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Review Articles

SALMONELLA SEROTYPES IN ANIMALS AND ANIMAL PRODUCTS IN MALAYSIA OVER 40 YEARS, 1980 – 2020 – A REVISIT

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SUMMARY

Salmonella is one of the most important pathogens affecting animal and human health. With more than 2600 serotypes ubiquitous in nature, *Salmonella* is found worldwide, causing salmonellosis in humans primarily through foodborne. This review highlights *Salmonella* serotypes belonging to *Salmonella enterica* subspecies *enterica*; in brief, the serotyping, nomenclature, host specificity, and typhoidal and non-typhoidal serotypes. It mainly reports on *Salmonella* isolations in Malaysia over 40 years, particularly from available reports by the Veterinary Research Institute and Regional Veterinary Laboratories under the Department of Veterinary Services, Malaysia. An interesting piece of information about 'geo-serotypes' is that one of the serotypes is named *Salmonella* Malaysia, and possibly three serotypes are named according to three locations in Malaysia.

Keywords: animals, Malaysia, non-typhoidal Salmonella, serotypes

INTRODUCTION

Salmonella is one of the most well-recognised and essential pathogens in animals and humans. It is reported to be one of the four key global causes of diarrhoeal diseases in humans. According to the World Health Organisation (WHO) (2018), diarrhoeal diseases are the most common illnesses as a result of unsafe food, due to being undercooked and contaminated, affecting 550 million people in contracting the diseases annually, including 220 million children under the age of 5 years old. A study estimated that each year, 94 million cases of gastroenteritis and 155,000 deaths occur in humans due to salmonellosis worldwide (Hoelzer et al., 2011).

Salmonella, a ubiquitous and hardy organism, can survive several weeks in a dry environment and several months in water (WHO, 2018). The organisms are widely distributed in domestic and wild animals. They are prevalent in food animals, which include poultry, pigs, cattle, goats, sheep; in companion animals, such as horses and in pet animals, such as cats, dogs, and birds; and rodents such as rats, rabbits, wild birds, reptiles such as turtles, lizards and snakes, in amphibians and fish and even in invertebrates. *Salmonella* can be found throughout the food chain from primary production in farms include in animal feed, slaughterhouses, processing plants and all the way to retail outlets and markets as well as food processing plants, food-service establishments and institutions, food retail outlets and households. In the US and Canada, it has been reported that commercial pet food and animal-derived pet treats can be sources of *Salmonella* infection not only to pet animals but also to humans (Hoelzer et al., 2011).

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Salmonella Serotyping and Serotypes

Genus *Salmonella* is a gram-negative rod belonging to the Enterobacteriaceae family. It consists of two species, namely *Salmonella* bongori and *Salmonella enterica*. *Salmonella enterica* is divided into six subspecies, namely S. *enterica* subspecies *enterica*, *S. enterica* subspecies salamae, *S. enterica* subspecies arizonae, *S. enterica* subspecies diarizonae, *S. enterica* subspecies houtenae and S. *enterica* subspecies indica.

Serotyping defines subtypes, i.e., serotypes or serovars, within a subspecies. This review mentioned typing only on *Salmonella enterica* subsp. *enterica*. The serotyping of *Salmonella* is by slide agglutination based on the Kauffmann-White, today known as the White-Kauffmann-Le Minor scheme (Ryan et al., 2017). This scheme is based on the agglutination of bacteria with specific sera to identify somatic (O) and flagellar (H) variants. These antigens are highly variable, with 64 O and 114 H variants identified; the O antigen determines it belongs to *Salmonella* while the H antigen determines the serotypes. The capsular (K) antigen is rare among *Salmonella* serotypes.

Today, the current nomenclature for *Salmonella* was updated by the Judicial Commission of the International

Committee for Systematics of Prokaryotes in 2005. Hence, the serotype name must not be italicised, and the first letter of the serotypes must be capitalised (e.g., Typhimurium) - an example: *Salmonella enterica* subspecies *enterica* serotype Typhimurium, for short as *Salmonella* ser Typhimurium, *Salmonella* Typhimurium or *S*. Typhimurium. Names are only given to subspecies *enterica* serotypes, representing 99.5% of all *Salmonella* strains, whereas the remaining *Salmonella* strains are named after their antigenic formula (Gossner, 2016; Ryan et al., 2017).

