# Prevalence and risk factors of *Isospora suis* infection in pig in Khon Kaen, Thailand

# ความชุกและปัจจัยเสี่ยงของการติดเชื้อ *Isospora suis* ในสุกร ในจังหวัดขอนแก่น ประเทศไทย

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**ABSTRACT**

Coccidiosis is an important disease, which causes substantial economic loss to the swine industry. A total of 300 fecal samples of pigs at different ages from 14 farms in Khon Kaen province were collected to investigate the prevalence of coccidiosis. The presence of *Isospora suis* in stool samples was examined with saturated floatation method and mean oocyst per gram was enumerated with McMaster method. Results showed that prevalence of coccidiosis at farm and individual levels were 50% and 4.3%, respectively. Pigs raised on cement and slatted steel floors are more positive to *Isospora suis* than those from tri-bar and plastic floors. Using anti-coccidia drugs, detergents disinfectants and high-pressured water did not have positive effect on reduction of coccidiosis prevelence in pigs. In conclusion, prevalence of *Isospora suis* in pig in Khon Kaen at farm level was high while that at individual was fairly low, and using different types of floors may affect the presence of *Isospora suis* in pigs.

**บทคัดย่ อ**

โรคบิดเป็นโรคสำคัญที่ก่อให้เกิดความที่ก่อให้เกิดความสูญเสียทางเศรษฐกิจในอุตสาหกรรมการเลี้ยงสุกรอย่างมากเก็บตัวอย่างอุจจาระจำนวน 300 ตัวอย่างจากสุกรช่วงอายุต่างๆ จากฟาร์มสุกร 14 ฟาร์มในเขตจังหวัดขอนแก่น เพื่อตรวจหาความชุกของการติดเชื้อ ตรวจตัวอย่างอุจจาระเพื่อหาเชื้อ *Isospora suis* โดยใช้วิธีลอยตัว และ หาความเข้มข้นโดยวิธีแมคมาสเตอร์ ผลการศึกษาพบอัตราการติดเชื้อในระดับฟาร์มและรายตัว ร้อยละ 50 และ 4.3 ตามลำดับ สุกรที่เลี้ยงบนพื้นคอนกรีตและลวดเหล็กมีอัตราการติดเชื้อสูงกว่าสุกรที่เลี้ยงบนพื้นพลาสติกและเหล็กสามเหลี่ยม การใช้ยาต้านเชื้อบิด น้ำยาซักล้าง น้ำยาฆ่าเชื้อ และ น้ำแรงดันสูงล้างพื้นคอกไม่มีผลต่อความชุกของการติดเชื้อบิดในสุกร สรุปผลจากการศึกษาพบว่า ความชุกของการติดเชื้อ *Isospora suis* ในสุกรในจังหวัดขอนแก่นในระดับฟาร์มอยู่ในระดับสูงในขณะที่ความชุกเป็นรายตัวค่อนข้างต่ำ และชนิดของพื้นคอกมีผลต่อความชุกของการติดเชื้อ *Isospora suis* ในสุกร

**Key words:** *Isospora suis*, pig, risk factor

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## Introduction

Coccidiosis is recognized as the most important protozoal disease in pigs because it is a common cause of economic losses in pig industry (Gualdi et al., 2003). It is common and widespread in sucking piglets and occasionally in pigs up to 15 weeks of age. There are several research articles reporting about porcine coccidiosis in different countries in the world (Holm, 2001; Karamon et al., 2007; Mundt et al., 2005; Niestrath et al., 2002; Soulsby, 1968; Weng et al., 2005; Zhang et al., 2012). The feces which are white to yellow and fatty or creamy, but can also be brownish or greyish, are the main clinical signs. Coccidia are recognized as a frequent and significant cause of enteritis in suckling piglets, it can cause hemorrhagic colitis mainly in the mucosal surface of the jejunum and ileum (Chae et al., 1998; Eysker et al., 1994; Sayd and Kawazoe, 1996). In this case, necrosis and atrophy of villi are common manifestations (Bergeland, 1977; Eustis and Nelson, 1981). This can be seen as the root cause of the secondary infections with other enteric pathogens and may also lead to mortality. In addition, mucosal injury of intestine also makes reduction of food absorption and reduced weight gain which lead to the economic losses (Driesen et al., 1993; Gualdi et al., 2003; Mundt et al., 2006; Niestrath et al., 2002).

