

CHAPTER 4

DISSEMINATION OF *Salmonella enterica* SEQUENCE TYPES AMONG ASEAN ECONOMIC COMMUNITY COUNTRIES

4.1 Abstract

Food-borne illness caused by *Salmonella enterica* remains a public health problem and results in economic loss worldwide. With the up-coming establishment of the ASEAN Economic Community (AEC) allowing unrestricted movement of labor and goods. This study characterized and investigated the spatial and temporal associations of *S. enterica* strains isolated in AEC countries compared with those isolated in northern-Thailand during 2011-2013. Of the 173 *S. enterica* strains examined, 68 sequence types (STs) and 32 clonal complexes (CCs) were identified by multi loci sequence typing. Twenty-one strains belonged to four sequence types new to AEC countries. Information will be important in formulating biosecurity measures, as well as in educating regarding the risk of disease transmission in AEC.

4.2 Introduction

Food-borne illness affected by *Salmonella enterica* is a public health problem and a cause of economic loss in many countries [11,15,49,80]. Consumption of contaminated food of animal origin, such as beef, egg, milk, pork, and poultry are known causes of food-borne illnesses [8,81-82]. In addition, direct contact between animal and animal, animal and humans, humans and humans as well as indirect contact via the environment are involved in the disease transmission [60]. Thus, there has been an increase in recognition of pathogen transmission through international travel international food chain supply, environmental changes and human migration as being important causes of disease dissemination [27,83-84].

Southeast Asia or ASEAN region is an area with high prevalence of salmonellosis due to *S. enterica*, persisting over several decades, indicating that this disease is quite difficult to eradicate [51,85-91]. The establishment in 2016 of ASEAN Economic Community (AEC), comprising of ten countries, will unrestricted movement of services, labor and goods [92] (Association of Southeast Asian Nations, 2008). This certainly will increase the chances of pathogens transmission among AEC countries.

Implementation of bacterial typing is an important argument for disease investigation and surveillance [33]. Accordingly, more understanding of the disease epidemiology is needed. Serotyping is the most common technique for *Salmonella* typing [42,47,81]. However, the technique has limited discriminatory power [60]. As a result, tracking of infectious agents are not effective enough [25]. On the other hand, multi locus sequence typing (MLST) is able to overcome this limitation of serotyping as it can differentiate bacterial strains from the sequences of multiple conserved housekeeping genes [93]. This technique is capable of accurately distinguishing bacterial genotypes and is more appropriate in epidemiological studies of bacterial pathogens such *S. enterica* [33].

From study of Boonkhot et al, 2015 [35] have employed MLST to characterize *S. enterica* strains from swine production chain in northern Thailand during 2011-2013. However, the dataset lacks comparisons of *S. enterica* genetic diversity in swine with other strains in other areas. The current study employed *S. enterica* MLST to (i) characterize the distribution, diversity and evolution of *S. enterica* circulating in AEC countries, and (ii) compare the spatial and temporal associations of strains obtained from previous study in northern Thailand with strains previously submitted to MLST database (<http://mlst.warwick.ac.uk/mlst/dbs/Senterica>) in order to expand existing knowledge of salmonellosis epidemiology in the ASEAN region.

4.3 Materials and methods

4.3.1 *S. enterica* strains

A total of 30 *S. enterica* strains isolated from swine production chain in northern Thailand during 2011-2013 were obtained [environment (n = 23), swine (n = 4) and

humans (n = 3)] and characterized by MLST [comprising *aroC* (encoding chorismate synthase); *dnaN* (DNA polymerase III beta subunit); *hemD* (uroporphyrinogenIII cosynthase); *purE* (phosphoribosylaminoimidazole carboxylase); *sucA* (alpha ketoglutarate dehydrogenase); *hisD* (histidinol dehydrogenase) and *thrA* (aspartokinase I/homoserine dehydrogenase)] from a previous study (Boonkhot et al, 2015). In addition, *S. enterica* strains (n = 143) analyzed by MLST from 7 AEC countries obtained during 1940 to 2012 (<http://mlst.warwick.ac.uk/mlst/dbs/Senterica>) were included. These strains were originated from Indonesia (n = 22), Lao PDR (n = 11), Malaysia (n = 45), The Philippines (n = 14), Singapore (n = 2), Thailand (n = 10) and Vietnam (n = 39), acquired from avian (n = 31), boar (n = 1), environment (n = 1), food (n = 2), humans (n = 87), reptile (n = 8), and unknown host origin (n = 13).