In 1934, only 44 *Salmonella* serotypes were identified; to date, there are 2639 serotypes in *Salmonella enterica*, predominantly in the subspecies *enterica*, which consisted of 1586 serotypes (Ryan et al., 2017). Most of these serotypes are found in humans and animals. A small number is said to be host-specific or host-restricted, while a very small number are host-adapted (Table 1).

Table 1: Classification of *Salmonella* serotypes as per their host specificity and diseases in the hosts

Group	Serotypes	Hosts
Unrestricted serotypes	*S*. Enteritidis *S*. Typhimurium *S*. Weltevreden Several others	Mainly in humans, poultry, pigs and many other animals. The organisms frequently cause gastroenteritis or food poisoning in humans.
Host specific / Host restricted serotypes	*S*. Typhi *S*. Parathypi A, B, C *S*. Sendai *S*. Hirshfeldii *S*. Pullorum *S*. Gallinarum *S*. Abortusovis *S*. Typhisuis *S*. Abortusequi	Humans (causing enteric fever or typhoid) Humans (causing enteric fever or paratyphoid) Humans (causing enteric fever) Humans (causing enteric fever) Poultry (causing pullorum disease) Poultry (causing fowl typhoid) Sheep (causing abortion and mortality of newborn lambs) Pigs (causing chronic paratyphoid) Horses (associated with abortion in mares, neonatal septicaemia, polyarthritis, testicular lesions in males)
Host adapted serotypes - may cause infection, though seldom, in other species, including humans	*S*. Dublin *S*. Choleraesuis	Cattle (causing cause clinical diseases, such as enteritis, septicemia, pneumonia, and reproductive losses) Pigs (causing typhoidal salmonellosis)

Thus far, no host adapted serotypes reported in cats and dogs. Rodents, reptiles and wild birds are the main reservoirs for Salmonella in the environment. Joseph et al., 1976; Uzzau et al., 2000, Singh (2013), Kuria (2023)

Geo-Serotypes *Salmonella*

Another interesting fact about *Salmonella*e is that, according to Gossner et al. (2016), most *Salmonella* serotypes have been named after geographic locations by tradition; they found that 1,475 (93%) of the 1,585 serotypes of S. *enterica* subsp. *enterica* could be categorised as geo-serotypes; the name refers to a geographic location. The three countries with the most geo-serotypes are Germany (n = 181), the United Kingdom (n = 157) and the United States (n = 148). Other serotypes' name refers to the name of a person, animal, tribe, or food item or are a composite of symptoms and host. For more interesting facts and details, refer to "Around the World in 1,475 *Salmonella* Geo-serotypes" (Gossner et al., 2016).

Upon searching in the comprehensive list containing 2557 *Salmonella enterica* serotypes by Grimont and Weill (2007) as to whether there are serotypes named after locations in Malaysia, these were found - *S*. Malaysia, *S*. Seremban, *S*. Taiping, *S*.. Melaka; *S*. Seremban was reported in man by Singh (1967), however reports on *S*. Malaysia, *S*. Taiping and *S*. Melaka were not available.

***Salmonella* Isolates in Malaysia**

Joseph et al (1978) reported *Salmonella* isolations in Malaysia were first made at the Veterinary Research Institute (VRI) in 1954, where *S*. Choleraesuis and *S*. Typhimurium were isolated from pigs, and the first isolation of S. Pullorum from chickens was made in 1956. Since then, the number of isolations of *Salmonella* serotypes and animal species has increased. Today, *Salmonella* isolations are also carried out in the five Regional Veterinary Laboratories (RVL) under the Department of Veterinary Services (DVS) Malaysia.

There is plenty of research and studies on *Salmonella* in Malaysia—in humans, animals, animal–based food products, vegetables, fruits, and the environment—including the serotypes identified. However, this brief history is on early *Salmonella* isolations and serotypes until quite recent reports, mainly those reported from VRI and RVL, 1971 - 2017, are shown in Table 2.

