NON-TYPHOIDAL SALMONELLA: RETROSPECTIVE STUDY ON DISTRIBUTION OF *SALMONELLA* SEROVARS IDENTIFIED FROM PIG SAMPLES IN VETERINARY RESEARCH INSTITUTE (VRI) BETWEEN 1986 TO 2017

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ABSTRACT. *Salmonella* serovars have been associated with foodborne outbreaks in humans due to the consumption of contaminated pig products in many countries. This paper provides a baseline data on distribution of *Salmonella* serovars in pigs from the year 1986 to 2017. A total of 1,020 positive *Salmonella* were isolated from pigs and pork and identified to 79 different serovars. The most predominant serovar identified were *S.* typhimurium (41%), followed by *S.* cholerasuis var. Kunzendrof (23%), S. Rissen (11%), *S.* Typhisuis and *S.* Weltevreden (5%). The other 74 serovars consist of the less frequently identified serovars including S. Tsevie (4%), *S.* Stanley (3%), *S.* Agona (2%) and *S.* Saintpaul (1%). Based on this data, *S.* Typhimurium was consistently found throughout for 31 years. This non-host adaptive serovar was frequently identified from diseased pigs previously. However, it is recently found in healthy pigs and pork meat as well. High numbers of positive isolation of host adaptive serovars such as *S.* Cholerasuis var. Kunzendorf and *S.* Typhisuis were reported in the year 1995 and 1996, but had rapidly decreased since the year 2000 and have never been isolated and identified until now. These two serovars have been noted to cause high mortality with prominent clinical signs among infected pigs. Emerging and shifting patterns from host adaptive to non-host adaptive serovars among pigs have been observed from this data collection and analysis. Keeping track of these circulating Salmonella serovars is important to study the disease epidemiology affecting the pig industry.

Keywords: Non-typhoid, salmonellosis, serovar, pigs

INTRODUCTION

Salmonellosis remains as the most important foodborne disease worldwide, specifically infections caused by non-typhoidal salmonella (NTS) (Campos *et al.*, 2019). The disease is characterised by mild to severe infections, with diarrhoea as its main symptoms. Gastrointestinal tract of animals, mainly food producing animals is the main reservoir for NTS infections as well in humans (Eng *et al.*, 2015). Humans might get infected with NTS through consumption of contaminated foods of animal origin, which is responsible for outbreaks leading to illnesses and death (Leekitcharoenphon *et al.*, 2019).

Several serovar including *S.* Enteritidis and *S.* Typhimurium are the major serovar associated with human infection in many countries based on National Enteric Disease Surveillance: Salmonella Annual Report, 2016 (Centers for Disease Control and Prevention (CDC) (2017) National Salmonella Surveillance Annual Report, 2016. Atlanta, GA). However, different salmonella serovars associated with salmonella infection such as *S*. Derby and *S*. Rissen also has been frequently reported to cause human infection, which also indicated the role of pigs and pork in salmonella transmission. Increase in international trade of pigs and pork meat due to increased demand in public consumption, leading to dissemination of clinically relevant serovars along the food chain, which drive new challenges to control salmonella. Potential risk transmission to humans associated with Salmonellae from animals, particularly non-host specific serovars have been reported (Hoelzer *et al.* , 2011). Worst, the emergence of virulent strains with multidrug resistance in non-typhoidal salmonella has been reported in many countries, causing a great deal of burden in widespread antibiotic resistant problems worldwide (Choe *et al.* , 2011).

First *Salmonella* isolation in pigs was reported in Malaysia in 1954 in the Veterinary Research Institute (VRI) involving *S*. Cholerasuis and *S*. Typhimurium serovar (Joseph, 1988). Since 1971, VRI has served as the national reference laboratory for *Salmonella* serotyping for animals and animal origin products in Malaysia and actively involved in surveillance and eradication of salmonella control programmes. However, the implementation and improvisation for the disease control strategies through baseline data are difficult due to very limited data in the country. Therefore, the purpose of this paper was to provide a baseline data on distribution of non-typhoidal salmonella serovars in the country for the period from 1986 to 2017, and to study the dissemination of potential clinically relevant pig-related serovars.