4.3.2 Discriminatory index

Simpson's diversity index was used to evaluate the discriminatory power of MLST method (<http://darwin.phylovis.net/ComparingPartitions/>). Simpson's diversity index is calculated according the formula [94]:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^s n_j(n_j-1)$$

where D is Simpson diversity index, N the overall number of sample population, S the total number of varieties and n_j the number of strains fitting into each variety. This index approximates the possibility that any two consecutively sampled strains from a sample population will fit to the same clutch. The index computes values in a range of 0.00 (certainly of no diversity) to 1.00 (immeasurably diverse).

4.3.3 Minimum spanning tree (MST) analysis

MST analysis is based on MLST characteristics in each locus using BioNumerics software version 7.1 (Applied Maths, Sint-Martens Latem, Belgium) to determine the relationships between sequence types and geographical areas (stratified by "advanced cluster analysis for categorical data" command). Sequence types that are closely related in loci characteristics are displayed in close proximities.

4.4 Results

Of the 173 *S. enterica* strains (30 strains obtained from swine production chain in northern Thailand and 143 strains acquired from *S. enterica* MLST database) examined, 68 sequence types (STs) were generated from analysis of 7 loci performed by MLST. Thirty-two clonal complexes (CCs) were available from 147 strains, while the remaining of 26 could not be identified in any CCs (Table 4.1). Of the 30 strains obtained from swine production chain in northern Thailand, 21 (70%) were represented by 4 new sequence types, namely, ST19, ST48, ST469 and ST1500, belonging to two clonal complexes, CC42 and CC66 (Boonkhot et al, 2015) (Table 4.1). The most frequent sequence type was ST2, followed by ST1541, then ST1, all of which belonged to CC13 or CC66. The strains belonging to CC3, CC13, CC56 and CC205, the groups of indistinguishable strains were isolated at different times, covering well over a decade. The discriminatory power value of Simpson's diversity index of MLST compared with serotyping, the most convenient method used for *Salmonella* characterization, was 0.959 (95% CI: 0.947-0.971) and 0.904 (95% CI: 0.882-0.925), respectively. In general, strains with common sequence types were demonstrated as the same serotypes, but strains congregated in *Salmonella* serotypes Weltevreden, *S. Typhi*, *S. Typhimurium*, *S. Enteritidis*, *S. Kentucky* and *S. Newport* were assigned to more than one sequence type.

MST analysis based on allelic profile characteristics revealed that strains discovered in Thailand were specific to a limited number of sequence types, namely, ST34, ST48, ST469, ST696, ST1500, and ST1541, the latter also found in Malaysia (Figure 4.1). Strains grouped in ST1, ST2, ST29 and ST198 were distributed among at least 3 AEC countries. In addition, > 40% of all strains had closely related allelic profiles (at least 5 loci) with similar CCs and were isolated from different host origins.

Table 4.1 Characterization and origins of *S. enterica* isolated through 1940-2013 in Asean Economic Community (AEC) countries.

Strain	Allelic profile							ST-type	ST-complex	Serotype	Host	Country	Year
	<i>aroC</i>	<i>dnaN</i>	<i>hemD</i>	<i>hisD</i>	<i>purE</i>	<i>sucA</i>	<i>thrA</i>						
139K	130	97	25	125	84	9	101	ST365	205	Weltevreden	Human	Indonesia	1940
M196	130	97	25	125	84	9	101	ST365	205	Weltevreden	Human	Philippines	2001
543053	130	97	25	125	422	9	101	ST1500	205	Weltevreden	Environment	Thailand	2012
543056	130	97	25	125	422	9	101	ST1500	205	Weltevreden	Environment	Thailand	2012
44	130	97	25	125	422	9	101	ST1500	205	Weltevreden	Environment	Thailand	2013
171	130	97	25	125	422	9	101	ST1500	205	Weltevreden	Environment	Thailand	2013
UI 22576	130	2	25	125	84	9	101	ST1797	205	-	Human	Laos	2011
541064	22	11	25	21	10	23	23	ST48	42	Panama	Environment	Thailand	2011
541069	22	11	25	21	10	23	23	ST48	42	Panama	Environment	Thailand	2011
22	22	11	25	21	10	23	23	ST48	42	Panama	Human	Thailand	2013
57	22	11	25	21	10	23	23	ST48	42	Panama	Swine	Thailand	2013
202	22	11	25	21	10	23	23	ST48	42	Panama	Environment	Thailand	2013
1933/77	48	209	148	250	219	202	204	ST764	-	Mississippi	Human	Indonesia	1977
UI 21074	140	392	40	125	140	9	18	ST1798	-	-	Human	Laos	2010
CDC 2906-58	33	26	30	26	21	87	308	ST1268	220	-	Reptile	Vietnam	1962
KT516	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1986
ST1165/87	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	1987
98531	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
98864	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
2	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
12	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
2219	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
184756	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Thailand	1989
E431	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	1989

