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Summary

An epidemiological survey of taeniasis/cysticercosis was carried out in one semi-urban and two urban villages in three districts in Bali, Indonesia in 2002 and 2004. A total of 398 local people from 247 families were diagnosed by anamnesis and clinical examinations, and 60 residents were suspected to be taeniasis carriers. Among 60 suspected carriers, 56 persons expelled a total of 61 taeniid adult worms after praziquantel treatment. From 398 residents, 252 stool samples were available for analysis of taeniid eggs, copro-antigens or copro-DNA for identification of taeniid species, and 311 serum samples were available for detection of antibodies against *Taenia solium* cysticercosis. Taeniasis prevalences were highly variable among three villages (1.1-27.5%) and only one cysticercosis due to *T. solium* infection was detected. All expelled tapeworms were confirmed to be *Taenia saginata* by mitochondrial DNA analysis. There was no *Taenia asiatica* human case in Bali. Case control analysis of 106 families chosen at random from 179 families in 2004 and another 106 families from non-endemic areas revealed that risk factors of *T. saginata* taeniasis for families were the level of education ($p < 0.01$), the consumption of beef *lawar* ($p < 0.01$) and the source of *lawar* ($p < 0.01$).

KEYWORDS: taeniasis, cysticercosis, *Taenia saginata*, *Taenia solium*, *Taenia asiatica*, questionnaire, direct smear, corpo-ELISA, copro-DNA, mitochondrial DNA, serology, Bali, Indonesia

1. Introduction

In Indonesia, there are three known endemic provinces for taeniasis/cysticercosis: Bali, Irian Jaya (Papua) and North Sumatra (Handali et al., 1997; Simanjuntak et al., 1997; Wandra et al., 2000, 2003; Margono et al., 2001; Ito et al., 2002, 2003, 2004). Taeniasis, either due to *Taenia saginata* and/or *Taenia solium* in East Nusa Tenggara and South-East Sulawesi were reported to be 7.0% (31/445) in 1975 (Simanjuntak et al., 1997) and 0.4% (1/243) in 1985 (Margono et al., 2004). Other surveys where Balinese people had been resettled revealed of taeniasis rates of 1.0% (5/476) in Lampung and 0.4% (1/245) in North Sulawesi in 1981 (Simanjuntak et al., 1997; Margono et al., 2004). Cases with taeniasis and/or cysticercosis have also been reported from Jakarta, West Kalimantan, East Kalimantan and East Java (Margono et al., 2001, 2004; Suroso, 2002).

Historically, the first report of *T. solium* infection in pigs in Bali was published in 1928. After 32 years, one case of subcutaneous cysticercosis (SCC) in a Balinese woman was reported (quoted from Sutisna, 1989, 2002; Margono et al., 2004). There are several reports on *T. solium* taeniasis, epileptic seizures, SCC, neurocysticercosis (NCC), and seroprevalence of cysticercosis in Bali (Ngoerah, 1975; Sutisna, 1989, 1990, 2002; Sutisna et al., 1999, 2000; Margono et al., 2001, 2004).

Prevalence rates of *T. saginata* taeniasis in Bali were reported during 1977-1990 to range from 0.4-23% and were distributed in all nine districts of the island (Sutisna, 1989, 1990; Suweta, 1991; Sutisna et al., 1999, 2000; Margono et al., 2001, 2004). A more recent survey performed in Gianyar District in Bali revealed a taeniasis prevalence of 1.3% by coproantigen detection test (Sutisna et al., 1999).

The present paper indicates a resurgence of *T. saginata* taeniasis related to consumption of local raw beef dish (*lawar*) through inadequate inspection of meat and food hygiene, and also reports on the present situation of *T. solium* cysticercosis in Bali.