MATERIALS AND METHOD

In this study, the data from 1986 to 2017 were retrieved from salmonella serotyping cases received by VRI. The data was based on number of positive *Salmonella* isolates received by VRI for serotyping during 31 years. All *Salmonella* isolated from diagnostic cases retrieved from pigs with history of significant clinical signs, rectal swabs from healthy pigs which were isolated from surveillance and monitoring work, while pork and meat products such as sausages collected from local abattoirs and wet market throughout Malaysia were submitted to VRI for further serotyping. For clinical cases, most of the cases were supported with significant clinical signs including high mortality, diarrhoea, pneumonia and other respiratory problems such as coughing and nostril discharge. Most of the isolates received from veterinary regional laboratories were isolated from visceral organs including lung, liver and intestines. However, the information was limited based on provided information in the available case registration sheet kept in VRI. Salmonella isolation and identification by conventional biochemical tests were conducted either in VRI or by veterinary regional laboratories for reference cases according to the standard protocols. Confirmed salmonella isolates were sent to VRI for purification and further serotyping onto slant nutrient agar. The isolates were subcultured on blood agar and MacConkey agar for purification before proceeding with serotyping. Serotyping of *Salmonella* sp. and determination of somatic ‘O’ antigen and flagellar ‘H’ antigen were as described in Roseliza *et al.*, 2020.

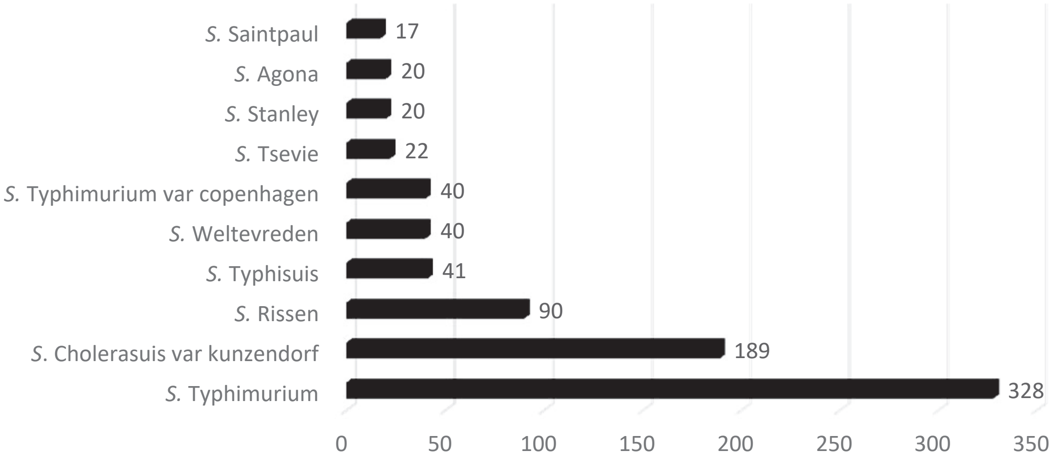

Figure 1. The ten most commonly identified non-typhoidal *Salmonella* serovars in pig from the year 1986 to 2017.

