Advances in diagnosis and spatial analysis of cysticercosis and taeniasis

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SUMMARY

Human cysticercosis, caused by accidental ingestion of eggs of *Taenia solium*, is one of the most pathogenic helminthiases and is listed among the 17 WHO Neglected Tropical Diseases. Controlling the life-cycle of *T. solium* between humans and pigs is essential for eradication of cysticercosis. One difficulty for the accurate detection and identification of *T. solium* species is the possible co-existence of two other human *Taenia* tapeworms (*T. saginata* and *T. asiatica*, which do not cause cysticercosis in humans). Several key issues for taeniasis/cysticercosis (T/C) evidence-based epidemiology and control are reviewed: (1) advances in immunological and molecular tools for screening of human and animals hosts and identification of *Taenia* species, with a focus on real-time detection of taeniasis carriers and infected animals in field community screenings, and (2) spatial ecological approaches that have been used to detect geospatial patterns of case distributions and to monitor pig activity and behaviour. Most recent eco-epidemiological studies undertaken in Sichuan province, China, are introduced and reviewed.

Key words: *Taenia solium*, taeniasis, cysticercosis, neurocysticercosis, epidemiology, transmission ecology, diagnosis.

INTRODUCTION

Taeniasis and cysticercosis (T/C) due to *Taenia solium* is one of the most pathogenic and potentially lethal but neglected parasitic diseases of rural areas in developing countries where local people eat raw or undercooked pork (WHO, 2010). As mentioned in the Preface of this special issue, *T. solium* is unique among other parasites including two other human *Taenia* spp. (*T. saginata* and *T. asiatica*). It infects humans with eggs (human cysticercosis) released from human tapeworm carriers, the only definitive host (human taeniasis), which develop into metacestodes in pork (porcine cysticercosis). Recent globalization has increased the risk of emergence of this complicated disease not only in developing countries but also in developed countries (Sorvillo *et al*. 2011; Yanagida *et al*. 2012). In China, pork is a favoured meat and in rural areas people frequently keep pigs as scavengers of human faeces. China is probably the biggest country endemic for *T. solium* and there are many hospitals and wards specialized for treatment of human cysticercosis (Yingkun *et al*. 1979; Chen *et al*. 2005; Ikejima *et al*. 2005; Li *et al*. 2006). T/C will remain an important public health issue in the future in China though the endemic areas may contract as urbanization increases. Indeed, through sustainable education of farmers about the risk of direct contact of pigs with human faeces, and the application of severe penalties for pig owners who owned cysticercotic pigs, T/C has become a more focal disease in rural China.

In this article, we summarize the recent methodological advances in detection and spatial analysis of *T. solium*, *T. saginata* and *T. asiatica* transmission that may allow efficient evidence-based control programmes to be designed. Consideration is given to the detection or identification of taeniasis carriers for differentiation of the three human *Taenia* species, for cysticercosis patients, either symptomatic or asymptomatic, and for cysticercotic pigs. For such approaches, immunological tools to detect antibody responses to cysticerci of *T. solium* in humans

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and pigs are essential. Also, copro-ELISAs for the detection of genus-specific antigens in human stool samples were introduced and later species-specific copro-DNA tests were developed. These molecular tools are highly useful for identification of the species and adult or larval stages of the parasite, as well as eggs in faeces. If these techniques are not available during the course of community-based field surveys, it may be very difficult to identify and treat taeniasis carriers before they move around or leave the community; in addition, pigs are easily killed and sold (Ito *et al*. 2011). Therefore, it is important to develop real-time detection systems for both cysticercosis and taeniasis. Geospatial quantified approaches will aid in determining the distribution of cases and whether local determinants affecting transmission are focal/aggregated or randomly distributed within an endemic community.

An international research consortium has studied transmission ecology of the zoonotic cestode *Echinococcus multilocularis* in various regions of China including Sichuan from 2001 to 2008, supported by US NIH-NSF funds (Giraudoux *et al*. 2013). This has, in parallel, allowed gathering of information about the situation of T/C in endemic areas in Sichuan and Yunnan Provinces (Ito *et al*. 2003) in close collaboration with Sichuan Centres for Diseases Control and Prevention (CDC). This consortium started epidemiological surveys of T/C in Sichuan from 2005. We now provide a review of the general field, with a focus on the epidemiology and transmission of T/C in several communities in Tibetan populations in western Sichuan, China.

TOOLS FOR AN ECO-EPIDEMIOLOGICAL APPROACH TO TAENIASIS/CYSTICERCOSIS

For community-based field work on taeniasis and cysticercosis, information on the evidence of NCC cases, adult *Taenia* carriers or pigs infected with *T. solium* cysticerci are essential starting points to focus an investigation at relevant location. In vast rural areas of developing countries such information may be made available from local health authorities (e.g. local CDCs in China) and hospitals. Also, it is essential to build and maintain a secured database of all families in the communities that include all the necessary information to be used for further epidemiological and spatial data analyses: how many family members, gender, age, occupation, history of expulsion of segments and of epilepsy, eating habits (e.g. uncooked pork or uncooked viscera), number of pigs owned, and the geographical location of houses.

Serology for cysticercosis diagnosis in humans and pigs

At present, diagnosis of neurocysticercosis is primarily based on imaging techniques including computed tomography (CT) and magnetic resonance (MR) in addition to clinical criteria (Ito and Craig, 2003). These imaging techniques are useful and accurate but sometimes limited by atypical images which are difficult to distinguish from neoplasms. Moreover, they are unsuitable for diagnosis in isolated communities. Therefore, immunological tests are considered to be important methods to confirm clinical findings and to help diagnostics. Two immunological tests, i.e. antibody detection of past and current infections and antigen detection of current infections, are available. Infection with *T. solium* leads to the production of a specific antibody, especially IgG, which can be detected in serum and cerebrospinal fluid. Thus, diagnosis of cysticercosis based on antibody detection is widely accepted because it can be done by using easily obtained serum samples. Numerous efforts have been directed towards identification and characterization of specific antigens for *T. solium* serodiagnosis. Gottstein *et al*. (1986) reported the species-specific antigens (8 and 26 kDa proteins) in crude extracts of *T. solium* metacestodes. Parkhouse and Harrison (1987) described glycoproteins in cyst fluids of *T. solium* and *T. saginata* using lentil-lectin affinity chromatography. Tsang *et al*. (1989) characterized glycoproteins in crude extracts of metacestode using lentil-lectin affinity chromatography and described the value of glycoproteins (seven glycoproteins ranging from 13 to 50 kDa) for differential serodiagnosis based on immunoblot analysis but not ELISA. Ito *et al*. (1998) have developed a simple method to purify glycoprotein antigens by preparative isoelectric-focusing electrophoresis (IEFE) from cyst fluid available for both ELISA and immunoblot analysis, and demonstrated the sensitivity and specificity for differential serodiagnosis of cysticercosis. Among specific antigens characterized, the glycoproteins (GPs) (10 to 26 kDa proteins under reducing conditions) in cyst fluid of *T. solium* metacestodes, which give close to 100% specificity and more than 95% sensitivity in patients with multiple cysts or 30–60% sensitivity in patients with a solitary cyst, have been widely accepted for serodiagnostic purposes (Ito and Craig, 2003; Deckers and Dorny, 2010).

Diagnostic performance is dependent on the quality of GPs while the quality of GPs antigens depends on the antigen purification methods (e.g. affinity purification using lentil lectin, preparative IEFE and affinity purification using GPs-specific antibody) and/or the quality of material used for antigen purifications (whole cyst or cyst fluid only). For preparation of native GPs, naturally infected pigs must be located in endemic areas from which to obtain cysts; alternatively, experimental infections can be used after identifying *T. solium* taeniasis patients and collecting infective eggs. These two strategies are not always feasible. To overcome this situation, molecular techniques have been applied

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to produce recombinant antigens because of easier management of the quality and quantity. Until now, several genes encoding serodiagnostic antigens have been identified. Most of these genes encode polypeptides of approximately 8 kDa, show homologies among each other, and have 0–3 predicted *N*-linked glycosylation sites (Deckers and Dorny, 2010). Using ELISA with recombinant 8 kDa family antigens, close to 90% of cysticercosis patient sera were judged as positive, which indicated the usefulness of recombinant protein as a serodiagnostic antigen (Chung *et al*. 1999; Greene *et al*. 2000; Sako *et al*. 2000; Hancock *et al*. 2003). When the recombinant antigen is used in the immunodiagnosis, the possibility that the difference between the native antigen and the recombinant antigen affects the serodiagnostic performance has to be considered. In this case, the difference is the existence of sugar chains. Native antigens might be highly glycosylated, and the carbohydrates were thought to be key antigenic parts for immunodiagnostic sensitivity. In fact, Obregon-Henao *et al*. (2001) demonstrated diminished antibody reactivity for the native antigen after deglycosylation. Nevertheless, the antigenicities of the recombinant 8 kDa family of antigens expressed in *Escherichia coli* or synthesized chemically (and thus not glycosylated) were comparable to those of native GPs, which indicates that antibodies to peptide, not carbohydrates, occur in patient serum and allow detection of *T. solium* infections (Sako *et al*. 2000; Hancock *et al*. 2003). Other antigenic proteins have also been characterized and used in the serology of cysticercosis (Hancock *et al*. 2004, 2006; Ferrer *et al*. 2005, 2007). In comparison to the 8 kDa family of antigens, their specificity and sensitivity seem to be lower.

