VETERINARY ASPECTS OF PORCINE CYSTICERCOSIS IN JAYAWIJAYA REGENCY, PAPUA PROVINCE, INDONESIA

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Abstract. *Taenia solium* cysticercosis is a significant public health problem in Papua, Indonesia. Porcine cysticercosis is highly endemic in areas of Jayawijaya due to the strong tradition of pig husbandry. This study determined seroprevalence using monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (MoAb-ELISA) of porcine cysticercosis between October 2009 and June 2011 among 104 pigs in eight districts of Jayawijaya. The highest prevalence (93%) was in Asolokobal District and the lowest (6%) in Wamena Kota District, with an overall prevalence of 37%. Pigs in the Asolokobal District were highly vulnerable to porcine cysticercosis. In Jayawijaya, pigs are cooked in a traditional manner using hot stones, the temperature of which is 300°C, for 90 minutes resulting in an interior meat temperature of 60-90°C that is unable to kill cysticerci. Thus, it will be necessary to incorporate an anthropological approach to the system of pig husbandry and pork cooking practice if porcine cysticercosis is to be reduced in Jayawijaya.

Keywords: porcine cysticercosis, sandwich-ELISA, seroprevalence, Indonesia

INTRODUCTION

Cysticercosis is a zoonotic disease caused by ingestion of eggs or proglotids of tapeworm *Taenia solium*. As the definitive host of this parasite, humans can suffer from cysticercosis if they accidentally ingest *T. solium* eggs. Cysticercosis in humans can be severe should the parasite infect brain and central nervous system (neurocysticercosis) or eyes (ocularcysticercosis) (Patel *et al*, 2011). Cysticercosis is a disease associated with poverty, indiscriminate defecation and free roaming of pigs (Rajshekhar *et al*, 2003).

In Asia, there are three *Taenia* spp that could infect humans, namely, *T. asiatica*, *T. saginata* and *T. solium* (Ito *et al*, 2003; Okamoto *et al*, 2007). While human cysticercosis is caused only by *T. solium*, pigs are the intermediate hosts of *T. asiatica* and *T. solium*, and many genetic similarities have been found between *T. asiatica* and

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T. saginata (Puchades and Fuentes, 2000; Ito *et al*, 2005; Anantaphruti *et al*, 2007; Okamoto *et al*, 2007).

Cysticercosis/taeniosis can be found in seven provinces of Indonesia, namely, Bali, Lampung, North Sulawesi, North Sumatra, Papua, Southeast Sulawesi, and West Kalimantan (Margono *et al*, 2006). The disease is endemic in Bali (*T. saginata* and *T. solium*), North Sumatra (*T. asiatica*), and Papua (*T. solium*). The first case of cysticercosis/taeniosis in Papua was reported in 1972 following the discovery of *T. solium* proglottids and eggs during a routine examination at Enarotali Hospital, Nabire Regency in the Central Highlands (Margono *et al*, 2006). Since then the disease has spread to other regions including Paniai and Jayawijaya. The spread of the disease is associated with the absence of latrines and poor personal hygiene, consumption of feces by straying pigs, and cultural practice of consuming raw or undercooked meat. Suroso *et al* (2006) and Margono *et al* (2006) reported the suspicion that cysticerci in pork cooked using "bakar batu" (a stone cooking pit), a traditional cooking method of Papua involving pre-heated stones, is a source of infection in humans.

Wamena, the capital city of Jayawijaya, is derived from the word "wam", meaning pigs. This suggests that pig is an important animal for the autochthonous population, the Dani ethnic group. The main types of pigs kept in Wamena are local breeds and some crossed with imported pigs, such as those of the large white variety (Kataren, 2008). Various ceremonies, performed in the public sphere, involve pigs, and religious ceremonies of the Dani focus on either welfare or warfare ceremonies were held in the past to invoke the tribe's welfare and to reinforce courage in battle (Department of Education, 1993). Because pigs are culturally important and an important food source for the indigenous community, efforts to improve the safety of pig meat consumption and to prevent the transmission of foodborne illnesses to humans are needed.

Research on cysticercosis/taeniosis in this region has mostly been conducted in humans (Handali *et al*, 1997; Margono *et al*, 2006; Salim *et al*, 2009). Recent seroepidemiological survey showed a high prevalence of human cysticercosis/taeniosis, 20.8% based on rT24H (cysticercosis antigen) enzyme-linked immunoelectrotransfer blot (EITB) and 7% for taeniosis based on rES33 (taeniosis antigen) EITB in Jayawijaya (Salim *et al*, 2009). These results indicated that cysticercosis and taeniosis are still prevalent and their infection rates have not changed since the first report 35 years ago.

