Parasitic Zoonoses in Asian-Pacific Regions 2012

EDITED BY
Masaharu Tokoro
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edited by

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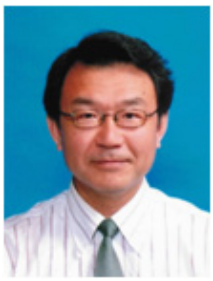

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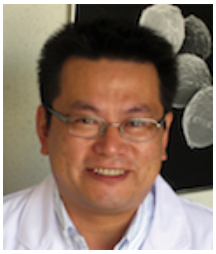

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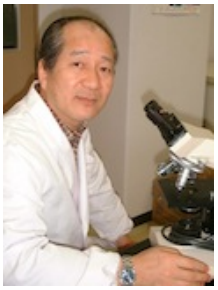

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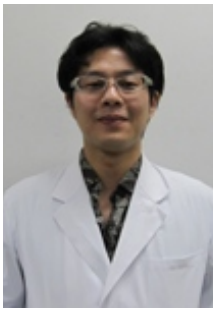

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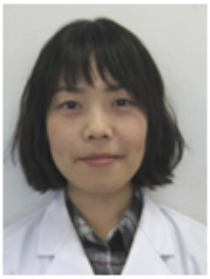

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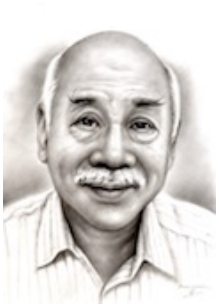

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Preface

The importance of zoonoses, or diseases that can be transmitted between humans and wild or domesticated animals, is increasingly recognized as a source of emerging infectious diseases (EIDs). In fact, more than half of EIDs have been estimated to be zoonotic pathogens. To effectively control the rising threat of infectious diseases, we need to monitor epidemiological status from the view of human and animal endemicity and using data from the surrounding ecological environments.

The crosstalk among professionals/researchers in different fields (e.g., human and veterinary doctors, clinical and basic researchers, parasitologists and entomologists, researchers and public health officers) that has traditionally take place at the Asian-Pacific Congress for Parasitic Zoonoses (APCPZ) provides an ideal opportunity to exchange and communicate this kind of information to each other for better disease control.

This book presents the proceedings of the 12th APCPZ held in Kobe, Japan, from October 6–7, 2012. The contributors to this book—the members of APCPZ—have kindly updated us on their own cutting-edge researches. Therefore, this book provides a catalog of a wide variety of valuable insights into the current status of parasitic zoonoses for professionals in many fields interested in this research area.

Masaharu Tokoro
March 2013

Cover Illustration: *The countries from which we welcomed attendees to the 12th APCPZ are highlighted on the map: Argentina, Bangladesh, China, Egypt, Indonesia, Iran, Japan, Korea, Malaysia, the Philippines, Taiwan, Thailand, the United States, and Vietnam.*

Section I
Original Papers

Present situation of parasitic zoonoses in Korea

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Abstract
Parasitic zoonoses are becoming more and more important in public health points of view around the world. In the Republic of Korea, major zoonotic parasitoses can be classified into protozoan diseases that include toxoplasmosis and cryptosporidiosis, nematode diseases such as anisakiasis, toxocariasis, trichinosis, and capillariasis, trematode diseases that include clonorchiasis, paragonimiasis, fascioliasis, heterophyidiasis (metagonimiasis, heterophyiasis, and others), echinostomiasis, neodiplostomiasis, and gymnophalloidiasis, and cestode diseases, namely, diphyllobothriasis, sparganosis, taeniases (solium, saginata, and asiatica), cysticercosis, and echinococcosis. For prevention of zoonotic parasitoses, it is strongly recommended to control animal infections together with early diagnosis and treatment of human patients. Proper disposal of human and animal excreta containing cyst, oocysts, and/or eggs is also needed for effective control.

Key Words: parasitic zoonosis, zoonotic parasite, protozoa, helminth, Korea

INTRODUCTION

Many species of protozoa and helminths have complex life cycles, in which specific intermediate or reservoir hosts are involved. These kinds of hosts may become the source of human or animal infections. When vertebrate animals, including birds and mammals, are involved in the transmission of parasites, the disease is called as a parasitic zoonosis and the parasite is called as a zoonotic parasite. Parasitic zoonoses are nowadays recognized as a highly important group of diseases in public health points of view. In the present paper, zoonotic parasites and the diseases that occur in the Republic of Korea are briefly reviewed.

ZOONOTIC PROTOZOAN DISEASES

Toxoplasmosis: The seroprevalence of *Toxoplasma gondii* infection had been generally reported to be 5-8% in Korea. However, the rate recently tend to increase in several areas. For example, in two townships of Ganghwa Island, Gyeonggi-do, the seropositive rates were 14.5% and 15.8% in 2010, and the rates increased to 23.8% and 25.8%, respectively, in 2011 [1]. In another area, a township of Cheorwon-gun, Gangwon-do, the average seropositive rate among residents during 2010 and 2011 was 17.0% [2]. It is presumed that toxoplasmosis is increasing because of more active maintenance of sylvatic life cycles, with increasing numbers of stray cats and wild boars.

Cryptosporidiosis: This protozoan disease is caused by *Cryptosporidium hominis* and *C. parvum* in Korea [3]. *C. parvum* is known to be zoonotic whereas *C. hominis* is not [4]. An extremely high prevalence of human cryptosporidiosis was reported in a rural village of Jeollanam-do, where 57% of 77 residents were found oocyst positive at least more than one time out of 12 monthly repeated examinations in a year [5]. In Seoul, the rate was 0.5%, and in rural areas of Jeollanam-do it was 10.6% [6]. There is little information in recent years.

ZOONOTIC NEMATODIASES

Anisakiasis: *Anisakis simplex, A. physeteris,* and *Pseudoterranova decipiens* are the three major anisakid species responsible for human infections, and *Anisakis pegreffii* has been recently added [7, 8]. Of more than 50,000 reported human cases around the world, over 90% were from Japan [8]. In Korea, about 300 cases, mostly due to infection with *A. simplex* followed by *P. decipiens* larvae, were documented [8]. The sea eel was one of the most common sources of human infections in Korea [8]. This disease tends to occur more frequently in Korea.

Toxocariasis: This disease is caused by the larvae of *Toxocara canis* or less frequently *T. cati*. The seropositive rate among healthy individuals was 5% in rural areas in Korea [9], and the disease is reported to be increasing recently [10]. The reason for high seropositive rates among the Korean people is reported to be frequent consumption of raw or undercooked cow liver or other animal livers [11].

Trichinosis: The presence of *Trichinella spiralis* life cycle was first confirmed in Korea in 2000, with discovery of an outbreak of human infections at least involving 3 cases [12]. Thereafter, 4 more small outbreaks have been reported in various localities [13]. The source of infection was the badgers or wild boars [13].

Capillariasis: A case of accidental *Capillaria hepatica* infection in the liver of a child was diagnosed by liver biopsy [14], and total five cases of intestinal capillariasis due to *Capillaria philippinensis* have been documented [15, 16].

ZOONOTIC TREMATODIASES

Clonorchiasis: The causative agent, *Clonorchis sinensis,* is currently the most important parasitic helminth of humans in Korea, both in terms of the prevalence and clinical significance, among those diagnosed by fecal examinations. The principal mode of human infection is

ingestion of raw or improperly cooked freshwater fish. The nationwide egg positive rate was 4.6% in 1971, 1.8% in 1976, 2.6% in 1981, 2.7% in 1986, 2.2% in 1992, 1.4% in 1997, and 2.9% in 2004 [17]. The Nakdong River is the most well-known endemic area, with 40-48% egg prevalence in the 1980s [18]. This figure appears quite constant in some tributaries of the river; for example, in 2006, among people living near the Nakdong River, 31.3% were found to be egg positive [19]. In this area, the prevalence of cholangiocarcinoma was estimated to be about 5.5 per 100,000 people [19]. The persistence of infection is due to difficulties in changing the traditional habit of eating raw freshwater fish.

Paragonimiasis: *Paragonimus westermani* is the only known lung fluke species causing human infections in Korea. The infection is contracted by eating raw or improperly cooked freshwater crabs or crayfish. The prevalence was high until the 1970s; however, after the 1990s it was reduced to one hundredth of that [20]. Nevertheless, the seropositive rate in a serology referral center in Seoul during 1993 and 2006 was 1.6% among examined, and new human cases continue to occur sporadically [21].

Fascioliasis: More than 30 biliary or ectopic *Fasciola hepatica* or *Fasciola gigantica* infections have been documented [22]. The ectopic fascioliasis cases involved the gall bladder, intestine, eye, and subcutaneous tissues [23]. Acute pancreatitis due to *F. hepatica* infection was also reported [23].

Metagonimiasis: Three species of *Metagonimus*, i.e., *M. yokogawai*, *M. miyatai* and *M. takahashii,* are known to cause human metagonimiasis in Korea [24-26]. About 240,000 Koreans have been estimated to be infected [27]. Large and small streams in eastern and southern coastal areas, where the sweetfish *Plecoglossus altivelis* (the major source of human infection) are available, are endemic foci of *M. yokogawai* infection [27]. Minnows and carps are the infection sources for *M. miyatai* and *M. takahashii*, respectively, and these infections are prevalent along the upper reaches of the big rivers [27]. The national figures for *Metagonimus* infection among randomly selected Korean people was 1.2% in 1981 and 1.0% in 1986, but this reduced to 0.3% in 1992, 0.3% in 1997, and 0.5% in 2004 [17].

Other heterophyidiases: *Heterophyes nocens, Pygidiopsis summa, Heterophyopsis continua, Stellantchasmus falcatus, Centrocestus armatus, Stictodora fuscata, Stictodora lari,* and *Haplorchis pumilio* are heterophyids other than *Metagonimus* spp. causing human infections in Korea [28, 29]. The estimated number of people infected with these parasites is 120 thousands [27]. *C. armatus* is transmitted by freshwater fish, but all others are transmitted by brackish-water fish, such as *Mugil cephalus, Acanthogobius flavimanus*, and *Lateolabrax japonicus* [27]. These brackish-water fish-borne trematodes are prevalent along coastal areas and off-shore islands [30, 31].

Microphalloidiasis: A species of the Microphallidae, *Gynaecotyla squatarolae*, an intestinal fluke of migratory birds, was recently found infected in a human case [29].

Echinostomiasis: Four echinostome (= Echinostomatidae) species, i.e. *Echinostoma hortense, Echinostoma cinetorchis, Echinochasmus japonicus,* and *Acanthoparyphium tyosenense,* are known to infect humans [27, 32]. About 60,000 Korean people have been estimated to be infected [27]. The most prevalent species is *E. hortense*, for which a southeastern inland area was found to be an endemic focus, with an egg positive proportion of 22.4% among villagers [33]. The sources of human infections with *E. hortense* and *E. cinetorchis* are freshwater fish, including the loach and carp, and the source of infection with *E. japonicus* is large freshwater snails [27]. The infection source of *A. tyosenense* infection is brackish-water bivalves or gastropods [34].

Neodiplostomiasis: The causative agent, *Neodiplostomum seoulense* (formerly *Fibricola seoulensis*), was originally described from house rats captured in Seoul [35]. Now it became a unique intestinal trematode that infects humans and rodents in Korea [35]. This species began to draw medical attention in 1982 when an infected young man complaining of acute abdominal pain, diarrhea, and fever was hospitalized [35]. He had eaten raw snakes seven days before admission to the hospital. Subsequently, the grass snake *Rhabdophis tigrina* caught his village was found to carry the metacercariae [36]. Further human infections were found in 25 soldiers who had eaten raw snakes during their survival trainings [35]. The second intermediate hosts are tadpoles and frogs, and the grass snake is a paratenic host [35]. It is noteworthy that this trematode is highly pathogenic and lethal to experimentally infected mice [37, 38].

Gymnophalloidiasis: The causative agent, *Gymnophalloides seoi*, was first discovered from a woman who complained of acute pancreatitis and gastrointestinal problems [39, 40]. A coastal village in the southwestern coast, where the patient resided, was found to be a highly endemic area of this fluke [41]. Now it is known that western and southern coastal villages and coastal islands are endemic areas of this fluke [40]. The source of human infection and the second intermediate host is the oyster *Crassostrea gigas*, and humans and wading birds, including the oystercatcher *Haematopus ostralegus*, are natural definitive hosts [40].

ZOONOTIC CESTODIASES

Diphyllobothriasis: About 50 worm-proven *Diphyllobothrium latum* (now it is proposed to be *D. nihonkaiense*) cases have been documented since 1971 until 2012 [42, 43]. The taxonomy of *D. latum* tapeworm in Korea is now put to a debate whether it should be *D. nihonkaiense* (as in Japan) or remained as *D. latum* [44]. The common sources of infection have been the salmon, trout, perch, and mullet [42]. The so-called *D. latum* parvum type (originally reported as *D. parvum* by

Stephens in 1908) was discovered in two Korean patients [45]. A case of *D. yonagoense* infection has been described in Korea [46].

Sparganosis: This larval tapeworm infection is caused by the metacestode of *Spirometra erinacei*, an intestinal tapeworm of dogs and cats. Several hundred human infections have been reported in Korea, including subcutaneous, breast, and cerebrospinal infections [47-49]. The source of infection is most commonly the snakes, but less commonly the frogs or water contaminated with infected cyclops may also be the source of human infections [47].

Taeniasis: Human taeniases due to *Taenia solium, T. asiatica,* and *T. saginata* had been quite common in various localities of Korea until the 1980s [50]. However, the national surveys on intestinal helminths every 5 years on randomly selected people revealed that the *Taenia* egg prevalence dropped from 1.9% in 1971 to 0.02% in 1997 and finally 0.0% in 2004 [17]. With the exception of 3 egg-positive cases in 2008 and 2 worm-proven cases in 2011, no more cases have been officially reporded. Jeju-do, where pigs were reared in a conventional way, was the highest endemic area of taeniases. However, even on this island, taeniases are no more reported at present [50]. Analysis of internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome *c* oxidase 1 (*cox1*) genes of 68 taeniases cases reported from 1935 to 2005 in Korea revelaed that the relative occurrence of the 3 *Taenia* spp. was as follows: *T. solium* (4.4%), *T. asiatica* (75.0%), and *T. saginata* (20.6%) [51].

Cysticercosis: The incidence of human cysticercosis in Korea was high before but is now showing a decreasing tendency [50]. For example, during 1968 and 1987, total 425 (0.24%) cysticercosis cases were diagnosed out of a total of 174,770 surgical biopsy specimens obtained in the Department of Pathology, Seoul National University Hospital [52]. In the Department of Pathology, Kyunghee University Medical Center (Seoul) also reported a similar figure, total 136 (0.30%) cysticercosis cases were detected out of a total of 45,651 surgical biopsies examined during 1972 and 1983 [53]. However, this ratio became much lower in a follow-up study (1984-2005) in Kyunghee University Medical Center; only 62 (0.029%) were found to be cysticercosis out of a total of 211,859 surgical biopsies [54]. The prevalence of cysticercosis was also estimated by serologic tests, in particular, ELISA. In 1993, Kong et al. [55] reported a seropositive rate for cysticercosis of 2.1% by ELISA among 750 general population selected from different areas of Korea, whereas 4.0% of 2,667 epileptic patients revealed positive reaction. A serologic reference center in Seoul reported an overall seropositive rate of 4.2% for cysticercosis during 1993 and 2006 and the seropositive rate of cysticercosis apparently showed decreasing trends, from 7.3-8.3% in 1993-1994 to 1.6-2.2% in 2005-2006 [21]. It is presumed that human cysticercosis will disappear within 10-20 years in Korea.

Echinococcosis: A total of 33 echinococcosis patients have been documented since 1983 [56]. Thirty-two cases were caused by *Echinococcus granulosus* and one was due to *E. multilocularis* [57]. Most of the *E. granulosus* case were imported from the Middle East and Asian countries [56]. However, the origin of two *E. granulosus* cases are uncertain and they are suspected to have been infected in Korea [56]. The patient infected with *E. multilocularis* had never been abroad [57]. Therefore, the domestic occurrence of both of the two species should be clarified.

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Present situation of parasitic zoonoses in Japan

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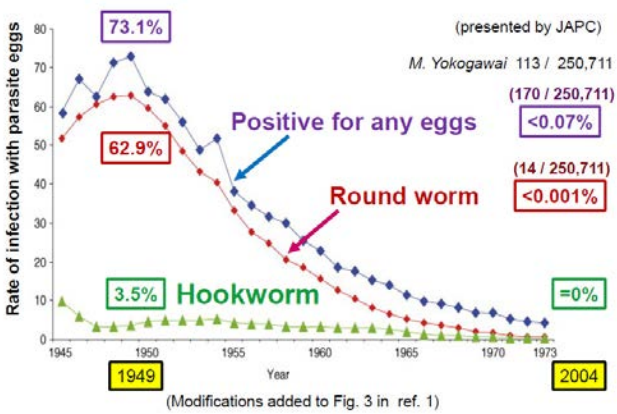
Abstract
Although Japan was previously known as a "paradise for parasites" because of predominant occurrences of ascariasis, trichuriasis, and hookworm disease, such soil-transmitted parasites were successfully eradicated following a nationwide campaign instituted under the Parasite Control Law (terminated in 1994). However, Japan seems to be presently facing a latent wave of parasitosis, mainly from imported and food-borne diseases. Most of the latter are either related to Japanese dietary habits or classified as parasitic zoonosis. Unfortunately, reliable numbers of patients with parasitic diseases, except for amebiasis, cryptosporidiosis, giardiasis, malaria, and echinococcosis, are largely unknown because of the current incomplete surveillance system. In order to elucidate the trends of parasitic diseases, we investigated case reports related to parasitic disease presented in the *Igaku Chuo Zasshi* (*Japana Centra Revuo Medicina*) database between 2000 and 2011. We found 644 reports of protozoan disease (Entameba 126, Plasmodium 113, Toxoplasma 95, Acanthoameba 61, Giradia 28, Cryptosporidium 14, Tricompnas 11, Leishmania 10, Isospora 4, amebic encephalitis 1) and 788 of helminthic diseases (Trematoda 161, Cestoidea 178, Nematoda 449). The majority of helminthiasis cases were shown to be food-borne zoonoses. In addition to eating marine and fresh water raw fish as *sushi* or *sashimi*, personal habits of consumption of other types of raw meat, such as wild boar and chicken/cattle liver, are underlying causes related to the prevalence of zoonotic parasitosis in Japan.
Key Words: food-borne disease, larva migrans, imported disease

I. Past status of parasitic diseases in Japan

Japan was previously known as an endemic country for acute and chronic infectious diseases including parasitic infection, with more than 70% of the Japanese population found to be infected with gastrointestinal parasites in 1949 [1] (Fig. 1). However, a survey performed by the Japan Association of Parasite Control (JAPC) in 2004 revealed that the infection rate had been reduced to less than 0.07%. Under strong supervision by central and local government agencies, successful control of parasitic diseases was nearly completed within the 3 decades after World War II in a nationwide campaign that involved school- and community-based health education and intervention.

Fig. 1. Infection rates of gastrointestinal parasites



(Modifications added to Fig. 3 in ref. 1)

II. Japan continues to be affected by many parasitic diseases

Under the present Infectious Diseases Control Law, 5 parasitic diseases are now required to be reported; amebiasis, cryptosporidiosis, giardiasis, malaria and echinococcosis (Fig. 2, 3). However, there are other parasitic diseases remaining in Japan outside of public awareness. Although the number of patients is quite small, our 3-year clinical experience with parasitic diseases at Nara Medical University has shown that parasitic diseases have not been eradicated (Fig. 4). The majority of these are food-borne, or caused by ingestion of cysts or eggs,

Fig. 2. Four protozoan diseases that require notification

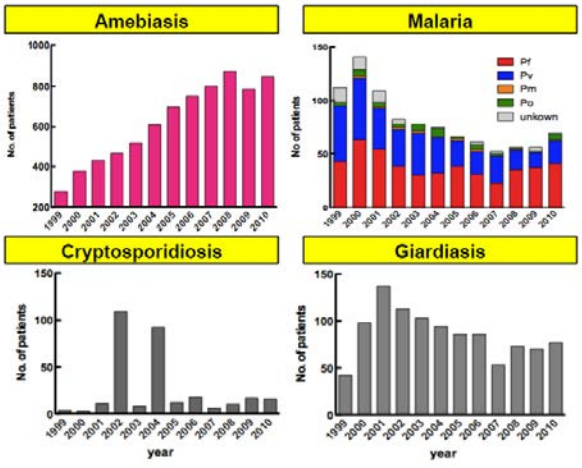


Fig. 3. Echinococcosis is the only helminthiasis that requires notification

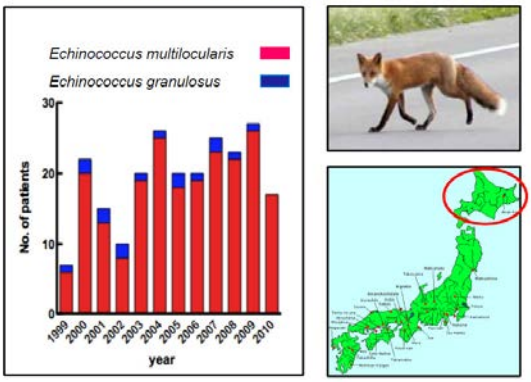


Fig. 4. Results from a clinic for parasitic diseases

Parasitic Diseases	No.	Parasitic Diseases	No.
Diphyllobothriasis	16	Ascariasis (biliary)	1
Toxocariasis	10	Trichuriasis	1
Anisakiasis	8	Loa filariasis	1
Amebiasis	5	Spiruriasis	1
Schistosomiasis	3	Sparganosis	1
Malaria	2	Giardiasis	1
Paragonimiasis	2	Leismaniasis	1
Echinococcosis(cystic)	1	Enterobiasis	1
		Total	**55**

(2008.Jul – 2011 Dec , Nara Medical University)

while toxocariasis should also be recognized as a food-borne disease [2, 3]. The remaining diseases encountered in our practice, including malaria, schistosomiasis haematobium, cystic echinococcosis, ascariasis (found in an immigrant from China), and leishmaniasis, were imported.

III. Survey of cases reported in literature

There are no reports showing the actual number of all parasitic patients in Japan, except for those with the 5 diseases noted above. Thus, we performed a survey to determine the number of case reports of parasitic disease. During the 11-year period from 2000 to 2011, more than 1400 reports were published, clearly indicating that Japan is still affected by these diseases (Table). Most of the helminthic diseases reported were food-borne, with the majority of those zoonotic.

IV. Summary: Present situation regarding parasitic diseases in Japan

Here is a brief summary of the current status of parasitic diseases in Japan based on a survey of published case reports.

1. Soil-transmitted helminthiasis is nearly eradicated, except for sporadic cases.
2. Food-borne parasitosis, generally related to raw fish consumption, is difficult to eradicate.
3. Imported parasitic diseases are increasing.
4. Zoonotic parasitosis, mostly food-borne, seems to be increasing.
5. Various factors, such as raising pets, sexual habits, and HIV infection, are related to parasitosis.

Table. Numbers of parasitic disease case reports in Japan found in Japana Centra Revuo Medicina Database (2000-2011)

Protozoa		Trematoda		Cestoidea		Nematoda	
Entamoeba	126	Paragonimiasis	76	Sparganum	51	Anisakis	136
Plasmodium	113	Schistosoma	59	Echinococcus	50	Strongyloides	76
Toxoplasma	95	Fasciola	10	Diphyllobothrium	41	Dirofilaria	58
Acanthamoeba	61	Clonorchis	8	Taenia solium	27	Spirurina	46
Giardia	28	Metagonimus	8	Taenia saginata	7	Toxocara	32
Cryptosporidium	14			Diplogonoporus	2	Gnathostoma	24
Trichomonas	11					Ascaris suum	21
Leishmania	10					Hookworms (A.d, N.a) A. lumbricoides	17
Isospora	4					Trichuris	14
Amebic encephalitis	1					Enterobius	9
						Angiostrongylus	6
						Trichinella	6
							4
Total	644		161		178		449

(1432 reports)

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Zoonotic Parasitic Diseases in Nepal

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Abstract

Status and spectrum of parasitic zoonosis in Nepal - the Himalayan country located in South Asia between Republic of India (in the south) and People's Republic of China (in the north) is presented. The most common zoonotic diseases studied/reported include toxoplasmosis, toxocariasis, cysticercosis, echinococcosis (hydatid cyst), cryptosporidiosis and others. The reported prevalence of these parasitic zoonosis varies from one to another according to study population in different geographical areas and ethnicity. With regard to toxoplasmosis, nearly half of the general population have antibody to *Toxoplasma gondii*. Prevalence is higher among pregnant women, women with bad obstetric history and patients with malignancy. However, up till now, only one case congenital toxoplasmosis has been reported. Major source of *Toxoplasma* infection appears to be meat/meat products as the seroprevalence among meat animals particularly in buffalo, goat/sheep and pig is very high (over two third). The reported seroprevalence of *Toxocara* antibody is very high (81.0%) though the clinical cases of toxocariasis (visceral and/or ocular larva migrans) are not very common. Cases of cysticercosis (ocular and/or neurocysticercosis) are on rise and is said to be due to the availability of diagnostic facilities (particularly imaging diagnostic such as CT and MRI) during recent years. The reported prevalence of cysticercosis in slaughtered pigs ranges from 11 to 29 percent. The reported teniasis in certain communities goes as high as 50 percent. Echinococcosis (hydatid cyst) occurs sporadically, but the prevalence in the community is not known. Hydatid cyst among the meat animals (buffaloes, sheeps, pigs and goats) ranged from 4 to 18 percent. Fifteen percent of stray dogs killed by poisoning are reported to harbour *Echinococcus* adult worms. The reported prevalence of cryptosporidiosis in man is less than 20 percent, but the prevalence among animals is not studied yet. Very rarely, cases of trichinosis, fascioliasis, paragonimiasis, sparganosis have also been reported. These findings indicate that different types of parasitic zoonosis are existed in Nepal with varying rates of prevalence and demands strict enforcement of existing meat inspection law and hygienic sale of meats/meat products so as to reduce/prevent the burden of parasitic zoonosis in this impoverished country. Also a large scale systematic study of zoonotic parasitic diseases is advocated for evidence based planning and decision making in tackling the parasitic zoonosis.
Key words: Parasitic diseases, Zoonosis, Nepal.

Introduction

Nepal is a roughly brick shaped land-linked least developed country located in the South Asia in between Republic of India (in the south) and People's Republic of China (in the north). Ecologically, it is divided into three regions (running east to west): (1) Mountains (3,001 to 8,848m), (2) Hills (1,001 to 3,000m) and (3) *Terai* or plain area (300 to 1,000m). Nepal is very rich in geography and biodiversity. Of the world's ten highest peaks (over 8,000m) eight including the Mt. Everest (*Sagarmatha*) - the highest peak in the world, lie in Mountain region. Because of these *Himalayas* Nepal is also known as Himalayan country. Administratively, the country is divided into 5 developmental regions that consist of 14 zones, which in turn, comprise 75 districts. The districts are divided into electoral constituencies and municipalities (n=58) and village development committees (VDC) (n=3,915). Since 2007, Nepal has been declared as Federal Democratic Republic of Nepal (*Sanghiya Loktantrik Ganatantra Nepal*).

The total population is 26.62 million (12.92 million males and 13.69 million females) [1]. Ethnically, the population belonging to different ethnics can be broadly grouped into two groups (a) *Tibeto-Burman* (characterized by round face, blunt nose, small eyes and no or very little facial hairs) and (b) *Indo-Aryan* (characterized by oval face, pointed nose, big eyes and presence of facial hairs) [2]. Most of the people (85 percent) still live in rural areas on agricultural subsistence. Though demographic indicators are on positive side [3], a lot have to be achieved to meet MDGs by 2015. Food insecurity, illiteracy/lack of awareness, undernutrition /malnutrition, lack of infrastructure, rugged topography, double burden of diseases are the major challenges in Nepal [4,5]. Because of these facts, study of zoonotic parasitic diseases is not in priority yet. However, whatsoever reports are available, they are reviewed in this paper.

Zoonotic Parasitic Diseases

The different kinds of zoonotic parasitic diseases studied/reported from Nepal include toxoplasmosis, toxocariasis (visceral and/or ocular larva migrans), cysticercosis (ocular and/or neurocysticercosis), teniasis, echinococcosis (unilocular hydatid cyst) and cryptosporidiosis. Of these, toxoplasmosis and hydatid diseases are reported relatively in large number. Very rarely, cases of trichinosis, fascioliasis, paragonimiasis, sparganosis have also been reported.

***(1) Toxoplasma infection*:**

Over dozen of reports on *Toxoplasma* infection in Nepalese are available [6-19]. A chapter entitled "*Toxoplasma* Infection in Nepal: An Overview" has also been published in a book called Asian Parasitology [18]. On an average, of the over 5,000 apparently healthy participants studied, nearly half (45.6%) of Nepalese possess anti-*Toxoplasma* antibody (Fig. 1). Seroprevalence, however, range from 24.0% to 67.1% in study populations [6-18]. This is associated with the difference in geography and ethnicity with their typical life-style/ food habits. The prevalence is significantly higher in females compared with males. This is attributed to the subsistence agriculture where women work both outside (in the farmland) and inside the house (while handling the stored grain) and are risk of exposure to the *Toxoplasma* oocysts [9,10] as the cat defecate both in the farmland and inside the house (in grain storage) [10]

Regarding the ethic group, a marginally higher seroprevalence is seen in *Tibeto-Burmans* (47.0%) compared with *Indo-Aryans* (44.0%) (Fig. 2). This is associated with the frequency and the type of meat (mutton, pork, buff and chicken) they eat [6-18] and the *Toxoplasma* seroprevalence observed in meat animals [20]. *Tibeto-Burmans* eat different types of meat (mutton, pork/wild boar meat, buff and chicken) whereas *Indo-Aryans* (also known as *Khas-Aryans*) are vegetarian traditionally or eat only mutton [9,10] though change is taking place during recent days. Furthermore, *Tibeto-Burmans* eat meat more frequently (once in a week to daily) than *Indo-Aryans* (once in three/four months to once in two weeks) [11,14]. However, the higher seroprevalence in *Tibeto-Burmans* was true only in Eastern and Central Regions and correlated well with the types and frequency of meat eating. On the contrary, higher prevalence in *Indo-Aryans* in Western Region was associated with the eating of raw wild boar meat [14].

On an average, one-third of Nepalese of 20 and less than 20 years have *Toxoplasma* antibody which increased with age attaining over two-third (67.3%) among elderly population of more than 60 years [16,18]. High prevalence appeared to be attributed to the sleazy environment as has been reported from developing countries elsewhere [21]. Highest overall *Toxoplasma* seroprevalence has been recorded in Western Region (56.9%) followed by Eastern Region (42.1%) and Central Region (32.9%), respectively [10,11,18]. Higher prevalence in Western Region was associated with eating raw meat of wild swine by *Indo-Aryans* (*Khas-Aryans*) in certain hilly districts [14]. *Indo-Aryans* in Nepal traditionally do not eat swine meat (pork).

The reported *Toxoplasma* seroprevalence on pregnant women and women with bad obstetric history (BOH) are 55.4% and 38.5%, respectively without significant difference among *Tibeto-Burman* and *Indo-Aryan* ethnic groups [12,18]. These findings indicated that nearly half of pregnant women are at the risk of primary *Toxoplasma* infection during pregnancy that may result into congenital toxoplasmosis. However, it was not known whether the BOH among these women were due to *Toxoplasma* infections. This was much higher compared with the prevalence reported in women with BOH in Delhi (India) (2.9%) [22] but lower than those reported from Bombay (India) (43.8%) [23] and Mexico (44.9%) [24]. It is, therefore, suggested a need for informing young and/or pregnant women about the preventive measures as to prevent primary infection during their pregnancy. Seroprevalence in Nepalese patients with ocular diseases (uveitis, retinitis etc.), malignancy, lymphadenitis including BOH is 50.7% [17,18]. *Toxoplasma* antibody positive subjects, 1.1% of the Toxoplasma antibody positive subjects [18].

First laboratory test confirmed case of congenital toxoplasmosis has also been reported recently (in 2011) [19]. This was supported by the mother's past abortion history, various clinical findings (abdominal distension due to hepato-splenomegaly, scar resembling ocular toxoplasmosis) of baby. Baby died after 6 days of birth. Immediate cause of death was cardiorespiratory failure.

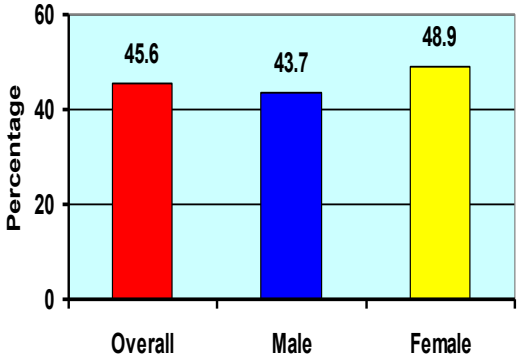

Fig. 1 *Toxoplasma* seroprevalence in two sexes in Nepal

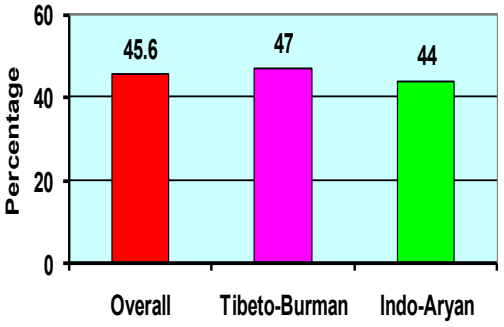

Fig. 2 *Toxoplasma* seroprevalence in two ethnic groups in Nepal

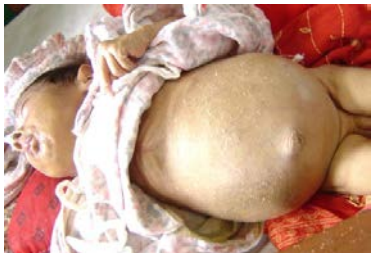

Fig. 3. First case of congenital toxoplasmosis (Ref. 19).

In Nepal, mainly mutton, pork, buff (water buffalo meat) and chicken are consumed [20]. Among the four meat animals studied, highest overall prevalence was observed in pigs (79.6%) followed by goats (68.8%), water buffaloes (63.4%) and chickens (41.1%). Highest seroprevalence in pigs was in agreement with the reports from elsewhere in world [25,26]. While, the prevalence reported from Nepal was higher than those reported from neighboring countries India [27,28] and China [29].

(2) Hydatid disease (Echinococcosis) and Cysticercosis:

Echinococcosis (hydatid cyst) in Nepal occurs sporadically, but the prevalence in the community is not known and many cases go unreported [30]. Of the reported cases, in some, unusual sites in the body are also involved [30,31]. A case of uncomplicated pulmonary (one, 8 cm) and hepatic (two, 7.5 cm) hydatid cysts in a 6 year old girl has also been reported [32]. The reported rate of hydatid cyst among the meat animals (buffaloes, sheeps, pigs and goats) ranged from 4 to 18 percent [33,34]. Fifteen percent of stray dogs killed by poisoning are reported to harbour *Echinococcus* adult worms [33].

Though there is no report of systematic study of cysticercosis, the available data suggest that the prevalence ranges from 0.002 to 0.1% in general population in Nepal [35]. In a retrospective study of 23,402 biopsied cases recorded at a hospital in Kathamndu Valley (KV), 62 (0.3%) were diagnosed as cases of cysticercosis (M=24, F=38) [30,36]. The cases of (ocular and/or neurocysticercosis) are on rise. One of factor contributing to this is said to be due to the availability of diagnostic facilities (particularly imaging diagnostic such as CT and MRI) during recent years. Cysticercosis in Nepal has different treatment outcomes [37]. Neurocysticecosis constitutes a major cause of seizure in Nepal [38]. Fourteen percent of slaughtered pigs have been found to be positive for cysticercosis (M pigs: 8.7%, F pigs: 24.0%) and it was slightly higher in KV (14.3%) than in eastern Nepal (11.1%) [36]. The reported teniasis in certain communities goes as high as 50 percent [39].

(3) Other zoonotic diseases:

There are sporadic reports on other zoonotic parasites. The reported seroprevalence of *Toxocara*

Fig. 4 Good situation for the spread of parasitic zoonosis (e.g. echinococcosis/hydatid cyst).

Fig. 5 Soil sampling in Kathmandu Valley.

antibody is very high (81.0%) [30,40] though the clinical cases of toxocariasis (visceral and/or ocular larva migrans) are not very common except sporadic cases of larva migrans [41]. The high seroprevalence was in agreement with the findings seen in Caribbean children (86.0%) of less than 6 years [42]. *Toxocara* eggs rank second (accounting 22.8%) among the helminth eggs contaminating the environment (soil) in Nepal [43]. The reported prevalence of cryptosporidiosis in man is less than 20 percent [30,44], but the prevalence among animals is not studied yet. Very rarely, cases of trichinosis, fascioliasis, paragonimiasis, sparganosis have also been reported [30].

These findings indicate that different types of parasitic zoonosis are existed in Nepal with varying rates of prevalence and demands strict enforcement of existing meat inspection laws [39]. Hygienic sale of meats/meat products and health promotion approaches for the control of food-borne parasitic zoonosis in Nepal are advocated [45]. A large scale systematic study of zoonotic parasitic diseases and surveillance is advocated for evidence based tackling the parasitic zoonosis in Nepal.

This is pertinent also from the view point of "one health" (human health, animal health and environmental health = one health) philosophy.

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Current situation of the most frequent human parasitic zoonoses in Iran

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Abstract

Zoonoses are at the present time more important than ever due to their magnitude and impact. This review presents up-to-date information on the distribution and control measures of the most important human parasitic zoonose in Iran. The prevalence of several protozoan infections appear to have fallen and this is most marked for some of the intestinal, blood and tissue protozoa. Approximately 90% of all cutaneous leishmaniasis are reported from eight countries including Iran. The highest rate of sero-epidemiology of toxoplasmosis is observed (55.7%) in the north of Iran. Recent research indicated that the frequency of *Cryptosporidium* spp. among human in our area was 7.7%. Fascioliasis is emerging as an important chronic disease of humans, especially in the northern province of Gilan**.** No cases of urinary schistosomiasis, a disease that once affected thousands of individuals in southwestern Khuzestan province, have been reported in Iran in recent years, and no cases of dracunculiasis have been seen in the country since the mid-1970s. Approximately 1% of all admissions to surgical wards are attributable to cystic echinococcosis, which is still considered endemic, but only a few cases of alveolar echinococcosis have been recorded. Recent estimates of the prevalences of ascariasis and strongyloidiasis, for example, lie between just 0.1% and 0.3%, and 1% of the population now appears to be infected with hookworm. Hymenolepasis and Enterobiasis remains fairly prevalent. In Iran, 5.6% of population is infected with *Toxocara* species. Just 10 cases of linguatulosis, 12 of dirofilariasis, one of gongylonemiasis, and three cases of acanthocephalan have been officially reported in Iran.

Introduction

Zoonoses are defined as infectious diseases naturally transmitted from vertebrate animals to humans. Both domestic and wild animal species can act as vectors for the spread of a large variety of zoonotic parasites. Zoonotic infectious agents are among the most prevalent on earth and are thought to be responsible for more than 60 percent of all human infections and 75 percent of emerging human infectious diseases [1]. A number of factors have led to the prevalence of zoonotic parasitic infections, these include: (1) changes in social, dietary or cultural mores which have led to the increased opportunity for exposure, (2) environmental changes and (3) the improved recognition of heretofore neglected infections, often coupled with an improved ability to diagnose infection [2].

Iran has an area of 1,649,000 km2, divided into 31 provinces, and lies in the Middle East. In the last (2012) census, the population was recorded at about 75 million people. In the present review, I will focus on some of the major zoonotic and foodborne parasite pathogens of humans and the recent prevalences of human parasitic diseases in Iran (Table1).

Zoonotic protozoa

(A) Malaria

Malaria is still a public health problem in southern part of Iran. Annually, about 15 to 20 thousand people are affected by malaria with a mortality rate of about one or two cases/year. Most cases of malaria are in the provinces of Sistan and Baluchestan, Hormozgan also the tropical part of Kerman. *Plasmodium vivax* is the most common type and *Plasmodium falciparum* only will form 7% [3].

(B) Leishmaniasis

Leishmaniasis is endemic in the Middle East, and both cutaneous and visceral forms are reported from the most of parts in Iran. At least, six endemic foci of VL have been known in Iran (Ardabil, East Azerbaijan, Fars, Boushehr, and recently from Qom) and CL is a major and increasing public health problem in 11 of 31 province of Iran. The annual occurrence of human visceral leishmaniasis cases to be 149 (annual incidence: 300 to 600) and about cutaneous leishmaniasis is 24,630 (annual incidence: 69,000 to 113,300) [4].

(C) Toxoplasmosis

Toxoplasma gondii is one of the most common parasitic infections in human. The sero-prevalence rate of *Toxoplasma*-IgG in north of Iran was reported 55.7%. High prevalence rates, reaching 82.2% have been described previously in Iran [5]. The high prevalence of toxoplasmosis may be due to consumption of contaminated water or vegetable (low socioeconomic condition).

(D) Cryptosporidiosis

Cryptosporidium is a single celled protozoan organism that is found worldwide, having been identified in more than 50 countries. The parasite infects the gastrointestinal tract of a wide range of vertebrates including humans, livestock, wild animals and birds. In various parts of Iran, the prevalence of the parasite was 4.1% in the west, 7% in the southeastern, 2.2% in the south, 7.7% in the northwest and 2.5% in the central parts [6].

Table 1 Important helminth and protozoan zoonoses reported from human in some areas of Iran

Infection	Organism
Malaria	*Plasmodium vivax, Plasmodium falciparum*
Leishmaniasis	*Leishmania major, Leishmania tropica, Leishmania infantum*
Toxoplasmosis	*Toxoplasma gondii*
Cryptosporidiosis	*Cryptosporidium paryum*
Hookworm	*Ancylostoma dudenale, Necator americanus*
Toxocariasis	*Toxocara canis, Toxocara cati*
Dirofilariasis	*Dirofilaria immitis*, *Dirofilaria repens, Dirofilaria conjunctive*
Capillariasis	*Capillaria philippinensis*, *Capillaria aerophilia*
Gongylonemiasis	*Gnathostoma pulchrum*
Pentastomiasis	*Linguatula serrata*
Acanthocephalans	*Moniliformis moniliformis*
Trichinellosis	*Trichenella spiralis*
Taeniasis/Cysticercosis	*Taenia saginata*
Echinococcosis	*Echinococcus granulosus*, *Echinococcus multilocularis*
Hymenolepiasis	*Hymenolepis nana*
Fasciolosis	*Fasciola hepatica, Fasciola gigantica*
Schistosomiasis	*Schistosoma haematobium*
Heterophyiasis	*Heterophyes heterophyes*

Zoonotic helminthes

1. Nematode infections

(A) Visceral & Intestinal

Previously recorded prevalence of ascariasis and strongyloidiasis are commonly low (0.1%-0.3%) and hookworm infection *Necator americanus* in the north and *Ancylostoma duodenale* in the south is merely detectable in less than 1% of the people. Several species of fish (long tail tuna and pikeperch) from Caspian Sea and Persian Gulf as well as Khuzestan province have been reported to be infected with *Anisakis* sp., but no reported human cases of Anisakiasis [7].

Problems in identifying have mistuned the accurate estimation of the prevalence of toxocariasis (VLM and/or OLM) in Iran. Using serological methods, the reported prevalence of canine infection with *Toxocara canis*, the main agent of human toxocariasis, vary from 1.35% to 65%. A study showed that 42.6% of cats to be infected with *Toxocara cati* and 6.3% of the soil samples as well [8].

(B) Dirofilariasis

Up to now, 12 patients approved of human dirofilariasis have been reported from 11 provinces in Iran (*Dirofilaria immitis*, *Dirofilaria repens* and *Dirofilaria conjunctive*) [9].

(C) Capillariasis

Just two cases of human capillariasis have known in Iran, one in a fisherman caused by *Capillaria philippinensis* and the other pulmonary in a 9-year-old son caused by *Capillaria aerophilia* [9].

(D) Gongylonemiasis

Gnathostomiasisis a foodborne zoonotic disease caused by several species of the nematode *Gnathostoma.* The first case of human infection with *Gnathostoma pulchrum* has been reported in a 35-year-old female patient in Iran [10].

(E) Pentastomiasis

This disease is caused by *Linguatula* sp., or tong worm belongs to the phylum Pentostomida. Nine cases of human infection with *Linguatula serrata* have been reported from various provinces of Iran [11].