It has been demonstrated that both native GPs antigens and recombinant antigens were applicable for the detection of specific antibodies in pigs by ELISA and immunoblot as well (Sato *et al*. 2003). Using ELISA, specific antibodies were detectable in experimentally infected pigs harbouring at least 16 *T. solium* cysts from 30 days after infection. Additionally, pigs naturally infected with the larval stage of *Taenia hydatigena* showed negative reactions to both antigens but the number of *T. hydatigena*-infected pigs examined was not sufficient to conclude that both antigens are highly specific to only *T. solium* infections in pigs. Although we had no information on or no interest in the numbers of *T. hydatigena* co-infected pigs, all pigs that were confirmed to be naturally infected with *T. solium* in Mexico, China and Indonesia showed reasonably good antibody responses to purified GPs (Ito *et al*. 1999). As these countries are expected to be highly endemic for *T. hydatigena*, we expect that purified GPs or recombinant antigens are more specific to *T. solium*. Further evaluations of both antigens using more sera from *T. hydatigena*- and other parasite-infected pigs are needed. Although antibody detection test using serum samples is a useful way to diagnose *T. solium* infection, the possibility that serum samples from patients who were unsuccessfully exposed and failed to be infected and/or already cured become positive (Garcia *et al*. 2001) must be considered.

Coprology for taeniasis diagnosis

A self-detection tool of tapeworm carriers and microscopy. Expulsion of proglottids is a specific symptom in taeniasis-infected persons. Carriers may report the presence of proglottids in faeces, in a toilet towel or, not uncommonly, felt in the undergarments (Ito *et al*. 2013). With *T. saginata* and *T. asiatica*, tapeworm proglottids are frequently expelled, and expulsion is spontaneous (independent of defaecation), whereas in *T. solium* taeniasis carriers expulsion of proglottids is passive (together with faeces) and occasional. *T. saginata*/*T. asiatica* carriers are often aware of the presence of a tapeworm, but this is not necessarily the case for *T. solium*. Therefore, questioning about the history of expulsion of proglottids can be used as an auxiliary method for the diagnosis of taeniasis infection. The feasibility of self-detection of tapeworm carriers has been previously confirmed not only for *T. saginata* and *T. asiatica*, but also for *T. solium* (Sarti *et al*. 1992; Schantz *et al*. 1993; Flisser *et al*. 2005; Li *et al*. 2012). The reliability of the clinical history of proglottid expulsion was largely different based on previous reports, and ranged from less than 50% in Honduras to over 80% in Sichuan, China (De Kaminsky, 1991; Li *et al*. 2012). The differences might be attributable to the predominant *Taenia* species, and habits/customs of local people. To improve reliability, it is advisable to carry out a public health education intervention in advance (Flisser *et al*. 2005).

Stool microscopy is one of the conventional techniques for diagnosis of taeniasis. It is generally agreed that microscopy lacks sensitivity (Pawlowski and Schulz, 1972), but repeated microscopic examinations are able to improve the diagnostic value (Hall *et al*. 1981). Concentration techniques such as ether sedimentation and formalin–ether concentration can be applied, which may detect about 62–68% of *T. saginata* and 38% of *T. solium* cases (Hall *et al*. 1981; Deplazes *et al*. 1991; Allan *et al*. 1996). In addition, 'Scotch' tape peri-anal swabbing is also used for detection of *T. saginata* and *T. solium* eggs (Pawlowski and Schulz, 1972; Schantz and Sarti-Guttierez, 1989; De Kaminsky, 1991), which is more sensitive than a single coprological examination with *T. saginata* (Pawlowski and Schulz, 1972). Anthelmintic treatment can detect many more tapeworm carriers than either coprological techniques or questioning (Hall *et al*. 1981; De Kaminsky, 1991; Allan *et al*. 1996).

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Coproantigen detection. Research in the 1980s/90s on experimental taeniases and hymenolepiasis in laboratory animals and dogs indicated that tapeworm-derived antigens could be detected by ELISA in host faeces (i.e. coproantigens) (Allan and Craig, 1989; Allan *et al*. 1990; Deplazes *et al*. 1990). Polyclonal antibodies from hyperimmunized rabbits against somatic or excretory/secretory (ES) tapeworm antigens were efficacious in robust detection of coproantigens before and during patency in those experimental infections, and showed that coproantigen levels in humans and animals rapidly declined (usually within 5–15 days) following successful anthelmintic treatment (Allan and Craig, 2006; Bustos *et al*. 2012).

Application of the coproantigen ELISA approach for detection of human taeniases quickly followed, and more than doubled the sensitivity compared to microscopy for the diagnosis of *T. solium*. Very high genus level (i.e. *Taenia* spp.) specificity was also apparent with specificities >98% when assessed against other non-*Taenia* spp. gut helminth infections including nematodiases and hymenolepiasis (Deplazes *et al*. 1991; Allan *et al*. 1992). Subsequent community application of *Taenia* copro ELISAs generally indicated, when compared to treatment/purge follow-up, very good detection ability for *T. solium* and *T. saginata* taeniases in Latin America (e.g. Garcia-Noval *et al*. 1996; Rodriguez-Canul *et al*. 1999; Lescano *et al*. 2009) and for all 3 human *Taenia* species, including *T. asiatica*, in SE Asia (Li *et al*. 2006; Somers *et al*. 2006; Wandra *et al*. 2006). A putative species-specific *T. solium* coproantigen ELISA has been reported which incorporated an anti-*T. solium* somatic capture antibody and an anti-*T. solium* ES detection conjugate; it showed no cross-reactivity with *T. saginata* infections, and had a sensitivity of 96·4% for *T. solium* carriers in Peru (Guezala *et al*. 2009).

Taenia and *Echinococcus* coproantigens are resistant to fixation in 5–10% formal saline, an advantage for community studies when stool samples cannot be frozen (Allan and Craig, 2006). Immunobiochemical analysis of *Echinococcus* coproantigens indicates they are highly glycosylated, with O-linked saccharides and N-linked glycans predominant being present on the surface glycocalyx of the tapeworm and shed in host faeces (Elayoubi and Craig, 2004; Hulsmeier *et al*. 2010). Interestingly, experimental *Taenia* spp. infections indicated detectable coproantigen levels by approximately 18–21 days post-infection (dpi) in dogs (Deplazes *et al*. 1990; Allan *et al*, 1992) and by 5 dpi in *T. solium* infected hamsters (Allan *et al*. 1990). However, in 5 *T. saginata* voluntary self-infections, coproantigens were detectable much later post-infection i.e. from 73 to 149 dpi (Tembo, 2010). Although it is most important to identify *T. solium* carriers, this assay would miss cases of *T. saginata* or *T. asiatica* in co-endemic areas, which might be a disadvantage for broader epidemiological studies on human taeniases.

Coproantigen detection and monitoring has proved very useful in epidemiological studies (reviewed by Allan and Craig, 2006), in evaluating the effects of health education (Sarti *et al*. 1997) and mass treatment (Sarti *et al*. 2000) on human taeniasis, but also at the clinical level, for example in detection of anthelmintic treatment failures for taeniasis in community intervention studies in Peru (Bustos *et al*. 2012). In addition, coproantigen ELISA has proved an important surveillance tool for post-intervention studies (Allan *et al*. 2002) and in geospatial analysis of porcine cysticercosis and taeniasis risk (O`Neal *et al*. 2012).