Table 4.1 (continued)

Strain	Allelic profile							ST-type	ST-complex	Serotype	Host	Country	Year
	<i>aroC</i>	<i>dnaN</i>	<i>hemD</i>	<i>hisD</i>	<i>purE</i>	<i>sucA</i>	<i>thrA</i>						
88405	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
99155	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
13-I	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
TY404	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Indonesia	1989
Ty10	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Vietnam	1993
CT18	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Vietnam	1994
E438	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	1995
TP3/97	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	1997
TP5/00	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	2000
TP60/05	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	2005
CR0063/07	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	2007
ST56880	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	2007
ST02/08	1	1	2	1	1	1	5	ST2	13	Typhi	Human	Malaysia	2008
ST495/87	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia	1987
2218	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Indonesia	1989
2233	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Indonesia	1989
99319	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Indonesia	1989
99282	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Indonesia	1989
ST286/90	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia	1990
ST306/91	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia	1991
9541	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Vietnam	1996
TP12/97	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia	1997
TP45/02	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia	2002
TP85/04	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia	2004

Table 4.1 (continued)

Strain	Allelic profile							ST-type	ST-complex	Serotype	Host	Country	Year
	BL196/05	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia
CR0044/07	1	1	1	1	1	1	5	ST1	13	Typhi	Human	Malaysia	2007
O6-9026	10	19	12	9	5	9	2	ST34	1	Typhimurium	Human	Thailand	2006
541006	10	19	12	9	5	9	2	ST34	1	Typhimurium	Environment	Thailand	2011
542068	10	19	12	9	5	9	2	ST34	1	Typhimurium	Environment	Thailand	2011
543010	10	19	12	9	5	9	2	ST34	1	Typhimurium	Environment	Thailand	2011
172	10	19	12	9	5	9	2	ST34	1	Typhimurium	Environment	Thailand	2013
193	10	19	12	9	5	9	2	ST34	1	Typhimurium	Swine	Thailand	2013
140	10	19	12	9	5	9	2	ST34	1	Typhimurium	Environment	Thailand	2013
542750	10	7	12	9	5	9	2	ST19	1	Typhimurium	Environment	Thailand	2011
18(retail)	18	14	12	9	5	18	21	ST36	138	Typhimurium	Avian	Malaysia	2005
05-572	18	14	12	9	5	18	21	ST36	138	Typhimurium	Human	Malaysia	2005
O6-4382	18	14	12	9	5	18	21	ST36	138	Typhimurium	Human	Thailand	2005
10C(SL_house)	18	14	12	9	5	18	21	ST36	138	Typhimurium	Avian	Malaysia	2012
11C(SL_house)	18	14	12	9	5	18	21	ST36	138	Typhimurium	Avian	Malaysia	2012
31(retail)	18	14	12	9	5	18	21	ST36	138	Typhimurium	Avian	Malaysia	2012
H2519	2	7	9	9	5	9	12	ST15	26	Heidelberg	-	Philippines	2001
H2520	2	7	9	9	5	9	12	ST15	26	Heidelberg	-	Philippines	2001
H2521	2	7	9	9	5	9	12	ST15	26	Heidelberg	-	Philippines	2001
H2523	2	7	9	9	5	9	12	ST15	26	Heidelberg	-	Philippines	2001
5-56	41	2	3	7	5	6	10	ST180	93	Enteritidis	Human	Vietnam	1956
50(retail)	5	2	3	7	6	6	11	ST11	4	Enteritidis	Avian	Malaysia	2012
71(SL_house)	454	2	3	7	6	6	11	ST1925	4	Enteritidis	Avian	Malaysia	2012
73(SL_house)	454	2	3	7	6	6	11	ST1925	4	Enteritidis	Avian	Malaysia	2012

Table 4.1 (continued)

