Original Article

Seroprevalence of *Toxoplasma gondii* in Cats and Pigs from Thua Thien Hue Province in Vietnam

Hiromi HOSONO^{*1}, Shigeru ITO², Hiroichi KONO² and Xuenan XUAN²

¹ Graduate School of Agriculture, Kyoto University, Kitashirakawaoiwake-town, Sakyo-ward, Kyoto-city, Kyoto 606-8502, Japan

² Obihiro University of Agriculture and Veterinary Medicine, West 2-11, Inada-town, Obihiro-city, Hokkaido 080-8555, Japan

(Received : 22 May, 2009/Accepted : 4 October, 2009)

Summary

The traditional small scale pig farmers in Vietnam keep animals in open backyard stalls and usually keep cats to catch rats and mice. Under these conditions, controlling for the exposure of pigs to toxoplasmosis, a significant zoonoses, is difficult. The purpose of this study is to investigate the seroprevalence of *Toxoplasma gondii* antibodies in domestic cats and pigs in T.T. Hue Province, Vietnam with commercial latex agglutination kits (Eiken-Toxocheck). A total of 155 cat and 260 pig sera samples (110 from a slaughterhouse and 150 from 6 pig farms) were collected in 2007. Among them, 112 (72.3%) cats, 62 (41.3%) live pigs and 76 (69.1%) slaughtered pigs tested positive for *T. gondii* antibodies (1 : 64 cut-off). The seroprevalence increases as the pig grows ; the seroprevalence of piglets less than 15 kg was 16.7% and 71.0% for those over 50 kg (OR=12.26, 95% CI=3.31, 45.34). We also have collected sera from the same herds of 6 farmers 2 times with 2 month interval. Of which, the prevalence rate at the first sampling was 28.4% (88 samples) and 2 months later in the 2nd sampling it was 64.0% (50 samples) (OR=4.48, 95%CI=2.14, 9.39). The results indicate a high prevalence of *T. gondii* in pigs under the current farming system in T.T. Hue Province, Vietnam.

Keywords : *Toxoplasma gondii*, Vietnam, pigs, cats, seroprevalence

Introduction

Toxoplasma gondii is an obligatory intracellular coccidian protozoan parasite that infects warm-blooded animals including humans. Cats and other felines are the definitive hosts of *T. gondii* as the sexual phase of its lifecycle occur only in the family Felidae and they shed unsporulated oocysts to the environment during acute infection that turned sporulate and survive for months⁽¹²⁾. Other infectious phases in *T. gondii* life cycle are tachyzoite and bradyzoite. Tachyzoites multiply rapidly in many types of host cells that finally develops cysts containing bradyzoites^(2,5,7,10). The

main routes of human infection are followings ; ingestion of sporulated oocysts shed in feces of infected felines that exists in the environment including water, soil and raw vegetables, ingestion of tissue cysts through raw or under-cooked meat of intermediate hosts such as pork and lamb, and transplacenta^(1,5,10). Human seroprevalence vary among countries, areas in one country and ethnic groups from less than 10% to more than 70%^(3,15). However, comparatively low prevalence, 4-39%, have reported in China, Korea and Thailand^(4,9,16). Although healthy human infection is generally asymptomatic or mild, immunocompromized persons, such as HIV-infected individuals, or congenitally infected children may suffer severe symptoms including loss of vision or fatal encephalitis.

On the other hand, toxoplasmosis in pigs may cause embryonic death, fetal death, abortion, stillbirth or neonatal death and loss in daily weight gain caused by serious diarrhea, all which can have a devastating affect on the financial situation of small scale farmers who keep the animals as

* Corresponding author :

Hiromi HOSONO

Graduate School of Agriculture, Kyoto University, Kitashirakawaoiwake-town, Sakyo-ward, Kyoto-city, Kyoto 606-8502, Japan.

