

Experimental Infection of *Taenia saginata* eggs in Bali Cattle: Distribution and Density of *Cysticercus bovis*

(INFEKSI EKSPERIMENTAL TELUR *TAENIA SAGINATA* PADA SAPI BALI: PENYEBARAN DAN KEPADATAN *CYSTICERCUS BOVIS*)

Nyoman Sadra Dharmawan¹⁾, I Made Damriyasa¹⁾, I Nengah Kapti²⁾,
Putu Sutisna²⁾, Munehiro Okamoto³⁾ and Akira Ito⁴⁾

¹⁾Center for Studies on Animal Diseases, Faculty of Veterinary Medicine, Udayana University, Bukit Jimbaran, Kuta, Bali, Indonesia, Phone/Fax: 0361-701808; E-mail: nsdharmawan@yahoo.com

²⁾Department of Parasitology, Faculty of Medicine, Udayana University, Bali, Indonesia;

³⁾Department of Parasitology, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan,

⁴⁾Department of Parasitology, Asahikawa Medical College, Midorigaoka-Higashi 2-1-1-1, Asahikawa, Japan.

ABSTRACT

The objective of this study was to observe the development, distribution, and infection density of *Taenia saginata* metacestodes in Bali cattle. Three Bali cattle were experimentally infected with *T. saginata* eggs which were collected from taeniasis patients. The experimental animal was inoculated with : i) 1000,00 *T. saginata*; ii) 500,000 eggs; and iii) 1,000,000 eggs, respectively 100,000 (cattle 1), 500,000 (cattle 2), and 1,000,000 (cattle 3) *T. saginata* eggs, respectively. To observe the development of cysticerci, all cattle were slaughtered at 24 weeks post infection. To observe their distribution and density, slicing was done to the cattle's tissues. The study results showed that cysts were found distributed to all muscle tissues and some visceral organs such as heart, diaphragm, lungs, and kidney of the cattle infected with 100,000 and 500,000 *T. saginata* eggs. Density of the cyst was in the range of 11 to 95 cysts per 100 grams of tissue. The highest density was noted in the heart (58/100 grams) and in diaphragm (55/100 grams). This study has confirmed that *T. saginata* eggs derived from taeniasis patient in Bali, if infected to Bali cattle can develop and spread to all muscle tissues and some visceral organs. From this study it was concluded that it is necessary to include the heart in the meat inspection at slaughter house for possibility of *T. saginata* cyst infection.

Keywords: *T. saginata* cyst; Bali cattle; experimental infection.

INTRODUCTION

A disease caused by infection of the tapeworm *T. saginata* is known as *T. saginata* taeniasis or beef tapeworm infection. Infection of this tapeworm in human has been known since pre-historical time. In this infection human is the definitive host, while cattle is the intermediate host. In human, the adult worm inhabits the intestine to cause taeniasis and the metacestode inhabits the tissue of cattle to cause cysticercosis (Margono *et al.*, 2005; Abuseir *et al.*, 2006; Kebede, 2008).

The tapeworm can cause a significant economic loss, especially in meat industries and for cattle breeders, besides the hazards caused on human community health (Abuseir *et al.*,

2006; Dorny and Praet, 2007; Flüttsch *et al.*, 2008). The infection is cosmopolitan in its distribution with the highest incidence being found in countries whose inhabitants like to consume beef. Its geographic distribution includes Europe, Middle East, Africa, Asia, North America, Latin America, Russia (Pawlowski and Schultz, 1972; Dharmawan, 2000; Ito *et al.*, 2005; Myadagsuren *et al.*, 2007). Human may be infected when they consume raw or half-cooked beef that contains cysticerci. On the other hand, cattle are infected with cysticerci when they eat grass that has been contaminated with eggs contained in feces of a taeniasis patient.

