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Morakot Nuntapaitoon

Sawang Katedangsakulwut

Rachod Tantilertcharoen

Napawan Bunpapong

Nanthiya Iampraphat

See next page for additional authors

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Authors

Morakot Nuntapaitoon, Sawang Katedangsakulwut, Rachod Tantilertcharoen, Napawan Bunpapong, Nanthiya Iampraphat, Suphadtra Therarachatamongkol, and Jeremy Gatine

Original Article

Potential risk factors for *Brachyspira hyodysenteriae*, *Lawsonia intracellularis* and *Salmonella* spp. infection and their prevalence in commercial swine farms in Thailand

Morakot Nuntapaitoon[1,2*] Sawang Katedangsakulwut[3] Rachod Tantilertcharoen[4]

Napawan Bunpapong[4] Nanthiya Iampraphat[4]

Suphadtra Therarachatamongkol[5] Jeremy Gatine[6]

Abstract

The aims of the present study were to evaluate the prevalence for *Brachyspira hyodysenteriae, Lawsonia intracellularis* and *Salmonella* spp. in pig feces and to identify their risk factors in 20 commercial swine farms in Thailand. The cross-sectional study was carried out from 20 farrow-to-finishing commercial swine farms in part of Thailand including the north (n=5), northeast (n=4), east (n=5), west (n=4) and south (n=1). A total of 589 fecal samples were randomly collected from 12 (n=200), 16 (n=196) and 20 (n=193) aged a week-old in each farm. All samples were analyzed for the detection of *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., in feces by multiplex PCR. The prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., in positive farms was 45.0% 85.0% and 10.0%, respectively (P>0.05). The prevalence in open housing system for *B. hyodysenteriae* and *L. intracellularis* (62.5%) had a tendency to be higher than a closed housing system (62.9% VS 33.3%, P=0.199 and 100.0% VS 75.0%, P=0.125, respectively). No effect of farm size, location of farm, number of sites and the diarrhea status on the prevalence for all pathogens was found. The pigs aged at 12 weeks (15.0%) had the prevalence for *B. hyodysenteriae* positive samples higher than the pigs aged at 16 weeks (3.3%, P=0.027) but did not differ significantly compared to the pigs aged at 20 weeks (6.7%, P=0.142). In conclusion, *L. intracellularis* is the main cause of intestinal bacterial infection in growing-finishing pigs in Thailand, especially in open housing system farms.

Keywords: *Brachyspira hyodysenteriae*, *Lawsonia intracellularis*, multiplex PCR, prevalence, risk factor, *Salmonella* spp.

[1]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[2]*Swine Reproduction Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[3]*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[4]*Veterinary Diagnostics Laboratory, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
[5]*AmcoVet Co., Ltd., Bangkok 10250, Thailand*
[6]*Patent Co., DOO. Vlade Ćetkovića 1a, 24211 Mišićevo, Serbia*
****Correspondence**: Morakot.N@chula.ac.th* (M. Nuntapaitoon)
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Introduction

The main bacteria intestinal diseases in growing-finishing pigs are porcine proliferative enteropathy, swine dysentery and salmonellosis which are caused by infection with *Brachyspira hyodysenteriae, Lawsonia intracellularis* and *Salmonella* spp., respectively. The infected pigs are subclinical to high clinical signs that affect growth performance and feed conversion (Hampson *et al*., 2015). All pathogens are transmitted via the fecal-oral route that is easy to infect in commercial swine farms. The all-intestinal bacterial infections have been reported in many countries worldwide (Bae *et al*., 2013; Dors *et al*., 2015; Hands *et al*., 2010). However, the prevalence for all pathogens in Thailand is limited within 10 years ago. The latest report of the prevalence for *B. hyodysenteriae* was shown in 2004 from 19 farms in the west region (Tummaruk and Prapasarakul, 2004). The prevalence for *L. intracellularis* in Thailand was reported in 2009 from 29 farms (Raphanaphraiwan *et al*., 2009). Some data of the prevalence for salmonellosis in swine commercial farms has been reported in Thailand. Recently, the prevalence for Salmonella in 2016-2017 was reported from backyard pigs in the north region of Thailand (Anuchatkitcharoen *et al*., 2020).

