アメーバ症
amebiasis

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I 赤痢アメーバ症

病態と診断

腸管寄生性の単細胞真核生物（原虫）である赤痢アメーバ（*Entamoeba histolytica*）による感染症である．その生活環は栄養体とシストからなる．ヒトは水や食物に混入した成熟シストの経口摂取により感染する．世界では毎年およそ4,000万人が罹患し，4万-5万人が死亡している．本邦での年間報告数は増加傾向にあり，2008年には861例を記録した．男女比は約8：1である．糞口感染による性行為感染症，輸入感染症としても注意が必要である．

Ⓐ 病態

経口摂取されたシストは小腸で脱嚢し，栄養体が大腸内に定着・増殖する．多くは不顕性感染に終わるが，およそ10%の感染例では栄養体が粘膜上皮に侵入しさまざまな症状を呈する．栄養体が血行性に肝・肺・脳などの実質臓器に転移すると膿瘍を形成し，放置すると致死的となる．

Ⓑ 診断

診断は検鏡によって検体中に栄養体を検出することによる．不顕性感染の場合は糞便中にシストを見出すことも多い．形態学的には非病原性アメーバ（*E. dispar*）との区別ができない．鑑別にはPCR，ELISAが有効である．

Ⓒ 感染症法に基づく届出

赤痢アメーバ症は5類感染症に指定されており，症状を有する患者を診断した医師は7日以内に最寄りの保健所に届け出る必要がある．

治療方針

Ⓐ 腸アメーバ症

メトロニダゾール（フラジール）による治療が第1選択である．

℞ 処方例

フラジール内服錠（250 mg）　6錠　分3　10日間

●治療終了の指標　副作用として末梢神経障害が出現したら投与中止．

投与1-2週後に糞便検査を行い陰性化を確認する．フラジールによる治療に反応しない症例に対して，パロモマイシン（アメパロモ）を使用すること

℞ 処方例

アメパロモカプセル（250 mg）　6カプセル　分3　10日間　腎機能障害がある場合は慎重投与

Ⓑ 腸外アメーバ症

メトロニダゾール（フラジール）による治療が第1選択である．

℞ 処方例

フラジール内服錠（250 mg）　6錠　分3　10日間

肝膿瘍からの排膿は原則不要である．経口摂取が困難な重症例では注射用フラジールを使用する（「熱帯病治療薬研究班」が保管している）．

℞ 処方例

Flagyl注（0.5%，100 mL）　1回500 mg　1日3回　7日間　静注　保外　国内未承認

II アメーバ性角膜炎

角膜炎を引き起こすアカントアメーバ（*Acanthamoeba* sp.）は土壌，水中をはじめ自然界に広く分布する自由生活性アメーバである．本来きわめてまれな病気であったが，コンタクトレンズの普及とともに近年急増してきた．

病態と診断

Ⓐ 病態

初期には角膜上皮・上皮下混濁を認め，毛様充血や眼痛を生じ，放置すると角膜潰瘍，穿孔に至る．

Ⓑ 診断

検鏡・培養・PCRによって病巣掻爬物内にアカントアメーバを証明することによる．

治療方針

特効薬がないために治療は困難である．基本的には病巣掻爬，抗アメーバ作用を有する薬剤〔アゾール系，ピマリシンなどの抗真菌薬，クロルヘキシジングルコン酸塩（CHX），ポリヘキサメチレンビグアナイド（PHMB）などの消毒薬〕の局所投与，抗真菌薬の全身投与を組み合わせる．近年，メチレンブルーを用いた光線力学療法（MB-PDT）が有効であり，治療効果の判定に5-cyano-2,3-tetrazolium chloride（CTC）染色が有効であることが報告された．CHX，PHMB，MB，CTCなどは医薬品ではないため，使用にあたっては十分なインフォームド・コンセントが不可欠である．

℞ 処方例　下記を併用する．

フロリードF注　0.1%に希釈して点眼　保外

イトラコナゾール錠（50 mg）　3-4錠　分1

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Chapter 27
Fertilization Mechanisms of the Rodent Malarial Parasite *Plasmodium berghei*

Makoto Hirai

Abstract Malaria, caused by *Plasmodium* spp., is transmitted by anopheline mosquitoes. When male and female gametes are introduced into the mosquito midgut, they reproduce sexually and proliferate, at which time the mosquito becomes infectious to vertebrates. It has been proposed that fertilization is a critical target in the parasite life cycle for the reduction of malarial prevalence. Although understanding parasite fertilization is crucial for the control of malaria, the precise molecular mechanisms involved have long remained unknown. Generative cell-specific 1 (GCS1) has been reported to be a critical fertilization factor in angiosperms. It was subsequently shown that the function of GCS1 is conserved in both the rodent malaria parasite *Plasmodium berghei* and the green alga *Chlamydomonas reinhardtii*. Moreover, a *GCS1*-like gene has been detected in the genomes of various organisms, suggesting that it plays a conserved role in gamete interaction. As GCS1 is thought to act as a membrane-anchoring protein in male gametes, a female counterpart is assumed to exist. To reveal the mechanisms involved in parasite fertilization, it is important to clarify the function of GCS1 and to identify GCS1 partners and other fertilization factors. In this review, I first describe the life cycle of malaria parasites, focusing on gametogenesis and fertilization and the underlying mechanisms. I then discuss the functions of GCS1 at the time of gamete interaction. Finally, I consider whether parasite fertilization factors, including GCS1, might be utilized in the development of antimalarial vaccines.