The prevalence of *T. saginata* in Bali is fairly high. In an epidemiological study done by Wandra *et al.*, (2006) in three locations in Bali namely

Gianyar, Badung and Denpasar, 60 of 398 persons belonging to 247 households were diagnosed to have taeniasis by the method of *questionnaire responses and demonstration of proglottids* (QDRP); and of these 56 persons (14.1%) expelled *Taenia* after being treated with praziquantel. From result of this study they concluded that the prevalence of *T. saginata* taeniasis in the village of Ketewel, Gianyar Regency, Bali had increased significantly from 2.1% in 1977 and 1.3% in 1999 to 14.1% in 2006 (Simanjuntak *et al.*, 1977; Sutisna *et al.*, 2000; Wandra *et al.*, 2006).

Until now studies on the biological development of *C. bovis* in Bali cattle remain rare. Dharmawan (2000) did an experimental infection of *T. saginata* gravid proglottids in Bali cattle and found that oncospheres of *T. saginata* successfully developed to become *C. bovis* in about eight weeks after infection. However, this experimental study did not observe in more detail about the distribution of cysts in the cattle's tissues. The following report contains results of our experimental study with the objective of observing the biological aspect of host-parasite relationship between *T. saginata* and Bali cattle, particularly on the distribution and density level of cysticerci in cattle. This experimental study was carried out using *T. saginata* eggs obtained from a taeniasis patient in Bali.

MATERIAL AND METHODS

Worm Collection

The field survey and treatment of patients were done in Banjar Pamesan, Ketewel Village, Gianyar Regency, Bali. During the survey a 45-year-old female patient was diagnosed as having taeniasis by QDRP method as done previously by Fan *et al* (1990a; 1990b) and Wandra *et al.*, (2006). The patient was treated with praziquantel and two hours later the patient was given MgSO₄ laxative in an adult dose of 30 grams.

The segments of the *Taenia* worm expelled from the patient were washed with tap water, kept in a bottle containing normal (0.9%) saline and taken to the laboratory of *Center for Studies on Animal Diseases* (CSAD), Faculty of Veterinary Medicine, Udayana University in Denpasar for species identification. After being confirmed it was *T. saginata*, its gravid proglottids were collected and carefully grounded to obtain the eggs. The eggs were counted under

the microscope using a McMaster counting chamber to prepare three different aliquots, each containing a different number of eggs in normal saline: 100,000 eggs (tube 1), 500,000 eggs (tube 2), and 1,000,000 eggs (tube 3).

Experimental Animal and Infection

For the experiment three Bali cattle were used, all female and 5-months old. The three cattle were confirmed to be free from infection of the gastrointestinal worms, including *Taenia*, based on negative result of coproscopic examination and on the fact that they had been bought very young from a cattle breeder having reasonably high level of environmental sanitation. Fourteen days before experimental infection the three cattle were given the anthelmintic Fenbendazole. The experimental cattle were kept in a separate, clean confinement. During the study the cattle were given feeds and drink *ad libitum* under strictly good hygiene and sanitation. The eggs were administered orally and directly into the cattle's stomachs. Cattle 1 was infected with 100,000 eggs, cattle 2 with 500,000 eggs, and cattle 3 with 1,000,000 eggs.

Necropsy of Experimental Animals and Examination of Cysticerci

The three experimental cattle were slaughtered 24 weeks after infection and possible development of cysticerci in the cattle were observed. The cattle were killed by piercing their jugular veins, the heads were decapitated from the bodies, the skin peeled off, and then the carcasses were cut and grouped in different classifications as commonly done in the slaughter house. Parts of the carcasses and visceral organs were carefully examined by slicing method (Collins, 1981) for the presence of cysticerci.

After locations of the cysts (*C. bovis*) in the carcasses and visceral organs such as heart, lungs and kidney, were recorded, the cysts were collected and brought to the CSAD laboratory for further examination. Locations of the cysts in the carcasses were classified into nine areas (see figure 1) as follows:

Area 1: *musculus longissimus dorsi*, *m. serratus dorsal post anterior* and *posterior*.

Area 2: *m. intercostalis exterior* and *interior*, *m. illiocolalis*, *m. levatores costarum*, *m. scalenus prima costae*, *m. obliquus abdominis externus* and *internus*, *m. transversus abdominis*.