Control of intestinal bacterial infection increased pig health, weight gain and production. In general, intestinal bacterial detection has been performed by many techniques that differ in terms of time or duration of detection, sensitivity and specificity. The multiple PCR was performed to detect *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. (Dors *et al*., 2015; Suh and Song *et al*., 2005). Moreover, knowledge of risk factors influencing the intestinal bacterial infection is beneficial for the commercial swine farms. Therefore, the aims of the present study were to evaluate the prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., in pig feces and to identify their risk factors in commercial swine farms in Thailand.

Materials and Methods

The present study was carried out using ethical principles and guidelines for the use of animals for scientific purposes published by the National Research Council of Thailand. All procedures were approved by the Chulalongkorn University Animal Care and Use Committee (animal use protocol number 2031010). The present study was approved by the Chulalongkorn University Biosafety Committee (institutional biosafety number 2031016).

Study design and farms: The cross-sectional study was carried out from 20 farrow-to-finishing commercial swine farms with 500-7,000 sows on production in Thailand between January and July 2020. On average, sows on production in collected farms were 2,110 sows. The farms were selected on a history of diarrhea in a growing and finishing farm or presence of diarrhea at the studying time.

Fecal collection: A total of 589 fecal samples from 20 swine farms was randomly collected from 12 (n=200), 16 (n=196) and 20 (n=193) weeks-old aged in each farm. All samples were collected from rectal examination or freshly defecated feces and were kept at -20 °C before being analyzed the detection of *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., in feces by multiplex PCR.

The detection of B. hyodysenteriae, L. intracellularis and Salmonella* spp., *in feces by multiplex PCR: From 1 to 4 collected fecal samples farm were pooled into 1 pooled sample, according to age and farm. The 3 pooled samples/age in each farm were detected for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in feces by multiplex PCR. Therefore, a total of 180 pooled samples were included in the present study.

The pooled samples were homogenized in plastic containers. The total DNA was extracted from each pooled sample using a commercial kit (QIAamp Fast DNA Stool Mini Kit, QIAgen, Hilden, Germany), according to manufacturing's instruction. The primers used in multiplex PCR for specific amplification of DNAs from of *B. hyodysenteriae L. intracellularis* and *Salmonella* spp., were performed according to the previous study (Suh and Song *et al*., 2005). The primers used in multiplex PCR for specific amplification of DNAs from *B. hyodysenteriae L. intracellularis* and *Salmonella* spp. were 5'-GCAGCACTTGCAAAC AATAAACT-3', 5'-TTCTCCTTTCTCAT GTCCCAT AA-3'; 5'-GCTGGAGATGATGCTTCTGG-3', 5'-GTCC AAGAGCTTGGCTGTTC-3'; 5'-TTGGTGTTTATGGG GTCGTT-3'; and 5'-GGGCATACCATCCAGAGAAA-3', respectively.

PCR amplification was conducted on a DNA Thermocycler (Thermal Cycler, Mastercycler, nexus gradient, Eppendorf, USA). The initial mixture was heated at 95°C for 1 min (Initial denaturation), 1 cycles 95°C for 15 secs (Denaturation), 56 °C for 15 secs (Annealing) and 72 °C for 30 secs (Extension) 35 cycles and 72 °C for 1o min (Final Extension) 1 cycles. The amplified DNAs were analyzed on 1.5% agarose gel electrophoresis immersed in Tris-borate-EDTA (1X TBE) and were stained using Redsafe™ Nucleic Acid Staining Solution (Intron biotechnology). The gels were visualized using UV transilluminator.

Statistical analysis: Data was analyzed by SAS program (SAS Institute Inc., Cary, NC, USA). A farm was defined as positive for *B. hyodysenteriae, L. intracellularis* or *Salmonella* spp. when at least one pooled fecal sample collected from the farm had a positive PCR result. Within-farm prevalence was defined as the number of positive pooled fecal samples divided by the total number of pooled samples collected in positive farms, according to previous study (Dors *et al*., 2015). The frequency of distribution of the within-farm prevalence for *B. hyodysenteriae, L. intracellularis* or *Salmonella* spp., in pooled fecal samples were performed by PROC FREQ of SAS. The prevalence was performed in a proportion and 95% confidence interval (CI) using Clopper-Pearson exact. The farms were in part of Thailand including the north (n=5), northeast (n=4), east (n=5), west (n=4) and south (n=1). The farm size was classified into 3 sizes including less than 1,000 (4 farms), 1,000-2,500 (10 farms) and more than 2,500 (6 farms). The type of farm included an evaporative cooling-housing system

(closed; n=12) and a conventional open-housing system (opened; n=8). The number of sites on reared pigs included 2 (n=6) and 3 (n=14) sites. Differences in the prevalence for all pathogens among farm size, farm location, the type of farm, diarrhea status and the number of sites on reared pigs were analyzed by X^2 test. Values with $P < 0.05$ were regarded as statistically significant.