(F) Acanthocephalans

Moniliformis moniliformis is an endoparasite found in the intestine of its definitive host and found in most parts of the world. Just three Iranian cases of human infection with *M. moniliformis* are known [12].

(G) Trichinellosis

Trichinellosis is a helminth infection having a wide geographical distribution. It is acquired by ingestion of raw or undercooked meat infected with *Trichinella* spp. Due to the dietary restrictions of Islam, lard is not consumed in Iran. Therefore, there is only one report of human infection with *Trichinella*, which reported some decades ago [9].

2. Cestode infections

(A) Taeniasis/Cysticercosis

Since Iran is a Muslim country, all human taeniasis caused by eating of raw or poorly cooked beef (contaminated to cysticerci of *Taenia saginata*). Human infection has been found in various regions of the country but only at prevalences of less than one percent. Only two cases of human cysticercus bovis have been detected in Iran, both by histological examination of biopsies collected during surgery [13].

(B) Echinococcosis

Cystic and alveolar echinococcosis (hydatid disease), caused by *Echinococcus granulosus* and *Echinococcus multilocularis*, respectively. Cystic echinococcosis is considered endemic throughout the Mediterranean region, including all the countries of Middle East, but within this region alveolar echinococcosis is known only from Iran, Iraq, Turkey and Tunisia. Between 2001 and 2005, 2083 cases of cystic echinococcosis were recorded in Iran. In sero-epidemiological study based on indirect ELISA, 19% of the subjects investigated in the western Iran, were found positive for cystic echinococcosis. Between 1946 and 1993, just 28 females and 9 males cases of alveolar echinococcosis were recorded in Iran [14, 15].

(C) Hymenolepiasis

Human infection with *Hymenolepis nana* remains fairly prevalent (between 0.7 and 8.3 percent) in all over Iran [9].

3. Trematode infections

(A) Fasciolosis

Fasciola hepatica and *Fasciola gigantica* are the major causative agents of fasciolosis in regions with temperate and tropical climates such as Bolivia, Peru, Iran, and Egypt. This infection is emerging as an important chronic disease of human in north of Iran (Gilan province). In local health clinics in this province between 68 and 223 cases of human fascioliasis/year have been detected over much of the last decade, although more than 7000 and more than 10,000 cases reported in massive outbreaks in 1989 and 1999, respectively. Another northern province regarded as an important area for disease is Mazandaran. Between 1999 and 2002, 107 cases form this region were found infected with *Fasciola*. In 2000, there was a minor emergence of fascioliasis with 17 people in the western province of Kermanshah. Fascioliasis with flukes in non hepatic sites such as the skin, eye or thyroid has been reported from Iran [9].

(B) Schistosomiasis

To date, no cases of intestinal schistosomiasis have been reported in Iran. Urinary schistosomiasis that caused by *Schistosoma haematobium* was common in the western province of Khuzestan until the 1980s. In this area, 11.3% of their subjectes infected with this trematode have been reported. Although, in a 10-year, between 1980 and 1990, field survey in this region, a total of 1518 subjects infected with *Schistosoma haematobium*, no Iranian cases of human schistosomiasis have been reported since 2001 Cercarial dermatitis or "swimmer's itch" a human disorder caused by the schistosome parasites of birds, is also known in Khuzestan and some northern provinces. In Khuzestan, the prevalence of cercarial dermatitis 1.1% has been reported and detected the cercariae of bird schistosomes, including *Trichobilharzia* sp., in 2.4% of the *Lymnaea gerosiana* that is examined [9].

(C) Other Trematodes

The eggs of heterophyid trematodes have been reported in the feces of 19 subjects from Khuzestan [9].

Conclusion

Zoonotic parasite infections remain endemic in some regions of Iran. Socio-economic conditions, population density, climatic and environmental conditions, and behavioural and occupational habits of humans are determinants of the incidence and prevalence of the disease.

Most cases of zoonotic infections are preventable. Host/reservoir control measures, environmental control programs and animal vaccination, in conjunction with a strong surveillance system may significantly reduce, if not eliminate, the disease. The education is necessary to inform the public of the ways in which parasitic infections can be acquired through the consumption of infected food products or direct contact through the skin or other tissues. Thus, an effective collaboration among Iranians in some areas is the key to successfully combating this public health problem.

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Prevalence of Parasitic Infection of Slaughtered Cattle and Buffalo in Municipal Abattoir at Ismailia, Egypt

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Abstract

Food-borne parasitic zoonoses have a major impact on the health and economy worldwide. In this study, an active survey of parasitic infections of cattle and buffalo was performed in a municipal abattoir located at Ismailia city, Egypt. The slaughter-halls were paid 5 visits weekly between March 21st, 2009 and March 20th, 2010. A total of 10055 cattle, 3811 buffalo and 2378 male buffalo calves, all of native breeds, were inspected according to the Egyptian legislations and parasitic infections were recorded. Results revealed that 3668 out of the slaughtered heads showed deviation from the normal in meat and/or organs as to render them repugnant to the consumers and consequently were condemned; 41.40 % of these condemned materials were referred to the parasites which giving a total prevalence of 9.35%. Three of tissue parasites cysts were detected macroscopically naming, *C. bovis*; hydatid cyst and *Sarcocystis* spp. cyst. Inspection of cattle carcasses revealed prevalence of 0.58% *C. bovis* and 0.49 % hydatid cyst. Buffalo's carcasses investigation revealed prevalence of *C. bovis* (0.18 %) and 1.50 % for Hydatid cysts; while 20.34 % of these buffaloes have been infected with *Sarcocystis* macro cysts which appeared as visible cylindrical bodies. Fascioliasis was one of the considerable leading causes of liver condemnation. Fasciola spp. was recorded in buffalo's liver with a prevalence of 3.23% which was relatively higher than that of cattle (1.46 %). Rumen of 168 (4.41%) buffalo was harboring *Paramphistomum spp.* However, the intestine of 133 (3.49 %) was harboring *Moniezia spp.* In this respect, the prevalence of those helminthes among 10055 cattle was 3.45% for *Moniezia spp.* and 1.44% for *Paramphistomum spp*. *Ascaris spp. (Toxocara vitulorum)* was the only gastrointestinal helminthes recovered from 302 calves with a prevalence of (12.70 %). In conclusion, parasitic infection among our beef production livestock have its hazardous zoonotic significance and provokes serious economic looses.

Key words: abattoir, Parasites, cysticercosis, Hydatidosis, Sarcocystosis, Zoonoses.

Introduction

Cattle and buffalo represent the majority of farm animals reared to meat and milk production. Such animals may suffer from some parasitic agents that may affect their live or their production either directly or indirectly. Direct adverse effects of parasites may include death in heavy infections or untreated animals, retarded growth rates and direct losses due to organ condemnations during meat inspection at slaughtering [1]. There are many techniques for detection of parasitic infections of livestock. However, slaughterhouses provide an excellent opportunity for detecting diseases of both economic and public health importance [1]. Records of ante-mortem and post-mortem inspections are useful epidemiological data for evaluation of diseases at farm level and verify the efficacy of prophylactic and therapeutic interventions. Moreover, knowledge of the extent to which the public is exposed to zoonotic diseases through meat consumption is useful in the preventive medicine.

The clinical effect of bovine cysticercosis on infected animals is generally not significant but it is more important with regard to high economic losses due to the condemnation of heavily infected carcasses and its public health impacts. On reviewing the literatures on muscular parasites among food animals in Egypt; *Cysticercus bovis* (*C. bovis*) have been studied. The recorded figures were ranged between 0.04 % up to 2.65 % for buffalo, but for cattle it was quite different; infection rates of 1.6 % up to 14.7 5% were reported [2, 3, 4, 5, 6].

Hydatidosis is an important cyclozoonotic disease caused by the larval stage of *Echinococcus granulosus (E. granulosus)*; tapeworm of dogs. The carnivores, where the adult worms develop, become infected through ingestion of raw offal infected with hydatid cysts. Subsequently, the spread of infection to the intermediate host animals takes place by ingestion of graved segments or egg of *E. granulosus*, which develop to hydatid cysts [7]. An intensive review of the literature has been published in Egypt through several decades, revealed the impacts of the disease on livestock animal production and hazard to the public health [8, 9, 10, 11, 12, 13]

Sarcocystis infection is prevalent infection in water buffalo (*Bubalus bubalis*). In Egypt, high prevalence of macroscopic and microscopic *Sarcocystis spp cysts* has been reported among buffalo carcasses [2, 5, 14, 15].

Fascioliasis is a serious disease in ruminants throughout the world with an enormous economic impact. *Fasciola* (*F.*) *hepatica* and *F. gigantica* are the most common trematodes affecting bovine liver. The former species has a worldwide distribution but *F. gigantica* is found in Asia and Africa. Of the two species, *F. hepatica* more commonly infects humans, estimate are given of 2.4 million human infections in over 56 country including Egypt [16]. Various studies about the prevalence of

fascioliasis have been conducted on different localities of Egypt; the lowest incidence recorded was 1.03% up to 67% for buffalo while that of cattle ranged between 2.63 % - 70% [13, 17, 18].

The gastro-intestinal tract (GIT) of livestock harbor a wide variety of parasites mainly helminthes; these parasites adversely affect the health status of livestock to be leading cause of condemnation and economic losses. GIT parasites are problems among bovine population where the incidence is variable depending upon the different intrinsic and extrinsic epidemiological and biological factors. Infection rates for various gastro-intestinal helminthes, *Paramphistomum spp.* (45.28 %); *Moniezia spp.* (8.33%) and *Ascaris spp.* (17.22%) were previously recorded among slaughtered animals [19]. In Egypt, infection rates of 47.13 % and 7.36 % for *Paramphistomum spp*. and *Moniezia spp*. respectively were reported among the slaughtered animals [13]. In buffalo calves less than 4 months of age slaughtered for veal meat, *Toxocara vitulorum* (*T. vitulorum*) was commonly infested intestine, with incidences ranged from 47.9 to 86.67% [20, 13].

During the thorough post-mortem inspection of bovine carcasses; there are huge of the information recorded which confirmed that abattoir is the biggest laboratory all over the world [1, 21]. Therefore, the present study was aim to screen the parasites exist among bovines slaughtered in Ismailia province abattoir.

Materials and Methods

The present study was conducted in the municipal abattoir of Ismailia province, east- north of Egypt. The slaughterhouse was paid 5 visits weekly between March 21st, 2009 and March 20th, 2010. A total of 16244 animals were inspected including, 10055 cattle, 3811 buffalo and 2378 male buffalo calves. The majority of the bovine intended for slaughtering were males, (98.25%) of cattle and (83.15%) of buffalo (table1). All the slaughtered animals were native breeds. The age was estimated by inspection of teeth eruption during ante-mortem examination. Ages were over 3 years for females, ranged between 1.5 – 3 years for males and 2 – 3 months for male buffalo calves. Regarding the capacity of the slaughtering in during the year, 54.72% of animals were slaughtered in worm season, which extends March to September, while 45.72% of animals were slaughtered in cold (Grazing) season which extend from October to February. Carcasses were examined according to procedures described by the Egyptian law [22]. Routinely all animals intended for slaughtering were physically examined a day before or shortly prior to slaughter. During the routine post-mortem meat inspection, a thorough visual examination, palpation and systemic incision of the carcasses and its viscera particularly, lungs, liver, kidney, heart, spleen were carried out. Affections of the organs were grossly diagnosed based on pathological changes of color, size, morphology, consistency, lesions and presence of parasites. The liver affection grossly diagnosed for presence of parasites based on the pathological changes of color, size, morphology, consistency, bile ducts, lesions, retro hepatic lymph node.

For detection of *C. bovis* and *Sarcocystis* macrocysts, efficient inspection with incisions was done in different organs including; a longitudinal incision of the under-side of the tongue, two antero-posterior incisions in the external and internal masseter muscles, longitudinal incisions in the heart from the base to apex to open the pericardium and cardiac muscles, inspection of the muscular portion of the diaphragm and deep incision above the point elbow in the shoulder muscles. A special inspection has been done for esophagus for detection of *Sarcocystis* macrocysts. For detection of GIT parasites, the bovine stomach was cleared from the content and inspected for rumen flukes and the stomach worms of the abomasums. Daily, at the end of meat inspection, the collected parasites and parasite cysts were labeled and transported in ice to the laboratories of Faculty of Veterinary Medicine, Suez Canal University for further investigation.

Results and discussion

In the present work, satisfactory inspection of 16244 carcasses revealed that 3668 (22.59%) out of the slaughtered heads showed deviation from the normal in meat and/or organs and considered as repugnant to the consumers and were obligatory condemned; 41.40 % of these condemned materials were referred to the parasites which giving a prevalence of 9.35% correlated to the total slaughtered animals. During the study, a total amount of dressed meat was prepared in Ismailia municipal abattoir was 2470578 kg, with total condemnation of 4690 kg (Tab.1, 2, 3).

Table 1 Annual distribution of the slaughtered heads according to species, gender and seasons

Season	Cattle			Buffalo			Male buffalo calves	Total
	Male	Female	total	Male	Female	total		
Worm	5641	113	5754	1628	416	2044	1091	8889(54.72%)
Cold	4239	62	4301	1541	226	1767	1287	7355(45.27%)
Total	9880 (60.82%)	175 (1.07%)	10055 (61.89%)	3169 (19.5%)	642 (3.95%)	3811 (23.46%)	2378 (14.63%)	16244

Table 2 The annual yield of saleable meat obtained from the different categories (wholesome meat and condemned)

Season	Cattle		Buffalo		Calves		Total	
	D*(Kg) Mean ±SD	C**(Kg)	D (Kg) Mean ±SD	C (Kg)	D (Kg) Mean ±SD	C(Kg)	D (Kg) Mean ±SD	C (Kg)
Worm	978180±33	1640	306600±20	830	70915± 9	70	1355695±60	2540
Cold	752675±29	1470	272118±25	680	90090± 12	0	1114883±66	2150
Total	1730855±61	3110	578718±45	1510	161005±21	70	2470578±55	4690

*D= Total weight of dressed meat (Kg) (Mean ±SD); C** = weight of total condemned meat and parenchymatous organs. (Kg)

These findings indicated high prevalence of parasitic infection among bovine with economic impacts due to condemnations and public health hazards of unsatisfactory meat inspection or lack of meat inspection by slaughtering outside the abattoirs that occasionally occurs particularly in festivals. Therefore, public awareness programs should be adopted. Based on the routine post-mortem inspection of the large ruminants, three of tissue parasite cysts were detected, naming *C. bovis*, hydatid cysts and *Sarcocystis spp.* macrocysts. Cysticercosis was detected in both species with a total prevalence of 0.47% (65/13866); *C. bovis* were detected in 0.57 % of cattle carcasses; however, markedly lower prevalence were recorded in buffalo (0.18%) (Tab.4a&b).

The records of the present study about *C.bovis* cysts were in agreement with the previous findings [2, 3, 23]; while it is lower than that recorded by [6]. It is worthy to state that the further inspection did not revealed any cases of heavy infection by *C. bovis* needed total condemnation of the whole carcass and only partial condemnation were judged by meat inspectors. The shape of *C. bovis* was appeared as clear transparent bladders 5 × 10 mm and degenerated, caseated and calcified (fig. 1).

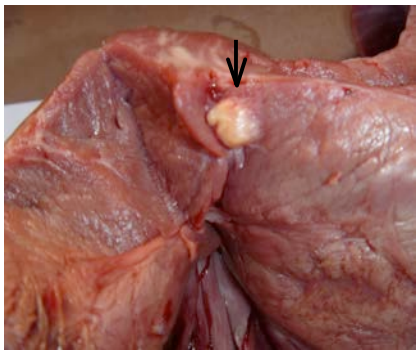

Fig. 1 *C. bovis* in heart muscle appeared as degenerated, caseated and calcified bladders 5 × 10 mm.

Bovine cysticercosis arise as a result of direct exposure to proglotids shed from farm workers, but there have been other reports of large scale outbreaks resulting from sewage contaminated feed or forage [24]. The existence of *C. bovis* in bovine carcasses indicates completing of such cycle between man and animals. Thus, prevention and control programs should be planned to break such cyclozoonosis. Although bovine cysticercosis usually does not cause much morbidity or mortality among cattle, it cause serious economic problems due to the condemnation of meat or downgrading of lightly infested carcasses which obligatory must treated to be safe as inferior quality [25]. The present study reflected both of the economic and zoonotic importance of cysticercosis which is in agreement with this statement.

Table 4 illustrated the data recorded about hydatidosis with infection rate of 0.49% in cattle and 1.5% in buffalo carcasses. The achieved results were relatively higher than those recorded by [6, 10, 12], but much lower than that revealed in other studies [26, 27]. The shape of the cyst was controlled by the organ in which it grows when uninfluenced by pressure it is oval-shaped or spherical as in lung (fig. 2).

Table 3 Comprehensive rates of abnormal conditions and diseases.

Season	Abnormal conditions	Parasitic	Total
worm	1184	807	1991
cold	966	711	1677
Total	2150 (58.60%)	1518(41.40%)	3668(100%)

Table 4a Prevalence of the parasitic affections encountered during the organized meat inspection of cattle.

Seasons	Cattle			
	Total No.	*C. bovis* No. (%)	Hydatid cysts No. (%)	Liver Flukes No. (%)
worm	5754	33(0.57%)	22(0.38%)	86(1.49%)
cold	4301	25(0.58%)	27(0.63%)	61(1.42%)
Total	10055	58(0.58%)	49(0.49%)	147(1.47%)

Table 4b Prevalence of the parasitic affections encountered during the organized meat inspection of buffalo.

seasons	Buffalo				
	Total No.	*C. bovis* No. (%)	*Hydatid cysts* No. (%)	Sarcocysts No. (%)	Liver Flukes No. (%)
worm	2044	5(0.24%)	28(1.37%)	388(18.98%)	73(3.57%)
cold	1767	2(0.11%)	29(1.64%)	387(21.90%)	50(2.83%)
Total	3811	7(0.18%)	57 (1.5%)	775(20.34%)	123(3.23%)

The size of hydatid cysts were frequently that of goose's egg and distributed on the surfaces and in paryncyma, it was noticed that their dimensions increased proportionally with the animal age. The cysts in sometimes showed degenerative changes, the vesicular fluid disappearing and the cavity contracted becoming filled with caseous matter by time replaced by gritty calcified mass. Besides the economic losses of hydatidosis of livestock animals, infection could contribute to completing the life cycle of the parasite when the dogs accesses to infected meat or organs that increasing zoonotic infection potentials.

Table 4 showed that the macroscopic *Sarcocystis spp.* macrocysts were detected only in the buffalo carcasses with prevalence of 20.34% (775 /3811) which was located within the previous levels recorded by [28]. However, higher rates of 72. 63 % up to 100% have been reported [14, 29, 30]. Macrocysts of *Sarcocystis spp.* appeared as clynder bodies which were usually larger size and superficially located in esophagus and smaller size in the tongue (fig. 3 a &b).

The main importance of sarcocystosis in the animals is a n economic one, for carcass of food animals showing seve re macroscopic infection are deemed unmarketable and are condemned. In addition, such infection has potential of pu blic health importance because man may acquire infection by consumption of meat from infected animals where eati ng raw or undercooked flesh containing mature sarcocysts cause intestinal sarcocystosis.

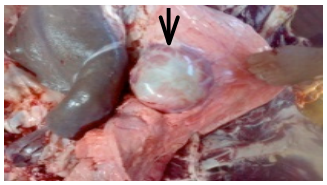

Fig. 2 Hydatid cyst in lung of cattle aged 3 year, spherical in shape with diameter 20cm.

Chronic fascioliasis was judged based on detection of the flukes in bile ducts and liver affection such as dark blue discolorations, pipness of the bile ducts and cirrhosis of the liver parenchyma (fig 4).

In this study, liver flukes were detected in 147 (1.47%) o f cattle livers and 123 (3.32) % of buffalo livers, with total prevalence of fascioliasis of 1.66% (table 4 A&B). Liver condemnations due to fascioliasis suggest its economic im portance in domestic ruminants [31, 32, 33]. Apart from it s veterinary and economic importance throughout the worl d, fascioliasis has recently been shown to be a re-emerging and widespread zoonosis affecting human population [16, 34, 35].

Paramphistomum spp. and *Moniezia spp.* were the gastrointestinal helminthes commonly observed during evisceration, with prevalence of 1.44 % and 3.45 % respectively in cattle. In this respect the rate of infection among buffalo was 4.41% for *Paramphistomum spp.* and 3.49 % for *Moniezia spp*. Concerning the prevalence of ruminal flukes, the results obtained were much lower than the previous records; [36] (44.7%); [37] (39.1%) and [13] (47.13%). The recorded prevalence of *Moniezia spp.* in present study was lower than that concluded by [38](8%), while it was relatively higher than the records of [39] (0.22%) and [40] (0.4%).

Results revealed that none of the slaughtered calves was found to be positive for tissue parasites and

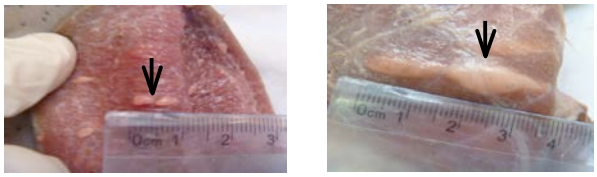

Fig.3 *Sarcocystis* Macrocysts in longitudinal section tongue muscle (left) and in superficial mucosa of oesophagus (right).

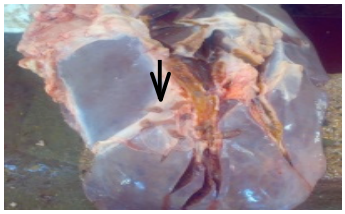

Fig. 4 Fasciolia infection of liver with chronic stage of affection characterized by dark blue discolorations, pipness of the bile ducts and cirrhosis of the liver parenchyma.

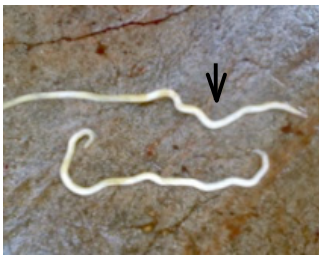

Fig. 5 *Toxacara vitulorum* recovered from intestine of two -month age male buffalo calf.

Ascaris spp. (Toxacara vitulorum) (fig. 5) was the only gastrointestinal helminthes recovered from the slaughtered calves at rate of 302/2378 (12.70 %). This result is lower than the findings of [13, 20, 41]. The differences in feeding habits and hygienic habitats beside the neglected attitude of some of the owners towards the management of buffalo male calves may illustrate such variance. Gastrointestinal parasites in calves lead to reduced growth and constantly hampering the development of livestock industry.

During in cold seasons, animals feed on green fodders eit her in their premises or in the field, which give more oppo rtunities for parasitic diseases transmission. However, resu lts revealed that generally there were no seasonal impacts on the prevalence of parasitic diseases. These findings mig ht be related to endemicity and/or choronicity of such para sitic diseases.

In conclusion, parasitic infection of bovine was prevalent among slaughtered bovine of Ismailia manucipal abattoir that indicated high burden in animal husbandry in Ismailia province or other areas of Egypt. Parasitic infections were a leading cause of economic loss through impairment of livestock production and condemnation of meat during meat preparations and provoke risk of zoonotic potentials.

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Caution on use of anti-malaria drag for control of mixed infection of malaria: Bayesian analysis of malaria data from Sarawak, Malaysia

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Abstract

It is necessary to consider the kind and dose of the drug, the species and the stage of *Plasmodium* in the blood of the patient when anti-malaria drugs are administered to the patients. It is generally thought that the effect of treatment can be observed if a clinician put these factors into consideration. However, the number of the factors increase and their relationships become complicated when a mixed infection or a super infection takes place. In this study, we considered this issue to see if an effective drug administration protocol can be devised by using statistical analyses, with special emphasis on Bayesian statistics. For this purpose, the data of malaria patients were adopted from those during 1997 – 2008 in three districts of Sarawak, Malaysia where high-frequency, complex mixed infections were reported. The results of the analyses suggested, unlike assessment of treatment of the infection by a single species of *Plasmodium* where intensive treatment at the specific stage of a known *Plasmodium* sp. is possible, those of mixed infection become more complicated; that the effect of the patient treatment with low drug dosage if evaluated as a community will be reduced, and that complete transfer of a malaria patient without further infection (mixed infection) by a drug-resistant *Plasmodium* parasite(s) or by other *Plasmodium* sp. including a monkey-malaria parasite, *Plasmodium knowlesi*, is difficult. We briefly overview the analytical methods used and the results of these analyses in this communication.

Key Words: malaria , anti-malarial drug, mixed infection, *Plasmodium knowlesi*, Bayesian statistics, MCMC, DAGs

1. Introduction

Infection rates of the people in Malaysia by *Plasmodium falciparum* and *Plasmodium vivax*, particularly in Sarawak, are relatively high. People in this area are also under additional and complicated threads of malaria: Recently, an outbreak of malaria of the drug-resistant type is becoming a worldwide problem and Sarawak cannot be an exception: past studies indicated that a mixed infection by *Plasmodium* spp., including a monkey-malaria parasite, *Plasmodium knowlesi,* is likely to spread more frequently than does a single infection. For these reasons, we need to note the following points in diagnosis of malaria, investigate the mechanism of mixed infection, and then consider effective treatment. In general, malaria is diagnosed from the presence in and/or the proportion of the erythrocytes invaded by *Plasmodium* parasites in a blood smear. However, if a physician diagnoses malaria in clinical practice, it is necessary to reveal the life-cycle stage of the malaria parasite(s).

Sarawak is the city in Borneo Island in Malaysia. It is the tropical area where outbreaks of malaria are frequent.

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Malaria continues to be a public health problem in Sarawak with most cases being reported. In this study, we used malaria data obtained in Sarawak, Malaysia, and calculated incidence and proportion of infection by *Plasmodium* spp., including mixed infections. It was reported previously that mixed infection by *Plasmodium falciparum* and *Plasmodium vivax* is frequent and that monkey-malaria parasite, *Plasmodium knowlesi,* can infect humans. We analyzed the data using a statistical approach, paying a particular attention to complicated issues associated with treatment of mixed infections. We constructed hierarchical models and used unknown parameters, some of which are factors that are difficult to measure in the model. We calculated the incidence and proportion of mixed infection by *P. falciparum* and *P. vivax* and used them as parameters in the above models.

In the analysis of local malaria data, we are able to consider the factor that may possibly be related high incidence rate of malaria. They include socio-economic factors, the life style and unique features of infected individuals [1][2]. But it is difficult to determine the main factor because these factors have various confounding effects to each other and data to analyze these factors are hardly available [3]. When dealing with highly uncertain and confounding or missing data, the methods in classical epidemiology affect the reliability and validity. As a result, a large amount of epidemiological evidence cannot be expected by this approach. For this reason, we estimated the factors involved in the generation of malaria by the use

of Bayesian statistics. Bayesian statistics is the new style statistics that is based on the concept of T. Bays. A key feature of this statistics is a mechanism that is similar to thinking of human, and therefore is widely used in fields of medicine and social sciences, natural sciences [4]. Formula (1) below indicates the Bayesian theorem. Here, θ indicates the density used in the general statistics, which corresponds to a likelihood. In addition, p(θ) is called a prior probability density of the right-hand side. This shows the probability density that is specific to Bayesian statistics. p(θ|X) is called the posterior probability density p of the left-hand side.

$$p(\theta \mid x) = \frac{p(x \mid \theta) p(\theta)}{\int p(x \mid \theta) p(\theta) d\theta} \qquad (1)$$

In addition, by combining the theory of decision making under uncertainty to Bays' theorem, one can derive the optimal action based on the data. Statistical decision theory called it, proves that the decisions based on the posterior distribution is optimal.

2. Materials and Methods

We analyzed malaria patients data that were collected in three districts of Sarawak, Malaysia, from1997 to 2008 [4]. We used Directed Acyclic Graphs (DAGs) to understand the cause for incidence of malaria. Bayesian networks are DAG where the nodes represent random variables and directed edges capture their dependence (Fig.1).

Causal graphs such as DAGs are a novel approach in epidemiology to conceptualize confounding and other sources of bias. DAGs visually encode the causal relations based on a priori knowledge among the exposure of interest and the outcome while considering several covariates. The application of formal rules on these diagrams enables the identification of the causal and non-causal structures in the DAGs. The causal effects are of interest and require no adjustment [5].

We further analyzed the data using WinBUGS as a Bayesian hierarchical model. Furthermore, we estimated, using Markov chain Monte Carlo (MCMC) method in the Gibbs sampler, the prior distribution. In addition, this sampling used (ver.1.4.3) WinBUGS. BUGS is a software package for performing Bayesian inference using Gibbs sampling (BUGS). BUGS project is related to the flexible software for Bayesian analysis of complex statistical models using MCMC method. The project began in 1989 in units of biostatistics MRC, and then WinBUGS software was developed in collaboration with Imperial College School of Medicine, St. Mary's, in London, which led to the first program in the `classic" BUGS program. The two programs are related simply by showing the relationship between the variables. The software to avoid the complexity of any (almost) and to specify a statistical model, the decision to analyze the model was made. This is specified as an appropriate MCMC scheme (based on the Gibbs sampler). The user that contains the "expert system" to control the execution of the scheme, and that can be freely selected from a wide range of output types [6] [7] were used. In addition," □ (the circle)" in Fg. 1 called a node was employed. The Bayesian network was dealt with as the conditional probability, the probability of stochastic causal relationship (Fig. 2).

Fig1 Simplest *A*□ *B* graph. *A* causes *B* or *B* is a consequence of *A*. We would say that *A* is a parent of *B*, *B* is a child of *A*, that *A* influences, or causes *B*, *B* depends on *A*. Also, *P*(*A*,*B*) = *P*(*A*)*P*(*B*/*A*).The independence of two nodes in a DAG depends on their relative position in the graph as well as on the knowledge of other nodes in the graph. The following simple example illustrates the influence of conditioning on the independence.

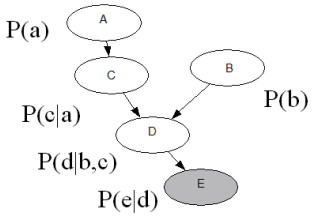

Fig. 2 The Bayesian network

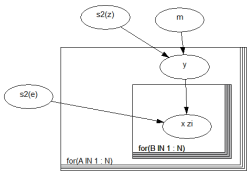

Fig. 3 A hierarchical model. The Doodles of DAGs consist of three elements: nodes, plates, and edges

Directed Acyclic Graphs is a directed graph with no directed cycles. That is, it is formed by a collection of vertices and directed edges, with each edge connecting one vertex to another, such that there is no way to start at some vertex v and follow a sequence of edges that eventually loops back to v again. In our study, we constructed the DAG shown in Fig4. Arrows in Fig.4 shows the probabilistic correlation. In this analysis, to move to the mosquito infection by blood-sucking mosquitoes in uninfected patients has not been considered. In addition, a possibility that this study may affect the ecology of mosquitoes to temperature is not considered.

The number of individuals in Kuching was based on the normal distribution. In two other cities their population was considered to be affected by economic conditions. The major difference between the two, for example, natural environments such as geographical factors was not considered. As a result, using a similar model, effects of test factors were analyzed by changing the economy and population.

3. Results

In the analysis that used WinBUGS, we can tell the variation of the parameters studied from the results of the analyses by five methods; Gelman-Lubin, Time series, Kernel density, Running quantiles, and Autocorrelation function and Dynamic trace[8].

In the analysis using WinBUGS, we can also tell the changes in the parameters from the results of the analyses by the six methods. In these methods, we have to see the effect of human sensitivity in infected persons and economic conditions (symptoms or no symptoms). Fig.4 shows the result of a time series analysis. This analytical method is a generic term for a method to predict the effect of time on expression of particular characteristics or changes. If a graph is dense, it generally ensures and validates that the order is effective. We can be confirmed by much of the graph at 10250- and 10750-times iterations in the graph of the top figure. In the middle figure, overlap of the graph is in a very dense state throughout the graph. On the other hand, overlap is very sparse in the graph of the bottom figure. Fig. 5 demonstrates the result of Gelman-Rubin method. This is a method to determine the convergence by comparing the variance between different Markov chain. If the value of the parameter R (an estimate of potential scale reduction) is near to 1, it indicates convergence [9]. The analysis of economic condition indicated it is under convergence. However, comparison between infected symptom and no symptom resulted in no convergence. Fig. 6 shows the result of the analysis of the Kernelel density curve. This shows the result of the analysis of the highest part of the density (equivalent to 97.5% - 2.5% points with the lower point of CI being 95% in the approximate maximum likelihood estimation method). That is, this corresponds to a 95% confidence interval of the maximum likelihood method. Figures 7, 8, and 9 show convergence of the parameters of the economic condition is most often encountered. In particular, the value of the parameter R is near 0. We also performed the convergence test of Marcov chain (Fig. 9). The result of this analysis suggests that value is likely to have converged to the invariant distribution. Comparing the results of these multiple analyses, we evaluated the reliability of the parameters of high uncertainty as above.

By a series of the approach explained as above, we analyzed the malaria data in Sarawak where mixed infection is frequent for important parameters and compared the results with those of the infection by a single species of *Plasmodium* where intensive treatment at the specific stage of a known *Plasmodium* sp. is possible. The results of the analyses suggested that assessment of treatment of the mixed infection become more complicated; that the effect of the patient treatment with low drug dosage if evaluated as a community will be reduced, and that complete transfer of a malaria patient without further infection (mixed infection) by a drug-resistant *Plasmodium* parasite(s) or by other *Plasmodium* sp. including a monkey-malaria parasite, *Plasmodium knowlesi*, is difficult.

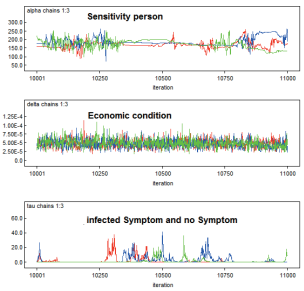

Fig. 4 Sarawak Directed Acyclic Graphs

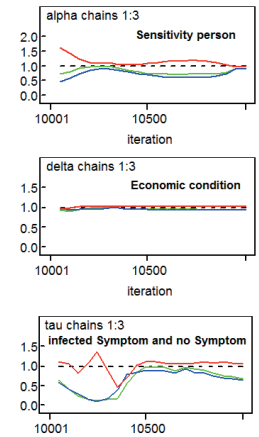

Fig.5 Gelman-Rubin

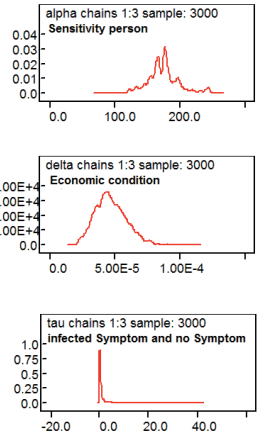

Fig.6 Kernel-density

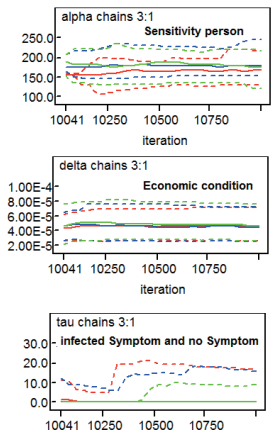

Fig.7 Running-quantiles

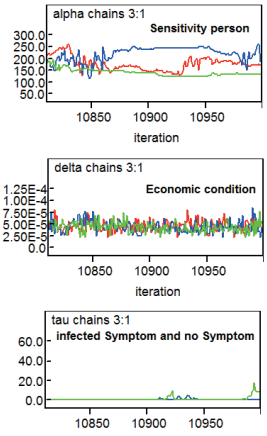

Fig.8 Dynamic-trace

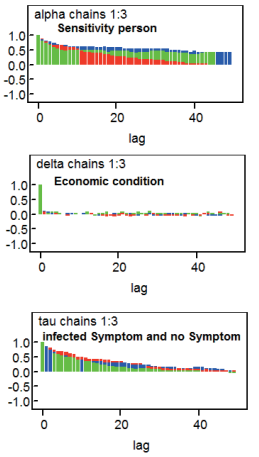

Fig.9 Autocorrelation function

4. Discussion

In 1911, Ross first introduced a mathematical model into epidemiological analysis of malaria. It was the first model using a simple differential equation. A variety of software based on this model has been developed for the analysis, and the result of such an analysis can be obtained very quickly today. However, the basic hart of the numerical formula has not changed greatly and it is used for the analysis of the patients suffering from various infectious diseases [8] and it has served as one of the effective methods in public health.

A recent study on epidemiology of malaria in Burundi adopted a Bayesian approach [10][11]. In this study we also used a Bayesian approach. Our study was performed to estimate the uncertain parameters using MCMC by WinBURG. As a result, parameters important for the control of malaria were indicated and the results suggest the patients with drug-resistant *Plasmodium spp*. or with mixed infection need to be treated with prudence. These are also important control measures in public health.

5. Conclusion

In the case of mixed infection of malaria, e.g., *P. falsiparum* and *P. vivax*, it is vital to consider a kind and the difference in each life cycle in drug to use for each extermination. We may promote an increase of plural malaria in a short time to participate in the spread of plural malaria at the same time when there is a patient of the mixed infection. In addition, when one is infected with a drug-resistant malaria parasite, our model predicted that the effect of the patient treatment with low drug dosage if evaluated as a community will be reduced, and that complete transfer of a malaria patient without further infection (mixed infection) by a drug-resistant *Plasmodium* parasite(s) or by other *Plasmodium* sp. including a monkey-malaria parasite, *Plasmodium knowlesi*, is difficult. It is therefore considered important that the patients with drug-resistant *Plasmodium spp*. or with mixed infection need to be treated with prudence.

In our study, we used DAGs basic of model for analysis. When we structure model, the non-causal effects have to be checked for confounding and for which covariates adjustment is necessary. The identification of the adjustment set depends on the causal relations among the variables. The consideration of these relations is valuable because adjusting for more variables increases the risk of introducing bias. Considering every single path of a DAG allows the systematic identification of the causal structures in the DAG, and the determination of minimally sufficient adjustment sets for estimating the causal effect of the exposure on the outcome based on the underlying DAG.

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Molecular phylogeny of *Blastocystis* isolates from rodents in the Sumba Island, Indonesia

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Abstract

Blastocystis is an intestinal protozoan parasite which infects a variety of animals including humans. Among *Blastocystis* isolates from mammalian and avian host species, all *Blastocystis* isolates have been classified into nine subtypes based on the small-subunit ribosomal RNA (SSU rRNA) gene sequences analyzed in the molecular phylogenetic tree. Commonly, in *Blastocystis* isolates from mammals and birds, multiple different subtypes have been detected from same host species. On the other hand, in *Blastocystis* isolates from rodents, 8 isolates are registered GenBank at present and all the isolates are classified into subtype 4.

This time, in our field research in the Sumba Island, Indonesia, several *Blastocystis* organisms were found in wild rodents. Then we amplified the SSU rRNA gene of three *Blastocystis* isolates and determined the sequence by cloning. Based on the sequence data, the three Indonesian rodent isolates with all other subtype 4 isolates registered in GenBank were phylogenetically analyzed. As a result, all three Indonesian isolates are classified into subtype 4 even though they are positioned at the different clade from other subtype 4 isolates.

Introduction

Blastocystis is an anaerobic eukaryotic unicellular organism that infects the caecum and large intestine. At present, *Blastocystis* organisms have been isolated from mammals including humans, birds, reptiles, amphibians, and insects. So far, genetic polymorphism among *Blastocystis* isolates has been analyzed with various molecular techniques, including arbitrary primers polymerase chain reaction, restriction fragment length polymorphism of the SSU rRNA gene and sequencing of the SSU rRNA gene. In 2007, *Blastocystis* isolates from mammalian and avian host species are classified into nine clades based on the SSU rRNA gene phylogeny and these clades are proposed as *Blastocystis* sp. subtypes from 1 to 9 [1]. Since most of the subtypes are comprised of different host origins, host-based speciation is impossible [2]. Interestingly, however, all 8 rodent *Blastocystis* isolates registered in GenBank showed subtype 4. These are isolated all over the world, Japan, USA, Singapore, and France. Therefore, it is important to reveal the host specificity of the subtype 4.

In our field research on Sumba Island, Indonesia, several *Blastocystis* organisms were found in wild rodents using the fecal culture method.

In this study, in order to clarify the genetic diversity among the subtype 4 *Blastocystis* organisms and host specificity of the subtype among the rodent isolates, we analyzed phylogenetically Indonesian rodent isolates with all other subtype 4 isolates in GenBank.

Materials and Methods

(1) Sources and isolation of *Blastocystis* from rodents
In our field research in the Sumba Island, Indonesia, from 2009 to 2011, we had captured wild rodents (*Rattus exulans*) and the caecum contents were cultured in the liquid medium. The culture medium used was Ringer's solution containing 10% horse serum and 0.05% asparagine [3]. After incubating the cultures at 37℃ for 3 to 4 days, we observed the culture sediments by a standard light microscopy and judged whether the cultures contained *Blastocystis* or not. When the typical vacuolar or granular forms of *Blastocystis* were observed, they were subcultured in a new medium. After one to two subcultures, *Blastocystis* suspensions were centrifuged at 3,000*g* for 1 minute, and then the DNA was extracted by DNAzol reagent (Invitorgen). A total 11 *Blastocystis* isolates were obtained from 67rodents from 2009 to 2011 in Indonesia. In this study, 3 isolates were analyzed an entire sequence of the SSU rRNA gene.

(2) SSU rRNA gene sequences used for phylogenetic analysis

The subtype 4 *Blastocystis* isolates registered in GenBank are listed in Table 1. In addition, the subtype 3 isolate HV93-13 (AB070986), was used as an outgroup, because the subtype 3 is the closest clade to the subtype 4 [4].

(2) Extraction of genomic DNA

The genomic DNA of the cultured *Blastocystis* was extracted by using DNAzol reagent (Invitrogen) according to the manufacturer's protocol. The extracted DNA was stored at -20ºC.

(3) PCR amplification and sequencing of the SSU rRNA gene of *Blastocystis*

Since the sequence of the SSU rRNA gene of *Blastocystis* is known to be genetically heterogeneous, two different combinations of the forward and reverse primers were constructed for PCR amplification as listed in Table 2.

PCR was performed by high fidelity Taq polymerase,

Table 1. *Blastocystis* isolates of subtype 4 in GenBank and 3 rodent isolates in Indonesia

Isolate	Host	Country of origin	GenBank accession no.
HG00-10	Human	Germany	AY244619
HG00-12	Human	Germany	AY244620
HJ01-7	Human	Japan	AY244621
DMP02-212	Human	Denmark	JN682513
WR1	Rat	Singapore	AY590113
WR2	Rat	Singapore	AY590114
S1	Rat	Singapore	AY590111
RN94-9	Rat	Japan	AB071000
clone1	Rat	France	AY135407
clone2	Rat	France	AY135408
NIH	Guinea pig	USA	U51152
No name	Guinea pig	USA	U26177
RI09-97	Rat	Indonesia	
RI10-21	Rat	Indonesia	
RI11-18	Rat	Indonesia	

Table 2. Primer sequences used for amplification of the SSU rRNA gene

Combination of primer	Name	Sequence
1F-416R	1F	5'-GCTTATCTGGTTGATCCTGCCAGT-3'
	416R	5'-CTGCTGCCTTCCTTGGATG-3'
355F-967R	355F	5'-TTTGGGTTCGATTTCGGAGA-3'
	967R	5'-CCCCTARCTTTCGTTCTTGATTAATG-3'
917F-1272R	917F	5'-GCGAAAGCATTTACCAAGG-3'
	1272R	5'-CCACCAACTAAGAACGGCCA-3'
1155F-1R	1155F	5'-GGCTTAATTTGACTCAACACGG-3'
	1R	5'-TTGATCCTTCCGCAGGTTCACCTA-3'
1F-618R	1F	5'-GCTTATCTGGTTGATCCTGCCAGT-3'
	618R	5'-CAACTACGAGCTTTTTAACTGCAAC-3'
539F-1149R	539F	5'-AAGTCTGGTGCCAGCAGCC-3'
	1149R	5'-CTCCACTCCTGGTGGTGCC-3'
1089F-1R	1089F	5'-GAGTATGGTCGCAAGGCTGAA-3'
	1R	5'-TTGATCCTTCCGCAGGTTCACCTA-3'

KOD-Plus-Ver.2 (ToYoBo). The PCR profile consisted of an initial denaturation at 94℃ for 2 min, followed by 35 cycles of 98℃ for 10s, 54℃ for 30s and 68℃ for 45s, with a final extension at 72℃ for 7 min. The amplified samples were conducted dA-addition using an A-attachment mix including a kit of Mighty TA-cloning Reagent Set for PrimeSTAR® (TaKaRa). Then, all the samples were immediately ligated with pMD20-T vector (TaKaRa) and transformed into *Escherichia coli* JM109 (ToYoBo) competent cells. In this study, at least 5 clones were chosen from each sample and the size of the insertion was confirmed by electrophoresis, and purified plasmid using a FastGene™ Plasmid Mini Kit (NIPPON Genetics Co.). Sequence PCR was performed using BigDye® Terminator V3.1. CycleSequencing Kit (Applied Biosystems) with primers (forward: 5'-GTTGTAAAACGACGGCCAGT-3', Reverse: 5'-GGAAACAGCTATGACCATGA-3') according to the manufacturer's protocol and analyzed sequence of the SSU rRNA gene with an ABI PRISM 3100® Genetic Analyzer (Applied Biosystems).

