

Laboratory Surveillance and Monitoring of African Swine Fever in Thailand from 2010 to 2015

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Abstract

African swine fever (ASF) is a viral disease which can cause devastating impact to worldwide swine industries. Unlike other diseases, neither a treatment nor a vaccination exists for ASF. The disease is endemic in sub-Saharan Africa and is now spreading to the Caucasus, the Russian Federation, and the European Union. ASF has the potential to spread to Southeast Asia because of expanding of global trade. Movement of subclinical animals or infected pork products from afflicted areas into Southeast Asia might happen if lack of potential screening and awareness.

To determine whether the African swine fever virus (ASFV) existed in domestic and native swine in Thailand during 2010 to 2015, we used indirect enzyme-linked immunosorbent assays (ELISA) to detect antibodies to ASFV. A retrospective study was conducted from 2010 to 2013 using a serum bank at the National Institute of Animal Health (NIAH). A cross-sectional study was conducted from 2014 to 2015. The sample size estimation was calculated to detect a disease using a prevalence of disease of 5%, a confidence interval of 95%, and an error of 5%. A total of 6,184 swine serum samples (230 farms) were tested; 3,240 (108 farms) were drawn from the serum bank, and 2,944 (122 farms) were collected in eight regions encompassing 32 provinces. Of these samples, 58 (1.97%) were collected from native swine, 441 (14.98%) from breeding swine, and 2,445 (83.05%) from fattening swine. No antibodies to ASFV were found in any of the samples. The results of this study support the argument that no ASF was circulating in domestic and native swine in Thailand between 2010 and 2015.

Keywords: African swine fever; Surveillance; Thailand; ELISA

Introduction

African swine fever (ASF) is a highly contagious swine disease caused by a double-stranded DNA virus which belongs to Asfarviridae family, genus *Asfivirus* (Andrew M.Q. King, 2012). It causes high fever, loss of appetite, and hemorrhages in the skin and internal organs. Mortality rates may be as high as 100%. On average, infected swine are death within 2-10 days. A natural reservoir of this virus is wild boar especially warthog, and this virus spread via the soft tick *Ornithodoros moubata*. Pigs usually become infected by direct contact with infected pigs or by ingestion of unprocessed infected pork products (OIE, 2016).

The disease is considered to be of economic importance because of its consequences, which include animal loss and trade restriction. There is no treatment or effective vaccination program to control ASF outbreaks (Spickler, 2015). ASF virus is not considered to be a zoonosis. It can persist in the environment and uncooked pork products for several weeks or months (FAO, 2008; Wilkinson, 1989).

African swine fever is endemic in sub-Saharan Africa. It was introduced to eastern Europe and Georgia in the Caucasus region in 2007 (Costard et al., 2009). There were several reports of ASF outbreaks in Ukraine and Belarus between 2012 and 2013. Moreover, since 2014, Lithuania, Poland, and Latvia have detected ASF in wild boar and domestic swine (Davies et al., 2015). ASF has persisted in Russian since 2008 and is continuing to circulate (FAO, 2013). There is lack of published data or studies concerning ASF situation in East and Southeast Asian countries. The disease could potentially spread to these countries, especially to China. This is because China shares a border with Russian. Furthermore, China is the biggest importer of swine and swine products and undertakes large investment as well as development projects in African countries. These led to increase exchanges of people and goods with ASF-endemic countries (LinkTADs, 2014). Although China does not have ASF outbreak yet, the Food and Agriculture Organization of the United Nations (FAO) collaborates with China to develop and implement a technical

cooperation program project (TCP) in order to assist China in the development of policy (ASF contingency plan), capacity, and awareness (FAO, 2014).

Thailand is located in the Southeast Asian region and has borders with Laos, Myanmar, Cambodia, and Malaysia. It is divided into nine livestock regions as figure 1 shows. According to the Department of Livestock Development (DLD) census of 2013, the overall swine population in Thailand was 9,511,389 head (249,047 farms), comprised of 61.78% fattening swine, 32.12% breeding swine, and 6.1% native swine. (DLD, 2013).

The DLD, a national veterinary authority, is in charge of animal disease surveillance, prevention, and control. Major swine diseases such as Foot and Mouth Disease (FMD), Porcine Reproductive and Respiratory Syndrome (PRRS), and Classical Swine Fever (CSF) are included in a national disease surveillance plan and in an emergency plan. However, ASF is not included in a national disease surveillance plan (as of January 2016) yet. The major aim of this study was to determine if antibody to ASF virus existed in domestic and native swine in Thailand during 2010 to 2015 using the ELISA technique.

