RETROSPECTIVE STUDY ON PERSISTENT *SALMONELLA* SEROTYPES IN MEAT SAMPLES TESTED IN THE VETERINARY PUBLIC HEALTH SECTION, REGIONAL OF VETERINARY LABORATORY BUKIT TENGAH, PENANG

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ABSTRACT. A retrospective study of laboratory data analysis on *Salmonella* serotypes was conducted in four states across the northern region of Malaysia including Perlis, Kedah, Penang and northern Perak. The increasing cases of antimicrobial resistant due to *Salmonella* spp. and various *Salmonella* serotypes being isolated from meat samples are alarming and this increases the chance of foodborne outbreak. Therefore, the aim of this study was to determine presence of various different *Salmonella* serotypes in meat samples received from year 2013 until 2017 by the Veterinary Public Health Section, Regional of Veterinary Laboratory Bukit Tengah, Penang. A total of 821 meat samples were sent for *Salmonella* isolations and serotyping and 117 different *Salmonella* serotypes were identified from poultry, buffalo, beef and pork meat. The most prominent serotypes were *Salmonella* ser. Enteritidis (10.23%), *Salmonella* ser. Typhimurium (8.89%), *Salmonella* ser. Senftenberg (7.43%) and followed by *Salmonella* ser. Brancaster (5.35%) and *Salmonella* ser. Albany (5.23%). Different patterns of predominance of the serotypes were shown for all the meat samples. For example, in poultry meat, the dominant serotype was *S.* Enteritidis (24.36%). Other serotypes such as *Salmonella* ser. Stanley (22.22%) and *Salmonella* ser. Jamaica (22.22%) were the most common serotypes identified in beef meat. In buffalo and pork meat samples, the predominat serotypes were *S.* Senftenberg (12.62%) and *Salmonella* ser. Rissen (30.77%) respectively. In conclusion, the identification of *Salmonella* serotypes will increase the effectiveness of control and prevention of an outbreak of salmonellosis.

Keywords: *Salmonella* serotypes, salmonellosis, meat, poultry, beef, buffalo, pork.

INTRODUCTION

Salmonella sp. was one of the main foodborne pathogens frequently reported worldwide (Kidanemariam *et al*., 2010) and has not declined for over a decade compared to others (Shafini *et al*., 2017). *Salmonella* infection known as salmonellosis is caused by non-typhoidal *Salmonella enterica* serotypes and characterised by a self-limiting gastroenteritis syndrome

(Antunes *et al*., 2016). The infected person may develop diarrhoea, fever and abdominal pain (Antunes *et al*., 2016; Sen, 2016), four to 72 h after infection and will usually last four to seven days (Sen, 2016). According to Majowicz *et al*. (2010), about 98.3 million illnesses and 155,000 deaths occur globally every year due to gastroenteritis caused by *Salmonella* species and has become the greatest foodborne burden that leads to impacts on human health (Kirk *et al*., 2015). Furthermore, consumption of meat products such as contaminated poultry meat has become the main source of infection globally (Antunes *et al*., 2016). It is in an agreement with Roseliza *et al*. (2011), where salmonellosis was found to be commonly associated with consumption of contaminated food products including poultry, beef and pork and further supported by Hsi *et al*. (2015), where poultry, beef, pork, or lamb had caused about one-third of *Salmonella*-related diseases in the US.

Various *Salmonella* serotypes isolated from the meat samples reflect the high chance of human infection since meat is the main source of protein. It is also an indication of the presence and widespread distribution of *Salmonella* of animal origin (Roseliza *et al*, 2011). Antimicrobial resistance and emergence of multidrug-resistance phenotypes among *Salmonella* serotypes are on the increase (Hur, Jawale and Lee, 2012), making it a major concern in terms of public health and its implications worldwide. In addition, as stated by Andino and Hanning (2015), animal food sources were found to express resistance to *Salmonella* strains. Thus, a better understanding of *Salmonella* serotypes from meat samples might help us prevent the potential spread of resistant strains by the implementation of more effective control to prevent outbreaks of salmonellosis. Therefore, the aim of this study is to determine the various *Salmonella* serotypes presence in meat samples.

