SEROPREVALENCE OF *LAWSONIA INTRACELLULARIS* ANTIBODY AMONG SWINE HERDS IN THAILAND, DECEMBER 2021-MARCH 2022

Mr. Thanh Nguyen Che

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ความชุกทางซีรัมวิทยาของแอนติบอดีต่อการติดเชื้อ *Lawsonia intracellularis* ในฟาร์มสุกรในประเทศไทย ในช่วงธันวาคม 2564 ถึง มีนาคม 2565

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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By	Mr. Thanh Nguyen Che
Field of Study	Veterinary Science and technology
Thesis Advisor	Associate Professor Doctor YAOWALAK PANYASING, D.V.M., Ph.D., D.T.B.V.P.
Thesis Co Advisor	Professor Doctor ROONGROJE THANAWONGNUWECH, D.V.M., Ph.D., D.T.B.V.P.

Accepted by the FACULTY OF VETERINARY SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

..... Dean of the FACULTY OF VETERINARY SCIENCE
(Professor Doctor SANIPA SURADHAT, D.V.M., Ph.D.)

THESIS COMMITTEE

..... Chairman
(Instructor Doctor ROONGTHAM KEDKOVID, D.V.M., Ph.D.)

..... Thesis Advisor
(Associate Professor Doctor YAOWALAK PANYASING, D.V.M., Ph.D., D.T.B.V.P.)

..... Thesis Co-Advisor
(Professor Doctor ROONGROJE THANAWONGNUWECH, D.V.M., Ph.D., D.T.B.V.P.)

..... External Examiner
(Assistant Professor Doctor SUPHATTRA JITTIMANEE, D.V.M., Ph.D.)

ทาน เหวียน เช : ความชุกทางซีรัมวิทยาของแอนติบอดีต่อการติดเชื้อ *Lawsonia intracellularis* ในฟาร์มสุกรในประเทศไทย ในช่วงธันวาคม 2564 ถึง มีนาคม 2565. (SEROPREVALENCE OF *LAWSONIA INTRACELLULARIS* ANTIBODY AMONG SWINE HERDS IN THAILAND, DECEMBER 2021-MARCH 2022) อ.ที่ปรึกษาหลัก : เยาวลักษณ์ ปัญญสิงห์, อ.ที่ปรึกษาร่วม : รุ่งโรจน์ ธนาวงษ์นุเวช

โรคติดเชื้อในระบบทางเดินอาหารที่มีสาเหตุจากการติดเชื้อแบคทีเรียถือได้ว่าเป็นปัญหาสำคัญในอุตสาหกรรมการเลี้ยงสุกรของประเทศไทยเป็นอย่างมาก Porcine proliferative enteropathy (PPE) หรือโรคอิลลิไอติส (porcine ileitis) เป็นโรคติดเชื้อที่มีสาเหตุมาจากเชื้อแบคทีเรีย *Lawsonia intracellularis* โรคอิลลิไอติสสร้างความเสียหายให้แก่อุตสาหกรรมการเลี้ยงสุกรทั่วโลก สุกรที่ติดเชื้อสามารถแสดงอาการท้องเสียได้หลากหลายรูปแบบ การวินิจฉัยทางซีรัมวิทยาเป็นวิธีการสำคัญที่สามารถนำมาใช้ในการประเมินความชุกของแอนติบอดีที่มีความจำเพาะต่อเชื้อ *L. intracellularis* และช่วงเวลาของการติดเชื้อภายในฝูงสุกรได้ สำหรับประเทศไทยนั้น ข้อมูลทางซีรัมวิทยาสำหรับโรคอิลลิไอติสยังมีอยู่จำกัดและยังมีการใช้ยาปฏิชีวนะในการเลี้ยงสุกรอีกด้วย ทำให้ไม่ทราบสถานการณ์ความชุกของโรคอิลลิไอติส การศึกษานี้มีวัตถุประสงค์เพื่อสำรวจความชุกทางซีรัมวิทยาของแอนติบอดีต่อการติดเชื้อ *L. intracellularis* ในฝูงสุกรขนาดใหญ่ของประเทศไทย โดยใช้วิธี blocking enzyme-linked immunosorbent assay (ELISA) ผลการทดสอบตัวอย่างซีรัมสุกรจำนวน 1,234 ตัวอย่าง จากฟาร์มสุกรที่ไม่มีการใช้วัคซีนโรคอิลลิไอติส ในทุกภาคของประเทศไทยพบความชุกของฝูงสุกร ที่ตรวจพบแอนติบอดีจำนวน 22 ฟาร์ม จากทั้งสิ้น 24 ฟาร์ม และพบความชุกของการติดเชื้อภายในฝูง 50.5% (95% CI: 47.7-53.3%) โดยพบความชุกของโรคมากที่สุดในกลุ่มสุกรที่มีอายุมากในฝูง นอกจากนี้พบความชุกของแอนติบอดีต่อการติดเชื้อมากที่สุดในฟาร์มสุกรในเขตภาคเหนือของประเทศไทย และพบความแตกต่างอย่างมีนัยสำคัญของความชุกทางซีรัมวิทยาภายในฝูงสุกรที่มีระบบการผลิตแบบพื้นที่เดียว (single-site) และแบบแยกพื้นที่เลี้ยง (multi-site) ($P < 0.001$) จากการศึกษานี้ยืนยันได้ว่าสามารถพบความชุกของแอนติบอดีต่อโรคอิลลิไอติสได้ในอัตราที่สูงในฟาร์มสุกรขนาดใหญ่ในประเทศไทย

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Thanh Nguyen Che : SEROPREVALENCE OF *LAWSONIA INTRACELLULARIS* ANTIBODY AMONG SWINE HERDS IN THAILAND, DECEMBER 2021-MARCH 2022. Advisor: Assoc. Prof. Dr. YAOWALAK PANYASING, D.V.M., Ph.D., D.T.B.V.P. Co-advisor: Prof. Dr. ROONGROJE THANAWONGNUWECH, D.V.M., Ph.D., D.T.B.V.P.

The gastrointestinal infectious diseases caused by bacteria have been a major problem in the Thai swine industry. Porcine proliferative enteropathy (PPE), also known as porcine ileitis, is an enteric infectious disease caused by *Lawsonia intracellularis*. Porcine ileitis has been an economic threat to the global pork production system due to various types of diarrheas. Serological diagnosis can be applied for determining pathogen-antibodies seroprevalence and the timing of *L. intracellularis* infection when introduced to the herd. A lack of serological data since 2009, as well as recent antibiotic usage restrictions, may have a negative impact on porcine ileitis seroprevalence in Thailand. Hence, the objective of this present study was to investigate the current serological status and the seroprevalence of antibodies against *L. intracellularis* in large-scale swine herds in Thailand using a commercial blocking enzyme-linked immunosorbent assay (ELISA). A total of 1,234 serums were sampled from 24 non-vaccinated commercial pig farms across Thailand as part of a monitoring program. The herd seroprevalence was obtained in a total of 22 out of 24 herds sampled, and the within-herd seroprevalence was 50.5% (95% CI: 47.7-53.3%), with higher seropositivity detected in older animals. Furthermore, the most *Lawsonia*-antibodies were found in the intensive larger farms in Northern Thailand. There was a significant difference in the within-herd seroprevalence between single-site and multiple-site production systems ($P < 0.001$). Here, the finding confirmed that in the absence of the *L. intracellularis* vaccine, PPE seroprevalence in large commercial swine herds in Thailand was prevailing.