2. Materials and Methods

2.1. Field survey and sample collection

An epidemiological survey was carried out in one semi-urban village, Ketewel (Gianyar District) in December 2002 and February 2004, and in two urban villages, Jagapati and Penatih (Badung and Denpasar Districts, respectively) in 2004. These three districts are located at the southern part of Bali Island and around and including the capital city, Denpasar. Ketewel village is just 7 km far east from Denpasar. The subjects voluntarily sampled were family heads and housewives and other persons ≥15 years in one Banjar (=sub-village) of each village. A Banjar was selected based on the local health center reports on taeniasis, and each Banjar consisted of 159-165 families and of 782-800 inhabitants. Additional sampled populations were from the neighboring Banjar(s), where they came after the Head of Banjar informed and invited them for a free check-up or treatment. After obtaining central and local government ethical approvals, 125 serum samples and 75 stool samples from 125 residents were collected in Ketewel in December 2002; 46 serum samples and 46 stool samples from 51 residents who were not examined in 2002 came in February 2004. Additional samples were collected one year later (February 2004) from 32 taeniasis patients in Ketewel treated with praziquantel (15 mg/kg single dose, Suroso, 2000) in 2002. Ninety one serum samples and 34 stool samples from 94 residents in Jagapati, and 49 serum samples and 97 stool samples from 128 persons in Penatih were also collected in February 2004. A total of 32 *T. saginata* taeniasis patients were confirmed and treated in 2002 in Ketewel. Taeniasis cases were suspected by Questionnaire Responses and Demonstration of Proglottids (QRDP), and a clinical examination including abdominal symptoms and history of expulsion of proglottids. Stool examinations for detection of *Taenia* spp eggs (direct smears) and morphological examinations of proglottids for identification of the tapeworm were carried out at Udayana

University, Bali. Stool samples available for coproantigen testings (copro-ELISA) were kept frozen. Stool samples for copro-DNA analysis and proglottid samples for mitochondrial (mt) DNA analysis were kept in 99.5% ethanol. Serological examination by both ELISA and immunoblot, and copro-ELISA, copro-DNA, multiplex PCR, and mtDNA sequencing were carried out at Asahikawa Medical College (Japan) in 2003 and in 2004 (Allan et al., 1992; Ito et al., 1998, 1999; Nakao et al., 2002; Yamasaki et al., 2004a, 2004b). Cysticercosis cases were suspected by questionnaires on the history of epileptic seizures, detection of subcutaneous nodule by palpation and serological examination for *T. solium* cysticercosis (Wandra et al., 2003). All tapeworms expelled from tapeworm carriers were confirmed by mtDNA analysis using cytochrome *c* oxidase subunit I (*cox1*) gene to differentiate *T. solium*, *T. saginata* and *T. asiatica* (Yamasaki et al., 2004a, 2004b, 2004c), since proglottids of *T. saginata* and *T. asiatica* are morphologically identical (Fan 1988; Eom and Rim, 1993; Ito et al., 2003; 2004) and *T. asiatica* was also expected to be distributed in Bali (Dharmawan, 1998). Additional data through questionnaires from 106 family heads were also collected in a non-endemic *Taenia* area (Banjar) in East Denpasar Sub-District (Denpasar District) as a family control for the 106 family heads living in the taeniasis endemic area (Banjar) in Ketewel and Penatih villages. Bivariate analysis was performed using Epi Info (version 6).

2.2. ELISA, immunoblot and copro-ELISA

Enzyme linked immunosorbent assay (ELISA) and immunoblot were carried out using glycoproteins (GPs) from a pH 8.1 fraction purified by preparative isoelectric focusing from *T. solium* cysts (Rotofor, BioRad, USA) (Ito et al., 1998, 1999) and a chimeric recombinant antigen (modified from Sako et al., 2000). Detection of antibody by ELISA was performed as described by Ito et al. (1998, 1999) and Sako et al. (2000). Copro-ELISA

for *Taenia* spp. based on the method described by Allan et al (1992) was carried out using a commercial ELISA kit according to the manufacturer's instructions (Genzyme Virotech, GmBh, Russelsheim Germany).

