klang Valley, Malaysia. The null hypothesis of this research is *Salmonella* sp. is not detected in the intestines of pet snakes in Klang Valley, Malaysia while the alternative hypothesis is *Salmonella* sp. is detected in the intestines of pet snakes in Klang Valley, Malaysia.

2.0 LITERATURE REVIEW

2.1 Aetiological agent

Salmonella is a Gram-negative bacterium which is characterised by the presence of flagella and is rod in shape. *Salmonella* was initially discovered by an American bacteriologist, D. E. Salmon from the swine intestines back in 1884. The nomenclature of *Salmonella* is rather complex although there has been immense amount of research revolving around this bacterium. *Salmonella* is a genus of the Enterobacteriaceae family that can be further classified into two species namely *Salmonella enterica* and *Salmonella bongori.* There are six subspecies under *S. enterica* specifically *enterica* (subspecies I), *salamae* (subspecies II), *arizonae* (subspecies IIIa), *diarizonae* (subspecies IIIb), *houtenae* (subspecies IV) and *indica* (subspecies V) and a plethora of serovars or serotypes. As reported by Andino and Hanning (2015), there are currently 2579 serovars. On other hand, there is only one subspecies which is the subspecies V under *S. bongori*.

S. enterica subsp. *enterica* is the perpetrator found in the majority of isolates that leads to disease in both humans and

animals. A few typhoidal serovars including *S. enterica* serovar *Typhi* (*S. Typhi*) and *S. enterica* serovar *Paratyphi* (*S. Paratyphi)* are pathogens that spread from human to human through direct contact and have no significant findings to proof that animals play a role as the reservoir. The non-typhoidal serovars namely *S. enterica* serovar *Typhimurium* (*S. Typhimurium*), *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica* and *S. bongori* are zoonotic or potentially zoonotic. These organisms are usually found in cold-blooded animals or poikilotherms such as reptiles and amphibians and in the environment (contaminated soil, water and vegetation) as well. Reported cases of salmonellosis in humans are occasionally linked with these pathogens.

2.2 Prevalence of *Salmonella* in snakes

The prevalence of *Salmonella* found in the gastrointestinal tract of pet snakes is expected to be high. It is estimated that 90% of all reptiles are the carrier of *Salmonella* in which they shed through their faeces (Woodward *et al*. 1997). A previous study done in Japan revealed that *Salmonella* was

detected in 23 of 23 (100%) snake samples collected from a pet shop (Nakadai *et al*., 2005). Lukac *et al*. (2015) reported that 8 of 90 snakes (8.9%) were tested positive for *Salmonella* in Croatia. In addition, *Salmonella* was detected in 51% of pet snakes that were presented for post-mortem in Canada (Onderka *et. al*, 1985).

2.3 Reptile associated salmonellosis

According to a study reported by Majowicz *et al.* (2010), it was estimated that 93.8 million cases of salmonellosis were reported annually in the entire world. Nonetheless, in the USA, it is hypothesised that interaction with reptiles and amphibians is the source of 6% of sporadic salmonellosis cases and 11% of cases in individuals under the age of 21 (Mermin *et al*., 2004).

According to the Centers for Disease Control and Prevention (CDC), 50,000 to 80,000 cases of reptile-associated salmonellosis are reported each year in the United States. In addition, CDC revealed that the rate of people infected with *Salmonella* after exposure to reptiles has increased seven times, from 96 cases in 1986 to 671 cases in 1995 (Constance and Wilkins, 1998).

3.0 MATERIALS AND METHODS

3.1 Sources of samples

Faecal materials and cloacal swabs regardless the species, sex and age were taken from individually owned snakes in Selangor area.

3.2 Sampling

Cloacal swabs were taken from one (1) California kingsnake and two (2) ball pythons using sterile cotton swabs. The swabs were inserted gently into the cloacas of the snakes to prevent unnecessary harm to the snake (Figure 1). It is important to restrain the snake correctly to prevent injury to both personnel and animal (Figure 2). Meanwhile, faecal materials (Figure 3) were collected from eight (8) ball pythons. A total of eleven (11) samples were acquired.

