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REVIEW

TAENIASIS/CYSTICERCOSIS IN BALI, INDONESIA

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Abstract. *Taenia solium* and *Taenia saginata* are found in humans in Bali, Indonesia. During a field survey of 660 people in Bali from 2002-2009 of taeniasis/cysticercosis cases using mitochondrial DNA confirmation of the species, we detected 80 cases of *T. saginata* taeniasis, 2 dual *T. saginata/T. solium* infections with *T. solium* metacestodes in the brain and 12 neurocysticercosis (NCC) cases at Sanglah Hospital, Denpasar. Although the prevalence of NCC in Bali is low, sporadic cases are still present. There is no *Taenia asiatica* in Bali. We summarize here the field survey findings of taeniasis, including 1 dual infection with taeniasis and cysticercosis in 2007, and the reason why there are no *T. asiatica* cases and we describe 3 NCC cases admitted to Sanglah Hospital, Denpasar, Bali in 2004. Diagnosis was based on anamnesis, clinical examination, including CT Scan, histopathological, serological and mitochondrial DNA examinations. In order to prevent unexpected symptomatic NCC after treatment with praziquantel, we recommend introducing a rapid test to confirm taeniasis carriers and cysticercosis cases as a tool for real time diagnosis.

Keywords: *Taenia solium, T. saginata,* taeniasis, cysticercosis, Indonesia

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INTRODUCTION

In Asia, there are 3 human *Taenia* species: *Taenia solium* (pork tapeworm), *Taenia saginata* (beef tapeworm) and *Taenia asiatica* (Chao and Fan, 1986; Fan *et al*, 1987; Fan 1988; 1988; Zarlenga *et al*, 1991; Eom and Rim, 1993; Bowles and McManus, 1994; Hoberg *et al*, 2000; Ito *et al*, 2003; Eom, 2006; Okamoto *et al*, 2010). The taxonomy of *T. asiatica* is still unclear, since hybrid *T. saginata/T. asiatica* worms

have been found in Thailand (Okamoto *et al*, 2010) and China (Nkouawa *et al*, in preparation).

Taeniasis in human due to *T. saginata* and *T. asiatica* is caused by eating uncooked or undercooked beef and viscera of swine contaminated with metacestodes of these species, respectively. Metacestodes of *T. asiatica* may develop not only in pigs but also in cattle and goats (Fan *et al*, 1987, 1989). In contrast, *T. solium* only causes two distinct clinical presentations: taeniasis due to the presence of adult tapeworm(s) in the small intestine of humans after eating uncooked or undercooked pork contaminated with the metacestodes of this species, and cysticercosis caused by the presence of metacestode(s) in parenteral tissue after accidental oral ingestion of eggs of the parasite. Cysticercosis caused by metacestode(s) in the central nervous system (neurocysticercosis, NCC) is one of the most important causes of epilepsy, and a leading cause of late-onset epilepsy (Takayanagui and Odashima, 2006; Ito *et al*, 2006).

Cases of *T. solium*, *T. saginata* and *T. asiatica* have been reported from Indonesia. To date, there have been 3 endemic provinces for taeniasis/cysticercosis in Indonesia: North Sumatra, Bali and Papua. North Sumatra is endemic for *T. asiatica* taeniasis (Kosin *et al*, 1972; Fan *et al*, 1990; Wandra *et al*, 2006). Bali is endemic for *T. saginata* taeniasis and *T. solium* taeniasis/cysticercosis (Sutisna *et al*, 1999, 2000; Wandra *et al*, 2006; Sudewi *et al*, 2008). Papua is endemic for *T. solium* taeniasis/cysticercosis (Tumada and Margono, 1973; Handali *et al*, 1997; Simanjuntak *et al*, 1997; Sutisna *et al*, 1999, 2000; Wandra *et al*, 2000, 2003; Subahar *et al*, 2001; Ito *et al*, 2002; Margono *et al*, 2003, 2006; Salim *et al*, 2009). Taeniasis and cysticerosis are reported sporadically in Lampung, Jakarta, East Java, West Kalimantan, East Kalimantan, North Sulawesi, South Sulawesi, South East Sulawesi, and East Nusa Tenggara Provinces of Indonesia (Simanjuntak *et al*, 1997; Margono *et al*, 2001; Wandra *et al*, 2003; Ito *et al*, 2004; Sudewi *et al*, unpublished).

Epidemiological surveys for taeniasis/cysticercosis were conducted in all 9 districts of Bali from 2002 to 2009. During the field surveys, we examined a total of 660 people and simultaneously collected data regarding NCC cases at Sanglah Hospital, Denpasar. In this review, we summarize 80 cases of *T. saginata* taeniasis, 1 dual infection with *T. solium*/*T. saginata*, in 2007 and 3 NCC cases admitted to the hospital in 2004.

TAENIASIS/CYSTICERCOSIS IN BALI

Taenia saginata taeniasis

During the epidemiological survey in Bali from 2002 to 2009, we detected a total of 80 cases of *T. saginata* taeniasis (32, 24, 7, 3, 3, 4, and 7 cases in 2002, 2004, 2005, 2006, 2007, 2008, and 2009, respectively) using mitochondrial DNA confirmation by multiplex PCR and DNA sequencings (Yamasaki *et al*, 2004; Ito *et al*, 2009). The risk factor for *T. saginata* taeniasis is consumption of "beef lawar", a traditional local food of raw minced beef. Butchers and their family members are often found to be infected with *T. saginata* in Bali and with *T. asiatica* in Lake Toba, North Sumatra (Wandra *et al*, unpublished).