Copro-DNA detection. Reliable epidemiological information on the prevalence of taeniasis, especially *T. solium* taeniasis, depends on the accurate identification of the causative parasite. The diagnostic methods based on the microscopic examination are inadequate. Because proglottids of *Taenia* species are morphologically similar to each other, technical skills are needed to identify species. In addition, there is no difference in the morphology of eggs among taeniid parasites. Furthermore, the sensitivity of this method is far from satisfactory.

To obtain precise information on *Taenia* infections in a definitive host, tools using molecular techniques including conventional PCR, PCR-restriction fragment length polymorphism (RFLP), multiplex-PCR and Loop-mediated isothermal amplification (LAMP) have been developed. PCR is a highly specific and sensitive method for amplification of a specific DNA target from a few copies to many. Mayta *et al*. (2008) have developed *T. solium*-specific nested-PCR assay targeted to Tso31, oncosphere-specific protein, gene. With faeces, the specificity and sensitivity of this assay were 100 and 97%, respectively, and the lower detection limit was 10 eggs per 250 mg of faeces. This assay cannot detect *T. saginata* and *T. asiatica*, which may be a disadvantage for epidemiological studies of the different taeniases. For differential identification of human *Taenia* parasites, RFLP analysis of PCR products is often carried out because this method is simple and gives unambiguous results (Mayta *et al*. 2000; Nunes *et al*. 2005). The PCR-RFLP for taeniasis can now differentiate *T. solium* from *T. saginata*, but whether these assays can differentiate *T. asiatica* from other two species is not clear. In addition, PCR plus restriction enzyme treatment is relatively time-consuming. To overcome this problem, differential identification by PCR assay only without restriction enzyme treatments of PCR products has led to the use of a multiplex-PCR which can amplify several target genes by using several primer sets in a single reaction (Yamasaki *et al*. 2004*a*, *b*; Gonzalez *et al*. 2004, 2010). Gonzalez *et al*. (2004) have demonstrated the usefulness of

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the multiplex-PCR targeted HDP2, non-coding repetitive DNA fragments in nuclear DNA, for differential identification of *T. saginata* and *T. solium*, but this assay lacked the ability to differentiate *T. asiatica* from *T. saginata*. Recently, these authors have developed a novel multiplex-PCR assay targeted to the HDP2 DNA fragment which can differentiate three *Taenia* parasites (Gonzalez *et al*. 2010). The sensitivity limit and the capability for detection of parasites in faeces with this novel assay are still unknown. Yamasaki *et al*. (2004*a*, *b*) have established the multiplex-PCR for a comprehensive identification of human taeniid cestodes based on *cox1* genes. Unlike HDP2-multiplex-PCR assay, this multiplex PCR method can differentiate not only three human taeniid parasites but two genotypes of *T. solium*. It has been demonstrated that this multiplex PCR method could provide reliable results if more than 50 eggs were present in 1 g of faeces. In this way, with the increase of genetic information available for the different *Taenia* species, higher specific and sensitive PCR assays should emerge.

It is not easy to exploit PCR techniques in the laboratories of developing countries because they require sophisticated equipment such as a thermal cycler. Furthermore, *Taq* DNA polymerase used in PCR is often inactivated by inhibitors present in biological material such as faeces, sometimes causing sensitivity and reproducibility problems. The recently developed LAMP method amplifies DNA with high specificity, sensitivity and rapidity under isothermal conditions (Notomi *et al*. 2000). This method requires a *Bst* DNA polymerase with strand displacement activity, four primers recognizing six regions on the target DNA and simple incubators such as water bath, block heater or thermos bottle. Unlike the *Taq* DNA polymerase, the *Bst* DNA polymerase resists many enzyme inhibitors in biological material, which means it is suitable for use with clinical samples (Mori *et al*. 2001; Mori and Notomi, 2009). For a differential detection and identification of *Taenia* species, Nkouawa *et al*. (2009) have developed the LAMP method targeting *cox1* and cathepsin L-like cysteine peptidase (*clp*) genes. This method is highly sensitive and specific for differential detection of *Taenia* species by using DNA prepared from proglottids, cysticerci and faeces of taeniasis patients in Indonesia and Sichuan (China). Evaluation using faeces from taeniasis patients has revealed higher sensitivity of the LAMP method than that of PCR (88·4 *vs* 37·2%, Nkouawa *et al*. 2010). Furthermore, Nkouawa *et al*. (2012) have demonstrated that differential identification of *Taenia* species by the LAMP method could be carried out successfully using a thermos bottle and hot water instead of an incubator requiring electricity if the reaction temperature could be maintained, indicating the usefulness of LAMP in the field. The fact that LAMP-positivity can be judged by the appearance of a large amount of white precipitate of magnesium pyrophosphate produced during DNA amplification visible to the naked eye is a great advantage in field (Mori *et al*. 2001), in contrast to PCR which depends on electrophoresis to detect the specific amplicon. Unfortunately, the multiplex-LAMP with several primer sets in a single tube is far from practical at present when compared with multiplex-PCR (Yamasaki *et al*. 2004*a*, *b*). Therefore, one primer set needs one reaction mixture. In the case of *Taenia*-LAMP, three reaction mixtures containing *T. solium*-, *T. saginata*- and *T. asiatica*-primers must be set up separately if the target gene is *cox1* gene. For future use, such complicated test methods should be simplified.

Molecular identification of the parasite. Several molecular tools have been developed and are now widely used for the species identification of human tapeworms. The molecular identification of *Taenia* spp. targets mainly the mitochondrial *cox1* gene, as it is variable enough to differentiate three human *Taenia* species. The above-mentioned multiplex PCR and LAMP methods are available for all the parasite stages (adult worm, metacestodes and eggs) in fresh, frozen and ethanol-fixed specimens (Yamasaki *et al*. 2004*a*; Nkouawa *et al*. 2009, 2010). These methods are highly sensitive and reliable, and the LAMP method is even possible for the identification of the adult worm without sophisticated equipment in field surveys (Nkouawa *et al*. 2012). DNA sequencing of mtDNA has also been used for the species identification. Although it requires more time, cost and labour, compared to the multiplex PCR and LAMP methods, DNA sequencing has two advantages. First, it is applicable to the formalin-fixed and paraffin-embedded (FFPE) histopathological specimens (Yamasaki *et al*. 2004*b*, 2006; Yanagida *et al*. 2010; Jongwutiwes *et al*. 2011; Swastika *et al*. 2012). In the case of cysticercosis, FFPE histopathological specimens are often the only available material for molecular identification. It is generally not suitable for molecular analyses because of the degradation of DNA in formalin-fixed materials. Therefore, short fragments of cox1 gene are amplified and sequenced for species identification (Yanagida *et al*. 2010; Jongwutiwes *et al*. 2011; Swastika *et al*. 2012). It is even possible to use the specimens after long-term preservation in formalin (Jeon *et al*. 2011). Secondly, DNA sequencing provides a clue to trace back the origin of the infection of *T. solium*. It is known that there are two mitochondrial genotypes which are geographically separated into Asian and African/Latin American countries (Nakao *et al*. 2002; Martinez-Hernandez *et al*. 2009). As *T. solium* generally has the unique mitochondrial haplotypes in each Asian country, it is possible to assess where the patient became infected (Yanagida *et al*. 2010, 2012). On the other hand, the

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African and Latin American haplotypes are often indistinguishable because of the recent introduction of the parasite from Europe (Nakao *et al*. 2002). Recently, sympatric distribution of both mitochondrial genotypes was demonstrated in Madagascar (Michelet *et al*. 2010; Michelet and Dauga, 2012).

The species status of *T. saginata* and *T. asiatica* is still a hot topic. The two species are genetically closely related and morphologically very similar, but their intermediate hosts are different. While metacestodes of *T. saginata* develop in the skeletal muscle of cattle, those of *T. asiatica* develop in pig viscera. Recently, evidence of hybridization between the two species has been reported from China and Thailand (Okamoto *et al*. 2010; Yamane *et al*. 2012). In the endemic regions of these two countries, several adult worms showed nuclear-mitochondrial discordance (e.g. *T. saginata*-type mtDNA with *T. asiatica*-type nuclear DNA). Thus, when both species are sympatric, we cannot exclude the possibility of hybrid worms by using mtDNA markers alone. In such cases, several nuclear genes can be used for confirmation of hybrid worms (Nkouawa *et al*. 2009; Yamane *et al*. 2012). However, it is not always possible to detect hybrids because the number of nuclear target genes is not sufficient. Apart from the debate whether *T. saginata* and *T. asiatica* are the same species or not, it is important to differentiate beef worms from pork worms. The intermediate host range of the hybrid worms is still not known. Further study is needed to clarify the host specificity of hybrid worms, by examining metacestodes from cattle and pigs in areas co-endemic for the two species using both mitochondrial and nuclear gene markers.

