

Original Research Article

Strongly Serological Evidence of Meat-Borne Disease Due to Cysticercosis and Toxoplasmosis Infections in the Provinces of Bali and East Nusa Tenggara of Indonesia in 2019

I Wayan Masa Tenaya^{1*}, Ida Bagus Swacita¹, Kadek Karang Agustina¹, Putu Henrywaesa Sudipa², Ni Made Arsani³¹Departement of Veterinary Publics Health, Faculty of Veterinary Medicine, Udayana University, Indonesia²Laboratory of Veterinary Bacteriology and Mycology, Faculty of Veterinary Medicine, Udayana University, Indonesia³Veterinary Disease Investigation Center Denpasar, Indonesia**Article History**

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Abstract: Cysticercosis and toxoplasmosis are parasitic diseases that are included in the list of 25 strategic infectious animal diseases in Indonesia based on the decree of the Minister of Agriculture No. 4026/Kpts/OT.140/4/2013. It emphasized the strategic policy to control mainly zoonotic diseases. This study aimed to investigate the current seroprevalence level of cysticercosis and toxoplasmosis in pigs in the provinces of Bali and East Nusa Tenggara. A cross-sectional study was conducted by taking 214 pig serum samples from smallholder farms. The samples were tested by specific ELISA for both cysticercosis and toxoplasmosis. Of the 214 serum samples tested, 147 (68.69%) were antibody positive to cysticercosis, and 111 (51.87%) were antibody positive to toxoplasmosis respectively. This finding suggested the strong evidence of infection of those parasitic agents where surveillance was conducted which potentially threaten human health. Therefore, food safety supervision must be tightened, including through counseling or the application of legal sanctions.

Keywords: Cysticercosis, toxoplasmosis, ELISA, antibody, Bali, Nusa Tenggara Timur.

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INTRODUCTION

Cysticercosis and toxoplasmosis are zoonotic-parasitic diseases that spread all over the world, including Indonesia. This disease is often found in areas where people do consume raw or undercooked beef or pork. In addition, poor environmental sanitation conditions where livestock food/drinking water sources can be contaminated with human feces is a risk factor by not deliberately transmit the disease. The first parasitic disease caused by the larvae (metacestode form) of tapeworms of the genus *Taenia* and some of its species are zoonotic and humans can act as definitive hosts, intermediate hosts, or both. Humans are the definitive host of *T. solium* and *T. saginata*, and also the definitive host of *T. Asiatica*. Moreover, humans can also act as intermediate hosts for *T. solium* and *T. Asiatica*. Apart from humans, the intermediate hosts for *T. solium* are pigs, while the intermediate hosts for *T. saginata* are cows. Cases of cysticercosis are often associated with people's habits of consuming raw or undercooked meat. Humans can become infected with cysticercosis/taeniasis by eating beef or pork that

contains larvae (cysticercus). Transmission of cysticercosis can also be through food or drinking water sources contaminated by *Taenia spp* worm eggs. Epilepsy and neurocysticercosis cases in humans are thought to be related to taeniasis and cysticercosis in animals. In Indonesia, cases of taeniasis/cysticercosis have been found in the provinces of North Sumatra, Papua, Bali, North Sulawesi, Southeast Sulawesi, NTT, and West Kalimantan [1]. In Bali, cysticercosis occurs, both in pigs and in cattle. Its prevalence in cattle in four districts in Bali including Badung, Gianyar, Klungkung, and Tabanan in 1977 was 3.3, 16.9, 1.2, and 8.3% respectively [1]. In a 1981 serological survey, 21% of serum samples from the Balinese population tested were positive for cysticercosis.

Toxoplasmosis is a blood circulating parasite caused by the sporozoa *Toxoplasma gondii*, an intracellular parasite that mostly infects humans and pets. Patients with toxoplasmosis often do not show clear clinical symptoms so that diagnosis of toxoplasmosis disease is often neglected. If the disease affects pregnant women in the third trimester, it can

cause hydrocephalus, chorioretinitis, deafness, or epilepsy. This parasite infects a variety of warm-blooded animals including cats, dogs, and humans. Infection with toxoplasmosis can occur due to ingestion of cysts in undercooked or raw meat tissue or by accidental ingestion of oocysts from the environment. *Toxoplasma gondii* only undergoes asexual (schizogony) and sexual (gametogony) proliferation in the definitive host, namely cats, and other *Felidae* species, so that the definitive host serves as the only place where oocysts are produced. Oocysts are stable in the environment after being excreted in the feces. Oocysts can be still infectious for approximately two years, and cause widespread contamination, and become a source of infection for humans and other intermediate hosts. Domestic cats are a major source of infection in humans and other potential hosts.