Field of Study:	Veterinary Science and technology	Student's Signature .....
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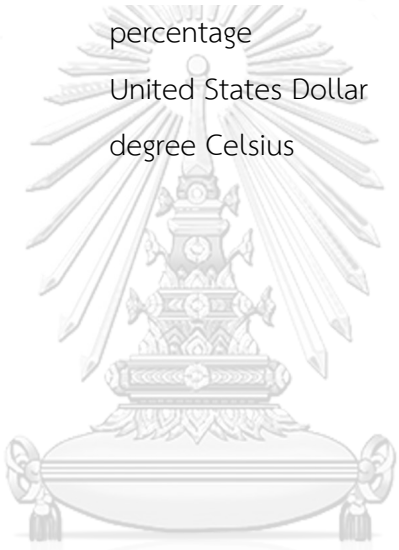
LIST OF ABBREVIATIONS

ASF	African Swine Fever
CI	confidence interval
DNA	deoxyribonucleic acid
DOC	sodium deoxycholate
dpi	day(s) post infection
ELISA	enzyme-linked immunosorbent assay
et al.	et alii, and others
FITC	fluorescein isothiocyanate
g	gram
H&E	hematoxylin and eosin
i.e.,	that is
IFAT	immunofluorescent antibody test
IFN-γ	Interferon-gamma
Ig	immunoglobulin
IHC	immunohistochemistry
IPMA	immunoperoxidase monolayer assay
ISH	*in situ* hybridization
L. intracellularis	*Lawsonia intracellularis*
LPS	lipopolysaccharide
M	molar(s)
min	minute(s)
mL	milliliter(s)
nm	nanometter(s)
OD	optical density
PBS	phosphate-buffered saline solution
PCR	polymerase chain reaction
PHE	Proliferative hemorrhagic enteropathy
PI	percent inhibition
PIA	Porcine intestinal adenomatosis

PPE	Porcine proliferative enteropathy
ppm	parts per million
rDNA	ribosomal deoxyribonucleic acid
SoELISA	sonicated enzyme-linked immunosorbent assay
TMB	Tetramethylbenzidine
U.S.A.	United States of America
WS	Warthin Starry
µL	microliter(s)
µm	micrometer(s)
%	percentage
$	United States Dollar
°C	degree Celsius



CHAPTER 1: INTRODUCTION

The gastrointestinal infectious diseases caused by bacteria have been a major problem in the porcine industry worldwide. Porcine proliferative enteropathy (PPE) is a typical and prevalent enteric disease in swine that is commonly referred to as "porcine ileitis". The causative agent, *Lawsonia intracellularis (L. intracellularis)* is an obligate intracellular bacterium, ubiquitous and highly pathogenic (McOrist et al., 1995). This microorganism engenders a great economic loss due to mild diarrhea, lower slaughter weight, feed conversion efficiency, space utilization in growing pigs, and high morbidity-mortality effects in fattening pigs and reproductive units (Kroll et al., 2005). Furthermore, diagnosis of *L. intracellularis* infection in swine herds remains challenging because symptoms mimic those of other intestinal diseases caused by several causative viruses (Porcine Circovirus type 2 enteritis) and bacteria (*Salmonella enterica serovar Typhimurium, Brachyspira hyodysenteriae*) (Baró et al., 2015; Borewicz et al., 2015; Suh and Song, 2005).

PPE has been detected in commercial swine herds all over the world using molecular detection of feces or infected tissues, and serological investigation of serum samples. With respect to molecular techniques, the microorganism was detected in 75% of Danish swine (Stege et al., 2004) and 48% of Swedish nursery farms (Jacobson et al., 2005). Serological data showed the seropositivity of PPE was nearly 91% of pork production in America (Armbruster et al., 2007), and 91.7% of swine herds in Europe (Arnold et al., 2019). Moreover, there were 74% of *Lawsonia*-antibody-positive pigs among highly intensive farms in Australia (Holyoake et al., 2010). This seropositive rate was in parallel with the finding of Wu et al. (2014) in China (77%). There has not been any research in Thailand specifically on porcine ileitis disease since 2009, when a cross-sectional study Raphanaphraiwan et al. (2009) found that the within-herd seroprevalence of *L. intracellularis* was 38.05%. This evidence indicated that PPE has also been exposed among Thai swine herds.

Porcine ileitis is common in a wide range of production cycle, affecting 30–40% of pigs and resulting in a variety of severe gross lesions in either natural or experimental infection (McOrist et al., 2003). Restrictions on the usage of antibiotics

have so far been implemented in some countries, such as Europe and America, as well as Thailand (Nuangmek et al., 2020). It may have an important impact, leading to a higher seroprevalence and the earlier seroconversion of *L. intracellularis* within a herd. (Arnold et al., 2019). Besides, the global swine industry's economy has been significantly affected since the *L. intracellularis* widespread onset. For instance, husbandry costs are increasing due to higher feed consumption and longer facility times to reach market weight (Mauch and Bilkei, 2005). Within an Australian-infected farm, it could cost from $3 to $11 for nursing expenditure and more than $25 per sow annually (Holyoake et al., 2010). Thus, porcine ileitis is a principal disease entity to contend with, but compared to other highly pathogenic infections, this disease receives relatively little attention from farmers, veterinarian committees, and animal health authorities (Wu et al., 2014).

Clinical diarrhea, specific gross lesions with thickening of the ileum wall at the abattoir, and microscopical estimation of the small and large intestines were used to diagnose *Lawsonia*-infection (Biester and Schwarte, 1931). But it required a postmortem examination to observe and evaluate the signature pathology. Polymerase Chain Reaction (PCR) is a molecular detection technique that has been used for determining the presence of bacteria in fecal materials and/or intestinal tissues. Animals with PCR-positive results indicated symptomatic cases of porcine ileitis, actively shedding the microorganism via feces at sampling time (Gebhart et al., 1993). Serology can be an effective strategic approach to evaluating the prevalence of swine disease, even with nonspecific clinical signs. *Lawsonia*-antibodies can be persisted in the host animal's serum for 13 weeks after exposure (Guedes and Gebhart, 2003b). Several serological methods have been commonly used to detect and measure the level of antibodies (Campillo et al., 2021); that is, the immunofluorescence antibody test (IFAT), the immunoperoxidase monolayer assay (IMPA), and the enzyme-linked immunosorbent assay (ELISA). The ELISA is viewed as a more realistic monitoring tool for herd surveillance, despite the IPMA having the highest sensitivity and specificity of those assays (Boesen et al., 2005).

In an attempt to provide information and raise awareness for the prevention and management of *L. intracellularis* infection after the era of antibiotic usage restrictions, the current exposure of PPE in the Thai swine industry should be investigated. Recently, the ELISA technique has been used as a routinely practical method for evaluating serological profiles in a swine production system. Since it has a high throughput, is simple to use, is inexpensive, and has a high accuracy rate. Thus, this study aimed to identify the seroprevalence and current serological status of *Lawsonia*-antibodies in the large-scale Thai swine herds using a commercial blocking ELISA.

Hypothesis

We hypothesize that there is prevalence of *Lawsonia intracellularis* infection among Thai large-scale swine herds with different seroconversion patterns.

Objectives of Study

1. To study the seroprevalence of *Lawsonia intracellularis* infection in commercial swine herds in Thailand.
2. To identify the seroconversion pattern within the seropositive farms.

Questions of study

1. What is the current exposure status of *Lawsonia intracellularis* infection in Thailand?
2. What is the seroprevalence and seroconversion pattern within the seropositive farms/regions?

CHAPTER 2: LITERATURE REVIEW

The *Lawsonia intracellularis* bacteria

For years, *Campylobacter spp.* was considered as a mainly pathogenic microbe causing porcine ileitis disease in swine due to successful isolation and cultivation from infected animals. However, the anticipated symptoms were not observed when the bacteria-inoculated experiment was performed with conventional pigs (Lawson et al., 1993). Until 1995, there was a proof about 16S ribosomal DNA (16S rDNA) gene sequencing by McOrist and his teammate; they classified and named the causative etiology as *Lawsonia intracellularis (L. intracellularis)* (McOrist et al., 1995). This organism is arranged systematically in the Proteobacteria's delta subdivision of the Desulfovibrionaceae family with 91% genetic similarity (Cooper et al., 1997; Gebhart et al., 1993). The bacterial morphologic studies have been described as rod-shaped, non-spore forming, 1.25–1.75 μm and 0.25–0.43 μm in length and width, respectively. Its fastidious characteristics include micro-anaerobic, obligate intracellular, and gram-negative that support organism to replicate by binary fission and extremely unavailable growth in cell-free media (McOrist et al., 1995).

Etiology and pathogenicity

Porcine proliferative enteropathy (PPE) is an endemic enteric disease in the global swine industry. Moreover, proliferative enteropathy also caused diseases in other animal species, including horses, foals, sheep, deer, foxes, emus, dogs, rabbits, hamsters, rats, and nonhuman primates (Cooper et al., 1997; Vannucci and Gebhart, 2014). Hence, these *L. intracellularis* infected animals can play a role as potential sources for spreading bacteria to the swine population. PPE has negative economic impacts because it has been reported on a wide range of age through oral challenge and natural infection, resulting in the varying degrees of clinical signs (Guedes and Gebhart, 2003b). In nursery-growing pigs, weight loss, a poor growth rate, stunning, and diarrhea were observed. The bloody diarrhea or/and sudden deaths were observed in finishing pigs or/and reproductive animals (McOrist et al., 2003; Stege et al., 2000).