To date, there are about 200 serotypes in Malaysia as reported in 1996 – 2001 by Maria et al. (2002), an increase from 24 serotypes reported in 1971 - 1975 by Joseph et al. (1976). Among the isolates being frequently reported in the last 20 years included *S*. Enteritidis, *S*. Typhimurium, *S*. Weltevreden, *S*. Newport, *S*. Agona, *S*S. Branderup, *S*. Seftenberg and *S*. Corvallis. Centers for Disease Control and Prevention (CDC) in US reported that approximately 60% of salmonellosis cases in human were caused by *S*. Enteritidis (24.7%), *S*. Typhimurium (23.5%), *S*. Newport (6.2%) and S. Heidelberg (5.1%) and that these same four serotypes represented 46.4% of

Table 2 *Salmonella* isolations and serotypes reported from VRI and RVL (DVS), 1976 to 2019

Authors	Reports
Joseph et al., 1976	● The earliest surveillance report on animal salmonellosis was for the period 1971 – 1975, reporting a total of 488 *Salmonella* isolations mainly from poultry (61%), followed by pigs (24%), cattle and buffalo (3%), others (2%) which included guinea pigs, parrots, horse, ducks. ● 24 serotypes were identified, and 11 new serotypes were recorded. The most common serotypes were *S*. Pullorum (60.7%), followed by *S*. Choleraesuis (10.9%) and *S*. Infantis (9.4%).
Joseph et al., 1986	● The next five-year period, 1976 – 1980, reported 652 *Salmonella* isolations from 24 sources, including from food-producing animals and their products, laboratory and wild animals, as well as the lizard, fish, and prawn. ● Fifty-six isolates were recovered, with 31 reported as new serotypes. The three most frequently serotypes isolated were *S*. Pullorum-gallinarum (26%), *S*. Blockley (7%) and *S*. Dublin (6%). ● *S*. Pullorum-gallinarum was significantly reduced from 1979 onwards due to successfully implementing the National Pullorum Disease Eradication Programme. ● It was also reported that *S*. Weltevreden replaced *S*. Typhimurium as having the widest zoological distribution.
Joseph et al., 1988	● The third surveillance report was for the period 1981 – 1985; there were 2322 *Salmonella* isolations, an increase of 356% compared to the previous five-year period ● Eighty-three serotypes were recorded, and of these, 34 were new serotypes, making a total of 97 serotypes in the list since 1971. The food-producing animals and edible products comprised the most isolations at 92%, with cattle and beef accounting for 70%. ● It is interesting to note that in this period, *S*. Dublin was the most frequently isolated serotype, while *S*. Pullorum-gallinarum was not isolated. This time *S*. Typhimurium had the widest zoological distribution
It was unfortunate that report for the period 1986 – 1990 was not available	
Zaliha et al., 1994	● A report on *Salmonella* in meat and meat products from 1990 – 1993 collected at entry points, abattoirs, processing plants and retail outlets found 11% positive and highest in frozen beef at 16.6%, followed by pork (10.3%), poultry meat (9.6%) and processed meat products (4.6%) ● The three most frequently isolated serotypes were *S*. Newport (10.1%), *S*. Anatum (8.6%) and S. Reading (5.9%).
Mokhtar et al., 1996	● A report on *Salmonella* in animals, livestock products and feed for the period 1991 – 1995 ● 8084 *Salmonella* isolations were reported with 1393 or 17% isolates identified as *S*. Enteritidis, followed by *S*. Typhimurium (6%), *S*. Weltevreden (5%), *S*. Braenderup (4.8%) and *S*. Newport (4.6%). *Salmonella* isolates were primarily from livestock products (46.7%), followed by food-producing animals (40.5%). ● Among food-producing animals, poultry accounted for 26.8%, followed by cattle at 5.4%, while among livestock products, beef/buffalo meat accounted for 30.4%, followed by chicken meat at 11.3%. ● It was also reported 5.9% of the environmental and feed samples were positive, with *S*. Enteritidis being most frequently isolated from the environment, mainly from hatcheries. ● In this period, 137 *Salmonella* serotypes were recorded, and 27 new serotypes were recorded.

At this time, saw a significant increase of *S*. Enteritidis from 1993 not only from poultry (36% of 2170 *Salmonella* positive poultry) but also from poultry meat (34% of 910 *Salmonella* positive chicken