Spatial approach to transmission

Spatial analysis of human and animal host infection distribution. Key questions in spatial eco-epidemiology are (1) is the distribution of cases in the geographical area surveyed similar to the one of non-cases (control) or (2) is there an abnormal concentration of cases in some locations? Location refers to household, village or any spatial unit at a given scale. This question implicitly involves considering the population of non-cases along with the population of cases in the framework of data analysis. Surprisingly, only very few studies dealing with *Taenia* spp. transmission have included a spatial approach to detect spatial patterns of infection. Two methods were developed: (1) the detection of spatial clusters of human and animal cases using Kulldorff spatial scan statistics, Ripley's *K* functions, or variance-to-mean ratio (VMR) and nearest neighbour index (NNI); (2) statistical modelling of seroprevalence or neurocysticercosis using the distance to the nearest tapeworm carrier as a covariate. Spatial scan statistics are used to detect clusters of cases by gradually scanning a circular window (centred on each individual, household and village) across space and/or time, computing the number of observed and expected observations inside and outside the window at each location (Kulldorff, 1997). Scan statistics use various probability models (e.g. Bernoulli, Poisson) to predict expected observations depending on the nature of the data. For each window, a likelihood ratio statistic is computed based on the number of observed and expected cases within and outside the window and Monte Carlo hypothesis testing provides a *P* value. Ripleys' *K* function is based on the concept that the expected number of events (cases, controls) in a circle radius *t* is some function of *t* that depends on the pattern of the events (Fortin and Dale, 2005). In the absence of clustering it is expected that *K* function for cases across a range of distance is not different from *K* function for controls.

Lescano *et al*. (2007, 2009) have undertaken a comprehensive investigation of the epidemiological position of *T. solium* in both humans and pigs in seven low-income rural villages of the northern coast of Peru (212 households comprising 898 permanent residents). Mass treatment of human taeniasis identified 11 adult worm carriers, and epidemiological screening revealed seroprevalences of human and pig cysticercosis of 24% (196/803) and 30·8% (280/908), respectively. Taking a household as the statistical unit and using distance to the nearest carrier (from 0 to 2000 m) as covariate, they (1) failed to show any relationship with prevalence of neurocysticercosis-related seizure, (2) evidenced a weak but significant relationship with human seroprevalence (21% >50 m from a carrier and 32% from 2–50 m), and (3) showed a strong relationship with pig seroprevalence (18% >500 m, 36% between 500 and 51 m, 69% within 50 m). Morales *et al*. (2008) showed that prevalence of porcine cysticercosis, as assessed by tongue examination, (13·3% out of 562 pigs) significantly varied among 13 villages of a rural area in Mexico. They investigated clustering patterns within each of the 13 villages comparing VMR and NNI of all pig-rearing farms, farms with healthy pigs and farms with infection-positive pigs. VMR indicated clustering of infected pigs in 3 villages only but the values were similar to VMR obtained for all farms and farms with healthy pigs, suggesting that clustering of pigs was due to clustering of farms and not to local determinants fostering transmission. Clustering of several indices of *T. solium* and *T. saginata* infection was determined using a spatial scan statistic in South India, North Tanzania and Eastern Spain, yielding contrasting outcomes. In Catalonia (Spain), Allepuz *et al*. (2009) disclosed two clusters of farms with bovine infection in an epidemiological investigation aiming at understanding the causes of a *T. saginata* outbreak. In their study area of approximately 200 km^2 in India, Raghava *et al*. (2010)

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detected clusters of human seropositivity to *T. solium* cysticercal antibodies, of positivity to circulating cysticercal antigens and of taeniasis, but failed to detect clusters of neurocysticercosis. In a Tanzanian study area of approximately 4000 km^2, Ngowi *et al.* (2010) assessed baseline prevalence of *T. solium* cysticercosis in 784 pigs (lingual examination) and incidence of infection in 295 sentinel piglets left on average for 4 months in households (lingual examination and Ag-ELISA). Local clusters of households were detected for prevalence ($n = 1$), seroincidence ($n = 1$) and incidence of lingual infection ($n = 2$), and all these clusters geographically overlapped partly, suggesting one large hotspot of transmission in the north-east part of the area. Spatial pattern analysis using Ripley's K functions supported the general clustering pattern for seroincidence and incidence of lingual infection but did not evidence clustering for seroprevalence. No evidence on the processes explaining patterns and differences between studies was provided. The authors hypothesized that several factors were involved including the mobility of people who acquired infection, the loss of adult worms, the possible long delay between contact with a tapeworm carrier and seizure appearance, the under-detection of cases, differences in defaecation practices, pig rearing systems, and food and drinking habits. Other sources of variation should also be considered. The spatial range of investigations may be a key issue. For instance, spatial clusters may not be detected if the range of the whole study area is included within a cluster within which random or regular distribution might be detected. Nevertheless, these results can have direct applications on a given scale. For instance, in Tanzania they served as baseline information to target health education efforts (Ngowi *et al.* 2010).

Monitoring pig behaviour and activity range. Several studies have identified free-roaming pig behaviour as a risk factor associated with increased pig cysticercosis (Pouedet *et al.* 2002; Sikasunge *et al.* 2007; Pondja *et al.* 2010), but some others have not (Morales *et al.* 2008; Jayashi *et al.* 2012). This apparent discrepancy may lie within differences in human defaecation facilities (e.g. open-air defaecation in a household might lead to infection of a pig even if it is retained) and behaviour (defaecation outside a household) or pig confinement efficiency. Very few data are available on the pig activity ranges and behaviour within villages or settlements to evaluate, for instance, how far from their owner's house pigs can potentially become infected. Copado *et al.* (2004) quantified the behaviour of free-ranging pigs, including the consumption of human faeces, in a rural setting with a population of 900 people living in 182 houses, located in the tropical area of Mexico (a place known to be endemic for *T. solium* transmission and where the frequency of outdoor defaecation by people is high). Their main findings were: (1) pigs spent more time feeding and moving around during the rainy season (1 h, 26 mn and 3 h, 01 mn, respectively) than during the dry season (43 mn and 1 h, 38 mn, respectively); (2) pigs walked 1023 ± 493 m per day during the dry season *vs* 2775 ± 1429 m during the rainy season; (3) the frequency of human faeces consumption was higher during the dry season (0·95 events/h *vs* 0·48 events/h); and (4) in both seasons adult females consumed human faeces more frequently (0·32/h during the dry season and 0·19/h during the rainy season) than pigs in the other age groups, and adult males consumed faeces more frequently than juveniles males. In an area of western Sichuan (China) where *T. solium* is suspected to be highly endemic in Tibetan communities (Li, unpublished data) we obtained original data on pig activity ranges during the course of an epidemiological investigation. Marihe village (Yajiang prefecture) comprises 21 households spread over 530 m along a banana-shaped valley at an altitude of 2550 m. In October 2011, a total of 22 pigs belonging to 16 households were individually marked using a paint code on both sides of the neck, and the geographical coordinate of the owner house was taken using hand-held GPS (Garmin GPSMAP 62). We then walked systematically in the village for 2 days and noted the geographical coordinates of each pig. Among the 22 marked pigs only 14 were observed outside their houses. The maximum Euclidian distance between the release point and the most distant observation ranged between 65 and 475 m with an average of 276 m (Fig. 1). This suggests that most pigs, at that time of the year (October), can occasionally walk across almost the entire area of Marihe village. Such behavioural data can be useful to understand the transmission system on a local scale in terms of seasonal patterns of pig infection and of the spatial scale at which pigs can become infected.