PPE was allocated to two distinct clinicopathologic forms, i.e., proliferative hemorrhagic enteropathy (PHE) and porcine intestinal adenomatosis (PIA). PHE is an acute form frequently related to adult pigs, 4–12 months old, during such period as the fattening or finishing stage, replacement gilts, breeders, and boars (Kroll et al., 2005). Recent studies indicated a new tendency: the potential for chronically infected replacement animals to appear in breeding units (Jacobson et al., 2010). PIA is an uncomplicated chronic and subclinical form that usually occurrs on most commercial swine farms and is influenced on young, post-weaning pigs, starting around 4 or 5–20 weeks old.

The pathogenesis of porcine ileitis has been described after ingestion of bacteria from an oral challenge (Karuppannan and Opriessnig, 2018). In most particular animals, the incubation stage was approximately 2–3 weeks, and a slow spread of this disease can be observed over a month or more after the first infected case was introduced into a group. The microorganism attacked the intestinal epithelium after 5 days of infection. Consequently, it located in the apical cytoplasm of the intestinal and proximal colon, which are the target organs of *Lawsonia*-infection. Fecal shedding was observed at 1–2 weeks, reaching a peak at 3 weeks, and persisting for 12 weeks post-infection (Guedes et al., 2002). The infected pigs showed clinical signs of diarrhea for 9-21 days. A hallmark of lesions appeared in the large intestine approximately 1–2 weeks after those in the small intestine (Guedes and Gebhart, 2003b; Karuppannan and Opriessnig, 2018). The study by Collins and Love (2007) revealed that *L. intracellularis* could survive in feces at 5–15^{o}C for more than 2 weeks, hence, it will become a beneficial condition to reintroduce the microorganism into farms.

Immunology

Infection with *L. intracellularis* triggers a different immunological response in the intestine, resulting in a distinct cellular and molecular immune response. Innate immunity is the initial line of defense. Pigs showed signs of a broad inflammatory response by 2–3 days post infection (dpi) (Mair et al., 2014). The absence of an inflammatory response may be a result of bacterial suppression in chronic porcine

proliferative enteropathy (PPE), whereas an increased macrophage population in the mucosa and elevated levels of proinflammatory cytokines are observed during acute PPE. Interferon-gamma (IFN-***γ***) was thought to be a controlled pattern of *Lawsonia*-infection for cell-mediated immune response. IFN-***γ*** was detected in infected pigs as early as 10 days after infection and peaked at 20 days; as a result, it could be a viable strategy for developing an early diagnosis in pigs (Riber et al., 2015). Specific antibodies were detected in the serum, intestinal mucosa, feces, and oral fluid in relation to humoral immunity (Gabardo et al., 2021). A previous study by Guedes et al. (2017) detected the highest titers of antigen-specific immunoglobulin (Ig)A in intestines between 15 and 29 dpi. Likewise, IgG and IgM response to microorganisms can be identified in serum 2 weeks after infection, peak at 3–4 weeks and persist for up to 13 weeks (Guedes and Gebhart, 2003b). However, they explored that the IgA concentrations are unrelated to serum IgG levels. An activated infection, immunization by vaccination, or colostrum consumption can all cause an IgG response; thus, it is impossible to distinguish between vaccinated and spontaneously infected pigs (Gebhart and Guedes, 2010).

Epidemiology

Porcine proliferative enteropathy (PPE) has been reported worldwide in highly intensive swine herds. By a blocking ELISA, the true within-herd prevalence of *L. intracellularis* antibodies in China was shown to be 77%, with fattening pigs, breeding sows, and boars in Northern or Southern parts having the highest frequency (Wu et al., 2014). This rate gained to 79.1% of 63 Chinese examined farms during 2019-2020 (Xiao et al., 2022). Another study, using the same technique, found high seroprevalence among high density pig population, with 71.7% and 88.9% in Western Australia and Queensland state, respectively; within the herds was 84.2% for the mean percentage of seropositive animals (Holyoake et al., 2010). In South Korea, the IFAT assay indicated 100% of herds had positive pigs with *Lawsonia*-specific IgG antibody, seroprevalence in growers was 45% and in finishers was 59% (Lee et al., 2001). On reproductive units, the difference of seroprevalence in sows by parity was not clear and ranged from 86-100% in Ontario, Canada (Corzo et al., 2005). At the

same, the seropositive gilts/sows in Brazil accounted for 36.61% (the 2nd rank after finishing pigs with 74.28%) (Resende et al., 2015). In association of Southeast Asian Nations (ASEAN) region, 10 out of 13 examined farms (76.9%) from Northern Vietnam showed IFAT-positive results for porcine ileitis disease (Vu et al., 2003). Raphanaphraiwan et al. (2009) used comparable method to investigate a 38.05% seroprevalence of PPE in commercial swine herds in Thailand. To be specific, the pigs in the Western area were likely exposed to *L. intracellularis* more than other areas with 90.5% seropositivity. Currently, very few prevalence surveys and risk factor analysis of swine enteric diseases, particularly PPE, have been conducted in Thailand.

The major transmission route of porcine ileitis is the fecal-oral route. Non-infected pigs ingest the bacterium into its mouth and gut after coming into contact with an infected pig's feces or contaminated environments and ingest the bacterium into its mouth and gut. Guedes and Gebhart (2003b) informed us that the infectious oral dose was quite low, but the fecal shedding may be high in experimental pigs. In addition, the contaminated areas can pose a risk of *L. intracellularis* exposure by transferring feces onto workers' boots, equipment, or trolleys. Bacteria can be possibly transferred via animal-vectors such as rodents, birds, and insects in contact with unhealthy pigs' feces to other cleaning areas of farm, commonly occurred on single-site farms (Bae et al., 2013). Rodents play a critical role as an intermediate host animal to introduce this pathogen in swine herds (Backhans et al., 2013). Gabardo et al. (2017) reported subclinical infection in pigs from the feces of infected mice. A survey presented up to 70.6% of wild rats captured in infected pig farms with *L. intracellularis,* and bacterial shedding in feces can last for 14 days at 5–15°C (Collins et al., 2000).

The exposure of *L. intracellularis* has been determined contrarily between farms, impacted by husbandry management systems, biosecurity, and antimicrobial usage. The dynamic usually occurs a couple of weeks after weaning stage in farms that apply a continuous flow (single-site production system) between each age stage of pigs/farm area/housing location, as the maternal antibodies fade and no prophylactic antimicrobial was given (Stege et al., 2004). Within production system that followed the rules of differentiation of age-group of post weaning and breeding

pigs following by age and site (all-in/all-out multi-site system), infection in growing/finishing pigs can be delayed until 14–20 weeks of age and occurred rarely in breeding stock (Marsteller et al., 2003). A few studies have illustrated a significant influence on hygiene of general- or batch-wise all-in/all-out system; maybe even better, it can reduce the circulation of PPE (Dors et al., 2015). Regarding the one health concern, it has been argued that prohibiting microbial growth promoters would affect the prevalence of *L. intracellularis*.

Diagnosis approaches

Microorganisms can be confirmed and the level of infection in microscopic lesions evaluated by immunohistochemistry (IHC), which utilizes specific monoclonal antibodies (Guedes and Gebhart, 2003a; Ladinig et al., 2009). A comparison study of the diagnostic sensitivity of IHC, hematoxylin and eosin (H&E) staining, and Warthin Starry (WS) silver staining showed that the IHC has higher sensitivity (86.8%) than WS staining (50%) and H&E staining (36.8%) (Guedes et al., 2002). The technique of *in situ* hybridization (ISH) is relatively similar to the IHC method, except for the use of *Lawsonia*-specific oligonucleotide probes (Jensen et al., 2010). Nonetheless, both IHC and ISH methods are considered the gold standard in PPE antigen detection, but they are not properly used to confirm an ongoing infection in live animals due to the postmortem examination requirement. Molecular techniques can find the bacteria's DNA in fecal materials, rectal swabs, or fresh intestinal mucosal tissues of infected animals. The polymerase chain reaction (PCR) is a common antemortem detection method for this microbe in early infection. However, these tests have changeable sensitivity (36–100%), specificity (50–100%), and are more costly than serology (Jacobson et al., 2004; Pedersen et al., 2012). The success of various PCR based assays in *L. intracellularis* diagnosis has been confirmed: conventional PCR (Suh et al., 2000), real-time PCR (Lindecrona et al., 2002), multiplex and quantitative PCR (Pedersen et al., 2010), and nested PCR (Jones et al., 1993). These methods show the ability to monitor the health status of animals by examining fecal material for signs of bacterial infection, which means non-shedding or subclinical infected animals can be missed.