Keywords Fertilization • Gametogenesis • GCS1 • Malarial parasite

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27.1 Sexual Differentiation and Gametogenesis of Malaria Parasites

In vertebrates, mature malaria parasites infect erythrocytes by egressing from older cells and invading newer ones. In asexually proliferating parasites, a subset of the parasites escapes from the asexual cycle and differentiate to produce male and female gametocytes (gametocytogenesis). When these gametocytes are introduced into the midgut of mosquitoes through feeding on malaria-infected vertebrates, they differentiate into gametes (gametogenesis). During gametogenesis, gametocytes shed the erythrocytic membrane within a few minutes of mosquito feeding. Subsequently, male gametes undergo three rounds of DNA replication within 2 to 10 min. Between 10 and 12 min, eight motile flagella (the motile form of male gametes) are released (Sinden et al. 1996) in a phenomenon termed exflagellation (Ross and Smyth 1997). For *Plasmodium*, an in vitro fertilization (IVF) assay has been established involving three factors required to induce gametogenesis: (1) a drop in temperature from 37 °C to 21 °C; (2) the presence of xanthurenic acid (XA), a previously determined mosquito-derived factor (Billker et al. 1998); and (3) alkaline conditions (pH 8.0). Through IVF, it has been demonstrated that XA activates membrane-bound guanylate cyclase (GC) (Muhia et al. 2001) and cGMP-dependent protein kinase (PKG) (McRobert et al. 2008), which are essential for male exflagellation. Moreover, *N*-methyl-hydroxylamine, an inhibitor of GC, inhibits exflagellation (Kawamoto et al. 1990). These findings demonstrate that cGMP is involved in XA-mediated signaling. In addition to cGMP signaling, it has been reported that intracellular Ca^{2+} concentrations in gametocytes are increased within 10 s after XA stimulation. Parasites lacking the *calcium-dependent protein kinase 4* gene are defective in genomic replication and mitosis, resulting in an absence of exflagellation (Billker et al. 2004). This finding suggests that XA may also activate calcium signaling. Furthermore, it has been reported that *P. berghei* homologues of atypical MAP kinase 2 (Map-2) (Rangarajan et al. 2005; Tewari et al. 2005) and cdc20 (Guttery et al. 2012) are required for male gametogenesis. Although the aforementioned molecules have been demonstrated to be involved in XA-mediated signal transduction, the most important question remains unanswered: How does XA induce gametogenesis? Given that membrane-bound GC in gametocytes is activated by XA in vitro (Muhia et al. 2001), a membrane-coupled XA receptor may exist to activate PKG and calcium signaling, thereby triggering gametogenesis.

27.2 Recognition, Attachment, and Membrane Fusion of *Plasmodium* Gametes

In general, the process of fertilization can be divided into three steps: recognition, attachment, and membrane fusion of gametes. In malaria parasites, it has been shown that male flagella swim freely, exhibiting no directed movement toward

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female gametes in vitro (Sinden 1983). Therefore, although many plants and animals utilize chemoattractants for gamete recognition when they are spatially isolated, malaria parasites may not possess such a system. Nevertheless, *Plasmodium* gametes must possess a molecular system for species-specific recognition. A recent study demonstrated that the gamete membrane-surface proteins P48/45, P47, and P230, which belong to the 6-cys family, are critical in gamete recognition. Mutant parasites lacking either P48/45 or P47 and P230 show male or female subfertility, respectively (van Dijk et al. 2010). Further phenotypic observation revealed that these mutants are unable to either recognize or attach to each other, demonstrating the vital role of this family of proteins in gamete recognition/attachment. Importantly, the fertility of these mutants is not completely impaired, suggesting that alternative pathways may compensate for the function of these proteins. Thus, definitive fertilization factors for malaria parasites have not been identified until recently.

In *Plasmodium*, the male gametes swim in a fashion similar to mammalian sperm and attach vigorously to female gametes. Interestingly, after entering female cells, the male gametes continue flagellar movement inside the cell for 1 min after fusion (Sinden and Croll 1975). A similar phenomenon has been observed in the fruit fly *Drosophila melanogaster*, whose spermatic membrane is detected in eggs until immediately after fertilization. Sneaky (Snky), a sperm membrane-specific protein, is responsible for sperm membrane degradation and is essential for successful fertilization (Wilson et al. 2006). Putative Snky homologues are present in vertebrate species, implying their functional conservation (Wilson et al. 2006). However, *Plasmodium* species do not possess an obvious homologue of this protein, suggesting that the molecular mechanism of *Plasmodium* fertilization is, at least in part, distinct from that of animals.