(4) Phylogenetic analysis

The sequences obtained in this study along with previously published SSU rRNA sequences of the subtype 4 *Blastocystis* isolates listed in Table 1 and the outgroup HV93-13 isolate were used for phylogenetic analysis. The sequences obtained in this study and previously published SSU rRNA sequences of *Blastocystis* isolates were aligned manually. Phylogenetic analyses were performed with maximum likelihood (ML) methods by using the PAUP 4.0β10 [5] program. Prior to the analyses, 56 different substitution models were compared by using the MODELTEST3.7 program [6]. The HKY [7] + Γ + I model was selected as the best model in each cases. Parameters estimated by the MODELTEST 3.7 program were used for inferring phylogenetic trees. The ML trees were reconstructed under heuristic searches by using tree

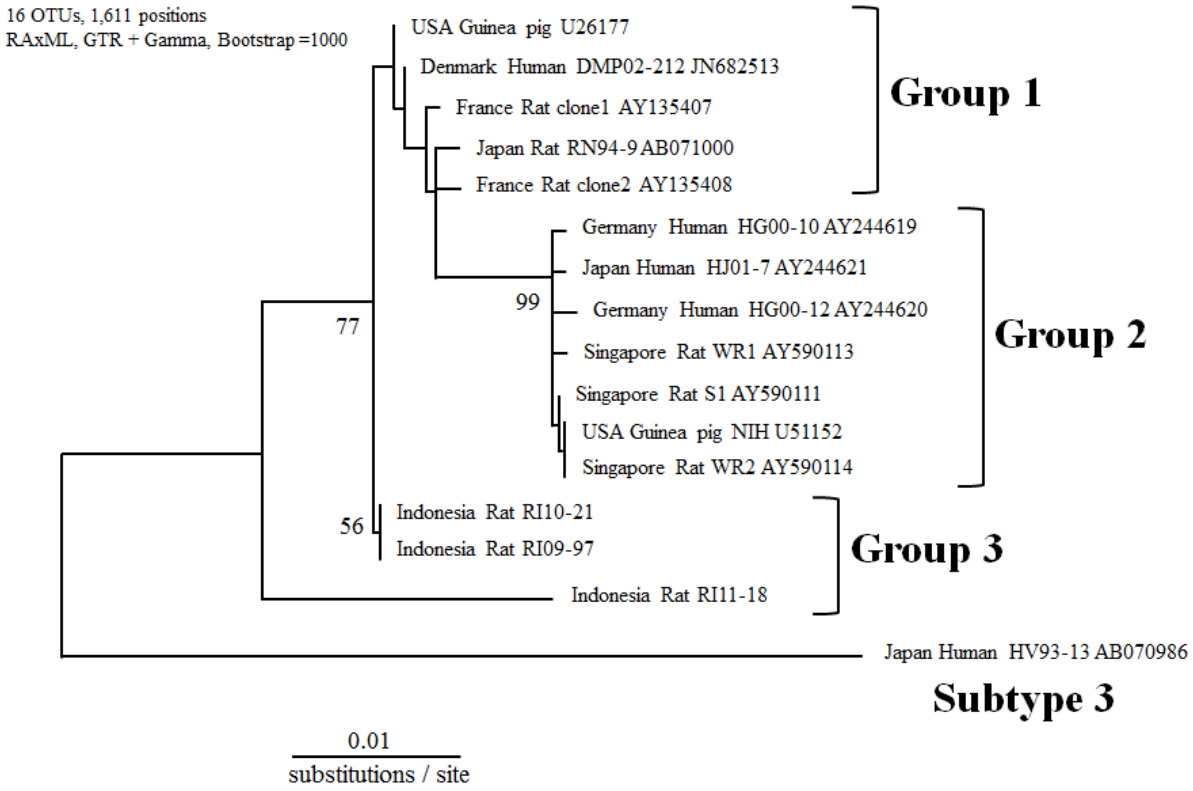

Fig. 1 Phylogenetic relationship among subtype 4 *Blastocystis* isolates inferred by maximum likelihood method. The isolates of subtype 4 were greatly classified into three groups. All Indonesian rodent isolates were positioned different clade from other subtype 4 isolates.

bisection reconnection (TBR) branch swapping in which the starting tree was obtained via stepwise addition. The bootstrap proportion of each internal branch was estimated by using nonparametric bootstrap [8] with 100 replications

Result and Discussion

So far, rodents isolates were only isolated from US, Singapore, France, and Japan, and all isolates showed the same subtype 4, hence *Blastocystis* isolates obtained from different region are essential for investigation of the genetic polymorphism among rodent isolates of *Blastocystis*. In this study, we had successfully sequenced the SSU rRNA genes of the 3 rodent *Blastocystis* isolates obtained in Indonesia (Table 1). Then, a phylogenetic tree was inferred with 1,611 unambiguously aligned positions selected from the 11 rodent isolates and the 4 human isolates listed in Table 1 with an outgroup HV93-13 isolate (Fig. 1).

All subtype 4 isolates were classified into three groups. Group 1 was comprised of rodent isolates in US, France, and Japan and a human isolate in Denmark. Group 2 was comprised of Singaporean and American rodent isolates, and German and Japanese human isolates. Group 3 was only comprised of all 3 rodent isolates in Indonesia in this study.

It is apparent that all the Indonesian rodent isolates are phylogenetically different from other subtype 4 isolates. Therefore, it is evident that the Indonesian rodent isolates are different from other subtype 4 isolates. It is interesting to note that two Indonesian rodent isolates, RI09-21 and RI10-97, obtained in 2009 and 2010, respectively were showed same sequence, while the remaining RI11-18 isolate obtained in 2011 was quite different from other two isolates. Therefore, further phylogenetic analysis is essential to reveal genetic polymorphisms among Indonesian rodent *Blastocystis*.

The isolates from Japan were the only subtype 4 isolates that were taken from both human and rodent hosts but were found to be located in different groups. However, these circumstances are only in Japan and the number of isolates from both human and rodent hosts is one case each. Therefore, it is essential to isolate more *Blastocystis* organisms from rodents and humans in the same country to see if zoonotic transmission of the subtype 4 will occur between human and rodents.

Acknowledgement

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Application of ELISA to detect urinary IgG4 for the mass survey of lymphatic filariasis in Bangladesh

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Abstract

In response to the WHO-led Global Programme to Eliminate Lymphatic Filariasis (GPELF) by 2020, the Bangladesh government made a national plan to eliminate the disease by 2015. Since the start of GPELF, many endemic countries have completed the planned anti-filarial mass drug administration (MDA) and the infection prevalence has been reduced significantly. In the post-MDA low endemic stage, the standard blood films to detect microfilariae are no more sensitive, and immunodiagnoses detecting filarial antigen or antibody will play a more significant role.

We reported ELISA using urine as samples (urine ELISA). The use of urine has advantages: sample collection is non-invasive and painless, and therefore people accept the method. This is a key factor in the post-MDA surveys, when most people are already parasite-free and not happy to be pricked for blood samples. In Sri Lanka, urine ELISA was tested for its usefulness in such circumstances. The ELISA was tested (i) with children less than 5 years of age, (ii) with pregnant mothers and their newborn babies, (iii) in an area where filariasis was said to be non-endemic, and (iv) in a 6-year long-term follow-up study. All these studies were carried out without problems. In China, urine ELISA was shown to be effective to confirm the elimination of filariasis. It is expected that urine-based immunodiagnoses would contribute a lot in the current and future filariasis elimination efforts.

We have developed a new urine–based ELISA which uses SXP1 recombinant antigen, and tested its diagnostic efficacy in Bangladesh. The sensitivity was 97% with urine samples from mf positive subjects as the positive standard, and the specificity 100% with urine samples collected in a non-endemic area as the negative standard. The new ELISA detected more positives than ICT antigen test (so-called the world standard diagnosis) with children aged 5-10 years, indicating the usefulness of the ELISA also in Bangladesh.

Key Words: Filariasis, Urine diagnosis, Recombinant SXP1, IgG4

Background information:

Bangladesh is among the first countries to start a national filariasis elimination programme under the WHO-led Global Programme to Eliminate Lymphatic Filariasis (GPELF). The main strategy of GPELF is the mass drug administration (MDA) of all people in endemic areas including non-infected people. The drugs used for MDA is the combination of diethylcarbamazine and albendazole (DEC/ALB), that are given once a year for minimum 5 years. With annual MDAs, Bangladesh achieved remarkable progress over the past decade. In 2001, 19 of the 64 total districts were selected as MDA implementation areas on the basis of microfilaria (mf) levels, and by 2010, 13 districts had completed five or more rounds of MDA [1], resulting in a significant reduction of infection as observed in other endemic countries [2-6]. In this post-MDA low endemic stage, mf densities have become low and the standard blood films are no more sensitive. Thus, the crucial issues needed to be addressed are methods of diagnosis/evaluation and their criteria to stop MDAs, to verify the interruption of transmission and to detect possible resurgence of the infection [7-10].

Among other requirements, identifying a highly sensitive and specific method to detect filarial infection will be of prime importance. Such method must be simple and easy in handling, not expensive and acceptable for people. Immunodiagnoses, which are relatively easy and applicable for mass surveys, have been used widely: ICT kits (immunochromatographic card test; Binax, Inc., USA) detecting *Wuchereria bancrofti* antigen [11] is considered as a preferred tool for monitoring MDA outcome due to its ease in handling and quick results in only 10 minutes. However, it is very costly, the result is only qualitative and the sensitivity was reported low in some occasions [12-14]. A more important disadvantage for ICT is the use of blood samples.

We have to realize that, in the post-MDA stage, the monitoring will have to be repeated with predominantly healthy subjects under the circumstance that filariasis has become a less important public health concern than in the past. People will not be willing to undergo painful invasive blood sampling, particularly for their children, who constitute an important sentinel group to know recent infection/transmission levels [15].

In order to ease sample collection we developed an ELISA that detects filaria-specific IgG4 in urine samples. *Brugia pahangi* crude antigen was used to capture the antibody. The ELISA were positive in 87 of 91 urine

samples obtained from *Wuchereria bancrofti* mf positive subjects (sensitivity 95.6%), and negative in 295 of 298 urine samples from non-endemic subjects in Thailand, Lao PDR and Japan (specificity 99.0%). Cross reactions with intestinal helminthes like hookworm, *Ascaris lumbricoides*, minute intestinal flukes, *Echinostoma* sp. and *Opisthorchis viverrini* were negligible [16].

Urine collection is applicable anytime of the day. In addition, urine samples added with sodium azide at 0.1% could be kept at 37°C at least for 4 weeks without deterioration of antibody titers [16].

The usefulness of the urine ELISA was confirmed in several studies in Sri Lanka and China.

(i) *Application of urine ELISA to children in Sri Lanka:*

Studies on the epidemiology of filariasis among young children were very limited. We carried out a study with 203 children aged less than 5 years of age, and their parents (n=292). There were 4 urinary IgG4 positive babies born within 58 days, suggesting trans-placental transfer of IgG4 from IgG4-positive mothers. Then, up to 417 days after birth, no positive baby was found. After day 1,000, the number of positives and their IgG4 titers increased quickly, indicating that filarial transmission (or antigen exposure through infective bites by mosquitos) was active [17].

In order to confirm trans-placental transfer of filaria-specific IgG4, 14 IgG4-positive pregnant mothers and their 14 new-born babies were followed up with urine ELISA for 2 years. IgG4 titers of mothers and their babies just after birth were positively and significantly correlated, and babies' IgG4 decreased gradually and became negative by day 339 after birth [18].

The use of urine ELISA enabled us to collect this kind of data relatively easily, because collecting urine was not as disturbing and uncomfortable as blood sampling.

(ii) *Study in Deniyaya region, Sri Lanka:*

In Deniyaya, where no endemic filariasis had been known, local people provided information that there were not a few hydrocele cases there. Urine ELISA was applied to study if Deniyaya is an endemic area or not. A total of 2,436 subjects were examined and 4.3% of them were found positive. The IgG4 titer analysis by age revealed that the youngest positive was 3 years old, and that the titers among children increased gradually as ages increased [19], indicating that filarial transmission was actually present in Deniyaya at a low level. This study showed that urine ELISA in "non-endemic" areas where people do not like blood sampling was possible, and that it was sensitive enough to detect a 'hidden' endemic focus.

(iii) *Long-term follow-up study in Deniyaya:*

Effects of 5 rounds of MDAs with DEC/ALB were studied for 6 years in Deniyaya using urine ELISA. The study subjects were children in 7 schools with the average age of 12 yrs. The pre-treatment IgG4 prevalence in 2002 was 3.2%, which decreased to 0.91% in 2003 after the first MA ($P < 0.001$), and finally to 0.36% in 2007 after the 5th MDA. IgG4 pattern analyzed by age indicated that transmission was most probably stopped in Deniyaya (Figure 1). This study showed that long-term monitoring was possible with urine ELISA, which is a difficult thing in areas where filariasis is not considered as a serious problem. Also the usefulness of urine ELISA to evaluate MDA effects was confirmed [20].

(iv) *Confirmation of elimination of filariasis in China:*

In two cities, Yongjia and Gaoan, where the elimination of filariasis had been confirmed based on Chinese criteria, urine ELISA was applied to study if it can reconfirm the elimination. In Yongjia, 2,411 schoolchildren aged 6-10 yrs were examined, and only 2 (0.08%) were found positive. In Gaoan, 7,998 children aged 5-16 yrs were examined and 28 (0.35%) were positive. All ELISA positive children and their family members were checked with several different diagnostic methods and all of them were found negative [21]. This study again showed the usefulness of urine ELISA to confirm the elimination of filariasis.

Another definite advantage of urine ELISA is its higher sensitivity than ICT antigen test. In a study in Sri Lanka, urine ELISA resulted in 2.0-2.7 times more positives compared with ICT positivity in children of 1-10 yrs old [22]. It seems that it takes much longer time for filarial antigen to become detectable in blood compared with antibody, implying that IgG4 detection would be more efficient to detect resurgence of filariasis.

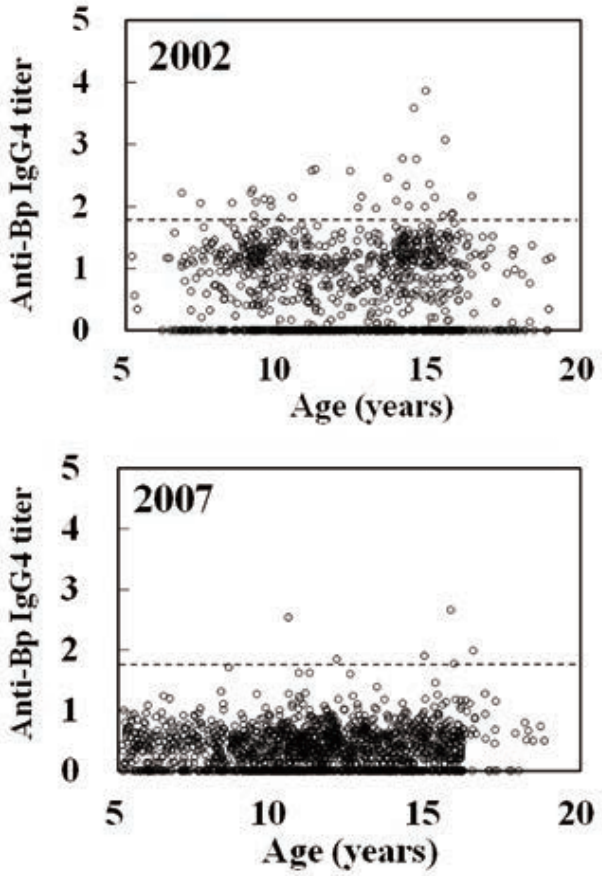

Fig. 1 Change in IgG4 titers before MDA (2002) and after 5 rounds of MDA (2007)
Note: Dotted horizontal line is the cutoff line IgG4 titers (vertical axis) are in log scale.

New ELISA and its application in Bangladesh

We have developed a new urine-based ELISA that uses recombinant SXP1 antigen instead of *B. pahangi* crude antigen, and a preliminary study to evaluate the diagnostic efficacy was conducted in Bangladesh.

Sensitivity study: The study was done in an area where multiple MDAs had been conducted. This is because any new diagnostic method needs to be effective in the post-MDA low endemic areas. The new ELISA with urine detected 30 of 31 mf positives (97% sensitivity). With the ICT positive subjects as a standard, the ELISA resulted in 85% sensitivity (89/105).

Specificity study: This study was done with urine samples obtained in a non-endemic area in Bangladesh. No positives were encountered.

Field application of the ELISA: After the sensitivity/specificity study, the new ELISA was used for a prevalence survey with more than 300 schoolchildren (5-10 yrs) in a post-MDA low endemic area. An interesting finding is that the ELISA detected much more positives than ICT test (2.2% vs. 0.3%), indicating that the new ELISA is sensitive enough to be applied in Bangladesh. The use of urine in Bangladesh was easily accepted.

Conclusion

The advantages of using urine ELISA have been confirmed in the field studies in Sri Lanka and China. We applied a newly developed urine-based ELISA in Bangladesh and obtained promising results. The urine tests were sensitive and specific enough and people accepted the use in various situations. With these favorable outcomes, urine-based immunodiagnoses will have a big potential not only in filariasis control but for other infections.

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Comparative Bio-efficacy of Three Formulations of Pyrethrod Insecticide Aerosol against lab-bred *Culex quinquefasciatus*

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Abstract

Comparative bio-efficiency of three formulations of pyrethroid insecticide aerosol coded as A. Prallethrin 0.090% w/w + d-phenothrin 0.050% w/w, B. d-transallethrin (75/25) 0.126% w/w + d-phenothrin 0.050% w/w, and C. transallethrin 0.040% w/w + cyfluthrin 0.025% w/w was evaluated against laboratory bred female adult of *Culex quinquefasciatus* mosquitoes in a 40 m^3 sized room. The insecticidal efficacy test was based in accordance with the WHO guidelines for efficacy testing of household insecticide product. Eight cages, each with 10 mosquitoes were placed in different places of the room with a height of one and two meter high. Prior to the experiments, the discharge rate of each aerosol can was determined. All the three aerosol formulations were sprayed for 5 seconds and the test mosquitoes were observed for knockdown and mortality at 5, 10, 15 and 20 munites of exposure after applying the aerosol. The mosquitoes were then transferred into a paper cup and fed with 10% sucrose solution. The mortality was determined after 24 hours of holding period. The present studies showed all the three aerosol formulations compared well with in its efficacy. Among the tested aerosol formulations, in terms of knockdown and mortality, code C was 59.17 and 26.25% respectively higher than code B, indicating a significant difference. This study would be useful while planning use of these aerosol formulations for the control of adult mosquito vectors.

Key words: Pyrethroid insecticide, aerosol formulation, vector mosquito

Introduction

The mosquitoes are well known for being vector of many dreaded human diseases worldwide such as malaria, lymphatic filariasis, dengue/dengue haemorrhagic fever. The process of rapid urbanization and unplanned growth of cities, lack of adequate drainage and water stagnation are promoting the breeding of *Culex quinquefasciatus* and the spread of filariasis due to *Wuchereria bancrofti* [1]. The mosquito-borne diseases are great public health importance in tropical, sub-tropical and temparate regions of the world [2]. Among the culicine, *Culex quinquefasciatus* is one of the most common mosquitoes found in human habitations in the Tropics and the Subtropics of the world. This mosquito is an important vector of lymphatic filariasis, commonly known as elephantiasis. Now more than billion people at risk and over 120 million people been affected by it [3]. It is one of the major public health problems in South East Asian countries and most of the countries in the region are recognized as endemic for filariasis [4]. Evaluation of vector management must regularly determine the rate at which the insecticides are contributing or enhancing resistance development [5]. To prevent mosquito transmitted diseases and to protect people from biting nuisance, chemical insecticides have been the backbone of insect pest control [6]. Insecticide aerosol spray is a major household product to control the domestic insect pests especially the adult mosquitoes. Currently the pyrethroid group of insecticides are being used as the main active ingredients in the household aerosol products to control insect vectors because of their biodegradable nature, low mammalian toxicity without any harmful residual effect and higher efficacy against the target species [8]. Although the pyrethroid compounds are very toxic even at very low concentrations, a limited informations to evaluate the range of toxicity of insecticide aerosol formulations to adult mosquitoes especially the *Culex quinquefasciatus* in Sabah, Malaysia. Hence this study was undertaken to evaluate the efficacy of three pyrethroid insecticide aerosol formulations applied at their recommended dosages against adult mosquitoes.

Materials and Methods

Test Aerosol

The tested aerosol insecticide containing the formulations coded A. Prallethrin 0.090% w/w + d-phenothrin 0.050% w/w, B. d-transallethrin (75/25) 0.126% w/w + d-phenothrin 0.050% w/w, and C. transallethrin 0.040% w/w + cyfluthrin 0.025% w/w, were purchased from a local supermarket. These

aerosols are known as household insecticide products refers to a range of ready to use products which are effective against specific target insects and are available in the cities and in the countryside.

Test Insect

The mosquitoes used in this experiment were laboratory bred non blood fed 3 days old female adult of *Culex quinquefasciatus*. Mosquito populations were maintained at insectarium conditions (27 ± 2 ºC and 70± 10% RH).

Mosquito sampling

Culex quinquefasciatus were sampled as larvae from three fixed larval breeding places located near human dwellings in Tanjung Kapur, Kudat, state of Sabah with coordinate (6º 54' 40'' N) and (116º 50' 35'' E). The larvae were collected by dipping method, stored in plastic boxes and transferred to insectarium of the Entomological Laboratory of Kudat District Health Office for rearing to the adult stage. Larvae collection was concentrated around floating debris and aquatic emergent vegetation, collected daily, for 20 days from 25 January until 16 February 2012. Different larval stages from 1st to 4th instar were reared, during this period larvae were fed with commercial aquarium fish food. When they pupate, picked and placed in emerging dish in cages. The collected larvae used in the first stage for identification and in the second stage reared to adult stage for adult bioassay. The adult females were kept in cages (30 X 30 X 30 cm) and daily provided with cotton wool soaked in 10% solution for a period of 3-4 days after emergence. A total of 80 adult mosquitoes was used for each spraying replication in the study.

Determination of Emission Rate

The emission rate of each aerosol (code A, B and C) was determined (shown in Tables-1, 2, and 3) for each experiment by first pre-weighing the new cans (to 0.1g). The can was then sprayed manually into the air in the room for 5 seconds and then re-weighted to compute the amount sprayed in 5 seconds. The procedures were repeated three times. The amount sprayed per second was averaged to obtain the mean emission rates. Table. 1-3 shows the average emission rate and the amount emitted from each formulaion. The total average emitted amount for 5 seconds was code A- 11.3 g, code B- 11.8 g and code C- 9.35 g. This indicates that the emitted amount was higher in code A and B than code C.

Test Room

The experiment was conducted in a square sized room (11x13x9 feet). 8 netted cages (measuring 6 X 6 X 6 inch each) with non blood fed 10 female adult mosquito for each aerosol test was transferred from the main cage.

Table 1. Emission rates of aerosol insecticide formulation **A**.(Prallethrin 0.09% w/w, d-phenothrin 0.05% w/w).

A	Weight of can (g)		Amount sprayed (g)	Spray Time (s)	Dosagerate (g/s)
	Pre	Post			
1	266.50	255.68	10.90	5	2.18
2	255.68	244.58	11.10	5	2.22
3	244.58	232.58	12.00	5	2.40

Average emission rate of A: 2.26 g/sec, test rate 5 sec/1430ft^3 or 40.5m^3

Table 2. Emission rates of aerosol insecticide formulation **B**.(d-trans allethrin 0.126% w/w, d-phenothrin 0.05w/w).

B	Weight of can (g)		Amount sprayed (g)	Spray Time (s)	Discharge rate (g/s)
	Pre	Post			
1	211.60	199.60	12.00	5	2.40
2	199.60	188.10	11.50	5	2.30
3	188.10	176.10	12.00	5	2.40

Average emission rate of B: 2.36g/sec, test rate 5 sec/1430ft^3 or 40m^3

Table 3. Emission rate of **C**. (Transfluthrin 0.040% w/w, cyfluthrin 0.025%w/w).

C	Weight of can (g)		Amount sprayed (g)	Spray Time (s)	Dosage rate (g/s)
	Pre	Post			
1	563.10	554.20	8.90	5	1.78
2	554.20	545.20	9.00	5	1.80
3	544.20	534.00	10.20	5	1.80

Average emission rate of C: 1.872g/sec, test rate 5 sec/1430ft^3 or 40.5m^3

Test Method

Space spraying was applied throughout the experiment. The basic idea of this method is to generate millions of droplets of insecticide, some of which strike the insect and kill it. A flying insect killer aerosol is designated to generate a cloud of small droplets containing insecticide. These droplets must remain airborne for a sufficient period of time to permit adequate collection by the insects as they fly through the aerosol mist ensuring they receive a lethal dose (LD).

Bio-efficacy test

The biological efficacy of the insecticidal aerosol spray against adult mosquitoes was followed the standard WHO guidlines for efficacy testing of household insecticide products [7]. The experiment was conducted in a square sized room mentioned before. 8 netted cages, each with 10 mosquitoes placed at 1 and 2 meter high for each aerosol test to see the effectivity between the position of this two heights. For each test a cage with 10 mosquitoes was kept as control in a seperate room. Prior to the experiment, doors and windows was closed, input fans turned off, room temperature and humidity was recorded. Each aerosol sprayed for 5 seconds from center of the room at a direction of clockwise, holding the can high as spraying commences. The number of mosquitoes knocked down were recorded at 5, 10, 15, and 20 minutes after spraying. Knockdown was determined as the mosquitoes dropping while still conscious, but unable to fly and unable to move the body. After 20 minutes (exposure period), all mosquitoes were transferred into clean paper cups with proper specification of the tested mosquito cages for mortality observation. The mosquito transfering were done by using insect aspirator. The cups were covered with netting and the mosquitoes were fed with 10% sucrose in soaked cotton, placed on the net. The mortality was recorded after 24 hours holding period.

Table 4. Knockdown efficacy of different formulation of aerosol.

CODE	Time			
	5 Minute (%)	10 Minute (%)	15 Minute (%)	20 Minute (%)
A	**63.61**	**76.25**	**90.42**	**92.92**
B	**12.50**	**25.42**	**33.75**	**40.83**
C	**75**	**87.08**	**97.08**	**100**

Result and Discussion

This study evaluated the comparative bio-effecacy of three different formulations of insecticide aerosol against adults of *Cx quinquefaciatus*. The test results showed code C is the most potent formulation to produce highest level of knockdown and mortality rate. Table 4. and Figure.1 indicates that at the final counting at 20 minutes, 100% knock down was found in **C**, which is 7.08% higher than **A**, and 59.17% higher than **B**. Table. 5 and Figure 2 represents the average results of the three replication of the mortalities at 24 hours of the three formulations. This results shows that code C had highest mortality 98.75% which is 2.08% higher than **A** and 26.25% higher than **B.**

For adult bioassays, resistant/susceptible status was defined according to WHO criteria, mosquitoes were considered susceptible if the mortality rates were greater than 97% and resistant if mortality rates were less than 80%. Mortality rates between 80-97% suggested possible resistance [9].

Table 5. Mortality at 24 hours of different formulations of aerosol.

CODE	Time
	Mortality at 24 hours (%)
A	**96.67%**
B	**72.50%**
C	**98.75%**

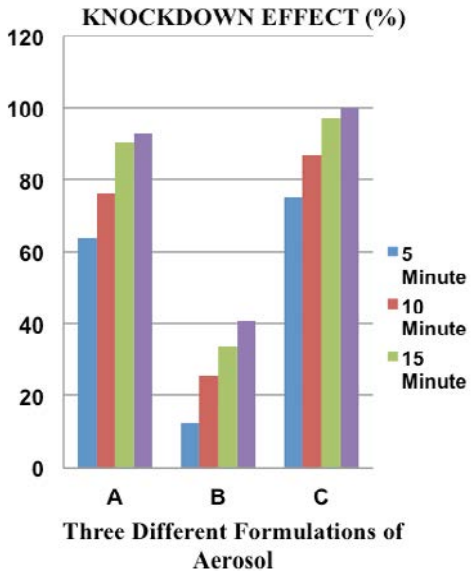

Fig. 1 Graph of knockdown efficacy of three different formulations

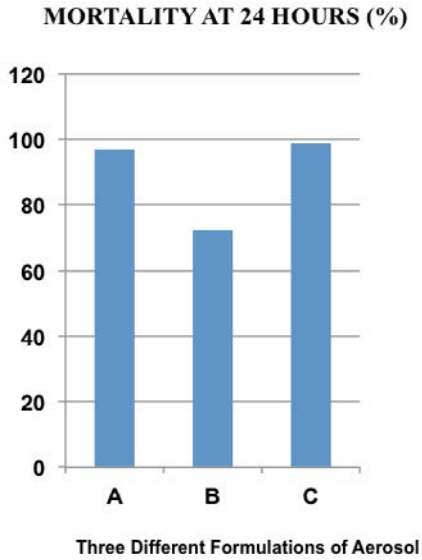

Fig. 2 Graph of mortality at 24 hours of different of aerosol

In conclusion, the findings of this study indicated that the aerosol containing the combination of formulation of transfluthrin 0.040% w/w, and cyfluthrin 0.025%w/w was most effective among three tested fomulations and *Cx quinquefasciatus* adults were most susceptible to this formulation. The aerosol product effectivity depends on application techniques. The factors of application techniques are like treated space and volume, aerosol emission timing, doors and windows should be closed, electric fan should be switched off during the period of application.

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Prevalence, Intensity and Viability of Tissue Parasites Infected Bovine Carcasses at Ismailia - Egypt with Special Reference to their Zoonotic implications

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Abstract:

The slaughterhouse represents a key control point of livestock production chain. It could be used to give a full picture about the zoonotic parasitic diseases. Therefore, this article aimed to determine the prevalence, intensity and viability of tissue parasites of bovines slaughtered at the main abattoir of Ismailia city, Egypt. From March 21st 2009 to March 20th, 2010, a total of 10055 cattle, 3811 buffalo carcasses were inspected, followed by parasitological and histopathological examinations. Stool specimens of 1200 farmers were examined for parasite eggs. Results revealed that the total prevalence of *Cysticercus bovis* was 0.47%, which was higher in cattle (0.57 %) than in buffalo (0.18%). 320 cysticerci were detected in 76 bovine carcasses, of which 103 (32.18%) were alive. The anatomical distribution of cysticerci was 55 (72.37%) heart, 13 (17.10 %) tongue, 7 (9.21 %) masseter, and 1 (1.31%) diaphragm. Hydatid cysts were detected in 106 (0.76%) carcasses. It was higher in buffalo 57 (1.49%) than cattle 49 (0.49%). A total 405 hydatid cysts were detected in 120 carcasses, of which 133 (32.83%) were viable. The predilection sites distribution of hydatid cysts were in the lung 84 (70%), 35 (29.17%) liver, 1 (0.83%) spleen. *Sarcocystis* macrocyst was detected grossly in buffalo carcasses only in 775 (20.33%). Macrocysts were identified to *Sarcocystis fusiformis*, and anatomically distributed as 403 (49.09%) in esophagus, 333 (40.56%) in tongue, 85 (10.35%) in skeletal muscles. The total prevalence of liver flukes was 1.94%, which was higher in buffalo (3.23%) than in cattle (1.46%). *Fasciola hepatica* and *Fasciola gigantica* were identified. Generally, females showed significantly higher infection rates because of elder ages. In human, taniid eggs were detected in 2 (0.16%), *Fasciola* eggs 4 (0.41%) in stool specimens. In conclusion, the occurrence of such affections throughout the edible organs reflects their economical and public health impacts in Ismailia province that might be prevalent in Egypt in large. These epidemiologic data could be a base of planning prevention and control programs.

Key Words: Parasites, Cysticercosis, Fascioliasis, Sarcocystosis, metacestodes, Zoonoses.

Introduction

Tissue zoonotic parasites of bovine are still prevalent in most regions of the world. In particular, the larval stage of two cestodes (*Echinococcus spp.* and *Taenia (T.) saginata)*, one trematode (*Fasciola spp.*) and a protozoon cyst (*Sarcocystis spp.*) occur worldwide causing considerable economic and public health consequences [1, 2].

Bovine cysticercosis is known as infection of cattle with metacestodes of the human tapeworm, *Taenia saginata,* or *Cysticercus (C.) bovis* [3]. The lifecycle involves humans as the definitive host for the tapeworm and cattle as the intermediate host for the larval stage. Infection in man is acquired by ingestion of raw or undercooked beef containing the larval cysts, *C. bovis,* while cattle become infected by ingesting tapeworm eggs passed with human stool [4]. Bovine cysticercosis has worldwide distribution; with occurrence in both developed and developing countries [5, 6]. In Egypt, various authors have reported the prevalence of cysticercosis in cattle [7, 8, 9], while few records of human taeniasis have been reported [7].

Cystic echinococcosis is caused by the larval stage of *Echinococcus granulosus*. The metacestodes usually form fluid filled cysts ('Hydatid') located in liver, lungs and other organs. It is one of the major zoonotic diseases in the world and it induces economic loses and public health problems [10]. Parasite definitive hosts are canids and intermediate hosts are mammalians, especially wild and domestic ruminants or humans [11]. High parasite prevalence was found in the Middle East as well as Arabic North Africa causes a particularly heavy burden in developing countries [10, 12]. In Egypt, cystic echinococcosis is also endemic disease with public health importance [13, 14].

Bovine fascioliasis is an economically important parasitic disease of cattle caused by Fasciolidae, which are trematodes of the genus *Fasciola* (*F.*). The two most important species of this genus are *F. hepatica* and *F. gigantica.* Animal fascioliasis is a significant factor limiting livestock production with economic losses due to fascioliasis are caused by mortality, morbidity, reduced growth rate, condemnation of liver, increased susceptibility to secondary infections and the expense of control measures [15]. Moreover, human fascioliasis is a serious public health challenge in many countries [16, 17]. In Africa, infection with fascioliasis represents a major animal and human health problem [18]. In Egypt, animal as well as human fascioliasis is a growing problem, as it is recorded in nearly all governorates [18, 19].

Sarcocystosis is a zoonotic and parasitic disease highly prevalent in livestock animals [20]. Species within genus *Sarcocystis* are heteroxenous, cyst-forming parasitic Coccidian belonging to the family Sarcocystidae. *Sarcocystis spp.* are obligate two-host parasites, generally alternating between a herbivorous intermediate host and a carnivorous definitive host [21]. Meat and organs that is heavily infected may be condemned as unfit for human consumption constituting much economic losses. The infection has potential of public health importance because man may acquire infection by consumption of

under-cooked meat containing well-developed tissue cysts containing bradyzoites [2, 22]. The disease is highly endemic in Egypt; macroscopic and microscopic sarcocysts are frequently detected during meat inspection [23, 24].

Abattoir records could provide baseline data concerning prevalence of the diseases that considered essential for contemplating rational control programs. Therefore, the aim of this study was to determine the magnitude, the localization and viability rates of tissue parasitic diseases in cattle and buffalo slaughtered in Ismailia Abattoir, Egypt.

Material and methods

Study area and animals under study: An active abattoir survey was performed on slaughtered cattle and buffalo at Ismailia municipal abattoir for a period of one year from March 21st, 2009 to March 20th, 2010. This abattoir is a central abattoir located at Ismailia city, Suez Canal area at the north-east of Egypt. During this study, a total of 16244 native breed bovine animals including; 10055 cattle and 3811 buffalo were slaughtered and inspected. All the females slaughtered aged over three years, and all animals were owned in household production.

Post-mortem inspection: The carcasses of bovine animals slaughtered were routinely examined according to procedures described by the Egyptian general guidelines on meat inspection of cattle (Egyptian Code no 517 for 1986). The presence of tissue cysts and its organ distribution were recorded. Tissue cysts were carefully removed and separately collected in clean containers and quickly delivered to the laboratory in ice for further characterization.

Detection and identification of *C. bovis*, hydatid cysts, liver flukes and sarcocysts: The observed *C. bovis* was categorized into live and calcified cysts. The cysts were regarded as viable if the scolex evaginated from the cyst membrane using 30% bovine bile after the incubation for 2h at 37°C [25]. The cysts were then identified as *C. bovis* if there were no hooks on the rostellum of evaginated scolex [26]. Hydatid cyst fertility and viability were determined by examining the cysts content. The presence of protoscolices either attached to the germinal layer in the form of brood capsule or its presence in the cyst fluid was considered as indicative of fertility. Fertile cysts were further subjected to viability test according to the procedures of [27]. The livers were examined for *Fasciola* by making length wise incisions of the ventral side of the liver in such a way that the bile duct is cut open. Then forceps was used to pick the exposed worms in the bile duct and gall bladder. Infected liver were classified according to the total number of worms recovered per liver into light (1-10), medium (11-50) and heavy (>50). Identification of the species involved was carried out using the size parameters described by [28]. Examination for macroscopic sarcocysts was done by the naked eye through meat inspection at the abattoir. Species identification of the *Sarcocystis* macrocyst was performed according to [24].

Histopathological examination: Freshly collected muscle samples from hydatid and sarcocysts were fixed in 10% neutral buffered formalin solution, dehydrated in graded ethyl alcohol, embedded in paraffin, cut at 5-µm thickness, processed routinely for hematoxylin and eosin staining and Stained sections were microscopically examined [29].

Coprological examination of human stool samples: Stool samples of 1200 of farmers were submitted for coprological examination for *Taenia spp.* eggs and *Fasciola* eggs were detected using the formalin-ethyl acetate sedimentation technique as previously described [30].

Data analysis: The significance of sex, age and seasons on the prevalence of tissue parasites among cattle and buffalo species was determined using Chi- square contingency with Fisher's exact test (two tailed). Statics were computed using GraphPad Prism (Version 5) software. P value of <0.005 was considered statistically significant.

Results

Prevalence of *C. bovis*, hydatid cysts, sarcocysts and liver flukes among slaughtered animals: As tabulated in table 1, the total prevalence of *C. bovis* was 0.47%, which was higher in cattle (0.58%) than buffalo (0.18%). The overall prevalence of hydatid cysts of the examined carcasses was 0.76%, with infection rate of 1.50% in buffalo and 0.49% in cattle. Sarcocysts was detected macroscopically in 775 (20.33%) of 3811 buffalo carcasses, however, it was not detected by similar macroscopic examination of cattle carcasses. Liver flukes were detected in liver of 3.23% of buffalo, which was higher than the detection rate in cattle (1.46%), indicating an overall infection rate of 1.94%.

Table1 Prevalence of C. bovis, Hydatid cysts and Sarcocysts and liver flukes among slaughtered cattle and buffalo

	Cattle No=10055	Buffalo No=3811	Total No= 13866
	no %	no %	no %
C. bovis	58 (0.58 %)	7 (0.18%)	65 (0.47%)
Hydatid cyst	49 (0.49%)	57 (1.50%)	106 (0.76%)
Liver flukes	147 (1.46%)	123 (3.23)	270 (1.95%)
Sarcocysts	0 (0%)	775 (20.33%)	775 (5.59%)

Table 2 Age, gender and seasonal distribution of tissue parasites among slaughtered cattle and buffalo.

	Adult Cattle and buffalo			Adult Buffalo
	C. bovis No (%)	Hydatid Cyst No (%)	Liver flukes No (%)	Sarcocyst No (%)
Sex/Age				
Male (2-3 years) (No=13049)	56 (0.43 %)	94 (0.72 %)	225 (1.72 %)	324/3169 (10.22%)
Female(>3 years) (No= 817)	9 (1.10%)	12 (1.47%)	45 (5.51%)	451/642 (70.25%)
Season				
Hot season (No=7798)	38 (0.49%)	50 (0.64%)	159 (2.04%)	388/2044 (18.98%)
Cold season (No=6068)	27 (0.44%)	56 (0.92%)	111 (1.83%)	387/1767 (21.90%)

Age, gender and seasonal distribution of tissue parasites among slaughtered bovine: Results shown in table 2 revealed that *C. bovis* infection was significantly higher in elderly females with age (more than 3 years) (1.1%) than fattening males aged (2-3years) (0.43 %) ($P< 0.05$). However, there was no significant difference between males and female infection by hydatidosis. Meanwhile, the prevalence of liver flukes was very significantly higher in females (5.5%) than males (1.72 %) ($P<0.01$). Macrocysts of *sarcocystis spp.* were detected visually by higher rates in female buffalo (70.24%) than in male buffalo (13.38%). Regarding seasonal effects, there was not significant effects on the prevalence of *C. bovis*, hydatid cysts and liver flukes between worm season which extends from March to September than cold which extends from October to February ($P>0.1$).

Organ distribution and viability of *C. bovis* and hydatid cyst among bovine carcasses: Table 3 displays the anatomical distributions and viability of *C. bovis* and hydatid cysts. Of the organs examined, the highest proportions of the *C. bovis* were observed in the heart (84.62%) followed by tongue (20%), masseter muscles (10.77%) and diaphragm (1.54%). The proportion of hydatid cyst was highest in the lungs (79.24%) followed by liver (33.02%) and spleen (0.94%). Of the total 320 *C. bovis* and 405 hydatid cysts collected, 103 (32.19%) and 7 (32.84%) were found to be alive, respectively. There rests were degenerated cysts (Table 3 and fig.1&2).

Table 3 The organ distribution and viability of *C. bovis* and hydatid cyst among bovine carcasses.

	C. bovis			**Hydatid cyst**		
Organs inspected	No. infected (%)	No. of cyst	No (%) of viable cyst	No. of positive (%)	No. of cyst	No (%) of viable cyst
Heart	55 (84.62%)	240	82 (34.17%)			
Tongue	13 (20%)	50	15 (30%)	0	0	0
Masseter muscles	7 (10.77%)	26	6 (23.08%)	0	0	0
Diaphragm	1 (1.54%)	4	0	0	0	0
spleen	0	0	0	1 (0.94%)	2	0 (0%)
Lung	0	0	0	84 (79.24%)	265	95 (35.85%)
liver	0	0	0	35 (33.02%)	140	38 (27.14%)
Total	76 (0.55%)	320	103 (32.19%)	120 (0.87)	405	133 (32.84%)

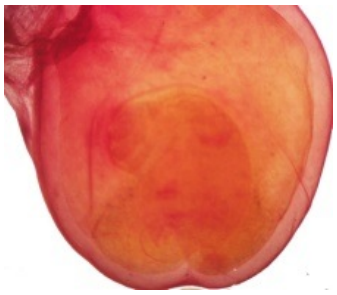

Figure 1 Microscopic picture for *C. bovis* showed invaginated scolex with four suckers, no rostellum and no hooks.

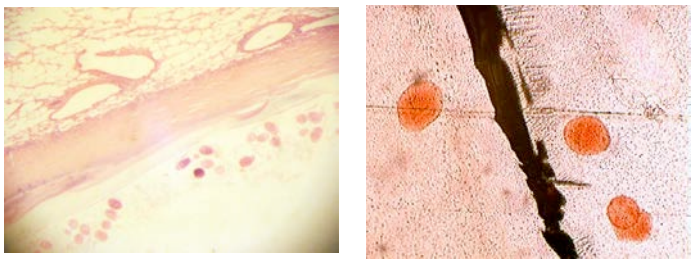

Figure 2 Several detached scolices in the cyst fluid stained by 0.1% eosin; each scolex is invaginated and armed with several hooks (left) Histopathological picture of hydatid cyst in lung tissue surrounded by thick fibrous pericyst and hyaline ectocyst (right).

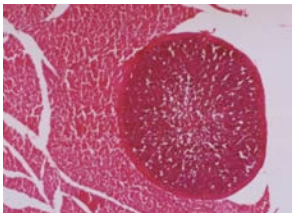

Figure 3 Histopathological Picture of sarcocyst in skeletal muscles showing a macrocyst which has thick capsule (H&E. X 100).

Organ distribution of sarcocysts in buffalo carcasses: Results revealed that the *Sarcocystis spp* macro-cyst could be identified to *Sarcocystis fusiformis* (*S. fusiformis*) (fig.3). The proportion of distribution of *Sarcocystis* macrocysts was (49%) esophagus, (40.6%) tongue and (10.4%) in skeletal muscles.

