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Seroprevalence and Risk Factors of Porcine Cysticercosis in Way Kanan District, Lampung Province, Indonesia

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Abstract: The study aimed to determine seroprevalence and risk factor of porcine cysticercosis. It was conducted in seven subdistricts of Way Kanan District from April 2012 until March 2013. Survey of this study was conducted through 4 stages. The first stage was collecting porcine blood serum, determining ordinate points and interview using questionnaire. The second stage was laboratory testing for porcine sera blood using *monoclonal antibody-based sandwich enzyme-linked immunosorbent assay* (MoAb-ELISA). The third stage was postmortem examination towards seropositive porcine. The last stage was statistical analysis. A hundred sixty nine (169) samples of porcines were tested serologically. The results of seropositives cysticercosis were found in 3 samples (1.78%), i.e. 2 samples (4.55%) from Negeri Agung Subdistrict and 1 sample (9.1%) from Pakuan Ratu Subdistrict. All seropositive results were obtained from female porcines. The age of porcine was a significant risk factor that affected cysticercosis (OR = 1.083; 95% CI = 1.023 - 1.142). The older porcine had higher risk of cysticercosis than the younger one. There are some ways to reduce the transmission risk of this disease, i.e. public awareness of animal health, increasing animal health service, monitoring pork distribution and conducting field surveillance continuously.

Key words: Porcine Cysticercosis · Seroprevelence · Way Kanan District

INTRODUCTION

Cysticercosis is a zoonotic parasite, causes considerable losses in public health, agricultural production and economy. Even though it is considered as a neglected disease in developing countries [1-5]. This zoonotic cestode has a global distribution, with main endemic regions including Latin America, China, South East Asia and sub-Saharan Africa [6-9]. Cysticercosis causes public health problem in developing countries with high porcine population, high pork consumption, poor education and poor sanitary facilities [10, 11].

Porcine is an intermediate host of *Tenia solium* and an infection source of *Cysticercus cellulosae* for human cysticercosis. Clinical signs of porcine cysticercosis are usually not seen and animals appear to be perfectly healthy, despite heavy infection. Meanwhile *Cysticercus cellulosae* in human causes a fatal health disorder [12, 13].

Taenia solium cysticercosis was reported in Vietnam, Kamboja, Laos, Thailand, China, Philipina and Indonesia [14, 15]. Indonesia is one of endemic cysticercosis country for three taenia species, i.e. *T. solium, T. saginata* dan *T. asiatica* [16-18]. The disease has been reported in Papua, Nusa Tenggara Timur, Nusa Tenggara Barat, Bali, East Java, Jakarta, Lampung, Riau, North Sumatera, Southeast Sulawesi, South Sulawesi, North Sulawesi and East Kalimantan [19].

Cysticercosis in Lampung Province occured sporadically. Non-moslem people in Lampung Province, especially at Way Kanan District, consume pork, gained from pig husbandry and wild boar hunt. Pig husbandries at Way Kanan District are run by Balinese and catholic society. Most of those pig husbandries categorized as traditionally small scale pig husbandry, consist of local and breed porcine. The study was conducted to determine cysticercosis in porcine and risk factors of it.

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MATERIALS AND METHODS

Location and Time of Survey: Survey was conducted on April 2012 until February 2013, located in seven subdistricts of Way Kanan District. The location of survey included Subdistrict Banjit, Baradatu, Bumi Agung, Buay Bahuga, Blambangan Umpu, Negeri Agung and Pakuan Ratu. Sera were tested in Medical Microbiology Division of Animal Public Health Department in Veterinary Medicine Faculty of Bogor Agriculture University.

Survey Design: This study used cross sectional design. The survey was conducted after receiving ethical approval from Medical faculty of University of Indonesia with register number 420A/PT02.FK/ETIK/2012. Survey of this study was conducted through four stages. The first stage was collecting porcine blood serum, determining ordinate points and interview using questionnaire. The second stage was laboratory testing for porcine sera blood using *monoclonal antibody-based sandwich enzyme-linked immunosorbent assay* (MoAb-ELISA). The third stage was postmortem examination towards seropositive porcine. The fourth stage was statistical analysis.

Samples size in each subdistrict was obtained systematic randomly and propotionally based on distribution of pigs population and farmers. The total sum of pigs population and farmers in Way Kanan District was calculated using formula $n = 4pq/L^2$, with level of confidence 95%, n =required sample size, p=prevalence (12%), L = precision (5%) and q = 1-p. Assumed that estimated prevalence of cysticercosis was 12%, the sample size for pigs was calculated 169 samples.