27.3 GCS1: An Ancient Fertilization Factor Conserved in Animals and Plants

Mori et al. reported a male gamete-specific protein, designated generative cell-specific 1 (GCS1), as a novel fertilization factor in angiosperms (Mori et al. 2006). GCS1 is a putative single-pass transmembrane protein with a putative N-terminal signal sequence but no functional domain (Mori et al. 2006; von Besser et al. 2006). *GCS1* knockout (KO) *Arabidopsis thaliana* plants show a severe form of male sterility in which male gametes can access the egg cell normally but cannot fuse with female gametes, suggesting that only the gamete interaction process is impaired in GCS1 KO plants (Mori et al. 2006; von Besser et al. 2006). Interestingly, functional GCS1 homologues are present in green algae and rodent malaria parasites (Mori et al. 2006; von Besser et al. 2006; Liu et al. 2008). Similar to angiosperm GCS1s, the expression of *P. berghei* GCS1 (PbGCS1) shows male gamete specificity, and loss of PbGCS1 results in male sterility, whereas female gametes remain fertile (Hirai et al. 2008; Liu et al. 2008). This situation is in sharp contrast to that in mutant parasites lacking any of the 6-cys family proteins, whose fertility is only

106 partially impaired, suggesting that no complementary molecule for GCS1 exists in
107 the parasite. In green algae, Liu et al. demonstrated the exact timing of GCS1 func-
108 tion. Algal GCS1 acts during the few seconds between the pre-fusion adhesion and
109 fusion steps, providing evidence that GCS1 may play a role in gamete membrane
110 fusion following gamete recognition (Liu et al. 2008). It is generally accepted that
111 the molecules that function in species recognition evolve rapidly, leading to repro-
112 ductive isolation and speciation among eukaryotes (Swanson and Vacquier 2002).
113 In this context, GCS1 has been shown to be conserved in various organisms, and the
114 functional timing observed during gamete recognition suggests that GCS1 may
115 have a role in membrane fusion following species recognition. A previous study by
116 our group demonstrated that male gametes lacking PbGCS1 can attach to their
117 female counterparts, suggesting that PbGCS1 is unnecessary for gamete recognition
118 (Hirai et al. 2008). To further confirm that GCS1 is not involved in gamete recogni-
119 tion, we generated transgenic *P. berghei* in which the endogenous *PbGCS1* gene
120 was replaced with that of *P. yoelii* (PyGCS1), another rodent malaria parasite.
121 Because *P. berghei* cannot fertilize *P. yoelii*, the PyGCS1-expressing *P. berghei*
122 male gametes are expected to fail in the fertilization of wild-type (WT) *P. berghei*
123 female gametes if the GCS1 protein plays any role in species-specific gamete rec-
124 ognition. Our results clearly demonstrated that the transgenic *P. berghei* male gam-
125 etes could fertilize WT *P. berghei* female gametes, indicating that *Plasmodium*
126 GCS1 is not involved in gamete recognition. Moreover, heterogeneous expression
127 of PyGCS1 in *P. berghei* did not result in any adverse effects on parasite develop-
128 ment, even after the zygote stage, suggesting functional complementation of
129 PyGCS1 in *P. berghei* (Hirai et al., unpublished data).
130 Given that GCS1 contains no predictable functional motif except for a signal
131 sequence and transmembrane domain, there are no available clues to allow specula-
132 tion regarding its function. As an initial step toward understanding the function
133 of PbGCS1, we generated a series of transgenic parasites in which endogenous
134 PbGCS1 was partially deleted to characterize the critical functional domains of
135 the PbGCS1 protein. Our results revealed that the putative cytosolic region in the
136 C-terminus of the protein is not necessary for successful fertilization but that all
137 other regions (e.g., the single-peptide transmembrane domain and recently defined
138 motifs such as HAP2/GCS1) are essential. Taken together, the results of this study
139 revealed that functional domains of PbGCS1 involved in gamete fusion reside in
140 putative extracellular locations and that PbGCS1 must be localized to the cell sur-
141 face to function properly (Mori et al. 2010). Interestingly, a similar analysis was
142 performed for *Arabidopsis* GCS1, generating results similar to those found for
143 PbGCS1 (Mori et al. 2010) and suggesting a conserved topology of GCS1 among
144 GCS1-possessing organisms. Additionally, the extracellular localization of GCS1
145 allows us to speculate that GCS1 may function on the surface of male gametes by
146 interacting with a partner molecule on female gametes. It is clear that the identifica-
147 tion of GCS1 partners and other fertilization factors will accelerate our understand-
148 ing of fertilization systems not only for malaria parasites but also for all
149 GCS1-possessing organisms.