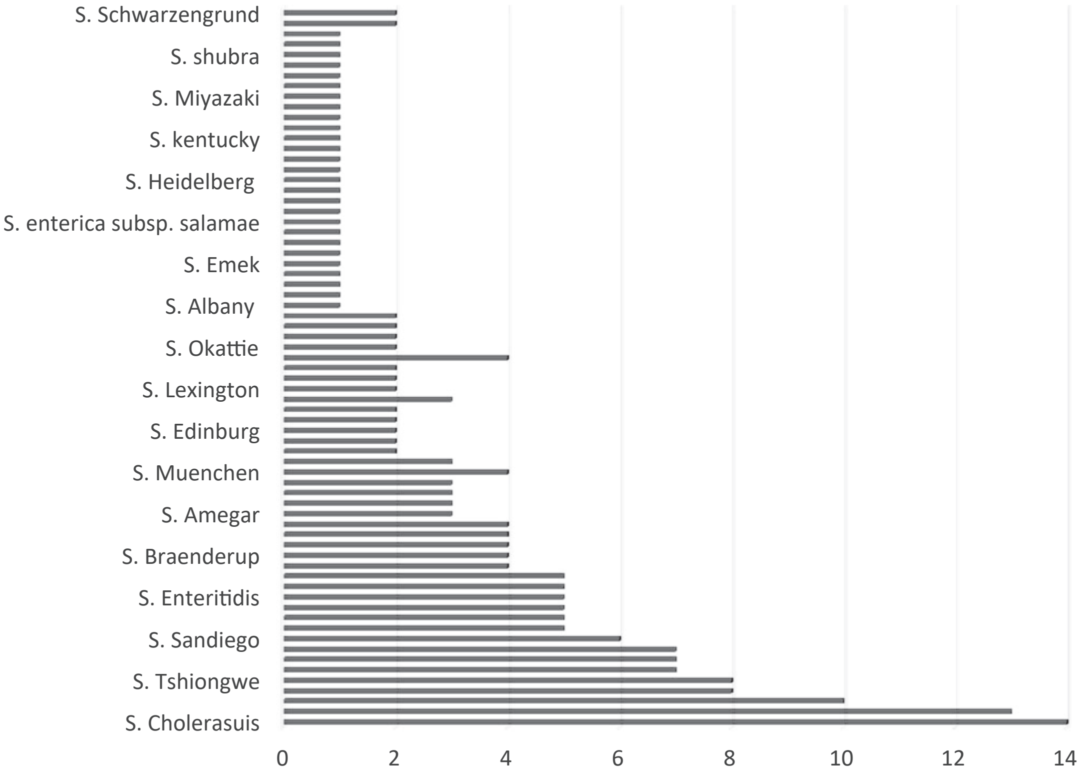

Figure 2. The less commonly identified Salmonella serovars in pig from the year 1986 to 2017.

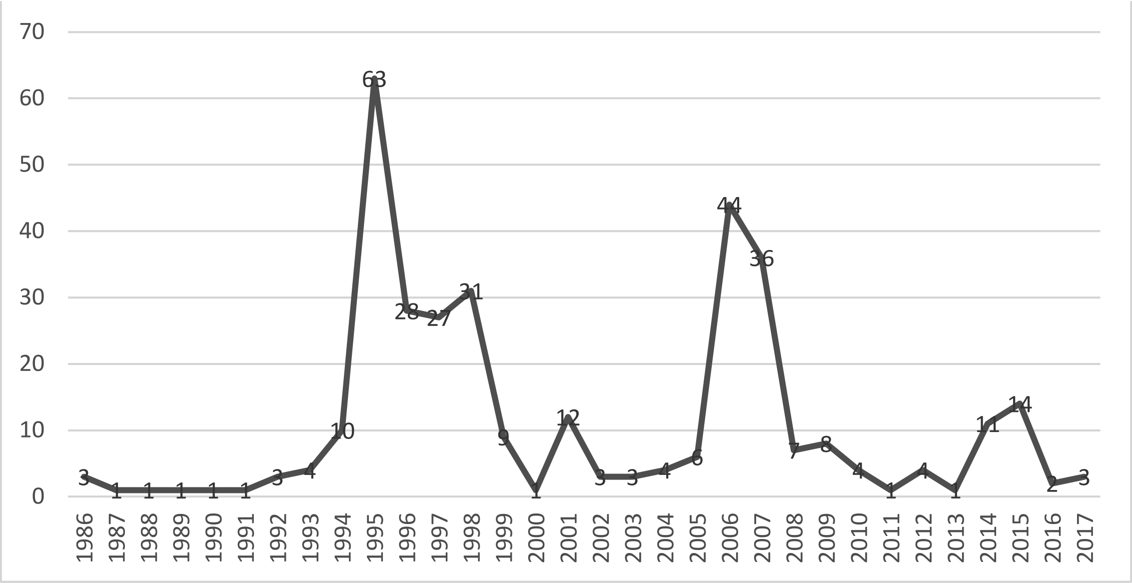

Figure 3. Distribution of positive Salmonella Typhimurium identified in pig.

Figure 4. Distribution of positive Salmonella Cholerasuis var Kunzendorf identified in pig.

RESULTS

Present analysis revealed that a total of 1020 culture confirmed Salmonella cases from pig received by VRI during 1986 to 2017, consist of 79 variety salmonella serovars were recorded from 1986 to 2017. S. Typhimurium found to be the most predominant serovar identified (41%), followed by *S.* Cholerasuis var. Kunzendorf (23%), *S.* Rissen (11%), S. Typhisuis, *S.* Weltevreden and *S.* Typhimurium var. Copenhagen (5%) respectively (Figure 1).

Other commonly identified serovars include *S.* Tsevie (4%), S. Stanley (3%), S. Agona (2%) and S. Saintpaul (1%). Another 45 serovar consist the less frequently serovar identified from cases submitted for serotyping to VRI along the period of study (Figure 2).

Meanwhile, Figure 3 showed the distribution pattern of *S.* Typhimurium, the most predominant serovar identified in pigs for past 31 years. Based on the analysis, *S.* Typhimurium was consistently identified from pigs every year with at least one case recorded. The incidence of S. Typhimurium was found to be higher in 1995 with 63 cases reported. Based on history provided in the case sheet submitted to VRI, most of the *S.* Typhimurium identified were obtained from pigs with significant clinical signs. Despite that, increase incidences of *S.* Typhimurium in the years 2006 and 2007 only involved healthy pigs.