Serology is a crucial tool for evaluating swine exposure to a certain agent, even in the absence of specific clinical signs. It is also feasible to ascertain the infection kinetics and suggest the optimal time for treatment or vaccination by knowing the age range in which the herd's peak seroconversion occurs (Resende et al., 2015). Serological methods are susceptible to detecting a humoral immune response from infection and/or indicating vaccinated animals and/or neonatal pigs have gotten antibodies through colostrum in a passive manner (Kroll et al., 2005). An immunofluorescence antibody test (IFAT) was investigated by using bacteria grown in pure culture stained with pig serum as the primary antibody and fluorescein isothiocyanate (FITC) labeled anti-swine IgG as the second antibody. The IFAT has a higher sensitivity of 90% than a PCR assay, but a lower specificity of 96% (Collins et al., 2012). Having a similar workflow, protocol, and purpose with IFAT, but the results of immunoperoxidase monolayer assay (IPMA) are read by using light microscopy, IFAT otherwise requires a fluorescent microscopy. The sensitivity and specificity of IPMA were validated at 89% and 100%, respectively. In controlled infection trials, both tests have a high level of agreement (98%) that indicates equivalent diagnostic utility in the diagnosis of *L. intracellularis* seropositive pigs (Guedes et al., 2002).

The enzyme linked-immunosorbent assay (ELISA) was developed for screening the appearance of *Lawsonia*-specific antibodies in the sera of pigs. In comparison to other serological methods, the ELISA can enable high sample throughputs to conduct disease seroprofiling, including in sub-clinically infected animals. The indirect ELISA, which uses whole cell antigen from sonicated bacteria (SoELISA) (Wattanaphansak et al., 2008), sodium deoxycholate (DOC) extracted antigen (Boesen et al., 2005), bacteria targets such as Lipopolysaccharide (LPS) (Kroll et al., 2005), LsaA protein ELISAs (Watarai et al., 2004). At present, "SVANOVIR® *L. intracellularis*/Ileitis-Ab" (Boehringer Ingelheim Svanova, Uppsala, Sweden) is the only commercial ELISA kit which can detect total IgA, IgG, or IgM without distinguishing each type of immunoglobulin. This ELISA kit procedure is based on a solid phase blocking ELISA in which the non-infectious *L. intracellularis* antigen is coated wells on microtiter plates. The anti-*L. intracellularis* horseradish peroxidase-conjugated antibodies are used as competitors with *Lawsonia*-antibodies in samples (Keller et

al., 2006). The accuracy of this ELISA kit was evaluated subsequently, but it has not been reliable depending on the cutoff of percent inhibition (PI)-value chosen. The study showed a higher sensitivity (72%) and specificity (93%) at cutoff PI 35. When the cutoff at PI 30 (value of manufacture's recommendation) was used to interpret results, the sensitivity and specificity were estimated at 75% (Jacobson et al., 2011). However, nonspecific, or cross reactivity reactions can occur if antibodies are formed towards other antigens with similar antigenic epitopes (Corzo et al., 2005). All serological tests cannot differentiate between vaccinated animal with live-attenuated or killed vaccine and those naturally infection.

Cumulatively, as the gap of each diagnostic tools noted previously, the diagnosis of *L intracellularis* exposure can be performed by using a combination of serological, histology and molecular techniques. These results are required to monitor disease prevalence and spread, as well as to implement appropriate management measures to treat or prevent occurrence of porcine ileitis. Finally, detection of sub-clinically infected animals is still a challenge for available diagnostic techniques, and further research is needed to find adequate diagnostic solutions.

Seroconversion

Several studies demonstrated seroconversion in infected/uninfected animals between production systems. Resende et al. (2015) conducted research in farrow-to-finishing system (single-site system) in Brazil, and their findings showed that seroprevalence decreased slightly from gilts and sows to nursery pigs before dramatically increasing and peaking at finishers using the IPMA technique. Screening the prevalence of positive porcine ileitis antibody levels from weaners to finishers on a conventional farm in Asia, the obtained maternal antibody persisted for 6 first-week post-weaning and dramatically appeared again around 8 weeks of life, and most pigs infected at 4 or 5 weeks when the maternal antibody was reduced (McOrist, 2005). The seroconversion of European pigs also started at the end of the nursery stage and persisted for 13 weeks, time for infection was examined about 2 weeks earlier (Hedge et al., 2005). Applying a similarly experimental model to a multi-site farm with an all-in/all-out procedure in North America, the clean breeding animals' offspring showed

delayed seropositivity until later in the finishing period, which indicated those piglets more susceptible to the acute form of disease (McOrist, 2005).

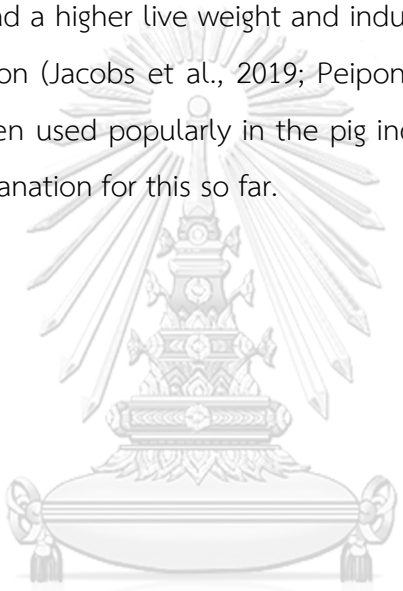
Prevention and treatment

Biosecurity is the most important factor in preventing the introduction of *L. intracellularis* into farms, and quarantine showed efficacy in keeping some herds free from PPE. To date, several commercially available disinfectants are based on various precautions and are designed to kill bacteria. Some of these include ammonium compounds, aldehydes, povidone-iodine, and oxidizing agents. Water hardness of less than 400 ppm is recommended for inactivating most disinfectants (Wattanaphansak et al., 2009). Therefore, implementing step-by-step cleaning and/or disinfection protocols, all-in/all-out procedures, and using older sows with higher parity in rotation are the keys to solving the PPE circulation problem in swine herds. Finally, the impact of constructed materials was researched, with a higher risk of infection associated with slatted concrete flooring, while straw bedding and solid flooring had a lower prevalence tendency (Bronsvoort et al., 2001).

The use of *in vitro* culture systems for the control of *L. intracellularis* has led to the discovery of novel antibiotics that can inhibit bacterial proliferation, for example, penicillin, erythromycin, difloxacin, chlortetracycline, tiamulin, and tilmicosin (McOrist et al., 1995). For prophylactic doses, tiamulin, tyrosine, tetracycline, and lincomycin were proven to be efficient controllers of porcine ileitis. During the clinical signs outbreak, infected animals were treated by using higher doses of all of the antibiotics listed above via feed or intramuscular administration (Guedes et al., 2009; Li, 2017).