Author's Proof

27.4 Application of Fertilization Factors to Anti-Malarial Vaccine Development

Malaria is a serious disease that was responsible for 655,000 deaths and 216 million reported cases in 2010 alone (Murray et al. 2012). The emergence of parasites that are resistant to drugs and vaccines necessitates the development of new strategies for malaria control. However, the development of antimalarial vaccines and drugs is difficult because both the antibodies elicited by vaccination and drugs provide selective pressure and induce recombination/mutation in the parasite genome, resulting in antigenic polymorphisms and the emergence of parasites that are tolerant to these treatments. In addition to antimalarial strategies aimed at the human blood-stage parasite, a transmission-blocking vaccine (TBV) that attacks the insect-stage parasite in mosquitoes has been proposed. Mosquitoes do not exhibit adaptive immunity and rely solely on innate immunity (Faye 1990), suggesting that insect-stage parasites do not face antibody-based immune pressure. It has therefore been speculated that an antibody-based immune attack could be effective against mosquito-stage parasites because they may not evolve any strategy for combating antibodies during the mosquito stage. Based on this assumption, TBV candidates have been selected from molecules expressed in gametocytes, gametes, zygotes, and ookinetes. Several cell-surface molecules (Pfs230, P48/45, P25/28) have been tested as TBV targets thus far, and the antibodies raised against them have succeeded in reducing the rate of parasite transmission (Barr et al. 1991; Healer et al. 1999; Williamson 2003). However, none of these studies has demonstrated complete blockage of transmission, most likely because the TBV candidate molecules are quite abundant in parasites and because the small quantity of antibodies administered may not be sufficient to completely block the activity of the candidate molecules. Even if the antibodies were able to fully block this activity, it is possible that the parasites would still be fertile, because parasites lacking any of these genes can still reproduce sexually in mosquitoes (van Dijk et al. 2010). Therefore, other molecules that display a definitive and crucial function in sexual reproduction represent ideal TBV candidates. We believe GCS1 best fits this criterion because PbGCS1 protein expression levels are quite low in the parasite, and a lack of PbGCS1 protein completely abrogates parasite fertility (Khan et al. 2005; Hirai et al. 2008). Recently, Blagborough et al. and our group performed experiments demonstrating that an anti-PbGCS1 antibody significantly, but not completely, blocked parasite reproduction based on IVF assays (Blagborough et al. 2013; Hirai et al. unpublished data). This failure to completely inhibit reproduction may arise from PbGCS1 possessing many cysteine residues and displaying a complex conformation that is not recapitulated by the recombinant protein, resulting in less effective antibody production. Such a requirement for correct folding of recombinant TBV proteins for effective antibody production has been observed in cases in which the TBV candidate is produced in yeast and algal systems (Kaslow et al. 1994; Gregory et al. 2013). In addition to this problem, the immunization protocol needs to be improved to generate higher antibody titers (Kubler-Kielb et al. 2007; Outchkourov et al. 2008).

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193 It is reasonable to assume that the use of multiple TBV targets would be effective
194 because the antibodies raised would simultaneously neutralize or block multiple
195 parasite molecules, leading to complete blockage of parasite fertilization. Thus,
196 we have employed a transcriptomic approach to identify new fertilization factors for
197 malaria parasites, and TBV candidates will be screened from this source.

198 27.5 Concluding Remarks

199 Since the draft genome sequences of the human malaria parasite, *P. falciparum*, and
200 its vector, *Anopheles gambiae*, have been published and reverse genetics has been
201 applied to the parasite, our knowledge of malaria biology has expanded. However,
202 this infectious disease is still life threatening. As I mentioned earlier, the main rea-
203 son for failure in eradication of this disease is the appearance of parasites tolerant to
204 drug and vaccine treatments, and we need to find new ways to eradicate this disease.
205 In this context, TBV might be a new weapon to combat the parasites. Because para-
206 site fertilization is the first stage in the mosquito life cycle, it is assumed that the
207 parasite fertilization factor would be the most effective TBV candidate. We under-
208 stand that there are many hurdles to overcome before a long-lasting and highly
209 effective TBV is obtained. Nevertheless, we believe that our attempt will result in
210 not only a discovery of new TBV but also in clearer understanding of the molecular
211 mechanisms underlying malarial parasite sexual reproduction.

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Uncorrected Proof

Chapter

INSIGHTS INTO ANIMAL SCHISTOSOMIASIS: FROM SURVEILLANCE TO CONTROL

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ABSTRACT

The role of animals in the transmission of schistosomiasis has not been given much importance, in fact massive efforts for the control of this disease have only been directed to humans. Other than human, more than 40 mammals are known to host the zoonotic *Schistosoma japonicum*. These animals somehow served as important "silent" sources of this parasitic disease in the transmission of the parasite and might be the loophole in the control of schistomiasis in endemic areas. However, minor steps toward animal schistosomiasis control have been taken, which include the use of recombinant antigens as a promising diagnostic tool for animals, as well as the admission of animals in the vaccination trials in China. This chapter will therefore tackle the importance of animals in the transmission of schistosomiasis, as well as the current updates on the zoonotic surveillance and control of this parasitic disease. It also aims to promote the integration of the public health and veterinary

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sectors which might lead to the possible elimination of the zoonotic schistosomiasis.

Keywords: S*chistosoma japonicum*, zoonotic surveillance, veterinary schistosomiasis, diagnostic tools, vaccine

ZOONOTIC SCHISTOSOMIASIS

The public health impact of animal schistosomiasis, such as the ones caused by *Schistosoma incognitum* among rodents [1], *S. currasoni* in sheeps [2], *S. hippopotami* and *S. edwardiense* found among hippopotamus [3] are very little compared to the zoonotic *S. japonicum*. *S. japonicum* is the major cause of zoonotic schistosomiasis in Asia, particularly in China, the Philippines and some parts of Indonesia. *S. japonicum* is known to infect over 40 different species of wild and domestic animals [4] belonging to the seven Orders in the Kingdom Mammalia (Figure 1).