CONCLUSION AND FUTURE DIRECTIONS

A number of methods are available for the diagnosis of T/C in human and animal hosts but several improvements are still needed to establish efficient evidence-based control programmes. In particular, an easy-to-use multiplex LAMP method that allows the discrimination of different *Taenia* species in faeces during the course of field community screening, as well as an improved ELISA to detect specific antibodies against *T. solium* or *T. hydatigena* in infected pigs would be valuable. Field investigations are required to identify which animals act as intermediate hosts of *T. saginata/asiatica* hybrids by using both nuclear and mitochondrial DNA markers. A few decades ago T/C was one of the national parasitic diseases in China, but it is now one of the NTDs, still highly endemic in remote and rural areas, especially in minority territories where meat

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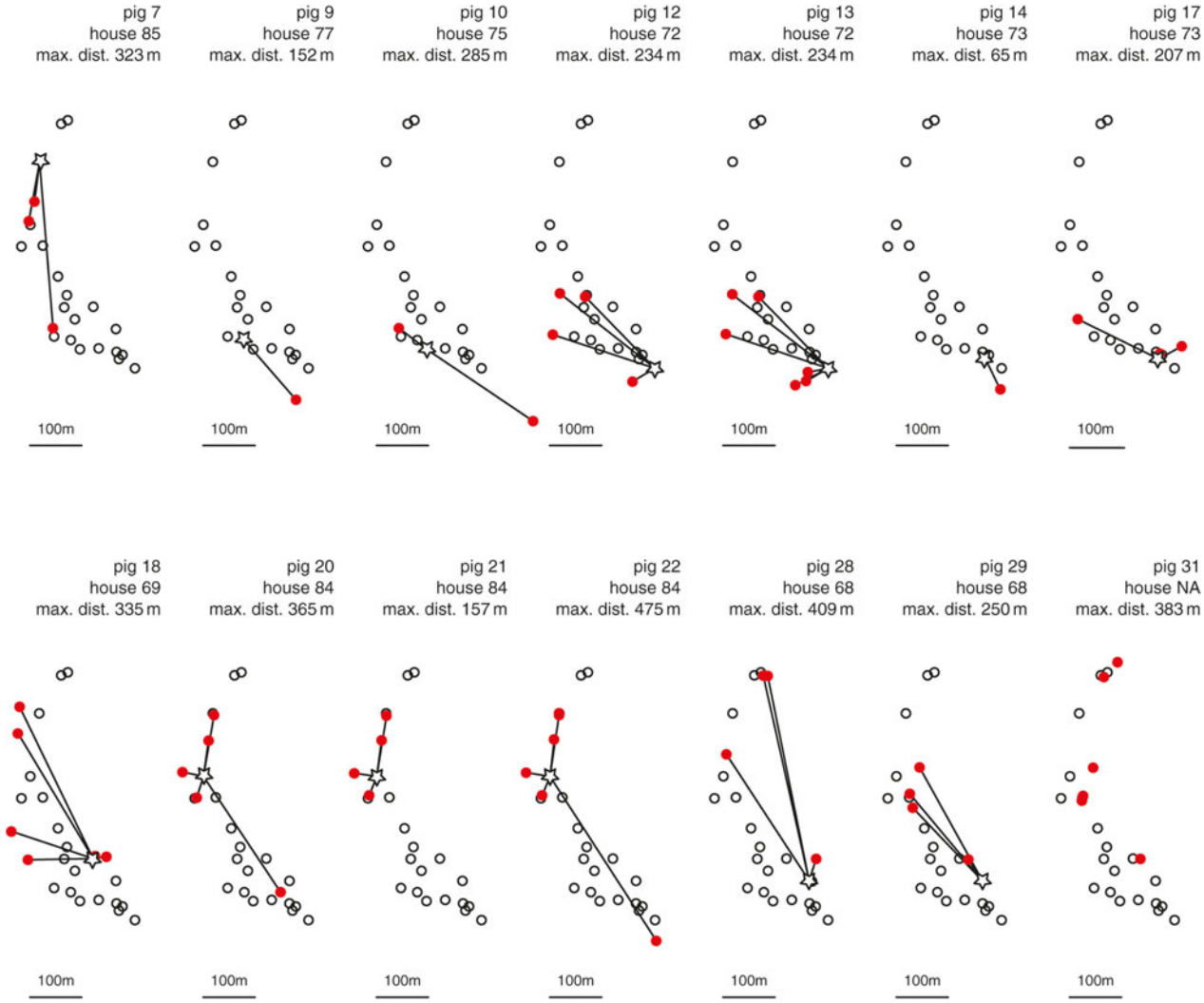

Fig. 1. Maps of observation locations of 14 colour-marked pigs in Marihe village (Sichuan, China) in 2011. White circle: village houses, White star: owner house where known, Red circle: observation location. Maximum Euclidian distance between the release point (owner house) and the most distant observation is given ('max. dist.'), except for pig 31 for which maximum Euclidian distance is calculated between the capture point and the most distant observation. Many pigs occasionally walk across the entire village.

inspection systems as well as sustainable education against T/C are lacking. The epidemiological picture is still largely under-evaluated in other regions/provinces of China (e.g. Yunnan) and elsewhere in the world (e.g. Indonesia) where raw meat consumption is normal. Spatial approaches have proven useful in identifying spatial clusters of human and animal cases and in assessing the spatial scale of pig contamination in villages. Such investigations are still barely reported in the literature, probably because of the lack of multidisciplinary research consortia involving ecologists, veterinarians, parasitologists, molecular biologists and public health practitioners.

Taeniasis carriers are a major force for infection of other people in the communities as well as themselves and additionally can migrate without any treatment from remote endemic areas to local cities and capital cities to seek employment. Therefore, T/C is no longer a local disease but in fact a neglected widely spreading disease which may cause sudden death outside endemic areas, a situation expected to be common in almost all countries. We expect that national campaigns for control of T/C and taeniases and cysticercoses in domestic animals caused by two other human *Taenia* spp. (*T. saginata* and *T. asiatica*) will be introduced for the prevention of this local disease spreading into urban areas. The most important prerequisite for the future control in endemic countries is to conduct more evidence-based-medicine, especially evidence-based-transmission ecological studies aimed at gathering direct evidence of infection in humans and in animals.

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REFERENCES

Allan, J. C., Avila, G., Garcia Noval, J., Flisser, A. and Craig, P. S. (1990). Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* **101**, 473–477.

Allan, J. C. and Craig, P. S. (1989). Coproantigens in gut tapeworm infections: *Hymenolepis diminuta* in rats. *Parasitology Research* **76**, 68–73.

Allan, J. C. and Craig, P. S. (2006). Coproantigens in taeniasis and echinococcosis. *Parasitology International* **55**, S75–S80.

Allan, J. C., Craig, P. S., Garcia Noval, J., Mencos, F., Liu, D., Wang, Y., Wen, H., Zhou, P., Stringer, R., Rogan, M. T. and Zeyhle, E. (1992). Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitology* **104**, 347–355.

Allan, J. C., Craig, P. S. and Pawlowski, Z. S. (2002). Control of *Taenia solium* with emphasis on treatment of taeniasis. In *Taenia solium Cysticercosis from Basic to Clinical Science* (ed. Singh, G. and Prabhakar, S.), pp. 411–420, 422–430. CABI, Wallingford.

Allan, J. C., Velasquez-Tohom, M., Torres-Alvarez, R., Yurrita, P. and Garcia-Noval, J. (1996). Field trial of the coproantigen-based diagnosis of *Taenia solium* taeniasis by enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene* **54**, 352–356.

Allepuz, A., Napp, S., Picado, A., Alba, A., Panades, J., Domingo, M. and Casal, J. (2009). Descriptive and spatial epidemiology of bovine cysticercosis in North-Eastern Spain (Catalonia). *Veterinary Parasitology* **159**, 43–48.

Bustos, J. A., Rodriguez, S., Jiminez, J. A., Moyano, L. M., Castillo, Y., Ayvar, V., Allan, J. C., Craig, P. S., Gonzalez, A. E., Gilman, R. H., Tsang, V. C. W. and Garcia, H. H. (2012). Detection of *Taenia solium* taeniasis coproantigen is an early indicator of treatment failure for taeniasis. *Clinical and Vaccine Immunology* **19**, 570–573.

Chen, Y. D., Xu, L. Q. and Zhou, X. N. (2005). *Cysticercosis cellulosae* in China. In *Asian Parasitology vol. 2. Taeniasis/Cysticercosis and Echinococcosis in Asia* (ed. Ito, A., Wen, H. and Yamasaki, H.), pp. 37–83. Federation of Asian Parasitologists, Chiba, Japan.