Control of coccidiosis is very essential in the pig industry. There are different treatments and strategies against coccidiosis in pig, but none of them provide absolute ability to eliminate the infection. In almost all countries, including Thailand, the drug that has been used for prevention of coccidian in piglets is toltrazuril (Driesen et al., 1995; Joachim and Mundt, 2011; Kreiner et al., 2011; Maes et al., 2007; Mundt et al., 2003; Skampardonis et al., 2010). However, control relies primarily on hygiene and housing design is the best way to prevent coccidian infection in pig. Unfortunately most commercial disinfectants used in pig farm are not effective against sporulated stages of coccidian (Mundt et al., 2007). Other preventive strategies including vaccination are not available. In Thailand, rare information about coccidian infection in piglets is available (Wijit et al., 1994). The objectives of this study were to investigate prevalence of coccidian infection in pigs in Khon Kaen province, Thailand, and to recognize the risk factors of coccidian infections in pigs.

## Materials and methods

### 2.1. *The study site*

The study area is Khon Kaen, which is the second-largest province in the Northeast of Thailand and locates between latitude 15°40′ to 17°5′N and longitude 101°45′ to 103°10′E. It covers a total area of 10,886 square kilometers (Office of National Statistic, 2000). Over the course of a year, the temperature typically varies from 17°C to 35°C and average rainfall per year ranges from 1,000 - 1,500 m.m. The rainfall is unevenly distributed during the rainy season (May to October) with over 80% occuring during August and September.

### *2.2. Sample and data collection*

Overall, 300 fecal samples were collected from 14 pig farms in Khon Kaen province during December 2012 to July 2013, fecal samples of 3-5 piglets/litters with age between 1 - 4 weeks old (at least 5 litters/farm) were randomly collected rectally from individual pigs. Fecal samples of the above piglets and from older pigs (nursery, grower, and fattener) and sows were also collected from

their pen floor. All samples were labeled individually, scored for consistency on a scale consisting of 3 levels (1 = nomal; 2 = pasty; 3 = diarrhea), and cooled in ice box during transport to the laboratory. They were kept at 4°C in the laboratory for maximum refrigeration for 7 days berore examination.

Information about farm, litter and forrowing house management was collected using a questionaire to investigate the risk factors associated with coccidian infections.

***2.3. Laboratory techniques***

Coccidian oocysts were detected using simple saturated salt solution flotation technique as described by Zajac and Conboy (2012) and identified based on their morphological characteristics. Coccidian oocysts per gram (OPG) of feces was then quantified using modified McMaster technique (Zajac and Conboy, 2012) for the positive samples. Sporulation technique using 2.5% (w/v) potassium dichromate solution were utilized to identify coccidian species as descripted by Karamon et al (2007). Feces was solubilized in 2.5% (w/v) potassium dichromate solution in order to stimulate the sporulation of oocysts. The samples were then evaluated for presense of sporulated oocysts under microscope during day $5^{th}$-$12^{th}$ after stimulation. *Isospora suis* was identifyed by peculiar species morphological characteristic such as the color, shape, length and width of oocysts, and sporocysts as well as sporozoites (Lindsay et al., 1982).

***2.4. Data analysis***

Prevalence of coccidian infection was analyzed on both farm and individual levels. Farm information, litter and farrowing house management data were classified and used as risk factors for analysis. Association between coccidian infection status and risk factors, e.g. floor type, disinfectant used, anticoccidian used, cleaning method, were analyzed using Chi-square tests. All tests were conducted in SPSS Statistical Software (version 20) with a statistically significant at $P\text{-}value \leq 0.05$.