Species identification and intensity of infection of liver flukes: The number of flukes/animals was varied, (1-10) (14.8%), medium (11-50) in (85.2%). Collected flukes were identified morphologically (fig.4) to *F. hepatica* and *F. gigantica* by proportion rate of (43.70%) and (56.3%) respectively.

Discussion

Although cysticercosis has little effect on the health of the animals, it is socially and economically important as a zoonosis, affected meat is very often condemned and control measures are usually expensive. The results of the present study revealed that the prevalence of *C. bovis* among the inspected bovine carcasses was 0.47% which was higher than that recorded by [31]; while it was much lower than the prevalence that has been documented at the same of our study area [32] and also in different areas of Egypt [7, 8, 9]. The variations of the prevalence of *C. bovis* infection with persistence of infection might be attributed to geographical distribution and cattle husbandry systems, which allow grazing on pastures, drinking from water systems and wastewater management. The predominance of *C. bovis* infection among cattle compared to buffalo was in agreement with previous studies [7, 8, 9, 32].

Figure 4 Microscopic picture of *F. hepatica* showing triangular shape, prominent shoulders, and converging borders (left). *F. gigantica* is grayish-brown, elongated, leaf like worms and up to 7cm, anteriorly there is a small cone-shaped projection and two shoulders (right).

However, species-related distribution of cysticercosis among slaughtered bovines could be related to the sample size and the predominance of cattle breeding in Egypt compared to buffalo. Regarding animal sex, bovine cysticercosis infection was significantly higher in females than in males, this finding was in agreement with the findings of previous studies in Egypt [7, 8, 32]; while it was inconsistent with other studies in Africa [26, 33]. The predilection sites of infection with *C. bovis* detected in this study were similar to that reported in many studies in Egypt [7, 32] and in Africa [26]. In this study, *C. bovis* wan not detected in muscular tissue that was inconsistent with the finding of other studies [34, 35]. It appears that several factors such as activity of muscles, age, and geographical area concerned determine largely the predilection sites in slaughtered cattle [26]. The prevalence of viable cysts reflected the actual risk of zoonotic transmission through consumption of inadequate meat harboring viable cyst. Results revealed that the percent of viable cysts was (32.18%), which was lower than that reported by [34] (44.2%), and higher than that reported by [35] (28.3%). Since the life cycle is dependent upon humans as the only definitive host, the detection of *C. bovis* among bovine tissue indicates the occurrence of *T. saginata* among human eating inadequately cooked infected beef meet containing viable cysticercai [4]. In the present work, the incidence of taniid eggs was (0.16%) which was lower that reported by [7] (1.6%). The infected persons are a potential source of animal infections by direct defecation in agriculture field or indirect through untreated sewage system used as plant fertilizer.

Hydatidosis is one of the most important zoonotic diseases in Egypt. In the current work, the overall prevalence of hydatid cyst was 0.76% which was higher than the findings of [36, 37] and lower than that recorded in another study (6.4%) [14]. Hydatidosis has been documented in different livestock with high fertility rate [37]. It was previously reported that buffalo are highly susceptible to hydatid infection [38]. Similarly, detection rate was higher in buffalo than cattle. It could be assumed that *E. granulosus* eggs disseminated by stray dogs are the common source to different livestock animals and human as well. In this study, no significant differences between males and females slaughtered, a similar finding was reported [39, 40]. The anatomical sites of distribution of hydatid cyst are related to mode of infection by *E. granulosus* eggs from dogs. The predominance of hydatid cysts in lung indicated that inhalation of infective eggs was the common mode of infection. In this work, 32.83% of the detected hydatid cysts were fertile and viable. Previous reports varied in fertility rate of hydatid cyst in cattle; viability rate of 1.7% [35], 10.66% [39] and 62.2% [40] were reported. From public health prospective, high burden of Echinococcosis/hydatidosis in animals increases the risk of human infections. Previous reports showed a low endemicity of human hydatidosis in Egypt [41].

In this study, the total prevalence of fascioliasis was 1.94%, which was much lower than the prevalence

reported in Ismailia governorate (29.2%) [42]. Although Ismailia city is semi-desert city, the occurrence of fascioliasis and their snail hosts were previously reported [43]. It has reported that during the years 1994 to 1997, the overall prevalence of fascioliasis among slaughtered cattle and buffalo in Egypt was 2.02% for cattle and 1.58% for buffalo [44]. The detection rate of fascioliasis among buffalo (3.23%) was much higher than cattle (1.46%). However, high prevalence of fascioliasis among buffalo could be attributed to age factor, sample number and pattern of feeding of dairy buffalo that mostly depends on green fodders and grazing, while fattening animals mostly fed dry ration. In Egypt, both *F. hepatica* and *F. gigantica* co-exist in domestic animals [28]; both species were identified in this study. Human fascioliasis in Egypt was very sporadic until the last three decades when clinical cases and outbreaks were reported [16, 18]. In this study, the prevalence of human fascioliasis detected by stool examination was 0.41%, which was much lower than that previously documented [42].

In this study, *Sarcocystis* macrocysts were detected macroscopically in buffalo carcasses by a prevalence of (20.33%) which was much lower than the previous reports in Egypt (94%) [23] and (78.9%) [24]. No macroscopic cysts of *Sarcocystis spp.* were detected visually among cattle carcasses during meat inspection. However, previous studies showed high prevalence of macroscopic and microscopic sarcocysts species among slaughtered cattle [23]. Visually detected macrocysts were identified to *S. fusiformis* species. A similar *Sarcocystis* species has identified in Upper Egypt governorates [23, 24] suggesting the endemicity of this species in different localities in Egypt. Detection of macroscopic cysts indicated occurrence of feline final hosts [24]. The predominance of infection in esophagus in the examined carcasses was in consistent previous reports [23, 24]. Humans can act as intermediate hosts, and are thus at risk when eating raw or improperly cooked meat from infected animals. This can result in intestinal sarcocystosis, and is potentially important in terms of public health [2].

Generally, females are prohibited for slaughtering in the abattoirs except for emergency or aged females. Therefore, sex and age factors are coupled as factors influencing infection rates in elderly females. In addition, old age exposed to the disease over a long period of time with an increased possibility of acquiring the infection. Regarding seasonal effects, there were no significant seasonal impacts on the prevalence of parasitic zoonoses that might be related to chronicity of the diseases.

In conclusion, the detection of such affections indicates their significance in our field of animal husbandry with relevance of human zoonoses. Proper implementation of meat inspection procedures during slaughter should be adopted. A systemic and large-scale surveillance of parasitic zoonotic diseases is essential for preventative and control measures.

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Detection of *Cryptosporidium* oocysts and *Giardia* cysts from drainage of the small-scale sewage disposal plants in Hyogo Prefecture.

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Abstract

We have been investigating *Cryptosporidium* and *Giardia* in the raw water and finished water of four water purification plants (WP) once per one or two months since 1998. Until now, in the result of regular investigations, the positive rates of *Cryptosporidium* per year in all WPs scored between 0 and 14%, and the detected number was between 1 to 18 per 10 liter. With regard to Sanda WP, the positive rates scored a little higher than 25%. Information on water source was collected, which showed a lot of pollution sources such as sewage disposal plants and livestocks near the catchments area of each WP. Among those, drainage samples of small-scale sewage disposal plants in the water source of Sanda WP and Funatsu WP were investigated several times. As the result, high levels of cysts of *Giardia* were detected, and they were confirmed to be the parts of the pollution sources. Eighteen oocysts were detected in the raw water of Inagawa WP on October 4, 2011. By another investigation in the same period, 155 oocysts were detected in the upper stream of the water purification plant, and 238 oocysts were detected in an upstream tributary. There is an area where household septic tanks are provided for sewage treatment in the upper part of this tributary, suggesting that this is the source of the contamination, and some patients existed there.

Key Words: Cryptosporidium, *Giardia*, sewage plant, prevalence, waterworks

Introduction

Cryptosporidium and *Giardia* are ubiquitous in the aquatic environment and their environmental stages (oocysts and cysts) may remain viable for several months under a range of environmental conditions [1]. In addition, oocysts and cysts are resistant to conventional disinfectants such as chlorine used at water purification plants. Due to the robust oocyst structure, the small size of this parasite and the low sedimentation rate, conventional water treatment is not totally effective, and oocysts may be present and infective in treated water, even if no treatment failure has occurred [2].

Therefore, they are strictly surveyed by waterworks. We also have surveyed them in the raw and finished water of our four water purification plants since 1998 and sometimes detected them in raw water samples.

In October 2011, an incident occurred that a lot of *Cryptosporidium* oocysts were detected in the raw water of a water purification plant, and we made an urgent survey of the catchments area of the water purification plant. In this paper, we report the result of our regular surveillance of these parasites and contamination status of the catchments areas.

Materials and Methods

1. Sample Preparation

A raw water sample (10 liters) was filtrated through a hydrophilic PTFE membrane filter, and the filter was put in a 50-ml centrifuge tube, followed by exfoliation using a vortex mixer [3]. Then the sample was concentrated by centrifugation and purified by immunomagnetic separation (IMS). For IMS, we used a magnet of our own making instead of manufacturer's offering product (MCP1). The purified sample was placed on a well-slide glass and stained with FITC-labeled antibody and DAPI solution. The well-slide was observed with an epifluorescent microscope. Objects stained with FITC-labeled antibody, confirmed with DIC imaging of the inner structure and its nuclei stained with DAPI, were taken into count. Overall protocol was according to the method proposed by Japanese Water Works Association [4] except for using the well-slide glass.

2. Regular Investigation

We have four water purification plants (WP), namely Tada, Sanda, Kande and Funatsu. We have made regular investigation on the raw and finished water of each WP since 1998 once a one or two months. *Cryptosporidium* oocysts and *Giardia* cysts were sometimes detected in the raw water samples, but they were not detected in the finish water samples. Therefore, we focused only on the result of the raw water.

3.Contamination sources and Selection of Sampling Points

We surveyed the catchment basin of each WP and found various facilities that can be contamination sources. At first, we found a lot of cow barns around the catchments basin of Sanda WP. We had surveyed the river nearby for several years. However, the result didn't suggest that these cow barns are the major sources. Therefore, we focused on small-scale sewage plants at the catchment basin, and found a lot of such plants. We selected sampling points of water, taking into account the influence and the distance to the WP. Consequently, we selected two points for the catchments basin of Sanda WP and three points for the catchments basin of Funatsu WP.

4 .Contamination incident

A contamination incident occurred at Tada WP in 2010. Eighteen *Cryptosporidium* oocysts were detected in the raw water of the WP. It was a large number never detected in the past. Therefore we made an urgent survey of the catchment basin of Tada WP.

Results and Discussion

1. Sample Preparation

Using the method proposed by Japanese Water Works Association, both *Cryptosporidium* oocysts and *Giardia* cysts were detected successfully. The recovery from sewage samples had some difficulties, as immunomagnetic beads were not collected well by the designated magnet (MCP 1). Therefore, we used a handmade magnet [5], with which both parasites were recovered without any difficulties.

2 .Regular Investigation

We have been investigating *Cryptosporidium* and *Giardia* in the raw water and finished water of four water purification plants (WP) once per 1 or 2 months since 1998. All results of the finished water were negative. The positive rates of all raw water samples per year were scored between 0 and 25% for *Cryptosporidium*. Until 2010, the detected number was between 1 and 8 per 10 liters. However, in 2011, 18 oocysts were detected in the raw water of Tada WP. It is described later. With regard to *Giardia*, a little low positive rate was observed (Table 1).

Table 1 Results of regular investigation (Raw Water)

Positive number of *Cryptosporidium* oocyst (positive / test)

WP	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Tada	1/8	0/5	1/11	0/12	0/12	0/13	1/16	1/12	0/6	0/7	2/7	0/6	0/6	1/7
Sanda	1/12	1/12	3/12	0/12	2/13	0/10	1/16	0/12	0/6	1/9	2/8	0/6	0/6	1/6
Kande	-	-	-		0/12	0/12	0/16	0/12	0/6	0/6	1/6	0/6	0/6	0/6
Funatsu	-	-	-		1/13	0/10	0/16	0/12	0/6	0/6	1/6	0/6	2/6	0/6

Positive number of *Giardia* cyst (positive / test)

WP	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Tada	-	-	-	-	-	0/13	0/16	1/12	1/6	0/7	1/7	0/6	0/6	0/7
Sanda	-	-	-	-	-	2/10	0/16	0/12	0/6	0/9	0/9	0/6	0/6	0/6
Kande	-	-	-	-	-	0/12	0/16	0/12	0/6	0/6	0/6	0/6	0/6	0/6
Funatsu	-	-	-	-	-	1/10	1/16	0/12	0/6	0/6	0/6	0/6	1/6	0/6

3. Contamination sources

The result of regular investigation suggests that there should exist contamination sources of *Cryptosporidium* and *Giardia* at the catchment basin of the water purification plants. When many oocysts or cysts are detected in the raw water or an epidemic occurs in the catchment basin, we have to establish an emergency system. So, it is important to identify possible contamination sources in advance. Therefore, we searched for facilities that can be the contamination sources and surveyed their effluent.

The results of investigations on small-scale sewage plants were summarized in Table 2. On the whole, the positive rate was 26% for *Cryptosporidium* and 100% for *Giardia*. Detected number of oocysts and cysts ranged 0 – 34 and 0 – 3000, respectively. Especially, *Giardia* cysts were detected in all samples and often reached high number.

Table 2 Result of investigation of sewage plant drainage

Area / Organism	Sewage Plant	Positive/Test	Detected Number Range
Mukogawa River (Sanda WP) / *Cryptosporidium*	Honjo	4/9	0-16
	Aimoto	0/8	0
	Aono	0/1	0
	Ono	0/1	0
Ichikawa River (Funatsu WP) / *Cryptosporidium*	Kitatsuneya	1/11	0-1
	Nakamura	1/2	0-34
	Kubata	1/5	0-20

Area / Organism	Sewage Plant	Positive/Test	Detected Number Range
Mukogawa River (Sanda WP) / *Giardia*	Honjo	9/9	1-1170
	Aimoto	9/9	4-1056
	Aono	1/1	113
	Ono	1/1	3000
Ichikawa River (Funatsu WP) / *Giardia*	Kitatsuneya	11/11	2-388
	Nakamura	2/2	4-32
	Kubata	5/5	144-700

The capacities of the sewage plants were given. Then the number of excreted parasites per day from the sewage plant service area was estimated by calculating from the detected number and the sewage plant capacity. If one patient excretes 1 hundred million parasites, most of these calculated numbers indicate less than one patient, but it is estimated that at least 3 giardiasis patients existed at the service area of Ono sewage plant (Table 3).

Table 3 Discharged number per day

Sewage Plant	Drain Vol. (m3/day)	From WP (km)	*Cryptosporidium* Detected (/10L)*	*Cryptosporidium* Discharged (/day)	*Giardia* Detected (/10L)*	*Giardia* Discharged (/day)
Kitatsuneya	125	5.4	34	4.25E+05	32	3.00E+05
Nakamura	105	6.5	1	1.05E+04	388	4.85E+06
Kubata	73	7.7	20	1.46E+05	700	5.10E+06
Honjo	505	5.1	16	8.08E+05	1170	5.91E+07
Aimoto	397	11.9	0	-	1056	4.19E+07
Aono	265	5.8	0	-	133	3.52E+06
Ono	451	7.7	0	-	3000	1.35E+08

*Maximum Number

These calculations were performed assuming that the recovery rates of oocysts and cysts were 100%. The actual recovery rate is about 50%, therefore the number of patients is considered to be larger.

4. Result of urgent survey of the catchment basin of Tada WP

High numbers of oocysts were detected continuously in the raw water of Tada WP (Fig 2). Much higher numbers were detected in the samples from upper river regions. In these area, most sewage flows to the river basin sewage system. So, it skips Tada WP and gives no effect. However, there are some areas where the sewage is treated by household septic tank and the drainage flows into the rivers (Fig. 1). Oocysts seemed to flow from these household septic tank areas. The flow rates of the river at Golf Bridge and at the point after two rivers join are given (stars in Fig. 1). The number of oocysts excreted per day was estimated from the number of detected oocysts and the river flow rate per day. The calculated number at Golf

Bridge reached 3 billion (Fig. 2). If one person excreted between one hundred million and one billion oocysts per day, there should have been 3 - 30 patients.

Fig 1 Inagawa *Cryptosporidium* Incident (October 2011)

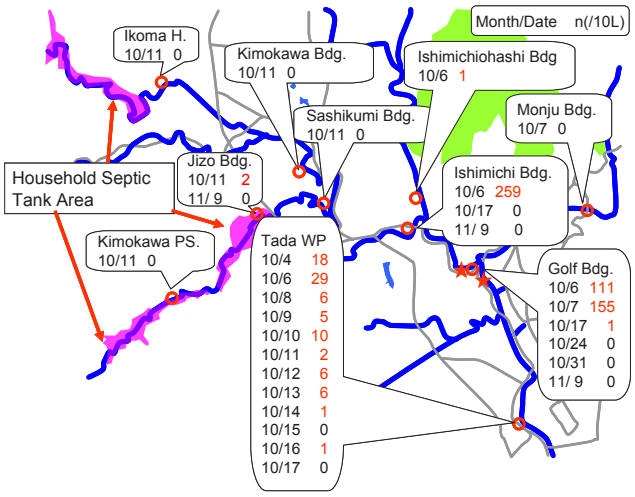

Fig 2 Estimated Number of Excreted Oocysts per Day

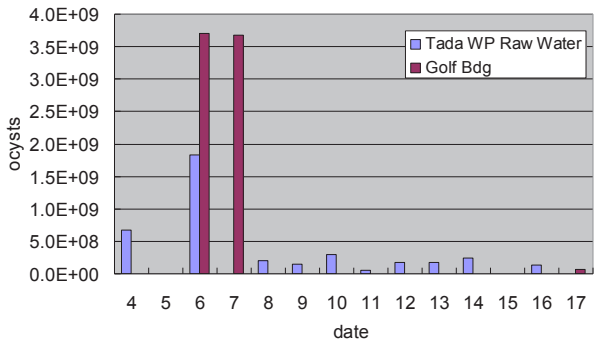

Table 4 shows the number of cryptosporidiosis and giardiasis reported [6]. They are very small numbers, therefore these diseases are considered to be rare and not prevalent. However, our data suggests a different situation. Although the present investigation was carried out in such a limited area, it suggested the existence of the diseases. This implies that there should be much more patients in the whole Hyogo prefecture, not to mention the whole nation. It is also suggested that the surveillance of the sewage plant drainage is a good tool which would indicate the existence of such diseases.

Table 4 Reported Number of Diseases

		1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Hyogo	C	0	0	1	61	0	0	0	0	0	0	0	0	0
	G	0	7	5	2	1	5	0	2	1	2	2	3	5
Japan	C	4	3	11	109	8	92	12	18	6	10	17	16	4
	G	42	98	137	113	103	94	86	86	53	73	70	77	42

C: cryptosporidiosis, G: giardiasis

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Occurrence of clinical cases diagnosed as diphyllobothriasis nihonkaiense between 1988 and 2012 in Kyoto, Japan

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Abstract
The occurrence of clinical cases diagnosed as diphyllobothriasis nihonkaiense between 1988 and 2012 clarified the incidence of human infection has been recently increasing from 1998 and reached to high level at 2008. especially with the increase of affected women , cases in younger and adult people, and in spring and early summer between 1998 and 2012 in Kyoto, Japan.

Keywords: diphyllobothriasis nihonkaiense; tapeworm: annual incidence in Kyoto, Japan

Introduction

The incidence of human infection with *Diphyllobothrium nihonkaiense* has been increasing in Japan. Moreover, clinical cases caused by *D. nihonkaiense* have been emerging even in European countries and others [1-3]. An imported case of diphyllobothriasis. nihonkaiense, possibly acquired in Switzerland, has been reported in Japan [4]. These cases highlight the globarization of *D. nihonkaiense*. The natural history and the geographic range of this tapeworm are still mysterious but recent studied have indicated that the Pacific salmon, *Oncorhynchus keta* (chum salmon) is now a high risk source for human infection in Japan and the brown bear in the northern parts of the Pacific coastal region is the natural final host [5-7]. Mitochondrial DNA divergence in populations of the tapeworm *D. nihonkaiense* in clinical cases and its phylogenetic relationship with *D. klebanovskii* from Russian brown bears and people are compared and analyses of mitochondrial DNA sequences of both tapeworms showed a high level of similarity, indicating synonyms of the two species [6]. Mitochondrial genomes demonstrated the distinct difference between *D. nihonkaiense* and *D. latum* [8-12]. The current situation of diphyllobothriasis nihonkaiense in Kyoto, Japan is not enough to remain to be elucidated. More knowledge about the occurrence of the patients is required. We examined the annual incidence of clinical cases, sex, seasonal occurrence, age distribution of patients, frequency of lineage of genotype of isolates and so on between 1988 and 2012, especially in two periods between 1998 and 2006, and between 2007 and 2012 in Kyoto, Japan.

Annual incidence of clinical cases of diphyllobothriasis nihonkaiense between 1988 and 2012.

Retrospective examinations of annual case numbers of diphyllobothriasis nihonkaiense revealed a total of 144 cases of diphyllobothriasis between 1988 and 2012. Case numbers represent all cases of *D. nihonkaiense* infection in Kyoto. Diphyllobothriasis nihonkaiense was diagnosed by its morphologic appearance and taxonomic characteristics of the strobila or gravid proglottids of tapeworms discharged in the feces of patients who had a history of eating salmon or a habit of eating sushi or sashimi (raw fish fillets), especially that derived from salmonids. Mitochondrial DNA (mtDNA) sequences of tapeworm *cox1* and/or *nad3* genes were also analyzed from most patient specimens obtained since 2004 and results clearly indicated the *D. nihonkaiense* species. Although the number of clinical cases has fluctuated some until 1998, the incidence was particularly high in 2008. So the annual incidence rates, sex, seasonal occurrence, age distribution and frequency of lineage A or B of genotype of isolates between 1998 and 2012. Moreover, the number of worms collected from feces after treatment was examined. The occurrence of clinical cases diagnosed as diphyllobothriasis nihonkaiense showed 27 cases between 1998 and 2006 in the past 9 years at an average number of 3.0 per year (average incidence of 0.3 cases per 100,000 people in Kyoto per year), and 70 cases were recorded between 2007 and 2012 in the present 6 years at an average number of 11.7 per year (average incidence of 1.2 cases per 100,000 people).

Sex of patients with diphyllobothriasis nihonkaiense between 1998 and 2012.

Twice as many men than women were affected in the past 9 years, but the number of women increased from 2007, where the number of men and women was 37 and 32, respectively.

Seasonal occurrence of patients with diphyllobothriasis nihonkaiense between 1998 and 2012

Clinical cases occurred in all seasons though a recent surge has been recognized in early and late summer. The pacific salmon, *Oncorhynchus keta* (chum salmon), caught in spring and early summer in the east coast of Hokkaido, Japan pose a higher risk for human infection than autumn-caught salmon. In mid and late summer, *O. keta*

is thought to return to the motherlands of Russian rivers.

Age distribution of patients with diphyllobothriasis nihonkaiense between 1998 and 2012

Age distribution of patients showed that every age group was affected, from 3 to 80 years. Most patients were 21-60 years of age between 1988 and 2006, but young cases were increasing with adult cases between 2007 and 2012, which probably reflects more frequent consumption of sushi and sashimi by people in these age groups than in other age groups.

Frequency of lineage A or B of genotype of isolates between 1998 and 2012

The DNA analysis of the isolates and then sequence divergences between lineages A and B were performed and examined as described before [6]. The DNA was extracted and purified using QIAamp DNA Mini Kit(50) (Qiagen GmbH, Hilden, Germany) according to the manufacture's instructions. The *cox1* and *nad3* regions of mtDNA were amplified by PCR. The PCR primers used were 5'- TTGATCGTAAATTTGGTTC-3' and 5'-AAAGAACCTATTGAACAAAG-3' for *cox1*, which yielded a 748-bp product; and 5'-AACTTTGTGTTTCATTGGTA-3' and 5'-GACAATAAGTTATTAGCAGT-3' for *nad3*, which yielded a 475-bp product. PCR amplication was performed in a volume of 50 ml containing 0.25ml of *Takara Ex Taq* Hot Start (5U/ml), 5 ml of 10x *Ex Taq* Buffer, 4ml of dNTP Mixture (2.5mM each) (TAKARA BIO INC., Otsu, Shiga, Japan), 2 ml of mixed primers, 1ml of DNA sample, and 37.75 ml of distilled water. PCR reaction was performed under following conditions: Samples were denatured at 94℃ for 5 min; then they were subjected to 35 cycles of 94℃ for 30 sec, 52℃ for 30 sec, and 72℃ for 30 sec, with final extension at 72℃ for 5 min. The PCR products were purified using MinElute PCR Purification Kit(50) (Qiagen GmbH, Hilden, Germany) and were sequenced. Analysis of mtDNA sequence polymorphisms revealed two deeply divergent lineages designated on A or B. The *cox1* sequences (700 bp) showed 22 polymorphic sites with 21 synonymous and one nonsynonymous substitutions. The *nad3* sequences (365 bp) showed 16 polymorphic sites with 7 sites of nonsynnymous substitutions. The difference of lineage A and B was strictly discriminative mainly in 6 sites of *cox1* and 5 sites of *nad3* sequences. Though lineage A of genotype of isolates increased in recent years; sixth as many isolates of lineage A than lineage B, the two cryptic lineages remain to be elucidated.

The number of worms collected from the feces after treatment with praziquantel and gastrografin in 95 cases between 1998 and 2012

An infected person may usually harbor a single worm; one worm in 89 cases; rarely two worms in 4, and three worms in one case. The greatest number of worms encountered in one individual appeared to be 7 in 2012; in this case (a 17-year-old boy), 7 scolices of worms were separately excreted in the feces after treatment with praziquantel. A single dose of praziquantel (10mg/kg) was also very effective for multiple infections.

A molecularly confirmed case of *D. latum* infection in a Japanese tourist

We experienced a molecularly confirmed *D. latum* case from a Japanese tourist who consumed a fresh water fish belonging to the *Coregonus* species from Russian rivers in 2010. The positions of polymorphic sites of cox1 (483bp) and also nad3 (369bp) sequence of this isolate were same to *D. latum* reported in DNA database.

Conclusion

The epidemiology of clinical cases clarified that the incidence of human infection with *Diphyllobothrium nihonkaiense* has been gradually increasing from 1998 and at high level in 2008. The incidence of diphyllobothriasis nihonkaiense, especially in young and adult people, has been recently increasing. The seasonal occurrence of the disease indicated most patients were demonstrated in all seasons, especially in spring and early summer and they regularly consumed raw fish fillets of pacific salmon. The number of affected women with men has also been increasing in recent years. Age distribution of patients showed the increase in younger age group with adult group. Genetic analysis of mtDNA sequences of isolates demonstrated the distinct difference between *D. nihonkaiense* and *D. latum* and confirmed first record of human infection with *D. latum* in a Japanese tourist who returned from Russian rivers. These results suggest that the incidence of *D. nihonkaiense* infection, especially in young and adult people, has been recently increasing.

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Newborn larvae of *Trichinella spiralis* have immature stichosome with mature granules

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Abstract

This contribution deals with ultrastructure of newborn larvae of *Trichinella spitalis*, which revealed presence of morphologically immature stichosome but with mature granules. This finding suggests that the stichosome is one of the origin of excretory and secretory products at the newborn larva stage as has been reported in the other stages.

Key Words: Trichinella spiralis, newborn larva, stichosome, stichocyte, granule, electron microscopy

Introduction

Newborn larvae (NBL) of *Trichinella spiralis* play a very unique role in terms of immunological and cell-biological response by the host. Once newborn larvae (NBL) enter the muscle tissue, NBL initiate a sequence of alterations in the infected muscle cells, transforming the cells into the nurse cells [1][2]. This early stage of NBL infection should receive more attention. However, deep investigation has yet been performed. The reason being is that the size of NBL is very small, and collecting a large quantity of NBL to perform biochemical and / or morphological analysis is quite difficult from methodological point of view. Although the biological function is remaining unknown, clones expressed predominantly specific to this stage have been already established [3][4].

Morphology of *Trichinella* at other stages, muscle larva and adult, has been extensively investigated and reported in many articles [5][6][7][8][9]. Examples of exocrine structures include the stichosome, intestinal gland, midgut, hypodermal gland, genital organs of adult worms, and midgut of the muscle larvae. Morphological information so far reported has provided deep insights to understand the origin of excretory and secretory proteins [10][11][12][13]. These proteins may be involved in the parasite-host interactions. Unfortunately, morphology of NBL has been poorly reported less than muscle larvae and adult worms,

In this contribution, we report the ultrastructure of stichosome at NBL stage of *Trichinella spiralis* seeking for the possible origin of excretory and secretory proteins.

Materials and Methods

Muscle larvae of *Trichinella spiralis* (ISS: 413) were isolated from infected mice by artificial gastric juice digestion. Adult worms were isolated from the mice intestine, 7 days after the oral infection. The worms were processed for the electron microscopic observation by the well-established procession. The female adults accommodate fetus worms in the vagina, which were just going to be delivered. These fetuses were supposed to be equivalent to NBL.

Results

The NBL have developed the body wall composed of cuticle, hypodermis, cords, and hypodermal muscle (see Figure). The internal organs were, however, poorly developed. The esophagus cuticle was identified. A very thin space was identified as the pseudocoelum. Between the esophagus and the pseudocoelum were supposed to be the stichosome (an organ with accumulated stichocytes). Based on this anatomical position, primordial cells of the stichosome were identified as immature stichocytes, where the rough endoplasmic reticulum system was less developed.

Although the stichosome was not fully developed, the primordial stichocytes had exocrine granules resembling β granules at the muscle larva stage and type I and II granules at the adult stages. Morphologically, these looked mature granules.

The canalicular tree structures in the stichocytes were not developed at this stage. Not-identified structures in the NBL included microvilli of the gut and genital primordium, which are identified in the muscle larvae.

Discussion

The NBL are more critical than the other stages because they enter intact normal muscle cells and likely initiate the transformation of host cells into the nurse cell [1][2]. The excretory and secretory products of NBL are the candidate molecules responsible for such host cell transformation [4]. Along this line of research interest, a couple of clones specific for this stage have been already established although the specific function is still remaining undermined [14][15].

The present study provided some hints about the origin of the excretory and secretory products of NBL. As shown in the results, the nematode *Trichinella spiralis* was composed of relatively simple structures including the body wall (cuticle, hypodermis, cords, and hypodermal muscle), digestive organs (esophagus, midgut, hindgut, stichosome, and intestinal gland), the nerve ring, and the genital primordium. The adult worm has additional structures such as hypodermal glands in the cord. The genital primordium develops into internal reproductive systems specific for male and female.

Among these structures, morphology of the stichocytes is intriguing as the origin of excretory and secretory products because the stichocytes have prominent secretion granules [12][13]. The ultrastructure of the stichocyte of *Trichinella* was revealed by our intensive studies [7]. The present study showed NBL stichocyte granules were

surrounded by the unit membrane and had round shape. The contents were middle in electron density and homogeneous without crystalloid inclusion. Thus NBL stichocyte granules morphologically resembled β granules of adult [12] and muscle larvae [7].

Stichocyte granules are supposed to be excreted through the specialized subcellular structures named canalicular tree (lined with the regular unit membrane), which opens to the esophagus lumen (lined with the thick cuticle) as reported by Takahashi and colleagues [6][9].

Stichocytes of NBL were devoid of the canalicular tree. Even without these specialized structures, it is possible for cells to excrete the contents outside the cell probably circumventing the route through the thick cuticle of the esophagus.

In conclusion, the NBL have the immature stichosome with mature exocrine granules, which may function to affect the host immune system and the infected muscle cells.

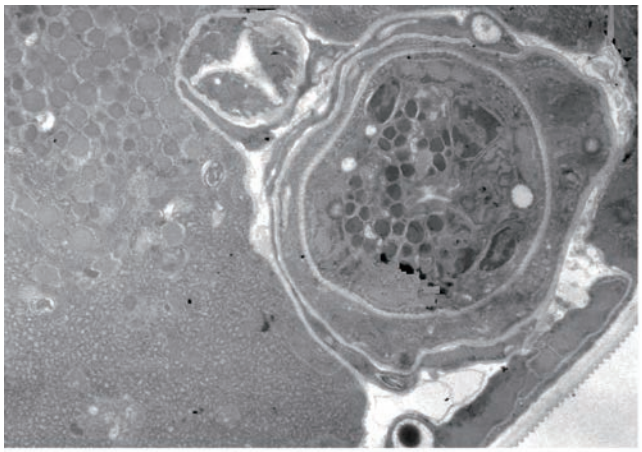

Figure A transverse section of an adult female through the stichosome (left), the esophagus (upper central) and the vagina (right) with a larva, The larva in the vagina is also cut transversely showing the body wall (cuticle, hypodermis, hpodermal muscles), the esophagus (in the center) and the immature stichocyte surrounding the esophagus. Note the many exocrine granules.

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Epidemiological survey of *Angiostrongylus cantonensis* in Port Island, Hyogo Prefecture, Japan

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Abstrct

Angiostrongylus cantonensis is a parasitic helminth that exploits snails (such as *Achatina fulica*) and slugs as intermediate hosts, while the definitive hosts for this parasite are rodents (*Rattus norvegicus* and *R. rattus*). Adult worms live in the pulmonary artery of parasitized rodents. *A. cantonensis* is distributed widely throughout Southeast Asia and the Pacific Islands. In Japan, *A. cantonensis* has been reported to be distributed mainly in the harbor areas of the Okinawa Prefecture and Amami Islands; however, a recent study showed that the parasite has spread to the mainland. Although Uga et al. (1981) [1] conducted an epidemiological survey of *A. cantonensis* in Port Island 30 years ago, no adult worms were found. In the present survey, because infection with *A. cantonensis* was seen in 19 out of the 41 rodents examined (46%), it was suspected that rapid invasion of the parasite had occurred over the past 30 years in Port Island. As a consequence, we sought to identify the intermediate hosts for *A. cantonensis* on this island. *Allopeas clavulinum* (snail), *Lehmannia valentiana* (slug), *Physa acuta* (snail), *Semisulcospira libertina* (snail), *Strobilops aenea* (snail), and *Zonitoides arboreus* (snail) were tested for *A. cantonensis* L3 parasites, but none were detected. Hence, we performed experimental laboratory infections to determine the intermediate hosts for *A. cantonensis*. The results showed that four (*A. clavulinum*, *L. valentiana*, *P. acuta*, and *Z. arboreus*) out of the eight gastropods tested were candidate intermediate hosts for *A. cantonensis* in Port Island.

Key Words: Angiostrongylus cantonensis, rodents, intermediate host, Port of Kobe, Port Island

Introduction

The definitive hosts for *Angiostrongylus cantonensis* are rodents, but many different types of snails and slugs can act as intermediate hosts [2]. Infection with *A. cantonensis* causes eosinophilic meningitis (Wang et al. 2008) and larva migrans in humans. The disease is transmitted by ingesting third-stage larvae (L3) in the raw or undercooked flesh of an intermediate host. Additionally, vegetable juices and salads have been identified as sources of infection, which has occurred following contamination with intermediate hosts [3]. After introduction of *A. cantonensis* during World War II, the parasite spread passively from East Asia to other regions [4], and is currently distributed in Southeast Asia, the Pacific Islands, and the Caribbean [5]. The recognized distribution of *A. cantonensis* has been increasing over time. Man-made spread of the giant land-snail *Achatina fulica*, which is the most important intermediate host for *A. cantonensis*, extension of the geographical distribution of slugs, snails and rodents as a consequence of global warming, and the unintentional transportation of *A. cantonensis*-infected rodents on vessels has caused expansion of this parasite in subtropical regions of the world. An outbreak of human angiostrongyliasis recently occurred in China [6], and several epidemiological surveys of *A. cantonensis* have been conducted in this country [7,8,9,10,11].

In Japan, cases of angiostrongyliasis have been reported successively in the Okinawa Prefecture since 1969, and *A. cantonensis* has been detected in rodents and certain types of slug in Japanese harbors, indicating that *A. cantonensis* has become an indigenous species on mainland Japan [12,13,14,15,16,17]. Epidemiological surveys of *A. cantonensis* in Japan peaked in the 1980s, and although the numbers of such studies have since decreased, new investigations started after an outbreak of angiostrongyliasis in Okinawa Prefecture [18], and the first fatal case of this disease was seen in Japan [19]. Recently, there have been reports that the prevalence of *A. cantonensis* is much higher in areas of Japan where it was not previously considered to be endemic. Indeed, the distribution of this parasite has extended outwards from the harbor areas and it can now be found inland [20,21]. Despite the importance of this situation in the context of public health, because most epidemiological studies on *A. cantonensis* in Japan have been published in Japanese, the significance of these studies has probably gone unnoticed by non-Japanese speaking scientists and clinicians.

In 1981 an epidemiological survey of *A. cantonensis* was conducted in Port Island, but no adult worms were detected in the rodents that were collected at that time [1]. Although recent epidemiological studies of *A. cantonensis* have revealed that the prevalence of this parasite has changed, its present status on Port Island is not clear. Therefore, the aim of the this study was to determine how the prevalence of *A. cantonensis* parasites has changed over time by comparing the data from the 1981 study with the data obtained in this study; both sets of data are based on the same sample collection area in Port Island.

Materials and Methods

1. Survey area and time period

The survey was carried out in Port Island, Kobe city, Hyogo Prefecture, Japan during July 2011 and October 2012. Port Island (8.26 km^2) is an artificial island located approximately 3 km south of the Port of Kobe. Port Island is linked to mainland Japan by a bridge and tunnel, has an airport and functions as a trade port. Port Kobe is one of the main international trade ports in Japan. The reclamation work needed to construct Port Island was conducted over two time periods. Construction of the first phase was from 1966 to 1981. During this time, the north side of the area (4.36 km^2), which included warehouses and residential areas, was constructed (Fig. 1-a). During the second phase (1987-2005), a 3.90 km^2 area in the south, which included a container terminal and airport, was constructed (Fig. 1-b). The total number of vessels entering Port Island was 36,638 in 2010 [22]. Of these, 7,259 were foreign vessels and 1,803 (25%) arrived via China. In the same year, the Port of Kobe handled 87 million tons of freight; about 50 million tons of it arrived from foreign countries (72% from China and Taiwan).

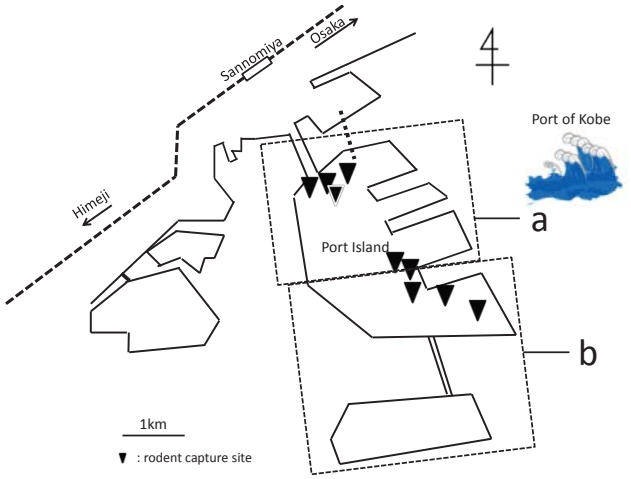

Fig. 1 Map of Port Island.

2. Survey of rodents in Port Island

a) Capture

Rodents (*Rattus norvegicus* and *R. rattus*) were captured by live-catch rat-traps. The traps were baited with deep-fried minced fish and placed in bushes or at the back of vending machines at around 8 p.m. Early the next morning, any rodents that had been captured were brought to the laboratory for examination.

b) Examination

Fecal samples from the captured rodents were examined by light microscopy to detect *A. cantonensis* first-stage larvae (L1) (approximately 250 μm in length). A few L1-positive rodents were maintained in the laboratory for use in the experimental infection study described below (section d). Body weight, body length, and the sex of the captured rodents were measured after euthanasia of the animals with carbon dioxide. The heart and lungs of each rodent were examined for the presence of adult *A. cantonensis* worms. In addition, the liver, stomach, small intestine, cecum, and large intestine were examined for signs of other helminths under a stereoscopic microscope and/or a light microscope.

3. Intermediate hosts on Port Island

a) Collection

Slugs, earthworms, terrestrial snails, and freshwater snails were collected from Port Island on cloudy or rainy days. All of the slugs, earthworms, and terrestrial snails were collected from under fallen leaves and stones, or from the surfaces of stone walls. In addition, a plastic container (90×15 mm) containing 15 ml of beer was placed on wet fallen leaves to attract slugs. After 1 hour, slugs that had been attracted to the beer were collected. Freshwater snails were collected from two ponds in Port Island.

b) Breeding

Some of the candidate intermediate hosts were kept in the laboratory for 3 weeks at room temperature for later use in the experimental infection study (section d). The containers used for breeding the invertebrates were 10 cm in diameter and 10 cm in height and were covered by cellophane in which 10 pinholes had been made. The breeding container was prepared appropriately for each invertebrate type and a maximum of 10 were kept in it. Two wet tissues and five filter papers were placed in each of the containers for humidity and for food, respectively. Earthworms and freshwater snails were kept in the laboratory under appropriate conditions.

c) Examination to detect third-stage larvae (L3)

After collection, the candidate intermediate hosts were chopped up into small pieces (using scissors) and digested for 2 hours in artificial gastric juice, using a magnetic stirrer (AS ONE Corporation, Osaka, Japan) for mixing. The sediment obtained was observed under stereoscopic and light microscopes. When larvae were found that were suspected of being *A. cantonensis* L3, their total length (approximately 470 μm) and existence of a rod-like structure on them were confirmed. Such larvae were used for the experimental infection study.

d) Infection study

Feces (0.5g) obtained from an *A. cantonensis*-positive rodent (containing L1 larvae) was fed to the candidate intermediate hosts. Feces were changed daily. After 3 weeks' infection with *A. cantonensis*, the invertebrates were digested and examined for the presence of L3 stage parasites. When larvae that appeared to be L3 were obtained, they were orally administered to 10-week-old male albino Wistar rats (CLEA Japan, Inc., Tokyo, Japan). After two months, the experimental rats were sacrificed and examined for the presence of adult worms.

4. Ethics approval

This study was approved by the Institutional Animal Care and Use Committee (Approval number: P121003) and was carried out according to the Kobe University Animal Experimentation Regulations.

Results

1. Rodents

1) Parasite species identified in rodents

A total of 41 rodents (including *R. norvegicus* (40) and *R. rattus* (1)) were captured in Port Island and examined for the presence of parasites. Ten genera and 10 species of parasite were detected and identified as either nematode or acanthocephalan worms (Table 1). Of the parasites detected, the prevalence rates for *Nippostrongylus brasiliensis* and/or *Orientostrongylus ezoensis* were the highest (87%). Although parasitism by both of these species was confirmed, the species prevalence rates were not examined; hence for the purposes of this study, *N. brasiliensis* and *O. ezoensis* were considered to belong to the Heligmonellidae family. Infection with *A. cantonensis* was seen in 19 out of 41 of the rodents examined (46%), and this rate was the third highest observed among the parasites that were detected (Table 1).

Table 1. Parasites found in rodents (2011 and 2012)

Examined helminth	No. of rodent examined	No. of rodent infected	Infection rate (%)
Angiostrongylus cantonensis	41	19	46
Capillaria sp.	39	3	8
Eucoleus bacillatus	39	1	3
Heterakis spumosa	39	19	49
Moniliformis sp.	39	3	8
Heligmonellidae*	39	34	87
Strongyloides ratti	39	1	3
Syphacia muris	39	1	3
Trichuris muris	39	6	15

**Nippostrongylus brasiliensis* or *Orientostrongylus ezoensis*

2) Infection with *A. cantonensis*

The mean number of adult *A. cantonensis* parasites per rodent was 11 (min. 1, max. 58) (Fig. 2-a). No significant interaction between the infection intensity and body weight in the rodents was observed (Fig. 2-b). Additionally, interactions between body weight, body length, and infection with *A. cantonensis* in the rodents was analyzed (Fig. 2-c). The results showed that all rodents (8/8) more than 140 g in weight and 19 cm in length were infected with *A. cantonensis*. In contrast, only 16% of the smaller rodents were *A. cantonensis*-positive.