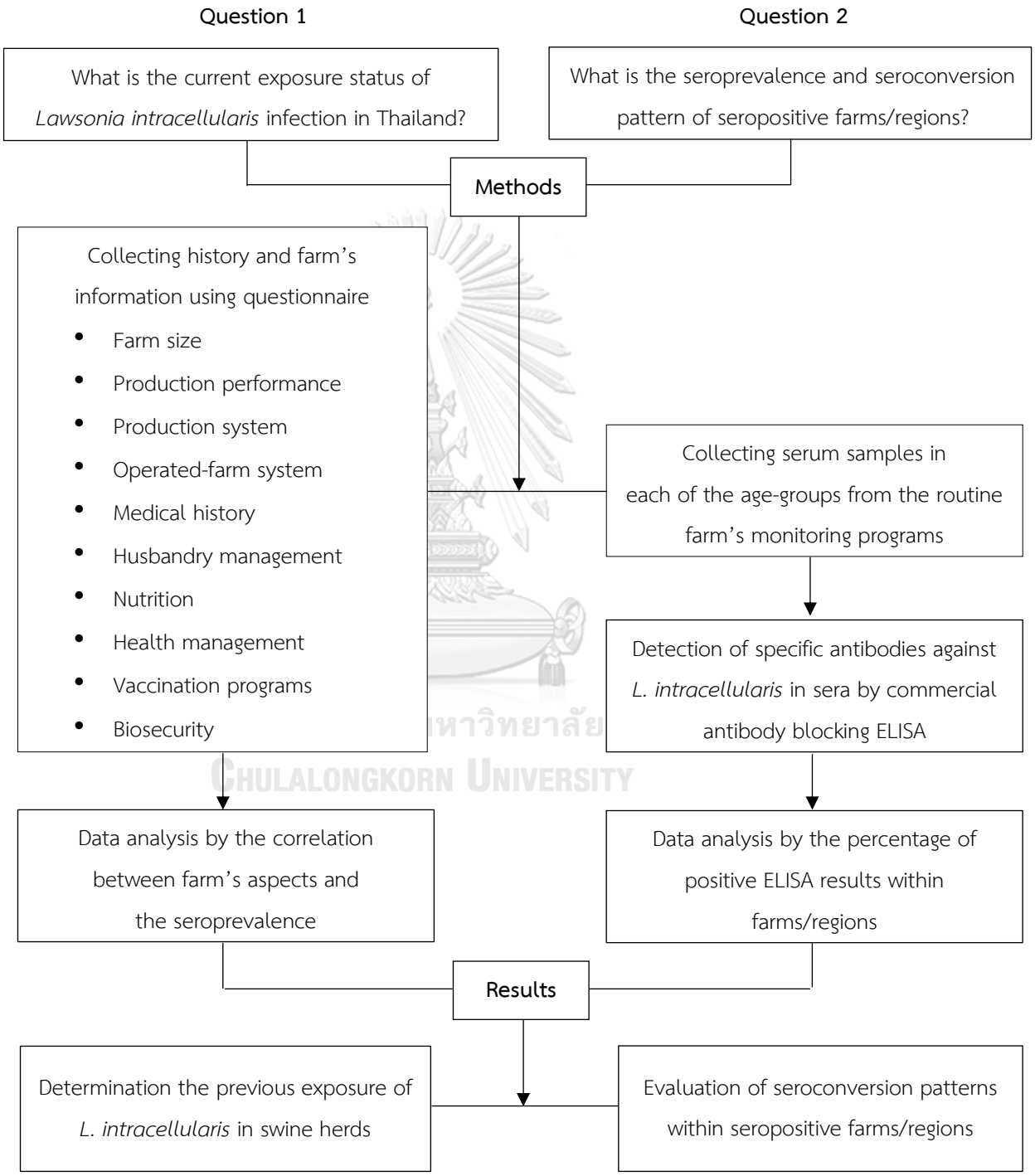
Vaccination is a key to preventing and controlling porcine ileitis. There are currently two commercially available vaccines for pigs, namely, an oral live avirulent vaccine Enterisol® Ileitis (Boehringer Ingelheim B.V., Alkmaar, The Netherlands), which has been introduced since 2004 (Almond and Bilkei, 2006), and an inactivated whole cell vaccine Porcilis® Lawsonia (Merck Animal Health, Madison, NJ, U.S.A.) from 2016 (Roerink et al., 2018). It has two implements affect the vaccination efficacy: route of administration and the type of vaccine. Research about the route of live attenuated

vaccination indicated that the IgG production between oral, intramuscular, and intraperitoneal was unsignificant. In addition, the live vaccine was the pigs' best option due to the fact that it mostly closely mimics natural infection, therefore they are more strongly protected later in life, and it elicits local mucosal immunity properly against the infection (Guedes and Gebhart, 2003b). Thus, oral live vaccination has been the best choice for PPE prevention because of stress reduction for pigs and labor saving. The efficiency of both vaccine products has been studied; those vaccinated animals (injected at 3 and 4 or 5 weeks of age for Eterisol® and Porcilis, respectively) had a higher live weight and induced significant protection from *L. intracellularis* infection (Jacobs et al., 2019; Peiponen et al., 2018). Nevertheless, vaccination has not been used popularly in the pig industry, particularly in Asia, and there has been no explanation for this so far.



CHAPTER 3: MATERIAL AND METHODS

Conceptual Framework



Study design

A questionnaire designed ad hoc for study was provided to a veterinarian or farmer, who eventually sampled the herds (Appendix). The questionnaire contains essential items, such as, general information (farm's size, production performance, and herd structure), nutrition, husbandry management, health measurement (vaccinated routine and antimicrobial usage), and biosecurity. Farms that participated in the study did not have a history of vaccination against *L. intracellularis*. The presence of at least one diarrhea outbreak for 3 to 6 months before the sample time point was an optional inclusion. "Diarrhea outbreak" is defined as already had or having clinical signs of diarrhea in any age-group of pigs: watery diarrhea, bloody diarrhea, and indigestible diarrhea. Any type, severity, and probable causes of diarrhea were included in the study.

After a preliminary survey, a total of 1,234 serum samples from a wide-ranged age of pigs were sampled and tested on a commercial blocking ELISA designed to detect IgG, IgA, and IgM against *L. intracellularis* (Jacobson et al., 2011). These samples were collected as a part of routine sero-monitoring of commercial swine herds in Thailand. Results (optical density) from ELISA were calculated as percentage inhibition, and qualitative results (positive/negative) were considered according to the manufacturer's cutoff. The herd and within-herd seroprevalence were calculated and determined by age, region, and production system.

Herd selection and sample collection

A cross-sectional investigation was performed for 4 months, from December 2021 to March 2022. The farms selected in this study were large-scale, closed-system commercial swine farms located in 6 geographical regions of Thailand, including Central, East, North, North-East, South, and West. These farms are single-site (farrow-to-finishing) and multi-site (site-1: breeding and site-2: weaning-to-finishing), with the size of the herd ranging from 1,200 to 4,800 sows for site-1 and 4,000 to 18,000 pigs for site-2. None of the pigs was immunized with the *L. intracellularis* vaccine.

The sampling procedure was allocated into multiple stages: herd-level (herd units) and animal-level (within-herd units). Using the WinEpi: Working in Epidemiology

(http://www.winepi.net/uk/index.htm, Faculty of Veterinary Medicine, University of Zaragoza, Spain) to estimate the number of required herd units. A total of 22 closed-system farms, which yielded a 95% confidence level of roughly 35% predicted prevalence and a 20% accepted error with an unknown population size (Dargatz and Hill, 1996), were estimated to be under study. Based on the most recent serological data available in 2009, the predicted prevalence percentage was surmised (Raphanaphraiwan et al., 2009).

For sampling within-herd units, the serum samples were collected as a part of the routine farm's monitoring program. These samples comprised from 5 to 20 animals from 6 distinct categories of production cycle, i.e., piglets (under 4 weeks old), nursery (4-10 weeks old), growers (10-18 weeks old), finishers (18–24 weeks old), gilts/sows, and boars (Wilson et al., 1999). We calculated the sample size per age-group with the goal of being 95% confident in our final estimate of 10-15% porcine ileitis seroprevalence (Sampaio, 2007). Moreover, this sampling plan could clearly illustrate the maternal antibodies' persistence and the fluctuation of serological status within each herd. Blood collection was conducted by the farm's veterinarians following protocol. Briefly, at least 6 mL of whole blood was collected from the external jugular, then submitted to the laboratory. After centrifugation at 2,500g for 10 min, the supernatant serum was assembled, aliquoted into sample tubes, and stored at -20°C until it was assayed.

Lawsonia-antibodies detection

A commercial blocking ELISA kit (SVANOVIR® *L. intracellularis*/Ileitis-Ab, Boehringer Ingelheim Svanova, Uppsala, Sweden) was used to detect *Lawsonia*-antibodies, following manufacturer's instructions. The component of the kit includes 96-well ELISA plate, sample dilution buffer, wash solution, conjugate solution, and stop solution (Figure 1). All reagents equilibrated to room temperature (18–25°C) for 30 min before testing. Negative and positive control, and samples were pre-diluted at 1:100 with sample dilution buffer in dilution plate, then 100 µL of diluted controls and samples were added into a 96-well microplate coated with non-infectious *L. intracellularis* antigen. Test plate was shake thoroughly and incubated at 37°C for 1

hour. The plate was washed 3 times with 350 µL of phosphate-buffered saline solution (PBS-Tween) 1X each well. Thereafter, 100 µL of diluted horseradish peroxidase conjugate (1:100) was added to the wells and incubated at 37°C for next 1 hour. The washing step was repeated. Next, 100 µL of substrate solution was added to the wells and incubate at room temperature (18–25°C) for 10 min. Subsequently, adding 50 µL of stop solution (sulfuric acid 2 M) is added to stop the reaction. ELISA antigen-coated plate is measured the optical density (OD) at 450 nm in a microplate photometer (Multimode Plate Reader EnVision® 2105, PerkinElmer, Waltham, MA, U.S.A.) immediately. The OD value was normalized and calculated as percent inhibition (PI) as the following formula:

$$PI = \frac{\text{OD (Negative control) - OD (Sample or Positive control)}}{\text{OD (Negative control)}} \times 100$$

Interpretation of PI values: PI ≥ 30 was considered as positive; PI < 20 as negative, and 20 < PI < 30 was scored suspicious. Doubtful samples were treated as negative. The investigated farm was concluded to be seropositivity when having at least one ELISA-positive result.