Chung, J. Y., Bahk, Y. Y., Huh, S., Kang, S. Y., Kong, Y. and Cho, S. Y. (1999). A recombinant 10-kDa protein of *Taenia solium* metacestodes specific to active neurocysticercosis. *Journal of Infectious Diseases* **180**, 1307–1315.

Copado, F., De Aluja, A. S., Mayagoitia, L. and Galindo, F. (2004). The behaviour of free ranging pigs in the Mexican tropics and its relationships with human faeces consumption. *Applied Animal Behaviour Science* **88**, 243–252.

Deckers, N. and Dorny, P. (2010). Immunodiagnosis of *Taenia solium* taeniosis/cysticercosis. *Trends in Parasitology* **26**, 137–144.

De Kaminsky, R. G. (1991). Albendazole treatment in human taeniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 648–650.

Deplazes, P., Eckert, J., Pawlowski, Z. S., Machowska, L. and Gottstein, B. (1991). An enzyme-linked immunosorbent assay for diagnostic detection of *Taenia saginata* copro-antigens in humans. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 391–396.

Deplazes, P., Gottstein, B., Stingelin, Y. and Eckert, J. (1990). Detection of *Taenia* copro-antigens by ELISA in dogs. *Veterinary Parasitology* **36**, 91–103.

Elayoubi, F. A. and Craig, P. S. (2004). *Echinococcus granulosus* coproantigens: chromatographic fractionation and characterisation. *Parasitology* **128**, 1–11.

Ferrer, E., Gonzalez, L. M., Foster-Cuevas, M., Cortez, M. M., Davila, I., Rodriguez, M., Sciutto, E., Harrison, L. J., Parkhouse, R. M. and Garate, T. (2005). *Taenia solium:* characterization of a small heat shock protein (Tsol-sHSP35·6) and its possible relevance to the diagnosis and pathogenesis of neurocysticercosis. *Experimental Parasitology* **110**, 1–11.

Ferrer, E., Gonzalez, L. M., Martinez-Escribano, J. A., Gonzalez-Barderas, M. E., Cortez, M. M., Davila, I., Harrison, L. J., Parkhouse, R. M. and Garate, T. (2007). Evaluation of recombinant HP6-Tsag, an 18 kDa *Taenia saginata* oncospheral adhesion protein, for the diagnosis of cysticercosis. *Parasitology Research* **101**, 517–525.

Flisser, A., Vazquez-Mendoza, A., Martinez-Ocana, J., Gomez-Colin, E., Leyva, R. S. and Medina-Santillan, R. (2005). Short report: evaluation of a self-detection tool for tapeworm carriers for use in public health. *American Journal of Tropical Medicine and Hygiene* **72**, 510–512.

Fortin, M. and Dale, M. (2005). *Spatial Analysis. A Guide for Ecologists*. Cambridge University Press, Cambridge, UK.

Garcia, H. H., Gonzalez, A. E., Gilman, R. H., Palacios, L. G., Jimenez, I., Rodriguez, S., Verastegui, M., Wilkins, P. and Tsang, V. C. (2001). Transient antibody response in *Taenia solium* infection in field conditions-a major contributor to high seroprevalence. *American Journal of Tropical Medicine and Hygiene* **65**, 31–32.

Garcia-Noval, J., Allan, J. C., Fletes, C., Moreno, E., de Mata, F., Torres-Alvarez, R., Soto de Alfaro, H., Yurrita, P., Higueros-Morales, H., Mencos, F. and Craig, P. S. (1996). Epidemiology of *Taenia solium* taeniasis and cysticercosis in two rural Guatemalan communities. *American Journal of Tropical Medicine and Hygiene* **55**, 282–289.

Giraudoux, P., Raoul, F., Pleydell, D., Li, T., Han, X., Qiu, J., Xie, Y., Wang, H., Ito, A. and Craig, P. S. (2013). Drivers of *Echinococcus multilocularis* transmission in China: host biodiversity, landscape or climate? *PLoS Neglected Tropical Diseases* **7**, e2045.

Gonzalez, L. M., Bailo, B., Ferrer, E., Garcia, M. D., Harrison, L. J., Parkhouse, M. R., McManus, D. P. and Garate, T. (2010). Characterization of the *Taenia* spp HDP2 sequence and development of a novel PCR-based assay for discrimination of *Taenia saginata* from *Taenia asiatica*. *Parasites and Vectors* **3**, 51.

Gonzalez, L. M., Montero, E., Morakote, N., Puente, S., Diaz De Tuesta, J. L., Serra, T., Lopez-Velez, R., McManus, D. P., Harrison, L. J., Parkhouse, R. M. and Garate, T. (2004). Differential diagnosis of *Taenia saginata* and *Taenia saginata asiatica* taeniasis through PCR. *Diagnostic Microbiology and Infectious Disease* **49**, 183–188.

Gottstein, B., Tsang, V. C. and Schantz, P. M. (1986). Demonstration of species-specific and cross-reactive components of *Taenia solium* metacestode antigens. *American Journal of Tropical Medicine and Hygiene* **35**, 308–313.

Greene, R. M., Hancock, K., Wilkins, P. P. and Tsang, V. C. (2000). *Taenia solium*: molecular cloning and serologic evaluation of 14- and 18-kDa related, diagnostic antigens. *Journal of Parasitology* **86**, 1001–1007.

Guezala, M. C., Rodriguez, S., Zamora, H., Garcia, H. H., Gonzalez, A. E., Tembo, A., Allan, J. C. and Craig, P. S. (2009). Development of a species-specific coproantigen ELISA for human *Taenia solium* taeniasis. *American Journal of Tropical Medicine and Hygiene* **81**, 433–437.

Hall, A., Latham, M. C., Crompton, D. W. and Stephenson, L. S. (1981). *Taenia saginata* (cestoda) in western Kenya: the reliability of faecal examinations in diagnosis. *Parasitology* **83**, 91–101.

Hancock, K., Khan, A., Williams, F. B., Yushak, M. L., Pattabhi, S., Noh, J. and Tsang, V. C. (2003). Characterization of the 8-kilodalton antigens of *Taenia solium* metacestodes and evaluation of their use in an enzyme-linked immunosorbent assay for serodiagnosis. *Journal of Clinical Microbiology* **41**, 2577–2586.

Hancock, K., Pattabhi, S., Greene, R. M., Yushak, M. L., Williams, F., Khan, A., Priest, J. W., Levine, M. Z. and Tsang, V. C. (2004). Characterization and cloning of GP50, a *Taenia solium* antigen diagnostic for cysticercosis. *Molecular and Biochemical Parasitology* **133**, 115–124.

Hancock, K., Pattabhi, S., Whitfield, F. W., Yushak, M. L., Lane, W. S., Garcia, H. H., Gonzalez, A. E., Gilman, R. H. and Tsang, V. C. (2006). Characterization and cloning of T24, a *Taenia solium* antigen diagnostic for cysticercosis. *Molecular and Biochemical Parasitology* **147**, 109–117.

Hulsmeier, A. J., Deplazes, P., Naem, S., Nonaka, N., Hennet, T. and Kohler, P. (2010). An *Echinococcus multilocularis* coproantigen is a surface glycoprotein with unique O-glycosylation. *Glycobiology* **20**, 127–135.

Ikejima, T., Piao, Z. X., Sako, Y., Sato, M. O., Bao, S., Si, D., Yu, F., Zhang, C. L., Nakao, M., Yamasaki, H., Nakaya, K., Kanazawa, T.

and Ito, A. (2005). Evaluation of clinical and serological data of *Taenia solium* cysticercosis patients in eastern area of Inner Mongolia autonomous region, China. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **99**, 625–630.

Ito, A. and Craig, P. S. (2003). Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. *Trends in Parasitology* **19**, 377–381.

Ito, A., Li, T., Chen, X., Long, C., Yanagida, T., Nakao, M., Sako, Y., Okamoto, M., Wu, Y., Raoul, F., Giraudoux, P. and Craig, P. S. (2013). Mini review on chemotherapy of taeniasis and cysticercosis due to *Taenia solium* in Asia, and a case report with 20 tapeworms. *Tropical Biomedicine* **30**, 164–173.

Ito, A., Okamoto, M., Li, T., Wandra, T., Dharmawan, N. S., Swastika, K. I., Dekumyoy, P., Kusolsuk, T., Davaajav, A., Davaasuren, A., Dorjsuren, T., Meconnen, S. M., Negasi, Z. H., Yanagida, T., Sako, Y., Nakao, M., Nakaya, K., Lavikainen, A. J., Nkouawa, A. and Mohammadzadeh, T. (2011). The first workshop on towards the control of cestode zoonoses in Asia/Africa. *Parasites and Vectors* **4**, 114.