Tel : 075-753-6338

E-mail : hiromix@kais.kyoto-u.ac.jp

important income source for their daily life¹⁴⁾. To control toxoplasmosis in pigs, it is essential to keep the animals away from cats and rodents, however in Vietnam it is difficult for small scale pig farmers to do this because they usually keep cats to protect their harvests and animal feed from rats and mice. In a study of pigs in Ho Chi Minh City, seroprevalence of *T. gondii* antibody were 23% in fattening pig, 30.4% in sows and 40% in boars, in total of 160 positive among 587 samples¹¹⁾. Previous small scale research in Thua Thien Hue Province have shown the prevalence of *T. gondii* antibody differ among animal husbandry systems, 5 out of 13 pigs were seropositive from traditional small scale pig farm, on the other hand, no sera from Hue University farm showed positive by quick inspection kit (TgICT)¹³⁾. In this research, we aimed to investigate the prevalence of *T. gondii* antibodies in pigs from a slaughterhouse, traditional small scale pig farms and cats from same area to investigate the prevalence and the risk factors of *T. gondii* infection of animal and human in Thua Thien Hue Province, Vietnam.

Materials and Methods

Pig and cat sera were collected in Thua Thien Hue Province in January in 2007. Pig sera sampling were done in

January and March and cat sera sampling were done in May and June. Cats were from farmers in Hue City, Phu Thanh and Phu Mau communes in Phu Vang District which is located next to Hue City, and the numbers of collected blood samples were 56, 55, and 51, respectively. For this blood sampling, we visited dwellers house one by one at each area to ask if they own cats or not. Then if they have and agreed to take blood with 30,000VND reward per cats, we collected blood from them until the number exceed 50 from each area. Each family were visited only once and one cat per family were applied for blood sampling. Therefore, collected 162 blood samples were from 162 individual cats. Along with the blood sampling, information concerning the cats, such as age of cats, number of cats keeping together, the reasons for keeping cats, if the cats ate rats/mice, if the cats had contact with other animals the farmers raised etc., was collected from the owners (Table 1). Out of 162 samples taken, 7 were eliminated from further analysis because of improper sera or stock tubes damage ; therefore 155 sera were tested for *T. gondii* antibody prevalence.

Pig sera sampling was done both at a slaughterhouse and at small scale pig farms. The slaughterhouse was located in the Phu Vang District where pigs were mainly from small scale

Table 1 Questions asked for cats blood sampling

No.	Questions	No.	Questions
1	Name of owner	10	Do other animals contact with mice?
2	Address of family		1) pig
3	The number of cats male__ head female__ head		2) buffalo
4	The age and sex of sampled cat		3) cow and cattle
5	Why do you keep cats?		4) chicken
	1) catch mice		5) duck
	2) for ornament		6) other
	3) other reasons, specify	11	Do other animals contact with cats?
6	What kind of other animals do you keep and how many?		1) pig
	1) pig		2) buffalo
	2) buffalo		3) cow and cattle
	3) cow and cattle		4) chicken
	4) chicken		5) duck
	5) duck		6) other
	6) other, specify	12	Where does your cat excrete urinary and feces?
7	Does your cat move around house and cage where above animals are kept?		1) in the house
	yes or no		2) in the basin
8	Does your cat contact with mice?		3) in the garden
	yes or no		4) other places, specify
9	Does your cat eat mice?	13	Do your animals eat cat feces?
	yes or no		yes or no
		14	Does your cat eat feces of cats and other animals?
			yes or no

pig farmers in Phu Mau and Phu Thanh communes. At the selected slaughterhouse, slaughtering activity is done every morning start from 2 A.M till around 6 A.M. The number of pigs slaughtered per day is about 40–50. These pigs are transported to the slaughterhouse in the previous day to be slaughtered. In January 2007, we collected 110 pig blood samples when the pigs arrived at the slaughterhouse with the corporation of the veterinary officer in Phu Vang District, the slaughterhouse manager and the security guard of the slaughterhouse. The origin of the pigs was traced back to individual farmers by asking the transporter of the pig to collect information of raising condition.