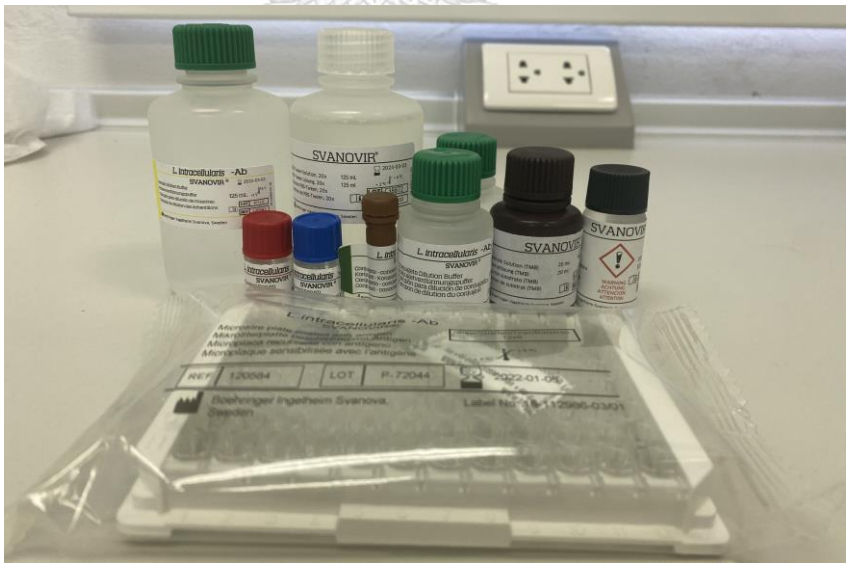


Figure 1 The component of SVANOVIR® *L. intracellularis*/Ileitis-Ab kit comprised 100X concentrated conjugate, substrate solution (TMB), stop solution, PBS-Tween solution 20X, positive control serum, negative control serum, conjugate dilution buffer and sample dilution buffer.

Statistical analysis

Raw data was filled in a spreadsheet program (Microsoft® Excel Office, version 16.5). An online software called Epitools - Epidemiological Calculators (http://epitools.ausvet.com.au, Ausvet, Canberra, ACT, Australia) was used to compute the apparent herd and within-herd seroprevalence. Particularly, the herd seroprevalence were calculated using the number of positive herds divided by the total number of tested herds. The overall within-herd seroprevalence were calculated using the number of positive samples divided by the total number of tested samples. Subsequently, with respect to age-groups, regions, and production site system, the apparent within-herd seroprevalence were calculated using the number of positive samples divided by the number of tested samples within each age-group, region, and production site system. To correct for the imperfect sensitive and specific ratio of the testing kit, the apparent seroprevalence was converted to true seroprevalence by applying the Rogan-Gladen correction (Rogan and Gladen, 1978). Binomial proportions' confidence limits were determined using Wilson score interval with a 95% confidence interval (CI) to obtain the probability of successful calculation (Reiczigel et al., 2010). Chi-square with Bonferroni post-hoc tests, and McNemar's test were conducted to compare the seropositive differences among seroconversion patterns, and between the apparent and true prevalence, respectively (GraphPad Prism 9, GraphPad Software Incorporated, San Diego, CA, U.S.A.). $P < 0.05$ was a statistically significant analysis.

CHAPTER 4: RESULTS

Over a four-month period of the current study, serum of wean-to-finish (n=850) and breeding (n=384) pigs were obtained from 24 non-vaccinated commercial swine herds among Thailand. The number of selected farms and tested samples for each province/region is displayed in Table 1 and Table 2, respectively.

The herd prevalence of *Lawsonia*-antibodies in Thailand

The apparent and true *L. intracellularis* seropositivity in herd units were indicated in Table 1. *Lawsonia*-antibodies was apparently found in 22 out of 24 investigated farms with one or more ELISA-positive animals. Two seronegative farms were in the North-eastern region, being site-2 of production system. Besides, the true herd prevalence showed only 20 seropositive farms, with two more seronegative farms located in the Central region, being single-site production systems. Nevertheless, no difference between the apparent and true herd seroprevalence was indicated.

Table 1 The apparent and true herd prevalence[†] of *L. intracellularis* in Thailand

Region	Number of farms (n)			
	Apparent prevalence		True prevalence	
	Positive	Tested	Positive	Tested
Central	4	4	2	4
East	7	7	7	7
North	2	2	2	2
North-East	5	7	5	7
South	1	1	1	1
West	3	3	3	3
Total	**22**	**24**	**20**	**24**

[†] The herd prevalence (herd units) indicates the percentage of positive herds per region divided by the total number of tested herds per region.

No statistical difference between the apparent and true herd prevalence by McNemar's test (P = 0.4795)

The within-herd prevalence of *Lawsonia*-antibodies by province/region

The average percentage of antibodies against *L. intracellularis* positivity within herds was 50.5%, leading to a true seroprevalence (51.0%) ranging from 45.4 to 56.6%. There was a significant difference in the true seroprevalence among geographical regions ($P < 0.001$). The Northern and Southern (100%, 95% CI: 100-100%) regions had significantly higher seropositivity than other areas, except for the Western Thailand. Similarly, the within-herd prevalence of the Eastern and Western parts was higher than in the Central and North-eastern regions of Thailand (Table 2).

Table 2 The within-herd seroprevalence[‡] of *L. intracellularis* by province/region

Region	Number of pigs (n)		Seroprevalence (%) (95% CI)	
	Positive	Tested	Apparent	True
Central				
Nakhon Pathom	2	40	5.0 (1.4 – 1.7)	0
Nakhon Nayok	18	30	60.0 (42.3 – 75.4)	70.0 (33.1 – 100)
Phetchabun	27	30	90.0 (74.4 – 96.6)	100 (97.4 – 100)
Saraburi	10	40	25.0 (14.2 – 40.2)	0
Sub-total	57	140	40.7 (32.9 – 49.0)	31.4 (15.6 – 48.6)[a]
East				
Chonburi	38	75	50.7 (39.6 – 61.7)	51.3 (28.6 – 74.1)
Prachin Buri	60	107	56.1 (46.6 – 65.1)	62.2 (42.5 – 81.0)
Rayong	52	70	74.3 (63.0 – 83.1)	98.6 (75.8 – 100)
Sub-total	150	252	59.5 (53.4 – 65.4)	69.1 (56.4 – 81.0)[b]
North				
Chiang Mai	90	103	87.4 (79.6 – 92.5)	100 (100 – 100)
Tak	34	40	85.0 (70.9 – 92.9)	100 (90.4 – 100)
Sub-total	124	143	86.7 (80.2 – 91.3)	100 (100 – 100)[c]
North-East				
Chaiyaphum	55	155	35.5 (28.4 – 43.3)	21.0 (6.6 – 37.1)
Kalasin	21	50	42.0 (29.4 – 55.8)	34.0 (77.0 – 62.1)
Nakhon Ratchasima	24	80	30.0 (21.1 – 40.8)	10.0 (0 – 32.4)
Yasothon	104	300	34.7 (29.5 – 40.2)	19.3 (9.0 – 30.1)
Sub-total	204	585	34.9 (31.1 – 38.8)	19.7 (12.2 – 27.8)[a]
South				
Phatthalung	35	35	100 (90.1 – 100)	100 (100 – 100)[c]
West				
Ratchaburi	53	79	67.1 (56.2 – 76.5)	84.2 (61.5 – 100)[bc]
Total	**623**	**1234**	**50.5 (47.7 – 53.3)**	**51.0 (45.4 – 56.6)**

CI: confidence interval

[‡] The within-herd prevalence (animal units) is the percentage of positive samples per province/region divided by the total number of tested samples per province/region.

[a, b, c] The significant difference in within-herd prevalence by region/province indicated by superscript.

The within-herd prevalence of *Lawsonia*-antibodies by age-categories

Within the positive herd, ELISA-positivity was detected in all stages of the production cycle. The true seropositive percentage varied, with mature animals showing higher positivity tendency (Table 3). The *Lawsonia*-seroconversion gradually increased from growers to breeders. Finishers (93.8%, 95% CI: 80.5-100%) and breeding animals, including gilts/sows (100%, 95% CI: 100-100%) and boars (100%, 95% CI: 68.2-100%), had a greater frequency rate compared to other groups (P < 0.001).