Strain	Allelic profile							ST-type	ST-complex	Serotype	Host	Country	Year
	74(SL_house)	454	2	3	7	6	6	11	ST1925	4	Enteritidis	Avian	Malaysia
UI 1184	5	2	3	381	6	6	11	ST1803	4	-	Human	Laos	2001
71-G-259	215	12	3	7	5	6	11	ST1863	-	Enteritidis	Avian	Vietnam	2010
M200	187	12	17	16	13	16	4	ST589	17	Javiana	-	Philippines	2001
71-G_395	60	429	17	16	13	16	4	ST1867	17	-	Avian	Vietnam	2010
1/50	13	11	16	15	12	15	4	ST23	41	Oranienburg	Human	Vietnam	1950
1359/73	22	104	48	231	114	107	4	ST1069	-	Poona	Food	Thailand	1973
M194	19	20	3	20	5	22	22	ST40	57	Derby	-	Philippines	2001
05-177	5	75	54	4	76	109	75	ST696	-	Kentucky	Human	Thailand	2005
08-6114	5	75	54	4	76	109	75	ST696	-	Kentucky	Human	Thailand	2008
0801W55	5	75	54	4	76	109	75	ST696	-	Kentucky	Human	Thailand	2008
UI 26273	5	85	79	88	76	95	85	ST1794	85	Tananarive	Human	Laos	2011
UI 24742	104	100	54	78	104	74	48	ST1796	45	-	Human	Laos	2011
1-63	76	14	3	77	64	64	67	ST198	56	Kentucky	Human	Vietnam	1963
1-66	76	14	3	77	64	64	67	ST198	56	Kentucky	Human	Vietnam	1966
8-76	76	14	3	77	64	64	67	ST198	56	Kentucky	Environment	Indonesia	1976
1-86	76	14	3	77	64	64	67	ST198	56	Kentucky	Human	Vietnam	1986
12-89	76	14	3	77	64	64	67	ST198	56	Kentucky	Human	Thailand	1989
10-93	76	14	3	77	64	64	67	ST198	56	Kentucky	Human	Indonesia	1990
92-6272	76	14	3	77	64	64	67	ST198	56	Kentucky	Human	Indonesia	1992
93-64	76	14	3	77	64	64	67	ST198	56	Kentucky	Human	Indonesia	1993
M202	76	14	3	77	64	64	67	ST198	56	Kentucky	-	Philippines	2001
182K	117	14	23	127	151	7	22	ST501	84	Singapore	Human	Singapore	1943
71-G-434	450	14	253	547	466	7	16	ST1861	-	-	Avian	Vietnam	2010

Table 4.1 (continued)

Strain	Allelic profile						ST-type	ST-complex	Serotype	Host	Country	Year	
	542003	92	107	79	156	64	151	87	ST469	66	Rissen	Environment	Thailand
542033	92	107	79	156	64	151	87	ST469	66	Rissen	Environment	Thailand	2011
542035	92	107	79	156	64	151	87	ST469	66	Rissen	Environment	Thailand	2011
543034	92	107	79	156	64	151	87	ST469	66	Rissen	Environment	Thailand	2011
40	92	107	79	156	64	151	87	ST469	66	Rissen	Environment	Thailand	2013
65	92	107	79	156	64	151	87	ST469	66	Rissen	Swine	Thailand	2013
107	92	107	79	156	64	151	87	ST469	66	Rissen	Human	Thailand	2013
115	92	107	79	156	64	151	87	ST469	66	Rissen	Environment	Thailand	2013
126	92	107	79	156	64	151	87	ST469	66	Rissen	Swine	Thailand	2013
176	92	107	79	156	64	151	87	ST469	66	Rissen	Environment	Thailand	2013
UI 21748	202	4	10	33	90	6	275	ST1799	-	-	Human	Laos	2010
UI 24712	202	20	10	33	3	6	275	ST1800	-	-	Human	Laos	2011
UI 10890	10	7	21	12	15	12	23	ST1801	3	-	Human	Laos	2008
15421	10	7	21	12	15	12	467	ST1802	3	-	Human	Laos	2009
FW10-56	10	7	21	12	15	12	12	ST46	3	Newport	Reptile	Vietnam	1952
FW13-56	10	7	21	12	15	12	12	ST46	3	Newport	Human	Vietnam	1952
FW15-56	10	7	21	12	15	12	12	ST46	3	Newport	Reptile	Vietnam	1956
FW18-56	10	7	21	12	15	12	12	ST46	3	Newport	Reptile	Vietnam	1952
FW23-56	10	7	21	12	15	12	12	ST46	3	Newport	Human	Vietnam	1956
FW8-56	10	7	21	12	15	12	12	ST46	3	Newport	Human	Vietnam	1956
FW19-66	10	7	21	12	15	12	12	ST46	3	Newport	Human	Thailand	1966
S/20032199	6	7	10	10	8	10	14	ST16	9	Virchow	Human	Thailand	2003
4C(retails)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
5C(retails)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012