Ito, A., Plancarte, A., Ma, L., Kong, Y., Flisser, A., Cho, S. Y., Liu, Y. H., Kamhawi, S., Lightowlers, M. W. and Schantz, P. M. (1998). Novel antigens for neurocysticercosis, simple method for preparation and evaluation for serodiagnosis. *American Journal of Tropical Medicine and Hygiene* **59**, 291–294.

Ito, A., Plancarte, A., Nakao, M., Nakaya, K., Ikejima, T., Piao, Z. X., Kanazawa, T. and Margono, S. S. (1999). ELISA and immunoblot using purified glycoproteins for serodiagnosis of cysticercosis in pigs naturally infected with *Taenia solium*. *Journal of Helminthology* **73**, 363–365.

Ito, A., Urbani, C., Qiu, J., Vuitton, D. A., Qiu, D., Heath, D. D., Craig, P. S., Feng, Z. and Schantz, P. M. (2003). Control of echinococcosis and cysticercosis: a public health challenge to international cooperation in China. *Acta Tropica* **86**, 3–17.

Jayashi, C. M., Arroyo, G., Lightowlers, M. W., Garcia, H. H., Rodriguez, S. and Gonzalez, A. E. (2012). Seroprevalence and risk factors for *Taenia solium* cysticercosis in rural pigs of Northern Peru. *PloS Neglected Tropical Diseases* **6**, e1733.

Jeon, H.-K., Kim, K.-H. and Eom, K. S. (2011). Molecular identification of *Taenia* specimens after long-term preservation in formalin. *Parasitology International* **60**, 203–205.

Jongwutiwes, U., Yanagida, T., Ito, A. and Kline, S. E. (2011). Isolated intradural-extramedullary spinal cysticercosis: a case report. *Journal of Travel Medicine* **18**, 284–287.

Kulldorff, M. (1997). A spatial scan statistic. *Communications in Statistics: Theory and Methods* **26**, 1481–1496.

Lescano, A. G., Garcia, H. H., Gilman, R. H., Gavidia, C. M., Tsang, V. C. W., Rodriguez, S., Moulton, L. H., Villaran, M. V., Montano, S. M. and Gonzalez, A. E. (2009). *Taenia solium* cysticercosis hotspots surrounding tapeworm carriers: Clustering on human seroprevalence but not on seizures. *PLoS Neglected Tropical Diseases* **3**, e371.

Lescano, A. G., Garcia, H. H., Gilman, R. H., Guezala, M. C., Tsang, V. C. W., Gavidia, C. M., Rodriguez, S., Moulton, L. H., Green, J. A. and Gonzalez, A. E. (2007). Swine cysticercosis hotspots surrounding *Taenia solium* tapeworm carriers. *American Journal of Tropical Medicine and Hygiene* **76**, 376–383.

Li, T., Craig, P. S., Ito, A., Chen, X., Qiu, D., Qiu, J., Sato, M. O., Wandra, T., Bradshow, H., Li, L., Yang, Y. and Wang, Q. (2006). Taeniasis/cysticercosis in a Tibetan population in Sichuan Province, China. *Acta Tropica* **100**, 223–231.

Li, T., Ito, A., Chen, X., Long, C., Okamoto, M., Raoul, F., Giraudoux, P., Yanagida, T., Nakao, M., Xiao, N. and Craig, P. S. (2012). Usefulness of pumpkin seeds combined with areca nut extract in community-based treatment of human taeniasis in northwest Sichuan province. *Acta Tropica* **124**, 152–157.

Martinez-Hernandez, F., Emiliano Jimenez-Gonzalez, D., Chenillo, P., Alonso-Fernandez, C., Maravilla, P. and Flisser, A. (2009). Geographical widespread of two lineages of *Taenia solium* due to human migrations: Can population genetic analysis strengthen this hypothesis? *Infection Genetics and Evolution* **9**, 1108–1114.

Mayta, H., Gilman, R. H., Prendergast, E., Castillo, J. P., Tinoco, Y. O., Garcia, H. H., Gonzalez, A. E. and Sterling, C. R. (2008). Nested PCR for specific diagnosis of *Taenia solium* taeniasis. *Journal of Clinical Microbiology* **46**, 286–289.

Mayta, H., Talley, A., Gilman, R. H., Jimenez, J., Verastegui, M., Ruiz, M., Garcia, H. H. and Gonzalez, A. E. (2000). Differentiating *Taenia solium* and *Taenia saginata* infections by simple hematoxylin-eosin staining and PCR-restriction enzyme analysis. *Journal of Clinical Microbiology* **38**, 133–137.

Michelet, L., Carod, J. F., Rakontondrazaka, M., Ma, L., Gay, F. and Dauga, C. (2010). The cause of cysticercosis: Biogeographic (temporal and spatial) origins in Madagascar. *Molecular Phylogenetics and Evolution* **55**, 744–750.

Michelet, L. and Dauga, C. (2012). Molecular evidence of host influences on the evolution and spread of human tapeworms. *Biological Reviews* **87**, 731–741.

Morales, J., Martinez, J. J., Rosetti, M., Fleury, A., Maza, V., Hernandez, M., Villalobos, N., Fragoso, G., De Aluja, A. S., Larralde, C. and Sciutto, E. (2008). Spatial Distribution of *Taenia solium* Porcine Cysticercosis within a Rural Area of Mexico. *PLoS Neglected Tropical Diseases* **2**, e284.

Mori, Y., Nagamine, K., Tomita, N. and Notomi, T. (2001). Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochemical and Biophysical Research Communications* **289**, 150–154.

Mori, Y. and Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *Journal of Infection and Chemotherapy* **15**, 62–69.

Nakao, M., Okamoto, M., Sako, Y., Yamasaki, H., Nakaya, K. and Ito, A. (2002). A phylogenetic hypothesis for the distribution of two genotypes of the pig tapeworm *Taenia solium* worldwide. *Parasitology* **124**, 657–662.

Ngowi, H. A., Kassuku, A. A., Carabin, H., Mlangwa, J. E. D., Mlozi, M. R. S., Mbilinyi, B. P. and Willingham, A. L. (2010). Spatial Clustering of Porcine Cysticercosis in Mbulu District, Northern Tanzania. *PLoS Neglected Tropical Diseases* **4**, e652.

Nkouawa, A., Sako, Y., Li, T., Chen, X., Nakao, M., Yanagida, T., Okamoto, M., Giraudoux, P., Raoul, F., Nakaya, K., Xiao, N., Qiu, J., Qiu, D., Craig, P. S. and Ito, A. (2012). A loop-mediated isothermal amplification method for a differential identification of *Taenia* tapeworms from human: application to a field survey. *Parasitology International* **61**, 723–725.

Nkouawa, A., Sako, Y., Li, T., Chen, X., Wandra, T., Swastika, I. K., Nakao, M., Yanagida, T., Nakaya, K., Qiu, D. and Ito, A. (2010). Evaluation of a loop-mediated isothermal amplification method using fecal specimens for differential detection of *Taenia* species from humans. *Journal of Clinical Microbiology* **48**, 3350–3352.

Nkouawa, A., Sako, Y., Nakao, M., Nakaya, K. and Ito, A. (2009). Loop-mediated isothermal amplification method for differentiation and rapid detection of *Taenia* species. *Journal of Clinical Microbiology* **47**, 168–174.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28**, E63.

Nunes, C. M., Dias, A. K., Dias, F. E., Aoki, S. M., de Paula, H. B., Lima, L. G. and Garcia, J. F. (2005). *Taenia saginata*: differential diagnosis of human taeniasis by polymerase chain reaction-restriction fragment length polymorphism assay. *Experimental Parasitology* **110**, 412–415.

Obregon-Henao, A., Gil, D. L., Gomez, D. I., Sanzon, F., Teale, J. M. and Restrepo, B. I. (2001). The role of N-linked carbohydrates in the antigenicity of *Taenia solium* metacestode glycoproteins of 12, 16 and 18 kD. *Molecular and Biochemical Parasitology* **114**, 209–215.

Okamoto, M., Nakao, M., Blair, D., Anantaphruti, M. T., Waikagul, J. and Ito, A. (2010). Evidence of hybridization between *Taenia saginata* and *Taenia asiatica*. *Parasitology* **59**, 70–74.