Pork is considered a major source of human infection for *Toxoplasma gondii* in Europe and the United States. The parasite has been isolated from unprocessed infected pig tissue as well as from processed meat cuts such as ham, bacon, and pork tenderloin. The possible source of naturally acquired porcine *Toxoplasma gondii* infection has been investigated in studies evaluating various risk factors for its infection in porcine. The direct access of cats to pig feed and high cat population on farms are positively associated with the rate of *Toxoplasma gondii* seropositivity in pigs. Inadequate rodent control was also associated with positive antibody levels of *Toxoplasma gondii* in pigs, indicating that infected mice are a possible source of *Toxoplasma gondii* infection for pigs. The seroprevalence of pigs in Estonia against *Toxoplasma gondii* is 5.8% [2], while the prevalence of IgM and IgG antibodies against *Toxoplasma gondii* in fattening pigs in Yucatan Mexico is 92.5% [3].

Another route of infection of *Toxoplasma gondii* in humans and animals is by ingestion of oocysts from cat feces. Oocysts are highly resistant to environmental conditions and contaminate water, soil, dust, vegetables, and fruits. However, infection through ingestion of tissue cysts in meat is considered to be one of the main sources of infection in humans. Between 30% and 60% of pregnant women who eat undercooked meat can develop acute toxoplasmosis. The low prevalence of toxoplasmosis found in a group of vegetarians (24%) confirms the suspicion that meat consumption is one of the most important modes of transmission of *Toxoplasma gondii* to humans. Today, after the *Toxoplasma* life cycle is discovered, it is hoped that prevention efforts will be easier to do. At this time the diagnosis of toxoplasmosis becomes easier to find because of the presence of IgM or IgG antibodies in the patient's blood.

The provinces of Bali and East Nusa Tenggara are the working areas of the Denpasar Veterinary Center with a fairly high pig population. The population of pigs in the province of Bali is estimated at 725,219, and the province of NTT 1,176,201 heads respectively (BPS, 2013). As an area with a non-Muslim majority population, pigs in those provinces are a leading commodity, as a source of animal protein and as a source of income for the community, especially livestock farmers. The existence of infectious animal diseases will of course greatly affect the social economy of the community, especially for zoonotic diseases in pigs which affect the public's sense of security in consuming materials of animal origin. Serological surveys of cysticercosis and toxoplasmosis have not been conducted. This survey is a preliminary study, with a special aim to estimate the seroprevalence of cysticercosis and toxoplasmosis in pigs. The results of this survey are expected to be used as a basis for the prevention and control of cysticercosis and toxoplasmosis in animals as well as actions and precautions that need to be taken so that they are not transmitted to humans.

MATERIALS AND METHODS

Surveillance activities were carried out to collect serum samples of pigs on smallholder farms from eight districts in Bali and Kupang, a district of East Nusa Tenggara respectively, based on Guidelines for surveillance [5]. A total of 214 serum samples were successfully collected (Table 1). The serum samples were obtained aseptically from the jugular vein and prepared for serum stored at -20° before being tested. The samples were tested at the same time and condition using Cysticercosis ELISA kit following the instruction procedures provided by the company test procedure listed on the Kit brochure (ApoDia, Ref.650501-Turnhout Belgium). Meanwhile, for serological testing to *Toxoplasma* antibodies, we used ELISA kit ID Screen® *Toxoplasmosis* Indirect Multi-species (rue Louis Pasteur-Grabels-FRANCE) according to the instruction manual in the Kit. All testing processing was done at the Denpasar Veterinary Center in 2019 under budgeting from the Ministry of Agriculture of Indonesia.

RESULT

ELISA test for both Cysticercosis and *Toxoplasmosis* serum samples showed that of the 214 samples tested, 147 (68.69%) were positive for cysticercosis and 111 (51.87%) were positive specific antibodies respectively (Table 1 and Table 2). The number of samples taken and the prevalence of antibodies to cysticercosis and toxoplasmosis in each district can be seen in Table 3 and Table 4.

Table-1: Prevalence of antibodies to Cysticercosis in Bali and East Nusa Tenggara Provinces

| Provinces | Sero (-) | Sero (+) | Total | Prev. (%) | 95% CI |
|-------------|----------|----------|-------|-----------|----------------------|
| Bali | 48 | 116 | 164 | 70.73 | 63.36 - 77.16 |
| NTT | 19 | 31 | 50 | 62.00 | 48.15 –74.14 |
| Grand Total | 67 | 147 | 214 | 68.69 | 62.19 – 74.53 |

Note: CI=Confiden interval.

