



JURNAL VETERINAR MALAYSIA

Vol. 25 No. 1 & 2 Dec 2013

Table of Contents

Journal Article

Potency and efficacy of a low pathogenic H5N2 inactivated vaccine against challenge with a Malaysian H5N1 highly pathogenic avian influenza virus

S.H. Sharifah, M.N. Surian, M. Maizan, G.H. Ong, D. Azizah, K. Suzana, A.R. Omar and I. Aini.....1

Effects of *Escherichia Coli* vaccination in gilts on piglet performance in a farm in Perak

W.C. Michelle-Fong, P.Y. Choo, B.L. Ong, C.Y. Tee, J.W. Lee, and P.T. Ooi.....7

Occurrence of *Salmonella* and other enteric microbes in faeces of house lizards (*Hemidactylus frenatus*)

Gwen Lo and Saleha A.A.....11

Review Article

Feline Hypertrophic Cardiomyopathy – Prevalence, risk factors and pathological aspect

K.H. Khor.....15

The role of omega-3 polyunsaturated fatty acids on brain cognitive function - Review of studies on laboratory animals

A. Hafandi22

Short Communication

Moderately-high humoral antibody responses to a H5N2 inactivated vaccine did not suppress shedding of highly pathogenic H5N1 Avian Influenza virus during challenge

S.H. Sharifah, K. Hasuzana, M.N. Suriani, M. Maizan, G.H. Ong, D. Azizah, A.R. Omar and I.Aini.....28

Jurnal Veterinar Malaysia is the official journal of the Veterinary Association Malaysia (VAM). It was published formally as *Kajian Veterinar* and the Malaysian Veterinary Journal.

Researcher papers on various aspects of veterinary medicine, animal science and research are invited for publication either as full articles or short communication. Review papers and abstracts of articles of local interest are also published from time to time. The publisher (VAM) does not hold itself responsible for statements made in the journal by contributors. Unless so stated, materials in the Journal do not reflect an endorsement or an official attitude of VAM or the Editorial Board of JVM.

THE VETERINARY ASSOCIATION MALAYSIA

ISSN 9128-2506

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OCCURRENCE OF *SALMONELLA* AND OTHER ENTERIC MICROBES IN FAECES OF HOUSE LIZARDS (*Hemidactylus frenatus*)

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SUMMARY

Reptiles have been shown to be natural reservoirs of *Salmonella* and other enteric bacteria and the reptile species close to homes and eateries are house lizards. This study aimed to determine the occurrence of *Salmonella* in house lizards at residential and eateries premises. Fresh faecal samples were collected from live 25 house lizards and 20 pooled dried droppings were collected around premises. None of the samples were positive for *Salmonella*. It was probable that a number of lizards may be carrying *Salmonella* as shown by other previous studies and in this case they were not shedding the bacteria in the faeces at the time and in the dried faecal droppings, *Salmonellae* was probably absent or did not survive the dry and hot condition. Enteric bacteria that were frequently isolated from fresh droppings were *Klebsiella pneumoniae* and *Citrobacter freundii* which were found resistant to amoxicillin + clavulanic and tetracycline.

Keywords: *Salmonella*, enteric bacteria, house lizards / geckos, antibiotic resistance

INTRODUCTION

Salmonella lives in the intestines of vertebrates and has been frequently reported in herpatofauna, particularly reptiles. The presence of *Salmonella* in reptiles was first reported in Gila monster and the regal horned lizard (Chambers *et al*, 2006). The increase in popularity of exotic reptiles, such as turtles, snakes and iguanas, as pet animals has led to increase in reptile-associated salmonellosis, such as in United States, which is estimated at 93,000 cases annually (Schroter *et al*, 2004; Pasmans *et al*, 2005). One reptile species that is of interest is the house lizards or geckos because they are a common sight in residential, commercial and eateries premises. These house lizards are native to Southeast Asia but are now invasively distributed worldwide in other tropical regions and also subtropical regions such as northern parts of Australia, Africa and America. They can be seen at night on walls and ceilings of houses, buildings, eateries, porches and balconies to hunt for insects that are attracted to lights.