Results

The prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., is presented in Table 1. Table 1 demonstrates that the percentage of prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in positive farms was 45.0% 85.0% and 10.0%, respectively ($P > 0.05$). The number and percentage of *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in positive samples was 8.3% 50.6% and 4.4%, respectively ($P > 0.05$).

The simultaneous presence of *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., is presented in Table 3 and Figure 1. Table 2 illustrates that 8 farms from 20 farms were detected with *L. intracellularis* only (40.0%), 7 farms with *B. hyodysenteriae* and *L. intracellularis* (35.0%), 1 farm with all pathogens (5.0%), with *L. intracellularis* and *Salmonella* spp. (5.0%) and *B. hyodysenteriae* only (5.0%). Moreover, 2 farms were not detected with any of the examined pathogens (10.0%) and none was detected with *Salmonella* spp., only and 2 pathogens (*B. hyodysenteriae* and *Salmonella* spp.)

The within-farm prevalence in positive farms for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., were 14.8% 49.0% and 11.1%, respectively. Moreover, 9 farms were detected with *B. hyodysenteriae,* 17 farms with *L. intracellularis* and 2 farms with *Salmonella* spp., the distribution of within-farm prevalence is presented in Figure 2.

The prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in difference farm size and location of farm is presented in Table 3. The prevalence in open housing system for *B. hyodysenteriae* and *L. intracellularis* (62.5%) was a tendency higher than closed housing system (62.9% VS 33.3%, $P = 0.199$ and 100.0% VS 75.0%, $P = 0.125$, respectively). No effect of farm size, location of farm, number of site and the diarrhea status for the prevalence for all pathogens was found.

The prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp., in difference pig age is presented in Table 4. There was a significant difference between pig age and prevalence for *B. hyodysenteriae*. The pigs aged at 12 weeks (15.0%) had a prevalence for *B. hyodysenteriae* positive samples higher than the pigs aged at 16 weeks (3.3%, $P = 0.027$) and have a tendency higher than the pigs aged at 20 weeks (6.7%, $P = 0.142$). No effect of age on the prevalence for *L. intracellularis* and *Salmonella* spp., was found.

Table 1 Prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in pooled fecal samples (n=180) from fattening pigs in selected 20 commercial swine farms in Thailand. The 95% confidence interval (CI) analyzed by using Clopper-Pearson exact.

Pathogens	No. of positive farms	%	95% CI	No. of positive samples	%	95% CI
B. hyodysenteriae,	9	45.0	23.0-68.5	15	8.3	4.7-13.4
L. intracellularis	17	85.0	62.1-96.8	91	50.6	43.0-58.1
Salmonella spp.	2	10.0	1.2-31.7	8	4.4	1.9-8.6

Table 2 Simultaneous presence of *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in pooled fecal samples (n=180) from fattening pigs in selected 20 commercial swine farms in Thailand. The 95% confidence interval (CI) analyzed by using Clopper-Pearson exact.

Pathogens	No. of positive farms	%	95% CI	No. of positive samples	%	95% CI
B. hyodysenteriae	1	5.0	0.1-24.9	4	2.2	0.6-5.6
L. intracellularis	8	40.0	19.1-64.0	76	42.2	34.9-49.8
Salmonella spp.	0	0.0	0-16.8	2	1.1	0.1-4.0
B. hyodysenteriae and *L. intracellularis*	7	35.0	15.4-59.2	10	5.6	2.7-10.0
L. intracellularis and *Salmonella* spp.	1	5.0	0.1-24.9	5	2.8	0.9-6.4
B. hyodysenteriae and *Salmonella* spp.	0	0.0	0-16.8	1	0.6	0-3.1
All pathogens	1	5.0	0.1-24.9	0	0.0	0-2.0
None	2	10.0	1.2-31.7	82	45.6	38.1-53.1