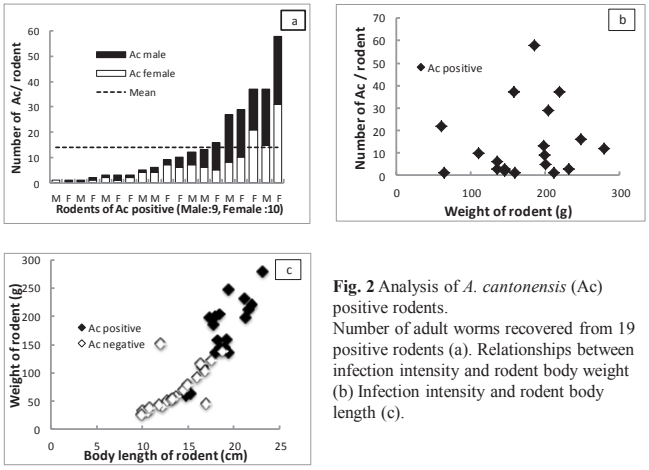

Fig. 2 Analysis of *A. cantonensis* (Ac) positive rodents.
Number of adult worms recovered from 19 positive rodents (a). Relationships between infection intensity and rodent body weight (b) Infection intensity and rodent body length (c).

2. Intermediate hosts

1) Collection of candidate intermediate hosts

Collections were conducted over two time periods, from September-November 2011 and April-November 2012. Six genera and six species (terrestrial snails: four species, freshwater snails: one species, slugs: one species) were collected (the numbers collected for each species were 12-200). Although a total of 396 individuals were digested and examined, no L3 stage *A. cantonensis* parasites were detected (Table 2).

Table 2. Gastropods infected with third-stage larvae of *A. cantonensis*

Species	No. of gastropod examined	No. of gastropod positive
Allopeas clavulinum	12	0
Lehmannia valentiana	134	0
Physa acuta	17	0
Semisulcospira libertina	20	0
Strobilops aenea	200	0
Zonitoides arboreus	13	0

These shellfishes were collected from Port Island and examined for *A. cantonensis* infection.

2) Experimental infections

Because no *A. cantonensis*-infected individuals were detected during the collection periods, we sought an alternative method of identifying intermediate host species in Port Island. This was achieved through the use of an experimental laboratory-based infection study. *A. cantonensis* L1s (Fig. 3-a) recovered from the fecal samples of the infected rodents were administered to eight candidate intermediate hosts (Table 3). After 3 weeks' infection, L3 larvae (Fig. 3-b, c), which had the characteristic features of *A. cantonensis* L3 (~480 μm in length with a rod-like structure at the anterior end), were detected in four genera (*Allopeas clavulinum*, *Lehmannia*

valentiana, *Physa acuta*, and *Zonitoides arboreus*). The L3 larvae obtained from these infections, which were injected into rats subcutaneously and then into adult worms (Fig. 3-d), were thereafter detected in the lungs and hearts of the infected rats. Hence, these four genera are candidate intermediate hosts for *A. cantonensis* in Port Island.

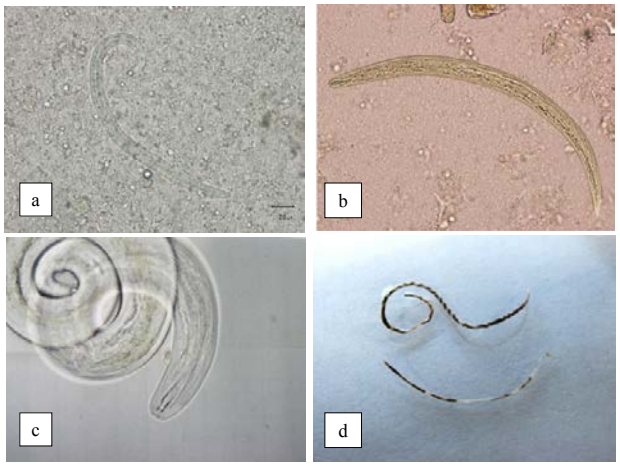

Fig. 3 Different developmental stages of *A. cantonensis*. a: First-stage larvae, b: Third-stage larvae, c: Details of anterior end of third-stage larvae showing rod-like tips and rod-like structure, d: Adult worms (upper: female, bottom: male)

Table 3. Experimental infections with *A. cantonensis*

Species	Examined	Result*
Allopeas clavulinum	26	+
Armadillidium vulgare	27	−
Lehmannia valentiana	23	+
Lumbricus terrestris	17	−
Physa acuta	6	+
Semisulcospira libertina	15	−
Strobilops aenea	36	−
Zonitoides arboreus	4	+

*The respective infection rates for each species are not shown.

Discussion

We conducted an epidemiological survey of *Angiostrongylus cantonensis* in Port Island, Hyogo Prefecture, Japan. A total of 41 rodents were captured throughout the survey period, and of those, 19 had lungs and hearts that were infiltrated by *A. cantonensis* adult worms (49%). Taira et al. (2002) [23] reported that the prevalence rate for *A. cantonensis* among *R. norvegicus* and *R. rattus* was 11% (5/46) in Okinawa, which is known to be an endemic area of Japan. Noda (2005) [24] reported that the *A. cantonensis* prevalence rate among *R. rattus* was 10.5% (2/19) in the Amami Islands, where the parasite is also endemic. In contrast, the *A. cantonensis* prevalence rates among rodents were reported as 68% (15/22), 100% (9/9), and 92% (22/24) in the Chiba Prefecture, the Tokyo Metropolitan area, and the Kanagawa Prefecture, respectively [21]. In addition, the *A. cantonensis* prevalence rate was 43.5% (10/23) in Kurume city, in the Fukuoka Prefecture [20]. These results are interesting because the parasite prevalence rates in the above-named places, which are known to be non-endemic, are higher than the rates for endemic areas of Japan; our results are consistent with these findings.

In Port Island where this survey was conducted, a similar epidemiological survey of *A. cantonensis* was performed by Uga et al. (1981) [1]. Although antibody-specific bands against the antigens of the adult worms were discovered in rodent sera using immunoelectrophoresis, no infections with adult *A. cantonensis* worms were found in the lungs and hearts of 144 *R. norvegicus*, *R. rattus*, and *Mus musculus* 30 years ago. From these results, we suspect that the spread of *A. cantonensis* occurred suddenly during the past 30 years. Yoneda et al. (2001) [20] and Ohta (2008) [21] reported that some infected rodents were captured at positions 10-35 km beyond the harbor area and therefore suggested that the habitat area had expanded. It was also suspected that invasion of *A. cantonensis* into the interior of Hyogo Prefecture was a possibility. These findings support the need for future epidemiological surveys of *A. cantonensis* to be conducted not only within the limits of the harbor areas, but also on mainland Japan.

The mean intensity of *A. cantonensis* parasites in the infected rodents captured in this survey was 11 (minimum=1, maximum=58). This result is consistent with the findings of Kinjo et al. (1993) [25] in the Nagoya Prefecture and Chen et al. (2011) [26] in China. Moreover, in the present survey, it was found that the *A. cantonensis* prevalence rate in large rodents was higher than that for small rodents. This result also accords with Kinjo et al. (1993) [25]. We suspect that changes in the feeding habits of rodents may occur during their growth, thus explaining the relationships between body length and the parasite prevalence rate.

A. fulica and particular slug species are important intermediate hosts for *A. cantonensis* in mainland Japan [27]. In this study, we were unable to find L3 larvae in any of the candidate intermediate hosts (snails and slugs) that were collected. Even though the number of the different types of invertebrates examined was limited, the prevalence rate, which was high among the definitive rodent host, contrasts with the lack of *A. cantonensis* L3 in the candidate intermediate hosts. The reason for this result is not completely clear; however, two possibilities exist. First, we suspect that the prevalence rate among slugs is probably not very high. One species of slug (*L. valentiana*) had the highest possibility of candidacy as an intermediate host among the gastropods collected in this survey. While studies on *L. valentiana* are limited, Asato

et al. (2004) [3] reported that the prevalence rate for *A. cantonensis* in *L. valentiana* was 3.8% (3/78) in the Okinawa Prefecture, while Lv et al. (2009) [6] reported a rate of 6.5% (349/5370) for slugs in China. Indeed, detection of infected intermediate hosts is known to be difficult [21,25]. Second, we suspect that some intermediate hosts are as yet undiscovered in Port Island. Nevertheless, the results of our experimental *in vivo* infections revealed that four genera are possible intermediate hosts. Because Lv et al. (2008) [11] reported that *A. cantonensis* has low intermediate host specificity, and Alicata and Jindrak (1970) [2] listed 56 species of Mollusca as intermediate hosts for this parasitic worm, the possibility exists that, in addition to the candidate intermediate hosts identified in our study, other hosts exist in Port Island. The data obtained from this survey area so far suggest that specific but as yet undefined species act as intermediate hosts, and some of these may also have low *A. cantonensis* prevalence rates. The epidemiology of the intermediate hosts for *A. cantonensis* in Okinawa Prefecture differs before and after 1990 [3], and knowledge that *R. norvegicus* as an omnivorous animal often eat insects and earthworms [28] supports our discussion.

In this survey, we found the prevalence rate for *A. cantonensis* was high in Port Island. Although cases of angiostrongyliasis have not been recorded in Port Island itself, the intermediate hosts should still be identified to provide greater awareness of how the disease is transmitted, which would help to prevent new cases in Japan. Identification of all of the intermediate hosts for *A. cantonensis* will require surveying a wider range of snails for L3 stage parasites; until then, what is a real intermediate host and what is not will remain unclear.

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Identification and diagnosis of *Acanthamoeba* with random amplified polymorphism DNA and 18S rRNA sequences-based PCR

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Abstrct

In the present study, we developed the specific primers based on the sequences of random amplified polymorphism DNA (RAPD) and 18S rRNA for the purpose to identify and diagnose the *Acanthamoeba* isolated from the patients in Tokyo and Gifu. DNA was extracted from the cultured trophozoit and cyst, as well as from the contact lens washing solution in one case. RAPD and 18S rRNA from several reference isolates which have been identified as *A. polyfaga*, *A. cultisoni* and *A. astronyxis*, were sequenced and the primers were developed based on these sequence. Three pairs of primers, SB216, SB162 and SB214 developed from the RAPD sequence, specifically amplified the DNA from *A. polyfaga*, *A. cultisoni* and *A. astronyxis*, respectively. One pair of primer, Aca1 developed from the conserved sequence of Acanthamoeba 18S rRNA, showed genus-specific and amplified the DNA from all patients, and this primer produced different PCR product size among *A. polyfaga*, *A. cultisoni* and *A. astronyxis*. In addition, the primer Aca1 amplified the DNA extracted from one patient's washing solution of contact lens. The present study indicated that these developed primers are useful in identifying species and/or isolates of *Acanthamoeba* and the molecular diagnosis of *Acanthamoeba* keratitis.

Key Words: Acanthamoeba, RAPD, 183RNA, PCR, specific primer

Introduction

Acanthamoeba is one of the most ubiquitous organisms in the environment, including in water sources and soil. Several species have been identified to be pathogenic to human, causing keratitis in contact lens cases which can be extremely serious and vision threatening. *Acanthamoeba* consists of many species and the taxonomy is complex. Morphological characteristics alone have been proven to be unreliable as identification criteria, due to the high polymorphism observed among individual isolates. In the present study, we developed the specific primers based on the sequences of random amplified polymorphism DNA (RAPD) and 18S rRNA for the purpose to identify and diagnose *Acanthamoeba* isolates.

Methods

1. *Acanthamoeba* isolates: 9 isolates from keratitis patients in Tokyo, 6 isolates from Gifu and one isolate from wash solution of contact lens of one keratitis patient. Three isolates which have been identified as *A. polyfaga*, *A. cultisoni* and *A. astronyxis* were used as reference.
2. RAPD-based PCR: AP-PCR was performed to amplify the DNA of the three reference isolates, and the RAPD were sequenced following the method of Nagano [1]. Three pairs of primers specific to the three reference isolates (SB216, SB214 and SB162) were developed.
3. 18S rRNA-based PCR: based on homology of 18S rRNA sequence, one pair of primer (Aca1) was developed in conserved region.
4. PCR detection: the specimens from patients were cultured and DNA was isolated with QIAamp DNA Mini Kit, and detected with the primers.
5. Sequencing: the PCR products by primer Aca1 were sequenced and blasted in GenBank for species identification.

Results

1. RAPD-based PCR can identify the isolates corresponding to the species of *A. polyfaga*, *A. cultisoni* and *A. astronyxis*, as shown in Fig 1.

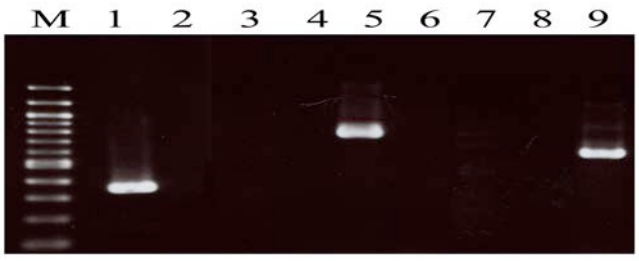

Fig. 1 Identification of Acathamoeba species with primer SB216, SB214 and SB162. The DNA of *A. polyfaga* (lanes 1, 4 and 7), *A. cultisoni* (lanes 2, 5 and 8), and *A. astronyxis* (lanes 3, 6 and 9) were amplified with the primer SB216 (lanes 1-3), SB162 (lanes 4-6) and SB214 (lanes 7-9).

2. 18S rRNA-based PCR can identify the genus of *Acanthamoeba*, and distinguish among the species of *A. polyfaga*, *A. cultisoni* and *A. astronyxis* based on the size of PCR products, as shown in Fig 2.

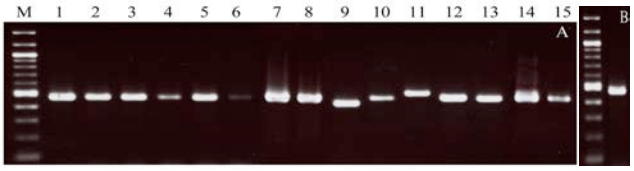

Fig. 2 Identification of *Acathamoeba* species with primer Aca1 which is genus specific to *Acathamoeba* . Lanes 1-6 are the samples isolated from patients in Gifu and lanes 7-15 are the samples isolated from patients in Tokyo. Lanes 9, 10 and 11 are *A. cultisoni*, *A. polyfaga* and *A. astronyxis*. The species were judged by the sizes of PCR products and confirmed by DNA sequence. Panel B showed the result of PCR detection of the DNA isolated directly from the wash solution of contact lens of one keratitis patient.

Discussion

The present study developed *Acathamoeba* specific primers based on the sequence of RAPD and 18S rRNA. The results, so far, indicated that these primers are useful in identifying isolates and species of *Acathamoeba*. Moreover, the Aca1 primer is promising in diagnosis of keratitis by detecting DNA isolated directly from contact lens washing solution. It is a matter of course that an additional study is necessary to further confirm the usefulness of these primers by testing more species and more samples of contact washing solution from the clinical cases.

References'

[1] Nagano I, Zhiliang W, Nakayama M, Takahashi Y. A simple method to design PCR primer to detect genomic DNA of parasites and its application to *Dirofilaria immitis*. Mol Cell Probes. 1996 Dec;10(6):423-5.

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Path traced to identify the transmission route of *Paragonimus skrjabini miyazakii* cercaria to its crab host, *Geothelphusa dehaani*

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Abstrct

The cercarial transmission route taken by *Paragonimus skrjabini miyazakii* to their crab host is a key factor to clarify the *Paragonimus* life cycle over successive subcultures of *Paragonimus* spp. in the laboratory. It was carried out in *P. s. miyazakii*. At first, we developed methods to collect a lot of *P. s. miyazakii* cercariae. Many laboratory experiments were carried out to observe cercarial behaviors. The results clearly showed that *P. s. miyazakii* cercaria penetrates its crab host percutaeneously.

Key Words: Paragonimus skrjabini miyazakii, cercaria, *Geothelphusa dehaani*, transmission route

Introduction

This study of how the cercaria *Paragonimus* spp. is transmitted to its crab hosts is important in that successive subcultures of *P.* spp. in a laboratory have brought forward the development of antihelmintics and diagnostic kits against human paragonimiasis. The present laboratory study was done to identify the route by which one species, *Paragonimus skjabini miyazakii* cercariae, which was obtained from its snail host, *Bythinella nipponica nipponica*, infects its crab host, *Geothelphusa. dehaani*.

Emerging cercaria of *P. s. miyazakii*

Preparing P. s. miyazakii cercariae to elucidate the transmission route to its crab host, G. dehaani.

In order to succesfully carry out cercarial transmission, minute snails, *B. n. nipponica*, which is a snail host for *P. s. miyazakii*, were infected with *P. s. miyazakii* miracidia. The snails were kept in a laboratory, using developed methods [1, 2] for over 85 days; the *P. s. miyazakii* cercariae, which emerged from them, were collected for the present study (Fig. 1).

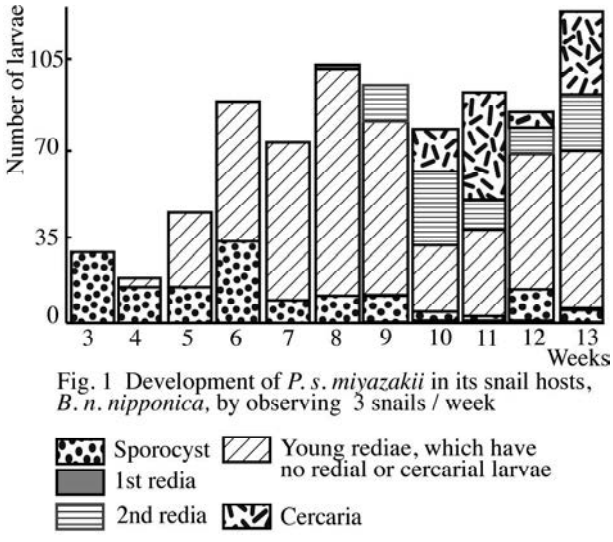

Fig. 1 Development of *P. s. miyazakii* in its snail hosts, *B. n. nipponica*, by observing 3 snails / week

These snails are very minute (1.5 mm long and 0.9 mm wide on the average), and the number of cercariae that emerged per snail per day was only a few. However, a sufficient number of cercariae were collected for the present study because a lot of snails were kept at the same time, and cercariae were continually discharged in water after 85 days of the miracidial infection (Fig. 2).

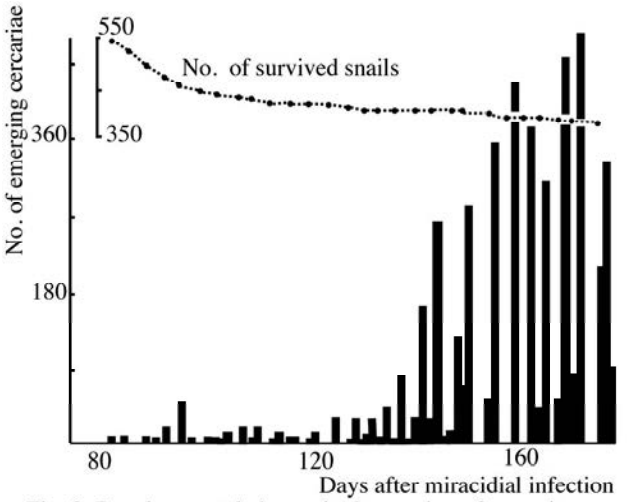

Fig. 2 Developmental change in the number of emerging *P. s. miyazakii cercariae* collected from surviving snail hosts, *B. n. nipponica* every three days in the course of the miracidial infection during the period from 80 to 180 days

Can emerging cercariae be transmited to their crab hosts, G. dehaani, and then develop into P. s. miyazakii metacercarie?

On the assumption that a cercaria is transmitted to its crab host via water, the transmission was attempted by putting crabs into water containing cercariae, and by injecting cercariae into the crab's gill chamber (Table 1,2) [2].

In the experiments, some of the cercariae used developed into *P. s. miyazakii* metacercariae. This showed that emerging cercariae had the same ability as a *P. s. miyazakii* cercariae transmitted to the crab host to maintain its life cycle in the natural world. The following studies were therefore carried out with the emerging cercariae to identify the cercarial transmission routes to the crab.

Table 1 Experimental infection for obtaining *P. s. miyazakii metacercariae* from the crab hosts put into water containing cercariae

Crabs Sex	Crabs Carapace width (mm)	Days after putting	No. of cercariae put	No. of metacercariae detected (%)	No. of metacercariae from the pericardial cavity	liver	genital organ	muscle
M	25	75	50	2 (4.0)	0	0	0	2
M	19	75	50	0	0	0	0	0
F	26	60	50	4 (8.0)	0	3	1	0
F	25	60	25	5 (20.0)	1	3	1	0
F	24	75	20	0	0	0	0	0
F	23	68	92	1 (1.0)	1	0	0	0
F	23	60	27	0	0	0	0	0
F	18	60	43	0	0	0	0	0
Total	18 to 26	60 to 75	357	12 (3.4)	2	6	2	2

F: Female, M: Male

Table 2 Experimental infection for obtaining *P. s. miyazakii* metacercariae from the crabs with cercariae inoculated into their gill chamber

Crabs Sex	Crabs Carapace width (mm)	Days after inoculation	No. of cercariae inoculated	No. of metacercariae detected (%)	No. of metacecariae in the pericardial cavity	liver	genital organ	muscle
F	17	1	348	1 (0.3)	0	1	0	0
F	19	1	50	0	0	0	0	0
F	18	20	50	0	0	0	0	0
M	22	20	50	1 (2.0)	0	1	0	0
F	17	40	50	0	0	0	0	0
F	18	40	50	0	0	0	0	0
F	19	40	50	0	0	0	0	0
F	19	40	50	1 (2.0)	1	0	0	0
F	21	40	50	0	0	0	0	0
M	23	40	50	1 (2.0)	0	0	1	0
M	23	40	50	0	0	0	0	0
F	25	40	50	2 (4.0)	0	2	0	0
F	19	60	50	1 (2.0)	1	0	0	0
F	19	90	19	1 (5.2)	0	1	0	0
Total	17 to 25	1 to 90	967	8 (0.8)	2	5	1	0

F: Female, M: Male

Percutaneous penetration of *P. s. miyazakii* cercaria to its crab host, *G. dehaani*

Portal of cercarial penetration in a crab

In order to identify the portal of cercarial penetration, the crabs were put into water containing cercariae (Photo. 1) at various times, and were then, killed, decalcified, and softened. After the muscles were removed, the crabs were preserved in 10% formalin for preparing Toluidin blue (TB)-stained whole specimens of the exoskeleton and the gill.

The TB-stained specimens were examined for cercariae. Almost all of the cercariae found were from the legs (Table 3, Photo. 2), with a few from the gills [3]. This shows that detailed information on the cercarial behavior on the surface of the legs and the body is needed to advance the present line of research.

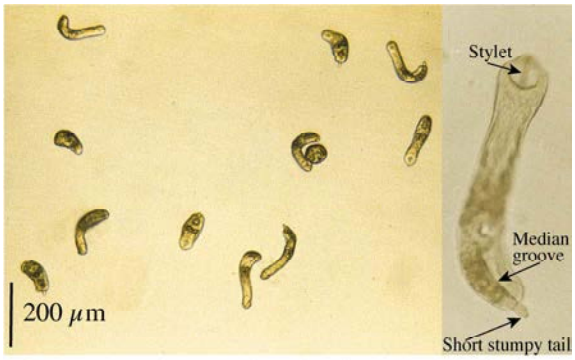

Photo 1 Emerging cercariae, showing morphological characteristics at a magnification

Table 3 Detection of *P. s. miyazakii* cercariae from the exoskeleton of the crabs, *G. dehaani*, put into water containing cercariae

Putting Hours	Crabs No. used	Crabs Carapace width (mm)	No. cercariae contained	Detection No. of Infected crabs (%)	No. of Cercariae from the leg	body	gill	un.	in total (%)
1	4	6.2 to 10.0	418	4 (100)	33	1	1	1	36 (8.6)
3	3	10.4 to 12.0	284	3 (100)	28	1	0	5	34 (12.0)
24	4	9.8 to 13.2	491	3 (75)	4	0	0	0	4 (0.8)
Total 1 to 24	11	6.2 to 13.2	1193	10 (91)	65	2	1	6	74 (6.2)

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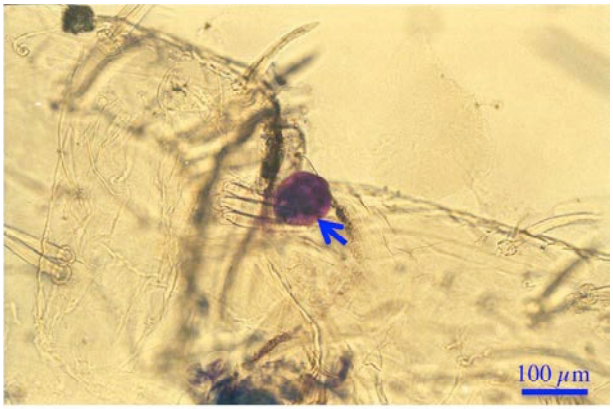

Photo 2 An attached cercaria (arrow) on the leg's exoskeleton of a crab, *G. dehaani*

Cercarial behavior toward a crab

In order to identify the mode of cercarial penetration in crabs, a living specimen of a crab put into water containing cercariae was observed under a stereoscopic microscope. Cercariae were seen to creep along the bottom or float on or swim near the surface of the water (Photo 1). At that time, when a crab's leg came into contact with cercariae、the cercariae became entangled with the leg (Photo 3).

Details of cercariae entangled with the leg were observed on a TB-stained whole specimen (Photo 4): the cercariae became entangled with the leg via a mucoid strand (ms)

arising from the mucoid coat (mc) covering the cercariae's body surfaces.

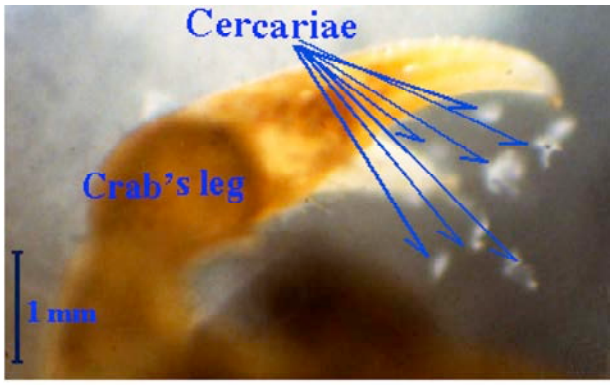

Photo 3 Cercariae entagled with the crab's leg (living specimens)

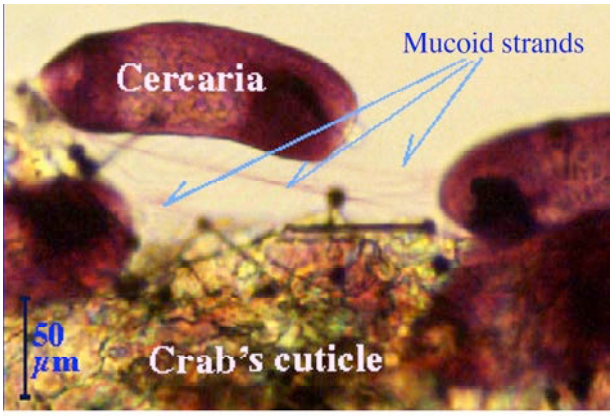

Photo 4 Cercariae entagled with the crab's leg by mucoid strands (A whole specimen stained with toluidine blue)

Cercarial penetration following the entanglement

Behavior observed on TB-stained serial sections of a crab's legs (Photos 5, 6, Table 4). Entangled cercariae attached to the leg's surface via mucoid coat, then became rounded. After that, the rounded cercariae penetrated into the leg, thrusting their body anterior extremity and thereby dissolving the leg's cuticle.

At 24 hours after having been put into the water, cercariae reached the crab's pericardial cavity, hepatic arltery, and muscle, all of which are the definitive habitats of *P. s. miyazakii* metacercaria (Table 5). Thus, this chain of findings from the cercarial emerging to the reaching of their difinitive havitats were considered to show a key route of cercarial transmission to crabs. Meanwhile, cercariae considered to have followed the route developed into mature metacercariae, which have infectivity in a mammalian host (Photos 7, 8; Table 6, 7)

These findings indicated that the percutaneous penetration was one of the transmission routes into the crabs, where a cercaria can develop into metacercariae. To date, it has been reported that some species of the gen *Paragonimus* other than *P. s. miyazakii* transmitted their crab host after having been put into water containing cercariae [5, 6]. These species should presumably have transmitted their crab host percutaeneously like *P. s. miyazakii*.

Another subject to be examined in the transmission route is the possibility of transmission by feeding a crab with an infected snail.

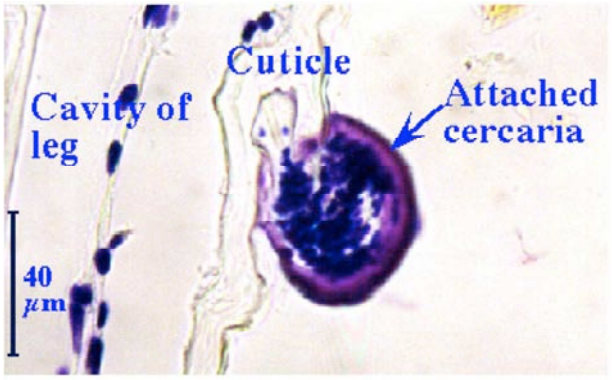

Photo 5 A cercaria attached to the cuticle of the crab's leg and starting to dissolve it (Sectioning and Toluidine blue staining)

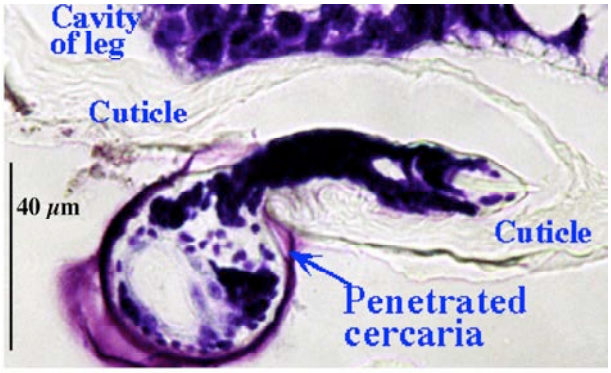

Photo 6 A cercaria having penetrated the crab's leg (Sectioning and toluidine blue staining)

Table 4 Cercarial behavior on / in the legs of the crabs, *G. dehaani*

Hours after entanglement	Legs	No. of cercariae				
	Length (mm)	entangled at start	detected (%)	in		
				free	attaching	penetrating
1	7.2	35	13 (37)	13	0	0
1	8.0	33	13 (39)	6	7	0
3	7.0	40	19 (48)	9	10	0
3	6.8	40	11 (28)	9	2	0
4	7.0	40	19 (48)	1	18	0
4	9.5	53	9 (17)	0	9	0
5	6.3	30	6 (20)	0	6	0
5	9.2	28	11 (39)	0	11	0
6	8.0	7	4 (57)	0	4	0
6	11.0	21	10 (48)	0	4	6
Total	6.3 to 11.0	327	115 (35)	38	71	6

Table 5 Detection of *P. s. miyazakii* cercariae from the internal organs of crabs, *G. dehaani,* put into water containing cercariae

Putting			Detection				
Hours after the start	Crabs Carapace width (mm)	No. cercariae contained	No. of cercariae from pericardial cavity	liver	b. m.	l. m.	in total (%)
18	4.8	95	0	0	2	0	2 (2.1)
18	4.5	100	0	0	3	1	4 (4.0)
24	4.6	103	3	5	2	2	12 (11.7)
Total	4.5 to 4.8	298	3	5	7	3	18 (6.0)

b.m.: body muscle, l.m.: leg muscle

Photo 7 A *P. s. miyazakii* metacercariae (a living specimen) obtained from the crab, *G. dehaani*, put previously into water containing the cercariae

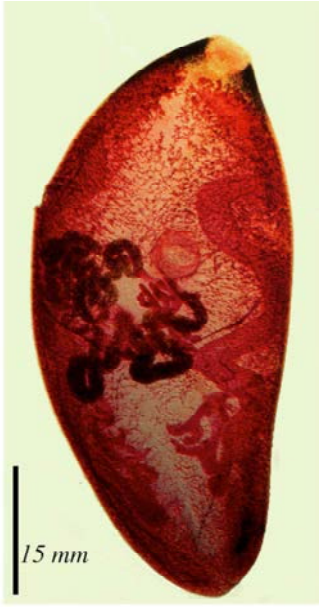

Photo 8 An adult (Borax carmine staining) of *P. s. miyazakii* derived from cecarial infection of a crab, *G. dehaani,* in a laboratory

Table 6 Detection of *P. s. miyazakii* metacercariae from the crabs, *G. dehaani*, put into water containing cercariae

Crabs Sex	Carapace width (mm)	Days after the start	No. of cercariae contained	No. of metacercariae detected from the pericardial cavity	liver	muscle	in total (%)
M	15	149	95	2	0	0	2 (2.1)
F	11	102	49	0	0	1	1 (2.0)
F	18	134	132	1	0	0	1 (0.8)
F	21	105	107	2	3	0	5 (4.7)
Total	11 to 21	102 to 134	383	5	3	1	9 (2.3)

F: Female, M: Male

Table 7 Infection of rats with *P. s. miyazakii* metacercariae obtained from the crabs, *G. dehaani*, put into water containing cercariae in a laboratory

Rats Sex	Body weight (g)	Days after given	No. of metacercariae given	No. of adult flukes obtained from the lung	pleural cavity	in total (%)
M	258	111	6	2	1	3 (50)
M	370	83	3	2	1	3 (100)

M: Male

Role played by feeding a crab with a snail in the cercarial transmission

Some studies have reported that a few species of *Paragonimus* cercariae other than *P. s. miyazakii* have become metacercariae within their crab hosts by feeding their snail hosts [7, 8].

Crab hosts, *G. dehaani,* of *P. s. miyazakii* were fed snails containing cercariae to identify the transmission routes during feeding [9].

The crabs were fed snails with cercariae at various times, and were then decalcified, softened and fixed for preparing serial sectioned specimens with hematoxylin-eosin stain。

Destruction of larvae in the alimentary canal of crabs

All *P. s. miyazakii* cercariae and rediae being in the alimentary canal of their host crabs were destroyed, becoming fragments (Table 8, Photo. 9). This indicated that none of the rediae or cercariae have viability in the alimentary canal, and can survive only if they enter the crabs through the skin or some other route.

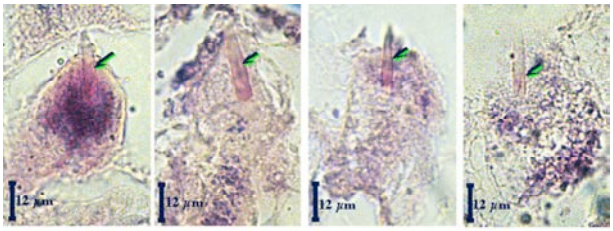

Photo 9 Digestion of *P. s. miyazakii* cercariae in the alimentary canal of crabs, with the stylet (arrow) remaining, Hematoxylin-eosin staining

Table 8 Feeding crabs, *G. dehaani*, with snails, *B. n. nipponica*, harboring *P. s. miyazakii* larvae

Min. after the feeding	Crabs		No. of snails* given	No. of larvae detected from the					
				Alimentary canal		Leg, carapace or gill			
	No. of used	Carapace width (mm)		Fragmental rediae	Fragmental cercariae	Released rediae	Released cercariae	Attached cercariae	Penetrated cercariae
15	4	4.8 to 5.6	3 to 5	2	10 (3)	3	16	1	0
30	8	4.3 to 8.5	4 to 10	4	17 (14)	4	15	2	0
45	3	4.9 to 5.6	All 3	0	2 (2)	0	2	0	0
60	2	4.1 to 7.5	9 or 10	1	2	0	6	1	0
90	2	7.5 to 8.5	All 6	0	1 (1)	0	6	2	0
180	2	4.3 to 5.4	All 10	1	1 (1)	2	2	0	0
360	4	8.0 to 9.4	All 10	0	0	1	11	9	1
Total	24	4.1 to 9.4	3 to 10	8	33 (21)	10	58	15	1

Figures in parentheses show the number of fragments with only the sylet
* The snails were assumed to have one to 19 (average, 15.6) rediae and six to 19 (average, 12.4) cercariae a snail. these values were obtained from 10 snails with the same miracidial infection as the present given snails.

Presence of Intact cercariae and rediae around or on the crab's leg after feeding

When intact cercariae and rediae were found on and around the crab's leg surface (Photo 10), they were believed to have been released from the snails, scattering around the crab, when the crab ate the snails.

Scattered cercariae attached to the leg's surface, dissolved the cuticle, then penetrated the leg (Photo 11).

This indicates that scattered cercariae do the same as emerging cercariae in that they penetrate the crab's leg, and seem to develop into the metacercariae. *P. s. miyazakii* cercariae can't penetrate into a crab through the alimentary canal. If they escape into the water around the crabs when the crab eat the snails, they can enter the crabs percutaneously.

However, this finding related percutaenous penetration isn't a major cercarial transmission route.

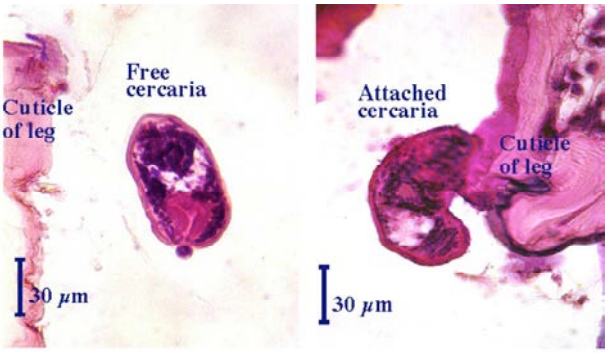

Photo 10 A released *P. s. miyazakii* cercaria near and an atttached one on the legs of crabs (Sectioning and toluidine blue staining)

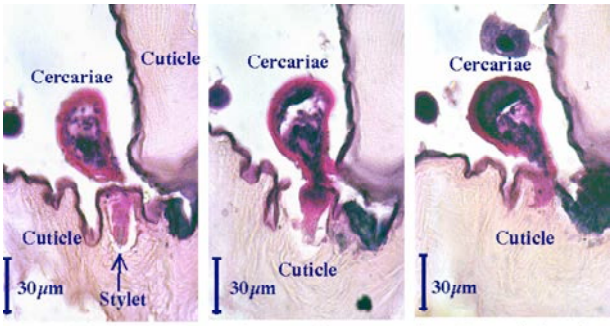

Photo 11 A *P. s. miyazakii* cecaria penetrating the cuticle of the leg after the crab ate snail hosts with cercariae, in serial sections (Toluidine blue stain)

A mucoid gland and a penetration gland supporting percutaeneous penetration

It became clear that two types of gland cells were involved in *P. s. miyazakii* cercarial transmission to its crab host.

Development of a mucoid gland (mg), formation of a mucoid coat (mc), and giving rise to a mucoid strand (ms)

A mg developed in an intraredial cercaria: as the cercariae were released from the rediae into the tissue of the snail body, mc were formed from the mucoid substances secreted by the mgs. After the cercariae covered their body with the mc competelly, they emerged from the snails into the water.

Some of the cercariae then came into contact with the crab's leg, attaching to it all around like a coat, then formed a ms like birdlime by the leg's movement. The ms entangled the cercaria with the leg, and is further attached to both of them via the mucoid coat. Ms thus aids cercarial penetration of a crab.

The Presence of a cuticle–solubility substance in cercariae

The Cuticle of the crab's leg was dissolved at any location where the leg come into contact with the anterior extremity of the cercaria's body. At the extremity, the ducts connecting the penetration glands have opened.

These finding suggested that the cercaria discharged a cuticle–solubility substance at the point of cercarial penetration of the crabs.

The presence of two types of gland cells helps us to understand the mechanism of the percutaeneous penetration of the crab by a cercaria

Conclusion

P. s, miyazakii cercarial emergence from its snail host, entanglement with the crab's leg via a mucoid strand, attachment to, and later penetration using a cuticle–solubility substance were all organically linked together to form a cercarial transmission route to its crab host. Some cercariae considered to follow the same transmission route developed into metacercariae in its crab host, while some of the metacercariae became adult flukes in a manmalian host.By being eaten, all of the cercariae ingested in the alimentary canal were destroyed; however, cercariae that escaped into the water after being released from the snails entered the crabs percutaeneously. These findings indicated that *P. s. miyazakii* cercarial transmission to the crab host took place by pecutaenious penetration.

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Molecular mechanism of tumorigenesis and biomarkers of opisthorchiasis-associated cholangiocarcinoma

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Abstrct

A solid relationship has been confirmed between *Opisthorchis viverrini* infection and tumorigenesis of cholangiocarcinoma (CCA) by epidemiological investigation and animal experiments. In the *O. viverrini* endemic area, opisthorchiasis-associated CCA has been becoming a serious public health problem. Understanding of the molecular mechanisms of tumorigenesis of the infection-induced CCA will be helpful to develop new biomarkers for diagnosis and therapy. This review addresses recent progresses in molecular mechanism and biomarker of opisthorchiasis-associated cholangiocarcinoma.

Key word: *Opisthorchis viverrini*, cholangiocarcinoma, tumorigenesis, biomarker

O. viverrini infection and cholangiocarcinoma

O. viverrini is a human liver fluke and mainly endemic in Thailand and its neighboring countries, including Lao PDR, Vietnam and Cambodia. Approximately there are 6 million in Thailand and 2.5 million in Lao PDR are infected with the parasite, somewhere the prevalence was as high as 64% (Furst et al, 2012; Sayasone et al, 2011; Sithithaworn et al, 2012). *O. viverrini* is a food-borne trematode. Individuals are infected by eating raw or uncooked fish infected with the metacercariae, associated with the custom in local endemic areas. The habitat of *O. viverrini* is in hepatobiliary tract, bile duct, and gallbladder, and opisthorchiasis is caused from mechanical irritation of the fluke and the excretory/secretory products released by the parasite that are immunogenic, mitogenic and toxic to the biliary epithelium, leading to chronic inflammation, desquamation, epithelial and adenomatous hyperplasia, goblet cell metaplasia, , periductal fibrosis and granuloma formation (Sripa, 2003; Sripa et al, 2007).

CCA arises from the neoplastic transformation of cholangiocytes. Many risk factors contribute to CCA development, including primary sclerosing cholangitis, congenital biliary cystic diseases, liver cirrhosis, and viral infections, exposed thorotrast and nitrosamine, and parasitic infection such as *O. viverrini* and *Clornorchis sinensis* (IARC 1994; 2011; Kim et al. 2009). The evidence from epidemiologic investigations and animal experiments has confirmed the definite relationship between *O. viverrini* infection and occurrence of CCA, combining with the traditional custom to eat fermented food containing potent carcinogens. Epidemiological investigation indicated that the CCA incidence is the highest in the regions of northeast Thailand with highest prevalence of *O. viverrini* infection, especially in Khon Kaen province where there was both highest CCA incidence and highest *O. viverrini* prevalence in the world (Shin et al, 2010; Srivatanakul et al, 1991; Vatanasapt et al, 1990). The evidence from animal experiment has also confirmed that *O. viverrini* infection can induce occurrence of CCA. An animal model for opisthorchiasis-NDMA-associated CCA has been established (Thamavit et al. 1993) and this model is now only available one for OV infection-induced CCA. In this model, infection with *O. viverrini* or administration with NDMA only does not induce formation of CCA, but combination of infection and NDMA administration dose cause the development of CCA. Therefore, *O. viverrini* was recognized as group 1 carcinogen by the International Agency for Research on Cancer (IARC) of the WHO in 1994, which means the parasitic infection is considered to be a direct risk factor for CCA.

Molecular mechanism of tumorigenesis of opisthorchiasis-associated CCA

The mechanism of CCA tumorigenesis induced by *O. viverrini* infection is still unknown. It is generally considered that mechanical injury and parasite-derived products is initial cause. The excretory-secretory (ES) products by adult worm are immunogenic, mitogemoc and toxic. Inflammation induced by the parasite products is linked with hepatobiliary pathology. NOS derived from inflammation caused by the infection is one of factors to cause the tumorigenesis. It has been demonstrated that *O. viverrini* caused the accumulation of NOS (Pinlaor et al, 2003). The accumulated NOS may induce the DNA damage, such as, pigenetic changes and genomic instability, which may initial the tumorigenesis of CCA. The oxidative DNA damage has been observed during *O. viverrini* infection, for example, 8-nitroguanine and 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxodG), an indicator of oxidative DNA damage, mediating iNOS dependent DNA damage in intrahepatic bile duct epithelium (Pinlaor et al, 2003; 2004; Kawanishi et al, 2006). cDNA microarray analysis indicated that in the

animal model of *O. viverrini* infection-induced CCA, the expressions of many antioxidative enzyme related genes were down-regulated, for example, Idh (isocitrate dehydrogenase), Hgd (homogentisate 1,2-dioxygenase), Gsta (glutathione S-transferase), Gss (glutathione synthetase), Prdx6 (peroxiredoxin 6) and Cyp (cytochrome P450) (Wu et al, 2011). Studies have revealed that the ES products of *O. viverrini* induced the proliferation of fibroblast cells in vitro (Thuwajit et al, 2004), and up-regulated the expression of proliferation factors, such as, Pdgf, Tgf, and EGF (Thuwajit et al. 2006).