To select the pig farmers for on farm sampling, we made list of pig farmers in Phu Mau Commune and selected comparatively large raisers who kept more than 5 fattening pigs in their backyard. Then we visited all the farmers who keep more than 5 fattening pigs to check the age of pigs to see if they would be suitable for re-sampling 2 months later. We assumed pigs weigh over 30 kg or age over 4 month might be sold in 2 month and not suitable for cohort study, therefore these herds were eliminated for sampling in January. All the weights of pigs were estimated with the observations of one local veterinarian who is working for Phu Vang district as a slaughterhouse inspector to check health condition of slaughtered pigs and to give certification stamp for sale. After selecting farms, we collected pig blood samples from 6 consenting farmers in January (88 samples) and March (62 samples), 2007. In order to avoid duplicate sampling, we marked the back of sampled pigs with black oil paint and moved out of the original stall. Of the 62 samples collected in March, 50 were from the same herds which had sampled in January. According to the pig farmers, 38 pigs either died of disease or were slaughtered because the pigs had reached to the slaughtering weights. Because we couldn't identify individual pigs which were still alive, 88 samples collected on farm in January and 50 samples collected on farm in March were applied for cohort study. On the other hand, all pig sera, 110 samples from slaughterhouse and 150 samples from 6 farms, were applied to examine cross sectional situation (Figure 1).

Sera were separated within a day of collection. Cats samples were labeled and stored at -20°C until tested for *T. gondii* antibodies, however pig samples were tested the same day of blood collection. Antibodies of *T. gondii* were examined with commercial agglutination latex test kits (Toxo-check-MT 'EIKEN'). A titer of $\geq 1:64$ were regarded as *T. gondii* antibody positive. Descriptive statistics of the results, calculation of odds ratio, correlation analysis, two sample *t*-test and ANOVA have done with Microsoft Excel 2007 and SPSS ver.15.

Fig. 1 Pig samples for cross sectional and cohort study

Results

The overall antibody prevalence of *T. gondii* in cats and pigs were 72.3% and 53.1%, respectively. The titers of detected *T. gondii* antibodies are shown in Table 2 based on cat age and pig weight. At the slaughterhouse, pig's age in months had not usually been reported, even the pigs' owner didn't know the age in month, therefore, we regarded pig weight as representative of age in this study. Regarding cats' age, it was reported in weeks, month and years by owners of cats but we categorized them into 4 groups to compare the prevalence. The antibody titers in positive cats and pigs varied 1 : 64 to 1 : 1,024. However, among positive cases, comparatively lower titers of antibodies were not observed in young cats less than 6 months of age or in pigs that weighed less than 15 kg. Through ANOVA analysis it was found that pigs weighing more than 70 kg with a titer of 1 : 64 or weighing between 50 and 70 kg with titer of 1 : 1,024 appeared significantly more often ($P < 0.05$ and $P < 0.01$, respectively) than other groups.

The relationship between *T. gondii* antibody prevalence and animal characteristics are shown in Table 3. Seropositivity in cats in the 3 areas varied from 64.8% (Phu Mau Commune) to 78.0% (Hue City). Of these, the differences were not significant ($P = 0.297$). The sex of the cat does not seem to affect the seroprevalence ; 71.4% in male cats and 74.2% in female cats. The factors which affect seropositivity of *T. gondii* are if the cats eat rats/mice and their age ($P < 0.01$). The seropositivity in cats more than 1 year old (both 1 to 3 year group and older than 3 year group) were significantly higher than the other groups ($P < 0.05$). Four of those cats reportedly did not eat rats/mice also were not in contact with rats/mice. All of them were *T. gondii* antibody negative and their ages were less than one year. The average number of cats kept in Hue city (2.55) was larger compared to that in Phu Mau Commune and Phu Thanh Commune, 1.74 and

Table 2 Prevalence of *T.gondii* antibody in cats by age and pigs by weight in T.T. Hue Province

age/weight		total	positive (%)	No. (%) of samples showing antibody titers at					
				1 : 32	1 : 64	1 : 128	1 : 256	1 : 512	1 : 1024
cats	≤ 6 month	19	10 (52.6)	0	0	2 (10.5)	3 (15.8)	3 (15.8)	2 (10.5)
	6 month to 1 year	34	17 (50.0)	3 (8.8)	2 (5.9)	6 (17.6)	3 (8.8)	4 (11.8)	2 (5.9)
	1–3 year	71	59 (83.1)	5 (7.0)	12 (16.9)	14 (19.7)	14 (19.7)	8 (11.3)	11 (15.5)
	> 3 year	31	26 (83.9)	4 (12.9)	4 (12.9)	6 (19.4)	9 (29.0)	4 (12.9)	3 (9.7)
	total	155	112 (72.3)	12 (7.7)	18 (11.6)	28 (18.1)	29 (18.7)	19 (12.3)	18 (11.6)
pigs	< 15 kg	18	3 (16.7)	0	0	0	2 (11.1)	0	1 (5.6)
	15–30 kg	65	25 (38.5)	6 (9.2)	8 (12.3)	6 (9.2)	2 (3.1)	5 (7.7)	4 (6.2)
	30–50 kg	70	34 (48.6)	6 (8.6)	4 (5.7)	8 (11.4)	7 (10.0)	5 (7.1)	10 (14.3)
	50–70 kg	82	57 (69.5)	3 (3.7)	5 (6.1)	9 (11.0)	6 (7.3)	9 (11.0)	28 (34.1)
	70 kg ≤	25	19 (76.0)	2 (8.0)	6 (24.0)	3 (12.0)	2 (8.0)	2 (8.0)	6 (24.0)
	total	260	138 (53.1)	17 (6.5)	23 (8.8)	26 (10.0)	19 (7.3)	21 (8.1)	49 (18.8)