meat), cattle and environment. The early report on the number of *S*. Enteritidis isolations was in 1966, 1967 (34) and 1974 (2), but mainly from guinea pigs (33), buffalo (2) and rabbits (1). Joseph et al (1986), isolation of *S*. Enteritidis in chickens (2) and duck (1) was made in 1976, 1977 and 1979 (1 each), and Joseph et al. (1988) reported four isolations of *S*. Enteritidis in chickens and ducks. An interesting hypothesis was put forth by Rabsch et al. (2000) that *S*. Enteritidis "filled the ecologic niche vacated by eradication of *S*. Gallinarium from poultry leading to an increase in human infections"; they tested this hypothesis through retrospective studies in Germany and found that *S*. Enteritidis cases in humans are inversely related to the prevalence of *S*. gallinarium in poultry and from other studies suggested that "*S*. Gallinarium competively excluded *S*. Enteritidis from poultry flocks early in the 20th century". It is also interesting to note that according to Rohani et al. (1995) on human salmonellosis in Malaysia for the period 1989 – 1994, S. Weltevreden was the most common isolate until 1993 when more than 30% of salmonellosis in humans was due to *S*. Enteritidis.		
Maria et al., 2002	●	A report for the period of 1996 – 2001. ● 12680 *Salmonella* isolates were serotyped. This period saw *S*. Enteritidis as most frequently isolated at 16%, and of this, chicken accounted for 43%, chicken meat at 17% and poultry farm environment at 27%. *S*. Weltevreden accounted for 4.8% and was isolated mainly from beef, followed by poultry, buffalo meat and poultry farm environment. *Salmonella* Typhimurium (4.8%) recovered mainly from beef, followed by pigs, ducks, poultry farm environments, buffalo meat, and poultry. ● Other common serotypes included *S*. Agona, *S*. Anatum, *S*. Mbandaka, *S*. Senftenberg, *S*. Braenderup, *S*. Newport and *S*. Hadar.
Rohaiza et al., 2006	●	A report for the period 2002 – 2005. ● Only 4652 *Salmonella* isolates were serotyped, and 93 serotypes were recorded. *S*. Enteritidis was most frequently isolated at 12.7%, followed by *S*. Typhimurium and *S*. Corvallis, *S*. Tennessee and *S*. Newport. *Salmonella* isolates mainly came from poultry (24.6%), followed by cattle, ducks, and buffalo. Among livestock products, *Salmonella* was isolated more from beef (8.3%), followed by chicken meat and buffalo beef.
Roseliza et al., 2010	●	In 2009, VRI received 425 *Salmonella* isolated from meat (buffalo meat, beef, pork, poultry meat) sampled in 38 retail plants in five states and provided by VPHL and RVL. ● 31 serotypes were identified. The most common serotype was *S*. Typhimurium, which was found in all types of meat, followed by *S*. Enteritidis, *S*. Corvallis, *S*. Senftenberg, and *S*. Indiana. ● The common isolates in poultry meat were *S*. Enteritidis and *S*. Corvallis; in beef, *S*. Senftenberg and *S*. Agona; in buffalo beef, *S*. Senftenberg; and in pork, *S*. Typhisuis (51%).
Thenamutha et al., 2013	●	218 *Salmonella* isolated from animal specimens received at VRI from 2008 – 2012; 13 serotypes were identified. ● It was reported *S*. Enteritidis, *S*. Albany, *S*. Tennesse, *S*. Typhimurium were isolated from poultry; *S*. Albany, *S*. Indiana, *S*. Kottbus, *S*. Dublin from bovine; *S*. Typhimurium from porcine and *S*. Weltreveden from caprine.
Marina et al., 2013	●	Apart from meat, meat-based products were also monitored. A report on poultry-based products, including nuggets, burgers and frankfurters from processing plants from 2010 to 2012, found *Salmonella* at 5.4% (145 of 2689 samples of poultry meat and poultry-based products). ● *Salmonella* Enteritidis was the most frequent serotype isolated, followed by *S*. Albany, *S*. Typhimurium, *S*. Agona and *S*. Corvallis.
Saira Banu et al., 2019	●	Poultry and buffalo meat, beef, and pork samples received at RVL at Bukit Tengah, Penang, from 2013 – 2017; a total of 14 % (117 of 821) were found positive for *Salmonella*e with buffalo meat accounting for the highest isolation at 51% with 91 serotypes identified. ● The most common serotypes isolated were *S*. Enteritidis (poultry meat at 24%) and *S*. Typhimurium (poultry meat at 12%; pork at 18%). Other serotypes included *S*. Senftenberg and *S*. Weltevreden (buffalo meat at 13% and 6% respectively), *S*.

Jamaica (beef at 18%) and *S.* Stanley (buffalo at 18%, pork 8%) and *S.* Rissen (pork at 31%).