In Gianyar District, *T. saginata* taeniasis cases were found yearly (32, 14, 5, 2, 3, 4, and 7 cases in 2002, 2004, 2005, 2006, 2007, 2008, and 2009, respectively). Approximately 84% of taeniasis cases (67/80) were from Gianyar District. There were no cases of reinfection with *T. saginata* after treatment with tapeworm(s). Once most

people are confirmed to have an infection, the majority will stop eating raw beef. The high number of cases in Gianyar District may be due to the low impact of health education campaigns advising the community to consume cooked beef only in order to prevent taeniasis. Further study is needed to determine the true prevalence of taeniasis in Gianyar District.

Nearly all taeniasis cases were suspected based on a question that asked about the expulsion of proglottids. Most people gave a reliable history, at least for *T. saginata*. *T. asiatica* is morphologically similar to *T. saginata* (Fan, 1988; Eom *et al*, 1993; Ito *et al*, 2003) and is fairly common in North Sumatra, Indonesia (Kosin *et al*, 1972; Fan *et al*, 1990; Wandra *et al*, 2006, 2007), but has never been reported from Bali. We believe the reason why Balinese people are not infected with *T. asiatica* is because they do not eat the uncooked viscera of swine, different from the people of North Sumatra (Wandra *et al*, 2006, 2007).

We found no cases of *T. solium* taeniasis in Bali. However, there are cysticercosis cases in Bali. In order to detect *T. solium*, we would have to develop a rapid diagnostic test to screen for taeniasis carriers. Serum samples of taeniasis cases may be useful for developing screening tests for taeniasis carriers (Wilkins *et al*, 1999; Levine *et al*, 2007; Nakao *et al*, unpublished), but fecal samples are useful to confirm taeniasis carriers and identify the species (Yamasaki *et al*, 2004; Guezala *et al*, 2009; Nkouawa *et al*, 2009).

Dual infection with *T. saginata* taeniasis and *T. solium* neurocysticercosis

In 2007, we found a 47-year-old- Balinese male *T. saginata* carrier who appeared to be healthy and had no neurologic symptoms of cysticercosis. We treated him with praziquantel 15 mg/kg BW. A single worm was expelled, examined morphologically and later confirmed to be *T. saginata* by multiplex PCR in Asahikawa, Japan. Unfortunately, he had seizure within 6 hours of treatment, requiring hospitalization for several days. A CT scan showed multiple cystic lesions in the brain. Serological examination carried out in Asahikawa, Japan, later showed weakly positive antibody responses to both native and recombinant antigens (Sako *et al*, 2000; Sato *et al*, 2006; Ito *et al*, 2009) before treatment, and a stronger response post-treatment (data not shown). This case shows asymptomatic NCC cases may become symptomatic after treatment even with a low dose of praziquantel (Flisser *et al*, 1993; Sarti *et al*, 2000). In 2009, we treated another dual infection case in Bali (Wandra *et al*, in preparation).

Cysticercosis due to *T. solium*

The first record of cysticercosis in Bali was reported from pigs more than 80 years ago (Le Coultre, 1928; Oemijati, 1977). Thirty-two years later, two human cases of subcutaneous cysticercosis (SCC) were reported (Soebroto *at al*, 1960). Since 1971, several cases of *T. solium*, epileptic seizures, SCC and NCC have been reported, including the seroprevalence of cysticercosis in Bali. Between 1960 and 1997, a total of six taeniasis cases due to *T. solium* have been confirmed. In contrast, a total of 44 cysticercosis cases have been reported. Both NCC and SCC are found in Bali, with a ratio of 2.6:1 for NCC (32 cases) and SCC (12 cases). However, there is no information about cases infected with both NCC and SCC (Soebroto *et al.*, 1960; Hadidjaya, 1971; Ngoerah, 1975; Simanjuntak *et al*, 1977, 1997; Coker-Vann *et al*, 1981; Bakta *et al*, 1983; Theis *et al*, 1993; Sutisna, 1994, 1999; Sudewi and Nuartha, unpublished quoted from Sutisna *et al*, 2000). The seroprevalence

of cysticercosis in Bali ranged from 5.2 to 21% during 1981-1997 (Sutisna *et al*, 2000). One risk factor was consumption of pork lawar, an uncooked minced pork mixed with fresh pigs blood, a traditional cuisine among the Balinese people (Wandra *et al*, 2006, 2007). Pork lawar is more popular than beef lawar in Bali. This is based on Balinese Hinduism, which differs from Indian Hinduism (Simanjuntak *et al*, 1997; Ito *et al*, 2003; Wandra *et al*, 2007).

The number of cysticercosis cases has decreased dramatically, since only two sero-positive cases were detected from 9 districts in Bali among 660 people during 2002-2009. We did not detect any taeniasis cases due to *T. solium*. The decrease in the number of NCC and SCC cases due to *T. solium* may be due to improvement in household sanitation and pig husbandry through sustainable public health education in Bali and improvement in the economy. Most families have latrines and pigs are generally reared indoors (Wandra *et al*, 2007).

Although the prevalence of cysticercosis is currently low, sporadic cases are still detected at hospitals, especially at Sanglah Hospital, Denpasar. NCC cases included 1 case of disseminated cysticercosis (2003) (Sudewi *et al*, 2008), 3 cases of NCC (2004) summarized in this review, 1 case of NCC (2005), 2 cases of dual infection (2007, 2009), and 5 cases of NCC (2009) (Wandra *et al*, unpublished).