Materials and Methods

(1) Study design and study population

This study included two phases: a retrospective study and a cross-sectional study. The retrospective study used serum samples from a swine serum bank at the NIAH in Bangkok, Thailand, from 2010 to 2013. These serum samples were collected under a Nipah virus surveillance project. The sampling frame of the Nipah project included sera of swine raised on 350 low-biosecurity farms from region one, two, seven, eight, and nine.

The cross-sectional study was conducted throughout eight regions of Thailand from 2014 to 2015. This study investigated breeding swine, fattening swine, and native swine in backyard or small scale farms (up to 500 head). Serum sampling was conducted in two stages: first, selected

provinces in each region and then selected farms in each province. The authors selected four provinces in each region based on their swine population density. Local veterinary officers selected at least three swine farms in each province in order to be a good representative of swine population in their provinces.

(2) Sample size

The retrospective study drew 1,080 samples (36 farms) out of 2,500 samples (80-100 farms) from the Nipah project each year. The sample size for cross-sectional study was calculated using an expected prevalence of disease of 5%, a confidence interval of 95%, and an error of 5%. The formula used to calculate the sample size conformed to the Principles and Methods of sampling in animal disease surveys (Graat et al., 1997).

(3) Sample and data collection

The swine serum samples were collected by either well-trained local veterinary officers or animal husbandry staff. Sample collection was focused on areas in each region with dense swine populations or on provinces with borders to neighboring countries. The swine density distribution and sample collection sites are demonstrated in figure 2.

All serum samples and questionnaires were collected at the Regional Veterinary Research and Development Center (RVRDC) in each region and then transferred to the NIAH in Bangkok following proper handling procedures. Participation in this study was voluntary, and no financial incentives were offered to the farmers.

(4) Diagnosis method

The commercial product ID Screen® African Swine Fever Indirect (ID.vet, France), an indirect ELISA, was used to detect ASFV antibodies in this study, which included both screening and confirmation tests. If screening tests were doubtful or positive, a confirmation test would be performed per manufacturer instructions. Each test kit was coated with three recombinant

proteins (p32, p62, and p72). Positive and negative controls were included in each test plate and tested along with the serum samples. The diagnosis sensitivity and specificity of the test kits were 96.31% and 99.73%, respectively (European Union reference laboratory for ASF, 2014). All of the serological tests were conducted at the NIAH within the DLD, which has considerable experience in the detection and confirmation of animal diseases as well as continuous programs for validation and quality control.

Results

A total of 6,184 swine serum samples from 230 farms were tested, including 3240 samples (108 farms) obtained from the bank from 2010 to 2013. No samples from 2012 were used because of their poor quality. During a cross-sectional study, the remaining 2,944 samples (122 farms) were collected from 32 provinces in eight regions (region one through eight). All serum samples were collected from backyard farms in densely populated areas with the exception of 270 samples (9.17% of the total) taken from nine non-backyard farms in region six. Of the 2,944 field samples, 58 (1.97%) were collected from native swine, mainly from region four and five; 441 (14.98%) were taken from breeding swine, mainly from region one and three; and 2,445 (83.05%) were obtained from fattening swine. No samples collected in region six and eight were taken from breeding swine. Table 1 shows the number of serum samples collected for this study. The number of samples taken from each type of swine operation is shown in Table 2.

Screening tests indicated no antibodies to ASFV were present in 6,113 of the 6,184 serum samples analyzed. Seventy-one of the tests (1.15%) produced unclear results. However, confirmatory tests yielded negative results for these 71 samples. The laboratory results are shown in Table 3.

Discussion

Although ASF is not endemic in Southeast Asia, raising awareness of the disease, monitoring its global progress, and preparing emergency plans are essential in order to protect swine industries.

This study intended to conduct surveillance on the disease throughout Thailand. However, a cross-sectional study of swine could not be carried out in region nine because political unrest in that region limited the time available to local officers to conduct operations. Additionally, because people in that area are predominantly Muslim, its swine population is not as dense as that of other regions. Serum samples of native swine were mainly collected in region four and five because of their high populations of indigenous swine. In region six, local veterinarian officers could not visit and collect samples from backyard swine due to disease outbreaks.

The swine serum bank proved beneficial to this study because it allowed researchers to utilize already-collected samples with minimal time and cost expended. Future studies on the early detection of emerging diseases should consider collecting serum samples at slaughterhouses.

A serological test using ELISA to detect antibodies of ASFV is a diagnostic technique recognized by the World Organization for Animal Health (OIE) (OIE, 2008). This study found no antibody titer to ASFV on domestic and native swine farms in Thailand from 2010 to 2015. Feral swine and swine at DLD breeding centers were not included in this study.