Figure 1. Cloacal swabbing of snake

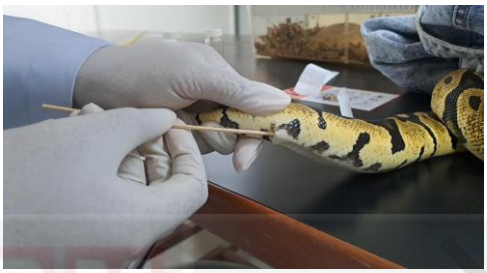

Figure 2. Restraining the snake

Figure 3: Faecal samples

3.3 Culture and identification of isolates

3.3.1 Pre-enrichment media

Upon reaching the Bacteriology Laboratory, the samples obtained were transferred into 9 ml of Buffered Peptone Water (BPW) followed by incubation at 37°C for 24 hours. In this research, Buffered Peptone Water (BPW) acts as pre-enrichment media as it is rich in nutrients that facilitates recovery of the bacteria and allows cell damage repair.

3.3.2 Enrichment stage

Following the pre-enrichment stage, Rappaport-Vassiliadis (RV) broth was used in the enrichment stage. Rappaport-Vassiliadis (RV) broth is a selective enrichment broth to isolate *Salmonella* sp. It inhibits the growth of enterococci and coliform bacteria and favours *Salmonella*, *Proteus* and *Pseudomonas*. After the previous stage, 1000µL of incubated Buffered Peptone Water (BPW) was transferred into 9 ml of Rappaport-Vassiliadis (RV) broth followed by incubation at 42°C for 24 hours.

3.3.3 Isolation media

After the enrichment stage, a loopful of incubated Rappaport-Vassiliadis (RV) broth was streaked onto Brilliant Green Agar (BGA) and Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24 hours. Presumptive *Salmonella* colonies (pink colonies on BGA agar and black colonies on XLD agar) were then subcultured onto nutrient agar to obtain pure colonies followed by incubation at 37°C for 24 hours.

3.3.4 Biochemical Tests

3.3.4.1 Triple Sugar Iron (TSI)

A single, pure colony from the nutrient agar was inoculated onto the TSI agar. The tubes were then incubated at 37°C for 24 hours. The function of TSI is to observe the ability of microorganisms to ferment sugar namely sucrose, lactose and glucose. *Salmonella* is a non-lactose fermenter and only ferments glucose. To detect glucose fermenter, the content of the glucose in the agar is 1/10 of sucrose and lactose. Since the fermentation on the slant causes it to rapidly oxidised, the slant remains red or alkaline. Whereas in the butt, since there is low oxygen tension, it remains acidic or yellow.

3.3.4.2 Urease

A single, pure colony from the nutrient agar was inoculated onto the urease agar. The tubes were then incubated at 37°C for 24 hours. Urease test is done to observe the ability of microorganisms to hydrolyse urea into ammonia and CO_2. Since *Salmonella* couldn't hydrolyse urea, negative results should be expected.

3.3.4.3 Citrate

A single, pure colony from the nutrient agar was inoculated onto the citrate agar. The tubes were then incubated at 37°C for 24 hours. Some serovars produce positive (green agar turns blue) result such as *S. enteritidis* and *S. typhimurium* while serovars such as *S. typhi*, *S. paratyphi* A, *S. pullorum* and *S. gallinarum* will produce negative (green agar remains green) results.

3.3.4.4 Sulfide-Indole-Motility (SIM) test

A single, pure colony from the nutrient agar was inoculated onto the SIM agar. The tubes were then incubated at 37°C for 24 hours. This test is used to differentiate the members of Enterobacteriaceae based on the H_2S, indole production and motility. Presence of H_2S is indicated by the black precipitation of

the medium while motility is indicated when the medium becomes completely turbid out of the stab line. Motility indicates the flagellar activity of the *Salmonella*. *Salmonella* does not produce indole because it does not harbour tryptophanase (tnaA) which is the enzyme responsible for tryptophan metabolism.

3.3.5 Serological test

This test was done by mixing together a drop of normal saline with a loopful of *Salmonella* colony from the nutrient agar on a glass slide. Then, a drop of *Salmonella* polyvalent 'O' was dropped onto the suspension. The glass slide was tilted back and forth in a swirling motion for one minute. It is then observed against a dark or black background with good illumination. A positive result will produce an agglutination or granular clumps within 60 seconds.

4.0 RESULTS

4.1 Isolation and identification

A total of one *Salmonella* isolate was acquired from 11 samples from the gastrointestinal tract of pet snakes.

4.2 Cell and colony morphology of *Salmonella*

Presumptive colonies on the BGA and XLD agar were subjected to gram staining. Under the microscope, *Salmonella* colonies appeared Gram-negative small rods. Typical *Salmonella* colonies on the BGA agar are characterised by formation of pink colonies. Colonies on XLD agar appeared as black dot colonies.