Table-2: Prevalence of antibodies to Toxoplasmosis in Bali and East Nusa Tenggara Provinces

| Provinces | Sero (-) | Sero (+) | Total | Prev. (%) | 95% CI |
|-----------|----------|----------|-------|-----------|---------------|
| Bali | 73 | 91 | 164 | 55.49 | 47.84 – 62.88 |
| NTT | 30 | 20 | 50 | 40.00 | 27.61 – 53.82 |
| Total | 103 | 111 | 214 | 51.87 | 45.20 – 58.47 |

Note: CI=Confiden interval

Table-3: Prevalence of antibodies to Cysticercosis based on district origins in Bali and East Nusa Tenggara Provinces.

| Provinces/Districts | Sero (-) | Sero (+) | Total | Prev.(%) | 95% CI |
|------------------------------|----------|----------|-------|----------|---------------|
| Bali | 48 | 116 | 164 | 70.73 | 63.36 - 77.16 |
| Badung | 9 | 11 | 20 | 55.00 | 34.21 – 74.18 |
| Bangli | 2 | 38 | 40 | 95.00 | 83.50- 98.62 |
| Buleleng | 1 | 3 | 4 | 75.00 | 30.06 – 95.44 |
| Denpasar | 7 | 13 | 20 | 65.00 | 43.29- 81.88 |
| Gianyar | 13 | 27 | 40 | 67.50 | 52.02-79.92 |
| Jembrana | 0 | 20 | 20 | 100.00 | 83.89-100.00 |
| Karangasem | 16 | 4 | 20 | 20.00 | 08.07 – 41.60 |
| East Nusa Tenggara Provinces | 19 | 31 | 50 | 62.00 | 48.15 –74.14 |
| Kupang | 19 | 31 | 50 | 62.00 | 48.15 –74.14 |
| Total | 67 | 147 | 214 | 68.69 | 62.19 – 74.53 |

Note: CI=Confiden interval

Table 4: Prevalence of antibodies to Toxoplasmosis based on district origins in Bali and East Nusa Tenggara Provinces.

| Provinces/Districts | Seronegatif | Seropositif | Total | Prev.(%) | 95% CI |
|---------------------|-------------|-------------|-------|----------|--------------|
| Bali | 73 | 91 | 164 | 55.49 | 47.84 –62.88 |
| Badung | 7 | 13 | 20 | 65.00 | 43.29- 81.88 |
| Bangli | 24 | 16 | 40 | 40.00 | 26.35 –55.40 |
| Buleleng | 2 | 2 | 4 | 50.00 | 1500 – 85.00 |
| Denpasar | 2 | 18 | 20 | 90.00 | 69.90-97.21 |
| Gianyar | 19 | 21 | 40 | 52.50 | 37.50 –67.06 |
| Jembrana | 12 | 8 | 20 | 40.00 | 26.35-55.40 |
| Karang Asem | 7 | 13 | 20 | 65.00 | 43.29- 81.88 |
| NTT | 30 | 20 | 50 | 40.00 | 26.35-55.40 |
| Kupang | 30 | 20 | 50 | 40.00 | 26.35-55.40 |
| Total | 103 | 111 | 214 | 51.87 | 45.20 –58.47 |

Note: CI=Confiden interval

DISCUSSION

The seroprevalence of cysticercosis and toxoplasmosis varies widely throughout the world. Of the 214 porcine sera tested from the provinces of Bali and East Nusa Tenggara showed a seropositive reaction to cysticercosis of 147 samples (68.69%). The prevalence of the diseases in Bali was 116 (70.73%), higher than those found in East Nusa Tenggara which was 50 (62.00%). The highest prevalence of seropositive in Bali were 100% and 95% detected from samples originated from Jembrana and Bangli

respectively, followed by the lowest prevalence of 20% those from Karangasem. This wide variation may be due to different levels number of factors mainly environmental conditions, livestock management systems, the density of pig population, and farmer knowledge. Similarly, research conducted in 2009 and 2011 in several districts and markets in Jayawijaya district Papua showed that seroprevalence in pigs was 14.29% to 92.86% [6]. Then [7] reported the seroprevalence of cysticercosis in pigs in Papua was only 23.5%, and the seroprevalence varied found in Jayawijaya district of 42.6%, Biak 22.5%, Nabire