O`Neal, S. E., Moyano, L. M., Ayvar, V., Gonzalvez, G., Diaz, A., Rodriguez, S., Wilkins, P. P., Tsang, V. C. W., Gilman, R. H., Garcia, H. H., Gonzalez, A. E. and Cysticercosis Working Group in Peru. (2012). Geographic correlation between tapeworm carriers and heavily infected cysticercotic pigs. *PLoS Neglected Tropical Diseases* **6**, e1953.

Parkhouse, R. M. and Harrison, L. J. (1987). Cyst fluid and surface associated glycoprotein antigens of *Taenia* sp. metacestodes. *Parasite Immunology* **9**, 263–268.

Pawlowski, Z. and Schulz, M. G. (1972). Taeniasis and cysticercosis (*Taenia saginata*). *Advances in Parasitology* **10**, 269–343.

Pondja, A., Neves, L., Mlangwa, J., Afonso, S., Fafetine, J., Willingham, A. L., Thamsborg, S. M. and Johansen, M. V. (2010). Prevalence and risk factors of porcine cysticercosis in Angonia District, Mozambique. *PLoS Neglected Tropical Diseases* **4**, e594.

Pouedet, M. S. R., Zoli, A. P., Nguekam, Vondou, L., Assana, E., Speybroeck, N., Berkvens, D., Dorny, P., Brandt, J. and Geerts, S. (2002). Epidemiological survey of swine cysticercosis in two rural communities of West-Cameroon. *Veterinary Parasitology* **106**, 45–54.

Raghava, M. V., Prabhakaran, V., Jayaraman, T., Muliyil, J., Oommen, A., Dorny, P., Vercruysse, J. and Rajshekhar, V. (2010). Detecting spatial clusters of *Taenia solium* infections in a rural block

in South India. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **104**, 601–612.

Rodriguez-Canul, R., Fraser, A., Allan, J. C., Dominguez-Alpizar, J. L., Argaez-Rodriguez, F. and Craig, P. S. (1999). Epidemiological study of *Taenia solium*/cysticercosis in a rural village in Yucatan State, Mexico. *Annals of Tropical Medicine and Parasitology* **93**, 57–67.

Sako, Y., Nakao, M., Ikejima, T., Piao, X. Z., Nakaya, K. and Ito, A. (2000). Molecular characterization and diagnostic value of *Taenia solium* low-molecular-weight antigen genes. *Journal of Clinical Microbiology* **38**, 4439–4444.

Sarti, E., Flisser, A., Schantz, P. M., Gleizer, M., Loya, M., Plancarte, A., Avila, G., Allan, J. C., Craig, P. S., Nijeyaratne, P. and Bronfman, M. (1997). Development and evaluation of a health education intervention against *Taenia solium* in a rural community in Mexico. *American Journal of Tropical Medicine and Hygiene* **56**, 127–132.

Sarti, E., Schantz, P. M., Avila, G., Ambrosio, J., Medina-Santillan, R. and Flisser, A. (2000). Mass treatment against human taeniasis for the control of cysticercosis: a population-based intervention study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 85–89.

Sarti, E., Schantz, P. M., Plancarte, A., Wilson, M., Gutierrez, I. O., Lopez, A. S., Robert, A. and Flisser, A. (1992). Prevalence and risk factors for *Taenia solium* taeniasis and cysticercosis in humans and pigs in a village in Morelos, Mexico. *American Journal of Tropical Medicine and Hygiene* **46**, 677–685.

Sato, M. O., Yamasaki, H., Sako, Y., Nakao, M., Nakaya, K., Plancarte, A., Kassuku, A. A., Dorny, P., Geerts, S., Benitez-Ortiz, W., Hashiguchi, Y. and Ito, A. (2003). Evaluation of tongue inspection and serology for diagnosis of *Taenia solium* cysticercosis in swine: usefulness of ELISA using purified glycoproteins and recombinant antigen. *Veterinary Parasitology* **111**, 309–322.

Schantz, P. M., Cruz, M., Sarti, E. and Pawlowski, Z. (1993). Potential eradicability of taeniasis and cysticercosis. *Bulletin PAHO* **27**, 397–403.

Schantz, P. M. and Sarti-Guttierez, E. (1989). Diagnostic methods and epidemiologic surveillance of *Taenia solium* infection. *Acta Leidensia* **57**, 153–164.

Sikasunge, C. S., Phiri, I. K., Phiri, A. M., Dorny, P., Siziya, S. and Willingham, A. L. (2007). Risk factors associated with porcine cysticercosis in selected districts of Eastern and Southern provinces of Zambia. *Veterinary Parasitology* **143**, 59–66.

Somers, R., Dorny, P., Nguyen, V. K., Dang, T. C. T., Goddeeris, B., Craig, P. S. and Vercruysse, J. (2006). *Taenia solium* taeniasis and cysticercosis in three communities in North Vietnam. *Tropical Medicine and International Health* **11**, 1–8.

Sorvillo, F., Wilkins, P., Shafir, S. and Eberhard, M. (2011). Public health implications of cysticercosis acquired in the United States. *Emerging Infectious Diseases* **17**, 1–6.

Swastika, K., Dewiyani, C. I., Yanagida, T., Sako, Y., Sudarmaja, M., Sutisna, P., Wandra, T., Dharmawan, N. S., Nakaya, K., Okamoto, M. and Ito, A. (2012). An ocular cysticercosis in Bali, Indonesia caused by *Taenia solium* Asian genotype. *Parasitology International* **61**, 378–380.

Tembo, A. (2010). Detection and diagnosis of human *Taenia saginata* taeniosis. Ph.D. thesis. University of Salford, UK.

Tsang, V. C., Brand, J. A. and Boyer, A. E. (1989). An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). *Journal of Infectious Diseases* **159**, 50–59.

Wandra, T., Depary, A. A., Sutisna, P., Margono, S. S., Suroso, T., Okamoto, M., Craig, P. S. and Ito, A. (2006). Taeniasis and cysticercosis in Bali and north Sumatra, Indonesia. *Parasitology International* **55**, S155–S160.

WHO (2010). *Working to Overcome the Global Impact of Neglected Tropical Diseases*. World Health Organization, Geneva.

Yamane, K., Suzuki, Y., Tachi, E., Li, T., Chen, X., Nakao, M., Nkouawa, A., Yanagida, T., Sako, Y., Ito, A., Sato, H. and Okamoto, M. (2012). Recent hybridization between *Taenia asiatica* and *Taenia saginata*. *Parasitology International* **61**, 351–355.

Yamasaki, H., Allan, J. C., Sato, M. O., Nakao, M., Sako, Y., Nakaya, K., Qiu, D., Mamuti, W., Craig, P. S. and Ito, A. (2004*a*). DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. *Journal of Clinical Microbiology* **42**, 548–553.

Yamasaki, H., Matsunaga, S., Yamamura, K., Chang, C., Kawamura, S., Sako, Y., Nakao, M., Nakaya, K. and Ito, A. (2004*b*). Solitary neurocysticercosis case caused by Asian genotype of *Taenia solium* confirmed by mitochondrial DNA analysis. *Journal of Clinical Microbiology* **42**, 3891–3893.

Yamasaki, H., Nagase, T., Kiyoshige, Y., Suzuki, M., Nakaya, K., Itoh, Y., Sako, Y., Nakao, M. and Ito, A. (2006). A case of intramuscular cysticercosis diagnosed definitively by mitochondrial DNA analysis of extremely calcified cysts. *Parasitology International* **55**, 127–130.

Yanagida, T., Sako, Y., Nakao, M., Nakaya, K. and Ito, A. (2012). Taeniasis and cysticercosis due to *Taenia solium* in Japan. *Parasites and Vectors* **5**, 18.

Yanagida, T., Yuzawa, I., Joshi, D. D., Sako, Y., Nakao, M., Nakaya, K., Kawano, N., Oka, H., Fujii, K. and Ito, A. (2010). Neurocysticercosis: assessing where the infection was acquired from. *Journal of Travel Medicine* **17**, 206–208.

Yingkun, F., Shan, O., Xiuzhen, Z. and Shulian, Y. (1979). Clinicoelectrocephalographic studies of cerebral cysticercosis, 158 cases. *Chinese Medical Journal* **92**, 770–786 (in Chinese).