Porcine Blood Sera Collection: 169 porcine sera were collected from 7 subdistricts (Table 1). Positive control serum was obtained from Bolakme Subdistrict in Jayawijaya District, Papua Province. It was cysticercosis positive for occuring in postmortem examination in meat. Negative control serum was obtained from pig husbandry in Central Java Province.

Blood was collected from porcine jugularis venous, left a while until it formed serum. Sera were then taken into microtube and stored at -20°C until tested. They were tested using ELISA method in Veterinary Medicine Faculty of Bogor Agriculture University.

ELISA of Metacestode Antigenic: Pre-treatment sera was conducted by mixing 150 µl trichloroacetic acid (TCA) (5%) solution and 150 µl serum sample. A similar step was conducted to positive control sera. A negative control sera was done by mixing 75 µl TCA (5%) solution and 75 µl serum sample. The mixture of solution was mixed by vortex and incubated for 20 minutes at ambient temperature. After that, the solution was mixed and centrifuged at 1200 g for 9 minutes. The mixture solution was neutralized by adding 75 µl (negative control) or 150 µl (other sample) of the supernatant into microtubes with the same amount of neutralization buffer [20].

After pre treatment step has done, the next step was coated the ELISA plate. All of the wells of plate were coated with 100 µl of capturing antibody (B158C11A10) (5 µg/ml coating buffer) except the 2 wells for the substrate control. A hundred micro liters of coating buffer was put into those wells. It was incubated at 37 °C while shaking for 30 minutes. Then, it washed once with washing buffer and blocked all wells with 150 µl of blocking buffer. It was incubated for 15 minutes at 37 °C while shaking. The step did not wash. A hundred micro liters of pre treated samples were put into designate wells. Blocking buffer (100 µl) was filled for substrate and conjugate controls. Then, it incubated for 15 minutes at 37 °C while shaking. It also washed 5 times with washing buffer. A hundred micro liters of detecting antibody (B60H8A4) (1.25 µg/ml blocking buffer) was put in all wells, except the 2 wells for 4 the substrate control. Then, put 100 µl blocking buffer in those wells and

Table 1: Number and location of porcine samples

			Sex			Species	
No.	Subdistricts	Number	Male	Female	Landrace	Cross breed	Local
1	Banjit	82	34		28	16	38
2	Negeri agung	44	22	22	14	0	30
3	Pakuan Ratu	11	2	9	0	0	11
4	Baradatu	10	9	1	10	0	0
5	Bumi Agung	10	6	4	0	0	10
6	Buay Bahuga	6	3	3	0	0	6
7	Blambangan Umpu	6	2	4	0	2	4
Total	169	78	91	52	18	99	

incubated for 15 minutes at 37 °C while shaking. It was washed 5 times with washing buffer. This was also the time to take the OPD out of fridge. 100 µl of peroxidase labeled streptavidin (1/10000 in blocking buffer) was put in all wells, except the 2 wells for the substrate control. Put 100 µl of blocking buffer in those wells and incubated for 15 minutes at 37 °C while shaking. It also was washed 5 times with washing buffer. 2.5 µl of H_2O_2 was added before put 100 µl of OPD on the plate. Then, incubated for 15 minutes at 30 °C in the dark and did not shake. The reaction stopped with the addition of 50 µl H_2SO_4 (4N) in each well. The last step was read the plate at 492 and 655 nm [20].

Postmortem Examination: Postmortem examination was conducted for positive serologic porcine. It aimed to find cysticercus in predilection organs of suspected cysticercosis porcine.

Microscopic Identification: Histopathology identification was conducted to scolex of cyst. Scolex was collected by pressing the cyst with scalpel. Scolex cutting was conducted as thin as possible. Then, it was washed in clove oil to get the transparent sample. It was soaked in 50%, 70% and 80% alcohol and absolute alcohol. Then, it was pressed using the cover glass and located in object glass, mounted and covered. Species identification was conducted by microscope.

Questioner Data Collection: Risk factors of cysticercosis were determined by interviewing questioner towards 42 farmers from 7 sub districts. The questioners' data consist of characterization of farmers, management of pig maintenance and farmers lifestyles.