20.6%, Mimika 17%, Jayapura 13, 5%, and Merauke 1.9%. In some areas of Brazil, the reported seroprevalence of cysticercosis in pigs was 4.4% in Salvador, 3.2% in Santo Amaro, and 23.5% in Jequié [8]. In Cambodia, it was 4.7% [9], while in Mozambique, [10] found that 34.9% of pig serum tested by Ag-ELISA was positive for cysticercosis. [11] reported the results of their study in Tanzania where 33.3% of pigs were positive for cysticercosis by the Ag-ELISA test, while [12] reported 4% of pigs in Kenya [13] also reported that the seroprevalence of cysticercosis in pigs in Maharashtra tested by ELISA was 8.5%.

The seroprevalence study was done in Bali and East Nusa Tenggara were included in the high category, which was 68.69%. This data suggested a high exposure of the disease-causing agent in the pig population in the sampled area. However, it does not rule out the possibility of false-positive results due to cross-reactions in the test. Because this was a preliminary study, these results can be used as lessons so that in future studies improvements can be made, among others, by increasing accuracy and skills in testing and selecting a more suitable ELISA kit. Currently available antibody detection tests cannot differentiate between active and inactive infection. False-positive results may also occur in cattle with other helminth infections (eg, *Echinococcus*).

The seroprevalence of toxoplasmosis in the provinces of Bali and NTT showed a seropositive of 51.87%, in Bali province, was 55.49% and 40% was detected in East Nusa Tenggara. However, compared to the prevalence of Cysticercosis within Bali provinces, the highest seroprevalence of Toxoplasmosis was reported from Denpasar district of (90%), followed by Badung and Karangasem with were similarly of 65%, while the lowest were samples originated Bangli and Jembrana districts which were 40 % each. Reported data by other workers, using the Indirect Haemagglutination Assay also reported varied results [14]. reported the presence of antibodies to *Toxoplasma gondii* in pigs in the Baliem Valley was 75.9% and in the Arfak Mountains of Papua was 25%. Then [15] found that the seroprevalence in West Lore District, Poso Regency, and Central Sulawesi was 24%. The results of a study reported in 2013 in Yucatan Mexico showed the prevalence of IgM and IgG against *Toxoplasma gondii* was 92.5% in fattening pigs and 33.7% in captive Danish sows [16]. In Central China, pigs had a seroprevalence of *Toxoplasma gondii* of 24.5% [17], while pigs in Jilin Province of China were 19.1% [18, 19]. Found the prevalence of *Toxoplasma gondii* in wild boars in Latvia was 33.2%, pigs in Ghana 39% [20], pigs in Guangdong Province China between 0 and 58.1% [21]. A possible source of infection in pigs can be due to constant contact with infective oocysts of *Toxoplasma gondii* present on farms, either in water, soil, or air sources, which are commonly found in swine

production systems. *Toxoplasma gondii* oocysts can survive for several years and tolerate extreme temperatures and humidity in the environment and they are capable of producing infection through contact with susceptible animals. Likewise, the presence of cats in agricultural production systems can increase the spread of oocyst pollutants. Infection rates should be reduced in pig farms with particular emphasis on cat and mouse control. Even if cats aren't present at the moment, oocyst contamination can persist on farms for a long time. It is important to consider that other risk factors such as cannibalism are another route of *Toxoplasma gondii* infection when pigs eat tissue cysts from mice or other pigs. Food storage is another factor to consider. Outside or in sheds without cat control, contamination of food by oocysts can occur.

CONCLUSIONS

There was strong evidence of meat-borne disease associated with parasite infections in some districts of Bali and East Nusa Tenggara Provinces. A serological based assessment conducted in the current survey showed a high level of 68.69% and 51.87% of cysticercosis and toxoplasmosis respectively. Although the level of seroprevalence of the parasite varied accordingly to different districts in the two provinces, it was considered as a risk factor that potentially threatened human health. A wide difference in the results of surveillance for both cysticercosis and toxoplasmosis in some regions may mainly be due to environmental conditions, and farmer knowledge. To prevent transmission of both zoonotic diseases that originated from pigs, it is necessary to educate the public so that pigs are kept in cages and try to keep pigs' feed and drinking water sources from being contaminated with human feces. Also, prevent the entry of cats and rats into the cage area and try to ensure that feed and drinking water sources for pigs are not contaminated with cat feces. The need to increase vigilance in consuming products made from pork to avoid cysticercosis and toxoplasmosis. Food safety supervision must also be tightened, including through counseling or the application of legal sanctions.

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