MATERIALS AND METHOD

There is a total of 821 samples of meat from slaughterhouses, abattoirs and Malaysian Quarantine and Inspection Services (MAQIS) located in Perlis, Kedah, Penang and Northern Perak were sent to the Regional Veterinary Laboratory of Bukit Tengah for *Salmonella* isolations and serotyping from year 2013 until 2017. The meat samples including poultry, buffalo, beef and pork. The total number of meat samples were respectively 312, 420, 11 and 78. *Salmonella* sp. was isolated and examined on the basis of cultural, morphological and biochemical reactions through four successive stages: pre-enrichment, selective enrichment, isolation and confirmation stages; based on the Manual of Veterinary Laboratory Testing for Veterinary Public Health (DVSM, 2016).

Pre-enrichment stage

About 25 g of meat sample was put into a sterile Stomacher® bag and 225 ml of non-selective liquid medium i.e. buffered peptone water (BPW) was added. The sample was then homogenised using Stomacher® blender for 1 to 2 minutes and was incubated at 37 °C for 24 hours.

Selective enrichment stage:

At this stage, 0.1 ml of the sample homogenate was transferred into a selective liquid medium i.e. the Rappaport-Vassilidiasis broth (RV) and was incubated at 42 °C for 24 hours.

Isolation stage

A loopful of selective enrichment cultures in RV was streaked onto Xylose Lactose Tergitol™ 4 (XLT-4) selective agar plate, followed by incubation at 37 °C for 24 hours. On examination, the appearance of black-centred colonies with yellow periphery were presumably *Salmonella* sp.

Confirmation stage

A biochemical test i.e. triple sugar iron (TSI), indole, urease and polyvalent O and H agglutination test was used to confirm *Salmonella* sp.

TSI test

The typical colonies of *Salmonella* sp. were picked using a sterile inoculating loop and was stabbed into the butt of the TSI and then streaked back and forth on the slant. The TSI was then incubated at 37 °C for 24 hours.

Indole test

Tryptone broth was inoculated with a loopful of TSI agar culture and was incubated at 37 °C for 24 hours. Then, Kovac's reagent was added. A negative reaction which is the absence of a deep red coloured ring at the surface of the broth would indicate the presence of *Salmonella* sp.

Urease test

The broth medium was streaked on the surface of an agar slant with a loopful of TSI agar culture and incubated 37 °C for 24 hours. A negative reaction with no colour changes would indicate the presence of *Salmonella* sp.

Polyvalent O and H agglutination test

A small amount of culture from the TSI agar slant was placed directly onto the slide and a drop of 0.85% sodium chloride (normal saline) solution was added and emulsified using a sterile loop. After that, a drop of polyvalent 'O' antiserum and polyvalent 'H' antiserum were added simultaneously. The mixture was tilted back and forth for 1 minute and then observed against a dark background for the presence of agglutination which would indicate the presence of *Salmonella* sp. A positive reaction would be shown by strong, rapid agglutination.

For transportation, the pure culture from TSI agar slant was inoculated into nutrient agar (NA) for all positive *Salmonella* sp. which had been confirmed through biochemical tests. All NA cultures were properly sealed, appropriately packaged, labeled and sent to the Veterinary Research Institute for complete identification and serological typing.

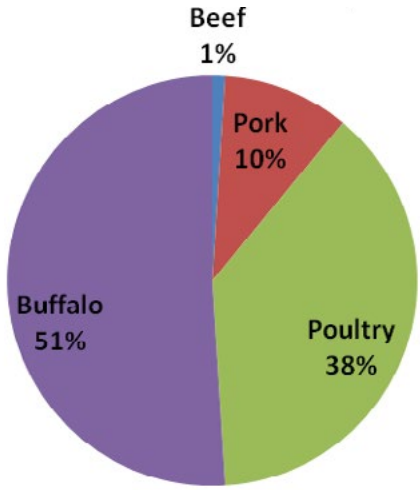

Figure 1. Percentage of total isolated *Salmonella* sp. in meats.

Table 1. Total number and percentage of *Salmonella* serotypes of different meat samples.