Statistical Analysis: Mean of absorbance value from each serum was calculated. Determination of cut off value was obtained from *t-student* value of negative control. Sera samples status were determined according to the mean ratio of absorbance value towards cut off value. If ratio value is more than 1, the interpretation of serum will be positive. If ratio value is less than 1, the interpretation of serum will be positive.The positive serum shows that there is *Cysticercus cellulosae* antigen in serum [20, 21]. Relation degrees between risk factor and disease case was got by calculating odds ratio value (OR) and Regression Logistic.

RESULT

General Description of Studying Location: Way Kanan District located on 104.17° - 105.04° EL and 4.12° - 4.58° SL (Figure 1). There is no sea boundary in Way Kanan District. The broad of Way Kanan area is 3.921,63 km^2. It consists of 14 subdistricts. The north of district adjacent with South Sumatera Province while the south adjacent with North Lampung District. The east of district adjacent with Tulang Bawang District and the west adjacent with West Lampung District [22].

In 2013, Population in Way Kanan District is 415.078 people with comparison of sex are 214.672 males and 200.406 females. Sex Ratio in 2013 is 107. The highest density is in Baradatu Subdistrict about 236 people in one km. Demography of the district classified into flat with 0-15% slope and wave area with 15-25% slopes. The slopes are steep with variety height between 450-1700 dpl [22].

Characterization of Pig Farmers: The result of the questioner to the pig farmers showed that the pig husbandry was more managed with male farmers (95.2%) than female farmers (4.8%). The farms were managed by themselves (88.1%) and managed by laborants (11.9%). Almost farmers were 50 years old (38.1%). Education backgrounds of them were senior high school degree (42.9%). Pig husbandry in Way Kanan District was a secondary occupation besides agriculture farming (78.6%). The data showed in Table 2.

Farm Maintenance Management: According to fields inspection and result of questioner showed that there were three types of pig species such as landrace, local and mix between landrace and local. Commonly, the maintenance type of pig was intensive. Pig farm

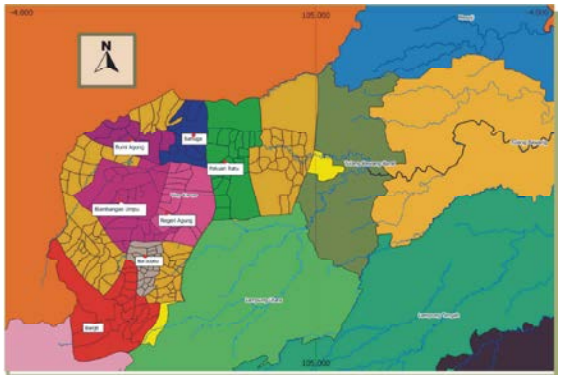

Fig 1: Geographic map of Way Kanan District

Table 2: Characterization of pig farmers in Way Kanan District

No	Data	Number	%
1	Sex		
	Male	40	95.2
	Female	2	4.8
2	Age		
	< 20	1	2.4
	20 - 29	1	2.4
	30 - 39	9	21.4
	40 - 49	15	35.7
	> 50	16	38.1
3	Education Background		
	Elementary School	8	19
	Junior High School	7	16.7
	Senior High School`	18	42.9
	Bachelor degree	5	11.9
	No education	4	9.5
4	Occupation		
	Agriculture Farmers	33	78.6
	Husbandry Farmers	4	9.5
	Business	3	7.1
	Government	2	4.8

management type in Way Kanan District was conducted with pen (81%), free-range (12%) and bound (7%). Although pigs managed in a pigpen, but most of them since born until weaned were wild. Piglets were find out their own feed and free to enter their pen and fields. The structure of pen was permanent that made from cement (75.74%). Bamboo and wooden pen were 11.24% and wild 13.02%. Environment around the pen is common clean (57%). The farmers washed the pen in the morning and evening (66.86%). Source of water availability in farm showed that 88.7% they have clean wash water treatment and 11.83% water in husbandry come from river. The habit of farmers defecation use toilet facilities (88.17%) and river (11.83%). Information from questionere show that the farmers were defecation in field in dry season. Supporting data about farm and animal health of pigs from local government has not been found.

The age of pigs that collected is from a month to 5 years old. Questioner data shows that most of pigs were sold between 3 until 6 months old. They consumed pork for ceremony of their religion and daily consumption. The distribution areas of pigs were located in Jakarta, South Sumatera and North Sumatera. Way Kanan societies consume pork rarely but wild boars.

Cysticercosis Seroprevalence: The result of ELISA towards 169 porcine sera from Way Kanan District shows that seropositive of cysticercosis found in 3 samples (Table 3). Two seropositive samples come from Negeri Agung Subdistrict and one sample come from Pakuan Ratu Subdistrict (Figure 1).