Salmonella presences in reptiles are often asymptomatic and they shed the organisms, continuously or intermittently, in the faeces. Humans and pet animals may be infected if they are in contact with *Salmonella*-laden faecal materials. Conditions associated with salmonellosis in humans include gastroenteritis, bacteraemia, meningitis, osteomyelitis, peritonitis and pleuritis (Chambers *et al*, 2006).

Salmonella are ubiquitous in nature and able to survive for weeks in water and for years in soil and dust in environmental conditions (such as temperature, humidity and pH) that are favourable (Todar, 2006). Thus, the bacteria can survive in dried faeces in the environment for a period of time. However, the occurrence of *Salmonella* in

the faeces was low compared to geckos that were actually carrying the bacteria (Chan *et al*, 1982).

Nonetheless, these faeces or droppings are still considered as route of transmission of the infection. Little is known on the faecal carrier status of *Salmonella* in the house lizards. Apart from *Salmonella*, not much is known regarding the occurrence of other pathogenic microorganisms in their faeces. This is because the droppings may contaminate food or water supply or the premises environments and that humans may be in contact with these faeces. Thus, the aim of this study was to determine the occurrence of *Salmonella* and other enteric microbes in the faeces of house lizards and the antibiotic resistance of the bacteria isolated.

MATERIALS AND METHODS

Samples collection

The premises identified to trap the live house lizards were two blocks of a residential college and two cafeterias in UPM, an apartment block and a water tank on the highest floor of an apartment building.

A total of 25 live lizards were caught. These lizards which crept on ceilings and walls were swept down and then captured using gloved hands. The lizards were then individually placed in a bottle (5L) containing a small box in which the lizard can hide inside. The lizards were kept until they defecated. Fresh faeces were collected within 12 hr after defecation using a sterile glove, placed in a plastic bag and brought to the Veterinary Public Health Laboratory, UPM. Most of the lizard defecated within a day after they were captured but a small number took two days. Those that did not defecate within two days were fed with a meal worm until they defecated. Once the faeces were collected, the lizard was released.

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Twenty (20) pooled lizard droppings were collected from different residential and eateries premises. The lizard droppings could not be differentiated physically in terms of age but were estimated to be more than 5 - 7 days old. Ten (10) of the droppings were collected from several eateries in Seri Kembangan and the other 10 were collected from various houses in Serdang. These droppings could be found on walls, furniture tops, window panes and corners and edges of rooms. The droppings on the floor were not collected.

Bacteria culture, isolation and identification

Unlike fresh faeces, isolation of enteric microbes was not done on the dried droppings, that is, isolation of *Salmonella* was only conducted on the dried faeces. Each faecal dropping was added into 10ml of buffered peptone water in a bottle for pre-enrichment. The mixture was incubated at 37°C for 24 hr. In the enrichment stage, 1ml of the pre-enriched sample was transferred into Rappaport Vassiliadis Enrichment Broth (Oxoid). The mixture was then incubated at 37°C for 24 hr. After incubation, a loopful of the enriched culture was inoculated onto Brilliant Green Agar (BGA) and Xylose Lysine Tergital 4 (XLT4) agar (Oxoid) and the plates were incubated at 37°C for 24 hr. Then, the plates were examined for bacterial growths and colonial morphology and the cellular morphology was examined by Gram staining. Different colonies were subcultured onto the same agar for purification. On BGA, typical *Salmonella* colonies appeared red whereas on XLT4, the bacteria colonies appeared black or black-centred with a yellow periphery. The suspected *Salmonella* colonies from pure cultures were subjected to biochemical tests for identification which consisted of inoculation into Triple Sugar Iron agar, Lysine Iron agar, Sulphide Indole Motility agar and subjected to citrase and urease tests. Then, suspected *Salmonella* colonies were subjected to a serological test, that is, a slide agglutination test using polyvalent O *Salmonella* antisera. To isolate other enteric microbes, the faecal samples were cultured on Blood agar (Oxoid) and MacConkey agar (Oxoid) to isolate Gram positive and Gram negative bacteria respectively. All the different types of colonies obtained were subjected to appropriate biochemical tests to identify the Gram positive and Gram negative bacteria as described in Jang *et al* (2008).

