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Short Communication

Gastrointestinal helminths and *Taenia* spp. in parenteral tissues of free-roaming pigs (*Sus scrofa indicus*) from hill-tribe village at the western border of Thailand

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[†]This research note is dedicated to Singkham Aueawiboonsri, the ex-director of Nong Bua District Health Promoting Hospital, who passed away in a car accident while performing his duty on March 16th, 2016. We sincerely express condolences to him with our great thanks for his friendship and smile. May his soul rest in peace
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Abstract. A serological survey of pig cysticercosis was conducted in a hill-tribe village at Thai-Myanmar border, Tak province of Thailand in 2012. Sixteen backyard pigs were examined for pig cysticercosis and gastrointestinal helminth infection. In addition to cysticerci of *Taenia solium* and *Taenia hydatigena* found outside the gut, nine other helminth species were found in guts: *Echinostoma malayanum*, *Pseudanoplocephala crawfordi*, *Ascarops dentata*, *Physocephalus sexalatus*, *Gnathostoma doloresi*, *Ascaris suum*, *Globocephalus* sp., *Oesophagostomum dentatum* and *Bourgelatia diducta*. The study presents a report for the first time of adult tapeworm, *P. crawfordi* infection in pigs from Thailand. For medical importance, *E. malayanum*, *P. crawfordi*, *G. doloresi and A. suum* have been confirmed as potentially zoonotic helminths and pigs may act as one of the reservoir hosts for human helminthiases. Pigs of both gender and all ages appeared to be exposed to the parasites equally and did not show any significant difference to these helminth species in richness and total intensity.

In Thailand, the main population of native pigs (*Sus scrofa indicus*) are distributed in the remote areas of northern and north-western highland, bordering Myanmar and Lao PDR, where hill-tribe villagers settle down (Davidson, 1957). In these poverty stricken areas of Thailand, pigs are kept in household-level as a source of supplying protein, generate income for family, and occasionally used in cultural sacrificed-meat in local spirit worship (Falvey, 1981). With indigent situation, most of the family keep their pigs unwell-managed, fed by leftover food scraps, and let them roam freely around houses and backyards scavenging for food by themselves. This poor husbandry may results in pig malnutrition, and affects their immune system leading them susceptible to parasite or microbial infections. This also increases the chance of people getting into contact with pigs through the sharing of the environment, and directly/indirectly transmitting some potentially zoonotic parasites, *i.e.*, *Fasciolopsis buski*, *Taenia solium*, *Taenia asiatica*, *Trichinella spiralis*, *Balantidium coli*, *Sarcocystis suihominis* (Kaewpitoon *et al.*, 2006; Wanda *et al.*, 2006; Conlan *et al.*, 2011; Khan *et al.*, 2013) as well as other pathogenic microorganisms, *i.e.*, Japanese encephalitis virus, Nipah virus, Swine Influenza virus, *Streptococcus suis*, *Leptospira interrogans* to human (Chua *et al.*, 2000; Carpenter *et al.*, 2005; Solomon, 2006; Nghia *et al.*, 2008; Pasma & Joseph, 2010).

As the International Collaboration for Detection, Surveillance and Control of Taeniasis/Cysticercosis and Echinococcosis in Asia Pacific (Ito *et al.*, 2006), the project has been carried out on the transmission ecological and epidemiological studies on *T. solium* cysticercosis in humans and pigs in a number of refugee villages in Kanchanaburi, a province along Thai-Myanmar border (Anantaphruti *et al.*, 2007). Through this work in Tak province, we conducted serological survey on 188 local pigs and had a chance to do necropsy on some of the pigs which showed antibody responses to diagnostic antigens purified from cyst fluid of *T. solium* by ELISA (unpublished). We report here the gastrointestinal helminths in those necropsied pigs.