Figure 4 showed incidence of *S.* Cholerasuis var kunzendorf which was the second most predominant serovar identified in pigs. Despite being identified consistently from 1986, however the number of incidence found to be declined, which no more *S.* Cholerasuis var kunzendorff were identified since 2001.

DISCUSSION

The high prevalence of *S.* Typhimurium serovar identified was in agreement with previous findings demonstrated in different countries. In Thailand and Laos, *S.* Typhimurium was the most predominant serovar found in pigs and pork, followed by *S.* Rissen (Sinawat *et al.*, 2016). While in Japan, serovars belonging to group B and E1 were commonly isolated from healthy pigs, with the most predominant serovars were, *S.* Typhimurium, *S.* Agona and *S.* Infantis in 1989 to 1999 period of study (Saito *et al.*, 2008). Another finding by Wales *et al.* (2013) showed that *S.* Typhimurium and *S.* Derby were most commonly found among integrated pig breeding and production in the United Kingdom. While in the Northern America region, this serovar was commonly isolated from pigs as reported by OIE (Poppe, 2009).

From this finding, *S.* Typhimurium was consistently identified throughout 31 years of study from 1986 to 2017 (Figure 3). *S.* Typhimurium, a non-adaptive serovar, as well *S.* Enteritidis in poultry remained the most predominant serovars in diverse industrialized countries and was most likely to be recovered in both clinical and subclinical samples (Evangelopoulou *et al.*, 2015). These non-host adapted serovars infect a wide range of hosts, and are significantly important in human and animal infections. Although they are usually associated with mild degree of infections, they become the ultimate challenges in the animal industry causing financial losses due to animal wasting and loss of productivity (Evangelopoulou *et al.*, 2015) when compared to those infections caused by host adaptive salmonella serovars. Often, they are found in healthy animals, therefore they are considered as 'symptomless excreter' which is the main reservoir for salmonella infections in animals. In this study, *S*. Typhimurium was most frequently isolated from visceral organs particularly the liver and mesenteric lymph nodes, which indicates that S. Typhimurium infections involve septicemia. However, the number of positive isolation of *S*. Typhimurium related to septicemia has decreased since the year 2000. Since then, it is found that the host unrestricted serovar, *S*. Typhimurium was frequently isolated from pork and the rectal swab of healthy pigs. The ability of the serovar to cause disease largely depends on the degree of adaptation in its host biological environment (Evangelopoulou *et al.*, 2015). Recent study also revealed that certain virulence genes that encode for colonisation, adhesion, intestinal invasion and survival in host tissues contributed to the enhanced ability of *S*. Typhimurium to adapt in a wide range of hosts (Campos *et al.*, 2019).

Another predominant serovar, which is *S*. Cholerasuis, including *S*. Cholerasuis var. Kunzendorf are highly adapted serovars that cause swine paratyphoid, particularly in young pigs. Resulting serovar Cholerasuis is highly pathogenic to humans and among the top 20th ranked non-typhoid salmonella serovar causing human infections particularly in Asian countries (Chiu *et al.*, 2004). *Salmonella* Cholerasuis is one of the virulent serovars associated with systemic disease in limited specific host such as S. Dublin in cattle (Evangelopoulo *et al.*, 2015).

In Taiwan, serovar Cholerasuis ranked among the 10th most common serovar that caused salmonellosis in humans, but the figure decreased since 1998 in Taiwan due to the outbreak of foot and mouth disease at that particular time (Chiu *et al.*, 2004). There is an increased finding of *S*. Cholerasuis positive isolation in 1995 and 1996 (Figure 4). This may be due to the occurrence of other viral infections at that time, as there is a synergistic action between the presence of viral agents and the stress factors predisposing either by poor sanitation, overcrowding and defective nutrition which trigger the infection caused by *S*. Cholerasuis (Wills *et al.*, 2000). Emergence of Nipah Virus infection among the pig population during that time caused a steep increase of *S*. Cholerasuis positive isolation.

These findings were further reinforced through the previous report by Joseph *et al.* (1978) which claimed that steep increase of salmonella isolation, particularly in pigs was due to stress factors resulted by swine fever virus vaccination. During that particular time, swine fever vaccine, especially non-japanese swine fever strain, was found to be the cause of salmonellosis in pigs. Since the early 1990's, even though several commercial vaccines were introduced to control *S*. Cholerasuis infection, occurrence of concurrent diseases such as Porcine Reproductive and Respiratory Syndrome (PRRS) contributed to decreased vaccine effectiveness, thus resulting in increase of *S*. Cholerasuis infection in the United States

(Schwartz, 1991). However, there was a sharp decline of positive isolation of *S*. Cholerasuis since the year 2000 (Figure 4). This might be due to the occurrence of the *Nipah* virus epidemic in the pig population in Malaysia, which at that time many pigs have been culled off in order to control the spread of the epidemic. Thus, as the pig population declined significantly, therefore resulting in a sudden reduction of swine adapted serovars including *S*. Cholerasuis, *S*. Typhimurium var. Copenhagen and *S*. Typhisuis.