Although the OIE WAHIS system does not show any report of ASF outbreaks in any country of Southeast Asian region yet, Thailand should increase awareness by including ASF in surveillance plans and import protocols.

The recommendation of this study is that the DLD should include disease surveillance for ASF in a national plan and collaborate with the Department of National Parks, Wildlife and Plant

Conservation to monitor the disease in feral swine. In addition, random testing of breeding swine and blood meal imported from European countries is necessary. Lastly, although there are no reports in Thailand of *Ornithodoros* ticks, which can serve as ASF vectors, research on ASF vectors should be pursued. This study could be cited in support of an application to the OIE for an official ASF-free declaration.

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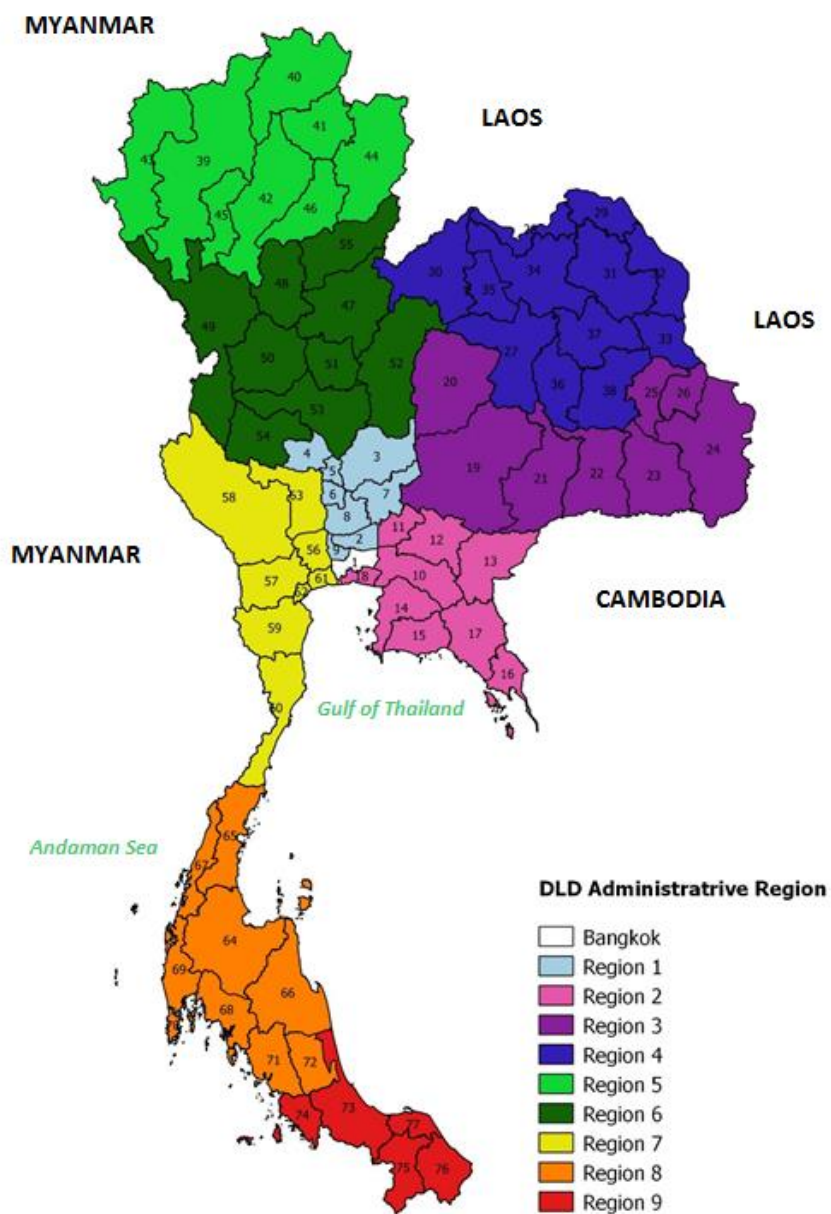


Figure 1: Map of Thailand showing 77 Provinces and 9 DLD Livestock Administrative Regions.