Case 1

A 50-year-old Balinese woman, from a rural village in Karang Asem District, eastern Bali was admitted to Sanglah Hospital on 4 September 2004. She complained of a headache and a history of epileptic seizures for two years duration, and presented with 2 subcutaneous nodules on her body. The subcutaneous nodules were mobile, non-tender, located at the fossa cubity sinistra and femoralis dextra, measuring 1 and 1.5 cm in diameter, respectively. There were no abnormalities of the respiratory or cardiovascular systems. On neurological examination she was unconscious with a GCS of E2V1M5, pupil anisocore (5 mm/3 mm). The Babinski's sign was positive bilaterally, funduscopy showed papiledema bilaterally. Electroencephalography showed bifrontal slow waves. Laboratory testing, including routine blood tests, eosinophils and fecal examination, were normal. Excision of the subcutaneous nodules was performed and histopathological examination revealed a characteristic structure of a taeniid metacestode (Fig 1a). A CT scan showed an active lesion with surrounding edema of the frontoparietal dextra et sinistra. ELISA results using native antigens for screening and recombinant chimeric antigens for confirmation of cysticercosis, 100% specific to cysticercosis, were positive [(OD values in native and recombinant antigen systems: 0.392 (cut-off value: 0.051) and 0.446 (cut-off value: 0.093), respectively] (Sako *et al*, 2000; Sato *et al*, 2006).

The patient was treated with 10 mg diazepam, 300 mg phenytoin (intravenous) and 50 mg dexamethasone (intravenous) and 800 mg albendazole daily for one month. On the 16th day, the patient was discharged in stable condition without neurological deficit and free from seizures. However, 8 months later (May, 2005), she had a focal seizure. The family stated she stopped taking phenytoin for the previous 4 months. After taking phenytoin orally again, she again became seizure free. A repeated CT scan 3 months later (August 2005) showed a calcified lesion of the frontoparietal dextra et sinistra. The serology results were negative (OD values in native and recombinant antigen

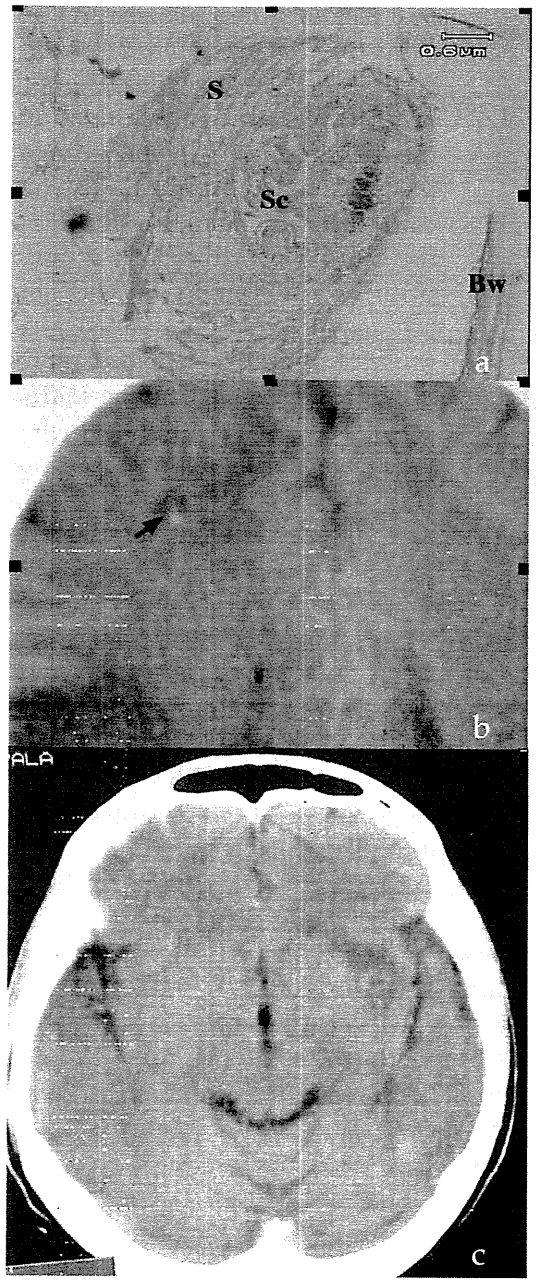

Fig 1–(a) Subcutaneous nodule showing a characteristic structure of a taeniid metacestode, scolex (S), spiral canal (Sc), and bladder wall (Bw). (b) CT scan showing an active lesion with scolex (arrow) with surrounding edema of the frontoparietal dextra et sinistra. (c) A calcified lesion after 3 months.

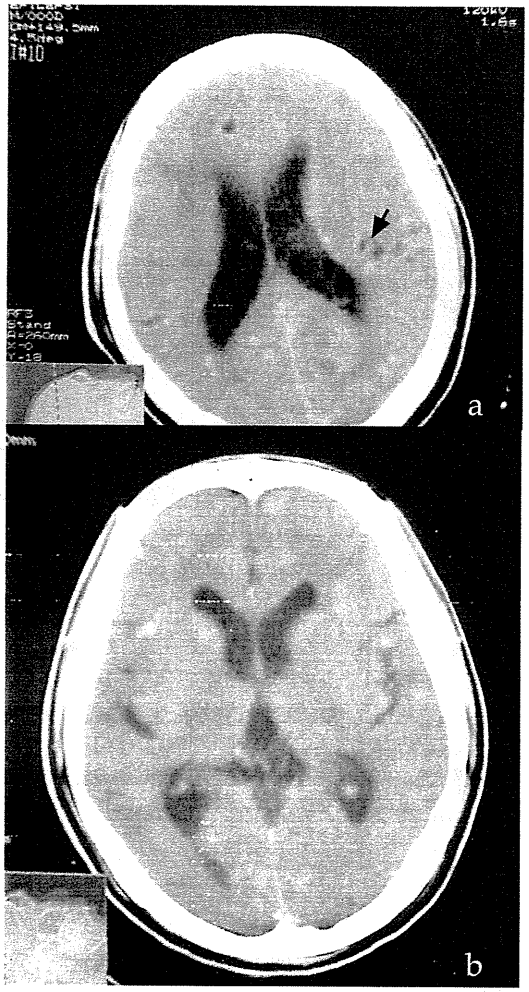

Fig 2–(a) CT Scan showing multiple cystic lesions with scoleces (arrow) and (b) multiple calcified lesions after 3 months.