The titer at $\geq 1 : 64$ are regarded as positive cases.

Table 3 The comparison of *T. gondii* antibody prevalence in cats and pigs between characteristics

Factor			n	Prevalence (%)	OR	95% CI
Cats	Sex	Male	56	71.4	1	
		Female	93	74.2	1.15	0.55–2.42
		Undetermined	6	50.0	0.40	0.07–2.19
	Eat	mice	28	50.0	1	
		No	127	77.2	3.38	1.45–7.89
		Yes				
	Area	P. Mau	54	64.8	1	
		P. Thanh	51	74.5	1.59	0.68–3.68
		Hue	50	78.0	1.92	0.81–4.60
	Age	≤ 6 month	19	52.6	1	
		6 month to 1 year	34	50.0	0.90	0.29–2.77
		1–3 year	71	83.1	4.43	1.48–13.21
		> 3 year	31	83.9	4.68	1.26–17.42
	Total		155	72.3		
Pigs	Weight	< 15 kg	18	16.7	1	
		15–30 kg	65	38.5	3.13	0.82–11.90
		30–50 kg	70	48.6	4.72	1.25–17.77
		50–70 kg	82	69.5	11.4	3.03–42.92
		70 kg ≤	25	76.0	15.83	3.39–74.04
	Place	farm	150	41.3	1	
		slaughterhouse	110	69.1	3.17	1.89–5.33
	Cohort	January	88	27.2	1	
		March	50	64.0	4.76	2.27–9.96
	Total		260	53.1		

Odds ratio (OR) is calculated comparing with the first of each factor.

1.52 respectively. And the number of cats kept by each family varied from one to 10 which was not affected the prevalence of *T. gondii* antibody in cats.

In pigs, seropositivity of *T. gondii* antibody increased as pigs' weights increased. Antibody prevalence in small pigs (less than 15 kg) was 16.7% which was significantly lower compared to those weighing 15–30 kg (38.5%), 30–50 kg (48.6%), 50–70 kg (69.5%) and over 70 kg (76.0%). Likewise, the results of forward cohort research showed the antibody positivity of the same herds increased 38% in the 2 month interval between samplings. Finally, seropositivity increased to 69.1% when pigs finished fattening and were shipped to the slaughterhouse (mean weight = 58.9 kg).

Discussion

High prevalence of *T. gondii* antibody both in cats and pigs sera were obtained in this research. Both the cross sectional and forward cohort study indicated the animals were infected while they were raised at households. The gap of cats' seroprevalence among 3 areas can partially be explained by the average age in each areas; 1.78 years in Phu Mau Commune, 2.56 years in Phu Thanh Commune, and 2.71 years in Hue City. The average age of the cats in Hue City was significantly higher compared to that in Phu Mau Commune and the prevalence of *T. gondii* antibody also was high in Hue city even it was not statistically significant. Because almost all of the farmers who keep animals also keep cats and most residents in surveyed area engaged in animal husbandry as well as agriculture, population density of cats was estimated to be high in the surveyed area. One reason for correlation between seroprevalence and cats' age is the increasing opportunity for exposure to soil, water and other environmental object that might be contaminated with oocysts shed by pig owners' and neighboring cats.