Table 3 The within-herd seroprevalence[‡] of *L. intracellularis* by age-categories

Age-categories	Number of pigs (n)		Seroprevalence (%) (95% CI)	
	Positive	Tested	Apparent	True
Piglets	2	60	3.3 (0.9 – 11.4)	0[a]
Nurseries	11	180	6.1 (3.5 – 10.6)	0[a]
Growers	121	400	30.3 (26.0 – 34.9)	10.5 (1.7 – 20.0)[b]
Finishers	151	210	71.9 (65.5 – 77.6)	93.8 (80.5 – 100)[c]
Gilts/Sows	316	356	88.8 (85.1 – 91.6)	100 (100 – 100)[d]
Boars	22	28	78.6 (60.5 – 89.8)	100 (68.2 – 100)[cd]
Total	**623**	**1234**	**50.5 (47.7 – 53.3)**	**51.0 (45.4 – 56.6)**

CI: confidence interval

[‡] The within-herd prevalence (animal units) is percentage of positive samples per age-group divided by the total number of tested samples per age-group.

[a, b, c, d] The significant difference in within-herd prevalence by age-group indicated by superscript.

The within-herd prevalence of *Lawsonia*-antibodies by site of production system

The within-herd prevalence by site of production system was identified (Table 4). There were significant differences in the true within-herd seroprevalence between single-site farms (34.7%, 95% CI: 23.6-46.0%) and multi-site farms (56.2%, 95% CI: 49.8-62.6%) (P < 0.001). Within the multi-site system, the breeding farms (100%, 95% CI: 100-100%) had a greater significant seroprevalence than the weaning-to-finishing farms (23.7%, 95% CI: 16.2-31.4%) (P < 0.001).

Table 4 The within-herd seroprevalence[‡] of *L. intracellularis* by site of production system

Site production system	Number of pigs (n)		Seroprevalence (%) (95% CI)	
	Positive	Tested	Apparent	True
Single site	127	300	42.3 (36.9 – 48.0)	34.7 (23.6 -46.0)[a]
Multiple site				
Site-1	268	315	85.1 (80.7 – 88.6)	100 (100 – 100)[*]
Site-2	228	619	36.8 (33.1 – 40.7)	23.7 (16.2 – 31.4)[**]
Sub-total	496	934	53.1 (49.9 – 56.3)	56.2 (49.8 – 62.6)[b]
Total	**623**	**1234**	**50.5 (47.7 – 53.3)**	**51.0 (45.4 – 56.6)**

CI: confidence interval

[‡] The within-herd prevalence (animal units) is the percentage of positive samples per site/subsite production system divided by the total number of tested samples per site/subsite production system.

[a, b] The significant difference in within-herd prevalence by the site production system of farm.

[*, **] The significant difference in within-herd prevalence by subsite of multiple site production system.

CHAPTER 5: DISCUSSION

To date, SVANOVIR® *L. intracellularis*/Ileitis-Ab is the only one commercially available ELISA kit for *Lawsonia*-antibodies detection. The PI 30 was used as a cutoff as the manufacture's recommendation to identify the current prevalence of antibodies against *L. intracellularis*. Different diagnostic performances of the test were reported with the use of different cutoff for PI value. Using the IFAT as a reference, Jacobson et al. (2011) reported that PI 35 was a more appropriate cutoff with a slightly lower sensitivity (72%) and higher specificity (93%) when compared to PI 30 (sensitivity and specificity are 75%). However, the study tested a small number of samples with an unknown history or infection status and applied the Bayesian modeling techniques to calculate the diagnostic performance of the test. Besides, with PI 30 and using the IPMA to be a competitor, 91% of sensitivity and 100% of specificity were measured with experimentally derived samples of known status (Magtoto et al., 2014).

The *Lawsonia*-antibody data obtained from this study showed that the large-scale swine farming systems in Thailand had a high rate (91.7% of herds selected, and 50.5% of animals selected) of natural exposure to *L. intracellularis*. The interpretation of these results was not interfered with by the antibodies derived from vaccination because all farms had not been vaccinated. This study's apparent seroprevalence results were expected to be similar to those of earlier studies using IFAT. In Argentina, 76.7% of herds were seropositive, with 31.2% pigs being positive (Machuca et al., 2009). In South Korea, 45% and 59% were the IgG-positive percentages in growing and fattening pigs, respectively, while 100% of herds had seropositive animals (Lee et al., 2001). Given that the IFAT assay has not been standardized and may result in significant variances across various laboratories, the Korean study's use of it to find antibodies specific for *L. intracellularis* should be interpreted with caution. With respect to the use of IPMA to detect *Lawsonia*-IgG antibodies, 75% of growing herds (11-92% for within-herd seroprevalence) and 78% of breeding herds (5-61% for within-herd seroprevalence) in the U.S.A. were seropositive (Marsteller et al., 2003). In Brazil, a total of 30 tested herds were

positive, and 34.7% of specimens from 2,999 sampled pigs showed positivity for anti-*L. intracellularis* IgG (Resende et al., 2015).

As the estimated apparent within-herd seroprevalence was relatively high, and to correct for the ELISA method's imperfect sensitivity and specificity, the Rogan and Gladen approach was applied to convert to the true seroprevalence (Rogan and Gladen, 1978). The true herd and within-herd seroprevalence were 83.3% and 51%, respectively in this study, which was consistent with the other serological investigations. Among a high-density pig population in Australia, a high seroprevalence was 84.2% for the mean percentage of seropositive animals (Holyoake et al., 2010). The true seroprevalence of antibodies against *L. intracellularis* in China was shown to be 77%, with fattening pigs, breeding sows, and boars having the highest frequency (Wu et al., 2014). An update of the serological data revealed that 2,837 out of 3,586 serum samples (79.1%) contained *Lawsonia*-antibodies in 63 farrow-to-finish farms in China. Moreover, Arnold et al. (2019) determined that 91.7% of swine herds and 31.6% of pigs had infected status in Europe. Regarding the research results in Ukraine, 94.2% of seropositive herds and 46.4% of seropositive animals (an increase of almost 10% compared to 2015) were found (Ermolenko et al., 2020). As mentioned above, the serological prevalence estimates found in Europe, Asia, and this study are varied. These disparities are caused by variations in the sensitivity and specificity of the diagnostic techniques employed to identify the presence of *L. intracellularis* (Jacobson et al., 2011) or the disease status in each area.

In Thailand, the current seroprevalence was higher by almost 15% than that of the study (Raphanaphraiwan et al., 2009), which found IFAT-positivity in 38.05% of 2,221 samples from 29 commercial swine herds. With respect to region, two Northern provinces (Chiang Rai and Chiang Mai) and three Southern provinces (Nakhon Sithammarat, Phatthalung, and Songkhla) were considered high pig density areas containing many intensive larger farms (Thanapongtharm et al., 2016). We hypothesized that this was one of the reasons for the highest circulation of *Lawsonia*-antibodies at 100% in the Northern and Southern parts. A study using the multiplex PCR method to identify morbidity and detect microbes in feces also

revealed the investigated farm in Southern had a high prevalence rate. However, this investigation was conducted with a small sample size, and the sampling period was long a year (Nitikanchana et al., 2010). These findings contrasted with the previous report that the Western part was likely exposed more than other areas, with 90.5% seropositivity (Raphanaphraiwan et al., 2009).

In terms of age categories, the expected bacterial exposure over animals' life spans in elder pigs and breeders could be described for their obviously higher seroprevalence of *Lawsonia*-antibodies. These observations corresponded well with the previous findings in other countries. The seroprevalence of *L. intracellularis* in Canada, was 90% and 56% for seropositive sows and finishers, respectively (Corzo et al., 2005). A pig was from 18-24 weeks of age showed the peak of seropositive rate in Midwest parts of America (Marsteller et al., 2003). In comparison between finishers and reproductive animals, there was a strategy for these antibodies to be more frequent in sows and boars. For breeding animals, sows were susceptible to infection via artificial insemination with infected semen (Wu et al., 2014). The source of replacement gilts from outside was a potential reservoir to introduce bacteria into the farms, giving them a better chance to expose the other production sites (Jacobson et al., 2010). High frequency of *Lawsonia*-antibodies found in growing-to-finishing pigs. The maternal antibodies were diminished after 5 weeks of age (Guedes and Gabhart, 2003b). The seroconversion occurred roughly 2 weeks after infection (starting from approximately 8 weeks of age), and it may persist for 13 weeks (Vannucci and Gebhart, 2014). Another reason that can affect the high seroprevalence in the finishing stage is related to antimicrobial resistance in humans. It can be caused by antibiotics that were contaminated in pork production (Lekagul et al., 2021; Nhung et al., 2016); for that reason, these farms did not mix antibiotics in feed (except for treatment) before the slaughterhouse period.