Prominant serotypes	Type of meat samples				Total	Percentage (%)
	Poultry	Buffalo	Beef	Pork		
S. Senftenberg	7	53	-	1	61	7.43
S. Enteritidis	76	8	-	-	84	10.23
S. Typhimurium	38	20	1	14	73	8.89
S. Brancaster	35	5	-	4	44	5.35
S. Albany	32	10	1	-	43	5.23
Others	124	324	9	59	516	62.85
TOTAL	312	420	11	78	821	100

Note: '-' represent no data

RESULTS AND DISCUSSION

A total of 117 *Salmonella* serotypes were isolated from a total of 821 meat samples.

Figure 1 shows that buffalo meat samples represent the highest total isolated *Salmonella* sp. accounting for 51%, followed by poultry meat 38%, pork 10% and beef 1%.

The most prominent serotypes were *S.* Enteritidis and *S.* Typhimurium, accounting for 10.23% and 8.89% respectively, which were identified from all the meat samples as shown in Table 1. Other serotypes were *S.* Senftenberg (7.43%), *S.* Brancaster (5.35%) and *S.* Albany (5.23%).

A total of 312 representing 38% of the isolated *Salmonella* sp. were obtained from poultry meat identified with 50 serotypes of *Salmonella* sp. Figure 2 shows the highest occurrence in poultry meat was *S.* Enteritidis

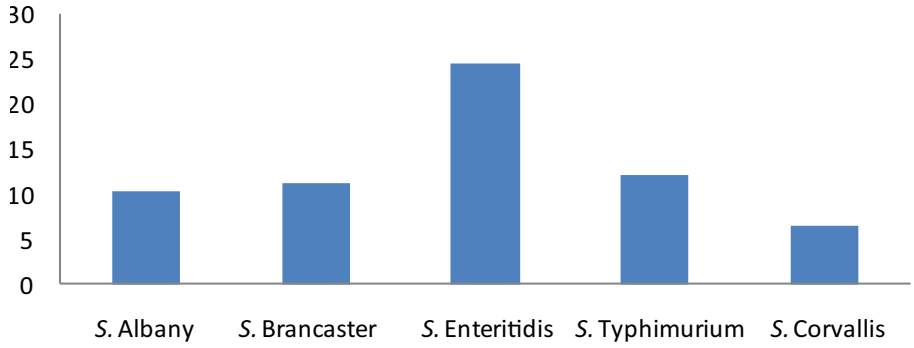

Figure 2. Percentage of *Salmonella* serotypes Isolated from poultry meat.

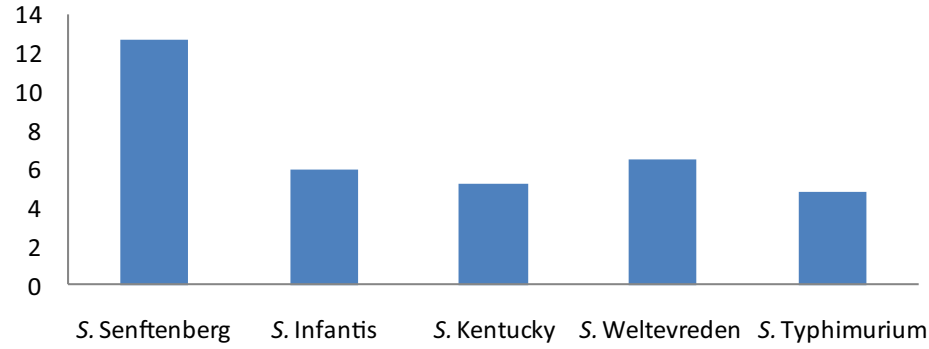

Figure 3. Percentage of *Salmonella* serotypes isolated from buffalo meat.