The study of cysticercosis in pigs as a source of human infection is very limited. The seroprevalence of cysticercosis in pigs in Jayawijaya from 1998 to 1999 ranges from 8.5 to 70.4% based on copro-ELISA of pigs (Ito *et al*, 2003, Wandra *et al*, 2007a,b). Maitindom (2008) conducting a post-mortem study in pigs sold at the Jibama Market, Wamena, Jayawijaya Regency reported a 77.1% prevalence of swine cysticercosis. Thus, it is important to study some of veterinary aspects of porcine cysticercosis in Jayawijaya Regency, Papua, Indonesia to determine the seroprevalence of porcine cysticercosis and pig farm management in the districts of the Regency. In addition, we also carried out a measurement of pork meat temperature traditionally cooked using "bakar batu" to determine risk associated with this cultural practice.

MATERIALS AND METHODS

Study area

The study was conducted in Jayawijaya Regency of Papua Province, Indonesia (138°30′ - 139°40′ East and 3°45′ - 4°20› South), and an altitude of 1,724 m above sea level. Data were collected from eight [Asolokobal, Assologaima, Bolakme, Homhom, Hubikosi, Kurulu, Musatfak, and Wamena Kota (the capital city)] out of 11 districts in the Regency based on their accessibility. The study was carried out during October 2009 – June 2011.

Permit for the research was obtained from the Livestock Service Office, Jayawijaya Regency.

Monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (MoAb-ELISA) detection of porcine cysticercosis

Blood serum samples were obtained from 104 pigs [Asolokobal (n = 14), Assologaima (n = 22), Bolakme (n = 6), Homhom (n = 11), Hubikosi (n = 7), Kurulu (n = 23), Musatfak (n = 4), and Wamena Kota (n = 17)] and 2 ml aliquots were stored at -20°C until used. MoAb-ELISA was performed as previously described (Dorny *et al*, 2004). In brief, 150 µl aliquot of serum was incubated with an equal volume of 5% trichloroacetic acid for 20 minutes at room temperature, centrifuged 12,000*g* for 9 minutes, and 150 µl aliquot of supernatant then was added to **75** µl of neutralizing buffer (0.156 M carbonate/bicarbonate pH 10). A 100 µl aliquot of capture anti-MoAb ($B158C_{11}A_{10}$; Institute of Tropical Medicine, Antwerp, Belgium) and 5 µl/ml 0.5 M bicarbonate buffer pH 9.8 were applied to wells of a 96-well microtiter plate pre-treated with 100 µl of coating buffer (0.05 M bicarbonate buffer pH 9.8) and incubated for 30 minutes at 37°C with shaking. Wells were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (washing buffer), followed by incubating with 150 µl of blocking buffer (NBCS, Antwerp, Belgium) for 15 minutes at 37°C with shaking. Then 100 µl aliquots of treated serum were added to the wells, incubated for 15 minutes at 37°C with shaking and then washed five times with washing buffer. Aliquots of 100 µl of detecting biotinylated MoAb ($B60H_8A_4$ (Institute of Tropical Medicine, Antwerp, Belgium) and 1.25 µg/ml blocking buffer were added and the plate was incubated as described above, followed by washing five times with washing buffer. Then 100 µl aliquot of orthophenylenediamine (Sigma, St Louis, MO) [one table spoon dissolved in 10 ml of aquabidest (Ikapharmindo putramas, Jakarta, Indonesia)], 2.5 µl of H_2O_2 and 100 µl of peroxidase-labelled streptavidin (Sigma) (1:10,000 in blocking buffer) were added and incubated for 15 minutes at the 30°C in the dark. The reaction was terminated by the addition of 50 µl of 8 M H_2SO_4 and absorbance measured at 492 and 655 nm (Microplate Reader, Benchmark, Bio Rad, London, UK). Appropriate positive and negative control samples were included in the assay.

Pig farm management survey

Replies to a questionnaire was obtained from 56 pig owners in the study areas to collect information on pig farm management. Interviews were conducted by Jayawijaya Regency Livestock Services officers using local Dani language.