Great progresses have been made in revealing the molecular mechanism of the tumorigenesis of opisthorchiasis-associated CCA by using animal model. Utilizing the CCA model, a systemic cDNA microarray analysis was performed, which listed up the candidate genes that may be involved in tumorigenesis. The microarray analysis indicated that the expression of a numbers of genes were alternated during the tumorigenesis, including the genes related to cell proliferation, differentiation and transformation, cell growth and cycle regulation, apoptosis, DNA repair, cytoskeletal structure, metabolic enzymes, tumor suppressor, and oxidative reduction response (Wu et al., 2011). Further studies confirmed several signal pathways to be likely involved in the tumorigenesis.

Proliferation, differentiation, and transformation are the key characterizations in tumorigenesis process. During the tumorigenesis of *O. viverrini* infection-induced CCA, some genes related to cell proliferation, differentiation, and transformation were reported to be up-regulated during the CCA development, for example, S100a6, Anxa2, Pdgfa, Frat1, Lifr, Npdc1, Enc1, Cgref1, Tgfb2, Klf4, Maff, Jund1 and Cebpd (Wu et al, 2011). Some of *O. viverrini*-derived factors have been identified to promote the proliferation of host cell, for example, granulin-like growth factor (Smout et al, 2009) and glutathione S-transferase (Daorueang et al, 2012)

NFkB signaling pathway is one of the important pathways to involve inflammation-induced carcinogenesis. The up-regulated expression of NFkB, NFkBIa, and NFkBIb, as well as cycle regulatory gene cyclin D1, cyclin E, and CDK4 was observed during tumorigenesis of *O. viverrini* infection-induced CCA, suggesting the involvement of this pathway in the tumorigenesis (Wu et al, 2011). Direct affects by ES products may be another cause because ES induced IkB degradation and activated NF-kB unclear translocation (Ninlawan et al, 2010)

RB pathway is critical in controlling G1/S transition in the cell cycle. RB1, $p16^{INK4}$, cyclin D1, and CDK4 are major components of the pathway. It is well-known that the RB pathway is inactivated in many kinds of human cancers. The analysis of kinetic expression of RB pathway genes in *O. viverrini* infection-induced CCA animal model indicated that the expressions of RB1 and $p16^{INK4}$ were down-regulated, and the expressions of cyclin D1 and CDK4 were up-regulated during the development of CCA (Boonmars et al., 2009).

c-Ski is an oncogene that was first identified as the transforming protein primarily defined by its ability to promote anchorage-independent growth of fibroblast when overexpressed, and by its roles in cell differentiation and transformation. C-Ski protein levels and subcellular localization were found to correlate with clininopathological parameters and tumor progression in several human malignancies. In the carcinogenesis of *O. viverrini* infection-induced CCA, the kinetic expression of c-ski and TGF-β signal pathway genes was corresponding to the time-dependent development of the CCA, suggesting that c-ski is likely involved the tumorigenesis as a repressor of TGF-β signaling pathway through inhibiting the pathway-induced growth barrier t (Boonmars et al., 2011).

More recently, it was reported that PDGF signaling pathway may play important roles in opisthorchiasis-associated CCA tumorigenesis (Boonjarasupinyo et al. 2012a; 2012b). PDGF/PDGFR complex engages several well known signaling pathways, such as Ras-MAPK, PI3K, and PLC. The ligand-activated receptors trigger downstream signal transduction pathways, including MAP kinase, PI3-kinase/AKT and JAK/STAT, playing the pivotal roles in cell proliferation, differentiation, transformation, invasion and survival. The overexpressions of PDGFs have been linked to different types of malignancies and demonstrated to involve in tumor angiogenesis, maintenance of the tumor microenvironment and implication in the development and metastasis of cancer (Kartha and Sundram, 2008; Lagonigro et al., 2006; Tamborini et al., 2006). The expression of Pdgfa and Pdgfra was found to be up-regulated during CCA tumorigenesis in *O. viverrini* infection-induced CCA animal model (Wu et al., 2011; Boonjaraspinyo et al., 2012a). Further analysis indicated that these factors were up-regulated in human CCA tissue (84.6 %) collected from heavy endemic area of *O. viverrini*. Positive PDGFA immunohistochemical staining was significantly correlated with status, stage and survival rate of CCA. Moreover, the serum level of PDGFA in CCA patients was significantly higher than that in healthy control, suggesting its potential as prognostic and diagnostic markers (Boonjaraspinyo et al, 2012b). Some other genes were found to be likely involved in CCA tumorigenesis, such as galectin-1. Galectin-1 is a beta-galactoside-binding lectin to function in cell adhesion, proliferation, differentiation, and might be involved in tumor progression and metastasis. Study has indicated that galectin-1 was greatly overexpressed at mRNA and protein levels with the tumor progression of opisthorchiasis-associated CCA. The mRNA expression was elevated in very early stage during tumorigenesis and the increase was time dependent. Galectin-1 protein expression profiles indicated that the increased expression was mainly located in the epithelium of extensively proliferated and hyperplasia small bile ducts at early stage of CCA development in model animal and mainly in the

extensive tumor stroma tissues in both model animals and human CCA cases at later stage (Wu et al., 2012).

Biomarkers of opisthorchiasis-associated CCA

CCA is a quite lethal cancer with a poor prognosis and limitation in therapeutic options because the early diagnosis is difficult due to lack of specific physical signs and symptoms, laboratory indexes, and tumor markers in early or premalignant stages. Different from the CCA in non-endemic area, majority of the CCA in *O. viverrini* endemic area belongs to intrahepatic characterized with the neoplasm in upper hepatoduodenal ligament, and extension into liver and vascular structure, which makes it necessary to employ different strategy for diagnosis. Complete surgical excision is the only chance for survival, but unfortunately, existence of distant and extensive metastasis before diagnosed usually excludes the option for surgical excision. The survival of patients with unresectable CCA is generally quite short, less than 12 months after diagnosis. Although improvement has been made in diagnosis techniques such as serum tumor markers, radiological and endoscopic imaging, and pathological analysis of biopsies or endoscopic brushings, the 5- year survival is still extremely low, which is now the problem confronted by doctors and patients in endemic areas of opisthorchiasis. Therefore, it is necessary to develop novel biomarkers for the diagnosis, prognosis, metastasis, and therapy of this malignancy.

Conventional serum markers, such as Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), is now used for the diagnosis of malignancies including in CCA. However, these markers are limited in diagnosis of CCA due to their insufficient sensitivity and specificity. Recent years, the progress has been made on the development of biomarkers of opisthorchiasis-associated CCA. Sripa et al (2012) reported that plasma level of IL-6 is associated with a significant increase in the risk of opisthorchiasis-associated CCA and advanced periductal fibrosis which is usually linked to high risk of CCA. *O. viverrini* infection alone did not cause the increase of IL-6 levels, as revealed by low level IL-6 in *O. viverrini* infected individuals without advanced periductal fibrosis or CCA, suggesting IL-6 may be a novel biomarker for diagnosis and monitoring the response to therapies in the lethal pathology induced by *O. viverrini* infection.

Variability of animal model of *O. viverrini* infection-induced CCA enhances the development of revealing tumorigenesis mechanism and exploring novel biomarkers for CCA. Several potential biomarkers have been identified in the animal model. PDGFA and PDGFRA were reported to be elevated in the animal model and the tumor tissue of CCA cases (Boonjaraspinyo et al. 2012b). Cox repression multivariate analysis indicated that positive immunostaining of PDGFA had a higher likelihood of risk of death, suggesting its potential as a prognostic factor. The serum level of this factor in CCA patients was higher than in healthy individuals, suggesting its potential as a diagnostic marker. The anti-cancer drug sunitibib malate inhibited the proliferation of CCA cell by suppressing PDGFRA signaling pathway, suggesting its potential as a target of genetic therapy. Similarly, the increased expression galectin-1 was observed during tumorigenesis of *O. viverrini* infection-induced CCA and in the tumor stroma of CCA cases (Wu et al. 2012). High positive rate of immunostaining and correlation with stage, metastasis and cumulative overall survival indicated that galectin-1 is likely involved in the tumorigenesis and expected to serve as a tumor stroma marker in diagnosis and prediction of metastasis and poor prognosis of the opisthorchiasis-associated CCA.

Many other biomarkers were also found to be promising as candidate markers of opisthorchiasis-associated CCA recently, for example hydroxyproline as a diagnostic marker (Prakobwong et al. 2012), ECPKA and its autoantibody as biomarkers (Loilome et al. 2012b), oxysterols (OSBP2 and OSBPL-7) as molecular markers for the identification of CCA metastasis in the bloodstream (Loilome et al. 2012a), and ANXA2 as prognostic marker (Yonglitthipagon et al. 2010).

Silsirivanit et al (2011) and Sawanyawisuth et al. (2012) reported an novel CCA antigens, an unidentified glycan epitope on MUC5AC and designated as CCA-associated carbohydrate antigen (CCA-CA), which was find to be up-regulated at early stage in CCA development, suggesting the possibility of this antigen as an early diagnostic and prognostic marker of CCA.

Perspective

Great progresses have been made in revealing molecular mechanism of tumorigenesis and developing biomarkers for diagnosis, prognosis and therapy, especially in recent years. However, we are still in its beginning. The situation of *O. viverrini* infection and CCA in endemic area is till serious and the lack of early diagnostic techniques and poor prognosis make the millions of people be in the risk of CCA. Moreover, as the second most common primary liver cancer in the non-endemic area of *O. viverrini*, the incidence and mortality of CCA are markedly increasing in recent decades. An unexpected case was reported in 2012 that the mortality of CCA of the workers in one printing company in Japan was about 2900 time high compared with normal population. Therefore, it is urgent to determine the tumorigenesis mechanism and develop novel biomarkers for early diagnosis. It is necessary to further confirm the identified biomarkers and the possibility in practical use. By utilizing new developed techniques in the research of opisthorchiasis-associated CCA, it will accelerate the progress to identify new biomarkers. More important is that unlike other type cancers, opisthorchiasis-associated CCA has its animal model which does closely parallel human CCA. By exploring in animal model and confirming in human CCA cases, it is believed that the

promising future on determination of tumorigenesis and development of biomarkers for diagnosis and therapy will come.

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Section II
Abstracts of the 12th APCPZ in Kobe
Oct. 6-7,2012

About APCPZ

Official name of the Asian-Pacific Congress for Parasitic Zoonoses (APCPZ) was given in 1990 when the 1st APCPZ was held in Sendai, Japan. The president of this memorial congress was Tomio Yamaguchi, emeritus professor of Hirosaki University. From then on this APCPZ has been held every two years either in Taiwan or in Japan. In 2010, markedly, the APCPZ was first time held outside of both Taiwan and Japan, due to the increase of participants of Korean parasitologist, the 11th APCPZ was held in Incheon, Korea. After this time 12th Kobe congress, the next 13th APCPZ (2014) will be held in Taiwan.

Table. APCPZ History

Place	Year (period)	Conference Chair
1st : Sendai (Japan)	July. 20-22,1990	Prof. Emeritus Tomio Yamaguchi (Hirosaki University) and Ogimoto Keiji (Tohoku University)
2nd : Taipei (Taiwan)	July. 24-26,1992	Prof. Emeritus Hsien-Chen Hsieh (Taiwan Society of Parasitology)
3rd : Tokyo (Japan)	July. 31-Aug.1,1994	Prof. Emeritus Tomio Yamaguchi (Hirosaki University) and Prof. Moriyasu Tuji (Kyorin University)
4th : Taichung (Taiwan)	Aug. 2-4,1996	Prof. Emeritus Hsien-Chen Hsieh (Taipei Medical College)
5th : Makuhari (Japan)#	Aug. 26,1998	Prof. Emeritus Tomio Yamaguchi (Hirosaki University) and Prof. Moriyasu Tuji (Kyorin University)
6th : Taipei (Taiwan)	July. 28-30,2000	Prof. Eng-Rin Chen (Kaohsiung Medical University)
7th : Okinawa (Japan)	Nov. 1-3,2002	Prof. Yoshiya Sato (University of the Ryukyus)
8th : Tainan (Taiwan)	Sep. 3-4,2004	Prof. Eng-Rin Chen (Kaohsiung Medical University)
9th : Gifu (Japan)	Aug. 24-25,2006	Prof. Yuzo Takahashi (Gifu University)
10th : Taipei (Taiwan)	Aug. 30-31,2008	Prof. Eng-Rin Chen (Kaohsiung Medical University)
11th : Incheon (Korea)	Oct. 26-28,2010	Prof. Jong-Yil Chai (Seoul National University)
12th : Kobe (Japan)	Oct. 6-7,2012	Prof. Shoji Uga (Kobe University)

#The fifth APCPZ was held in Makuhari, Chiba as a satellite meeting of the 9th ICOPA (International Congress of Parasitology) joint with APCO (Asia Parasite Control Organization).

The 12th Asian-Pacific Congress for Parasitic Zoonoses
(12th APCPZ 2012) Oct. 6-7, 2012. Kobe, Japan

S-1

Present Situation of Parasitic Zoonoses in Taiwan

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There are more than 50 species of zoonotic parasites reported in Taiwan. With the advance of education and improvement in public health, soil- and mosquito- transmitted parasites are basically under low transmission level or extinct. In the past two decades, zoonotic parasitic infections of *Angiostrongylus cantonensis*, *Strongyloides stercoralis*, *Capillaria phillippinensis*, sparganum, *Anceylostoma ceylanicum*, etc. still were reported in the literature from time to time. *Angiostrongyliasis* is highly endemic in southern and eastern Taiwan. Since the world first human case was reported in Taiwan in 1945, over 400 cases have been reported. Prior to 1991, over 80% of these patients were children under 15 years old and the major infecting agent was the giant African snail (*Achatina fulica*). After year 2000, however, among the 60 patients only one is a 10-year-old boy. About half of them are Thai laborers who got infected by consuming raw or undercooked golden apple snail (*Ampularium canaliculatus*). Other ways of contracting the infection include drinking raw vegetable juice and ingestion of raw frogs. Strongyloidiasis and intestinal capillariasis are also endemic in southern and eastern Taiwan. Strongyloidiasis averaged 2-3 cases per year. Many of the patients are elderly farmers over 60 years of age, severe cases are often related to chronic obstructive pulmonary disease or the use of corticosteroids. About 33 cases of intestinal capillariasis have occurred in Taiwan since it was first reported in 1989, but the source of infection has not been identified yet. About 28 sparganosis cases (3 were proliferative type) have been reported in Taiwan since 1922, subcutaneous tissue was most often affected, then followed in sequence by central nervous system, eye and pleural cavity. Thanks to the popularity of enteroscopy, 6 cases of *Anceylostoma ceylanicum* infection have been identified in Taiwan since 2004. Clonorchiasis used to be highly endemic in certain areas in Taiwan where freshwater fish are consumed raw, only sporadic cases are seen in recent years. Six sporadic cases of human hydatid disease were reported in the literature in Taiwan between 1995-2005. Four of them were males who most likely got infected in India, Nepal or Tibet before coming to Taiwan. The other two cases were female patients with no history of traveling abroad and hence the origin of infection remained obscure. Exotic parasites such as *Diphyllobothrium* spp. are emerging due to traveling abroad or importation of vectors from endemic areas.

Present situation of parasitic zoonoses in Korea

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Abstract: Parasitic zoonoses are becoming more and more important in public health points of view around the world. In the Republic of Korea, major zoonotic parasitoses can be classified into protozoan diseases that include toxoplasmosis and cryptosporidiosis, nematode diseases such as anisakiasis, toxocariasis, trichinosis, and capillariasis, trematode diseases that include clonorchiasis, paragonimiasis, fascioliasis, heterophyidiasis (metagonimiasis, heterophyiasis, and others), echinostomiasis, neodiplostomiasis, and gymnophalloidiasis, and cestode diseases, namely, diphyllobothriasis, sparganosis, taeniases (solium, saginata, and asiatica), cysticercosis, and echinococcosis. For prevention of zoonotic parasitoses, it is strongly recommended to control animal infections together with early diagnosis and treatment of human patients. Proper disposal of human and animal excreta containing cyst, oocysts, and/or eggs is also needed for effective control.

INTRODUCTION

Many species of protozoa and helminths have complex life cycles, in which specific intermediate or reservoir hosts are involved. These kinds of hosts may become the source of human or animal infections. When vertebrate animals, including birds and mammals, are involved in the transmission of parasites, the disease is called as a parasitic zoonosis and the parasite is called as a zoonotic parasite. Parasitic zoonoses are nowadays recognized as a highly important group of diseases in public health points of view. In the present paper, zoonotic parasites and the diseases that occur in the Republic of Korea are briefly reviewed.

ZOONOTIC PROTOZOAN DISEASES

Toxoplasmosis: The seroprevalence of *Toxoplasma gondii* infection had been generally reported to be 5-8% in Korea. However, the rate recently tend to increase in several areas. For example, in two townships of Ganghwa Island, Gyeonggi-do, the seropositive rates were 14.5% and 15.8% in 2010, and the rates increased to 23.8% and 25.8%, respectively, in 2011 (Yang et al., 2012). In another area, a township of Cheorwon-gun, Gangwon-do, the average seropositive rate among residents during 2010 and 2011 was 17.0% (Ahn et al., 2012). It is presumed that toxoplasmosis is increasing because of more active maintenance of sylvatic life cycles, with increasing numbers of stray cats and wild boars.

Cryptosporidiosis: This protozoan disease is caused by *Cryptosporidium hominis* and *C. parvum* in Korea (Park et al., 2006). *C. parvum* is known to be zoonotic whereas *C. hominis* is not (Morgan-Ryan et al., 2002). An extremely high prevalence of human cryptosporidiosis was reported in a rural village of Jeollanam-do, where 57% of 77 residents were found oocyst positive at least more than one time out of 12 monthly repeated examinations in a year (Chai et al., 2001). In Seoul, the rate was 0.5%, and in rural areas of Jeollanam-do it was 10.6% (Chai et al., 1996). There is little information in recent years.

ZOONOTIC NEMATODIASES

Anisakiasis: *Anisakis simplex*, *A. physeteris*, and *Pseudoterranova decipiens* are the three major anisakid species responsible for human infections, and *Anisakis pegreffii* has been recently added (Chai et al., 2005; Sohn and Chai, 2011). Of more than 50,000 reported human cases around the world, over 90% were from Japan (Sohn and Chai, 2011). In Korea, about 300 cases, mostly due to infection with *A. simplex* followed by *P. decipiens* larvae, were documented (Shin et al., 2008). The sea

eel was one of the most common sources of human infections in Korea (Sohn and Chai, 2011). This disease tends to occur more frequently in Korea.

Toxocariasis: This disease is caused by the larvae of *Toxocara canis* or less frequently *T. cati*. The seropositive rate among healthy individuals was 5% in rural areas in Korea (Park et al., 2002), and the disease is reported to be increasing recently (Noh et al., 2012). The reason for high seropositive rates among the Korean people is reported to be frequent consumption of raw or undercooked cow liver or other animal livers (Choi et al., 2008).

Trichinosis: The presence of *Trichinella spiralis* life cycle was first confirmed in Korea in 2000, with discovery of an outbreak of human infections at least involving 3 cases (Sohn et al., 2000). Thereafter, 4 more small outbreaks have been reported in various localities (Rhee et al., 2011). The source of infection was the badgers or wild boars (Rhee et al., 2011).

Capillariasis: A case of accidental *Capillaria hepatica* infection in the liver of a child was diagnosed by liver biopsy (Choe et al., 1993), and total five cases of intestinal capillariasis due to *Capillaria philippinensis* have been documented (Lee et al., 1993a; Kim et al., 2009).

ZOONOTIC TREMATODIASES

Clonorchiasis: The causative agent, *Clonorchis sinensis*, is currently the most important parasitic helminth of humans in Korea, both in terms of the prevalence and clinical significance, among those diagnosed by fecal examinations. The principal mode of human infection is ingestion of raw or improperly cooked freshwater fish. The nationwide egg positive rate was 4.6% in 1971, 1.8% in 1976, 2.6% in 1981, 2.7% in 1986, 2.2% in 1992, 1.4% in 1997, and 2.9% in 2004 (Korea Association of Health Promotion, 2004). The Nakdong River is the most well-known endemic area, with 40-48% egg prevalence in the 1980s (Seo et al., 1981). This figure appears quite constant in some tributaries of the river; for example, in 2006, among people living near the Nakdong River, 31.3% were found to be egg positive (Lim et al., 2006). In this area, the prevalence of cholangiocarcinoma was estimated to be about 5.5 per 100,000 people (Lim et al., 2006). The persistence of infection is due to difficulties in changing the traditional habit of eating raw freshwater fish.

Paragonimiasis: *Paragonimus westermani* is the only known lung fluke species causing human infections in Korea. The infection is contracted by eating raw or improperly cooked freshwater crabs or crayfish. The prevalence was high until the 1970s; however, after the 1990s it was reduced to one hundredth of that (Cho et al., 1997). Nevertheless, the seropositive rate in a serology referral center in Seoul during 1993 and 2006 was 1.6% among examined, and new human cases continue to occur sporadically (Lee et al., 2010).

Fascioliasis: More than 30 biliary or ectopic *Fasciola hepatica* or *Fasciola gigantica* infections have been documented (Chai, 2007a). The ectopic fascioliasis cases involved the gall bladder, intestine, eye, and subcutaneous tissues (Woo et al., 2006). Acute pancreatitis due to *F. hepatica* infection was also reported (Woo et al., 2006).

Metagonimiasis: Three species of *Metagonimus*, i.e., *M. yokogawai*, *M. miyatai* and *M. takahashii*, are known to cause human metagonimiasis in Korea (Chai, 2007b; Yu and Chai, 2010, 2013). About 240,000 Koreans have been estimated to be infected (Chai and Lee, 2002). Large and small streams in eastern and southern coastal areas, where the sweetfish *Plecoglossus altivelis* (the major source of human infection) are available, are endemic foci of *M. yokogawai* infection (Chai and Lee, 2002). Minnows and carps are the infection sources for *M. miyatai* and *M. takahashii*, respectively, and these infections are prevalent along the upper reaches of the big rivers (Chai and Lee, 2002). The national figures for *Metagonimus* infection among randomly selected Korean people was 1.2% in 1981 and 1.0% in 1986, but this reduced to 0.3% in 1992, 0.3% in 1997, and 0.5% in 2004 (Korean Association of Health Promotion, 2004).

Other heterophyidiases: *Heterophyes nocens*, *Pygidiopsis summa*, *Heterophyopsis continua*,

Stellantchasmus falcatus, *Centrocestus armatus*, *Stictodora fuscata*, *Stictodora lari*, and *Haplorchis pumilio* are heterophyids other than *Metagonimus* spp. causing human infections in Korea (Chai et al., 2009; Chung et al., 2011). The estimated number of people infected with these parasites is 120 thousands (Chai and Lee, 2002). *C. armatus* is transmitted by freshwater fish, but all others are transmitted by brackish-water fish, such as *Mugil cephalus*, *Acanthogobius flavimanus*, and *Lateolabrax japonicus* (Chai and Lee, 2002). These brackish-water fish-borne trematodes are prevalent along coastal areas and off-shore islands (Chai et al., 2004; Park et al., 2007).

Microphalloidiasis: A species of the Microphallidae, *Gynaecotyla squatarolae*, an intestinal fluke of migratory birds, was recently found infected in a human case (Chung et al., 2011).

Echinostomiasis: Four echinostome (= Echinostomatidae) species, i.e. *Echinostoma hortense*, *Echinostoma cinetorchis*, *Echinochasmus japonicus*, and *Acanthoparyphium tyosenense*, are known to infect humans (Chai and Lee, 2002; Chai, 2009). About 60,000 Korean people have been estimated to be infected (Chai and Lee, 2002). The most prevalent species is *E. hortense*, for which a southeastern inland area was found to be an endemic focus, with an egg positive proportion of 22.4% among villagers (Lee SK et al., 1988). The sources of human infections with *E. hortense* and *E. cinetorchis* are freshwater fish, including the loach and carp, and the source of infection with *E. japonicus* is large freshwater snails (Chai and Lee, 2002). The infection source of *A. tyosenense* infection is brackish-water bivalves or gastropods (Chai et al., 2001).

Neodiplostomiasis: The causative agent, *Neodiplostomum seoulense* (formerly *Fibricola seoulensis*), was originally described from house rats captured in Seoul (Seo, 1990). Now it became a unique intestinal trematode that infects humans and rodents in Korea (Seo, 1990). This species began to draw medical attention in 1982 when an infected young man complaining of acute abdominal pain, diarrhea, and fever was hospitalized (Seo, 1990). He had eaten raw snakes seven days before admission to the hospital. Subsequently, the grass snake *Rhabdophis tigrina* caught his village was found to carry the metacercariae (Hong et al., 1982). Further human infections were found in 25 soldiers who had eaten raw snakes during their survival trainings (Seo, 1990). The second intermediate hosts are tadpoles and frogs, and the grass snake is a paratenic host (Seo, 1990). It is noteworthy that this trematode is highly pathogenic and lethal to experimentally infected mice (Kook et al., 1998; Chai et al., 2000).

Gymnophalloidiasis: The causative agent, *Gymnophalloides seoi*, was first discovered from a woman who complained of acute pancreatitis and gastrointestinal problems (Lee et al., 1993b; Chai et al., 2003). A coastal village in the southwestern coast, where the patient resided, was found to be a highly endemic area of this fluke (Lee et al., 1994a). Now it is known that western and southern coastal villages and coastal islands are endemic areas of this fluke (Chai et al., 2003). The source of human infection and the second intermediate host is the oyster *Crassostrea gigas*, and humans and wading birds, including the oystercatcher *Haematopus ostralegus*, are natural definitive hosts (Chai et al., 2003).

ZOONOTIC CESTODIASES

Diphyllobothriasis: About 50 worm-proven *Diphyllobothrium latum* (now it is proposed to be *D. nihonkaiense*) cases have been documented since 1971 until 2012 (Lee et al., 2007; Choi et al., 2012). The taxonomy of *D. latum* tapeworm in Korea is now put to a debate whether it should be *D. nihonkaiense* (as in Japan) or remained as *D. latum* (Jeon et al., 2009a). The common sources of infection have been the salmon, trout, perch, and mullet (Lee et al., 2007). The so-called *D. latum* parvum type (originally reported as *D. parvum* by Stephens in 1908) was discovered in two Korean patients (Lee et al., 1994b). A case of *D. yonagoense* infection has been described in Korea (Lee SH et al., 1988).

Sparganosis: This larval tapeworm infection is caused by the metacestode of *Spirometra erinacei*,

an intestinal tapeworm of dogs and cats. Several hundred human infections have been reported in Korea, including subcutaneous, breast, and cerebrospinal infections (Cho et al., 1975; Shin et al., 2008; Park et al., 2011). The source of infection is most commonly the snakes, but less commonly the frogs or water contaminated with infected cyclops may also be the source of human infections (Cho et al., 1975).

Taeniasis: Human taeniases due to *Taenia solium*, *T. asiatica*, and *T. saginata* had been quite common in various localities of Korea until the 1980s (Chai, 2012). However, the national surveys on intestinal helminths every 5 years on randomly selected people revealed that the *Taenia* egg prevalence dropped from 1.9% in 1971 to 0.02% in 1997 and finally 0.0% in 2004 (Korea Association of Health Promotion, 2004). With the exception of 3 egg-positive cases in 2008 and 2 worm-proven cases in 2011, no more cases have been officially reporded. Jeju-do, where pigs were reared in a conventional way, was the highest endemic area of taeniases. However, even on this island, taeniases are no more reported at present (Chai, 2012). Analysis of internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome c oxidase 1 (cox1) genes of 68 taeniases cases reported from 1935 to 2005 in Korea revelaed that the relative occurrence of the 3 *Taenia* spp. was as follows: *T. solium* (4.4%), *T. asiatica* (75.0%), and *T. saginata* (20.6%) (Jeon et al., 2009b).

Cysticercosis: The incidence of human cysticercosis in Korea was high before but is now showing a decreasing tendency (Chai, 2012). For example, during 1968 and 1987, total 425 (0.24%) cysticercosis cases were diagnosed out of a total of 174,770 surgical biopsy specimens obtained in the Department of Pathology, Seoul National University Hospital (Chi et al., 1988). In the Department of Pathology, Kyunghee University Medical Center (Seoul) also reported a similar figure, total 136 (0.30%) cysticercosis cases were detected out of a total of 45,651 surgical biopsies examined during 1972 and 1983 (Cho et al., 1998). However, this ratio became much lower in a follow-up study (1984-2005) in Kyunghee University Medical Center; only 62 (0.029%) were found to be cysticercosis out of a total of 211,859 surgical biopsies (Choi et al., 2010). The prevalence of cysticercosis was also estimated by serologic tests, in particular, ELISA. In 1993, Kong et al. (1993) reported a seropositive rate for cysticercosis of 2.1% by ELISA among 750 general population selected from different areas of Korea, whereas 4.0% of 2,667 epileptic patients revealed positive reaction. A serologic reference center in Seoul reported an overall seropositive rate of 4.2% for cysticercosis during 1993 and 2006 and the seropositive rate of cysticercosis apparently showed decreasing trends, from 7.3-8.3% in 1993-1994 to 1.6-2.2% in 2005-2006 (Lee et al., 2010). It is presumed that human cysticercosis will disappear within 10-20 years in Korea.

Echinococcosis: A total of 33 echinococcosis patients have been documented since 1983 (Byun et al., 2010). Thirty-two cases were caused by *Echinococcus granulosus* and one was due to *E. multilocularis* (Kim et al., 2011). Most of the *E. granulosus* case were imported from the Middle East and Asian countries (Byun et al., 2010). However, the origin of two *E. granulosus* cases are uncertain and they are suspected to have been infected in Korea (Byun et al., 2010). The patient infected with *E. multilocularis* had never been abroad (Kim et al., 2011). Therefore, the domestic occurrence of both of the two species should be clarified.

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S-3

Present situation of parasitic zoonoses in Japan

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Although Japan was previously known as a "paradise for parasites" because of predominant occurrences of ascariasis, trichuriasis, and hookworm disease, such soil-transmitted parasites were successfully eradicated following a nation-wide campaign instituted under the Parasite Control Law (terminated in 1994). Presently, Japan seems to be facing a latent wave of parasitosis, including imported (malaria, giardiasis, cysticercosis) and food-borne diseases, some of which are related to Japanese dietary habits (anisakiasis, diphyllobothriasis, gnathostomiasis, sparganosis), while others have a strong zoonotic nature (toxocariasis, *Ascaris suum*-related larva migrans, paragonimiasis). Reliable numbers of patients with parasitic diseases, except for amebiasis, cryptosporidiosis, giardiasis, malaria, and echinococcosis, are largely unknown because of the current incomplete surveillance system. In order to elucidate the trends of parasitic diseases, we investigated case reports related to parasitic disease presented in the Igaku Chuo Zasshi (Japana Centra Revuo Medicina) database between 2000 and 2011 (Table). Our analysis showed that the majority of helminthiasis cases are caused by food-borne zoonoses. In addition to eating marine and fresh water fish as sushi or sashimi, cultural habits of consumption of other types of raw meat, such as wild boar (paragonimiasis) and chicken/cattle liver (toxocariasis, *Ascaris suum*-related larva migrans), are underlying causes related to the prevalence of zoonotic parasitosis in Japan.

Table. Numbers of parasitic diseases case reports in Japan found in Japana Centra Revuo Medicina Database (2000-2011)

Protozoa		Trematoda		Cestoidea		Nematoda	
Pneumocystis	177	Paragonimus	76	Sparganum	51	Anisakis	136
Entoamoeba	126	Schistosoma	59	Echinococcus	50	Strongyloides	76
Plasmodium	113	Fasciola	10	Diphyllobothrium	41	Dirofilaria	58
Toxoplasma	95	Clonorchis	8	Taenia solium	27	Spirurina	46
Acanthamoeba	61			Taenia saginata	7	Toxocara	32
Giardia	28			Diplogonoporus	2	Gnathostoma	24
Cryptosporidium	14					Ascaris suum	21
Trichomonas	11					Hookworms	17
Leishmania	10					Trichuris	9
Isospora	4					Enterobius	6
Amebic encephalitis	1 4					Angiostrongylus Trichinella	6 4
Total	644		153		178		435

S-4

Zoonotic Parasitic Diseases in Nepal

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Status and spectrum of parasitic zoonosis in Nepal - the Himalayan country located in South Asia between Republic of India (in the south) and People's Republic of China (in the north) is presented. The most common zoonotic diseases studied/reported include toxoplasmosis, toxocariasis, cysticercosis, echinococcosis (hydatid cyst), cryptosporidiosis and others. The reported prevalence of these parasitic zoonosis varies from one to another according to study population in different geographical areas and ethnicity. With regard to toxoplasmosis, nearly half of the general population have antibody to *Toxoplasma gondii*. Prevalence is higher among pregnant women, women with bad obstetric history and patients with malignancy. However, up till now, only one case congenital toxoplasmosis has been reported. Major source of *Toxoplasma* infection appears to be meat/meat products as the seroprevalence among meat animals particularly in buffalo, goat/sheep and pig is very high (over two third). The reported seroprevalence of *Toxocara* antibody is very high (81.0%) though the clinical cases of toxocariasis (visceral and/or ocular larva migrans) are not very common. Cases of cysticercosis (ocular and/or neurocysticercosis) are on rise and is said to be due to the availability of diagnostic facilities (particularly imaging diagnostic such as CT and MRI) during recent years. The reported prevalence of cysticercosis in slaughtered pigs ranges from 11 to 29 percent. The reported teniasis in certain communities goes as high as 50 percent. Echinococcosis (hydatid cyst) occurs sporadically, but the prevalence in the community is not known. Hydatid cyst among the meat animals (buffaloes, sheeps, pigs and goats) ranged from 4 to 18 percent. Fifteen percent of stray dogs killed by poisoning are reported to harbour *Echinococcus* adult worms. The reported prevalence of cryptosporidiosis in man is less than 20 percent, but the prevalence among animals is not studied yet. Very rarely, cases of trichinosis, fascioliasis, sparganosis have also been reported. These findings indicate that different types of parasitic zoonosis are existed in Nepal with varying rates of prevalence and demands strict enforcement of existing meat inspection law and hygienic sale of meats/meat products so as to reduce/prevent the burden of parasitic zoonosis in this impoverished country. Also a large scale systematic study of zoonotic parasitic diseases is advocated for evidence based planning and decision making in tackling the parasitic zoonosis.

Good situation for the spread of parasitic zoonosis (e.g. echinococcosis/hydatid cyst)

S-5

Parasitic Zoonoses in the Philippines: Current Status and Issues

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Parasitic zoonoses arise from parasitic infections transmitted from vertebrate animals to humans. These animals may be domesticated (livestock or companion) or wild. These diseases are called "neglected" because they are not adequately addressed nationally and internationally. Millions of people are affected worldwide, but in the Philippines it is one of the leading causes of morbidity. Examples of zoonotic parasites in the country can be classified as water-borne such as *Giardia* and *Cryptosporidium*, food-borne such as trematodes and cestodes, vector-borne such as malarial and filarial parasites, and water contact-borne such as *Schistosoma*. These infectious diseases affect millions of people who live in remote, hard-to-reach areas of the country where basic health services and correct information remain difficult for the people at risk to access. These areas are among the poorest in the country and belong to the 5th-6th class municipalities with meager and inadequate financial resources to implement health programs. Commonly affected population groups are farmers, fishermen, migrant workers, indigenous cultural groups, communities affected by armed conflicts and women and children. This situation not only poses a huge economic, social and health burden to these people but generates an enormous and persistent challenge to the health deliverers of the health programs in the country.

However, mortality and morbidity due to parasitic diseases in the Philippines have been in steep decline at the national level. The early part of 1980s saw the beginning of a multi-disciplinary approach in the study of the parasitic zoonoses in the country. Research in disease control, dynamics of transmission and understanding of disease morbidity and pathology contributed to the decline in prevalence rates. This was also made possible with input from WHO/TDR and the Rockefeller Foundation and research collaborators from various institutions local and abroad. Due to government extensive program and campaign, the prevalence rate of zoonotic diseases in the country has decreased. For instance, due to Roll Back Malaria Project, from 2005 to 2010 the country has experienced 50% reduction of malaria transmission, morbidity and mortality in high incidence provinces of Mindanao and the 90% reduction from the Visayas. On the other hand, national prevalence of Schistosomiasis has decreased from 3% in 1996 to less than 1% in 2010. However, a new endemic site of Schistosomiasis was found in the northern region of the country. Given the high mobility of the population, not to mention hydrological connectivity, it could just be a matter of time before it is discovered in other unexpected provinces and this continues to worry authorities in the country. Meanwhile, the population at risk for filariasis is estimated to be 23.5 million people in 40 out of 77 provinces with microfilaremia rates ranging from 0.05% to 29.2%. The country has a 20-year strategic plan for the elimination of lymphatic filariasis, with the vision of all endemic communities free of transmission of lymphatic filariasis by 2020. The Department of Health aims to eliminate this disease through mass drug administration (MDA) using diethylcarbamazine and albendazole tablets given to the eligible population. Other zoonotic parasites such as geo-helminthes (i.e., *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) and food-borne trematodes (e.g., heterophyids, *Taenia* spp., *Paragonimus westermani,* and echinostomes) were recorded in various surveys conducted in the Philippines.

Although there has been an overall reduction in parasitic disease incidence and deaths in the Philippines, other factors such as environmental changes, changes of land cover, wider animal

reservoirs, human and animal demography, pathogen changes and changes in farming practice may pose threats to the emergence and re-emergence of zoonotic parasites. Social and cultural factors such as food habits and religious beliefs play a role too. Emerging zoonotic diseases have potentially serious human health and economic impacts. The current status may be declining, but given the environmental changes and anthropogenic activities, the trend is likely to increase again in the country especially if off guarded. Hence, this paper attempts to create public awareness for concerted efforts, both by private and public constituents, to control parasitic zoonotic diseases by preventing their occurrence in humans and by controlling transmission in animal reservoirs.

S-6

Situation and problems of Parasitic Zoonoses in Vietnam

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Parasitic diseases have been the major problems of public health of population and widely distributed in Vietnam. The main important is Parasitic Zoonoses with high prevalence and on the burden of disease for communities.

The Soil-transmitted helminthiasis is high prevalence in hold country. Most of surveys show that the prevalence of soil-transmitted helminthiasis in some areas and varied from place to place as it was higher in the North than in the South. The prevalence of Ascariasis of communities in most provinces was 10%-95%. It is high heavy endemically infection rate were 80%-95% in Red-river delta region, the light in the South & highland regions (10% - 40%). Distribution of Trichuriasis is as wide as Ascariasis, prevalence rate was 0,5%-89% in the surveys. The infection rate in the North is higher than that in the South. The infection rate of Hookworm was 30%- 85% in the North the and 47%-68% in the South.

Fish borne trematode infection included small liver flukes and small intestinal flukes. Clonorchiasis is distributed in over 21 provinces in the North and opisthorchiasis distributed in 11 provinces in the South of the country, highest prevalence of 40%. The infection rate of *Clonorchis/Opisthorchis* in dog and cat (reservoir hosts) was 28.6% and 64.2% respectively. Small intestinal flukes (Heterophyidae and Echinostomatidae) is distributed in more than 18 provinces, highest prevalence of 64%.

Paragonimiasis distributed in 10 provinces. The highest infection rate of *Paragonimus* was 15% in Son La province. Most of 175 patients of paragonimiasis, children were 69.7%. The infection rate of *Paragonimus* in dogs was 34.5%. Adult worms were found in dogs and infected-cats identified by morphology and molecular methods as *Paragonimus heterotremus*.

Fascioliasis has been distributed in 52 provinces of Vietnam including 26 provinces in the South and 26 provinces in the North. Samples of *Fasciola* eggs and adult worms collected from the patients were analyzed and identified by molecular method as *Fasciola gigantica*. It is suggested that the Vietnamese *F. gigantica* has been hybridized with *F. hepatica*.

Fasciolopsis buski is widely distributed in more than 16 provinces. By morphological and molecular methods, *Fasciolopsis buski* is identification.

Taeniasis was very common in Viet Nam. The infection rate of *Taenia* was 0.5-2% in plain area, 3.8% in highland and 2-6% in mountain area. Most of Taeniasis was *Taenia saginata* and *Taenia asiatica* (78-80%). Cysticercosis have distributed in many provinces (over than 50 provinces), the prevalence of it was 5-7% at some villages.

Trichinellosis with 5 outbreaks in Yen Bai 1970, in Dien Bien in 2002 and 2004, in Son La in 2008 and in Thanh Hoa province in 2012 with over 100 cases in total.

Hundreds of Gnathostomiasis and hundreds of Toxocariasis, Strongylosis and Sparganosis were report in Vietnam. Particulaly, the first time *Dirofilaria repens* and *Thelazia callipaeda* in Vietnamese people were reported.

The habit of eating raw food and pollutions of environmental sanitation by pathogens of parasites are common in Vietnam. However, the Parasitic Zoonoses are big problems up to now.

GP1-1

Current status of human taeniasis in Lao People's Democratic Republic

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Human taeniasis was investigated in Lao PDR during the years between 2000 and 2011 as a part of the activities of nationwide helminthiases surveys which were funded by Korea Association of Health Promotion, Korea International Cooperation Agency and Korean Foundation for International Healthcare. A total of 66,643 inhabitants including 29,846 schoolchildren were examined by Kato-Katz method, Scotch tape anal swab and morphological observation of adult worms. Molecular identification of *Taenia* tapeworms was performed by multiplex PCR and DNA sequence analyzed for mitochondrial cox1 gene

corresponding to position 90-530 of cox1 gene. The cox1 sequences (440 bp) of the code number 1678, 1685 and 1687 showed 99% similarity with the reference sequence of *T. solium* (GenBank AB086256) and the remaining samples were showed 99% similarity with *T. sagianta* (GenBank AY684274). In multiplex PCR, a 474 bp diagnostic band was detected in the *T. solium* samples of the code numbers 1678, 1685 and 1687. The overall Taenia egg positive rate was 1.3% (904/66,643). The adult tapeworms were identified all *T. solium* or *T. saginata* (n=126): 3 specimens from Luangprabang were *T. solium* and 123 specimens were *T. saginata* from Attapeu, Bokeo, Bolikhamxay, Champassak, Huaphan, Khammmouane, Luang Namta, Luang Prabang, Oudomxay, Phongsaly, Saysomboune, Saravan, Savanakhet, Xayaboury, Xe Kong, Xieng Khouang and Vientiane Municipality. T. solium infections found in the present study were from inhabitants in Lathahea district, Luang Prabang which is located in the northern Lao PDR. A male patient with neurocysticercosis also was found from one of our survey regions, Oudomxay, in 2005. In that region, we could find a pig having *T. solium* metacestode of more than 2,000 from a slaughter in Oudomxay in 2007. All of 3 human *Taenia* tapeworms, i.e., *T. solium, T. saginata* and *T. asiatica* have been found from the near countries of Lao PDR like Vietnam, Thailand and Yunnan Province of China which just locates on the border of Lao PDR and *T. asiatica* has. However, the distribution of *T. asiatica* in Lao PDR is yet to be cleared. *T. asiatica* is found to distribute in many Asian countries: Korea, China, Indonesia, the Philippine, Vietnam, Thailand, and Japan. Recently, molecular diagnostic methods have been developed for rapid and accurate detection of these tapeworms including the use of formalin fixed specimens. If the molecular diagnostic methods are more activity applied to the field surveys, we could get more precise epidemiological status of tapeworm infections in the near future.

