Infection, Genetics and Evolution 12 (2012) 227–235

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Review

Ascariasis in people and pigs: New inferences from DNA analysis of worm populations

Weidong Peng [a,*], Charles D. Criscione [b]

[a] Department of Parasitology, College of Basic Medicine, Nanchang University, Nanchang 330031, People's Republic of China
[b] Department of Biology, Texas A&M University, 3258 TAMU, College Station, TX 77843-3258, USA

ARTICLE INFO

Article history:
Received 7 November 2011
Received in revised form 12 January 2012
Accepted 14 January 2012
Available online 28 January 2012

Keywords:
Ascaris
Molecular epidemiology
Host affiliation
Cross infection
Hybridization
Genetic structure

ABSTRACT

Ascaris is a large parasitic roundworm (nematode) of the small intestine of humans and pigs. These roundworms cause the socioeconomically important disease, ascariasis. For the past 20 years, molecular markers have been used in studies on *Ascaris* and ascariasis, and added valuable information to the understanding of these roundworms. Here, we provide a review of these studies on human and pig roundworms. We begin with a summary of studies using molecular phenotypic markers to compare *Ascaris* from humans and pigs, followed by a synopsis of comparisons using genetic markers. We then draw forth inferences in the aspects of host affiliation and infection success, transmission between and among humans and pigs, evolutionary history of *Ascaris*. We also highlight additional topics such as mating dynamics, diagnostics, and paleoparasitology where molecular epidemiological approaches have been utilized.

Contents

1. Introduction

Ascaris lumbricoides Linnaeus, 1758 and *Ascaris suum* Goeze, 1782 are two of the most common intestinal geohelminths of humans and pigs, respectively. Ascariasis in humans was reported in at least 150 countries with most in the developing world, particularly in Asia and sub-Saharan Africa (Crompton, 2001). The global prevalence in the 1990s was estimated to be approximately 1.5 billion with 100–200 million people, many of which were children, clinically affected (Chan et al., 1994; Crompton, 1989a; O'Lorcain and Holland, 2000; Peng et al., 1998a; WHO, 1987). The updated global infection is down to 1.2 billion (de Silva et al., 2003; WHO, 2006), which is likely linked to China's large-scale 'deworming' programs (Peng et al., 1998a; Sun et al., 2008). However, it is still of major public health importance in many parts of the world

* Corresponding author. Address: Department of Parasitology, College of Basic Medicine, Nanchang University, Xuefu Dadao 999, Nanchang 330031, People's Republic of China. Tel.: +86 13698418201; fax: +86 791 86361335.
E-mail address: pwdjxmu@hotmail.com (W. Peng).

1567-1348/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2012.01.012

and is considered a neglected tropical disease (Dold and Holland, 2011a; Hotez et al., 2008). A large body of research across multiple countries indicates that people, especially children, with this infection can suffer from some degree of nutritional deficit, cognitive impairment, intestinal complications, and occasionally death (e.g., Crompton and Nesheim, 2002; Bundy and Guyatt, 1996; Stephenson, 1987; Thein-Hlaing, 1993; Zhou et al., 1999). Importantly, the global disability-adjusted life-years (DALYs) lost as a result of infection from *A. lumbricoides* is estimated at 10.5 million (Stephenson et al., 2000).

Ascariasis of pigs is of major economic significance due to production losses linked to reduced feed conversion efficiency and losses to the meat industry associated with the condemnation of 'milk-spot' livers (Roepstorff and Nansen, 1998; Stewart and Hale, 1988). The prevalence of *A. suum* depends on management practices of pig farms and also varies with geographical regions, but few swineries are completely free of infection (Boes et al., 2000; Nansen and Roepstorff, 1999; Nganga et al., 2008; Weng et al., 2005). Infections of *A. suum* can cause similar problems in pigs as in people infected with *A. lumbricoides*. Briefly, some degree of migration of larval *A. suum* through the liver and lungs of pigs appears to be associated with decreased food intake, increased nitrogen loss and acute allergic asthma. Infections of adult *A. suum* in the intestine of pigs can cause chronic decreased food intake and increased nutrient excretion as well as mal-absorption, accompanying villus atrophy, impaired absorption of vitamin A and temporary lactose intolerance (Copeman and Gaafar, 1972; Forsum et al., 1981; Pérez et al., 2001).