systems: 0.027 and 0.034, respectively). Immunoblot also became negative (data not shown).

Case 2

A 31-year-old Balinese man, from a rural village in Gianyar District, Bali was admitted to Sanglah Hospital on 31 October 2004. He complained of headache and gave a history of epileptic seizures for 10 years duration with paresthesias. He had 3 generalized tonic-colonic seizures

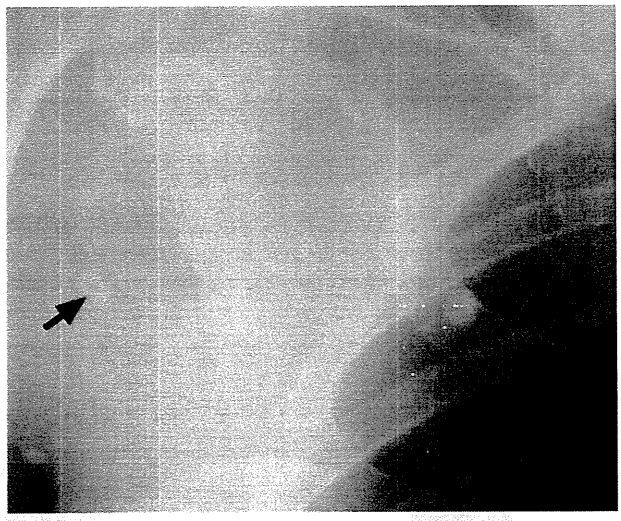

Fig 3–Chest radiograph showing multiple cigar-shaped calcified lesions (arrow).

3 hours prior to admission. On examination, he was conscious and had left sided paresthesia of his whole body. There were no abnormalities of the respiratory or cardiovascular systems. Funduscopy showed papiledema bilaterally. Laboratory data, including routine blood tests for eosinophils and fecal examination, were normal. A CT scan of the brain showed multiple cystic lesions with a scolex and multiple calcified lesions (Fig 2a). ELISA, using native and recombinant antigen systems, was positive (OD values: 0.338 and 0.879, respectively). He was treated with diazepam 10 mg/day orally, followed by phenytoin 300 mg/day (intravenous), albendazole 800 mg/day and dexametasone 40 mg/day for 1 month. He was free of seizures during hospitalization. A CT scan of the brain performed 3 month later showed the cystic lesions had disappeared and there remained multiple calcified lesions (Fig 2b). Antibody responses remained positive after treatment.

Case 3

A 60-year-old Balinese woman from a rural village of Denpasar Municipality, Bali was admitted to Sanglah Hospital on 29 December 2004. She complained of a headache and a history of 3 focal seizures involving the right side of her body during the previous 3 months. She also had dysarthria and hemiparesthesias of the right side of the body 6 hours prior to admission. She was conscious with right hemiparesis, had muscle strength of 4/4 based on the Medical Research Council (MRC) scale, but had right supranuclear facial and hypoglossal nerve palsies, and right hemiparesthesia. The Babinski sign was negative and she had decreased deep tendon reflexes. Funduscopy showed bilateral papiledema. Laboratory data, included routine blood tests for eosinophils and fecal examination, were normal. A CT scan showed a calcified lesion with surrounding edema in the parietooccipital region. A chest radiograph showed multiple cigar-shaped calcified lesions (Fig 3). Serological results with ELISA using native and recombinant antigen systems were positive (OD values: 0.092 and 0.371, respectively). She was treated with piracetam 12 grams, dexametasone 10 mg followed by 20 mg daily, and acetosal 100 mg, phenytoin 300 mg daily for one month. She gradually improved and was discharged from the hospital without neurological deficits on 4 January 2005. Antibody responses in this case remained weakly positive after treatment.

These 3 confirmed NCC cases in 2004 showed active *T. solium* cysts in the brain. Several retrospective studies of a Japanese NCC case reveal cysts can survive at least 10 years (Ito *et al*, 1999; Yanagida *et al*, 2010). Therefore, it is important to check antibody responses in NCC suspected or confirmed cases before and after treatment

in order to monitor progression.