GP1-2

The present status of parasitic zoonoses in Iran

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Zoonoses are defined as infectious diseases that can be transmitted naturally between humans and wild or domestic animals. Iran has an area of 1,650,000 km2, divided into 31 provinces, and lies in the Middle East. In the last (2012) census, the population was recorded at about 75 million people. Over the last few decades there have been several marked changes in the zoonotic helminth infection of human found in Iran. Fascioliasis is emerging as an important chronic disease of humans, especially in the northern province of Gilan (where outbreaks in 1999 involved more than 10,000 cases). In contrast, no cases of urinary schistosomiasis, a disease that once affected thousands of individuals in south–western Khuzestan province, have been reported in Iran in recent years, and no cases of dracunculiasis have been seen in the country since the mid-1970s. Approximately 1% of all admissions to surgical wards are attributable to cystic echinococcosis, which is still considered endemic, but only a few cases of alveolar echinococcosis have been recorded. Recent estimates of the prevalences of ascariasis and strongyloidiasis, for example, lie between just 0.1% and 0.3%, and 1% of the population now appears to be infected with hookworm. In contrast, human infection with *Hymenolepis* and *Enterobius* remains relatively common. In Iran, 5.6% of population is infected with *Toxocara* species. Just 10 cases of linguatulosis, 12 of dirofilariasis, one of gongylonemiasis, and three of moniliformiasis have been formally recorded in Iran. In the last few decades, for example, the prevalences of several protozoan infections appear to have fallen and this is most marked for some of the intestinal, blood and tissue protozoa. Approximately 90% of all cutaneous leishmaniasis are reported from eight countries including Iran. The highest rate of sero-epidemiology of toxoplasmosis is observed (55.7%) in north of Iran. Recent research indicated that the frequency of *Cryptosporidium* spp. among human in our area was 7.7%.

GP1-3

Occurrence of clinical cases diagnosed as *Diphyllobothriasis nihonkaiense* between 1988 and 2012 in Kyoto, Japan

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We retrospectively examined annual case numbers of diphyllobothriasis nihonkaiense. Between 1988 and 2012, a total of 97 cases of diphyllobothriasis were recorded. *Diphyllobothriasis nihonkaiense* was diagnosed by its morphologic appearance and taxonomic characteristics of the strobila or gravid proglottids of tapeworms discharged in the feces of patients who had a history of eating salmon or a habit of eating sushi or sashimi (raw fish fillets), especially that derived from salmonids. DNA sequences of tapeworm cox1 and/or nd3 genes were also analyzed from most (92) patient specimens obtained since 2004 and results clearly indicated the *D. nihonkaiense* species. Molecularly confirmed *D. latum*, from a Japanese tourist who consumed a fresh water fish belonging to the *Coregonus* species from a Russian river, was reported in 2010. Case numbers represent all cases of *D. nihonkaiense* infection in Kyoto. The occurrence of clinical cases diagnosed as Diphyllobothriasis nihonkaiense showed 27 cases between 1998 and 2006 in the past 9 years at an average number of 3.0 per year (average incidence of 0.3 cases per 100,000 people in Kyoto per year), and 70 cases were recorded between 2007 and 2012 in the present 6 years at an average number of 11.7 per year (average incidence of 1.2 cases per 100,000 people). Twice as many men than women were affected in the past 9 years, but the number of women increased from 2007, where the number of men and women was 37 and 32, respectively. Age distribution of patients showed that every age group was affected, from 3 to 80 years. Most patients were 21-60 years of age between 1988 and 2006, but young cases of 1-20 years of age were increasing with adult cases of 21-40 years of age between 2007 and 2012, which probably reflects more frequent consumption of sushi and sashimi by people in these age groups than in other age groups. An infected person may usually harbor a single worm; one worm in 89 cases; rarely two worms in 4, and three worms in one case. The greatest number of worms encountered in one individual appeared to be 7 in 2012; in this case (a 17-year-old boy), 7 scolices of worms were separately excreted in the feces after treatment with praziquantel. These results suggest that the incidence of *D. nihonkaiense* infection, especially in young and adult people, has been recently increasing.

GP1-4

Cerebral angiostrongyliasis cantonensis in Taiwan

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Eosinophilic meningitis or meningo-encephalitis caused by Angiostrongylus cantonensis is commonly seen in Southern and Eastern parts of Taiwan, especially in summer rainy season. The main intermediate host are African giant snail (*Achatina fulica*), *Ampullarium*, and slug. Peoples lived in South and East Taiwan like to eat Afican giant snail not well cooked as a delicious food.

There are 109 cases diagnosed as eosinophilic meningitis or meningoencephalitis enrolled in this study from year of 2001 till 2011. Ninty eight (90%) cases of total were below 14 years of age. Three fourth were presented as eosinophilic meningitis. Twenty cases complicated with the VI cranial nerve palsy. The most common symptoms and signs were fever, headache, vomiting, anorexia, etc. All the cases of their eosinophil count in CSF (cerebral spinal fluid) were more than 10% of the total WBC. We used the pumping method to collect the parasite by way of lumbar puncture. The worm recovery rate with the pumping method was 68% which was higher than our previous report. The most effective drugs used in this study were albendazole and levamisole without the use of steroid.

It is concluded that eosinophilic meningitis or meningoencephalitis is still seen in rural or mountainous areas of Taiwan. Parasitic meningitis or meningoencephalitis should be suspected when the eosinophil count of CSF was higher than 10% of WBC. Albendazole and levamisole are effective for these patient. Steroid is used only for life-threatened or unconscious cases.

GP2-1

Transcriptional regulation of encystation-specific genes in *Giardia lamblia*

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The protozoan *Giardia lamblia* parasitizes the human small intestine to cause diarrheal disease worldwide. It differentiates from a pathogenic trophozoite into a resistant walled cyst for transmission. During encystation, cyst wall proteins (CWPs) are coordinately synthesized and targeted to *cyst* wall. It is of interest to identify factors involved in up-regulation of *cwp* genes. Multiprotein bridging factor 1 (MBF1) from human and fly promotes cell proliferation and differentiation by interacting with other transcription activators. BLAST searches of the *Giardia* genome database identified one gene (*mbf1*) encoding a putative MBF1 protein with a helix-turn-helix domain. We found that *mbf1* gene expression levels increased significantly during encystation. MBF1 was found to localize in cell nuclei and cytoplasm with higher expression during encystation. Recombinant MBF1 specifically bound to the *cwp* gene promoters. Interestingly, overexpression of MBF1 resulted in a significant increase of the levels of *cwp* gene expression and cyst formation. Mutation of the second helix in the helix-turn-helix domain resulted in a decease of DNA binding activity and transactivation activity of MBF1. We also found MBF1 can interact with E2F1, Pax2, and WRKY, transcription factors that up-regulate the *cwp* genes during encystation. Our results suggest that MBF1 family has been conserved during evolution and that MBF1 is an important transcription factor in regulation the *Giardia cwp* genes, which are key to *Giardia* differentiation.

GP2-2

Inhibition of IL-2 production by novel regulatory CD4$^+$ T-cells during infection with malaria parasites

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We have been investigating cytokine production of CD4$^+$ T-cells during malaria infection using a model of rodent malaria parasite, *Plasmodium berghei* ANKA (PbA). We found that CD4$^+$ T-cells from mice infected with PbA showed severe defects in IL-2 production in response to T cell receptor (TCR)-stimulation, while they produced higher levels of IFN-γ, IL-4 and IL-10 to the same stimulation. Antigen-specific proliferation of CD4$^+$ T-cells *in vivo* during infection with PbA was also impaired, which was reversed by the reconstitution of IL-2. When CD4$^+$ T-cells from uninfected mice were co-cultured with those from PbA-infected mice, their IL-2 production in response to anti-TCR mAb was severely reduced depending on the dose of CD4$^+$ T cells from the infected mice, suggesting that the defect in IL-2 production was due to the inhibitory CD4$^+$ T cells. This inhibitory effect was seen in CD4$^+$ T cells after the depletion of foxp3$^+$ cells, suggesting that it is not due to foxp3$^+$ Tregs. The inhibition was mediated by soluble mediator(s), since the culture supernatant of CD4$^+$ T cells from the infected mice inhibited IL-2 production of normal CD4$^+$ T-cells. To identify cell type that produce this mediator, we sorted CD4$^+$ T cells from PbA-infected mice to CD11a^{hi}CD49dhi and CD11a^{lo}CD49d^{lo} cells, and showed that only CD11a^{hi}CD49d^{hi} cells produced these factors, suggesting that the malaria specific CD4$^+$ T cells produce these mediators. We next tried to determine the inhibitory factor. Monoclonal Abs specific for IL-10, TGF-β, IL-4 or IFN-γ were unable to neutralize the inhibitory effect of the culture supernatant. Thus, we examined other inhibitory cytokines of IL-12 family, IL-27 or IL-35, which are heterodimeric proteins containing EBI3. CD4$^+$ T cells from PbA-infected EBI3 KO mice produced IL-2 at levels similar to those from uninfected mice, while CD4$^+$ T cells from p28 KO mice and p35 KO mice did not produce IL-2 after infection. In addition, culture supernatant from PbA-infected CD4$^+$ EBI3 KO T cells did not inhibit IL-2 production of normal CD4$^+$ T-cells. Furthermore, TCRβ KO mice that were transferred with CD4$^+$ T cells from EBI3 KO mice showed improved resistance to the PbA-infection when compared with control TCRβ KO mice that received wild-type CD4$^+$ T cells. These results suggest that malaria-specific CD4$^+$ T cells produce an inhibitory EBI3$^+$ cytokine and regulate their protective immune responses during infection with PbA.

GP2-3

Is *Acanthamoba* pathogenicity associated with endosymbiotic bacteria?

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Background: *Acanthamoeba* is a free-living protist pathogen isolated from a wide range of environments including soil or river water and rarely corneal scraping, capable of causing a blinding keratitis, although the exacerbating factors that contribute to *Acanthamoeba* infections are still missing. Meanwhile, it has been shown a high prevalence of endosymbiotic bacteria found in *Acanthamoeba*, approximately 20□25% of clinical or environmental isolates, emphasizing amoebae as a Trojan horse of endosymbiotic bacteria and furthermore potential role of the bacteria into *Acanthamoeba* pathogenicity. Since we have previously isolated and characterized *Acanthamoeba* harboring endosymbiotic bacteria, we therefore assessed potential role of endosymbiotic bacteria on amoebal growth speed and motility, compared to aposymbiotic amoebae eliminated by antibiotic treatment. Furthermore, we found an amoebal endosymbiotic bacterium causing apoptosis through mitochondria pathway on human immortal cells, probably connecting exacerbation on *Acanthamoeba* pathogenicity.

Methods: Amoebae and endosymbiotic bacteria: Two distinct *Acanthamoeba* strains (R18 and S13) harboring endosymbiotic bacteria, *Protochlamydia* and *Neochlamydia*, respectively, were used for this study. Both *Acanthamoeba* strains have been originally isolated from Hokkaido, Japan, and the bacteria have been classified through phylogenetic analysis with 16S rRNA sequences. Establishment of aposymbiotic amoebae: Aposymbiotic amoebae (R18DOX and S13RFP) were established from *Acanthamoeba* R18 and S13 strains, treated with the antibiotics doxycycline (DOX; 64 μg/mL) and rifampicin (RFP; 64 μg/mL), respectively, for 9 days. Assessment of amoebal growth and motility: The growth speed and motility with morphological changes were compared between the symbiotic and aposymbiotic amoebae. The change of number of *Acanthamoeba* as a marker of growth speed was determined by the trypan blue exclusion assay. The motility of *Acanthamoeba* was also estimated through scratching assay. Cytopathic effect of an amoebal endosymbiotic bacterium (*Protochlamydia*) on human immortal HEp-2 cells: The purified bacteria were incubated with HEp-2 cells at MOI 100 for up to 24 hours. The cell death was accessed with DAPI staining, transmission electron microscopy (TEM), and western blotting with anti-poly (ADP-ribose) polymerase (PARP) antibody. In several experiments, caspase inhibitors were also used.

Results: Effect of amoebal endosymbiotic bacteria on amoebal growth speed and mortility: The growth speed of R18DOX amoebae was significantly decreased, although that of S13RFP was increased. A marked change in motility was observed only for R18DOX amoebae. We observed a significant change in the phalloidin staining pattern and morphological changes in R18DOX (but not S13RFP) amoebae. Apoptosis induction on HEp-2 cells by an amoebal endosymbiotic bacterium (*Protochlamydia*): After incubation of HEp-2 cells with *Protochlamydia*, several apoptotic features including nuclear fragmentation and cell blebbing were seen when DAPI staining or TEM observation. Western blotting analysis also showed PARP cleavage in HEp-2 cells incubated with the bacteria. Furthermore, we found that caspase-3 and -9 inhibitors blocked the apoptosis. These results indicated that *Protochlamydia* induces apoptosis on HEp-2 cells through mitochondria pathway.

Conclusion: Our findings indicated that amoebal growth speed and motility significantly altered depending upon the present of endosymbiotic bacteria with cytopathic force, possibly connecting *Acanthamoeba* pathogenicity.

GP2-4

Larvicidal activity of a novel yeast species *Geotrichum silviloca* against *Aedes aegypti* mosquito vector

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Biological control is a major alternative to avoid using synthetic insecticides in mosquito vector control programs. Although many organisms were reported to have mosquito larvicidal activities, finding the new and better ones is of great importance. In 2011 we found that the yeast species *Geotrichum silvicola* possesses larvicidal activity against *Aedes aegypti* mosquito (Diptera: Culicidae). In this work we studied further various aspects of *Ge. silvicola* larvicidal mechanism: the most susceptible stage of mosquito larvae, the effective concentration of yeast strain and the possible cause of larval death. It was found that the 3rd stage larva was the most susceptible stage and 5.77 x 10^6 cells/ml final yeast concentration caused the highest larval mortality rate after 24 hour exposure. Based on microscopic observation, the cause of larvae death might be an obstruction of its midgut. Although we demonstrated clearly that *Ge. silvicola* is capable to kill *Ae. aegypti* larvae the detailed mechanism, the cause of larval death, the activity against other important mosquito species have to be investigated, and the field study should be performed.

GP3-1

Use of polycarbonate-track-etch (PCTE) membrane filters in detecting *Cryptosporidium* oocysts from drinking water

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We developed filtration and concentration method of high recovery using polycarbonate-track-etch (PCTE) membrane filters for detecting *Cryptosporidium* oocysts from drinking water. Two hundred liters of sample water spiked with approximately 95 oocysts were filtered through a PCTE membrane (diameter, 90 mm ; pore size, 3.0 μm). Then oocysts captured on the filter were eluted by sonication. Finally, oocysts in the suspension were purified by the immunomagnetic separation (IMS) method and stained by IFA. The mean recovery for finished water was 80% and higher than that of tap water (50%). Under microscopical observation, oocysts recovered tap water were coated by debris of iron rust. The debris was released from pipe wall, and adsorbed to oocysts during the filtration process. As a result, it caused interference of combination of antibodies with oocysts. Adsorption of debris could be also enhanced by centrifugation. Therefore, we concluded that, filtration through large pore size membrane, and avoidance of centrifugation is essential for high recovery efficiency of oocysts from water samples.

GP3-2

Detection of *Cryptosporidium* oocysts and *Giardia* cysts from drainage of the small-scale sewage disposal plants in Hyogo Prefecture

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We have been investigating *Cryptosporidium* and *Giardia* in the raw water and finished water of four water purification plants (WP) once per one or two months since 1998. Until now, in the result of regular investigations, the positive rates of *Cryptosporidium* per year in all WPs scored between 0 and 14%, and the detected number was between 1 to 18 per 10 liter. With regard to Sanda WP, the positive rates scored a little higher than 25%. Information on water source was collected, which showed a lot of pollution sources such as sewage disposal plants and livestocks near the catchments area of each WP. Among those, drainage samples of small-scale sewage disposal plants in the water source of Sanda WP and Funatsu WP were investigated several times. As the result, high levels of cysts of *Giardia* were detected, and they were confirmed to be the parts of the pollution sources. Eighteen oocysts were detected in the raw water of Inagawa WP on October 4, 2011. By another investigation in the same period, 155 oocysts were detected in the upper stream of the water purification plant, and 238 oocysts were detected in an upstream tributary. There is an area where household septic tanks are provided for sewage treatment in the upper part of this tributary, suggesting that this is the source of the contamination, and some patients existed there.

GP3-3

Isolation and characterization of *Giardia*-like microorganism detected in reservoir

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Giardia-like microorganisms were found as a result of the detection assay for *Cryptosporidium* in reservoir samples in Kobe City. We investigated seasonal variation in abundance and isolated the microorganism for characterization.

They showed clear seasonal change, and drastically increased in January and April. The abundance in January was higher than that of April. The maximum abundance of the microorganism was 430 cells per litre.

Sequences of 18S rDNA and ITS region showed high similarity to those of *Lunulospora curlvula*, mitosporic Ascomycota.

Giardia-like microorganisms in surface water were inoculated on several media for fungi. Most of them germinated successfully and showed steady growth. However, no spore was found. Spores were formed only after the colony was inoculated to distilled water. They were exogenous and clearly stained by immunofluorescent reagent for *Cryptosporidium*/*Giardia*. Therefore, the *Giardia* like microorganism was confirmed to be a spore (conidium) of fungi. A severe condition such as nutritional limitation may cause the spore formation in reservoirs. Care should be taken in the examination during winter and spring. These findings will be very helpful in avoiding false positives in future assays.

GP3-4

Detection of *Babesia microti*-like parasites in feral raccoons and a Japanese badger in Wakayama Prefecture in Japan

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Babesiosis is an intraerythrocytic protozoan parasitic disease like malaria. Etiological pathogens of babesiosis, genus *Babesia* species, are transmitted by ticks and found in a variety of wild and domestic mammals. Unlike malaria, human specific species do not exist and only a few zoonotic species of more than 100 *Babesia* species have been known to infect humans. In immunocompetent human individuals the disease has been caused almost solely by *Babesia microti*, which has long been regarded as a *Babesia* species that infects a wide range of small mammals including rodents, insectivores and also rabbits. Most human babesiosis cases caused by B. *microti* have been reported from the northeast and the upper Midwest regions of the United States of America.

Since we found the first Japanese human babesiosis case in Kobe in Hyogo Prefecture attributed to the blood transfusion caused by autochthonous *B.* microti in 1999, we have started the epizootiological survey for rodent B. *microti*-infection in Japan. The survey revealed that *B. microti* is widely and commonly parasitizing Japanese rodents. There are at least four SSUrDNA (small subunit ribosomal RNA gene) –types, Kobe- Otsu- Nagano- and US-types.

Recently, *B. microti*-like parasites have been found in dogs, raccoons, squirrels and monkeys, although the genus *Babesia* has been believed to be highly host specific. It is necessary to reconsider the classification (taxonomy) based mainly on the hosts species. Instead, *Babesia* spp. parasitizing various mammals should be reclassified based on the SSUrDNA sequences.

We, thus, investigated intraerythrocytic protozoan parasites infecting various large or middle wild mammals captured in Wakayama Prefecture from November 2008 to October 2009. We found *B. microti*-like parasites in two feral raccoons and a Japanese badger. The SSUrDNA sequences of the parasites infecting the two feral raccoons were the same and different by 43bp nucleotides from that of the Japanese badger in Wakayama. Phylogenetic analysis showed that B. *microti*-like parasites in the two feral raccoons were the closest relative of *B. microti*-like parasites found in feral raccoons in Hokkaido. The parasite infecting the Japanese badger in Wakayama was placed closest to *B. microti*-like parasites found a Spanish dog and a European badger. *B. microti*-like parasites were not detected in any other animals in this survey.

It should be noted that *B. microti*-like parasites were parasitizing these large animals, although it remained unknown whether these parasites are infective or pathogenic to humans.

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GP3-5

A comprehensive genetic identification approach revealed endemic status of intestinal protozoan parasites in Indonesia

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Advances in molecular parasitology including molecular taxonomy of protozoan parasites provide a unique opportunity to understand the parasite variations in endemic areas, though it remains unclear how the parasites maintain those diversity within the populations. The data may give us a promising potential to establish novel intervention strategies on the front lines of the war against parasitic organisms.

To take such valuable data including spatio-temporal distribution patterns of genotypes/species of parasites and molecular epidemiologic baselines in a parasite endemic area, we have been conducted a series of fieldwork in Indonesia. From 2009 to 2011, cross-sectional screening studies for intestinal protozoan parasites in humans and also in domestic/wild animals were conducted using morphological and molecular examination methods in Sumba Island, Indonesia.

In *Giardia intestinalis* analyses, we detected assemblage A and B from humans, while assemblage E from pigs, assemblage G and also *Giardia muris* from rodents were confirmed. Recent proposal of a novel species "*Giardia enterica*" for the assemblage B has still been argued, and a driving force behind the speciation has not been revealed. In this study, we could confirm those geographical and age layered different distributions between assemblage A and B in the site. The results indicated that some habitat fragmentation or life cycle segregation of the population seems important to evolve those two genotypes.

In *Entamoeba* spp. analyses, we could detect various species such as *E. dispar*, *E. moshkovskii*, *E. plecki, E. coli* and *E. hartmanni* from humans. The detection was conducted using species-specific PCRs, thus complex status of mixed infection with 2 to 3 *Entamoeba* species in the investigation site was also confirmed. Considering the highly endemic conditions, we should pay more attention to the mixed infections in epidemiologic investigations targeting those amebic species.

For trichomonad, we tried culture detection by modified Chiba-Tanabe medium and molecular analyses. From humans, only *Pentatrichomonas hominis* was confirmed. The same species was detected also from bovines, goats, dogs and pigs. While various other trichomonad species such as, *Hypotrichomonas acota* from dogs, rodents and pigs, *Simplicimonas similis* from rodents, *Tetratrichomonas* sp., *Trichomitus batrachorum* from pigs were detected. Due to the non-pathogenic feature of *P. hominis*, epidemiologic data of the human infection has been quite limited; however, using molecular methods, the potential of zoonotic transmission and other trichomonad infection to humans might be assessable now.

In this study, we challenged a comprehensive approach to detect genotypes and species of intestinal protozoan parasites in the target area, and revealed various intra and inter species diversity in the population of parasites. Using the baseline data, intervention trials are on going, and the improvement of infection status will be measured using the same method.

GP4-1

Sarcocystis singaporensis as Biological Control Agent against Rodent Pest in Philippine Rice

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Rat problem has been associated with agriculture ever since man started cultivating. They are considered to be an omnivore species as they feed on almost all of human foods. In many areas, scarcity is attributed/partly attributed to rodent outbreak. Yield loss can reach up to 60% due to this pest. Different management strategies are currently employed in the field by farmers at individual or community level such as chemical, cultural, and mechanical methods. The most widely used method is through chemical-based specifically through the use of acute rodenticide such as zinc phosphide. The use of biological method has not been fully utilized in the Philippines. A protozoan parasite, *Sarcocystis singaporensis* has been looked into as a potential biological rodent control agent in Asia. The organism has been found virulent to rodents at certain concentrations and different isolates shows specificity to different rodent pest species. There were four isolates used namely; SSPR 201207 (P2)020608 Surattani, SSPR 170107 (P1)171207 Banglen, SSPR201207 (P1)060308 Surattani, and SSPR060709 (P1)230209 Borneo, assigned as treatments 1, 2, 3, and 4, respectively. Bait feeding method of infection was done using bait pellets with *S. singaporensis* isolates. A drop of *S. singaporensis* solution with approximately 200,000 sporocysts was incorporated with each bait pellet. Two sites with large rice production areas in the Philippines were selected for the field test. An area of 100 hectares (50 ha. treated and 50 ha. untreated) were selected in each site for the field evaluation. *Sarcocystis singaporensis* can be lethal to rats given at higher dose. This can reduce the damage and yield losses as the result of lessen number. The use of *Sarcocystis singaporensis* as an alternative to chemical rodenticide has a great potential in the Philippines. This can help in reducing hazard to environment such as poisoning of non-target species. This can also be applied in all crops with rodent pest problems especially with export crops as this produces absolutely no chemical residues.

GP4-2

Chronic infection of *Toxoplasma gondii* in the brain induces M2 microglia phenotype by reducing inducible Nitric oxide synthase

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Chronic infection of *Toxoplasma gondii* is mainly dependent on the concentration of IFN-γ in the CNS. IFN-γ polarizes microglia toward the M1 phenotype. Microglia activated with IFN-γ is involved in parasitism control and in tissue pathologies. M2 microglia (CD163+) has an important role in tissue remodeling during a chronic infection of *T. gondii*. The present study investigated the polarization of microglia in the brain after T. gondii infection. A chronic strain of *T. gondii* (ME49) was infected to C57BL/6 mice. After the infection with 10 ME49 cysts, cytokine levels and NO production in the brain were determined using ELISA. In addition, cytokines in the primary cells and BV-2 microglia were also examined using qPCR and ELISA. Results in the brain and primary microglia showed that anti-inflammatory cytokine levels were increased and NO production was decreased after *T. gondii* infection or antigen treatment.

In this study, we suggest that NO production of IFN-γ-activated microglia is inhibited during a chronic infection of *T. gondii*, and the decreased IFN-γ and the increased anti-inflammatory cytokines (IL-10 and TGF-β) may contribute to neuron viability in the brain after *T. gondii* infection. In the early phage of *T. gondii* infection, microglia shows M1 phenotype mainly depending on the IFN-γ and NO production in the CNS. However, during the chronic infection, microglia shows M2 polarization by secreting anti-inflammatory cytokines and low NO production. This study also suggests that the plasticity of microglia during a chronic infection of T. gondii may be toward a neuronal protection. Therefore, *T. gondii* is considered as a well-adapted parasite to avoid host immune defense system.

GP4-3

Entamoeba moshkovskii is Associated with Diarrhea in Infants and Causes Diarrhea and Colitis in Mice

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Entamoeba moshkovskii is prevalent in developing countries and morphologically indistinguishable from pathogenic *Entamoeba histolytica* and non-pathogenic *Entamoeba dispar*. However, it is not known if *E. moshkovskii* is pathogenic. The purpose of the study was to elucidate the pathogenicity of *E. moshkovskii*. Mice were intracecally challenged with the trophozoites of each Entamoeba spp. to test the ability to cause diarrhea, and infants in Bangladesh were prospectively observed to see if newly acquired *E. moshkovskii* infection was associated with diarrhea. *E. moshkovskii* and *E. histolytica* caused diarrhea and weight loss in susceptible mice. *E. dispar* infected none of the mouse strains tested. In Mirpur, Dhaka, Bangladesh, *E. moshkovskii, E. histolytica* and *E. dispar* were identified in 42 (2.95%), 66 (4.63%) and 5 (0.35%) respectively out of 1426 diarrheal episodes in 385 children followed prospectively from birth to one year of age. Diarrhea occurred temporally with acquisition of a new *E. moshkovskii* infection: in the two months preceding *E. moshkvskii*-associated diarrhea, 86% (36/42) of monthly surveillance stool samples were negative for *E. moshkovskii*. *E. moshkovskii* was found to be pathogenic in mice. In children, the acquisition of E. moshkovskii infection was associated with diarrhea. These data are consistent with *E. moshkovskii* causing disease, indicating that it is important to re-examine its pathogenicity.

GP4-4

Biomarkers of opisthorchiasis-associated cholangiocarcinoma

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Definitive relationship has been confirmed between *Opisthorchis viverrini* infection and tumorigenesis of cholangiocarcinoma (CCA) by epidemiological investigation and animal experiments. In endemic areas of *O. viverrini*, especially in northeastern of Thailand, there is the highest CCA prevalence in the world. *O. viverrini* infection can induce occurrence of CCA in hamster animal. CCA arises from the neoplastic transformation of cholangiocytes with a poor prognosis and limitation in therapeutic options because of the difficulty in early diagnosis, due to the lack of specific physical signs and symptoms, laboratory indexes, and tumor markers in early or premalignant stages. Complete surgical excision is the only chance for survival, but unfortunately, existence of distant and extensive metastasis before diagnosed usually excludes the option for surgical excision. The survival of patients with unresectable CCA is generally quite short, reportedly less than 12 months after diagnosis, which is now the problem confronted by doctors and patients in endemic areas of opisthorchiasis. Therefore, it is necessary to determine the molecular mechanism of CCA tumorigenesis and develop novel biomarkers for the diagnosis, prognosis, metastasis, and therapy of this malignancy. In the present study, by using animal model of opisthorchiasis-associated CCA, a kinetic analysis of cDNA microarray was performed to screen the candidate genes that may involve in the development of opisthorchiasis-associated CCA. Microarray analysis revealed that the expressions of 131 genes were upregulated during the development of CCA, including the genes relative to cell proliferation, differentiation and transformation, cell growth and cycle regulation, apoptosis, DNA repair, and cytoskeletal structure. The expressions of 145 genes were down-regulated, including the genes relative to metabolic enzymes, tumor suppressor, apoptosis, and oxidative response and oxidation reduction. Further analysis using human CCA cases from endemic region has revealed that some of these candidate genes can be promising biomarkers in diagnosis and therapy of *O. viverrini* infection-induced CCA, for example, galectin-1 which was overexpressed during CCA progression and its overexpression was correlated with advanced stage and metastasis and with shorter cumulative overall survival of the patients, and was of independent prognostic significance for CCA. Another promising biomarker is PDGFA. This factor expression was up-regulated during tumorigenesis of *O. viverrini* induced CCA in animal model. Analysis in human CCA indicated the up-regulation in CCA tissue, and was correlated with status, stage, metastasis and short survival rate. Moreover, the serum level of PDGFA in CCA patients was significantly higher than those of healthy control. With similar analysis, several other genes, for example, Mfge8, S100A2, S100P and Staminth 1 are now in studying and some promising results have been obtained. In conclusion, with cDNA microarray analysis of *O. viverrini*-CCA animal model, we listed up the candidate genes which may involve in tumorigenesis of CCA and as biomarkers for diagnosis and therapy. Further study with human CCA cases identified some genes as promising biomarker of opisthorchiasis-associated CCA

GP4-5

Spirometra erinacei infection in stray cats (*Felis catus*) from Korea: prevalence and chemotherapy

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Feline sparganosis is caused by plerocercoid stage larvae (spargana) of the genus *Spirometra*, which exploit copepods as the first intermediate host, and a wide range of amphibians, reptiles, birds, and mammals serve as the second intermediate hosts. Cats as well as dogs serve as the definitive host and can potentially be a threat to human transmission indirectly. Present study was performed to survey *S. erinacei* infection in stray cats from riverside areas of 5 major rivers in the Republic of Korea. A total of 568 stray cats were captured with live-traps from 10 provinces/metropolitan cities from 2009 to 2011. Small intestines resected from cats were opened with a scissors in a beaker with 0.85% saline and examined with naked eyes and under a stereomicroscope. Adult worms of *S. erinacei* were found in 44 (7.7%) cats. This study also aimed to determine the efficacy of emodepside (3 mg/kg) and praziquantel (12 mg/kg) topical solution (Profender®, Bayer AG, Leverkusen, Germany) in the treatment of *S. erinacei* infection in cats. Thirty five cats were orally infected with 3 plerocercoids of *S. erinacei*. Infected cats were randomly allocated to three groups in which 15 cats in the test group were treated with Profender® once topically, and 10 cats in the positive control group were administered once orally with praziquantel alone (Distocide® 12 mg/kg, Shinpoong, Korea) on day 14 post infection. Ten cats allocated in the negative control group were left untreated. All cats were necropsied on day 24 post infection and the number of adult worms in the small intestine was counted. Results showed that the test product was 73.3% (4/15) effective against adult stage of *S. erinacei* while the oral administration of praziquantel alone to cats in the positive control group resulted in 80% removal of the worms (8/10). An average of 2 adult worms was collected from the ten cats in the negative control group (0%, 10/10).

GP5-1

Prefabricated trap: Application to monitor fly populations in global change

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Inevitably, the effect of global warming will trigger the physiological and/or behavioral responses of terrestrial organisms, thereby increasing their populations, including those of some insects in particular areas. The increment of insect populations, especially in those of medical importance, may affect humans by means of involvement with more vectors of disease pathogens. To understand population changes in medically important flies, population monitoring in the natural environment should be performed regularly by using a sweeping or trapping technique. Although several fly trap models have been constructed and utilized, some of them are impractical for fly survey strategy, for example, traps that are too large and heavy for easily transferring collected specimens to the laboratory for investigation. Therefore, we hereby introduce a lightweight and inexpensive apparatus for assembling a fly trap, consisting of 3 parts: 1) frame, made from polyvinylchloride pipe (1.25 inch in diameter), 2) net for fly collection, and 3) entrance dome. Tests of this novel trap, using fresh and tainted pork viscera as bait in field experiments, clearly indicated the great practicality of this device, both for assembling to form a trap and transporting to and from the laboratory. The flies collected revealed 10 species in the family Calliphoridae, 6 species in the family Muscidae, and 3 unidentified species in the family Sarcophagidae.

GP5-2

Comparative Bio-Efficacy Test of Insecticide Aerosol against *Culex quinquefasciatus*

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A comparative bio-efficacy test of aerosol insecticide with three different formulations coded as formulation A. Prallethrin 0.090% w/w + d-phenothrin 0.050% w/w, B. d-transallethrin (75/25)..0.126% w/w + d-phenothrin 0.050% w/w, and C. transfluthrin 0.040% w/w + cyfluthrin 0.025% w/w. was evaluated against laboratory bred female adult of *Culex quinquefasciatus* mosquitoes in a simulated room conditions. The insecticidal efficacy test was based in accordance with the WHO guideline for efficacy testing of household insecticide product. Eight cages, each with 10 mosquitoes were placed in different places of the room. Test mosquitoes were observed for knockdown at 5, 10, 15, and 20 minutes of exposure after applying the aerosol. The mortality was determined after 24 hours of holding period. The present studies showed all the aerosol formulations compared well with in its efficacy. Among all the formulation tested the highest knockdown and mortality was obtained from the formulation C.

GP5-3

Toxocara canis eggs isolated from blow fly *Chrysomya megacephala* (Diptera: Calliphoridae) in Ubon Ratchathani Province, Northeast Thailand

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Filth flies are natural carriers of pathogens and play a considerable role in transmission of pathogens such as viruses, fungi, bacteria, and parasites in various regions of the world. *Chrysomya megacephala* and *Musca domestica* have been recognized as common filth flies and medically-important insects of Thailand. The objective of this study was to examine the potential of these flies for obtaining the parasites from several areas in Ubon Ratchathani Province, Northeast Thailand. The flies were collected individually from fresh-food markets, garbage piles, restaurants, school cafeterias and paddy fields in Muang and Warinchamrap districts of Ubon Ratchathani Province by using insect sweeping nets. We totally collected 555 adults of *C. megacephala* and 439 adults of *M. domestica*. *T. canis* eggs were isolated from only *C. megacephala* collected in almost of all sites, except for the restaurants in Muang district. Other helminthes and protozoa were not found in both fly species. These data suggest that *C. megacephala* is more likely to be a carrier of *T. canis* eggs associated with human habitation in this region of Thailand than *M. domestica*. Moreover, visceral larva migrans in human toxocariasis, an important parasitic zoonosis caused by *T. canis*, should be monitored in these areas further.

GP5-4

Signal Regulation of mosquito reproduction

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Mosquito-borne diseases are the most devastating agents for human being, due to its high diversity of transmissible pathogens like protozoan and viruses. Despite the efforts from government agencies that have contributed the eradication of the mosquito-borne diseases for several decades, the goal has not been achieved yet. Therefore, many research institutes turn their attentions toward the mosquito life cycle and immune system to halt the disease transmission. Previous studies have already demonstrated that Target of Rapamycin (TOR) signaling pathway plays an important role in mosquito vitellogenesis, whereas Wnt signaling pathway has been shown to be participated in the embryonic development and cell polarity in *Drosophila*. However, the interactions between these pathways are poorly understood. In this study, we propose a hypothesis that factors of TOR and Wnt signaling pathway play synergistically in the mosquito vitelloginesis. We attempt to characterize Wnt signaling components in the mosquito, *Aedes aegypti*. Our results showed that silencing of *Aedes aegypti* Frizzled2 (AaFz2), a transmembrane receptor of Wnt signaling pathway, resulted in the decrease of *Aedes aegypti* survival fitness against *S. aureus* and *E. coli* infection. Interestingly, the fecundity of female mosquitoes was inhibited in the absence of AaFz2. Also, we demonstrated that AaFz2 is highly expressed in the mosquito fat body at 6 hours post blood meal in turns of transcriptional and translational level, suggesting the amino acid-stimulated feature of AaFz2. The activation of TOR signaling is reduced in the absence of AaFz2. Our results showed that AaFz2 is critical in the regulation of mosquito vitellogenesis.

GP6-1

Intensity and Viability of Tissue Parasites Infested Bovine Carcasses at Ismailia - Egypt with Special Reference to their Zoonotic implications

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The slaughterhouse represents a key control point of livestock production chain and it could used to give a full picture about the zoonotic parasitic diseases. Therefore, this article dealt with some of the principal factors that concerning *Cysticercosis*, *hydatidosis*, *Sarcocystosis and Fascioliasis* in bovines slaughtered at the main abattoir of Ismailia city, Egypt. The comprehensive studies based on inspection of the carcasses which were routinely examined according to Egyptian Code for Inspection of ruminants followed by parasitological, histopathological studies. Questionnaire survey hospitals clinic laboratories were also conducted that consider one of the corner stone of the zoonotic process.

The main study conducted on a total of 10055 cattle, 3811 buffaloes and 2378 male buffalo calve. The total prevalence of C bovis was (0.47%), it was higher in cattle 0.57 % than in buffalo 0.18%. From a total of 13866 samples inspected, 320 cysticerci were detected in 76 samples, of which 103 (32.18%) were alive. The anatomical distribution of cysticerci was 55(72.37%) heart, 7 (9.21 %) masseter, 13 (17.10 %) tongue, 1 (1.31%) diaphragm samples.

Out of the total 13866 adult bovine examined, 106 (0.76%) were found harboring *Hydatid* cysts. It was higher in buffalo 57 (1.49%) than cattle 49 (0.49%). A total 405 hydatid cyst with varying sizes were detected in 120 samples, of which 133 (32.83%) were alive. The predilection sites of cysts were in the lung 84 (70%), 35 (29.17%) in the liver, 1 (0.83%) in the spleen.

Sarcocystis was detected in adult buffalo only in 775 (20.33%). Of 821 Sarcocysts detected, 403 (49.09%) in esophagus, 333 (40.56%) tongue, 85 (10.35%) skeletal muscles. Histological sections were done on positive cases. Two species of Sarcocystis were detected of which *S. cruzi* and *S. fusiformis*. Regarding other diseases, The total prevalence of liver flukes were 1.94% it was higher in buffalo 3.23% than in cattle 1.46% the number of flukes/animals was varied, (1-10) (14.8%), medium (11-50) in (85.2%).

Generally, females showed higher prevalence than male 2 times more because of increasing older ages. Calves were negative to all expect for intestinal *ascariasis* (12.69%). In human, by stool examination of 1200 specimens, taniid eggs were rarely detected 2 (0.16%), fasciola eggs 4 (0.41%).

The detection of such affections throughout the edible organs indicated the significance of such parasitic diseases in our field of animal husbandry with relevance of human zoonoses via consumption route. Therefore, proper implementation of meat inspection procedures during slaughter should be a vital part of the national public health protection program in Egypt.

GP6-2

Zoonotic helminth infections in small intestines of stray cats (*Felis catus*) from riverside areas of Seomjingang, Republic of Korea

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Present study was performed to survey the zoonotic intestinal helminth infections in stray cats from riverside areas of Seomjingang (River) in the Republic of Korea. Total 203 stray cats were captured with live-traps in 7 sites from June to October, 2010. Each small intestines resected from cats were opened with a scissors in a beaker with 0.85% saline and examined with naked eyes and under a stereomicroscope. More than 21 species of helminth were detected from 144 (70.9%) cats. As the members of nematode, *Toxocara cati* and hookworms were found in 31 (15.3%) and 34 (16.7%) cats respectively. More than 16 species of trematodes were also recovered and 9 out of them were members of Family Heterophyidae. The members belonging to each families were as follows (positive rate: %). **Heterophyidae:** *Metagonimus* spp. (30.0), *Pygidiopsis summa* (16.3), *Heterophyes nocens* (14.3), *Stellantchasmus falcatus* (3.0), *Heterophyopsis continua* (2.5), *Acanthotrema felis* (2.5), *Centrocestus armatus* (2.0), *Cryptocotyle lingua* (1.0) and *Stictodora lari* (0.5); **Echinostomatidae:** *Echinochasmus japonicas* and *E. perfoliatus* (9.4), *Echinostoma hortense* (1.0), *Echinoparyphium recurvatum* (0.5) and unidentified echinostome larvae (4.4); **Plagiorchiidae:** *Plagiorchis muris* (3.9). Two species of cestode, i.e., *Spirometra erinacei* and *Taenia taeniaeformis*, were detected in 23 (11.3%) and 13 (6.4%) cats respectively. From the above results, it was confirmed that stray cats in riverside areas of Seomjingang are highly infected with zoonotic helminthes, especially with intestinal trematodes.

GP6-3

Zoonotic helminth infections in small intestines of stray cats (*Felis catus*) from riverside areas of 4 major rivers in the Republic of Korea

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Present study was performed to survey the zoonotic intestinal helminth infections in stray cats from riverside areas of 4 major rivers, i.e., Hangang, Geumgang, Nakdonggang and Youngsangang, in the Republic of Korea. We captured 61, 38, 57 and 41 stray cats with live-traps in riverside areas of above 4 rivers respectively from June to October, 2011. Each small intestines resected from cats were opened with a scissors in a beaker with 0.85% saline and examined with naked eyes and under a stereomicroscope. More than 11 species (3 namatode, 2 cestode and more than 6 trematode spp.) of helminth were recovered in 38 (62.3%) cats from Hangang areas. Four major species, i.e., *Toxocara cati*, hookworms, *Metagonimus* spp. and *Spirometra erinacei*, were collected from 29.5%, 23.0%, 21.3% and 14.8% cats respectively. More than 7 species (2 namatode, 2 cestode and more than 3 trematode spp.) were detected in 18 (47.4%) cats from Geumgang areas. As the dominant species, *T. cati* and hookworms were recovered in 21.1% and 23.7% cats respectively. More than 11 species (1 namatode, 1 cestode and more than 9 trematode spp.) were found in 32 (56.1%) cats from Nakdonggang areas. Four major species, i.e., *T. cati, Heterophyes nocens*, *Metagonimus* spp. and *Pygidiopsis summa* were recovered in 21.1%, 21.1%, 17.5% and 17.5% cats respectively. More than 13 species (2 namatode, 1 cestode and more than 10 trematode spp.) were detected from 25 (61.0%) cats from Youngsangang areas. As the dominant species, *H. nocens, Metagonimus* spp. and hookworms were collected from 19.5%, 12.2% and 12.2% cats respectively. Conclusively, the above results suggested that the infection status, i.e., infection rate, intensity of infection and number of helminth species infected, with helminth is more or less different in stray cats by the surveyed areas, 4 major rivers in the Republic of Korea.

PGS-1

Comparative morphology of minute intestinal fluke eggs that can occur in human stools in the Republic of Korea

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The egg morphology of minute intestinal flukes (MIF) that can occur as human infections in the Republic of Korea, i.e., *Metagonimus yokogawai*, *M. miyatai*, *M. takahashii*, *Heterophyes nocens*, *Heterophyopsis continua*, *Stellantchasmus falcatus*, *Stictodora fuscata*, *Pygidiopsis summa*, and *Gymnophalloides seoi*, was studied in comparison with *Clonorchis sinensis*. The adult worms were obtained from residents of endemic areas, and their intrauterine eggs were studied and measured using light microscopy; the length, width, length-width ratio (LWR), and Faust-Meleney index (FMI). Several specimens were processed for scanning electron microscopy (SEM), and before gold-coating, the uterine portion of each fluke was etched with a sharp pin in order to expose the eggs. The MIF eggs were ovoid, pyriform, or elliptical with a size range of 21-35 x 12-21 μm. *S. fuscata* eggs revealed the highest FMI (largest in the area) and lowest LWR, whereas *P. summa* eggs showed the lowest FMI and medium LWR. SEM revealed that *G. seoi* and *S. fuscata* had remarkably clean shell surface lacking the muskmelon-like structure which is prominent in *C. sinensis* eggs. In *Metagonimus* spp., *H. continua*, *H. nocens*, and *S. falcatus* eggs, minute surface ridges were recognizable though less prominent compared with *C. sinensis*. On the surface of *P. summa* eggs, thread-like curly structures were characteristically seen. The results revealed that important differential keys for MIF eggs include the length, width, area (FMI), shape of the eggs, and the extent of the muskmelon-like structure or ridges on their shell surface and operculum.