**Results**

Coccidian oocysts were observed in 7 out of 14 pig farms in Khon Kaen province, the result of survey are presented in table 1. Out of the 14 pig farms, 50% (7/14) were positive for coccidian oocysts and they were found in both breeding farm and breeding to finishing farm. The overall prevalence of *Isospora suis* in pigs of all ages is 4.3% (13/300). There was no significant difference in prevalence of coccidian infection in pigs among or between different types of farms ($P\text{-}value > 0.05$).

**Table 1** Prevalence of coccidian infection in pigs in Khon Kaen, Thailand

| Farm | Number of tested Farms | Farm prevalence (%) | Number of individual samples | Individual prevalence (%) |
|---|---|---|---|---|
| **Breeding** | 8 | 37.5 (3/8) | 126 | 4.8 (6/126) |
| **Farrowing to finishing** | 6 | 66.7 (4/6) | 174 | 4.0 (7/174) |
| **Total** | 14 | 50.0 (7/14) | 300 | 4.3 (13/300) |

Coccidian oocysts were found in three age groups in pigs out of five age groups (Table 2). Of the pigs examined in pig farms, 5.5% of piglet, 4.5% of fatteners and 4.3% of weaners were infected with coccidian oocysts. In the 22 growing pigs and 33 sows, no cases of coccidian infections were detected. The intensity of oocyst shedding in feces ranged from 1,993 to 54,667 per gram of feces (OPG). The mean OPG values in pigs were: 23,329 for piglets, 8,600 for weaning pigs and 1,993 for fattening pigs. Chi-square test showed that there ware no significant differences (*P-value* > 0.05) in prevalences among different age groups.

**Table 2** Prevalence and intensity of coccidian infection in pigs at different age

| Pig Category | Number of samples | Number of positive samples | Prevalence (%) | Mean OPG (range) |
|---|---|---|---|---|
| **Piglets** | 200 | 11 | 5.5 | 23,329 (5,333 – 54,667) |
| **Weaners** | 23 | 1 | 4.3 | 8,600 |
| **Growers** | 22 | 0 | 0 | 0 |
| **Fatteners** | 22 | 1 | 4.5 | 1,993 |
| **Sows** | 33 | 0 | 0 | 0 |

**OPG**: Oocysts per gram of feces.

The presence of oocysts was correlated with the type of floors in the farrowing crates and pig management, statistical analysis showed significantly higher prevalence in pigs from cement & slat steel floors than that in pigs from tri-bar & plastic floor in the farrowing houses (Table 3). Other factors such as usage of disinfectant, anticoccidian period time for empty crates before farrowing were not associated with the prevalence of oocysts in pigs (*P-value* > 0.05).

**Table 3** Risk factors association with coccidian infection in pigs

| Type of factor | Category | Number of samples | Number of positive samples (%) |
|---|---|---|---|
| Type of floor | Cement & slat steel | 72 | 9 (12.5) |
| | Tri-bar & plastic | 228 | 4 (1.8) |
| Crates were left empty for | ≥ 5 days | 71 | 5 (7.0) |
| | < 5 days | 229 | 8 (3.5) |
| Use of detergent | Yes | 48 | 4 (8.3) |
| | No | 252 | 9 (3.6) |
| Use of disinfection | Yes | 300 | 13 (4.3) |
| | No | - | - |
| Use of high pressure | Yes | 151 | 6 (4.0) |
| | No | 149 | 7 (4.7) |
| Use of anticoccidia | Yes | 183 | 8 (4.4) |
| | No | 117 | 5 (4.3) |