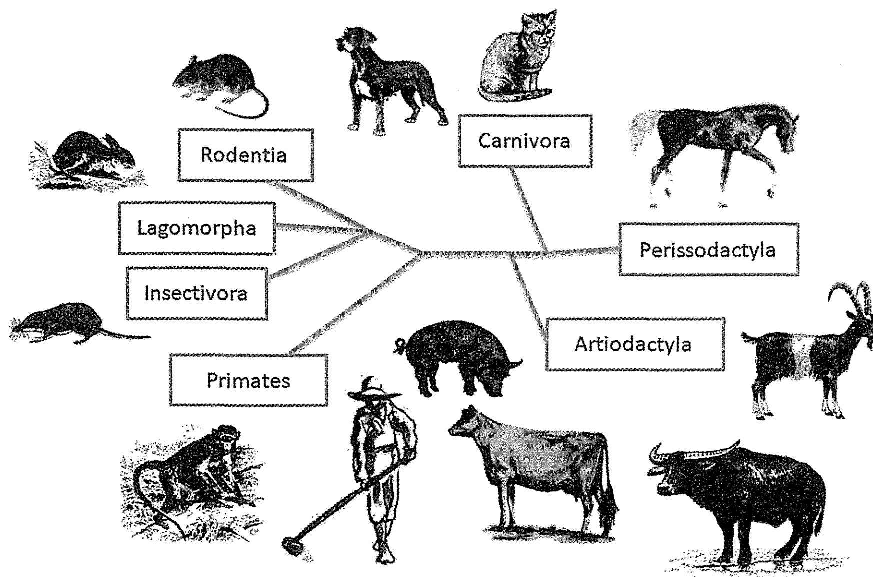

Figure 1. *Schistosoma japonicum* is known to infect a wide variety of animals belonging to different orders in the Kingdom Mammalia which includes human and other domesticated animals.

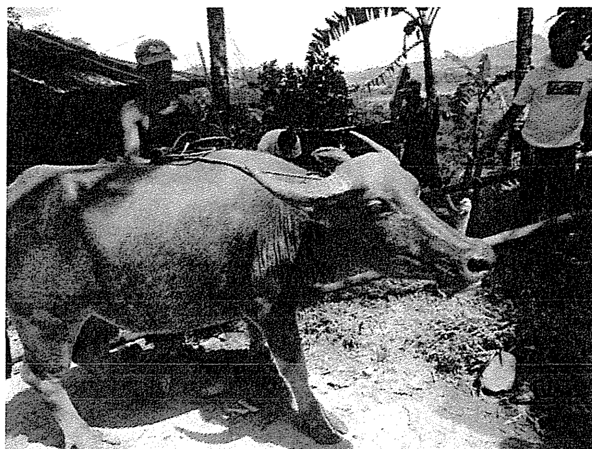

Figure 2. Domesticated animals including water buffaloes live so close to the human hosts that they even share infection caused by zoonotic parasites like *S. japonicum*.

These animals serve as reservoir hosts for the schistosome parasite which considerably complicates the control programs done in the endemic countries. These reservoir hosts play a significant role in maintaining the parasite, especially in areas where there is close co-existense between dense populations of humans and dense snail population. The close proximity of domesticated animals such as the dogs and water buffaloes to the human hosts as seen in Figure 2 is an important factor that can contribute in maintaining this parasite in endemic localities like the ones seen in rural agricultural areas. However, despite of this important role of animals in the parasite transmission, disease surveillance and control are only directed to the human hosts which might be a hindrance in obtaining the elusive disease elimination. On the other hand, the minor human schistosome *S. mekongi* was also found in animals such as dogs [5] and pigs [6]. Whether there is an active transmission between these animals and humans are not indicated so far with *S. mekongi*. The African *S. mansoni* is different with these two zoonotic schistosome species as the infected animals such as dogs most often do not shed the eggs in their excreta [7].

THE ROLE OF ANIMALS IN SCHISTOSOMIASIS

Animals have already been implicated as possible sources of the schistosome parasite in schistosomiasis-endemic countries. In China, the most

important reservoir hosts for *S. japonicum* are the bovines including the cattle and water buffalo [8-9]. Bovine defecation owned a large proportion of the environmental contamination for *S. japonicum* in China's schistosomiasis-endemic areas [10]. In Guanghui village, the relative transmission index (RTI) for schistosomiasis was calculated and found that water buffaloes were accounted for nearly 90% of the transmission, even higher than the 80.4% RTI from humans [11]. A study done using microsatellite markers has found that *S. japonicum* larval samples examined were divided into two clusters: one from the isolates from humans, cattle and water buffalo; and the other from dogs, cats, goats and pigs [12]. This suggested that transmission in China is more closely associated with bovines than the other domesticated animals.

In Indonesia, Izhar et al., have described that a small portion of domestic animals like water buffaloes and wild animals were found to be infected with the schistosome parasite [13]. On the other hand, a wide range of animals in the Philippines such as rats, cats, dogs, pigs, cattle and water buffaloes were found to be potential hosts for schistosomiasis using parasitological and immunological assays [14-16]. Furthermore, a population genetic study among these animal reservoir hosts, performed in one endemic province in the Philippines, suggests that there is a very high level of transmission between humans and dogs [17]. Water buffaloes, on the other hand, were examined in two separate studies and half of the examined population was found to be infected with *S. japonicum* [18-19]. These results strongly suggest that dogs and water buffaloes are the major reservoir hosts of schistosomiasis in the Philippines.