CONCLUSION

T. solium and *T. saginata* have been described in Bali. Recent field surveys from 2002 to 2009 showed *T. saginata* taeniasis is still endemic. The prevalence of cysticercosis is now low, but sporadic cases are still detected at Sanglah Hospital, Denpasar, Bali. Clinicians should remember symptomatic NCC may be induced by treatment with low dose praziquantel for the treatment of *T. saginata* taeniasis, some may also have asymptomatic NCC. Cysticercosis outbreaks may occur even with a single taeniasis carrier of *T. solium* in the community (Schantz *et al*, 1992; Hira *et al*, 2004). We need to develop new tools for detection of taeniasis carriers, such as rapid tests to detect taeniasis and cysticercosis during field surveys in endemic areas (Handali *et al*, 2010a, b). In order to prevent unexpected seizures induced after treatment with praziquantel, we need to develop rapid serology to detect cysticercosis. One drug based on pumpkin seeds has been developed for the treatment of intestinal cestode infections in China, and it apparently, does not induce seizures (Li *et al*, in preparation). The recombinant antigens 100% specific for detection of cysticercosis (Sako *et al*, 2000; Sato *et al*, 2006) have been used in Indonesia (Sudewi *et al*, 2008) China, Thailand and Cameroon to detect NCC cases in the community (Li *et al*, 2006; Anantaphruti *et al*, 2010; Nkouawa *et al*, 2010). We need to introduce rapid immunochromatography test kits or other rapid tests to detect cysticercosis and taeniasis (Handali *et al*, 2010a, b) as was done for echinococcosis (Sako *et al*, 2009). Sustainable campaigns to eradicate of cysticercosis are important for people to know the risk factors for this disease.

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REVIEW

SEROLOGICAL AND MOLECULAR TOOLS TO DETECT NEUROLOGIC PARASITIC ZOONOSES IN RURAL CAMEROON

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Abstract. Parasitic helminthiases, such as toxocariasis, cysticercosis and paragonimiasis are a public health threat, since they can affect the brain leading to neurological disorders. Epilepsy and paragonimiasis are common in southwestern Cameroon. We reviewed the literature for studies using antigens to diagnose toxocariasis, cysticercosis, and paragonimiasis. Serology revealed that 61 (36.3%), 26 (15.5%) and 2 (1.2%) of 168 persons examined [78 males (15.2 ± 8.2 years old), 90 females (12.9 ± 5.9 years old), 143 persons < 20 years old] had antibody responses to toxocariasis, paragonimiasis and cysticercosis, respectively. Of the 14 people with epilepsy, 5 were seropositive for *Toxocara* antigens and 1 was positive for both *Toxocara* and *Paragonimus* antigens. Two children were serologically confirmed to have cysticercosis. Serologic screening for cysticercosis may be feasible to detect asymptomatic cysticercosis in children in endemic areas leading to early treatment. The causative *Paragonimus* species was confirmed to be *P. africanus* by molecular sequencing. Education, screening and confirmation test for these diseases may be needed for control in Cameroon.

Keywords: epilepsy, parasitic zoonoses, toxocariasis, paragonimiasis, cysticercosis, immuno- and molecular-diagnosis, Cameroon

INTRODUCTION

Parasitic zoonotic infections have a potentially serious impact on human health and economy. Paragonimiasis, cysticercosis and toxocariasis represent a public health threat in developing countries, since they can affect various tissues and organs including the brain leading to neurological disorders, such as epilepsy (Nicoletti *et al*, 2002; Ito *et al*, 2006b; Garcia and Modi, 2008). Cysticercosis has been considered to be a major cause of the late-onset epilepsy in developing countries (Zoli *et al*, 2003; Montano *et al*,

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2005; Villaran *et al*, 2009). Other helminthic diseases such as toxocariasis, paragonimiasis, and onchocerciasis and protozoan diseases, such as malaria and toxoplasmosis, can also cause neurological disorders and idiopathic epilepsy (Bachli *et al*, 2004; Akyol *et al*, 2007; Garcia and Modi, 2008; Kaiser *et al*, 2008; Ngoungou and Preux, 2008). Therefore, early detection of these pathogens in humans using immunological and molecular tools is important for early treatment and prevention of the neurological sequelae (Ito, 2002; Ito and Craig, 2003; Margono *et al*, 2003; Ito *et al*, 2006b).

In general, ELISA using crude antigens is suitable for screening and the immunoblot using crude antigens or semi-purified antigens is for confirmation in the diagnosis of these helminthes. With pure antigens the ELISA and immunoblot may have a better specificity. Both these tools might be used to screen and confirm infections in endemic areas (Ito *et al*, 1998, 1999; Sako *et al*, 2000; Sato *et al*, 2003, 2006).

Tombel Health District, Southwest Province, Cameroon is endemic for paragonimiasis and epilepsy is common (Moyou-Somo *et al*, 2003; Moyou-Somo and Tagni-Zukam, 2003). However, there is a paucity of information about the role of parasitic zoonoses among people with neurological disorders in this area. There is little information about cysticercosis in children, although cysticercosis is relatively common in adults and epileptic patients (Nguekam *et al*, 2003; Zoli *et al*, 2003). There is no data regarding the prevalence of toxocariasis in human in Cameroon, although toxocariasis is prevalent in dogs (Komtangi *et al*, 2005). Paragonimiasis is diagnosed by the detection of eggs in the sputum but control of this disease has only been partially successful.

We reviewed the literature regarding the prevalence of toxocariasis, paragonimiasis and cysticercosis, and their association with neurological diseases among children in southwestern Cameroon using immunological techniques.

TOXOCARIASIS

Toxocariasis is a zoonotic infection of humans caused by dog and cat roundworms, *Toxocara canis* and *Toxocara cati*, respectively. Humans become infected by ingestion of embryonated eggs from contaminated sources. Children often become infected because they put objects in their mouths or eat dirt. This disease can be found in developed and developing countries (Hayashi *et al*, 2005; Fernando *et al*, 2007; Nicoletti *et al*, 2008; Yoshikawa *et al*, 2008; Zarnowska *et al*, 2008). We expect *T. canis* infections are more common than *T. cati* infections due to the defecation habits of dogs, but there is no information about this disease in humans in Cameroon. The prevalence of this disease in dogs is high in Cameroon (Komtangi *et al*, 2005). We expected the prevalence of this infection in children might also be high especially in rural areas where risk factors are common in Cameroon.