The average age of cats those ate rats/mice and those didn't eat rats/mice were 2.59 and 1.18 years respectively. The difference between the group were significant at less than 1% level of risk. Although there remained the possibility that those cats declared not eating rats/mice might actually eat them but not observed by owners, however as shown above, the prevalence of *T. gondii* antibody was significantly higher among the cats declared to eat rats/mice. According to Dubey et al., pigs and mice infection can be developed by ingesting even one or less than one oocysts while cats are not so susceptible to oocysts^{6,8)}. On the other hand, bradyzoites are more infective for cats than mice through oral route⁸⁾. Considering this with the observed high prevalence in cats, higher rate of rats/mice *T. gondii* infection developed by ingestion of sporulated oocysts can be presumed even it was not investigated. And the results combined with above hypothesis indicates another reason of the high prevalence in

cats, especially older cats, is the regular eating of rats/mice, which are an intermediate host. The high prevalence in cats kept by farmers is possibly an important risk factor for human and animal infection even though the seropositivity in humans is not yet clear in the study area.

According to interviews with the pig farmers from areas where the pig sera for cohort study were collected, all 6 farmers keep cats to hunt rats/mice. These cats are permitted to walk around freely and access to pig pens, the water well, kitchen, where pig feed and/or human meals are prepared, and the compound feed for pigs is not protected. Under these conditions, combined with the high prevalence in cats, pigs may easily be infected with *T. gondii* due to close contact with infected cats and rodents, ingestion of well water and feed contaminated with environmentally resistant oocysts and contaminated soil and grass. Consequently, horizontal cyclic infection of *T. gondii* between cats-rats/mice and cats-pigs at traditional pig farming area in central Vietnam were indicated to be common in this study. In accordance with our previous research in the same area, all pig sera from the Hue University farm, where pigs are raised under better management practices (inaccessible to cats, slatted pens, well-managed and stored feed), were *T. gondii* antibody negative¹³⁾. To manage the risk to pigs of *T. gondii* infection, it is recommended that farmers control cat access to pig stalls, feed and water supplies, however it is difficult to do under existing conditions. Farmers kept cats as an inexpensive way to control rats/mice populations, which damage their crops, eat their food and animal feed, not as pets.

Although, the rate of *T. gondii* infection in humans is not yet clear at the study area, the conditions observed in this study should be of concern due to lower farmer productivity as well the impact on general public health. In this study area, seropositivity of *T. gondii* antibody was 72.3% in cats and 69.1% at slaughtering pigs which was significantly higher than the result at Ho Chi Minh City, 22%¹¹⁾. According to Buchy et al., *T. gondii* antibody prevalence in pregnant women in Nha Trang and intravascular drug users in Ho Chi Minh City were 11.2% and 7.7% respectively³⁾. Even the animal situation at Nha Tran is not known, assuming farmers have closer contact with cats and infected pork meat is regarded as one of the main factor of human infection, residents in T.T. Hue Province are facing high risk. Further research, including human infection and socio-economic analysis, should be conducted to find ways to reduce animal and human infection and to improve productivity and public health status by providing information of the disease and the current situation of *T. gondii* infection.

Acknowledgement

This research is supported by the Program of Founding

Research Center for Emerging and Reemerging Infectious Disease proposed by the Ministry of Education, Culture, Sports Science and Technology of Japan. We'd like to express our deep appreciation to Dr. Hung, the director of Hue Provincial Department of Animal Health, the veterinary officers, slaughterhouse staffs and the collaborated farmers in Hue Province.