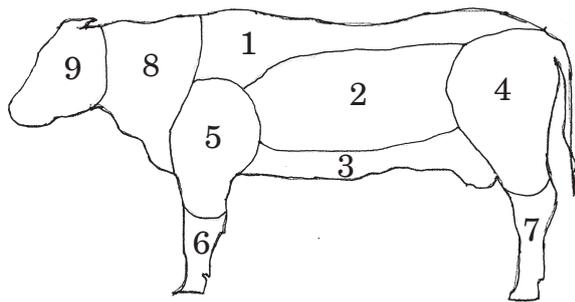


Figure 1. The diagram of carcass areas for cysts examination.

Area 3: *m. pectorial profundus posterior*, *m. pectorial profundeus humeralis*, *m. rectus abdominis*.

Area 4: *m. gluteus superfisialis*, *m. biceps femoralis*, *m. semitendinius*, *m. tensor latae*.

Area 5: *m. deltoideus*, *m. tricep brachi*, *m. trapezius trocalis*, *m. sipraspinatus*.

Area 6: *m. extensor carpi radialis*, *m. flexor carpi radialis*, *m. flexor carpi ulnaris*.

Area 7: *m. extensor dig pedis longus*, *m. flexor hallucis longus*, *m. flexor dig pedis profundus*.

Area 8: *m. sterno mandibularis*, *m. splenius*, *m. cledio mastoidius*, *m. rhomboideus cervicis*.

Area 9: *m. masseter*, *m. buccalis*, *m. auricularis buccalis*, *m. zigomaticus*, *m. nasolabialis*, dan *m. maxilaris*.

Evaluation of cyst density was done by taking several grams of infected carcasses/organs that were found infected and the number of cysts was counted in every 100 grams of tissue, with three

times repetition. Density level was calculated by dividing the average number of cysticerci identified with the average weight of the muscle tissues/organ.

RESULTS AND DISCUSSION

Two of the three infected with cattle developed cysticercosis. The two cattle were the ones that were infected with 100,000 and 500,000 eggs, respectively. Six months after being infected, the two cattle were killed and on examination cysticerci were found in all parts of the carcasses (Fig. 2 and 3) and in several visceral organs. The finding of cysticerci to have had developed in all parts of the carcasses agrees with the statement of Soulsby (1982) that the oncospheres that hatch in the intestine penetrate the intestinal mucosa and enter blood circulation. By means of blood circulation the oncospheres will get into different tissues and subsequently develop into cysts. Infection with 100,000 eggs resulted in formation of cysticerci, which were distributed to all muscle tissues and several visceral organs. Minozzo *et al.*, (2002) inoculated a smaller number of eggs (20,000) and found cysticerci developed in all muscles and in several organs such as heart, diaphragm, lungs, liver, tongue, and kidney. In our present study we did not find any cysticerci in the liver. In cattle 3 infected with 1,000,000 eggs we did not note any cysticerci formation in any of the tissues or organs. This finding may be due to technical

Table 1. Distribution and Density of *C. bovis* in Bali Cattle Infected with *T. saginata* Eggs.

No.	Part of Carcass/Organ	Density of Cysticercus (cyst/100 grams)			
		Cattle 1	Cattle 2	Total	Average
1.	Carcass area 1	21	21	42	21
2.	Carcass area 2	36	36	72	36
3.	Carcass area 3	25	38	63	31
4.	Carcass area 4	13	12	25	12
5.	Carcass area 5	11	22	33	16
6.	Carcass area 6	11	28	39	19
7.	Carcass area 7	11	18	29	14
8.	Carcass area 8	11	48	59	29
9.	Carcass area 9	14	20	34	17
10.	Diaphragm	59	51	110	55
11.	Lungs	0	11	11	5,5
12.	Heart	21	95	116	58
13.	Kidney	0	4	4	2



Figure 2. Showing two cysticerci in muscle tissue of the dorsal area



Figure 3. Some of the individual cysticerci isolated from muscle tissue

fault that might have occurred during administration of the eggs or it might be possible that the eggs were vomited any time after being administered. In this study we did not do around-the-clock observation on the three cattle after treatment. Table 1 shows distribution and density of the cysts in cattle 1 and 2.