4.3 Biochemical Test

Salmonella was isolated in one sample. From the TSI test, a positive growth of alkaline (red) slant, acidic (yellow) butt, presence of hydrogen sulfide (H_2S) with the presence of gas formation. The isolate showed negative for both urease and citrate tests. SIM test showed sulphide positive, indole negative with positive motility.

This presumptive *Salmonella* colony was then subjected to polyvalent 'O' antisera for confirmation. Rapid agglutination

was formed within 60 seconds as soon as the *Salmonella* suspension was mixed with the antisera.

5.0 DISCUSSION

A zoonosis is an infectious disease that can spread from animals to people or vice versa. Those who are immunosuppressed or immunocompromised, such as those receiving immunosuppressive therapy (patients undergoing chemotherapy or taking glucocorticoids such as prednisolone and dexamethasone), newborns, young children, and elderly people with chronic illnesses, are those who are most at risk. It is advisable for those at high risk of manifesting severe symptoms after contracting *Salmonella* to avoid contact with reptiles as the bacteria can be lethal to humans.

Many issues in pet snakes can be attributable to management. When in captivity, reptiles are rarely kept in ideal environmental conditions. This occurs as it can be laborious to the owner to maintain the enclosure of the snakes as close as possible to their natural habitat in which it can result in a weakened immune system and the invasion of opportunistic pathogens like *Salmonella*. *Salmonella* could also infect pet snakes after they are fed with contaminated raw foods such as chicken and mouse.

To reduce the risk of acquiring *Salmonella* from pet snakes, personal hygiene should be practised by all owners. A study done by Saravanan in 2004 concluded that good sanitation practices such as washing hands could reduce the transmission of *Salmonella* as much as 67%. The awareness about the danger of keeping pet snakes also should be taught to buyers and owners by veterinarians and pet shop operators. Certification that could prove the snakes are *Salmonella*-free during importation could also protect the general population from acquiring reptile-associated salmonellosis.

As there is a high risk for owners to acquire *Salmonella* from their pet snakes, Association of Reptilian and Amphibian Veterinarian (ARAV) had developed guidelines in order to minimise the risks of the owners to get infected with reptile-associated salmonellosis. Some of the suggested precautions were:

- Reptile veterinarians should inform owners or potential buyers to take an appropriate step to minimise the risk of spreading *Salmonella* to people
- Suitable actions to lower the risk of *Salmonella* transmission between reptiles and people should be

followed by all veterinarians, employees, and clients who handle these animals

- It should be assumed that all reptiles and amphibians carry *Salmonella* in their digestive systems and may shed it in their faeces
- Antimicrobial drugs should not be administered to healthy reptiles and amphibians with the goal of removing *Salmonella* from the digestive system as emergence of antimicrobial-resistant *Salmonella* strains might cause a massive health risk to people

6.0 CONCLUSION

Pet snakes are potential carriers for *Salmonella*. Healthy adults are generally less likely to get infected with *Salmonella* if compared to young children or elderly people. A clear understanding should be established in order to handle the animals safely. Education and public awareness are the responsibility of veterinarians, pet store owners and pet owners in reducing the risk of contracting reptile-associated from pet snakes.

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8.0 APPENDICES

Table 1: Laboratory results of samples collected from Selangor area

Sample	TSI	UREA	CITRATE	SIM	POLY 'O'	RESULT
1	Alkaline/ Alkaline H_2S	-ve	-ve	+ve	-ve	Neg
2	Alkaline/ Alkaline H_2S	-ve	-ve	+ve	-ve	Neg
3	-	-	-	-	-	Neg
4	-	-	-	-	-	Neg
5	Alkaline/ Acidic	-ve	+ve	-ve	-ve	Neg
6	-	-	-	-	-	Neg
7	Alkaline/ Acidic H_2S Gas	-ve	-ve	+ve	+ve	Pos
8	-	-	-	-	-	Neg
9	Alkaline/ Acidic	-ve	+ve	-ve	-ve	Neg
10	-	-	-	-	-	Neg
11	-	-	-	-	-	Neg

Table 2: Common and scientific name of the sampled snakes

Sample	Common (English) name	Common (Malay) name	Scientific name
1-2	Ball python	Ular sawa bola	*Python regius*
3	California kingsnake	Ular raja	*Lampropeltis getula californiae*
4-11	Ball python	Ular sawa bola	*Python regius*