- Interestingly, *S.* Rissen was reported as common worldwide, causing salmonellosis outbreaks and being among the top three serotypes in pigs and pork products in Europe and Southeast Asia.

the isolates from nonhuman sources that year (Demirbilek, 2017).

Humans generally contract salmonellosis through consuming contaminated food of animal origin (mainly eggs, meat, poultry, and milk), either raw or undercooked, and other foods, including green vegetables contaminated by manure or contaminated soils, have been implicated. Salmonellosis in humans can also occur when individuals have contact with infected animals, including pets and captive animals in public settings such as petting zoos, and with contaminated surfaces and environments in zoological gardens. Infected animals most often do not show signs of disease. Person-to-person transmission can occur through the faecal-oral route. A study estimated that about 55% of *Salmonella* cases in humans are foodborne, 14% are travel-related, 13% are acquired through environmental sources, 9% are attributable to human-to-human transmission, and 9% are due to direct animal contact (Hoelzer et al., 2011).

Among food of animal origin, poultry meat is popular worldwide, including in Malaysia, being readily available, somewhat cheaper compared to other types of meat, and almost acceptable to all. Khoo et al. (2023) reported that DVS had conducted the *Salmonella* National Surveillance Programme and found an increasing trend in the isolations of *S.* Brancaster from chickens in the country, being isolated along the processing line, which included the floors, chopping boards, wash water, and chicken cuts. In Europe and West Africa, it was reported that *S.*. Brancaster had been isolated from patients with diarrhoea and implicated in fatal cases in infants and elderly patients (Khoo et al., 2023).

Chuah et al. (2018) reported from various studies that poultry and environment samples obtained from wet markets were to be consistently contaminated with *Salmonella*. Moreover, their study was on three predominant serotypes, namely *S.* Albany, *S.* Brancaster and *S.* Corvallis isolates; they found these isolates colonised various sites in the processing environment by producing biofilms on food contact surfaces or food processing equipment. Producing biofilms can lead to the *Salmonella* adaptation and long-term survival at these various sites in wet markets and poultry processing plants. Several researchers have also reported biofilm-forming *Salmonella* serotypes on the surfaces of equipment and premises in plants where food animals are slaughtered and processed. *Salmonella* are also found to form biofilms on plastic, cement and stainless steel. Biofilm formation is considered an environmental adaptation strategy by other microorganisms, as it provides protection to the bacterial cells against various environmental challenges such as desiccation, pH and osmotic changes, disinfectants, antimicrobials and UV light radiation (Chuah et al., 2018). In addition, their study found the *Salmonella* isolates were resistant to several antibiotics; thus, the presence of these multidrug-resistant (MDR) *Salmonella* is a serious and significant public health concern.

According to Gantois et al. (2009) reported that *S.* Enteritidis, contaminates chicken eggs more successfully than any other *Salmonella* serotype and that the contamination of the eggs is by "the passage from the hen's intestinal tract to the reproductive tract and is not by penetration through the shell; from the reproductive tract, *Salmonella* gets incorporate into the forming egg on the vitelline membrane, in the egg white or the shell membranes". Hence, such eggs can cause salmonellosis if consumed raw or semi-cooked (egg-borne salmonellosis).

Salmonellosis in humans is usually characterised by acute onset of fever, abdominal pain, diarrhoea, nausea and sometimes vomiting. The onset of symptoms is about 6–72 hours (usually 12–36 hours) after ingestion of food contaminated with *Salmonella*, and the illness may last about 2–7 days. The symptoms of salmonellosis are relatively mild, and in most cases, infected persons usually recover without specific treatment. However, in some cases, particularly in children and elderly patients, the dehydration and infection can become severe and life-threatening. It is interesting to note that large *Salmonella* outbreaks in humans usually attract media attention; however, 60–80% of all salmonellosis cases are classified as sporadic cases, or are not diagnosed as such at all.

CONCLUSION

Salmonellosis causes great health and economic impacts resulting from treatment and health care, prevention and control, and surveillance and monitoring of *Salmonella* in humans and animals. The presence of *Salmonella* in the environment and food products is worrisome because they may cause diseases, and in cases of food products, it is due to the product recall. Moreover, the development and spread of multidrug resistant *Salmonella* has become a significant public health concern. Thus, concerted efforts and strategies are very much needed for prevention and control of *Salmonella* in animals, animal products and the environment.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

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