Toxocariasis affecting the organs is called visceral larva migrans, and the disease restricted to the eye and the optic nerve is called ocular larva migrans (Logar *et al*, 2004; Yoshikawa *et al*, 2008). Neurological disorders, especially epilepsy have been associated with toxocariasis (Bachli *et al*, 2004; Moreira-Silva *et al*, 2004; Akyol *et al*, 2007; Nicoletti *et al*, 2008). Epilepsy is common in southwestern Cameroon. Serology using a recombinant antigen of *T. canis* second-stage larvae (Yamasaki *et al*, 2000) was performed on serum samples collected from children in Southwest

Table 1
Clinical and serological results of individuals investigated.

Clinical/immunological diagnosis of persons with symptoms	Age/gender (male/female)			Total (%) (n = 168)
	0-10 years (31/49)	11-20 years (35/28)	> 21 years (14/11)	
Cough	64 (31/33)	51 (29/22)	20 (12/8)	135 (80.3)
Headache	49 (22/27)	40 (22/18)	17 (7/10)	106 (63.0)
Eye disorder	7 (3/4)	9 (5/4)	14 (6/8)	30 (17.8)
Epilepsy	2 (2/0)	7 (5/2)	5 (3/2)	14 (8.3)
Chest pain	33 (15/18)	30 (15/15)	17 (7/10)	80 (47.6)
Hemoptysis	8 (3/5)	7 (3/4)	3 (1/2)	18 (11.3)
Crab eaten	60 (26/34)	60 (34/26)	17 (11/6)	137 (81.5)
Pork eaten	64 (26/38)	49 (28/21)	22 (12/10)	135 (80.3)
Microscopy for *Paragonimus* spp eggs[a]				
Sputum	9 (2/7)	5 (3/2)	2 (0/2)	16 (9.5)
Feces	0 (0/0)	0 (0/0)	0 (0/0)	0 (0.0)
ELISA and/or immunoblot				
Paragonimiasis	13 (4/9)	9 (5/4)	4 (2/2)	26 (15.5)
Toxocariasis	31 (13/18)	24 (14/10)	6 (4/2)	61 (36.3)
Cysticercosis	0 (0/0)	2 (2/0)	0 (0/0)	2 (1.2)

[a]The eggs were later confirmed to be *P. africanus* using molecular tools.

Cameroon. ELISA results showed that 61 persons (36.3%) exhibited antibody response to this antigen (Table 1) (Nkouawa *et al*, 2010a). It suggests that children are the most exposed people (Hayashi *et al*, 2005; Fernando *et al*, 2007; Zarnowska *et al*, 2008; Sviben *et al*, 2009). Among the 14 persons with epilepsy, 5 were seropositive for specific *Toxocara* antigen (Fig 1). More epidemiological data of toxocariasis are needed to determine its association with epilepsy in this area, and in other regions in Cameroon to determine the distribution of this disease.

PARAGONIMIASIS

Paragonimiasis is a parasitic disease of humans and other mammals caused by *Paragonimus* spp (lung flukes). Humans are infected after ingestion of raw or undercooked fresh water crabs harboring the metacercariae. Pulmonary paragonimiasis is the most common, but cerebral paragonimiasis is not rare. Since both paragonimiasis and epilepsy are highly prevalent in the Southwest Province of Cameroon, it is necessary to investigate the correlation between epilepsy and paragonimiasis in this area. Microscopic examination of eggs in feces is generally the most reliable direct method. However, *Paragonimus* eggs were mostly found from sputa but not from fecal samples collected in Southwest Cameroon (Nkouawa *et al*, 2009a). This might be due to the people's attitude to vomit sputa rather than swallowing them. Therefore, the diagnosis of paragonimiasis in Cameroon by detecting *Paragonimus* eggs from feces lacks sensitivity (Ripert *et al*, 1992; Ollivier *et al*, 1995; Moyou-Somo *et al*, 2003; Moyou-Somo