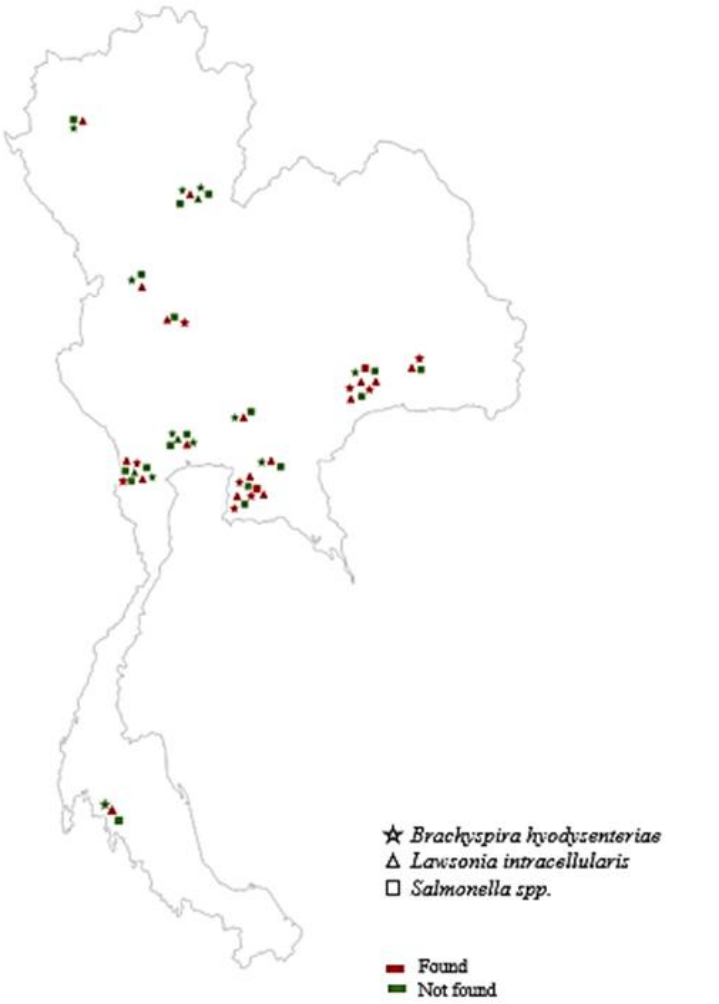
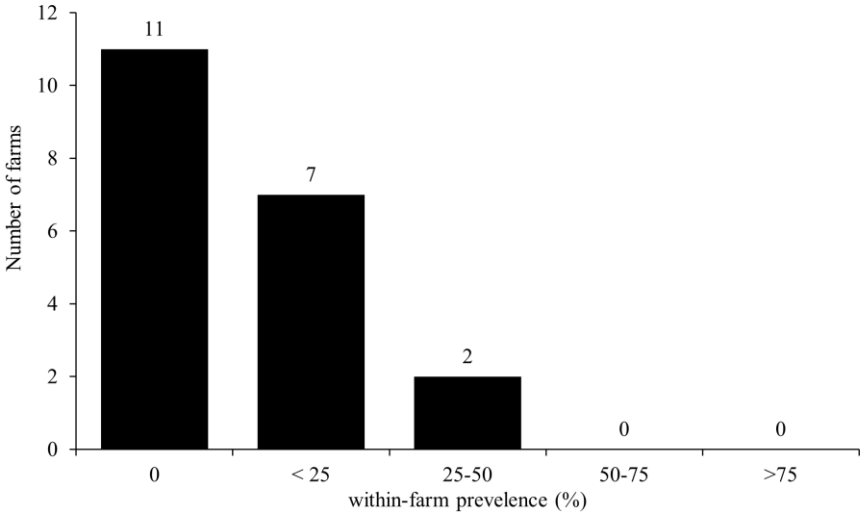


Figure 1 Geographic distribution of fattening pigs in selected 20 commercials swine farms in Thailand 20 farms were investigated the prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in pooled fecal samples (n=180).