PGS-2

Contamination of soil by parasite eggs in Barangay Bayog Los Baños, Philippines

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Infectious diseases caused by soil-transmitted helminths (STHs) are important diseases of humans, which affect about one third of the world□s population. Examination of soil can be used to estimate the risk of STH infection in humans because STH eggs grow to the infectious stage in soil. We carried out this survey to clarify the current status of soil contamination by parasite eggs and to assess the risk of STH infection of nearby residents. Surveys were conducted from September to October, 2011 and from February to March, 2012. During these periods, we examined soil by using sucrose centrifugal flotation method, feces by using formalin-ether sedimentation method, and lifestyle of residents by questionnaire. Six genera and eight species of parasite eggs including *Ascaris lumbricoides*, *Toxocara cati*, *Toxocara canis*, and *Trichuris trichiura* were recovered from 85 out of 120 soil samples (71%). Contamination of soil by parasite eggs had spread widely throughout the village, and 50% of eggs recovered had already developed into fertilized eggs. It is remarkable that *Ascaris* eggs were recovered from inside the houses. Prevalence rate of STH in school children was 63% and more than 80% of school or preschool children had witnessed defecation by their friends. This may indicate that school or preschool children cause soil contamination. Some of the eggs recovered were not only from humans but also from dogs and cats. From the results obtained, the need for health education with regard to zoonoses was revealed because 77% of fertilized *Toxocara* spp. eggs were detected. We conclude that the risk of STH infection in residents was extremely high, because the soil in this village was highly contaminated by infective parasite eggs.

PGS-3

Molecular phylogeny of *Blastocystis* isolates from rodents in the Sumba Island, Indonesia

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An intestinal parasite, *Blastocystis* is one of the most frequently encountered microorganisms in human and animal fecal samples. Although many *Blastocystis* isolates have been isolated from a variety host species, most of the isolates had been classified into the known subtypes 1 to 9 based on the small subunit rRNA gene sequence or PCR-based amplification of subtype-specific sequence tagged-site (STS) primers. Interestingly, most of the subtypes have comprised isolates from human and several different animal hosts, while subtype 4 is only identified from rodent hosts and rarely in humans. To date, only 8 sequence data of rodent origins are available in GenBank and these isolates were distributed in geographically different countries; namely USA, Japan, Singapore, and France. Since the data of rodent isolates are limited in the world, we had surveyed *Blastocystis* infection in rodents in Sumba Island, Indonesia from 2009 to 2011. A total 11 isolates were established and genotyping with RFLP analysis combined with the known 7 kinds of the STS primers. Most of the isolates showed subtype 4, while one isolate showed subtype 5 and several isolates showed mixed subtypes. We are now sequencing the entire sequence of the SSU rRNA gene of the all isolates to confirm the subtypes. In this presentation, we show phylogenetic tree of the rodent isolates and discuss on the diversity among the isolates.

PGS-4

Survey of Zoonotic Helminthes of Wild Carnivores in Korea

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Carnivores are definitive hosts of diverse parasites, and especially are well-known as hosts of food-born zoonotic parasites. Carnivores that are sharing same foods with humans who are sharing the same localities have enough chances of getting parasitic infections that parasitize local peoples.

Eradication of such parasites from humans by treatment alone is mostly difficult due to the vast range of reservoir host animals.

In this context, surveying reservoir hosts are important in terms of public health. In Korea, there are only some survey reports on parasitic infections in domestic animals-dogs and cats-are available for public health concerns and epidemiological figuring out of parasitic zoonoses. Regarding on the parasitic infections in wild animals, however, are not much surveyed like in wild carnivores such as raccoon dogs, leopard cats, and weasels, etc. In the present survey, we investigated the zoonotic helminthes of wild carnivores in Korea. From March 2010 to August 2012, carcasses of 33 raccoon dogs, 3 leopard cats, 11 weasels and 5 stray cats were donated by "The Wildlife Center of Chungbuk", "Chungnam Wild Animal Rescue Center", "Conservation Genome Resource Bank for Korean Wildlife" and "Gangwon-do Veterinary Service Laboratory(Pyeongchang)". We necropsied those subjected animals and collected parasitic worms from internal organs of them. Recovered worms were collapsed, stained with Semichon's acetocarmine(trematodes and cestodes) or were cleared with glycerol-alcohol(nematodes). As a result, numbers of helminth parasites were found, i.e., *Toxocara tanuki, Arthrostoma miyazakiense, Ancylostoma sp., Trichuris vulpis, Spirometra decipiens, Mesocestoides sp., Echinochasmus japonicus, Echinochasmus sp. Echinostoma sp.* and *Alaria sp.* from *raccoon* dogs, *Toxocara cati, Spirometra decipiens, Ancylostoma sp., Centrocestus armatus, Clonorchis sinensis* and *Metagonimus miyatai* from leopard cats. Weasels have *Isthmiopora sp.* and stray cats were infected with *Toxocara cati, Taenia taeniaeformis* and *Metagonimus miyatai.* Number of natural definitive hosts of zoonotic helminthes were also confirmed.

PGS-5

Prevalence of *Opisthorchis viverrini* infection in humans and fish in Kratie Province, Cambodia

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Opisthorchis viverrini is a medically important foodborne parasite in the Indochina Peninsula. In Cambodia, the prevalence of this trematode has been reported in Takeo Province, but not in other areas. In this study, we investigated the prevalence of *O. viverrini* infection among people in seven riparian villages along the Mekong River, Kratie Province. We also examined the status of metacercarial infection in fish hosts. Fecal specimens were collected from 2,101 residents and schoolchildren, and were examined by the Kato-Katz technique. The average *O. viverrini* egg positive rate was 4.6%, with the highest prevalence found in Roka Kandal A village (10.4%) followed by Talous village (5.9%). In these villages, adult residents showed higher prevalences (19.4% and 9.0%, respectively) than schoolchildren (6.4% and 1.4%, respectively). *O. viverrini* adult worms were recovered from 2 egg-positive cases (18 and 4 specimens) after praziquantel treatment and purgation. In addition, three of seven freshwater fish species caught near the villages were positive for *O. viverrini* metacercariae. A total of 367 metacercariae were harvested from 19 infected fish (metacercarial density; 19 per fish). The species of the metacercariae was confirmed through adult worm recovery by experimental infection to hamsters. The results provide evidence that the surveyed areas of Kratie Province, Cambodia, are endemic for *O. viverrini* infection.

PGS-6

Isolation of *Leishmania infantum* from symptomatic and asymptomatic cases of canine leishmaniasis of Argentina

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Leishmaniasis is a zoonotic disease, caused by kinetoplastid flagellates of the genus *Leishmania*, the visceral form is the severest one and it is fatal if not treated appropriately. Visceral leishmaniasis (VL) is found across South America, where the main causal agent is *Leishmania* (*L.*) *infantum* (= *L. (L.) chagasi*), and dogs are the main domestic reservoir. In Argentina, VL has recently emerged, although not defined in detail. According to clinical signs, infected dogs are divided into three categories: those with more than three clinical symptoms are *Symptomatic*, *Oligosymptomatic* are those from one to three clinical symptoms and *Asymptomatic*. Parasitological diagnostic methods, based on observation of amastigotes, are very specific but lack of sensitivity. Contrary to this, serological methods, such as rK39, have good sensitivity but lack of specificity. The isolation of promastigotes *in vitro* culture is the only method able to prove infection with alive, viable and transmissible parasites and it is necessary for applying characterization by biochemical or molecular methods. We examined symptomatic and asymptomatic dogs from Misiones and Salta provinces. Spleen and lymph node aspirates were taken for culture in USAMARU medium with PBSS. Eleven *Leishmania* stocks (seven from Misiones and four from Salta endemic areas) were isolated from eight asymptomatic and symptomatic dogs and were typified by PCR and *Cytochrome b* gene sequencing as *L. (L.) infantum*. We found two genotypes *LiA1* and *LiA2*. This is the first report of the isolation of L. (L.) *infantum* from Argentinean canine leishmaniasis cases. The parasite/strain isolation from symptomatic and asymptomatic dogs would mean that both may play an important role in the *L. (L.) infantum* transmission cycles, although symptomatic and asymptomatic dogs would have different force of infection rates in the given endemic areas.

PGS-7

Improvement of molecular detection methods for intestinal protozoan parasites

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[Introduction]

In molecular epidemiology, the rates of positive for some pathogens are directly affected by the detection sensitivity used for. Therefore it is quite important to optimize and improve the methods including DNA extraction and PCR sensitivity/specificity. Moreover, an epidemiologic research screening using a large number of samples requires a simple and cost-effective method.

To improve the molecular analysis procedures for intestinal protozoan parasites, we evaluated some points as below.

1) Additives effect in the process of DNA extraction and PCR.
2) DNA amplification efficiency of different taq polymerases and PCR kits.

[Materials and methods]

Human fecal samples including cysts of *Giardia intestinalis* and *Entamoeba coli* were used for the target material. DNAzol® (Invitrogen), a complete and ready-to-use organic reagent for the isolation of genomic DNA from solid and liquid samples, was used for the base of the trials. This ethanol precipitation based reagent is comparatively cheap and easy to use kit and thus quite convenient for the treatment of a large number samples, however the efficiency of DNA extraction is slightly low. Addition to the original protocol, we assessed the effect of freeze and thaw, protease K and RNase treatment, and linear polyacrylamide as a co-precipitation. As a control kit for DNA extraction, an affinity-based column kit, ZR Genomic DNA Kit® (Zymoresearch) was used. For PCR analyses, PrimeSTAR® HS (TAKARA) and TaKaRa LA Taq® with GC buffer (Takara) were evaluated, and effect of dimethyl sulfoxide (DMSO) as an additive for PCR was also assessed.

[Results and Discussions]

Compared with ZR Genomic DNA Kit®(ZR kit), the DNA extraction efficacy of DNAzol® was clearly low; however the price of ZR kit is three times expensive than DNAzol. The improvement trials using freeze and thaw, protease K, RNase treatment and linear polyacrylamide addition to the original protocol of DNAzol was quite effective, and reached almost the same level of DNA extraction efficacy as ZR kit.

In PCR optimization, the DMSO as additive for PCR reaction could improve the amplification efficacy of high GC containing DNA such as various genes of Giardia intestinalis. While, TaKaRa LA Taq® with GC buffer was actually the best choice for *G. intestinalis* template PCR, though PrimeSTAR® was difficult to use for it, even using GC buffer.

Using the best and appropriate combination of procedures for molecular analyses, we could improve the sensitivity and the specificity dramatically.

PGS-8

Impact of intestinal protozoan parasites as opportunistic infections in HIV patients

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Human immunodeficiency virus (HIV)-infected individuals have greater susceptibility to infections by intestinal protozoan parasites, which can cause significant morbidity and mortality to the host compared to immune-compromised individuals. Prevalence of opportunistic infections (OIs) caused by intestinal protozoan parasites also vary according to the geographical area and the endemic levels in each location.

To assess the status of opportunistic protozoan infection in developing areas, we conducted an intestinal protozoan molecular screening for HIV/AIDS patients at Bali Island, Indonesia. The 83 patients maintained by highly active anti-retroviral therapy (HAART) were recruited to this study. From the fecal samples, genomic DNA was extracted, and subjected to the PCR/DNA sequencing analyses for intestinal protozoan parasites.

Within those 83 samples, 7 (8.4%) *Cryptosporidium* spp. (2 *C. hominis*, 1 *C. parvum* and 4 *C. meleagridis*), one of AIDS related OIs, were detected. While, 27 (32.5%) *Giardia intestinalis*, and amebic infections such as 2 (2.4%) *Entamoeba dispar*, 3 (3.6%) *E. coli* , 5 (6.0%) *E. hartmanni* were also detected

The high prevalence of certain opportunistic parasites among HIV positives is well known. Such co-infections present with more severe clinical symptoms compared to parasite infections of otherwise healthy people, and are more difficult to treat. Parasite-HIV co-infections are one of the neglected areas in HIV research although HIV generally has become a major public health concern and research topic in Indonesia. Even since the concerns regarding opportunistic parasite infections among HIV positives have been widely recognized, there is still lack of available data among HIV-infected individuals have been reported in Indonesia.

PGS-9

Genetic diversity of clinical isolated *Acanthamoeba* spp. from keratitis cases in Japan

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[Introduction]

To assess the genetic diversity within *Acanthamoeba* spp., which is known as a causal agent of keratitis, we have analyzed those clinical isolates of keratitis cases using a nuclear and a mitochondrial gene loci.

[Materials and Methods]

Totally 27 isolates of *Acanthamoeba* spp. were collected from the patients at clinical sites as direct corneal scrapes and/or preservation solutions of contact-lens containers by an amebic saline culture containing inactivated *Escherichia coli*. As target gene loci, we analyzed a 550bp fragment of nuclear small subunit ribosomal RNA (18SrRNA) gene and a 1540bp mitochondrial small subunit ribosomal RNA (16SrRNA) gene.

[Results]

As a result of phylogenetic reconstruction of the sequences of 18SrRNA gene fragments, the most of the *Acanthamoeba* isolates were clustered into T4 genotype (23 isolates), and others were belonging to T3 (3 isolates) and T5 (single isolate). Within those T4 genotypes, sub-genotype levels of variations, which were including homologues of multiple species such as *A. polyphaga* and *A. castellanii*, were confirmed; however those monophyletic clusters of sub-genotypes in T4 were not supported statically by bootstrap values. To identify those ambiguous T4 variations and to revalidate the results, all isolates were subsequently analyzed by the 16SrRNA gene locus. The result confirmed those genotypes of 26 isolates as the same genotypes with significant bootstrap values of T3, T4 and T5 as observed in 18sRNA analysis. While, one remaining isolate, identified to T3 in 18sRNA, was confirmed as T4 in 16SrRNA gene analysis.

[Conclusions]

1) The genotyping mismatches depending on gene loci have been reported in other protozoan parasites, and we now know that such mismatch could also take place in the analyses of *Acanthamoeba* species.
2) The phylogeny analyses resolution was clearly higher in 16SrRNA gene than that in 18SrRNA gene locus.
3) It might be also noteworthy that this is the first detection of T5 genotype from a keratitis case.

PGS-10

Application of ELISA to detect urinary IgG4 for the mass-survey of lymphatic filariasis in Bangladesh

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In response to Global Programme to Eliminate Lymphatic Filariasis (GPELF) by 2020 led by World Health Organization, the Bangladesh government made a national plan to eliminate the disease by 2015. Since the start of GPELF, many endemic countries have completed the planned MDAs and the prevalence of filarial infection has been reduced significantly. At this point of post-MDA low endemic stage, when microfilaria (mf) densities have become low, the standard blood films are no more sensitive; immunodiagnoses that detect filarial antigens or specific antibodies will play a more significant role. In Sri Lanka, an ELISA which detects filaria-specific IgG4 in urine showed high sensitivity and specificity. It also showed much higher positive rates than antigen tests in prevalence studies with young children. In this study, we have confirmed the usefulness of SXP1 recombinant antigen-based urine ELISA in the field of Bangladesh. The urine ELISA detected 30 of 31 (97%) most certain positives (mf and ICT positives) in endemic areas. With a more practical positive standard (ICT positive subjects), the ELISA gave 85% (89/105) sensitivity, which was considered satisfactory for use in Bangladesh field. All of 104 ICT negative people in a non-endemic area were ELISA negative (100% specificity). In a prevalence study with 319 young children (5-10 years) from a low endemic area after five rounds of MDA, seven (2.2%) were detected as positive by the present urine test, against only one (0.3%) by the ICT (p = 0.075). Besides the higher sensitivity, the quantitative urine ELISA will provide more information. The urine ELISA with satisfactorily high sensitivity and specificity will be an effective tool in the post-MDA surveys to confirm elimination or to detect resurgence in Bangladesh

PGS-11

Relationship between species identification and observed parasitemia using thin blood smears of four types of malaria

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Optical microscopy is, to date regarded as the "gold standard" for malaria diagnosis in the field. However, there are number of complications and errors that can occur during slide examination that can lead to misidentification. This study assessed the relationship between parasitemia and blood stages as observed in thin blood smears and determined how this relationship effects species identification. It was undertaken by examining 7 to 20 thin blood smears of each human *Plasmodium* spp. at 1000X magnification in oil immersion. In each slide 30 randomly chosen microscopic fields were observed for: 1) total number of erythrocytes, 2) total number of infected erythrocytes, and 3) the number of each developmental blood stage along with any respective characteristics. *P. falciparum* had the highest mean parasitemia at 1.06 followed by *P. vivax* (0.66), *P. ovale* (0.25), and *P. malariae* (0.15). The proportion of the mean parasitemia correlated to the blood stages and characteristics used only for species identification was 0.72, 0.37, 014, and 0.07 in *P. falciparum, P. vivax, P. ovale*, and *P. malariae* respectively. The percent reduction between the mean parasitemia and the proportion related to identification was the lowest for *P. falciparum* at 32%. All other species had a percent reduction of ≈50% or more. While a low percent reduction suggests that most of the blood stages and characteristics observed can be used for identification, a reduction of one half or more implies there is a higher chance of misdiagnosis. This demonstrates one of the main challenges for the accurate identification of *Plasmodium* spp. in the field.

PGS-12

Development of free-living stages of *Strongyloides ratti* under different temperature conditions

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It is well known that *Strongyloides* species has two different developmental courses, direct and indirect, and that selection of these courses is affected by various environmental factors. Although many factors that affect larval development have been reported, we focused on the effect of temperature on the development of first stage larvae of *Strongyloides ratti*. The purpose of this study was to clarify how the larvae adapt and survive in an unsuitable environment. We have previously observed the development of larvae between 15 and 25°C (Minato et al, 2007). In this study, we found that larvae kept at 4 or 10°C for five days could not develop at all. However, they developed into third-stage larvae (L3s) when they were transferred to 25°C and were cultured for an appropriate period thereafter. We have previously demonstrated that most larvae cultured at 25°C adopted the indirect course of development. In contrast, larvae subjected to low temperature stimulation showed a tendency towards direct development in spite of being cultured at 25°C. This stimulation for one minute was sufficient to trigger direct development. We observed the morphology of the low-temperature-stimulated L3s, and compared them with those of control larvae (L3s without low-temperature stimulation). Although the L3s stimulated at 4°C (L3s-4) showed less development, those stimulated at 10°C (L3s-10) developed as well as the control larvae ($p<0.05$). Furthermore, we revealed that the L3s-10 showed similar infectivity to the controls when they were injected subcutaneously into rats as the final host. These results indicated that L3s-10 grew normally. We conclude that *S. ratti* has a survival strategy: when it is excreted in inadequate cold conditions; it becomes durability type and ceases development, but it develops normally via the direct developmental pathway if the inadequate conditions change.

PGS-13

Depressed neuronal growth associated protein (GAP)-43 expression in the small intestines of mice experimentally infected with *Neodiplostomum seoulense*

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Neodiplostomum seoulense (Digenea: Neodiplostomidae) is an intestinal trematode that can cause severe mucosal pathology in the small intestines of mice and even mortality of the infected mice within 28 days after infection. We observed neuronal growth associated protein-43 (GAP-43) expression in the myenteric plexus of the small intestinal wall of *N. seoulense*-infected mice until day 35 post-infection (PI). BALB/c mice were infected with 200 or 500 *N. seoulense* metacercariae isolated from naturally infected snakes and were killed every 7 days for immunohistochemical demonstration of GAP-43 in the small intestines.

N. seoulense-infected mice showed remarkable dilatation of intestinal loops compared with control mice through days 7-28 PI. Conversely, GAP-43 expression in the mucosal myenteric plexus was markedly reduced in the small intestines of *N. seoulense*-infected mice during days 7-28 PI and was slightly normalized at day 35 PI. From this study, it is evident that neuronal damage occurs in the intestinal mucosa of *N. seoulense*-infected mice. However, the correlation between intestinal pathology, including the loop dilatation, and depressed GAP-43 expression remains to be elucidated.

PGS-14

Laboratory infection of second intermediate host, freshwater fish, by *Centrocestus armatus*

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Centrocestus armatus (Heterophyidae) is a small trematode that uses *Semisulcospira libertina* and cyprinid fish such as *Nipponocypris temminckii* as its first and second intermediate host, respectively. Although the final hosts of this parasite are water birds and mammals, we think that this parasite should be considered as a causative agent of zoonoses in Japan because human cases have been occasionally reported. It is known that this trematode is widely distributed in Asian countries such as Japan, Korea, and Thailand. In our previous study, we have reported that there is a high prevalence of both the first and second intermediate hosts living in endemic river in our region. We revealed that the number of cercaria recovered from the endemic river was seven per liter of river water. However, little is known about the relationship between the cercarial number and the infection rate in an endemic river. Therefore, we conducted an experiment to clarify the dynamics of infection. Infection of fish with cercariae was carried out in a glass container that contained 150 ml of water. Cercariae used for this study were freshly excreted from infected snails and the concentration was adjusted to one cercaria per milliliter of water. The infection rate depended on the duration of infection, and the infection rates after 1, 10 and 30 minutes were 37%, 64% and 79%, respectively. The (meta)cercariae were found not only in muscles and internal organs, but also in the brain and eyes. In particular, the respective infection rates in the brain and eyes were 13% and 5% of the total number of metacer-cariae recovered.

PGS-15

Effects of *Centrocestus armatus* infection on physiology and behavior of the second intermediate host, freshwater fish

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It is known that some parasites control the behavior of intermediate hosts for their own survival. This phenomenon is called parasite manipulation and has been demonstrated for some types of trematodes, however, studies on *Centrocestus armatus* (Heterophyidae) regarding this phenomenon have not been fully appreciated. Therefore, we conducted an ecological study of *C. armatus* infection to clarify parasite manipulation by this species. Fish were captured from different rivers in Hyogo prefecture and were used for our study. Fish with <10 metacercaria in their brain were regarded as negative (control), and those with >20 were regarded as positive. Physiological and behavioral changes in the fish were measured by three indicators: school distribution, body color, and response time against stimulation. These results showed that (1) an individual positive fish swam apart from the control fish; (2) body color adjustment declined in the positive fish; and (3) positive fish showed a slow reaction against a stimulation of a predatory bird. These behavioral changes in infected *Nipponocypris temminckii* indicated that *C. armatus* induce fish to take action by which it is easy to be eaten by a definitive host. In conclusion, we demonstrated the parasite manipulation phenomenon by *C. armatus*. Further study of behavioral change before and after *C. armatus* infection is needed.

PP-1

Penetration of crab hosts by *Paragonimus cercariae* : Identifying a transmission route of *Paragonimus skrjabini miyazakii* cercaria to its crab host, *Geothelphusa dehhani*

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Transmission of crab hosts by *Paragonimus* cercariae occur when crab hosts are put into the water containing the cercariae or when the snail hosts harboring the cercariae are fed to the crab hosts (Yokogawa, 1953; Yoshida, 1961), but the details of how the cercariae are transmitted to the crab hosts are not known. Establishing cercarial transmission in a host is required to enable *Paragonimus* undergo a complete life cycle in the laboratory. Successive culture of *Paragonimus*. in a laboratory set forward development of antihelmintics and diagnostic kits against human Paragonimiasis. This study was done to identify the route by which one species, *Paragonimus skrjabini miyazakii* cercaria which were obtained from snail hosts, *Bythinella nipponica nipponica*, infects its crab host, *Geothelphusa dehaani* in a laboratory. The details are as follow.

When cercariae of *P. s. miyazakii* reached maturity in the snail hosts, they emerged from the snail hosts into the water, after which they creep along the bottom or float on or swim near the surface of the water, they survive for one or two days in the condition. During the period the cercariae that came into contact with the crab legs became entangled with it with strands that arose from the mucoid coat on the body surface of the cercariae, cercariae found then had become rounded and attached to the leg surface. They have penetrated the cuticle by 6 hrs after the cercarial exposure started, the stylet first, reached the cavity of the leg, after which cercariae are found in the hepatic altery of the crab hosts 18 hrs latter. Some develop into mature metacercariae in the crab hosts 90 days after the cercarial exposure. Some of them develop into adults in cysts of the lung of dogs or rats. When crabs feed snails with *P. s. miyazakii* cercariae, almost of cercariae are ingested in the crab's alimentary canal, and then destroyed, becoming fragments, but a few cercariae released from the snails into the water around the crabs. The released cercariae do the same as the emerging cercariae penetrate the interior of the legs through the cuticle, as mentioned above. Transmission of *P. s. miyazakii* to the crab host believe to occur by percutaneous penetration of a emerging cercaria in water. Eating the snail host by the crab host is anything more than help with percutaneous penetration.

PP-2

Murine gene expression of host peripheral tissue of *Echinococcus multilocularis* in the liver

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The larval stage of *Echinococcus multilocularis* causes alveolar hydatidosis, locates in the liver, and proliferates unlimitedly. The parasites are covered with host tissue in the cyst-mass. Including fibrocytes, macrophages, giant cells, lymphocyte, neutrophils, eosinophils, mast cell etc. We analyzed the gene expression during the time course of infection in the primary echinococcosis by oral infection with eggs. RNA expression profiles of susceptible DBA/2 mice and resistant C56B/6 mice were assessed using Agilent DNA microarray analysis (Whole Mouse Genome 4x44K v2), at 4, 8 and 16 weeks after the infection. Histopathological examination was also curried out. In the both mice, about 4,400 differentially expressed genes increased at 2 or more time, and 226 genes decreased by 1/4 or below. Most increased mRNAs were tissue inhibitor of metalloproteinase 1, resistin like alpha, chemokine (C-C motif) ligand 8, chitinase 3-like 3, Immunoglobulin heavy chain C gene, amiloride binding protein 1, matrix metallopeptidase 13, chemokine (C-C motif) ligand 11, serine (or cysteine) peptidase inhibitor etc. Analyses of the upregulated genes were done by VLAD and GOTermfinder. The results suggested the activation of immune system process (leukocyte-differentiation, -proliferation, -activation, and -chemotaxis, inflammation, cytokines, apoptosis, cell adhesion, signal transduction, wound healing, antigen processing and presentation, et al.). mRNAs of IL-4, 5, 13, 33, 1, 6 and 11 and chitinase 3-like 3, resistin like alpha, chemokine (C-C motif) ligand 17, and coagulation factor XIII, A1 subunit increased significantly. These results suggested responses of Th2 lymphocyte and M2 type macrophage. Fibrosis around the cyst were corresponding with the increased expression collagens (type I-alpha 1 and -alpha 2, type III-alpha 1, type XII-alpha 1, type V-alpha 1 and -alpha 2, type VI-alpha 1 and - alpha 2 and type XV-alpha 1), matrix metallopeptidase 13, 12, 2, 3. 9 and 23, a disintegrin and metallopeptidase domain 8, 19 (meltrin beta) and 2, fibroblast activation protein and fibroblast growth factor 18 etc. Prominently increased chemokine ligands were chemokine (C-C motif) ligand 8, 11, 7, 12, 17, 6, 24, 3, 4, 2 and 1, chemokine (C-X-C motif) ligand 5, 3 and 14 and chemokine (C-X3-C motif) ligand 1, etc. Eosinophil, neutrophil and mast cell accumulation were corresponded with increased expression of chitinase 3-like 3 (ECF-L), amiloride binding protein 1 (amine oxidase, copper-containing), proteoglycan 2 bone marrow (MBP), integrin alpha X (CD11c), chemokine (C-C motif) ligand 11 (eotaxin), chemokine (C-C motif) ligand 12 (MCP-5), eosinophil peroxidase, neutrophil cytosolic factor 1, 2 and 4, neutrophilic granule protein, myeloperoxidase, mast cell protease 1, 2 and 4, chymase 1, mast cell (Mctp5), and carboxypeptidase A3, etc. The gene expression profiles during 4 and 16 weeks were not change significantly in both mice. C56B/6 mice showed more increase of mRNA for immune system process than DBA/2. Downregulated genes were included monooxygenase activity, oxidoreductase activity, G-protein coupled receptor activity, especially cytochrome P450 and olfactory receptor.

PP-3

Toxocara spp. Eggs Soil Contamination in Los Baños, Laguna, Philippines and Seroprevalence of its Infection among Public School Children

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The soil-transmitted nematode *Toxocara* sp. is one of the neglected parasites due to lack of epidemiological information. In this study, the extent of soil contamination of *Toxocara* eggs and seroprevalence of *Toxocara* infection among public school children in Los Baños, Laguna, Philippines were determined. Soil samples from public schools, backyards, and empty lots in the five barangays of Los Baños were examined for the presence of *Toxocara* spp. eggs through modified sucrose flotation technique; and serum samples from public school children in the three barangays of Los Baños were examined for *Toxocara* infection through ELISA test. From a total of 200 samples, 85 (43%) were found positive for *Toxocara* eggs with a level of contamination of 1 egg/g soil sample, wherein 42% of the public school samples, 45% of the backyard samples, and 40% of the empty lot samples were positive. Moreover, from a total of 75 children, 37 (49%) were found positive for *Toxocara* infection which was relatively high and alarming. There was also a positive correlation with the intensity of *Toxocara* spp. eggs and seroprevalence of *Toxocara* infection. The results of this study provide baseline information on the soil prevalence of *Toxocara* spp. eggs and seroprevalence of *Toxocara* infection among children in Los Baños, Laguna, Philippines.

PP-4

Prevalence of parasitic infestation among the bovine species slaughtered in municipal abattoir at Ismailia - Egypt. An abattoir's survey

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The present study had been carried out on 10055 cattle, 3811 buffaloes and 2378 male buffalo calves; all were of native breeds and examined at Ismailia municipal abattoir, east-north of Egypt The slaughterhouse was paid 5 visits weekly between March 21st, 2009 and March 20th, 2010. 3668 out of the slaughtered heads showed deviation from the normal as to render them repugnant to the consumers and obligate the condemnation; 41.40 % of these condemned materials were referred to the parasites which giving an overall prevalence rate of 9.35%. Inspection of cattle carcasses revealed infestation with *C.bovis* and *Hydatid cysts* at rates of 0.58 % & 0.49 % respectively.

Buffalo carcasses showed minimal incidence for *C.bovis* (0.20%) & incidence of 1.50 % for *Hydatid cysts*; while 20.34 % of these buffaloes have been infested with Sarcocysts macro cysts which appear as visible cylindrical bodies. Fascioliasis recorded in cattle liver at rate of 3.23% which was quite higher than that of buffaloes (1.47 %). Rumen of 4.41% buffaloes was harboring *Paramphistomum* spp., also the intestine of 3.49 % harboring *Moniezia spp.* In this respect the rate of infestation among 10055 cattle was 3.45% for *Moniezia spp.* and 1.44% for *Paramphistomum spp. Ascaris spp.* was the only gastrointestinal helminthes recovered from 302 male calves with an incidence of 12.70 %.

PP-5

Involvement of PI 3 kinase/Akt-dependent Bad phosphorylation in *Toxoplasma gondii*-mediated inhibiton of host cell apoptosis

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Toxoplasma gondii-infected cells are resistant to various apoptotic stimuli, however, the role of the pro-apoptotic BH3-only Bad protein in *T. gondii*-imposed inhibition of host cell apoptosis in connection with the phosphoinositide 3-kinase (PI3K)-PKB/Akt pathway was not well delineated. Here, we investigated the signaling patterns of Bad, Bax and PKB/Akt in *T. gondii*-infected and uninfected THP-1 cells treated with staurosporine (STS) or PI3K inhibitors. STS treatment, without *T. gondii* infection, reduced the viability of THP-1 cells in proportion to STS concentration and triggered many cellular death events such as caspase-3 and -9 activation, Bax translocation, cytochrome c release from host cell mitochondria into cytosol, and PARP cleavage in the host cell. However, *T. gondii* infection eliminated the STS-triggered mitochondrial apoptotic events described above. Additionally, *T. gondii* infection *in vitro* and *in vivo* induced the phosphorylation of PKB/Akt and Bad in a parasite-load-dependent manner which subsequently inhibited Bax translocation. The PI3K inhibitors, LY294002 and Wortmannin, both blocked parasite-induced phosphorylation of PKB/Akt and Bad. Furthermore, THP-1 cells pretreated with these PI3K inhibitors showed reduced phosphorylation of Bad in a dose-dependent manner and subsequently failed to inhibit the Bax translocation, also these cells also failed to overcome the *T. gondii*-imposed inhibition of host cell apoptosis. These data demonstrate that the PI3K-PKB/Akt pathway may be one of the major route for *T. gondii* in the prevention of host cell apoptosis and *T. gondii* phosphorylates the pro-apoptotic Bad protein to prevent apoptosis.

PP-6

Glycerophospholipid metabolism in *Blastocystis hominis*

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Next generation sequencing (NGS) is a high throughput sequencing can sequence up to one billion bases in a single day. Many researchers are forging ahead with projects to sequence a range of species using this new technologies. However, the new technology produce read lengths as short as 35□40 nucleotides, posing challenges for genome assembly and annotation. *Blastocystis hominis* is a neglected intestinal protozoa, with a prevalence in developing countries of up to 50%. Clinical manifestations include non-specific gastrointestinal symptoms (such as diarrhea, abdominal pain, anorexia, vomiting, flatulence, tenesmus) and rarely hepatosplenomegaly, allergic-type cutaneous rash, and pruritus; lower gastrointestinal bleeding as a complication of this infection is uncommon. In this study, we observed that *B. hominis* has lipid storage by using Sudan black B stain isolated from patients and with several different types of lipid droplets. These are also found in cultured *B. hominis*. Several photomicrograph observations in cultured *B. hominis* that lipid will be accumulated from several small droplets into large ones. That the morphology similar as *B. hominis* isolated from patient. In order to know the lipid accumulation and genes regulation of lipid metabolism in *B. hominis*. The RNA-seq by using NGS technology was used for the *B. hominis*. There are 71% were annotated by using P_{fam} blast in 6,020 CDS of *B. hominis*. That are 0.3% proteins effected of lipid metabolism in 6,020 CDS. We found 5,683 unnamed protein products in first annotation. The 2,937 proteins were annotated of 5,683 unnamed protein products in second annotation. There are 15 glycerophospholipid genes can be mapped to KEGG, and over 45% and 75% were similar to *Entamoeba histolytica* and *Saccharomyces cerevisiae* in respectively. The storage and utility of lipid droplets in *B. hominis* will be studied in the further by using system biological approach and analytical chemistry strategy in the further.

PP-7

Caution on use of anti malarial drag for mixed infection and infectious control of super infection of malaria particular: Baysian analysis of malaria data from Sarawak, Malaysia

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Past studies indicated that a mixed infection by Plasmodium spp. is likely to spread the infection more frequently than does a single infection. From these reasons, we need to note the following points in diagnosis of malaria and the mechanism of mixed infection and considering the treatment. In general, malaria is diagnosed from proportion of erythrocytes invaded by plasmodium in a blood smear. However, if a physician diagnoses malaria in clinical practice, it is necessary to exam the life cycle of malaria parasites. In this study, we used malaria data obtained in Sarawak, Malaysia, and calculated incidence proportion and proportion of mixed infection. Then, we analyzed the effect of anti-malarial drug using calculated proportion. It is difficult that we measure immunological effect and interaction of plasmodium needed in this analysis. For this reason, we constructed hierarchical model and used unknown parameter some factors that were difficult to measure in the model. We calculated the incidence proportion for *P. falciparum* and *P. vivax* and two mixed and introduced these parameters to the abobe model. The model and unknown parameters were analysed by WinBUGS, ver.1.4.3. Conventionally, in super infection, it is difficult to see what is involved in such super infection interactions between the two spaces of Plasmodium. As the result of the analysis, without receiving the effect of the action of each other and from the environment in blood, was considered to be raised by proliferation-specific symptoms. In addition, *P. falsiparum* and *P. vivax* have different living space and life cycle. From this point of view, the possibility of their super infection were suggested. In this case, we constructed hierarchical model with normal distribution .But considering the time as factor, it is consider important to concentrate on a certain time of the day, in other words, if we can observe the growth of *P. falciparum* or *P. vivax* such as to concentrate on a certain time, it estimated by another probability distribution is needed.

PP-8

Developmental rate of the blow fly, *Hypopygiopsis tumrasvini* Kurahashi (Diptera: Calliphoridae): forensic entomology application

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Blow flies of the genus *Hypopygiopsis* are considered important forensically, since the second and third instars of *Hypopygiopsis* violacea Macquart were collected from a human corpse at a crime scene in Malaysia. In this study, the developmental rate of *Hypopygiopsis* tumrasvini was determined in the egg, larval and puparial stages in chamber reared at 25±2°C and 80±5% relative humidity. The embryonation period of this fly was 10-12 hours, as observed under light microscope. A video recorder employed to observe the hatching process, demonstrated continuous movement of the embryo during the 10-12 hours. The embryo shrunk vigorously just prior to hatching, causing collapse of the latero-anterior margin of the eggshell. Separation of the anterior hatching line allowed the first instar to exit. The developmental time of the first, second, third instar (feeding phase), third instar (post-feeding phase) and puparia was 8 hours, 10 hours, 34 hours, 22 days and 9-10 days, respectively. Video recording of pupariation initially revealed the pupal respiratory horn beneath the larval integument at 27 hours; whereas later observation saw it protrude through the hole of the integument at 27.55 hours. Dissection of an elderly puparium revealed that the base of the pupal respiratory horn is connected to the anterior spiracle of the adult, indicating the respiratory channel of the adult within puparia.

PP-9

House fly, *Musca domestica*, and blow fly, *Chrysomya megacephala*, as sources of pathogenic bacteria associated with human habitations in Northeast Thailand

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The house fly, *Musca domestica*, and the blow fly, *Chrysomya megacephala* are synanthropic flies which are adapted to live in close association with human habitations and are capable of transmitting human pathogens either mechanically or biologically through this close relationship. The objective of this study was to investigate the potential of these flies for acquiring pathogenic bacteria from human habitations including fresh-food markets, garbage piles, restaurants, school cafeterias and paddy fields in Muang Ubon Ratchathani and Warinchamrap district of Ubon Ratchathani province for 12 months. Bacteria were isolated from flies collected in these habitats, using standard bacterial isolation techniques from the external surfaces of 994 flies (439 *M. domestica and* 555 *C. megacephala*). A total of 15 bacterial genera were isolated, comprising ten gram-negative bacterial genera and five gram-positive bacterial genera. The most common bacterium isolated from both fly species was coagulase-negative staphylococci, followed by *Streptococcus* group D non-enterococci. Human pathogenicenteric bacteria isolated were *Salmonella* sp*.*, *Shigella* sp., *Escherichia coli* O157:H7, *Sallmonella typhi*, *Bacillus* sp. and *Enterococcus* sp. In addition, other human pathogens were identified including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The number of *C. megacephala* positive for bacteria was significantly higher than for *M. domestica* in both districts ($P<0.0001$). Among flies collected from five sites in Muang Ubon Ratchathani, the number of *M. domestica* positive from the restaurant sites was significantly higher than any of the other sites ($P<0.008$). Otherwise, no significant difference was found when comparing *C. megacephala* positive flies collected from Muang Ubon Ratchathani and Warinchamrap district ($P=0.166$ and $P=1.000$, respectively). The total bacteria isolated from *C. megacephala* was highest rate in February and June in both districts; while the highest number of *M. domestica* positive for bacteria was in June. These data suggest that these fly species have the potential to act as carriers of enteric bacteria associated with human habitation in this region of Northeast Thailand.

PP-10

Functional analysis of Notch in the regulation of development in the mosquito, *Aedes aegypti*

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Mosquitoes are important vectors for several infectious diseases such as malaria, dengue fever, Japanese encephalitis, West Nile fever, yellow fever…etc. All together kill more than 2 million people a year, due to the unavailability of effective vaccines for malaria and other mosquito-borne diseases and the development of insecticide and drug resistance to vectors and pathogens. Therefore, there is an urgent need to explore every possible avenue for developing novel control strategies against these mosquito-borne diseases. Notch signaling pathway is an evolutionary highly conserved cell-cell signaling pathway, which regulates many events during development. In *Drosophila*, Notch signaling was shown to be participated in the wing development and the regulation of the activity in nervous system. To investigate the roles of Notch signaling in the mosquito *Aedes aegypti*, we made use of the RNA interference approach to silence *Aedes aegypti* Notch (AaNotch). To our surprise, RNAi-mediated silence of Notch resulted in a significant reduction of egg tanning. The majority of these non-melanized eggs are unable to hatch. To characterize the Notch expression in the mosquito, we showed that Notch is highly expressed in the female ovary 24 hours post a blood meal. Immunofluorescent assay revealed that Notch is located in the surface of the epithelial cell of the follicles. Detail characterization of mosquito Notch will provide new insights into the evolutionary conserved features of Notch. Information gathered in this study will pave the way toward the establishment of efficient strategies for vector control.

PP-11

Essential role of Wnt signaling in the regulation of vitellogenesis in the mosquito, *Aedes aegypti*

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Signaling transduction of Wnt-Frizzled is an important aspect of tissue/organ development and maintenance. Wnt and Frizzled family genes were first cloned and characterized in *Drosophila* as a key event in development. However, the roles of Wnt signaling in the mosquito remain largely unknown. Recently, we identified nine Wnt ligands and four Frizzled receptors in the mosquito *Aedes aegypti*, the major vector of dengue virus. All of these Wnt ligands harbor potential sites for glycosylation and palmitoylation, which appear to be useful for signal transduction. Our previous results demonstrated that *Aedes aegypti* Frizzled 2 (AaFz2) is expressed in the mosquito fatbody at 6 hr after a blood meal. Silencing of AaFz2 reduced the fecundity in the mosquito. In this study, we showed that Wnt is highly expressed in the mosquito ovary in terms of transcriptional and translational level. Also, silence of Wnt significantly reduced the egg production. Intriguingly, Wnt is specifically expressed in the oocyte of the follicle cells before a blood meal and only shortly after the blood meal. Activation of Wnt signaling by RNAi-mediated silence of GSK-3 resulted in the phosphorylation of S6K at the pre-vitellogenic stage. Data revealed by this proposal will be crucial for future studies on vector competence and vector control in the field.

PP-12

Matrix metalloproteinase-9 leads to claudin-5 degradation via the NF-κB pathway in BALB/c mice with eosinophilic meningoencephalitis caused by *Angiostrongylus cantonensis*

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The epithelial barrier regulates the movement of ions, macromolecules, immune cells and pathogens. The objective of this study was to investigate the role of the matrix metalloproteinase (MMP)-9 in the degradation of tight junction protein during infection with rat nematode lungworm *Angiostrongylus cantonensis*. The results showed that phosphorylation of IκB and NF-κB was increased in mice with eosinophilic meningoencephalitis. Treatment with MG132 reduced the phosphorylation of NF-κB and the activity of MMP-9, indicating upregulation of MMP-9 through the NF-κB signaling pathway. Claudin-5 was reduced in the brain but elevated in the cerebrospinal fluid (CSF), implying that A. cantonensis infection caused tight junction breakdown and led to claudin-5 release into the CSF. Degradation of claudin-5 coincided with alteration of the blood-CSF barrier permeability and treatment with the MMP inhibitor GM6001 attenuated the degradation of claudin-5. These results suggested that degradation of claudin-5 was caused by MMP-9 in angiostrongyliasis meningoencephalitis. Claudin-5 could be used for the pathophysiologic evaluation of the blood-CSF barrier breakdown and tight junction disruption after infection with *A. cantonensis*.

PP-13

A transcriptomic study on the pepsin-activated infective larvae of *Angiostrongylus cantonensis*

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To investigate the pepsin-activated infective (third-stage) larvae of Angiostrongylus cantonensis at the transcriptomic level, 1,496 ESTs were generated from a cDNA library and clustered into 161 contigs and 757 singletons. Among these unigenes, 54.5% had significant sequence homology with known proteins. The most abundantly expressed transcripts were cathepsin B-like cysteine protease 1 and 2, metalloprotease I, metalloprotease 1 precursor, and extracellular superoxide dismutase. Protein complex was the most common Gene Ontology classification within the 'cellular component' category, embryonic development ending in birth or egg hatching within 'biological process', and protein binding within 'molecular function'. Moreover, 280 clusters were mapped to 158 KEGG pathways and 134 had unique EC numbers. These findings suggest that treatment with pepsin–HCl not only digests the tissues of the snail host but also activates the infective larvae.

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About APCPZ

Official name of *the Asian Pacific Congress for Parasitic Zoonoses (APCPZ)* was given in 1990 when the 1st APCPZ conference was held in Sendai city, Miyagi Prefecture. The president of this memorial conference was Tomio Yamaguchi, emeritus professor at Hirosaki University. Since then, APCPZ has been held every two years, in either Taiwan or Japan. The year 2010, marked the first time the conference was held in a third country—due to the increasing participation of Korean parasitologists, the 11th APCPZ was held in Incheon, Korea.

The 12th APCPZ was held on October 6–7, 2012, in Kobe, Japan, with attendees from all over the world: Argentina, Bangladesh, China, Egypt, Indonesia, Iran, Japan, Korea, Malaysia, the Philippines, Taiwan, Thailand, the United States, and Vietnam (see the cover map showing the countries). Although APCPZ members work in various fields—as human and veterinary doctors, clinical and basic researchers, parasitologists and entomologists, researchers and public health officers—all the members share an interest in and passion for parasitic zoonoses.

Gathering the fruits of the face-to-face discussions that took place at the 12th APCPZ between those professionals and researchers, this book provides a wide variety of interesting insights into the current status of parasitic zoonoses, constituting a valuable record of crosstalk between highly diverse specialties.

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