**Discussion and conclusions**

Literature reports that *Isospora suis* is the most important factor causing diarrhea in piglets. In one report, *Isospora suis* oosysts were present in 53.8% of the samples from diarrheic piglets, while *Escherichia coli* was present in 18.2% of samples, and rotavius was found in 16.9% of samples (Driesen et al., 1993). *Isospora suis* in piglet feces were detected in many countries with prevalences between 40% and 100% on farm level (Wijit et al., 1994; Mundt et al., 2005; Karamon et al., 2007; Zhang et al., 2012). Similar results were obtained in our investigations carried out in Khon Kaen, Thailand, where 50% of *Isospora suis* oocystes were found. Considerably high percentages of pigs infected with *Isospora suis* is probably due to the short development cycles of *Isospora suis.* Namely, in the environment, the time period needed for *Isospora suis* oocysts to become infective (the sporulation time) is very short (about 24-48h). Moreover, a microclimate in farrowing pens (high temperature – about 30ºC) can shorten the sporulation time of *Isospora suis* oocysts to a mere 12h. This situation enables a quick spreading of the *Isospora suis* infection in pigs.

The uneven prevalence of coccidian infection among different age groups is a common phenomenon, *Isospora suis* mainly affects pigs up to three weeks of age. This is confirmed by various studies showing a low prevalence in older pigs (Vetterling., 1966) but a high incidence in piglets (Stuart et al., 1982). It was maximized at 10-19 day-old piglets in Brazil (Sayd and Kawazoe., 1996). Yang et al. (2004) reported the highest prevalence of coccidian infection at the third week of age in Sichuan, China. Similarly, in the present study 5.5% (11/200) of piglets were positive while no sows (0/33) were infected with *Isospora suis*. Our results also demonstrated a lack of correlation between the presence of oocysts in the sows, and the *Isospora suis* infected piglets born from them. In a study by Farkas et al. (2004), no such correlation was observed in the study carried out in Hungary where no cases of coccidian infections were detected in sows.

The lack of *Isospora suis* oocysts in feces of most of the examined sows observed in our and other authors' investigation indicates that contaminated farrowing pens are an important source of coccidian infection in pigs. Moreover, our findings suggested that usage of anti-coccidia drugs, detergents disinfectants and high-presured water had no actions on the prevention of coccidiosis in piglets. Similarly, keeping pens empty before delivery did not decrease the seroprevalence of *Isospora suis* in piglets (P-value > 0.05).

Interestingly, while other factors (usage of anti-coccidia drugs, detergents disinfectants high-presured water and keeping crates empty before delivery) did not significantly influence the prevalence of *Isospora suis*, interestingly, the types of floors in the farrowing crates were associated with the risk of I. suis infection. The farrowing crates with cement & slatted steel floor have been associated with an increased risk of coccidian infection (*P-value < 0.05*). This could be related to the fact that cement & slatted steel floor tends to be porous and therefore are more difficult to clean than tri-bar & plastic floor. Therefore, pigs raised in pens with cement and slatted steel floor have higher risk to expose to the source of infection, and subsequently, are more likely to be infected with *Isospora suis*. Sayd and Kawazoe (1996) also reported that prevalence of *Isospora suis* in pigs raised in dirty-cement floors was higher than pigs raised in clean-cemented or self-cleaning floors. On the contrary, a previous study did not find an association between the

use of detergents, disinfections, treatment with amprolium, furaxolidone, diclazuril as well as toltrazuril and the prevalence of *Isospora suis* (Girard and Morin, 1987; Mundt et al., 2003; Mundt et al., 2007). Although hygiene measures, types of floors and other factors such as usage of anti-coccidia drugs, detergents disinfectants, high-presured water and keeping crates empty before delivery are not sufficient to eradicate absolutely coccidian infection in pig farms, they should be considered as an important aspect to restrict oocysts spreading in the farm which can lead to reduce prevalence of coccidian infection in pigs.

In conclusion, prevalence of *Isospora suis* in pigs in Khon Kaen was high (50%) at farm level and low (4.3%) at individual level. Types of floors in the farrowing crates is associated with *Isospora suis* infection in pigs.

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