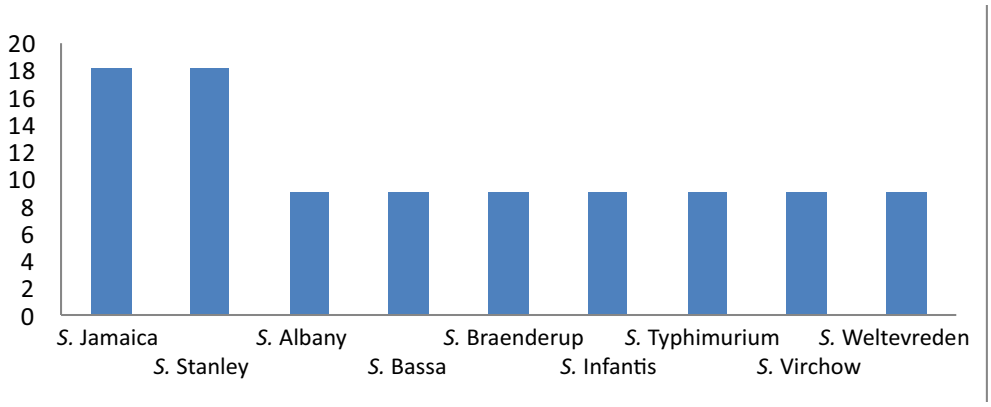

Figure 4. Percentage of *Salmonella* serotypes isolated from beef.

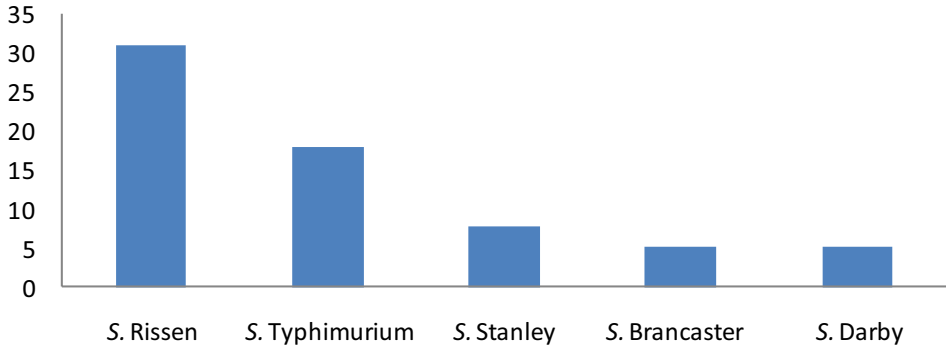

Figure 5. Percentage of *Salmonella* serotypes Isolated from Pork.

(24.36%), followed by *S*. Typhimurium (12.18%), *S*. Brancaster (11.22%), *S*. Albany (10.26%) and *S*. Corvallis (6.41%).

A total of 420 representing 51.16% of the isolated *Salmonella* sp. were obtained from buffalo meat samples. 91 *Salmonella* serotypes were identified. *S*. Seftenberg (12.62%) was the highest number isolated followed by *S*. Weltevreden (6.43%), *S*. Infantis (5.95%), *S*. Kentucky (5.24%) and *S*. Typhimurium (4.76%) as shown in Figure 3.

Figure 4 shows that beef was found to have only 1.34% of isolated *Salmonella* sp. of all meat sampled. Only 9 *Salmonella* serotypes were identified where the highest occurrence, 18.18%, were of *S*. Jamaica and also *S*. Stanley (Grimont and Weill, 2007).

In addition, there was a total of 78 *Salmonella* sp. isolated from pork representing 9.50% of total *Salmonella* sp. About 22 *Salmonella* serotypes were identified. The highest occurrence was *S*. Rissen (30.77%), followed by *S*. Typhimurium (17.95%), *S*. Stanley (7.69%), *S*. Brancaster (5.12%) and *S*. Darby (5.12%) as shown in Figure 5.

In Malaysia, salmonellosis outbreaks has become a major concern since a lot of salmonellosis related illnesses happened nowadays. Food safety through thorough screening either microbiological or chemical contamination of each food or food products of animal origin is tremendously important.

S. Enteritidis and *S*. Typhimurium with 1.34% difference was found to be the most dominant *Salmonella* serotypes. This concurs with studies by Roseliza *et al*. (2011) and Centers of Disease Control and Prevention (2007), where the commonly isolated serotypes were the same. They will remain as the most frequently isolated *Salmonella* serotypes in the future (Rohaiza *et al*., 2005).