The ELISA seropositivity detected in piglets and nurseries, may correspond to maternal antibodies or transmitted pathogens from sows to their suckling piglets (Jacobson et al., 2010). The offspring of infected gilts had an intensified risk of being seropositive after 5 to 26 weeks (Mauch and Bilkei, 2005). Unfortunately, the clarification of seropositivity from either the natural exposure or the maternal

antibodies cannot be conducted in this study, especially in younger animals. The information about suckling piglets from which parity of sows was also missed, resulting in an unavailable analysis. To avoid this interference, it is suggested that pigs older than 5 weeks should be sampled in future studies. Due to the announcement of an African swine fever (ASF) outbreak in early 2022 (WOAH, 2022), only one breeding farm from the Southern part of Thailand was sampled for this study. Hence, there was not enough data to conclude the Southern region had the highest within-herd seroprevalence. In addition, a greater number of samples and an equal sample size between regions and age categories should be conducted.

The husbandry management has been a crucial issue affecting the high within-herd seroprevalence against *L. intracellularis* in Thailand, particular structured systems. These studied farms were designed according to a modernized model (such as a closed-system indoor system with concrete or slatted flooring), which led to a higher exposure opportunity than backyard farm systems (Bronsvoort et al., 2001). Modern intensive herds tend to intensify production by adding more animals in limited spaces, thus providing ideal conditions for *L. intracellularis* to spread through animal-to-animal contact or through feces (Hagen and Bilkei, 2003; Stege et al., 2004). There was a high prevalence in herds that have single-site (Bea et al., 2013; Raphanaphraiwan et al., 2009). However, the current study found that the multi-site system had a higher seropositive rate. Pig flows can explain this discrepancy, almost farrow-to-finish herds adequately applied continuous flow facilities, which were known to be a high-risk factor for infection transmission among different age-groups more than all-in/all-out facilities (Corzo et al., 2005). Interestingly, even all selected herds had used antimicrobial protocols as promoters in growing stage and/or to prevent diseases in reproductive animals. There was a high seroprevalence, indicating that antibiotic usage did not prevent *L. intracellularis* infection in this study.

In summary, with the absence of attenuated, live vaccine in the Thai market, the infectious diarrhea caused by PPE or porcine ileitis has been a major concern in Thai intensive swine farms. For monitoring the serological status of the herd, the commercial blocking ELISA is considered a promising diagnostic tool. The *L. intracellularis* exposures were widespread among large-scale commercial farms in all

6 regions of Thailand with a high rate of seroprevalence. There was a high within-herd seroprevalence in the elderly and breeders in the *L. intracellularis* endemic herds. Husbandry management, biosecurity, and hygiene are the keys to preventing and controlling the *Lawsonia*-infection in the swine herds.

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APPENDIX

QUESTIONNAIRE

I. General information:

- Farm name:..... Mobile phone:.....
- Province:..... Region: .....
- Production size: ..... Breed:.....
- Veterinarian name:..... Veterinarian phone: .....
- Housing: o Partially closed (Semi-closed/Open)
  o Totally closed (Evaporative cooling system/Tunnel)
- Production system: o One site: Farrow to Finish
  o Two site: Breeding herd-nursery and Growth-finishing
  o Three site: Breeding herd; Nursery and Growth-finishing
- Any diarrhea **reported** during 6 months before? o Yes, age: .....o No
- Did you cull pigs because of diarrhea? o Yes o No
- How many percentages of **mortality** because of diarrhea (last time)? .....
- How many percentages of **morbidity** because of diarrhea (last time)? .....

II. Facilities

	Contact between batches		Flooring		Temperature
Nursery	o Yes	o No	o Slatted	o Concrete	
Growing	o Yes	o No	o Slatted	o Concrete	
Finishing	o Yes	o No	o Slatted	o Concrete	

- Air flow: o Natural o Mechanical Specify: .....

III. Herd practical procedures

1. Colostrum and milk management

- Newborn assistance at farrow and piglets' first colostrum intake: o Yes o No
- Do **all piglet** suckle in the first hour after birth: o Yes o No
- Do you use sow milk replacer: o Yes o No
- Do you keep a colostrum/milk storage: o Yes o No

2. Weaning management

- Piglet weaning age: o Classical–21 days o Early segregated
  o Medicated early segregated

- Cross-fostering:
  - o **Low** transference rate
  - o **High** transference rate
  - o Transference **in the first 24h** after birth
  - o Transference **after 24h** after birth

3. Nursery, growing, finishing management

	Nursery	Growing	Finishing
Are pigs from different **age** mixed?	o Yes o No	o Yes o No	o Yes o No
Are pigs from different **source** mixed?	o Yes o No	o Yes o No	o Yes o No
Do you measure the size of pig?	o Yes o No	o Yes o No	o Yes o No
Do you have **special management with wasted pigs**?			
What is **size of batches**?			
What is **the number of pigs/batches**?			

IV. Nutrition

- Do you use farm-made feed? o Yes o No
- Do you use antimicrobials in the feed? o Yes o No
- Nucleus, microminerals, premix and vitamins: o Farm-made
  o Use commercial products
- How is the feed provided? .....
- What is the water source? .....
- What is type of drinking system? o Nipple o Bowl o Trough
- How many drinkers/pigs? .....

V. Health management

Vaccine	Age of vaccination/frequency	Application*
(name in appendix)		

(*) i.m. = intramuscular s.c. = subcutan i.d. = intradermal p.o. = per os(water)/(feed)/(drenching)

- What are the infectious pathogens diagnosed in the herd? .....
- What actions do you have for their control? .....
- Which level of ***Salmonella*** in farm? o None o Minor (<50%) o Major (>50%)
- Do you treat sick pig and remove to separate pen? o Yes o No
- Do you mix recovered pigs (from sick pen) with other pigs? o Yes o No
- Do you use antimicrobials: o Yes o No

Antimicrobials	Purpose	Age of pigs	Using length	Dosage
(name in appendix)	*e.g Treat or Prevent*			

VI. Biosecurity

1. *Pig flow in the property:* o Continuous flow o All-in, all-out o Farrowing batches
   o Nursery o Growing o Finishing
2. *Cleaning program* o Daily cleaning o Cleaning after moving pigs

- What detergents/disinfectants do you use? .....
- How do you use them? .....
- How long for downtime period? .....
- What are other strategies do you use for cleaning or disinfection? .....
- How to manage the carcass? .....

3. *People, vehicles and pig entry procedures*

a. People

- Are the employees required to take a shower before entrance? o Yes o No
- What kind of clothes does the employee wear during the working time?
  o Property uniform o Regular clothes
- Do you use different shoes between outside and inside the barns? o Yes o No
- Do you disinfectant before and after go inside the barns? o Yes o No
- Are workers separated by the age of the pigs or not? o Yes o No

b. Vehicles

- Are the trucks disinfected before the entrance of farm? o Yes o No
- How long for keeping the trucks are dried? .....

4. Pigs

- How do you replace your breeding pigs?

  o Auto-replacement o Acquired replacement animal from multipliers

- How many percent of sow replacement per year? .....

- Do you have quarantine period for new pigs? o Yes, in.....days o No

Immunoprophylaxis

Vaccine	Code
Actinobacillus pleuropneumoniae	A
Bordetella bronchiseptica	B
Coronavirus	C
Escherichia coli	D
Haemophilus parasuis	E
Lawsonia intracellularis	F
Mycoplasma hyopneumoniae	G
Pasteurella multocida	H
Porcine circovirus type 2	I
Porcine reproductive and respiratory syndrome virus	J
Salmonella spp.	K
Streptococcus suis	L
Swine herpesvirus type 1	M
Swine influenza virus	N
Autogenous vaccine and others*	O

Antimicrobials

Antimicrobial	Code
Amoxicillin	1
Amoxicillin/ Clavulanic acid	2
Ampicillin	3
Apramycin	4
Cefquinome	5
Ceftiofur	6
Cephalothin	7
Chlortetracycline	8
Clindamycin	9
Colistin	10
Colistin sulphate/ Polymyxin B	11
Danofloxacin	12
Enrofloxacin	13
Erythromycin	14
Florfenicol	15
Gentamycin	16
Josamycin	17
Lincomycin	18
Neomycin	19
Oxytetracycline	20
Penicillin	21
Spectinomycin	22
Sulfonamid + Trimethoprim	23
Tetracyclin	24
Tiamulin	25
Tilmicosin	26
Trimethoprim sulfamethoxazole	27
Tulathromycin	28
Tylosin	29
Tylvalosin	30
Valnemulin	31

VITA

NAME	Thanh Nguyen Che
DATE OF BIRTH	25 September 1996
PLACE OF BIRTH	Vietnam
INSTITUTIONS ATTENDED	Nong Lam University Chulalongkorn University
HOME ADDRESS	At Ratchathewi, 488/18 Petchaburi Soi 18, Ratchathewi, Bangkok, Thailand 10330
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