Heated stone meat cooking technique

The "bakar batu" cooking technique consists of heated stones to roast pork and vegetables. These large hot stones are placed into a pit (about 70 cm deep) using a special clamp ("apando") and then covered with weeds (Fig 1). The raw pork was

covered with grass and tied with rattans to prevent steam generated from the heat of the hot stone from evaporating. An infrared (Digital Infrared Thermometer IR 102, PPACAM Instruments, Shenzhen, China) and thermocouple (Digital Thermometer D55, Hanyoung NUX, Incheon, Korea) thermometer located in the pork was used to measure the temperature of the stones and pork, respectively.

Data analysis

The ratios of pig serum sample absorbance from MoAb-ELISA to that of the cut-off value obtained from negative serum were processed using the *t*-test with a *p*-value ≤0.05 considered significant (Sokal and Rohlf, 1981).

RESULTS

Prevalence of porcine cysticercosis

Swine cysticercosis prevalence based on presence of serum *T. solium* antigen determined using MoAb-ELISA. Positivity, defined if the ratio between absorbance of sample/cut off value >1 in 44/104 (37%) samples, with the highest seroprevalence found in Asolokobal District (13/14, 93%), followed by Musatfak (3/4, 75%), Kurulu (15/23, 65%), Bolakme (2/6, 33%), Asologaima (7/22, 32%), Homhom (2/11, 18%), Hubikosi (1/7, 14%) and Wamena Kota (1/17, 6%) (Fig 2).

Pig farm management

Forty-eight percent of respondents reported good swine husbandry consisted of caging pigs in the evening, but releasing them in the morning and afternoon; 34%that pigs should be caged at all times; and18% that pigs should not be caged but should be allowed to roam for food. Two thirds of residents who had a cage or enclosure chose to allow their pigs to roam free during the day, and those without cages allowed their pigs free movement. Of respondents with a pig pen, 88% had a semi-permanent enclosure, 2% a permanent enclosure and the remaining 10% were not aware of the concept of a permanent enclosure. All respondents provided root crops and vegetables to their pigs. When asked about the role of the government in educating them on swine husbandry, 79% of respondents had never received any government assistance, while 14% voiced that the government should be involved in such activity. Jayawijaya communities practice pig husbandry using methods handed down by their parents and forefathers. When pigs suddenly die, farmers undertook a range of actions, with 85% of the respondents butchering and eating the pigs, 13% selling the carcasses and 2% burying the carcasses.

The highest percent of farms with cages were in the districts of Asolokobal, Bolakme and Musatfak (100%), followed by Kurulu (52%), Homhom (36%), Wamena Kota (18%), Hubikosi (14%) and Asologaima (9%). The percent of farms that did not provide cooked feeds to their pigs were highest in the districts of Hubikosi and Asolokobal (100%), followed by Kurulu (83%), Musatfak (75%), Asologaima (50%), Wamena Kota (23%), Bolakme (17%) and Homhom (0%).

Observations on "bakar batu" meat cooking technique

Using an infrared thermometer, the temperature of the hot stones ranged from 170ºC to 300°C (data not shown). The temperature of pork cooked over the stones over a period of 90 minutes ranged from 60ºC to 90°C (Fig 3). According to the respondents, the cooked pork meat was placed directly on grass (85%) or ground (13%) before being consumed. Most of the respondents (89%) ate the meat using bare hands without previously washing them.

Fig 1–"Bakar batu" cooking technique. A. Stones are placed at the bottom of a 70 cm deep pit and covered with weeds. B. "Hipere" (sweet potato). C. A thermocouple thermometer is inserted in the pork. D. The cooking stove is covered with grass and tied with rattans.

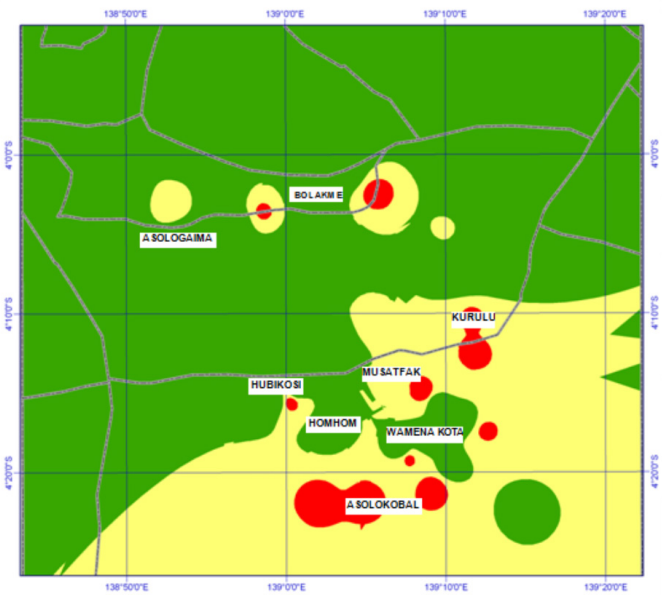

Fig 2–Map of porcine cysticercosis prevalence in Jayawijaya Regency, Papua, Indonesia. Red area, highly vulnerable (Asolokobal, Kurulu); yellow, vulnerable (Bolakme, Hubikosi and Musatfak); green, not vulnerable (Asologaima, Homhom, Kota, and Wamena).