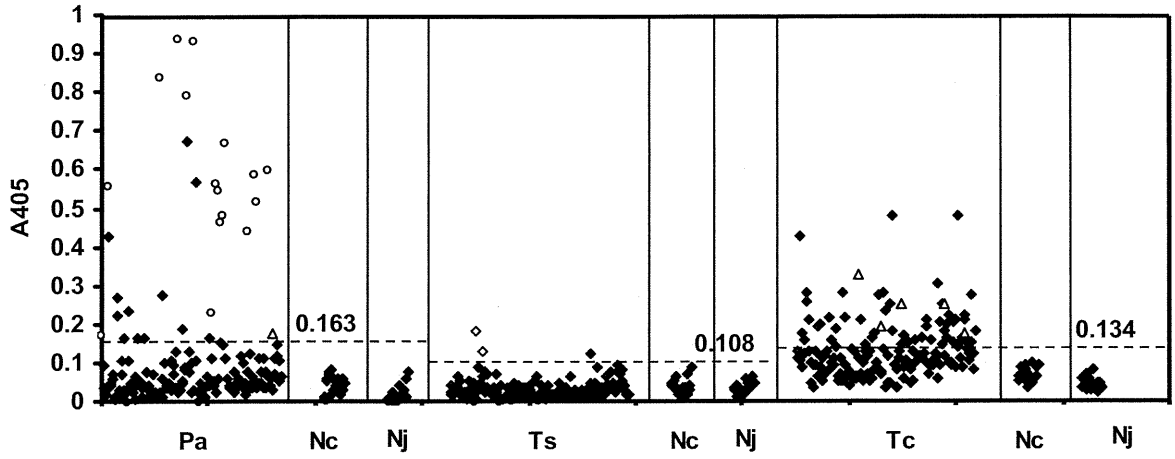

Fig 1–ELISA results modified from Nkouawa *et al* (2010a) for paragonimiasis (Pa), cysticercosis (Ts) and toxocariasis (Tc) from 208 persons [168 and 20 (Nc) with and without symptoms from Cameroon, respectively and 20 from Japan (Nj)]. ○, samples with *Paragonimus* eggs in the sputum. Serology could detect more than all cases with eggs in the sputum and was expected to be more sensitive for detection of paragonimiasis including immature adult stage (Nkouawa *et al*, 2009a). ◇, positive against the recombinant antigen 100% specific to cysticercosis by immunoblot (Sako *et al*, 2000). △, patients with history of seizures were positive to *P. africanus* somatic antigen and to recombinant antigen of *T. canis* second-stage larvae. The broken line denotes the respective cut-off value for each disease.

and Tagni-Zukam, 2003). Furthermore, it is not possible to detect eggs during pre-patent period, in single infections, or extra-pulmonary paragonimiasis. Serological tests, which have been confirmed to be useful for detection of paragonimiasis in Asia and Latin America (Ikeda *et al*, 1996; Kong *et al*, 1998; Blair *et al*, 2007), are more sensitive than detection of eggs in the sputum for diagnosis of paragonimiasis in Cameroon (Nkouawa *et al*, 2009a). A positive antibody responses to crude antigens of *P. africanus* adult worms were observed in 14.8% ($n = 25$) and 15.5% ($n = 26$) of 168 sera collected from persons living in Southwest Cameroon by enzyme-linked immunosorbent assay (ELISA) and immunoblot analysis, respectively (Table 1). Sera from other parasitic infections (schistosomiasis haematobium, schistosomiasis japonicum, fascioliasis, clonorchiasis, cysticercosis and sparganosis) showed weak cross-reaction to crude antigens (Nkouawa *et al*, 2009a). Immunodominant bands of 33- and 35-kDa of *P. africanus* antigens recognized by most patients' sera were similar to those of *P. westermani* adults exhibiting strong reactions with IgG4 (Kong *et al*, 1998). Crude antigens of *Paragonimus* spp are useful for detection of paragonimiasis, since these antigens appear to be genus specific (Kong *et al*, 1998). Sera from persons infected with *P. africanus* showed strong antibody response to the Japanese *P. westermani* and *P. miyazakii* adult antigens, and *vice versa* sera from persons infected with the Japanese *P. westermani* and *P. miyazakii* strongly cross-reacted with the antigens of *P. africanus* adult (Nkouawa *et al*, 2009a). This indicates that differentiation of *Paragonimus* species cannot be achieved serologically. Since at least ten *Paragonimus* species have been reported to infect

humans in endemic areas in Asia (Sripa *et al*, 2010), antigens from any species are expected to provide reliable results.

In Cameroon, immunological tests detected more paragonimiasis cases (15.4%) than microscopic examination of eggs in the sputum (3.0-9.6%) (Ripert *et al*, 1992; Ollivier *et al*, 1995; Moyou-Somo *et al*, 2003; Moyou-Somo and Tagni-Zukam, 2003). Therefore, we adopted immunological tests for the surveillance of this disease. Although the number of cases has decreased in adult populations in endemic areas in Southwest Cameroon (Moyou-Somo *et al*, 2003; Moyou-Somo and Tagni-Zukam, 2003), our data have demonstrated that children, especially those < 10 years old are still highly infected with *P. africanus* due to their food eating habits and poor hygiene and sanitation (Table 1). Therefore, control measures including mass screening by immunological method and education of children are an urgent task to prevent this disease. Out of 14 epileptic patients, only one showed positive antibody responses to antigens specific to paragonimiasis (Fig 1), this suggests that paragonimiasis might not be the major cause of epilepsy in this area.

Since four species, *P. africanus*, *P. uterobilateralis*, *P. westermani*-like and *Euparagonimus* sp, are distributed in Cameroon (Voelker and Sachs, 1977; Cabaret et al, 1999), it is necessary to confirm the parasite species causing paragonimiasis by molecular tools. Adult worms recovered from the lungs of a cat after experimental infection with metacercariae collected from crabs in Southwest Cameroon were identified as *P. africanus* by sequence data from the internal transcribed spacer 2 (ITS2) region and cytochrome *c* oxidase subunit 1 (cox1) genes (Nkouawa *et al*, 2009a). Therefore, molecular sequencing can be used to detect *P. africanus*, the causative agent of paragonimiasis in Southwest Cameroon. PCR using specific primer designed from *P. africanus* ITS2 and cox1 sequences was also used to detect target DNA from fecal samples. It is more sensitive than microscopic examination of eggs in feces (Nkouawa *et al*, 2009a).

Detection of target DNA in the sputum provides more sensitive results, especially to identify the *Paragonimus* species, since eggs are often found in cases of pulmonary paragonimiasis. This method cannot be applied for extra-pulmonary cases or cerebral paragonimiasis.