Statistical analysis using Kruskal-Wallis test showed that distribution and density of cysticerci in cattle 2 infected with 500,000 eggs were significantly greater than in cattle 1 infected with 100,000 eggs ($P < 0.05$).

Areas of cysts distribution in visceral organs in the cattle infected with 100,000 eggs also differed from those in the cattle infected with 500,000 eggs. In cattle 2 infected with 500,000

eggs, cysticerci were found in diaphragm, lungs, heart and kidney, while in cattle 1 infected with 100,000 eggs, cysticerci were only found in diaphragm and heart. It can be concluded that the level of cysts distribution is dependent on the number of eggs ingested.

The highest average density of cysts was in the heart (58/100 grams), followed by diaphragm (55/100grams), carcass area 2 (36/100 grams), and carcass area 8 (29/100 grams). Overall, the average density in carcasses and visceral organs ranged from 2 – 58 cysts/100 grams tissue. The finding that heart had the highest density of cysts suggests that heart is the most important visceral organ to examine for possible presence of *C. bovis* in cattle. This finding is similar to result of the study by Maeda *et al.*, (1996) on distribution of *T. saginata* cysts in cattle naturally infected in Tanzania. In their study they found that heart was also most heavily infected by *T. saginata* cysts. In our present study we did not find cysts in the liver. This differs from results of other studies done by Belino (1975) in Nigeria, Kozakiewich (1977) in Poland, Nyaga and Gathimo (1979) in Kenya and Minozzo *et al.*, (2000) in Brazilia, in which all found that cysticerci had developed in the liver.

The difference in the manner of cysts distribution in our present study as compared with that in previous studies done in Africa and Latin America may be due to some factors such as geographical difference, breed, and level of muscle activity, as suggested by Pawlowski and Schultz (1972). Other influencing factor could be difference in the strain of *T. saginata* found in Africa and in Bali. This agrees with Fan (1988) who pointed out that the cysts isolated in cattle's liver that developed from *T. saginata* eggs obtained from Africa belonged to a new strain of *Taenia*, which he then called *Taenia taiwanensis*.

CONCLUSION

From our present study it can be concluded that heart is an important organ of predilection for *T. saginata* cysticerci. This fact suggests that the heart of cattle must be always examined during routine meat inspection in slaughter houses to detect *T. saginata* cysts.

Moreover, our present study has shown that *T. saginata* eggs derived from taeniasis patients in Bali, if infected to Bali cattle can readily develop into cysticerci in the cattle's tissues or organs. This finding indicates that Bali cattle

are a potential source of taeniasis infection for the Balinese community, regardless the fact that until now no epidemiological data are available concerning cysticercosis in Bali cattle. Therefore, we recommend that an epidemiological study be conducted on cysticercosis in Bali cattle, in accordance with the prevention of human taeniasis cases in the Balinese community.

ACKNOWLEDGEMENTS

We sincerely thank all individuals or parties who have given assistance during the study. This study was conducted under the collaboration of Udayana University with the Department of Parasitology, School of Veterinary Medicine Faculty of Agricultural Sciences, Tottori University, Tottori 680-8553, Japan and Department of Parasitology, Asahikawa Medical College, Asahikawa 078-8510, Japan; with a grant from the JSPS-Research Fund (No.18406008).