Cysticercosis Seroprevalence Based on Sex: According to porcine sex, there were 95 male porcine and 104 female porcine (Table 4). Three of the samples were female porcine seropositives.

Postmortem Examination: Postmortem examination was conducted by seropositive porcine. This study found out 3 seropositives. Necropsy was only done in 2 porcine, while the last porcine has been sold by the farmers. Cysticercus was found out of first porcine. It showed white color in intercoastal muscles. It formed whitish, semitransparent vesicle that measures around 1 cm.

Risk Factors: Risk factors which are cause porcine cysticercosis are age, sex, maintenance management, farming system, water availability, feed, pork consumption, house slaughtering availability and latrine availability [23-25].

Risk factors which affected Cysticercosis in this study were age (OR = 1.083; 95% CI = 1.023 - 1.142). The older porcine has otentially risked 1.083 times than the younger.

Table 3: Seroprevalence of porcine cysticercosis in Way Kanan District

			Result		
No.	Subdistricts	Number	Positive	Negative	Prevalence (%)
1	Banjit	82	0	82	0
2	Negeri Agung	44	2	42	4.55
3	Pakuan Ratu	11	1	10	9.1
4	Baradatu	10	0	10	0
5	Bumi Agung	10	0	10	0
6	Buay Bahuga	6	0	6	0
7	Blambangan Umpu	6	0	6	0
Total		169	3	166	1.78

Table 4: Seroprevalence based on sex

			Result			
No.	Sex	Number	Positive	%	Negative	%
1	Male	78	0	0	78	100
2	Female	91	3	3.3	88	96.7
Total		169	3	1.78	166	98.22

DISCUSSION

The study showed that Way Kanan District was non endemic area of porcine cysticercosis with seroprevalence 1.78% in this study. Cysticercus of first porcine was found in intercoastal muscles at postmortem examination and serologically was seropositive. In second porcine, cysticercosis was negative. According to Dorny *et al*. [15], sensitivity and specificity values obtained in postmortem examination were 38.7% and 100%. There were difference between sensitivity and specificity values obtained in antibody detection of ELISA and antigen detection of ELISA. Sensitivity values of antibody detection (45.2%) was lower than antigen detection (64.5%). Meanwhile, specificity values of antibody detection of ELISA (88.2%) was higher than antigen value (91.2%). The low sensitivity of post mortem examination related to none cysticercus. Optical density in second porcine was lower than first porcine. It showed that cysticercosis transmission in first porcine was higher than the second one. According to Gavidia *et al*. [26], existence of antibody towards *T. solium* does not related on cyst finding in necropsy. It caused by maternal antibody transfer for months, prior effective treatment, past infection that has been cleared (degenerated or caseous cysts), exposure to *T. solium* eggs without development of observable cysts (not enough time for the cyst to develop at the time of the necropsy), or the ingestion of non-viable or infective eggs among other possible explanations.

Pigs that had seropositive results were female but no significant difference statistically. It appropriated with the study of Garcia *et al*. [13] that sex did not affect significantly with porcine cysticercosis seroprevalence. Ngwing *et al.* [25] studied of 499 porcine in Kamerun and showed that sex did not lead to significant difference towards cysticercosis cases. The result of Morales' study [27] towards pregnancy in sows and castration of male pigs showed that cysticercosis case increase 20 to 50%. It caused by difference of androgen and progesterone hormone. According to Escobedo *et al.* [28], progesterone gave positive correlation in scolex evagination in tissue and oncosfer motility. Higher progesterone lead to scolex evagination and cysticercosis motility were higher too.

Porcine age was a significant risk factor in this study. The older porcine has cysticercosis infection about 1.083 higher than the younger porcine. Porcine in this study were aged between 1 month and 5 years old. Pouedet e*t al*. [29] conducted study in Kamerun and got result that adult pig significantly had higher seroprevalence (15%) that younger pigs (8.4%). The same result occurred in study of Pondja *et al.* [24] in Mozambique and Sarti et al. [30] in Meksiko. Pigs could become infected at any age. They pick up eggs at the age of 2 to 4 weeks and the metacestodes are present in the liver. In older animals aged 4 to 6 months, the larvae were also found in the muscles. In a 6-month-old pig larvae were found in the muscles and brain [31]. Breeders have a significantly higher prevalence than weaners. Similarly fatteners had a significantly higher prevalence than weaned pigs [32]. The frequency of human faeces consumption was higher during the dry season. In both seasons the leaders of the groups, which were usually adult females, ate human faeces significantly more frequently than other pigs [33]. Older pigs might have had more chance to get exposure to *T. solium* eggs than younger ones and more time for cyst to develop and trigger the production of circulating antibodies. Older pigs were more than 2 times likely to be seropositive as compared with younger ones. Besides, it could be possible that younger pigs are protected during their first months of life against parasite infection, perhaps due to the presence of maternal cysticercus-antibodies (passive immunity) and become susceptible later after the slow clearance of those antibodies [26].