Table 4.1 (continued)

Strain	Allelic profile							ST-type	ST-complex	Serotype	Host	Country	Year
	197	87	10	234	8	65	22						
43(retails)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
46(retails)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
53(retails)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
59(retails)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
4C(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
6C(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
7C(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
8C(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
62C(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
64(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
68(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
69(Sl_house)	197	87	10	234	8	65	22	ST1541	236	Corvallis	Avian	Malaysia	2012
2876	81	69	36	12	68	12	17	ST203		Bareilly	Food	Vietnam	2001
M199	16	16	20	18	8	12	18	ST29	29	Stanley	-	Philippines	2001
543003	16	16	20	18	8	12	18	ST29	29	Stanley	Environment	Thailand	2011
543044	16	16	20	18	8	12	18	ST29	29	Stanley	Environment	Thailand	2012
17(etail)	16	16	20	18	8	12	18	ST29	29	Stanley	Avian	Malaysia	2012
27(etail)	16	16	20	18	8	12	18	ST29	29	Stanley	Avian	Malaysia	2012
9C(sl_house)	16	16	20	18	8	12	18	ST29	29	Stanley	Avian	Malaysia	2012
65(sl_house)	16	16	20	18	8	12	18	ST29	29	Stanley	Avian	Malaysia	2012
66(sl_house)	16	16	20	18	8	12	18	ST29	29	Stanley	Avian	Malaysia	2012
29	16	16	20	18	8	12	18	ST29	29	Stanley	Human	Thailand	2013
UI 11516	16	16	20	18	8	12	11	ST1795	29	-	Human	Laos	2008
10128	16	16	12	18	8	12	18	ST1550	29	-	Human	Vietnam	2007

Table 4.1 (continued)

Strain	Allelic profile							ST-type	ST-complex	Serotype	Host	Country	Year
1395K	324	31	10	375	36	12	303	ST1248	-	Vietnam	Reptile	Vietnam	1961
iNTS_2113	93	135	26	370	36	78	70	ST1864	-	-	Human	Vietnam	2012
M197	18	2	18	12	25	7	12	ST434	173	Rhydyfelin	-	Philippines	2001
FW50-3	2	2	15	14	15	20	12	ST31	7	Newport	Human	Vietnam	1946
1066AK	2	14	24	14	2	19	8	ST43	5	Paratyphi B	Human	Indonesia	2004
06_4953	2	14	24	14	2	19	8	ST43	5	Paratyphi B	Human	Indonesia	2006
8892/98	36	31	35	14	26	6	8	ST145	6	Cholerasuis	Human	Singapore	1998
M193	36	31	35	14	26	6	8	ST145	6	Cholerasuis	-	Philippines	2001
M201	188	112	121	14	176	183	1	ST590	183	Amsterdam	-	Philippines	2001
71-V-114	15	14	341	8	7	19	18	ST1868	-	-	Avian	Vietnam	2010
20074	41	4	23	14	16	19	140	ST1545	32	-	Human	Vietnam	2009
C2374	41	393	79	490	76	169	177	ST1546	-	-	Human	Vietnam	2010
20213	60	12	17	16	13	16	4	ST1547	17	-	Human	Vietnam	2010
123NDN	10	14	15	495	25	20	33	ST1548	65	-	Human	Vietnam	2007
20114	347	394	78	496	426	9	102	ST1549	-	-	Human	Vietnam	2009
71-H-71	10	60	21	125	8	7	182	ST1866	-	-	Boar	Vietnam	2010
71-H-54	115	175	8	115	8	116	182	ST1865	-	-	Environment	Vietnam	2010
M203	5	21	18	9	6	12	17	ST50	14	Saintpaul	-	Philippines	2011
M195	10	60	58	66	6	65	16	ST155	237	London	-	Philippines	2001
983K	13	63	16	104	40	23	4	ST287	160	Quinhon	Reptile	Vietnam	1959
2940_80	162	148	117	179	93	162	96	ST475	-	Itami	Human	Thailand	1980
2907-58/168	285	26	30	55	21	87	28	ST1197	10	-	Reptile	Vietnam	1958
149NHL	418	14	224	499	89	313	2	ST1561	-	-	Human	Vietnam	2007
50NTD	2	4	40	43	66	2	425	ST1562	-	-	Human	Vietnam	2007