2.3. Multiplex PCR and DNA sequencing

According to Yamasaki et al. (2004a, 2004b, 2004c), mitochondrial DNA samples from stools from taeniasis cases and from expelled tapeworms were analyzed by multiplex PCR for differentiation of *T. saginata*, *T. asiatica* and *T. solium* using stool samples fixed in ethanol from the tapeworm carriers.

3. Results

3.1. Taeniasis Survey

Population sample size and data on *T. saginata* taeniasis from three villages in three districts in Bali are summarized in Table 1. A total of 60 persons among 398 local people in three villages of three districts in Bali were suspected to be *T. saginata* taeniasis cases by QRDP (questionnaire responses and demonstration of proglottids) and 56 taeniasis cases were confirmed after treatment. The history of expulsion of proglottids from four suspected persons who did not harbour any taeniid worms when they were treated with praziquantel was that they expelled proglottids one or two years before but no more evidence past one year. Stool samples from these four persons were negative by direct smears. So, these persons were expected to have had taeniid worms but lost them more than one year before. After QRDP, a total of 252 stool samples and 311 serum samples were obtained. A total of 15 from 252 stool samples (5.95%) were positive by direct smears, whereas only 82 faecal samples were available for copro-ELISA, since the volume of some fecal samples were too small and no more samples were available for

copro-ELISA after direct smear tests. Eighty-two faecal samples included seven (8.54%) copro-ELISA positives and four direct smear positives. These direct smear positive cases were not applied for re-confirmation by copro-ELISA any more, since we had data that direct smear positive cases were all copro-ELISA positive (Wandra et al. unpublished data). So, a total of 11 (13.41%) of 82 faecal samples were confirmed to be taeniasis by direct smears and copro-ELISA. All other samples (n = 71) from direct smear negative were confirmed to be copro-ELISA negative. Taeniasis prevalence ranged from 25.6% (32/125) in 2002, to 27.5% (14/51) in 2004 in Ketewel village, whereas prevalence was lower at 1.1% (1/94) in Jagapati village and 7.0% (9/128) in Penatih village in 2004.

The number of *T. saginata* taeniasis cases was significantly higher in males (82.1%, 46/56) than in females (17.9%, 10/56), respectively ($p<0.01$). Most of the *T. saginata* carriers were in the 30-44 year age group (51.8%, 29/56), followed by that of 45 years or older group (30.4%, 17/56), and that of the 15-29 year group (17.8%, 10/56). The youngest *T. saginata* carrier was a 19 year old male and the oldest was a 70 year old male.

In Ketewel village, the number of families consuming raw beef (*lawar*) increased from 19.2% (14/73) in 2002 to 32.4% (12/37) in 2004. When 32 taeniasis patients in this village, who expelled tapeworms after treatment with praziquantel in December 2002, were re-examined one year later (in February 2004), they all had no more history of additional expulsion of proglottids by QRDP and they all were direct smear negative.

Taeniid egg positive stool samples available (n=10) and all proglottid samples from 56 tapeworm carriers were analyzed by copro-DNA and multiples PCR, respectively and all were confirmed to be *T. saginata* (data not shown). Additional mtDNA sequencing was carried out for 32 of 56 proglottid samples in order to re-confirm if they were really *T. saginata* or *T. asiatica* which might be distributed in Bali. All were re-confirmed to be *T. saginata* and no *T. asiatica* was found (sequencing data not shown).

During 2002-2004, there was no person with indication of *T. solium* cysticercosis including history of epileptic seizures or presence of subcutaneous nodules except one seropositive against both *T. solium* GP antigens and a chimeric recombinant antigen in Ketewel in 2002 (data not shown). In this village, serological examination of 125 serum samples revealed that 1/125 (0.8%) of sera was seropositive (a 76 years old male). There was no other seropositive cysticercosis case from all residents examined in 2004.

