นิพนธ์ต้นฉบับ

# Swine Trichinellosis in Northern Thailand : Detection by ELISA

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*ทริคิโนซิสเป็นโรคสัตว์ติดคนซึ่งมักเกิดกับคนในภาคเหนือเป็นประจำเกือบทุกปี สุกรขาวเขาเป็นพาหะแพร่โรคที่สำคัญที่ทำให้เกิดโรคในคน ได้มีการนำวิธีอีไลซ่ามาตรวจโรคทริคิโนซิส โดยทำการตรวจในสุกรขาวเขาในปี 2533 จำนวน 456 ตัว พบว่าให้ผลบวก 14.4% ที่จังหวัดน่าน 1.5% ที่จังหวัดเชียงราย และ 21.9% ที่จังหวัดเชียงใหม่ ในปี 2535 มีการเกิดโรคทริคิโนซิสกับคนในจังหวัดดังกล่าว ทำการตรวจซีรั่มสุกรขาวเขาในหมู่บ้านที่เกิดโรคพบว่า ให้ผลบวกต่อการตรวจด้วยวิธีอีไลซ่า 2 ตัวจากจังหวัดเชียงราย 5 ตัวจากจังหวัดน่าน แต่ตรวจพบตัวพยาธิจากสุกรเพียง 5 ตัว โดย 2 ตัวเป็นสุกรที่ให้ผลบวกอีไลซ่าจากจังหวัดเชียงราย และ 3 ตัวเป็นสุกรที่ให้ผลบวกจากจังหวัดน่าน จากการศึกษาจึงสามารถใช้วิธีอีไลซ่าตรวจวินิจฉัยโรค ซึ่งจะเป็นประโยชน์ต่อด้านระบาดวิทยาและการควบคุมป้องกันโรคในคน*

**คำสำคัญ :** *ทริคิโนซิส, อีไลซ่า; สุกรขาวเขา*

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*Abstract :* **C.Vitoorakool, I.Chaichanapunpol, N.morakote, and C.Khamboonruang. 1993. Swine Trichinellosis in Northern Thailand : Detection by ELISA. Thai J Hlth Resch 7(2) : 61-65**

*Trichinellosis is endemic in northern Thailand. Outbreak of human trichinellosis occurs almost every year, and hilltribe pigs have been considered as an important vehicle for transmission of human trichinellosis. An ELISA has recently been introduced for field detection of Trichinella spiralis larvae in these hilltribe pigs. In 1990 we tested 456 porcine serum samples colleted from 3 provinces in Northern Thailand using crude somatic larval antigen(SA). Positive rates were as follows: 14.4% in Nan, 1.5% in Chiang Rai, and 21.9% in Chiang Mai. In 1992, following an outbreak of human trichinellosis in the same provinces as mentioned above, 46 serum samples from hilltribe pigs in Chiang Mai, 41 in Chiang Rai, and 40 in Nan were tested by ELISA using SA. Seven from total (127) found to be positive (Chiang Mai 0, Chiang Rai 2, Nan 5). Positive pigs were sacrificed and examined for larvae by digestion method. Two pigs from Chiang Rai were infected whereas only 3 of 5 pigs from Nan were positive. The ELISA using excretory-secretory antigen (ES) was repeated. Only 3 pigs from Nan were true positive. The above findings seem to indicated that the ELISA using ES antigen appears to be a promising laboratory tool for epidemiological study and for control of human trichinellosis.*

**Key words :** *Trichinellosis, ELISA; Hilltribe pigs.*

## Introduction

Human trichinellosis in Thailand was first reported in 1962 at Mae Hong Son province involving 56 patients and 11 of them died (Boonthanom and Nawarat, 1963). Subsequently, outbreaks have been reported every year and most of them were in Northern Thailand (Dissamarn and Indrakamhang, 1985). Most of outbreaks were have consuming meat of illegally-slaughtered hilltribe pigs. During 1962-1964, 70 hilltribe pigs from 15 villages of 4 provinces, i.e., Chiang Mai,Chiang Rai, Phrae and Nan were examined parasitologically and 8 of them (11.4%) were found infected (Dissamarn and Indrakamkang, 1985). Since then, little has been done in surveillance of infected hilltribe pigs in order to control trichinellosis. One obstacle in attributable to high cost of buying pigs for parasitological examination, either by compression or digestion method.

With an advent of ELISA, it is now possible to screen pigs with lower cost (Gamble *et al.*, 1983; Seawright *et al.*, 1983; Murrell *et al.*, 1986; Ko and Yeung, 1989; Arriga *et al.*, 1989; Su and Prestwood, 1991; Serrano *et al.*, 1992). In this study, we conducted a field survey for detection of infected hilltribe pigs by ELISA.

## Materials and Methods.

**Serum samples :** Blood was collected from hilltribe pigs in Chiang Mai, Chiang Rai, and Nan Provinces. Sera were separated and stored at $-20°C$.

**Antigen :** Crude somatic antigen (SA) of muscle larvae was prepared as previously described (Morakote *et al.*, 1991). Excretory-secretory antigen (ES) was kindly provided by Dr.H. Ray Gamble, Helminthic Diseases Laboratory, Animal Parasitology Institute, Department of Agriculture, Beltsville, MD 20705, USA.

**ELISA :** ELISA using SA was basically similar to that described by Morakote *et al.* (1991). Briefly, each well of microtiter plate was coated with 4.5 μg of SA overnight, pig serum was tested at 1:100 dilution, the second antibody was 1:6,000 dilution of peroxidase-conjugated anti-swine Ig, and the substrate was TMB. The reaction was stopped 5 min later by addition of 50 μl of 1.25 M sulfuric acid. Absorbance (A) at 450 nm was recorded.