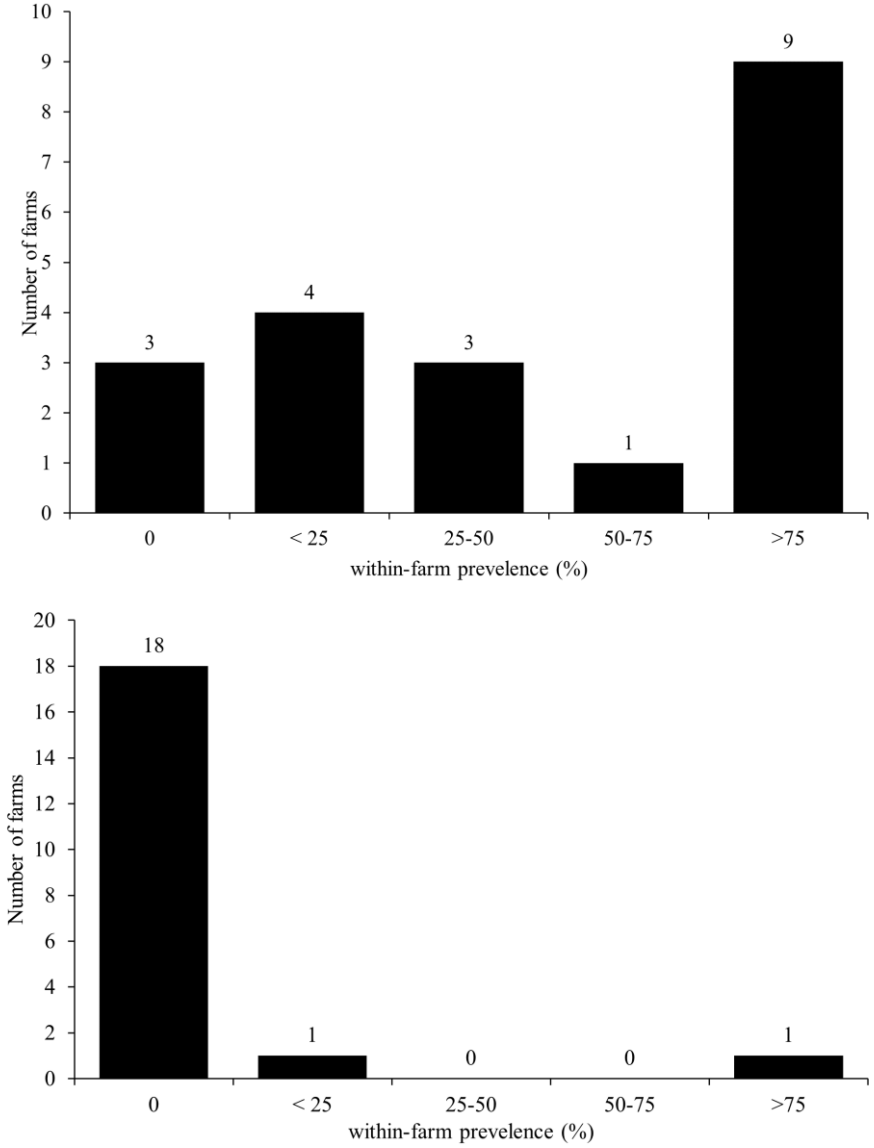


Figure 2 The within-farm prevalence for a.) *B. hyodysenteriae*, b.) *L. intracellularis* and c.*)* *Salmonella* spp. in pooled fecal samples (n=180) from fattening pigs in selected 20 commercial swine farms in Thailand.

Table 3 Prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in pooled fecal samples (n=180) from fattening pigs in selected 20 commercial swine farms in Thailand by farm level. The 95% confidence interval (CI) analyzed by using Clopper-Pearson exact.

Regions	**No. of farms**	***B. hyodysenteriae***			***L. intracellularis***			***Salmonella* spp.**		
		No. of positive farms	**%**	**95% CI**	**No. of positive farms**	**%**	**95% CI**	**No. of positive farms**	**%**	**95% CI**
Farm size, sows										
<1,000	4	3	75.0	19.4-99.4	4	100.0	39.8-100.0	0	0.0	0.0-60.2
1,000-2,500	10	3	30.0	6.7-65.3	9	90.0	55.5-99.8	2	20.0	2.5-55.6
>2,500	6	3	50.0	11.8-88.2	4	66.7	22.3-95.7	0	0.0	0.0-45.9
Region										
West & South	6	2	33.3	4.3-77.7	4	66.7	22.3-95.7	0	0	0-45.9
North	5	3	60.0	14.7-94.7	4	80.0	28.4-99.5	0	0	0-52.2
Northeast	4	3	75.0	19.4-99.4	4	100.0	39.8-100.0	1	25.0	0.6-80.6
East	5	1	20.0	0.5-71.6	5	100.0	47.8-100.0	1	20.0	0.5-71.6
Farm status										
Opened	8	5	62.5	24.5-91.5	8	100.0	63.1-100.0	1	12.5	0.3-52.7
Closed	12	4	33.3	9.9-65.1	9	75.0	42.8-94.5	1	8.3	0.2-38.5
Diarrhea										
Yes	17	7	41.2	18.4-67.1	15	88.2	63.6-98.5	2	11.8	1.5-36.4
No	3	2	66.7	9.4-99.2	2	66.7	9.4-99.2	0	0.0	0-70.8
Number of sites										
2 sites	6	3	50.0	11.8-88.2	5	83.3	35.9-99.6	1	16.7	0.4-64.1
3 sites	14	6	42.9	17.6-71.1	12	85.7	57.2-98.2	1	7.1	0.2-33.9