3.2. Risk factors for *T. saginata* taeniasis

Socio-economic data for 56 *T. saginata* taeniasis cases and 192 families showed that 87.5% (49/56) defecated in a toilet, while 12.5% (7/56) defecated in a river. No taeniasis carriers admitted to defecating in the space behind the house (*teba*). All taeniasis cases consumed raw pork and/or beef (*lawar*). Furthermore, 96.9% (186/192) of families had a sanitary facility, and 3.1% (6/192) defecated in a river. All pig owners (56 of 192 families) kept their pigs indoor. Five of 11 cattle owners kept their cattle in a fenced field, other cattle owners (6 families) kept their cattle in open common pasture. All families reported consumption of raw pork and/or beef.

During 2002-2004, we found that three of 56 taeniasis cases were *lawar* sellers, and had suffered from *T. saginata* taeniasis for 1-10 years. Several of the other taeniasis carriers also bought *lawar* from these sellers. Biviarate analyses of 106 family heads from endemic and non-endemic areas each in 2004 showed significant association of *T. saginata* taeniasis and level of education ($p < 0.01$), consumption of beaf *lawar* ($p < 0.01$), and source of *lawar* ($p < 0.01$) (Table 2).

4. Discussion

According to National Socio-Economic Survey, 2003, the majority of the 3 million population of Bali is Hindu (Hindu 93.4 %, Muslim 5.1 %, Buddhist 0.5 %, Protestant 0.5 %, Catholic 0.4 %) with a total population density of 585 per km^2. The number of Muslims is however steadily increasing through immigration from Java, Lombok and other areas of Indonesia who seek work in Bali. Most people live in the coastal areas in the south, and the island's largest town and administrative center is located in Denpasar with a population of over 370,000.

A total prevalence of 14.1% for *T. saginata* taeniasis was detected during 2002-2004 in two urban areas and one semi-urban area around Denpasar in Bali. Although sample collection was not completely random, it was suggested that the number of cases of *T. saginata* taeniasis had increased dramatically in Ketewel village compared with previous surveys in 1977 (2.1%) and 1999 (1.3%) (Simanjuntak et al., 1977; Sutisna et. al., 2000). Only seven *T. saginata* taeniasis cases were found in Gianyar District in 2002 (unpublished information from Gianyar District Health Office Services). Sutisna et al. (1999) reported that among 33 taeniasis patients in one village of Denpasar District, only 21.2% (7/33) visited the health facilities. These strongly suggest that such report was much lower than real prevalence through active case findings.

Several interesting questions still remain on the existence of taeniasis in Bali over the years. Previous surveys carried out in Gianyar, Badung, Denpasar Districts showed that the local people consumed pork more frequently than beef. However, *T. saginata* taeniasis was more common. It was suggested therefore that the taeniasis cases reported to be due to *T. saginata* might be caused by *T. asiatica* (Dharmawan, 1998; Sutisna et al., 1999; Ito et al., 2003, 2004). Based on the current study, however, there is no evidence for the existence of *T. asiatica* in human in Bali. We tried to get information on this issue from local people and got the information that they do not like the taste of uncooked viscera. This might be

the reason why we cannot find any *T. asiatica* where local people like uncooked meat with blood as a traditional local dish (pork *lawar*) and *T. solium* cysticercosis cases are still sporadically found in Bali. This is thoroughly different from the ethnic Batak people in Samosir Island, North Sumatra who like the taste of uncooked viscera and where taeniasis of *T. asiatica* is still very common (Wandra et al., unpublished). Further surveys are necessary to get conclusion on this issue in different areas in Bali.

The number of cases with *T. saginata* taeniasis was higher in males than in females and most of the patients were in the 30-44 year age group. Similar findings were reported in Renon village, Denpasar and in other areas of Bali by Sutisna et al. (1999): In the traditional communities in villages, adult male groups often enjoy raw beef (*lawar*) with spirit/toddy (*tuak*). It is expected to be one of the factors why the number of cases in males is higher especially in the 30-40 year age group.