Antibiotic susceptibility test

Bacteria that were found predominant in the lizards caught in each premises were subjected to antibiotic susceptibility test using the disc diffusion method of Kirby Bauer as described by NCCLS (2002), now known as Clinical and Laboratory Standards Institute or CLSI. Six (6) antibiotics were used, namely streptomycin (S, 10µg), chloramphenicol (C, 30µg), amoxycillin + clavulanic acid (AMC, 30µg), tetracycline (TE, 30µg), trimethoprim-

sulfamethoxazole (SXT, 25µg), and enrofloxacin (ENR, 5µg). Briefly, each isolate was suspended into 2mL nutrient broth and standardized to 0.5 Mac Farland standard. A sterile swab was dipped into the suspension and swabbed over the entire surface of Mueller Hinton (MH) agar (Oxoid) plates in three overlapping directions - horizontally, vertically and obliquely. The plates were allowed to dry for approximately 5 min. The six antibiotic discs were dispensed onto the inoculated MH agar plate by using an antibiotic disc dispenser. After incubation, the plates were examined against a dark background to clearly visualize the zone of inhibition around each antibiotic disc. The zone of inhibition was measured using a calliper. The measured zones were compared to the zone diameter interpretive standards breakpoints (NCCLS, 2002) or following the manufacturer instructions to determine the susceptibility of the isolates to the antibiotics.

RESULTS AND DISCUSSION

From the study, *Salmonella* was not isolated from all the fresh and dried samples. Nine (9) out of 20 dried droppings that were collected from various residential and eateries premises showed no bacteria growth. It is probable that the lizards may be carrying *Salmonella* but not shedding the bacteria in the faeces at the time, hence it would not be present in the sampled faecal droppings. This is because the study by Fazhana *et al* (2007) on 32 house geckos found 31.0% positive, with 43.0% and 28.0% from houses and eateries, respectively. In that study, *Salmonella* was isolated from the gastrointestinal tracts of the house lizards. A study by Calaway *et al* (2011) in Townsville, North Australia found 7% of the house geckos carried *salmonellae* in the large intestines. Otokune *et al* (2003) reported *Salmonella* carriage rate of 32% in the gastrointestinal tracts of wall gecko (Geckonidae) and 35% in pooled lizard droppings from various sources. A number of works had reported that *Salmonella* is able to survive in adverse environment for a considerable period of time; however in the dried lizard droppings sampled, *Salmonellae* was probably absent or did not survive the dry and hot condition. However, according to Otokune *et al* (2003), *Salmonella* can survive longer in dry as compared to wet environments - 4 weeks in tap water and up to 6 and 8 weeks in droppings left exposed directly to air and mixed with dry sand respectively. The frequency of isolation of different species of enteric bacteria from the faeces of the house lizards sampled is shown in Table 1.

The predominant enteric bacteria from house lizards at the residential and eateries premises were *Klebsiella pneumoniae*, followed by *Citrobacter freundii*. Other bacteria that were isolated from the residential premises were *Klebsiella oxytoca* and *Proteus penneri* whereas from the eateries were *Enterobacter cloacae*, *Staphylococcus sp* and *Bacillus sp*. Apart from *Salmonella*, the enteric organisms which Gugnani *et al* (1986) isolated from wall geckos (*Hemidactylus brookei*) in Nigeria, included *Proteus*

mirabilis, *Pseudomonas aeruginosa*, *Escherichia coli*, *K. pneumoniae* and *E. cloacae*. A number of these enteric bacteria are opportunistic pathogens that can cause infections to those who are immunocompromised such as the young, old, people with debilitating disease, cancer or Acquired Immunodeficiency Syndrome.