Generally, pig farms management in Way Kanan District was better. It showed by maintenance of pig in permanent pen. Routinely, pen was cleaned twice a day in the morning and evening. Farmers' lifestyles were also better than they used to be. They had habits to wash their hands after defecation. They were also defecation in toilet. Water source came from draw well. The habit of defecation in field on dry season could cause

environment contamination. Probably it caused by Taenia eggs and assumed a carrier among them. The risk of pigs to contact with Taeniid eggs, however, is reduced by the high number of farmers use toilet facilities.

Farm management and good sanitation will decrease risk of porcine cysticercosis case. It appropriated with the study of Suroso *et al*. [19] that found the good sanitation and management would decrease cysticercosis and taeniasis significantly. A study in Kamerun found that there were no significant differences on porcine cysticercosis which were managed in house with toilet [29]. The study which conducted by Secka *et al*. [34] in Gambia dan Senegal showed that availability of toilet was not risk factor in cysticercosis case. According to Margono *et al*. [14], infection frequently occurs in populations living in poor sanitary conditions and people infected with *T. solium* can initiate the spread of proglottids into an endemic environment. Cysticercosis is, therefore, a communicable infectious disease among humans residing in poor and unhygienic communities. Defecation in field caused existence of *T. solium* in environment [35].

This study used specific Ag-ELISA method to detect antigen of other metacestode based on cross reaction. Antigen of other cysticercosis could be detected such *Cysticercus bovis* (*T. saginata*), metacestode of *T. asiatica* and *Cysticercus tenuicollis* of *T. hydatigena* [16]. Cross reaction among antigens were caused by similarity of antigenic component of cysticercosis which could be catched from antibody [36, 37]. The cross reaction will disturbed accuracy of test if it used to pig population in endemic area of *Taenia sp.* except *T. solium*. *Cysticercus tenuicollis* of pig in Way Kanan District had not been reported. The Ag-ELISA, which has been validated by postmortem examination. Cysticercus of porcine was found in postmortem examination. The cysticercus is a whitish, semitransparent vesicle that measures around 1-2 cm and has a spherical scolex inside [38]. C ysticercosis hook that found in this study was similar with *T. solium* [39]. In pigs, cysticerci are mainly found in the muscles of neck, shoulder, thigh, tongue, heart and brain. In heavily infected cases, cysts are also found in the liver, lungs and intercoastal muscles [40]. *T. asiatica* cysticercus was not found in muscle but in liver, omentum, serosa and lung [41]. It concluded that seroprevalence of this study probably was *Cysticercus cellulosae*.

Cysticercosis seroprevalence in Lampung on 1981 was 12.3% [42]. Seroprevalence value in this study was declined because some factors. They were better pig maintenance management, sanitation and farmer lifestyle. Recently, habits of defecation in fields are not occurred to cut off hooks life cycle. *T. solium* cysticercosis in Lampung was occurred sporadically [43].

Way Kanan District does not have pig abattoirs and no inspection of porcine carcases by the veterinary or medical personal. Many pigs are slaughtered for consumption during family festivities or sold to customers on demand without any carcase inspection. Under this kind of situation that evidence of porcine cysticercasis has been demonstrated, humans are risk to get taeniasis/cysticercosis.

Cysticercosis that transmitted in Way Kanan District was found in Pakuan Ratu and Negeri Agung Subdistricts. Balinese village location is close tor each other, so that the parasite would be existed. By this reason, controlling and preventing of the parasite should be done with noticed of religion, culture, education background and socio economic aspects. There are some ways to reduce diseases transmision risk such as increasing public awareness animal health, increasing animal health service, monitoring pork distribution and continuing field surveillance.

CONCLUSION

Seroprevalence of porcine Cysticercosis in Way Kanan District was 1.78%. It showed that Way Kanan District is an endemic area. Risk factors of cysticercosis were the age of porcine, farmers' lifestyle and farm maintenance management.

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