Another S. Typhimurium var. Copenhagen, a monophasic strain which lacks somatic O: 5 antigen was last identified in 2000. This serovar was found in young animals, 4 to 6 weeks old with prominent clinical signs including diarrhoea, respiratory distress and history of high morbidity and mortality rate. Retrospective review of data also revealed that this serovar was consistently emerging in EU countries and was among the most frequently reported in pigs and pork products until 2011 (Evangelopoulou *et al*., 2015). However, there is no available data on emergence of this serovar in Malaysia although there were still cases reported in other countries until now (Bonardi *et al.,* 2017).It is suggested that the infections only involved live animals in the farms, and because of the strict procedure to ensure safety of export pork meat products implemented by those countries. Unlike *S*. Typhimurium, serovar var. Copenhagen only adapted at swine host and contains swine –associated phage such as phage type DT104 which is a common cause of human salmonellosis (Gebreyes and Altiers, 2002).

Nevertheless, a changing trend in NTS associated with foodborne salmonellosis has been observed in last decades, with the worldwide expansion of less common serovars in both pig and pork such as *S*. Rissen , *S*. Derby, *S*. Infantis, *S*. Brandenburg and *S*. serotype 4,[5],12:i:- (a monophasic variant of *S*. Typhimurium) in United States and European countries (Guerra Filho *et al*., 2016; Bonardi, 2017; Campos *et al*., 2019). Less importance serovars including Copenhagen, Derby, Infantis and Virchow were found important and potentially replacing currently predominant serovars (Evangelopouo *et al*., 2015). In this study, at least about 68 less common serovars have been reported in pigs and pork meat based on positive salmonella isolation received by VRI.

Surprisingly, based on these findings showed that all the salmonella isolates identified in current years were isolated from healthy pigs based on history, which involved less common NTS serovars. Frequent colonisation of less common serovars in pigs can occur either through horizontal or vertical transmission (Campos *et al*., 2019). Environmental contamination on a farm through pig shedding contributed to the pig colonisation by salmonella (Campos *et al*., 2019). Infection with less common NTS usually results in asymptomatic healthy animals, which is the main factor introduction of these bacteria in pig production chains including during slaughter and processing of meat carcasses (Campos *et al*., 2019). In fact, high levels of salmonella contamination can occur in slaughterhouses through various routes including contaminated slaughter environment, equipment and meat handlers (Campos *et al*., 2019).

Based on the data presented here, previous salmonellosis outbreak reported in the country caused by host adaptive serovars which is more invasive in pigs causing high morbidity. From this report, we found that NTS does not cause disease in pigs with significant clinical signs. Although certain serovars seem diminished and were not reported for almost 20 years, however there is a possibility that it will emerge hence causing an outbreak. However, pigs remain as relevant reservoir for NTS in Malaysia, which might contribute to the occurrence of salmonellosis outbreaks caused by new serovars, with unknown characters. Enriched adaptive features through gene transfer including resistance mechanism among the strains might pose a major challenge for the spread of the disease in both humans and animals.

CONCLUSION

Pig and pork products were reported as an important source of NTS salmonellosis in humans in many countries. While in animals, it poses a major threat to the control measures of salmonellosis as most of the cases did not involve animals with clinical signs. Emergence of new serovars, which received much less attention in veterinary field should not be neglected, because of their potential to trigger invasive infections in comparison with highly prevalent serovars of Salmonella such as *S.* Typhimurium, *S.* Cholerasuis and *S.* Typhisuis. On the other hand, high frequency of non-host adaptive serovars in pigs can become a great challenge to control the disease. Therefore, it should be highlighted that these non-host adaptive serovars hold the ability to adapt into the host defense system thus infected animals do not show significant clinical signs, or with minimum symptoms. Most of the salmonella infections remain unreported till today. The continuous control and monitoring of Salmonella along the food chain including implementation of good farm management is crucial to minimise their introduction to the food-animal production.

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ACKNOWLEDGEMENT. The authors would like to thank the Director-General of the Department of Veterinary Services Malaysia (DVS), Director of Veterinary Research Division DVS for their kind permission to publish the paper. Special thanks to the Director of VRI and staff of the Bacteriology section, VRI for their contributions in this study. This study was financially supported by VRI Ipoh.