In 2012, the field survey was conducted in three nearby-Karen-tribe villages: Bann Nong Bua, Bann Tala Orka and Bann Khue Khoe, Mae Usu sub-district, Tha Song Yang district, Tak province, Thailand (Latitude: N 17° 20' 23.291'', Longitude: E 98° 6' 32.579''). The field study was a part of the project to establish immunological and molecular diagnostic techniques for taeniasis/cysticercosis in humans and pigs. Blood samples were collected from free-roaming pigs (usually younger than 2 years old approximately), and from older pigs in pigsties (>2 years old). Then, serological diagnosis (ELISA) of cysticercosis was applied in the field for screening of pig-positive cases using glycoproteins purified by cation exchange chromatography (Sako *et al.*, 2013). Subsequently, 11 pigs with the ELISA medium to strong-positives as well as 5 negative individual were killed by owners themselves followed their common traditional method. Pig bodies were brought to Nong Bua Health Promoting Hospital, Tha Song Yang district, Tak province, and examined for evidence of cysticerci of *Taenia* spp. Meanwhile, gastrointestinal tracts (GI-tracts) were then examined in order to investigate the helminth infection status in the pig population. All procedures contributing to animal examination and specimen collection were controlled and permitted by district livestock officer who joined in this research study following the cooperation document number TMHM 0517.116/00442.

A total of 16 pigs were examined. Individual host attributes, gender and age (juvenile < 8 months and adult ≥ 8 months) were recorded for further statistical analysis. Firstly a post-mortem examination was carried out on the pig's tongue, conjunctiva, brain, viscera ,including liver, and all musculatures for potential infection of cysticerci of *T. solium* from the whole carcass, and *T. asiatica* and *T. hydatigena* in the livers. Intestinal mesenteries were checked for attachment of *T. hydatigena* cysticerci. Detailed report on *Taenia* spp. and serology will be published elsewhere. Three main parts of GI-tracts were divided into stomach, small intestine and large intestine. Each organ was then opened in separated containers so that all the intestinal contents were collected as individuals. In addition to the contents, stomach and intestinal mucosa were also cleaned and scraped off. Then, the contents were subjected for cleaning and sedimentation by mixing the contents with water in buckets, let them stand for 15 minutes, and supernatants were discarded. The sedimentation was repeated 3 times aiding clarity of the sediments. The sediment sub-samples were slightly poured into a black tray with some water to check for a present of large and medium-sized worms. Stereomicroscope was also used to examine any small-sized worms in the sediments. The isolated worms were identified morphologically following the description keys (Soulsby, 1982; Schmidt, 1986). The parasites were counted separately regarding to each helminth species and individual host infection to access helminth intensity and abundance. Total helminth intensities were calculated by sum of helminth intensity in an individual pig, whereas total number of helminth species infected in each pig was also taken as representative for helminth species richness (HSR).

The prevalence, mean abundance, mean intensity and range of each helminth infection were estimated by Quantitative Parasitology software, version 3.0 (Rozsa *et al.*, 2000). Effects of sex and maturity on HSR and total helminth intensity were tested by using Welch two sample t-test in R freeware (R Core Team, 2013).

Totally, we found 9 GI helminths (Table 1, Figures 1 and 2) along with cysticerci of *T. solium* and *T. hydatigena* in the examined pigs, indicating that even in the three small villages and small host sample size (n=16), pigs in this studied area harboured several helminth species. Individually, pigs were infected with multiple helminths, ranging from 2 – 6 species. Apart from *T. solium*, *T. asiatica* and *T. hydatigena* which were expected to be detectable by ELISA (will be reported elsewhere), we suggest that the pig hookworm *Globocephalus* sp. was the dominant parasite found in the pigs, followed by *Oesophagostomum dentatum* (nodular worm) and *Ascaris suum* (pig roundworm), (Table 1).

There was no significant difference observed in HSR (log-transformed) between pig genders ($t = -0.66$, $df = 11.68$, $p = 0.51$) and age ($t = -0.57$, $df = 8.48$, $p = 0.58$). Similarly, total helminth intensity (log-transformed) did not significantly differ between genders ($t = 0.81$, $df = 13.98$, $p = 0.43$) and ages ($t = -0.81$, $df = 12.83$, $p = 0.44$), inferring that there was no difference in gastrointestinal helminth infection pattern between pig gender and maturity in the studied area. They appeared to have exposed to the parasites equally.