However, an important factor in the transmission of schistosomiasis is the number of schistosome eggs being passed in the excreta from the animal hosts that contaminates the environment, in addition to the duration of egg excretion and egg viability. Previous studies have shown that the number of eggs passed in the feces per female worm for each day differs between species [20]. Horses, goats and sheep shed the fewest, while the largest number of excreted eggs is seen in dogs and pigs as shown in Table 1. However, water buffaloes are more susceptible in acquiring *S. japonicum* than the other reservoir hosts as they spend more time in water, which might be contaminated with the infective *cercaria*. This water activity of the buffalo also largely contributes to the spread of the parasite making them important disease-transmitting hosts in the marshland areas of China. Furthermore, miracidial hatchability should be considered in assessing the actual contribution of the infected animals. It has been shown that excreted eggs from different host species have different hatching rates [21]. Therefore, it is more recommended to determine the

number of miracidia per gram of stool rather than counting the number of eggs per gram of stool.

Table 1. Differences in the development of *Schistosoma japonicum* among domesticated animals [4]

Host	Worm establishment after one single infection (%)	No. of eggs shed in excreta per female worm per day	Persistence of egg excretion period post-infection
Dog	50-77	300-2000	8-12 months
Rabbit	37-75	100-500	1 year
Goat	39-76	50-100	1 year
Sheep	7-65	50-200	1 year
Pig	2-15	200-2000	6-10 months
Cow	41-65	150-600	>1 year
Water buffalo	5-16	150-600	6-8 months
Horse	<1	Few	1-4 months

Availability and abundance of the permissive hosts in an endemic area are also important in the transmission of the parasitosis. This makes the domesticated animals such as dogs, pigs and bovines to play a more significant role in the transmission than wild animals such as field rats, rabbits and monkeys. *S. japonicum* is also known to exist in Taiwan but the Formosan strain is zoophilic and cannot infect humans [22]. In early epidemiological studies done in Taiwan, domestic animals [23] including dogs, buffaloes, goats and pigs, as well as the wild *Rattus norvegicus*, *Crocidura murina*, *R. losea*, *Mus formosanus* and *R. rattus* [24] were found harboring the schistosome parasite. In 1986, an outbreak of schistosomiasis occurred at a cattle farm in Hanpao Fram, Fangyuan District, Chonghua County and have killed at least 15 cattle [25]. This indicates that this schistosome strain is a threat to the livestock animals not to humans.

Schistosome Infection in Animals

Unlike in the human host, the pathology caused by *S. japonicum* among the various animal reservoir hosts still remains to be cleared and is not much unraveled. Bovine schistosomiasis caused by *S. bovis* [26], *S. matthei* [27] and *S. spindale* [28] was known to show various lesions in the livers, intestinal tracts and other organs. On the other hand, canine schistosomiasis is mainly

caused by the non-human schistosome *Heterobilharzia americana* in the North America which causes chronic intermittent dysentery, loss of weight, anemia and lymphadenopathy in dogs [29]. The pathological conditions seen with *S. japonicum* infection however differ among the reservoir host species.

In China, He et al., examined and compared the development rates of *S. japonicum* in mice, rats, guinea pigs, rabbits, goats, sheep, pigs, water buffalo, yellow cattle, horses and another 12 kinds of animal under identical conditions for up to 60 weeks [30]. The results presented that the development rates of the zoonotic schistosome in these hosts were quite different, with the highest infection rate of 60.3% seen in goats, 43.6% in yellow cattle, and 1% in water buffalo and horses. These differences in the parasite development inside the animal hosts are mainly due to the parasite clearance by the animal's immune system and non-immune system factors, called the self-cure phenomenon. The sharp drop in the worm burden after a period of *S. japonicum* infection was only seen in some animal hosts, such as rats, pigs [31] and water buffalo [32], but not in other susceptible animals like mice and yellow cattle.

Another study was done by Yang et al., comparing the development of *S. japonicum* in two important bovine hosts in China [33], namely the yellow cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*). The authors showed that the yellow cattle was found to be more suited to the development of *S. japonicum* than the water buffalo. Moreover, the cattle had more serious pathological damage. This high incidence of infection among the cattle might therefore possibly explain the significant mortality in this animal seen in most of the endemic areas. It was also shown that there is a decreased number of CD4+ T cells in yellow cattle, an increased proportion of CD8+ T cells in water buffalo, and a decreased CD4/CD8 ratios in both species after schistosome challenge, suggesting that CD4+ T cells could be an integral component in the immune response against the parasite and might explain the worm development in yellow cattle. Results of an earlier study on the schistosome-sensitive mice were consistent with the above reported observations [34]. Yang et al., also compared the general morphology and ultrastructure of adult schistosomes derived from the two bovine hosts as well their gene expression profiles using high-throughput microarray technology [35]. Results of the ultrastructure observations showed that the schistosomes from water buffalo differ from those of the yellow cattle as the adult worms have a collapsed and loose surface, with fewer spines and sensory papillae, dissolved cytoplasmic organelles and more vacuole structures in the male worm tegument. On the other hand, their results also identified genes that were differentially expressed in worms from the two natural hosts. Schistosomes

extracted from water buffalo overexpressed genes associated with the stimulus response, and lipid and nucleotide metabolism, and protein kinase and phosphatase; genes associated with reproduction, anatomical structure morphogenesis and multifunctional motif were underexpressed. These differentially expressed genes (mainly involved in nucleotide, energy and lipid metabolisms, transcription, transport and signaling pathways) greatly affect the survival and development of schistosomes in different natural host species. These results provide significant information that will possibly help in understanding the differences in the developmental rates among different reservoir hosts as well as the serious pathological damage seen in some animals. Further elaboration of the mechanism of this self-cure phenomenon might also be important in the development of schistosome vaccine.