S. Typhimurium infects many types of animals and has been associated with contamination in a wide range of foods (Thorns, 2000). Besides that, *S*. Senftenberg has become the next most dominant serovar at 7.43%, after *S*. Typhimurium and *S*. Enteritidis. Although *S*. Typhimurium and *S*. Enteritidis were the main causative agents, *S*. Senftenberg had been reported to cause foodborne gastroenteritis (L'Ecuyer *et al*., 1996; Kumar and Kumar, 2003) and has become an important persistent contaminant in slaughterhouses (Sogaard and Nielsen, 1979; Liebana *et al*., 2001).

In poultry, the most important and dominant serotype in poultry meat was *S.* Enteritidis (24.36%), found most prevalent in poultry carcasses (European Food Safety Authority, 2011). Arumugaswamy *et al*. (1995) reported that 39.4% of chicken portions, 35.3% chicken liver and 44.4% chicken gizzard were contaminated with *Salmonella* sp. Therefore, poultry meat serves as the main vehicle of salmonellosis outbreak. In the study by Busani *et al*. (2005), *Salmonella* sp. was frequently detected in meat products, and among them the highest contamination rates were in poultry, followed by pork.

In this study, 51.16% of *Salmonella* sp. was isolated in buffalo meat which is the highest compared to other meat samples. According to Sen and Garode (2016) and Muller *et al.* (2012), *Salmonella* sp. was able to survive under harsh conditions, including persisting for a year or more in frozen meat. Hence, the source of *Salmonella* sp. in exported frozen buffalo meat could be due to lack of hygiene especially during processing prior to being frozen for exported. The commonest serotype isolated from buffalo meat was *S*. Senftenberg (12.62%) concurring with the study by Roseliza *et al*. (2011).

In beef, 1.34% of *Salmonella* sp. was isolated, the lowest case among other servars. *Salmonella* serotypes such as *S*. Jamaica and *S*. Stanley were the highest isolated. *S*. Typhimurium was usually isolated from beef as *S*. Typhimurium is a natural pathogen of cattle, and beef is the reservoir for human infection (Santos *et al*., 2001; Costa *et al.*, 2012). *S*. Stanley was isolated from food including beef and was among the 20 most frequently reported serotypes in Malaysia from 2001 until 2007 (ECDC-EFSA, 2014).

In this study, *Salmonella* Rissen was the highest serotype isolated from pork. *S*. Typhimurium was the predominant serotype reported by Boyen *et al.* (2008). Egan *et al.* (2017), found *Salmonella* Rissen as a common serotype in pigs around the world and the most prevalent in salmonellosis outbreaks worldwide. Schmid *et al*. (2012) found *S*. Rissen amongst the top three serotypes in pig and pork products in Europe and Southeast Asia.

CONCLUSION

In Malaysia, a lot of cases have been found involving salmonellosis outbreak in humans and it will keep increasing if there is no effort to control the outbreak. For example, the average American acquires *Salmonella* related illnesses from consuming poultry about 1 in 40 lifetimes, while the risk is 1 in 100 lifetimes from consuming beef and pork (Hsi *et al*., 2015). Therefore, it is tremendously important to educate people about this foodborne related illness. Efforts to control *Salmonella* in food-producing animals are also necessary as many food animals are natural hosts for *Salmonella sp*. (Bedale and Milkowski, 2015). In conclusion, the five serotypes found in meat samples (in order of dominance) are *S*. Enteritidis, *S*. Typhimurium, *S*. Senftenberg, *S*. Brancaster and *S*. Albany. Different patterns of predominance were shown for different types of meat samples i.e. *S*. Enteritidis in poultry, *S*. Stanley and *S*. Jamaica in beef, *S*. Senftenberg in buffalo and *S*. Rissen in pork. The presence of *Salmonella* sp. in food would

be one of the important indicators for the the effectiveness of efforts in controlling the outbreak. It is also important to acquire further information on *Salmonella* serotypes to enable identification of new or resistant strains for effective control and prevention of outbreaks.

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