Table 4.1 (continued)

Strain	Allelic profile							ST-type	ST-complex	Serotype	Host	Country	Year
	10	59	23	549	38	9	477						
rep38	10	59	23	549	38	9	477	ST1870	-	-	Reptile	Vietnam	2009
Hue_39	10	7	20	9	5	7	18	ST1871	-	-	Human	Vietnam	2009
7875	45	4	35	119	27	56	8	ST1872	-	-	Human	Laos	2006
1346	16	347	146	219	8	9	2	ST1873	-	-	Human	Vietnam	2011
Hue_70	16	2	8	18	8	9	339	ST1874	-	-	Human	Vietnam	2010

Light-grey strips represent the strains which were isolated from swine production chains in northern-Thailand (2011-2013).

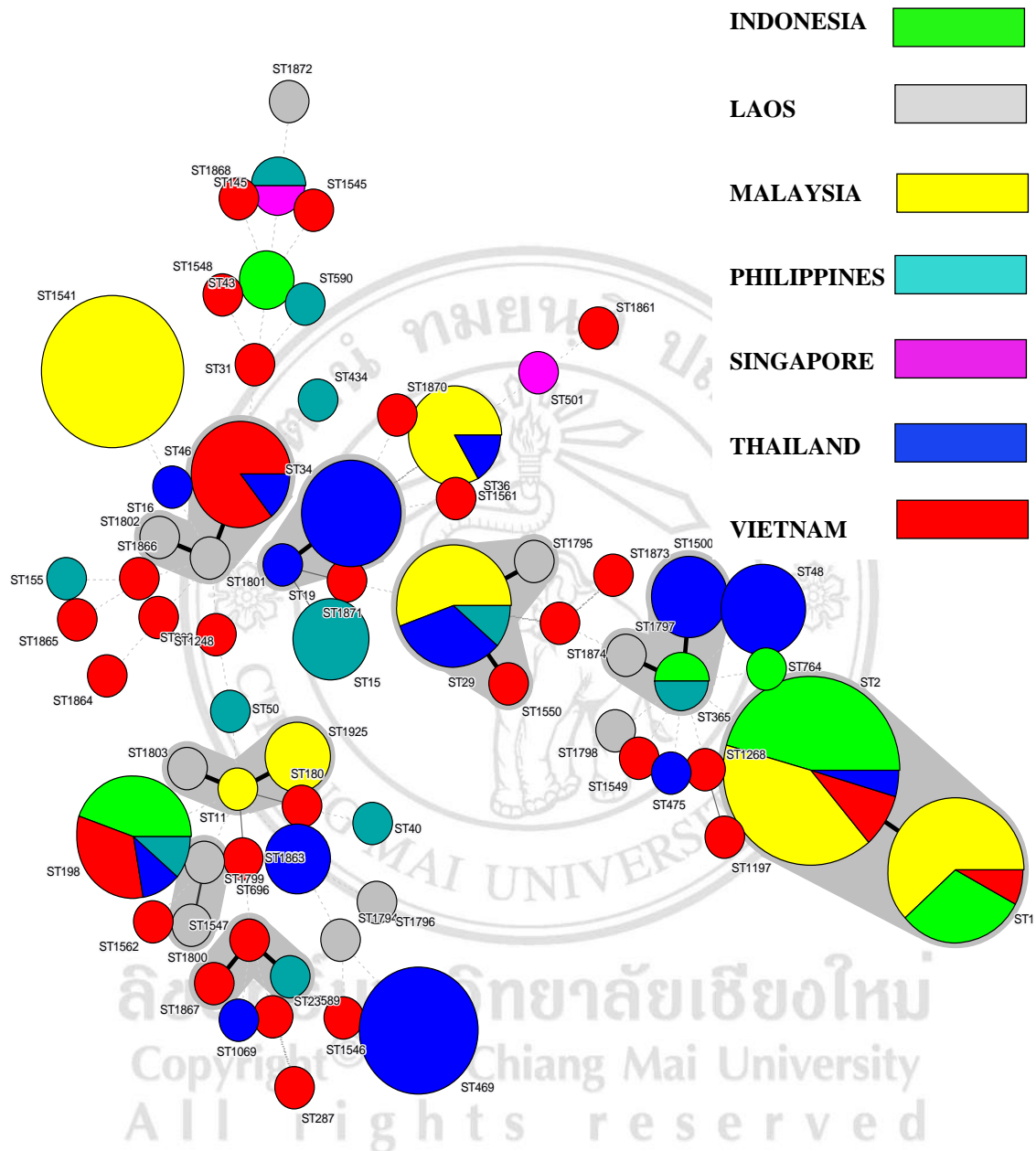


Figure 4.1 MLST analysis of 173 *S. enterica* strains divided in geographical distribution.

The Minimum Spanning Tree (MST) analysis, each circle represents each STs, the size reveals the numbers of strains. In the branch styles coding: thick solid lines connect between ST-type with one locus difference; solid lines, thin solid lines and dotted lines

connect between ST-type with double loci, three loci and four loci differences, respectively; dashed lines connect between ST-type with above four loci differences. The grey shade areas represent the related at least five loci between STs (belonging in common CCs).

4.5 Discussion

An investigation of the epidemiology of 173 *S. enterica* strains and their relationships isolated from AEC countries over the past several decades revealed the existence of 68 sequence types (from analysis of 7 house-keeping gene loci), the majority of which could be grouped into CCs. Strains whose allelic profiles did not match with the others suggest that they contained infrequent sequence types due to rare recombination or mutation events, horizontal gene transfer or microevolution occurrences [32,93]. Application of Simpson's diversity index indicated that MLST was superior than serotyping in differentiate the relatedness of *S. enterica* strains. This finding is in agreement with previous studies on typing of *S. enterica* [27,33,95], as well along with the other organisms, including *Streptococcus suis* [96] and *Vibrio cholera* [97]. In this study, a number of *S. enterica* serotypes were associated with more than one sequence type, a phenomenon also observed *Salmonella*, in which serotypes Weltevreden, Typhi and Typhimurium are correlated with common CCs [27,95] confirming the high resolution power of MLST technique.