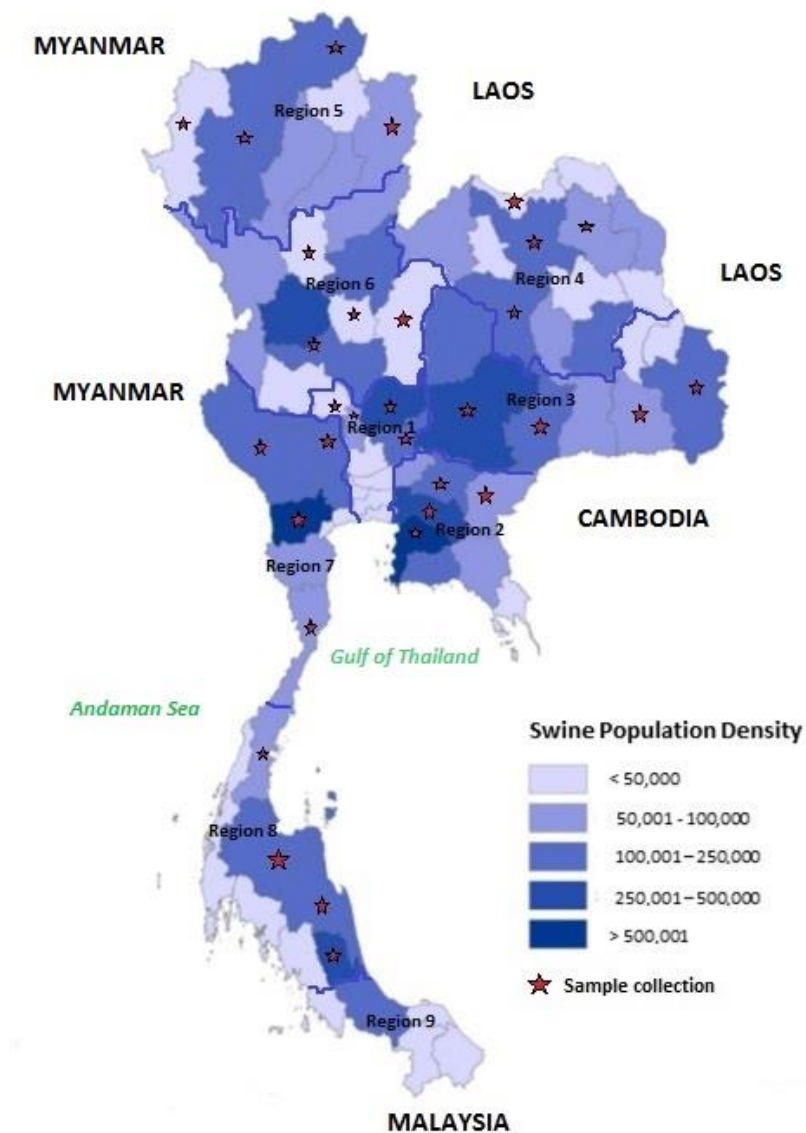


Figure 2: The swine density distribution and location of sample collection from 2014 to 2015.

187 Dark blue indicates provinces with high density. Light blue indicated provinces with low density. 188 Sample collection was done in provinces marked with a star.

Table 1: Number of samples* used in this study by year and region.

Year	2010	2011	2013	2014	2015	Total
Region 1	0	0	240 (9)	349 (15)	0	589 (24)
Region 2	180 (6)	270 (9)	250 (8)	360 (12)	0	1,060 (35)
Region 3	0	0	0	0	364 (14)	364 (14)
Region 4	0	0	0	0	453 (15)	453 (15)
Region 5	0	0	0	0	330 (17)	330 (17)
Region 6	0	0	0	0	360 (12)	360 (12)
Region 7	300 (10)	270 (9)	250 (8)	364 (22)	0	1,184 (49)
Region 8	360 (12)	270 (9)	250 (8)	364 (15)	0	1,244 (44)
Region 9	240 (8)	270 (9)	90 (3)	0	0	600 (20)
Total	1,080 (36)	1,080 (36)	1,080 (36)	1,437 (64)	1,507 (58)	6,184(230)

*serum samples (no. of farms)

** serum samples of 2012 were not included in this study because they were not in good condition.

Table 2: Number of serum samples collected from each type of swine operation from 2014 to 2015.

Region	Type of swine			
	Breeder	Fattening	Native	Total
1	105	244	0	349
2	60	300	0	360
3	105	259	0	364
4	6	434	13	453
5	68	217	45	330
6	0	360	0	360
7	97	267	0	364
8	0	364	0	364
Total	441 (14.98%)	2,445 (83.05%)	58 (1.97%)	2,944

Table 3: Laboratory results are summarized below. Seventy-one (71) doubtful results from screening test were retested with a confirmation test. All samples provided negative results.