Despite the fact that most Balinese people are Hindi, they do consume beef. In Ketewel village, the number of families consuming raw beef (*lawar*) increased from 19.2% (14/73) in 2002 to 32.4% (12/37) of families in 2004. There was no difference in the human taeniasis rate in 2002 (25.6%, 32/125) and 2004 (27.5%, 14/51) between several Banjars in Ketewel village. However, when 32 taeniasis patients in this village, who expelled tapeworm after treatment with praziquantel in December 2002, were re-examined one year later (in February 2004), there were no cases of re-infections with *T. saginata.* All of them reported and stressed that they stopped eating beef *lawar* anymore after they recognized that they harbored big tapeworms but few persons could not afford to stop eating beef *lawar* after one-three moths of the shocking evidence of expulsion of big tapeworms. This is a problem for control program especially for health education of these local people who like traditional local food.

The source of infection for *T. saginata* taeniasis in the survey areas is not clear, and could be from other areas in Bali, since these survey areas are around Denpasar and semi-urban areas, whereas most of the rest areas are rural and mountainous areas. According to unpublished information from Provincial Livestock Office Services in 2004, there is one slaughterhouse in Gianyar, Badung, Denpasar Districts. The personnel consisted of one veterinarian and one assistant veterinarian (*mantri hewan*) in each district. The number of livestock slaughtered ranged from 6-20 cattle/pigs per day in Gianyar, 20-21 in Badung, and 50-60 in Denpasar. However, many cattle/pigs from areas in Bali are also slaughtered in *illegitimate* slaughterhouses, and then distributed to the markets. The main problem is how to check the quality of beef/pork in these *illegitimate* slaughterhouses as well as the quality of meat hygiene in the markets.

The pork tapeworm *T. solium* now appears to be rare in Bali. Between 1975-2001, only six *T. solium* taeniasis carriers were detected (Simanjuntak et al., 1977; Sutisna 1989, 1990; Sutisna et al., 1999; Margono et al., 2004). Generally *T. solium* is rare in Indonesia as a whole because of Islam taboo on eating pork. However in a number of areas eg. Papua and Timor it is a public health problem, as it has previously been in Bali (Suroso, 2002; Margono et al., 2003, 2004; Wandra et al., 2003; Ito et al., 2003, 2004). Human cases of epileptic seizures in Balinese communities was reported to range from 1.1%-8.0% in Trunyan (Bangli District), Sukawati (Gianyar District) and Padangsambian (Badung District) in 1977 (Sutisna, 2002). At Wangaya Hospital in Denpasar, 68 and 368 new cases of epileptic seizures were reported in 1980 and 1984, respectively, whereas at Sanglah Hospital in Denpasar, 74 new cases were reported within 3 month in 1991 (Sutisna, 2002). Margono et al. (2001) reported that 10 of 74 (13.5%) patients with epilepsy in Bali were diagnosed to be due to neurocysticercosis (NCC) based on ELISA serology (Margono et al., 2001). Both SCC and NCC cases were reported in Bali during 1960-1997 with the

frequency of NCC cases approximately three times more than SCC cases (Ngoerah, 1975; Sutisna, 2002; Margono et al., 2004). Seroprevalence of cysticercosis in humans in Bali ranged from 5.2% up to 21% since more than 20 years ago (Coker-Vann et al., 1981). Another survey of 746 people revealed that 94 persons (12.6%) were positive by the more specific glycoprotein immunoblot (Theis et al., 1994). A previous immunoblot survey for cysticercosis performed in Ketewel village revealed a seroprevalence of 5.2% (6/115) (Sutisna et al., 2000).

In the current survey (2002-2004), there was no person detected in the community survey with a history of epileptic seizures or subcutaneous nodules, and only one cysticercosis case was diagnosed as seropositive (in Ketewel village)(data not shown).