Seventy percent of *S. enterica* strains recently isolated from northern Thailand [35] were represented by 4 sequence types (ST19, ST48, ST469 and ST1500) new to AEC countries. *S. enterica* ST469 and ST1500 were isolated originally in AEC-nearly areas such as China and India, and ST19 was found first in China [27], demonstrating that the strains which are categorized in similar STs may be associated with the common ancestors as well as common sources of infection [60]. Conversely, most of strains belonging to ST48 are derived from Europe [33]. It may be the first time that this ST has been discovered in AEC region. Of the 143 strains deposited in *S. enterica* MLST database, the highest frequency was observed in ST2, followed by ST1541 and ST1, respectively. ST2 and ST1 have been reported as being the dominant sequence type

isolated from humans in the AEC and nearby regions for decades [98]. On the other hand, *S. enterica* ST1541 was very rare among AEC countries, being reported only in Malaysia. For that reason, it is difficult to conclude the epidemiology for this STs. Moreover, the presence of indistinguishable strains, discovered at different times well over a decade, is a strong evidence for the persistence of certain *S. enterica* strains in the AEC areas [60].

MST analysis based on allelic profiles and geographical distributions in the AEC countries revealed that Thailand contained unique *S. enterica* sequence types, almost all of which were isolated recently in northern Thailand, thus suggesting that the notion of facile disease spread of disease across international border in this region may not be relevant [60]. Nevertheless, such strains as ST1, ST2 and ST198 have been discovered in various AEC countries (The information is demonstrated in MST) (Figure 4.1). International travel, food chain supply and/or trade may be playing a key role on this aspect circumstances [27,83-84].

4.6 Conclusions

MLST approach provides precise typing of *S. enterica*, which allows ready comparison of the results among different laboratories. It is accepted as a standard tool in routine genotyping suitable for diseases surveillance and investigation. This study provided information on *S. enterica* epidemiology in AEC countries that will be important for implementing biosecurity measures and for educating awareness of the risks of disease transmission in AEC.

4.7 Acknowledgements

The authors thank the National Science and Technology Development Agency (NSTDA), Thailand (Project ID: P-10-10409 and P-11-00792) for support and Chiang Mai University for *S. enterica* strains and valuable contributions.