Generally, the larval stage (metacestodes) such as hydatid cysts (*Echinococcus* spp.) and cysticerci (*Taenia* spp.) were mainly found in pigs as intermediate host, while relatively low incident of adult tapeworm has been reported. According to the 'Host-Parasite Database' (www.nhm.ac.uk), some evidences of adult tapeworms in pigs, including wild boar *Sus scrofa leucomystax* were sporadically recorded, *i.e.*, *Moniezia expansa* and *Moniezia benedeni* were found in Indian region (Chowdhury, 1994); *Pseudanoplocephala crawfordi* in India, China and Vietnam (Jiang *et al.*, 1990; Nama, 1990; Krivolutsky *et al.*, 1991); *Pseudanoplocephala nipponensis* in Japan (Sato *et al.*, 2008); *Diphyllobothrium latum* in Palearctic region and Mozambique (Govorka *et al.*, 1988; Afonso-Roque, 1992); *Spirometra erinaceieuropaei* in Australia, Indian and Palearctic region (Govorka *et al.*, 1988; Parlov *et al.*, 1992; Chowdhury, 1994); *Spirometra mansoni* and *Spirometra ranarum* in Indian region (Chowdhury, 1994). In Southeast Asia, *M. expansa* and *P. crawfordi* infection was only reported in

Table 1. Prevalence (%), mean intensity (MI) and range of helminth infection in free-roaming pigs (n=16) from a minority tribe-villages in Tak province, Thailand

Group	Helminth species	Organ	Prevalence (No. infected host)	MI	Range
Cestode	*Pseudanoplocephala crawfordi*	Small intestine	25.0 (4)	6.5	0–12
Trematode	*Echinostoma malayanum*	Small intestine	12.5 (2)	6.0	0–9
Nematode	*Ascarops dentate*	Stomach	12.5 (2)	1.5	0–2
	Physocephalus sexalatus	Stomach	12.5 (2)	2.5	0–4
	Gnathostoma doloresi	Stomach	6.3 (1)	6.0	0–6
	Ascaris suum	Small intestine	31.3 (5)	1.6	0–3
	Globocephalus sp.	Small intestine	68.8 (11)	36.7	0–210
	Oesophagostomum dentatum	Large intestine	37.5 (6)	33.0	0–150
	Bourgelatia diducta	Large intestine	12.5 (2)	1.0	0–1