The apparent significant decrease in transmission of *T. solium* in Bali is probably due to improvement in sanitation and pig husbandry at least in urban and semi-urban areas where we did the field survey. In the surveyed areas, almost all families had sanitary facilities, and no person said that they defecated in the backyard (*teba*). In addition all pig owners interviewed kept their pigs indoors. By contrast, only 64.1% of families had sanitary facilities, while 42.4% of taeniasis carriers and 12.6% of families defecated in the backyard, and pigs were allowed to roam free in 1990 (Sutisna, 1990, 2002; Margono et al., 2004).

In conclusion, the current survey for human taeniasis and cysticercosis in Bali indicates that *T. saginata* taeniasis has increased in number of cases, while *T. solium* taeniasis and cysticercosis is now rather rare compared to 10-20 years ago. In order to control taeniasis/cysticercosis, several strategies have been proposed (Ito et al., 2003; Flisser et al., 2003; Gonzalez et al., 2003; Sarti et al., 2003; Schantz and Tsang, 2003). However, based on our survey results, (1) active case finding (active surveillance) and treatment of the tapeworm carriers, (2) check of beef *lawar* hygiene in the market including *lawar* sellers' health, periodically, (3) establishment of a system to check the quality of beef/pork and

look for distribution of infected animals in Bali, and (4) sustainable public health education through treatment are expected to have the priorities.

We are planning to do similar work in other villages including western part of Bali and far north from Denpasar where traditional rural life style is still well conserved. Therefore, such areas are expected to be relatively high risk areas for both *T. saginata* in humans and cattle and *T. solium* in humans and pigs.

Conflicts of interest statement.

The authors have no conflicts of interest concerning the work reported in this paper.

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Table 1 Number of *Taenia saginata* taeniasis cases detected by anamnesis and stool examination by either direct smear or copro-ELISA test in urban and semi-urban areas in Bali, 2002 and 2004

Year	Village (District)	A[a]/B[b]/C[c] (% of A/C)	No. of positive by direct smear/ no. of samples (%)	No. of positive by copro-ELISA/ no. of samples (%)
2002	Ketewel[d] (Gianyar)	32[g]/35/125 (25.6)	11/75 (14.66)	Not tested
2004	Ketewel (Gianyar)	14/14/51 (27.5)	3/46 (6.52)	5/40 (12.50)
	Jagapati[e] (Badung)	1/1/94 (1.1)	0/34 (0.0)	0/11 (0.0)
	Penatih[e] (Denpasar)	9/10/128 (7.0)	1/97 (1.03)	2/31 (6.45)
	T o t a l	56[f] /60/398 (14.1)	15/252 (5.95)	7/82 (8.54)
2004	Ketewel (Gianyar)	0/0/32[g]	0/32 (0.0)	Not tested

[a] No. of persons who expelled taeniid tapeworms

[b] No. of suspected taeniasis persons through QRDP

[c] No. of residents examined

[d] Semi-urban area

[e] Urban areas

[f] 56 persons expelled a total of 61 taeniid adult worms after praziquantel treatment (15 mg/kg).

[g] Persons who expelled tapeworm after treatment with praziquantel in December 2002 and were reexamined one year later in February 2004.

Table 2 Risk factors associated with *Taenia saginata* taeniasis in the communities (2004)

Variables	Endemic area[a] (n=106)		Non-endemic area[b] (n=106)		p
	n	%	n	%	
Levels of education					
Low[c]	62	59.0	33	31.1	<0.01
Middle and/or High[d]	44	41.0	73	68.9	
Consumption of beef *lawar*					
Yes	63	59.4	37	35.0	<0.01
No	43	40.6	69	65.0	
Source of *lawar* (beef and/or pork)					
Market	55	51.9	30	28.0	<0.01
Home made	51	48.1	76	72.0	

[a]: Banjar Saba, Banjar Pamesan and its surroundings, Sukawati Sub-District, Gianyar.

[b]: Banjar Paang Kelod, East Denpasar Sub-District, Denpasar.

[c]: No formal school education or only primary school.

[d]: Junior/senior high school (middle), Academy/university (high).