Region	Total serum tested* (2010-2015)	Screening Test			Confirmation Test	
1	589	0	563 (95.59%)	26 (4.41%)	0	26
2	1,060	0	1,046 (98.68%)	14 (1.32%)	0	14
3	364	0	364 (100%)	0	0	0
4	453	0	453 (100%)	0	0	0
5	330	0	330 (100%)	0	0	0
6	360	0	360 (100%)	0	0	0
7	1,184	0	1,159 (97.89%)	25 (2.11%)	0	25
8	1,244	0	1,242 (99.84%)	2 (0.16%)	0	2
9	600	0	596 (99.33%)	4 (0.67%)	0	4
Total	6,184	0	6,113 (98.85%)	71 (1.15%)	0	71

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โครงการสำรวจและเฝ้าระวังโรค African Swine Fever ทางห้องปฏิบัติการ ในประเทศไทย ระหว่างปี 2553-2558

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บทคัดย่อ

โรคอหิวาต์แอฟริกัน หรือ African swine fever (ASF) เป็นโรคติดต่อร้ายแรงในสุกร และส่งผลกระทบต่อธุรกิจสุกรในระดับโลก โรคนี้ที่เกิดจากเชื้อไวรัสที่ยังไม่พบแนวทางการรักษา อีกทั้งการป้องกันโรคโดยวัคซีนยังไม่ประสบผลสำเร็จนัก ASF เป็นโรคประจำถิ่นในกลุ่มประเทศตอนใต้ทะเลทรายซาฮาราในทวีปแอฟริกา ปัจจุบันโรคนี้ได้แพร่ระบาดมายังประเทศแถบทวีปยุโรป และบริเวณคอเคซัสของประเทศรัสเซีย ปัจจุบันการค้าขายระหว่างประเทศได้ขยายตัวขึ้น การเคลื่อนย้ายสัตว์หรือสินค้าปศุสัตว์ที่มีเชื้อโรคแฝงเป็นการเพิ่มโอกาสการแพร่ระบาดของโรคเข้ามาสู่ภูมิภาคเอเชียตะวันออกเฉียงใต้ หากไม่มีมาตรการป้องกันและการเฝ้าระวังที่ดีเพียงพอ

การศึกษานี้เพื่อสำรวจและเฝ้าระวังโรค ASF ในประเทศไทย โดยศึกษาในสุกรพื้นเมืองและสุกรที่เลี้ยงหลังบ้าน ตั้งแต่ปี 2553 จนถึง ปี 2558 ทีมวิจัยสำรวจโรค ASF โดยการตรวจหาระดับแอนติบอดีในซีรัมสุกร ด้วยวิธี indirect ELISA การวิจัยแบ่งเป็น 2 ส่วน โดย (1) การศึกษาย้อนหลัง โดยใช้ตัวอย่างที่เก็บในธนาคารเลือดของสถาบันสุขภาพสัตว์แห่งชาติ ระหว่างปี 2553-2556 และ (2) การศึกษาเชิงรุกแบบตัดขวาง โดยลงพื้นที่เก็บตัวอย่าง ระหว่างปี 2557-2558 การคำนวณจำนวนตัวอย่างเป็นการคำนวณเพื่อค้นหาโรคที่ระดับความซุก 5% ความเชื่อมั่น 95% และความผิดพลาดที่ยอมรับได้ 5%

ทีมวิจัยทดสอบตัวอย่างรวมทั้งสิ้น 6,184 ตัวอย่าง จาก 230 ฟาร์ม โดยมีตัวอย่างจากธนาคารเลือด 3,240 ตัวอย่าง (108 ฟาร์ม) และตัวอย่างจากพื้นที่ทั้ง 32 จังหวัดในเขตการเลี้ยงปศุสัตว์ทั้ง 8 เขต (เขต 1-8) รวม 2,944 ตัวอย่าง (122 ฟาร์ม) ในจำนวนตัวอย่างทั้งหมดนี้ มีตัวอย่างจากสุกรพื้นเมือง 1.97% (58 ตัวอย่าง) สุกรพ่อแม่พันธุ์ 14.98% (441 ตัวอย่าง) และสุกรขุน 83.05% (2,445 ตัวอย่าง) ผลการทดสอบไม่พบแอนติบอดีต่อโรค ASF ในทุกตัวอย่างที่ทดสอบ การศึกษานี้จึงเป็นข้อมูลสนับสนุนว่าในช่วงระหว่างปี 2553-2558 ไม่พบโรค ASF ในสุกรพื้นเมืองและสุกรที่เลี้ยงหลังบ้านในประเทศไทย

คำสำคัญ: โรคอหิวาต์แอฟริกัน สำรวจและเฝ้าระวัง ประเทศไทย ELISA