Table 4 Prevalence for *B. hyodysenteriae, L. intracellularis* and *Salmonella* spp. in pooled fecal samples (n=180) from fattening pigs in selected 20 commercial swine farms in Thailand by farm characteristic. The 95% confidence interval (CI) analyzed by using Clopper-Pearson exact.

Parameters	No. of samples	*B. hyodysenteriae*			*L. intracellularis*			*Salmonella* spp.		
		No. of positive	%	95% CI	No. of positive	%	95% CI	No. of positive	%	95% CI
Age, wk										
12	60	9	15.0[a]	7.1-26.6	29	48.3	35.2-61.6	2	3.3	0.4-11.5
16	60	2	3.3[b]	0.4-11.5	31	51.7	38.4-64.8	2	3.3	0.4-11.5
20	60	4	6.7[ab]	1.9-16.2	31	51.7	38.4-64.8	4	6.7	1.9-16.2

[a, b] Statistically significant differences among row in characteristic group ($P > 0.05$)

Discussion

The present study is the first report in over 10 years on the prevalence for intestinal bacterial infection in growing-finishing pigs in Thailand. It was found that *L. intracellularis* is the main cause of intestinal bacterial infection in growing-finishing pigs in Thailand. The *L. intracellularis*, *B. hyodysenteriae* and *Salmonella* spp., are the most enteric bacterial infection in pig worldwide. The present study indicated that *L. intracellularis* is the main causes of intestinal bacterial infection and followed by *B. hyodysenteriae,* while *Salmonella* spp., is the minor causes of intestinal bacterial infection, in agreement with previous report in Canada in 1990 (Wilson *et al*., 1990). On average, the prevalence for *L. intracellularis* was 20.0-100.0% in the Asia and Europe region (Dors *et al*., 2015; Hands *et al*., 2010; Jacobson *et al*., 2003; Lee *et al*., 2001; Stege *et al*., 2000). This study demonstrated that a high prevalence for *L. intracellularis* was found in Thailand both farm (85.0%) and within-farm levels (50.6%) when compared with the previous report in other counties. In Asia, the data were reported by Chang *et al*. (1997), the prevalence in farm and within-farm levels was 30.0% and 5.5%, respectively in Taiwan. In addition, the researchers reported that the prevalence in South Korea farm and within-farm levels was 20.0% and 3.3%, respectively in 1998 and 46.5% and 19.9%%, respectively in 2005 (Kim *et al*., 1998; Suh and Song, 2005). In Europe, the mean of *L. intracellularis* prevalence in farm level was 20.0%, 48.4%, 44.6% and 31.8% in Brazil, German, Italy, and Czech Republic, respectively (Chiriboga *et al*., 1998; Cizek *et al*., 2006; Merialdi *et al*., 2003; Reiner *et al*, 2011). However, the prevalence in farms in Thailand was lower than in pig farms in Hungary and Denmark (Biksi *et al*., 2007; Stege *et al*., 2000) that high pig production countries. The prevalence for *L. intracellularis* in Thailand in 2009 was 38.1% from 29 farms (Raphanaphraiwan *et al*., 2009). The data was collected from the same age pig and from farm with diarrhea history. It can be concluded that intestinal bacterial infection in the farm may rapidly enhance because pigs have no clinical signs and/or chronic infection.