Field rats, on the other hand, were also known to have high percentages of infection in extensive field surveys done in China, the Philippines and Indonesia [36-37]. Rodents were seen to have the highest infection prevalence in hill areas within Anhui Province of China whereas the cattle had the highest infection prevalence in the marshlands [38]. However, the risk of transmission from these rodents seems to be minimal as the schistosome worms were found to be trapped in the lungs and only rarely mature to produce eggs [39]. This suggested that although the rodents are infected with the parasites, the importance of these animals in the disease transmission is definitive low. In contrast to this, epidemiological studies done in endemic areas in China and the Philippines showed excretion of many viable eggs in the feces from field rats [39-40]. Although field rats at individual level might not contribute significantly to transmission, the presence of enormous number of field rats in endemic areas might pose a potential problem for the spread of the parasite.

As shown in Table 1, worm establishment is generally low in pigs. There are also no visible clinical signs among the infected pigs except from the diarrhea at the onset of patency [41-42]. Due to acquired immunity and short life span, the role of the pigs in the disease transmission is somehow negligible. However, sows infected at 10 weeks of gestation showed transplacental infection [43]. This proved that pigs can still be a threat in transmitting the disease in endemic areas where pig-raising is usually done.

Animal Surveillance

Detection of the schistosome eggs in the stool samples through microscopy is still the gold standard for schistosomiasis diagnosis in both

humans and animals. Stool microscopy is known to show low sensitivity in diagnosing low infection and in areas with low endemicity. Therefore, more accurate coprological techniques were developed for schistosomiasis diagnosis such as the Danish Bilharziasis Laboratory (DBL) technique [44] and the formalin-ethyl acetate sedimentation-digestion (FEA-SD) technique. Carabin et al., measured and compared the specificity and sensitivity of the filtration and sedimentation DBL using one, two, three, four or five consecutive days of stool samples from various animal hosts in the Philippines [14]. Results of this study showed that the DBL technique has at least 96% sensitivity when three stool samples or more are collected on three separate days and at least 91% specificity when three stool samples or less were collected on three separate days. This therefore suggested that the maximum sensitivity and specificity DBL can achieve is by collecting three stool samples on separate days and results based on only one stool sample underestimate the true prevalence of animal infection.

On the other hand, FEA-SD has shown to have improved visualization of *S. japonicum* eggs in bovine feces [45]. This technique includes filtration, sedimentation, potassium hydroxide digestion and centrifugation steps prior to microscopy discriminating nearly 70% of debris from the fecal samples and rendering the remaining debris translucent. This will be useful in rural endemic localities as it is not costly and does not require expensive equipments.

As a better alternative for the less sensitive coprological methods, crude antigen-based immunodiagnostic techniques like circum-oval precipitin test (COPT) and enzyme-linked immunosorbent assay (SEA-ELISA, SWAP-ELISA) might be preferably used. However, crude antigens are known to cause cross-reactions with other helminthiasis, affecting its specificity. In addition, massive production of crude egg antigen for large scale diagnostic purposes such as epidemiological surveys is somewhat difficult and tedious. To possibly replace the crude antigens, recombinant antigens which are easier to produce have been characterized and studied for their diagnostic potentials. Only a few defined antigens have been identified so far for serological diagnosis of animal schistosomiasis (see Table 2). These antigens, which are tested using animal samples, yielded remarkable sensitivity and specificity with a range of 78 to 100%. These recombinant antigens therefore can be used in improving the schistosomiasis surveillance in endemic areas.

However, the success of zoonotic surveillance cannot rely on sensitivity and specificity. The universality of the antigens for schistosomiasis diagnosis is another criterion that needs to be considered.

Table 2. List of promising recombinant *S. japonicum* antigens characterized and tested for the diagnosis of animal schistosomiasis

Antigen	Species
26 kDa Glutathione-S-transferase (Sjc26GST)	Water Buffalo [46]
Fructose-1,6-biphosphate aldolase (FBPA)	Water Buffalo [47]
Gynecophoral canal protein (GCP), tetraspanin fragment (Sj23) and 28 kDa GST (rSjGCP-Sj23-Sj28)	Cattle [48]
Thioredoxin peroxidase-1 (SjTPx1)	Cattle [48], human [49], water buffalo [18]
Elongation factor 1-alpha (rSjEF1)	Cattle [48]
Large hydrophilic domain of Sj23 (LHD-Sj23)	Cattle [50]
Dentin sialophosphoprotein (Sj1TR)	Human [49], water buffalo [18]

The majority of these antigens were examined in either the cattle or water buffalo. Tandem repeat protein Sj1TR was tested for both humans [49] and water buffaloes [18] but results showed that it was only good for diagnosing the latter. Among the antigens tested, only thioredoxin peroxidase-1 (SjTPx-1) showed a promising diagnostic capability as it was shown to have remarkable diagnostic potentials in a number of species which includes human [49], cattle [48], water buffalo [18] and dog (Angeles et al., unpublished data) serum samples. Examining the potential of SjTPx-1 on other animal reservoir hosts like pigs and rats to develop a more universal diagnostic test for both human and animals is now underway. A multi-species diagnostic test will surely strengthen the disease surveillance for schistosomiasis, ensuring the detection of all active transmission mechanisms involved in one endemic area.