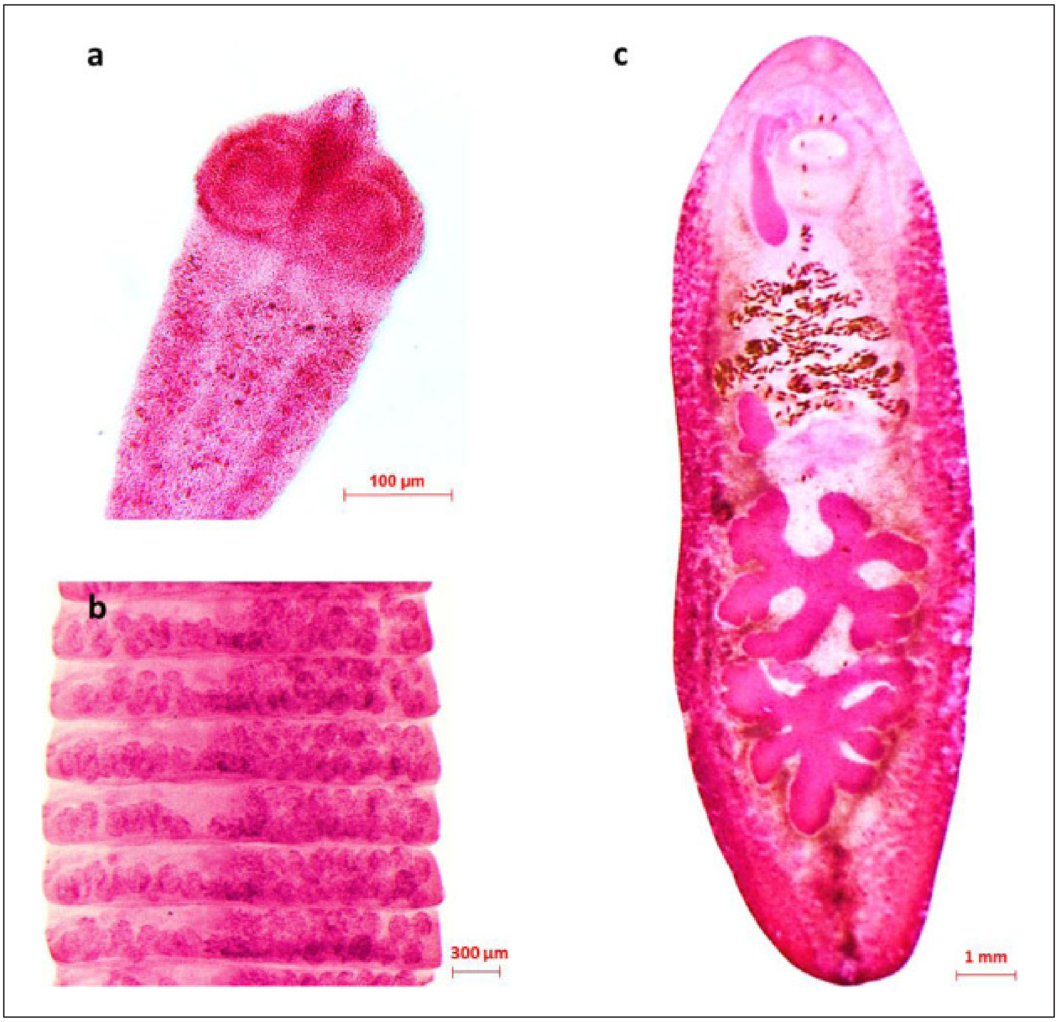

Figure 1. Cestode and trematode from pig intestine: (a) unarmed scolex of *Pseudanoplocephala crawfordi*; (b) mature segments of *P. crawfordi*; (c) whole body of *Echinostoma malayanum.*

domestic pig from Vietnam (Krivolutsky *et al.*, 1991). Accordingly, our discovery of *P. crawfordi* is the first report in Thailand, expanding the information on geographical distribution of the cestode species.

As mentioned previously, the ELISA method used in this study was genus-specific, not limit only to *T. solium* but also may detect *T. asiatica* and *T. hydatigena* (Swastika *et al.*, 2016). *T. asiatica* was thus expected to be found in pigs from this study area, since there have been three human *Taenia* species reported in Kanchanaburi, near-by province along Thai-Myanmar border (Anantaphruti *et al.*, 2007; Ito *et al.*, 2016). However, there was no evidence of pig infected with *T. asiatica* in these villages so far. This was in accordance with human taeniasis status in the villages which all the cases were diagnosed as *T. solium* infection by both morphology and molecular identification of all tapeworms expelled through anti-helminthic treatment (unpublished).

Echinostoma malayanum, *P. crawfordi*, *A. suum* and *Gnathostoma doloresi* are the gastrointestinal worms that potentially infect human (Nawa *et al.*, 1989; Belizario *et al.*, 2007; Arizono *et al.*, 2010; Zhao *et al.*, 2015), suggesting that the pigs may act as reservoirs of several zoonotic helminthiases in this area.