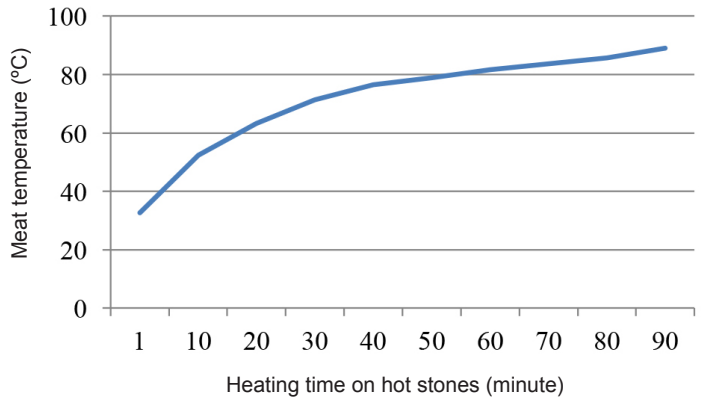

Fig 3–Temperature, measured using a thermocouple thermometer, of inner part of pork meat cooked using hot stone ("bakar batu") technique.

DISCUSSION

Seroprevalence of swine cysticercoid in Jayawijaya Regency during October 2009 - June 2011 was conducted using MoAb-ELISA and mapped to determine levels of exposure to cysticercosis. Margono *et al* (2006) reported Papua as having one of the highest (50.6%) endemic rates of *T. solium* in the world, and this rate has not much changed. High prevalence of cysticercosis in general occurs in areas far from cities where most of the houses have latrines, whereas in rural communities many homes still do not have access to latrines and feces becomes easily accessible to free roaming domestic animals, including pigs.

Non-caged pigs can eat feces contaminated with *Taenia* eggs allowing transmission of cysticercosis (Garcia *et al*, 1998). Suroso *et al* (2006) found cysticercosis/taeniosis decreases significantly in Bali with improved sanitation and pig husbandry. However, a number of rural districts in Jayawijaya are located in parts of Papua with difficult access to veterinary facilities and animal health professionals. Sikasunge *et al* (2007) suggested pigs not caged are significant risk factors for cysticercosis transmission and Garcia *et al* (2007) recommended the most convenient way to eliminate the transmission of porcine cysticercosis is to cage pigs and noted farmers in developing countries do not generally cage pigs due to economic factors. Maitindom (2008) also pointed out that allowing pigs to roam free could reduce feeding cost.

As part of this study we assessed how the traditional "bakar batu" method of pork cooking could impact on zoonotic transmission of cysticercosis as this practice is highly popular and an important cultural feature of the Dani community in Jayawijaya (Kataren, 2008). Pork roasted on hot cooking stones is usually not completely cooked and this cooking method has been suspected as a risk factor for the spread of cycsticercosis/taeniosis in humans (Handali *et al*, 1997; Suroso *et al*, 2006; Wandra *et al*, 2007b). However, the inner temperature in pork cooked by "bakar batu" method of 60-90°C over a 90-minutes period is sufficient to kill cysticerci (Pawlowski and Murrell, 2001; EFSA, 2004). This heating process could take longer because most respondents stated that the hot stone pit cooking usually lasts longer than two hours. However, poor personal and food hygienic practices could allow food to become contaminated with *Taenia* eggs.

In conclusion, this study shows a high prevalence of solium cysticercosis among pigs in Jayawijaya Regency, Papua, especially in Asolokobal District. Contrary to

previous reports from the same region, this study demonstrates that traditional hot stone pit ("bakar batu") cooking technique provided sufficient temperature and cooking time to destroy pork-infected *Taenia* eggs, and the actual cause(s) of human cysticercosis should stem from other behavioral and environmental risk factors, which require urgent investigation. In the meantime, local communities should be made aware of good swine husbandry practices.

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