In the case of epidemiological studies and surveillance of animal infection in areas that have reached elimination level, a more sensitive and specific diagnostic test is highly required. Molecular techniques like polymerase chain reaction (PCR) have been developed for detecting animal infection. Wu et al., have compared stool microscopy with a real-time PCR detection of *S. japonicum* mitochondrial DNA in water buffalo stool samples [19]. Their results showed a very big difference in prevalence between the two techniques and suggested that microscopic-based techniques dramatically underestimate the prevalence of *S. japonicum* infection among the water buffaloes. Therefore there is really a need for better and improved diagnostic techniques to reach the real picture of animal transmission for schistosomiasis.

SCHISTOSOMIASIS CONTROL IN ANIMALS

Control measures done in schistosomiasis-endemic countries consist mainly of community-based praziquantel chemotherapy, sanitation, supply of safe water, provision of personal protection, snail elimination and health education.. However, schistosomiasis control is complicated such that these parasites are so thoroughly integrated into the ecosystems, in which they occur, making the control at the community level very difficult, despite available control mechanisms. In Japan, *S. japonicum* was eliminated in 1977 just before the drug praziquantel became available [51]. This success story was largely based on ecological approaches strongly implemented by the Japanese government. In contrast, most underdeveloped countries have neither the political will nor the infrastructure, nor enough funding for the control measures leading to the steady existence of schistosomiasis until this very day.

Despite over 50 years of concerted efforts for its control, schistosomiasis still remains to be a major public health concern in China [52]. Transmission interruption had reached 60% and transmission control 14.5% of all the schistosome-endemic areas in China, until the mid-80's. Ecological factors like flooding, however, led to snail diffusion and re-emergence of schistosomiasis in the Yangtze River in recent years [53].

In the Philippines, chemotherapy has made the prevalence and morbidity of schistosomiasis dropping significantly in many areas. [54]. But in the past few years, emergence of two new endemic foci has been reported primarily due to the mobility of the people and other ecological factors [55]. New effective control strategies against schistosomiasis therefore should be studied and applied to eradicate this disease of antiquity.

Inclusion of zoonotic interventions in the national control programs done in these endemic countries is a much-needed strategy. Previous studies have shown that animal interventions can successfully cause decline in the prevalence of *S. japonicum* infection among humans [56-57]. In a five-year praziquantel-based intervention study done around the Poyang Lake in Jiangxi Province, China, simultaneous treatment of the bovine hosts has proven to be effective in the reduction of human schistosomiasis cases. This has significantly changed the epidemiological situation in China, such that they now consider schistosomiasis primarily as a bovine disease that can be transmitted to humans and, therefore, bovine schistosomiasis should be managed first before the human one.

Rapid re-infection is indeed possible for schistosomiasis. This requires repeated treatments, which raise concerns on the development of drug resistance. Resistance for the anti-schistosome drug praziquantel has only been reported for *S. mansoni* in Africa [58-62], but not yet for the zoonotic *S. japonicum*. Seto et al., measured the efficacy of praziquantel for the treatment of *S. japonicum* in humans by conducting a cross-sectional survey in 33 villages of Sichuan Province and concluded that praziquantel remains to be an effective drug for treating this parasitosis [63]. Nevertheless, other strategies should be done to complement existing control measures and therefore, development of a protective vaccine against human and animal schistosomiasis might be an essential component on the long-term disease control. In addition, on one hand zoonotic transmission made the control programs more complex, on the other it also provides opportunities for novel approaches in vaccine development to prevent the human disease.

Development of transmission-blocking veterinary vaccines that can be used for livestock animals is a complementary approach in schistosomiasis control. Radiation-attenuated cercarial vaccines have been successfully tested in water buffaloes [64] and pigs [65] under field conditions in China. However, many inherent problems were found using the cercarial vaccines, such as difficulty on producing quality-controlled, reproducible batches of these vaccines. Thus, vaccine researches resorted to the use of either the protein or DNA vaccines that might be effective [66-69]. Table 3 shows the list of the *S. japonicum* protein and DNA vaccines tested in domesticated animal reservoir hosts.

The protective efficacy of the schistosome-derived glutathione S-transferase (GST) family has been thoroughly investigated. The schistosome GST enzymes, which exist as 26- (Sj26GST) and 28-kDa (Sj28GST) molecules, have demonstrated remarkable effects on the fecundity of the adult parasites. Vaccination of sheep with native unfractionated *S. japonicum* GSTs has induced 53% partial protection [76]. Recombinant Sj26GST (reSj26GST) is capable of stimulating anti-fecundity immunity in pigs [79], water buffaloes [77] and sheep [75] with more than 50% egg reduction. Wu et al., demonstrated that vaccination of cattle with reSj26GST could elicit protection for at least 12 months and a 30% worm burden reduction [78]. Natural field challenge of recombinant Sj28GST (reSj28GST) has shown 33% worm reduction and 36% fecal egg reduction in water buffaloes, but not in cattle [80]. In sheep reSj28GST has induced 34-69% worm reduction, 56-69% liver egg reduction and 43-60% fecal egg reduction [75].