ELISA using ES was essentially as described by Gamble *et al.* (Cited by Murrell *et al.*, 1986). Neagative control serum was pool serum from farm pig and positive control serum was from experimentally infected pig. Results were expressed as absorbance at 405 nm.

**Parasitogical examination :** ELISA-positive pigs were killed. Pieces of muscle from tongue, diaphragm and other body parts were pooled to yield 200-300 g samples and subjected to pepsin digestion for the presence of muscle larvae of *Trichinella.*

**Data analysis :** In ELISA using SA, a serum which gave A twice that of negative control serum was considered positive. In the same way, serum which gave A five times that of negative control serum would be considered positive in ELISA using ES.

## Result

A survey in 1990 involving 456 hilltribe pigs using SA as antigen revealed that 23 of 160 (14.4%) pigs in Nan, 1 of 68 (1.5%) pigs in Chiang Rai, and 50 of 228 (21.9%) pigs in Chiang Mai were positive. However, seropositive pigs were not confirmed parasitologically.

In 1992, outbreak of trichinellosis occurred in Amphoe Hot, Chiang Mai; Amphoe Mae Chan, Chiang Rai; and Amphoe Tha Wang Pha, Nan province. Epidemiological investigation revealed that source of infection was hilltribe pigs bought from (1) Ban Pae, Huay Hin Phon District, Amphoe Mae Cham, Chiang Mai province, (2) Ban Wiang Sa, Sri Kum District, Amphoe Mae Chan, Chiang Rai province, and (3) Ban Doi Tew, Sri Phum District, Amphoe Tha Wang Pha, Nan province. Sera were than collected from 46 hilltribe pigs in Ban Pae, 40 in Ban Wiang Sa, and 41 in Ban Doi Tew, and examined by ELISA using SA as antigen.

The result shows that 7 of them were ELISA-positive. These pigs were bought, killed, and examined further for the presence of muscle larvae by digestion method. As showed in Table 1, five of them were infected by *T.spirallis.*

**Table 1.** Details of 7 hilltribe pigs which were positive by ELISA using somatic larval antigen.

| No. | Origin | Larval densities | Absorbance SA | Absorbance ES |
|---|---|---|---|---|
| 1. | Chiang Rai | 4 larvae/300 g | 0.576(+) | 0.092(−) |
| 2. | Chiang Rai | 2 larvae/200 g | 0.529(+) | 0.196(−) |
| 3. | Nan | 11,500 larvae/250 g | 0.705(+) | 0.337(+) |
| 4. | Nan | 1 larva/250 g | 0.583(+) | 0.226(+) |
| 5. | Nan | None/250 g | 0.629(+) | 0.006(−) |
| 6. | Nan | None/250 g | 0.540(+) | 0.052(−) |
| 7. | Nan | 6,380 larvae/300 g | 0.695(+) | 0.307(+) |
| | Negative control | | 0.18 | 0.040 |

(+) = positive, (−) = negative

Two pig sera from Ban Wiang Sa, Chiang Rai province and five sera from Ban Doi Tew, Nan province were re-examined by ELISA using ES antigen which found that only three from Ban Doi Tew were positive (Table 1).

In 1993, outbreak of human trichinellosis occurred in Amphoe Chiang Kum, Pha Yao province and Amphoe Mae Cham, Chiang Mai province. Serum samples from 27 hilltribe pigs in Ban Doi Tew, Amphoe Tha Wang Pha, Nan province, 17 pigs in Amphoe Mae Cham, Chiang Mai province, and 3 pigs in Ban Wiang Sa, Amphoe Mae Chan, Chiang Rai province, were examined by ELISA using both SA and ES. All sera were found negative.

## Discussion

The use of crude somatic antigen in ELISA produced 2 false-positve pigs while the use of ES produced 2 false-negative pigs. Pigs which were negative with ES had light infection, i.e., less than 1 larva per gram muscle. This has been previously reported (Murrell *et al.*, 1986; Ko and Yeung, 1989; Su and Prestwood, 1991). Another cause of false negativity may be immune responsiveness of pigs and time lag of infection (Su and Prestwood, 1991). Alternatively, calcified cysts of *Trichinella* may be dissolved in pepsin digestion produing negative results (Murrell *et al.*, 1986; Su and Prestwood, 1991).

False positivity in ELISA using SA could be due to other parasitic infection. Anti-*Trichuris suis* and anti-*Metastrongylus apri* were found to react with SA (Ko and Yeung, 1989). Pigs with *Sarcocytis, Oesophagostomum, Ascaris suum,* or *T.suis* gave high absorbance (Arriga *et al.*, 1989; Serrano *et al.*, 1992). False positivity can produce economic loss due to unnecessary destruction of uninfected pigs. On contrary, false negativity allows naintenance of *Trichinella* in the environment although it could be argued that larval density of 1 per gram meat will not produce clinical disease in man (James, 1989).

The high larval density in pig number 3 is stunning. This pig should have induce an outbreak of human trichinellosis if it was illegally slaughtered and consumed. Detection of this infected pig then emphasized the importance of ELISA. Later examination of hilltribe pigs sera in areas which infected pigs have been previously detected revealed no seropositive pigs. This would suggest that an intermittent screening of hilltribe pigs by ELISA should be able to reduce source of infection and as a result reduces outbreak of human trichinellosis in Thailand.

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