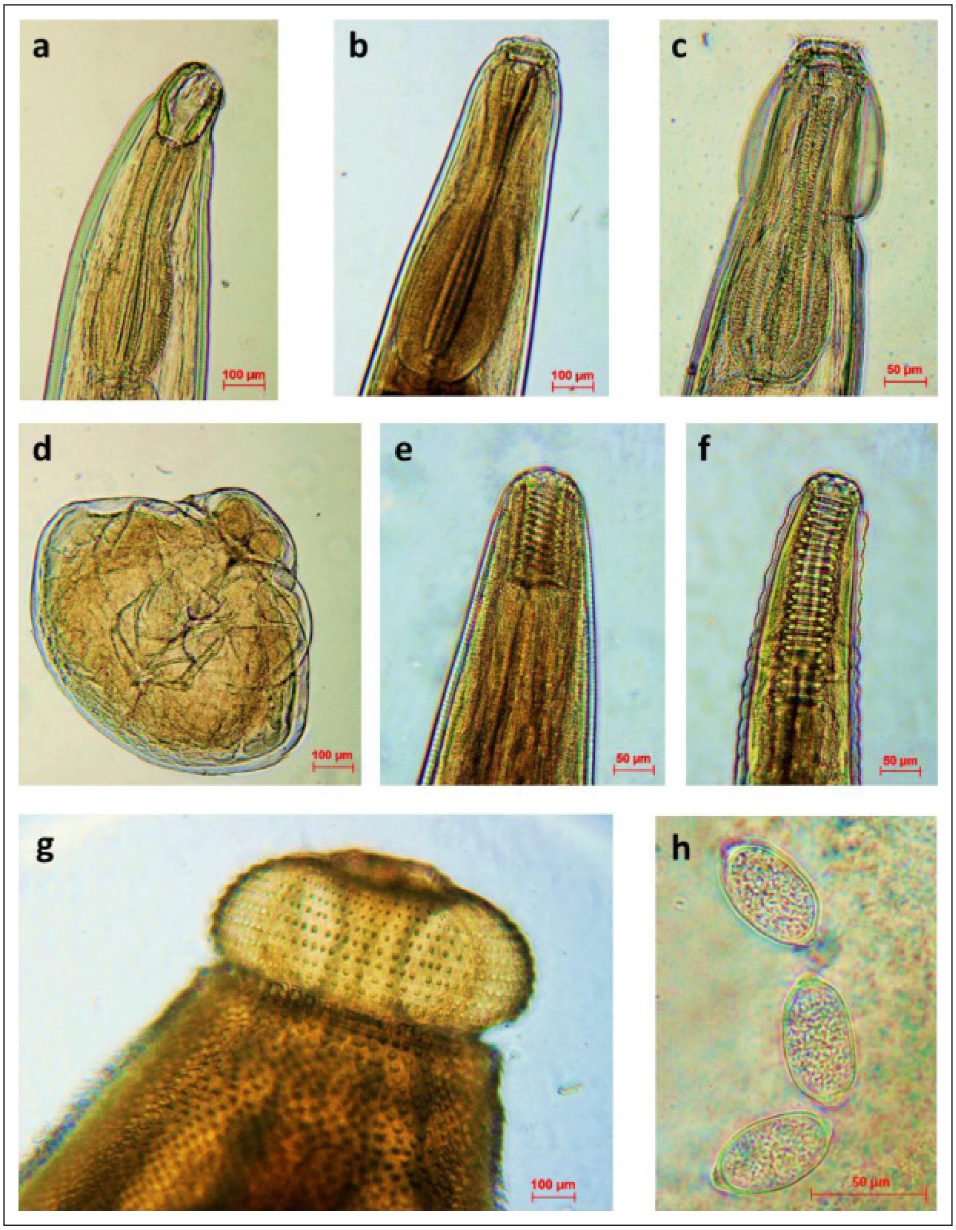

Figure 2. Anterior part of nematodes in pigs: (a) *Globocephalus* sp.; (b) *Bourgelatia diducta*; (c) *Oesophagostomum dentatum*; (d) *Ascaris suum* (tri-lobed lips); (e) *Ascarops dentata*; (f) *Physocephalus sexalatus*; (g) *Gnathostoma doloresi* and (h) eggs of *G. doloresi* in uterus.

In conclusion, a number of gastro-intestinal helminth species were detected in cysticercus-seropositive pigs raised by hill-tribe villagers from remote area of Thailand. The ELISA-positive pigs were co-infected with cysticerci of *T. solium* and *T. hydatigena*, and there was no evidence of *T. asiatica* cysticerci in this area. High diversity of helminth infections similarly found in any pig ages and genders, as well as multiple helminth infections occurred in individual pigs, reflexing their free-roaming habit might be the key role of pig highly exposed to the infective stages circulating in the environment.

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