The prevalence for *B. hyodysenteriae* in Thailand was reported in 2004 from 19 herds in west region included Phetchaburi and Nakornpathum province as west and south in the present study (Tummaruk and Prapasarakul, 2004) by bacteria culture technique. On average, the prevalence for *B. hyodysenteriae* was 20.3% and was the highest in pigs at 20–24-week-old (Tummaruk and Prapasarakul, 2004). On the other hand, the present study found that the prevalence for *B. hyodysenteriae* was 8.3% and was the highest in pigs at 12-weeks-old. Moreover, Tummaruk and Prapasarakul (2004) reported that the prevalence for *B. hyodysenteriae* was 47.3% and this study found was 33.3%. It can be concluded that that the high prevalence for *B. hyodysenteriae* in Phetchaburi and Nakornpathum provinces still occurred. However, the *B. hyodysenteriae* detection was found in younger pig because of different technique between multiplex PCR in the present study and bacterial culture in Tummaruk and Prapasarakul (2004). In general, bacterial detection was performed by culture technique and time was spent for about 10-12 days in anaerobic conditions while multiplex PCR was more famous because of fast, high sensitivity and specificity. In line with this, the multiplex PCR method was recommended as the gold standard for detection. In accordance with Jung *et al*. (1994) and Soh and Song, (2005) reported the prevalence for *B. hyodysenteriae* in South Korea was 30.7-37.2% and 10.6-10.8%, both herd and within-herd level, respectively.

Porcine proliferative enteropathy and swine dysentery was highly prevalent in large herd sizes (Holyoake *et al*., 1994; Kramomtong *et al*., 1996). However, the prevalence for *L. intracellularis* and *B. hyodysenteriae* in farm size > 2,000 sows in South Korea was 41.7% and 16.7%, respectively. The result of the present study found that the prevalence in farms < 1,000 sows was the highest prevalence for both *L. intracellularis* and *B. hyodysenteriae*. The high within-farm prevalence in positive farms for *L. intracellularis* was found in the study. In total, 11 farms were within-farm prevalence more than 25.0% and 7 farms were within-farm prevalence more than 75% in all pigs of all ages. The only 2 farms have within-farm prevalence more than 25%. Factor influencing all bacterial infection related coinfection and biosecurity in the farms. In accordance with the result in previous study, simultaneous presence of *B. hyodysenteriae* and *L. intracellularis* was 37.5% in farm level (Soh and Song, 2005) and 40.0% in the present study. In line with this, risk factor of *B. hyodysenteriae* and *L. intracellularis* infection in Thailand may be similar. On the other hand, simultaneous presence of *B. hyodysenteriae* and *L. intracellularis* was only 1.4% in Poland and 50.0% of farm had only *L. intracellularis* infection (Dors *et al*., 2015). None farm of *L. intracellularis* infection in Poland have 34.3% while in Thailand and South Korea have 10.0% and 13.4%, respectively. In line with this, the prevalence for *L. intracellularis* was higher than Poland. Farm system was highly influencing all bacterial infections (Dors *et al*., 2015). The present study found that high prevalence for all pathogens was found in the open housing system. All intestinal bacteria were infected by fecal-oral route. The water consumption for

cleaning and reducing heat stress in the farm may be increased infection. Moreover, animal grouping, buying in replacement stock and hygiene induced high prevalence in swine commercial herds. Therefore, prevention and control strategies should be provided to reduce the occurrence of the bacteria in swine production. Efficient disinfectants, antimicrobial drugs and vaccine were used for preventing together with high biosecurity management (e.g., used cleaned water, all-in-all-out, separated sick pig and reduced stress) (Karuppannan and Opriessnig, 2018). Moreover, the phytogenic feed additive supplementation was applied to control bacterial infection in fattening pigs (Bošnjak-Neumüller *et al*., 2019; Delić *et al*., 2018; Draskovic *et al*., 2018; 2020). Additionally, copper-bearing montmorillonite (Cu-MMT) reduced the number of *Salmonella* spp., colonies in vitro (Ting *et al*., 2020). The Cu-MMT are an effective bactericide in the digestive tract. One of limitations of the present study is that no data of the number of pigs per housing and pen was observed. The pig population may be affected by bacterial infection in swine commercial herd. Therefore, the number of pigs in per housing and pen should be investigated in further studies.

In conclusion, the present study indicated that *L. intracellularis* is the main causes of intestinal bacterial infection in growing-finishing pigs in Thailand. The pigs reared in conventional open housing system farms have high risk for intestinal bacterial infections from *B. hyodysenteriae, L. intracellular* and *Salmonella* spp., regardless of location and size of farms in Thailand. The 12- and 20-week-old pigs was high *B. hyodysenteriae* infection. Management to decrease intestinal bacterial infection in Thailand should be emphasized at growing pig (12-week-old pigs) reared in opened housing system.

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