Reference

- 1) Aize, K and Erik, J. : Control of the risk of human toxoplasmosis transmitted by meat. *Int. J. Parasitol.*, **38**, 1359–1370, 2008.
- 2) Astrid M.T., Anja R.H. and Louis M.W. : *Toxoplasma gondii* : from animals to humans. *Int. J. Parasitol.*, **30**, 1217–1258, 2000.
- 3) Buchy P.J., et al. : Serologiccal study of toxoplasmosis in Vietnam in a population of drug users (Ho Chi Minh City) and pregnant women (Nha Trang). *Bull. Soc. Pathol. Exot.*, **96** (1), 46–47, 2003.
- 4) Chintana T., et al. : *Toxoplasma gondii* antibody in pregnant women with and without HIV infection. *South-east Asian J. Trop. Med. Public Health.* **29**, 383–386, 1998.
- 5) Dubey, J.P., Beattie, C.P. : *Toxoplasmosis of Animals and Man*. CRC Press, Inc., Boca Raton, Florida, 1988.
- 6) Dubey, J.P., et al. : Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs., *J. Parasitol.*, **82**, 438–443, 1996.
- 7) Dubey, J.P. : The history of *Toxoplasma gondii*-the first 100 years, *J. Eukaryot. Microbiol.*, **55** (6), 467–475, 2008.
- 8) Dubey, J.P. : Comparative infectivity of oocysts and bradyzoites of *Toxoplasma gondii* for intermediate (mice) and definitive (cats) hosts. *Vet. Parasitol.*, **140**, 69–75, 2006.
- 9) Hou J.H., et al. : A study on IHA examination of *Toxoplasma* infection in pregnant and post partum women in Lanzhou district, endemic Dis. *Bull.* **12**, 78–79, 1997.
- 10) Hill D., Dubey J.P. : *Toxoplasma gondii* : transmission, diagnosis and prevention. *Clin. Microbiol. Infect.*, **8**, 634–640, 2002.
- 11) Huong L.T.T. and Dubey J.P. : Seroprevalence of *Toxoplasma gondii* in pigs from Vietnam. *J. Parasitol.*, **93** (3), 951–952, 2007.
- 12) Jones, J.L., et al. : Congenital toxoplasmosis : a review, *Obstet Gynecol Survey*, **56** (5), 296–305, 2001.
- 13) Kono H., et al. : Infection of *Toxoplasma gondii* in Vietnamese pig production. *J. Vet. Epidemiol.*, **11** (2), 96–101, 2007. (in Japanese)
- 14) Luft, B.J., et al. : Toxoplasmic encephalitis in patients with acquired immune deficiency syndrome. *J. Am. Med. Assoc.*, **252**, 913–917, 1984.
- 15) Petersen E. : Toxoplasmosis. *Semin. Fetal Neonatal Med.* **12**, 214–223.
- 16) Ryu J.S., Min D.Y., Anh M.H., et al. : *Toxoplasma* antibody titers by ELISA and indirect latex agglutination test in pregnant women. *Korean J. Parasitol.*, **34**, 233–238, 1996.

原 著

ベトナム国フエ省における猫と豚のトキソプラズマに関する血清疫学的研究

細野ひろみ^{*1}・伊藤 繁²・耕野拓一²・玄 学南²¹ 京都大学大学院農学研究科² 帯広畜産大学

要旨

ベトナムの伝統的な小規模養豚農家では、ブタは裏庭の開放的な豚舎で飼育されており、ネズミを捕獲するためにネコが飼われている。このような状況の下では、重要な人獣共通感染症の1つであるトキソプラズマ症へのブタの感染をコントロールすることは難しい。本研究の目的は、ベトナム国フエ省における家ネコおよび肉用豚のトキソプラズマ抗体の血清陽性率をラテックス凝集反応（トキソチェック，栄研）により把握することにある。2007年に155匹の家ネコおよび肉用豚（と畜場から110検体，6件の養豚農家から150検体）から血液を採取した。ネコでは112検体（72.3%），養豚農家では62検体（41.3%），と畜場では76検体（69.1%）が *Toxoplasma gondii* 抗体陽性であった（1：64以上を陽性と判定）。ブタ

では体重の増加に伴い陽性率が上昇し，15 kg 以下では16.7%であったのに対し，50 kg 以上では71%が陽性であった（OR=12.26, 95% CI=3.31, 45.34）。養豚農家からのサンプリングでは，2ヶ月後に同群から再度血液の採取を行った。1度目に採取した検体では陽性率が28.4%であったのに対し，2ヶ月後に実施した2度目の検体では64%に増加していた（OR=4.48, 95% CI=2.14, 9.39）。本調査結果により，現在ベトナムのフエ省において一般的にみられる伝統的な養豚システムの下では，肉用豚のトキソプラズマ症への感染率が高いことが示された。

キーワード：トキソプラズマ，ベトナム，豚，猫

連絡先：細野ひろみ*

京都大学大学院農学研究科 寄附講座 食と農の安全・倫理論

〒606-8502 京都市左京区北白川追分町

Tel : 075-753-6338

E-mail : hiromix@kais.kyoto-u.ac.jp