CYSTICERCOSIS

Cysticercosis caused by *T. solium* larvae is one of the most serious diseases in many developing countries (Schantz *et al*, 1998; Murrell, 2005; Ito *et al*, 2006a). Previous studies have focused on adult population and pigs in the western and northwestern regions of Cameroon (Pouedet *et al*, 2002; Vondou *et al*, 2002; Nguekam *et al*, 2003; Shey-Njila *et al*, 2003; Zoli *et al*, 2003), and on swine cysticercosis in the North Province (Assana *et al*, 2010). There is little or no information from other regions, especially where epilepsy is relatively high and where the risk factor of this disease such as pig farming with free access of pigs to human feces remains. For human cysticercosis, detection of antibody responses has been combined with clinical criteria and/or imaging figures (Ito *et al*, 2006c; Willingham *et al*, 2010). As the CT or MRI is too expensive and is not affordable to many people in developing countries, immunodiagnosis using specific antigens is the method of choice for screening of cysticercosis due to its simplicity and reliability.

Among several characterized specific antigens for *T. solium*, the glycoproteins

(GPs) have been widely used for serodiagnosis of cysticercosis (Ito *et al*, 1998; Sako *et al*, 2000; Wandra *et al*, 2003; Ito *et al*, 2006c; Li *et al*, 2006; Sato *et al*, 2006; Wandra *et al*, 2006). We applied GPs and recombinant antigen (Sako *et al*, 2000; Li *et al*, 2006; Sato *et al*, 2006; Anantaphruti *et al*, 2010) for diagnosis of cysticercosis in serum samples collected in Southwest Cameroon (Nkouawa *et al*, 2010a). More than half of epileptic adult patients in West and North West provinces of Cameroon exhibited strong antibody responses. It suggested that cysticercosis was the major causative agent of the late-onset epilepsy in this area (Zoli *et al*, 2003). However, out of 168 samples including 143 persons < 20 years old, collected from Southwest Cameroon, 1 girl aged 4 years old and 2 boys aged 11 and 13 years old without symptoms or any history of seizures exhibited antibody responses against GPs by ELISA. Immunoblot using the same GPs and recombinant antigens of *T. solium* revealed that the 2 boys were seropositive. Therefore, these 2 children are asymptomatic cysticercosis (Nkouawa *et al*, 2010a). As it has been shown that cysticercus of *T. solium* can survive for many years in patient's body (Yanagida *et al*, 2010), it is necessary to follow up these two children.

The follow-up of asymptomatic healthy but serologically positive people for cysticercosis in Papua, Indonesia, revealed that most of them had detectable subcutaneous nodules (Wandra *et al*, 2003; Ito *et al*, 2004). However, subcutaneous cysticercosis is not common in Africa as in Asia (Ito *et al*, 2003). Therefore, follow-up antibody titers may be another indicator to confirm active cysticercosis in Africa. Serology using highly specific antigens is useful for detection of asymptomatic cysticercosis and symptomatic cases in endemic areas.

TAENIASIS

To achieve the control and prevention of cysticercosis, the detection of cysticercosis in humans, pigs, and dogs (Ito *et al*, 2002) together with the detection of taeniasis carriers are required in endemic areas. Although cysticercosis is still a serious problem in some areas in Asia, Africa and Latin America, the disease has been well controlled (Ito *et al*, 2005; Garcia *et al*, 2007; Wandra *et al*, 2006). Nonetheless, the control in Cameroon is mainly focused on the detection of cysticerci in humans and pigs, which are the end stage of the parasite in the intermediate hosts. There is few information on adult tapeworms and the risk factor for cysticercosis in pigs and humans in many endemic areas. A previous study reported that *T. solium* taeniasis was diagnosed by microscopic examination of *Taenia* egg in fecal samples collected from people in endemic areas of West Cameroon (Vondou *et al*, 2002).

The microscopic examination of *Taenia* eggs is not sensitive and eggs of all *Taenia* species are morphologically identical. More reliable molecular tools such as multiplex PCR and loop-mediated isothermal amplification (LAMP) have been introduced for identification of eggs, cysticerci, adult worms and for feces without eggs or proglottids (Nkouawa *et al*, 2009b; Yamasaki *et al*, 2004). LAMP is more sensitive to detect taeniasis than multiplex PCR (Nkouawa *et al*, 2010b) in endemic areas in Cameroon. For future studies in endemic areas in Cameroon and other countries, the detection of adult tapeworms as a screening of taeniasis carriers of *T. solium* using both copro-ELSIA (Guezala *et al*, 2009) and copro-DNA methods (Nkouawa *et al*, 2009b, 2010b; Nakao *et al*, 2010) is necessary.

CONCLUSION

In this review, highly specific recombinant antigens for both toxocariasis and cysticercosis, and crude antigens of *P. africanus* showing no or little cross-reaction with other helminth infections were used. Toxocariasis (36.3%), paragonimiasis (15.5%) and cysticercosis (1.2%) have been confirmed among surveyed persons. Among the seropositive persons, 11 were found to have dual infections of both *Toxocara* and *Paragonimus*. Of 14 epilepsy cases, 5 were seropositive for toxocariasis and one was simultaneously positive for paragonimiasis. This demonstrated that 1) paragonimiasis among children still remains a serious health problem in this endemic area, 2) asymptomatic cysticercosis cases were detected in 2 children, and 3) toxocariasis was serologically confirmed for the first time in Cameroon with relatively high prevalence. The serological tools are highly useful for screening of these diseases having great consequences on the brain. Therefore, early diagnosis at the early stage is one of the key strategies to control and prevent the spread of these diseases in people, especially in children in endemic areas. For future strategies, detail epidemiological studies to screen young and adult populations for taeniasis/cysticercosis, and pigs for cysticercosis and also other helminthic infections are necessary to establish the correlation between epilepsy and parasitic diseases for the early treatment and prevention of these diseases in this area and other regions in Cameroon and any other countries.

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