REFERENCES

- Abuseir S, Epe C, Schnieder T, Klein G, Kühne M. 2006. Visual diagnosis of *Taenia saginata* cysticercosis during meat inspection: is it unequivocal? *Parasitol Res* (99): 405-409.
- Belino ED. 1975. Some observation on *Taenia saginata* cysticercosis in slaughter cattle in Nigeria. *Int. J. Zoonoses*. 2: 92-99.
- Collins FV. 1981. Meat Inspection. Rigby Publishers Limited. Sydney.
- Dharmawan NS. 2000. (Experimental infection of *Taenia saginata* in Bali cattle). *Maj. Kedokteran Udayana*. 31(110): 240-243. (in Indonesian).
- Dorny P and Praet N. 2007. *Taenia saginata* in Europe. *Vet. Parasitol*. 149(1-2): 22-24.
- Fan PC. 1988. Taiwan *Taenia* and taeniasis. *Parasitol. Today*. 4: 86-88.
- Fan PC, Chung WC, Lin CY, Wu CC. 1990a. Experimental infection of Thailand *Taenia* (Chiengmai Strain) in domestic animals. *Int. J. Parasitol*. 20(1): 121-123.
- Fan PC, Chung WC, Lo CT, Lin CY. 1990b. The pig as an experimental host of *Taenia saginata* (Ethiopia and Madagascar Strain). *Ann. Trop. Med. Parasitol*. 84(1); 93-95.
- Flütsch F, Heinzmann D, Mathis A, Hertzberg H, Stephan R, Deplazet P. 2008. Case-control study to identify risk factors for bovine cysticercosis on farms in Switzerland. *Parasitol*. 135(5): 641-646.
- Ito A, Nakao M, Wandra T, Suroso T, Okamoto M, Yamasaki H, Sako Y, Nakaya K. 2005. Taeniasis and cysticercosis in Asia and the Pacific: present state of knowledge and perspectives. *Southeast Asian J. Trop. Med. Public Health*. 36(suppl 4): 123-130.
- Kebede N. 2008. Cysticercosis of slaughtered cattle in Northwestern Ethiopia. *Res. Vet. Sci*. 85(3): 522-526.
- Kozakiewicz B. 1977. Studies on cattle cysticercosis, I. Experimental cysticercosis in calves infected with eggs and oncospheres of *Taenia saginata*. *Acta Parasitologica Polonia*. 24: 357-369.
- Maeda GE, Kyvsgaard NC, Nansen P, Bogh HO. 1996. Distribution of *Taenia saginata* cysts by muscle group in naturally infected cattle in Tanzania. *Prev. Vet. Med.* (2): 81-89.
- Margono SS, Wandra T, Suroso T, Ito A. 2005. Taeniasis and cysticercosis in Indonesia, in: Ito A., Wen H, Yamasaki H. (Eds): *Taeniasis/Cysticercosis and Echinococcosis in Asia*. Asian Parasitology Vol 2. AAA Committee/Federation of Asian Parasitologists, Chiba, Japan, pp. 115-134.
- Minozzo JC, Gusso RLF, de Castro EA, Logo O, Soccol VT. 2002. Experimental bovine infection with *Taenia saginata* eggs: recovery rates and cysticerci location. *Brazilian Arch. Biol. Tech*. 45(4): 451-455.
- Myadagsuren N, Davaajav A, Wandra T, Sandar T, Ichinkhorloo P, Yamasaki H, Sako Y, Nakao M, Sato MO, Nakaya K, Ito A. 2007. Taeniasis in Mongolia 2002-2006. *Am. J. Trop. Med. Hyg*. 77(2): 342-346.
- Nyaga PN and Gathimo JM. 1979. Some observation on *Taenia saginata* cysticercosis in Kenya slaughter cattle. *Bull. Anim. Health Prod. Africa*. 27: 51-59.
- Pawlowski Z and Schultz MG. 1972. Taeniasis and cysticercosis (*Taenia saginata*). *Adv. Parasitol*. 10: 269-343.
- Simanjuntak GM, Margono SS, Sachlan R, Haryono C, Rasidi R, Sutopo B. 1977. An investigation on taeniasis and cysticercosis in Bali. *Southeast Asian J. Trop. Med. Public Health*. 8: 494-497.

- Soulsby E JL. 1982. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th Ed. Bailliere Tindall. London.
- Sutisna P, Kapti IN, Allan JC, Rodriguez-Canul R. 2000. Prevalence of taeniasis and cysticercosis in Banjar Pamesan, Ketewel Village, Gianyar, Bali. Maj. Kedokteran Udayana. 31: 226-234. (in Indonesian).
- Wandra T, Sutisna P, Dharmawan NS, Margono SS, Sudewi R, Suroso T, Craig PS, Ito A. 2006. High prevalence of Taenia saginata taeniasis and status of Taenia solium cysticercosis in Bali, Indonesia, 2002-2004. Trans R Soc Trop Med Hyg. 100: 346-353.