Figure 1 depicts the antimicrobial resistance among all the *K. pneumoniae* and *C. freundii* isolates. The two bacteria were found resistant to amoxicillin + clavulanic acid while 80% of *K. pneumoniae* isolates were resistant to tetracycline. The bacteria were susceptible to enrofloxacin, chloramphenicol, trimethoprim + sulfamethoxazole and streptomycin. The isolated bacteria showed low antibiotic resistance with no multiple drug resistance (resistant to four or more antibiotics) detected; it is most likely due to lack of exposures of the house lizards to environment that were contaminated with resistant bacteria (Pasmans *et al*, 2005).

The house lizards are a common sight in residential, commercial and eateries premises and they indiscriminately litter the premises with their droppings, also reported by Otokune *et al* (2003). Although in this study *Salmonella* was not isolated, other studies had shown otherwise. The presence of house lizards in close proximity to man and the enteropathogens they may carry could cause infections in man should man come in contact with enteropathogen-laden faeces or by contaminating food, water and the floors. A more detailed epidemiological study with larger sample size, more premises and wider locations and to include the zoonotic viruses and enteric parasites would paint a better picture into the possible role of the house lizards in sporadic zoonotic infections such as salmonellosis in households and eateries.

Table 1: Enteric microbes isolated from faeces of house lizards

<i>Bacteria</i>	<i>No. of sample with isolates</i>	<i>Percentage (%)</i>
<i>Klebsiella pneumoniae</i>	15	44.1
<i>Klebsiella oxytoca</i>	3	8.9
<i>Citrobacter freundii</i>	10	29.4
<i>Proteus penneri</i>	1	2.9
<i>Enterobacter cloacae</i>	2	5.9
<i>Staphylococcus sp</i>	2	5.9
<i>Bacillus sp</i>	1	2.9
TOTAL	34	100.0

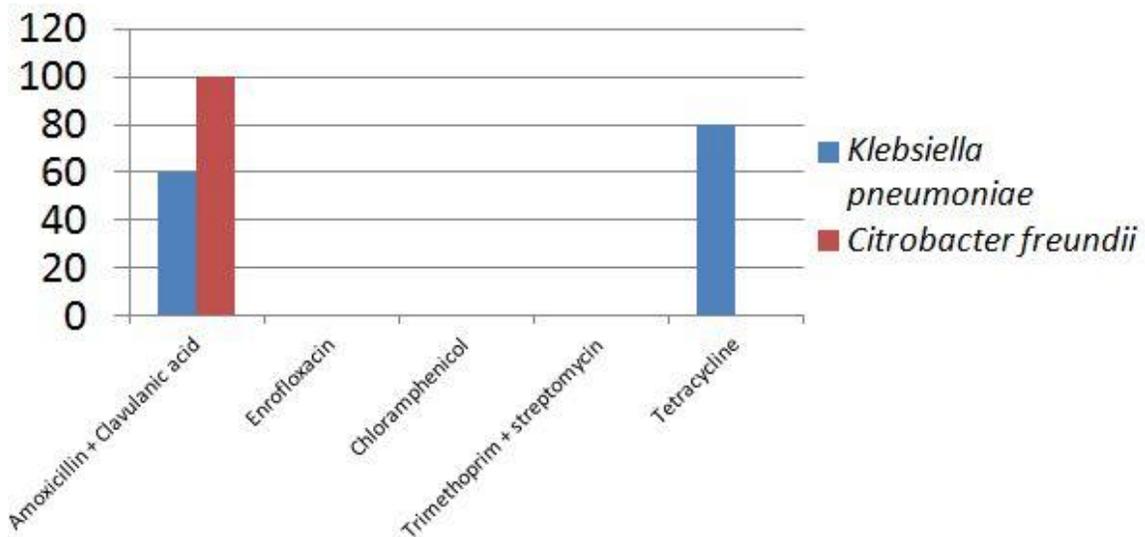


Figure 1: Antimicrobial resistance among *Klebsiella pneumoniae* and *Citrobacter freundii* isolated from house geckos

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