Because of the economic and health importance of *Ascaris* infections, there has been much interest in elucidating the epidemiology/epizoology of ascariasis transmission in natural (including farmed host) populations or via controlled experimental infections. Classically and necessarily this entails quantifying worm burdens (i.e., number of worms per host, a.k.a., infection intensity, or egg counts per gram of feces (EPG) as an indirect measurement of infection intensity). Many factors have been examined as potential correlates that explain the variation in worm burdens among individuals. Such variables have included host genetics or immunological response (Dold and Holland, 2011b; Nejsum et al., 2009a; Williams-Blangero et al., 1999), individual or household predisposition, swine raising conditions, and the age, sex, and social/economic status of the human host (e.g., Holland, 2009; Peng et al., 1996; Roepstorff et al., 1999; Walker et al., 2011). Such epidemiological studies based on worm burdens have proven instrumental in elucidating variables related to the distribution of *Ascaris* parasites among hosts and are necessary to help reduce, control, or eliminate worm burdens. However, worm counts provide little insight into such questions as cross-infection between humans and pigs, patterns of exposure to infection (clumped infection or trickle infection), detection of early infection in the host (tissue-migratory juvenile stages), dynamics of transmission and evolution of ascariasis, and population structure of *Ascaris* spp. On the other hand, molecular markers and population genetic analyses of the parasites, for example, can help elucidate parasite dispersal at several scales (e.g., among host individuals, populations and species), and thus complement traditional epidemiological studies to provide a thorough understanding of *Ascaris* transmission. Population genetic analyses also provide important parameters for understanding the evolutionary potential of parasite populations. For instance, estimates of parasite inbreeding are critical for predicting the dynamics of drug-resistance evolution (e.g., Schwab et al., 2006). Here we provide a review of how molecular markers have been used to elucidate the ecology, evolution, infection dynamics, and epidemiology of *Ascaris*.

2. Comparisons of *Ascaris* from humans and pigs

2.1. Molecular phenotypic markers

There is still a longstanding debate with regards to the separate species status of *A. lumbricoides* and *A. suum* (Anderson, 2001; Crompton, 2001; Macko and Dubinsky, 1997; Peng et al., 2007). Taxonomic uncertainty stems from the limited and inconsistent morphological distinction between the two roundworms (Kurimoto, 1974 and references therein). This problem is not just a taxonomic issue, but also an important epidemiological concern with direct implications for the development and implementation of any control program for ascariasis. For example, without a diagnostic marker, how is it possible to ascertain if there is any shared ascarid transmission between humans and pigs? Consequently, in areas of human–pig sympatry, how can one determine if control measures should target only infected people (as is usually carried out) or both infected humans and pigs at same time? Cross-infection experiments have shown that *Ascaris* of pig origin can infect humans and vice versa (Galvin, 1968; Takata, 1951; reviewed in Crompton, 1989b). These experiments highlight both humans and pigs are possible hosts for each other's *Ascaris* parasite; however, these experiments do not reveal transmission in natural settings nor do they reveal if there is any cross breeding between the host-associated worms.

In attempts to find a diagnostic character or ascertain species status, several studies have examined protein, antigenic, or expressed DNA profiles between worms obtained from humans and those from pigs (Abebe et al., 2002a,b; Alba et al., 2009; Kennedy et al., 1987; Kurimoto, 1974; Wu et al., 2010). Although a few differences have been noted, several of these studies are cofounded with geographic sampling (e.g., Abebe et al., 2002a,b; Kennedy et al., 1987; Kurimoto, 1974). For example, Kennedy et al. (1987) note that their samples may represent geographic genetic differentiation as human worms came from India and pig worms came from Scotland. As discussed below, *Ascaris* populations (among humans or pigs) across broad geographic locations do indeed show high genetic structuring when examined with neutral genetic markers.

Enzyme electrophoresis also has been used as a technique to examine for diagnostic differences between human and pig *Ascaris*. Nascetti et al. (1979) examined pig worms from Italy and human worms from Italy, Egypt, and India. Of the 21 loci, 4 (malate dehydrogenase-3, glucose-6-phosphate dehydrogenase, esterase-2, and aldolase) showed fixed allelic differences between human and pig *Ascaris*. Nadler (1987) examined human and pig worms from the USA and found 15 (out of 18) monomorphic loci and 1 locus (superoxide dismutase, which was not studied by Nascetti et al., 1979) that was fixed between host species. However, malate dehydrogenase-3 and glucose-6-phosphate dehydrogenase could not be resolved by Nadler (1987), and aldolase, a locus shown to be fixed in Nascetti et al. (1979), was monomorphic (Nadler, 1987). No fixed differences were found in the 13 loci tested in Anderson et al. (1993). Interestingly, malate dehydrogenase-3 was one of the eight monomorphic loci found in the sympatric samples of humans and pigs in Guatemala. Thus, enzyme markers that were once thought to be diagnostic turned out not to be so as additional studies were conducted. Allele frequency differences in allozymes have been used to determine if there is genetic differentiation between sympatric human and pig samples of *Ascaris* (e.g., Anderson et al., 1993). However, the low polymorphism of allozymes has largely limited their utility in this regard (Anderson, 2001).

An important caveat to using the above mentioned phenotypic markers (i.e., morphology and expressed proteins) as diagnostic markers is that they are subject to environmental induced

variation or expression. For instance, human worms are often collected after treatment with an antihelminthic drug, whereas pig worms are often collected from untreated slaughtered pigs (e.g., Abebe et al., 2002a,b; Alba et al., 2009; Kennedy et al., 1987; Kurimoto, 1974; Nascetti et al., 1979; Wu et al., 2010). As Abebe et al. (2002a) discussed with regard to a protein assay, "it is difficult to assert at this stage whether the appearance of the different spots encountered in human *Ascaris* is due to the effect of the drug". Importantly, the host environment itself could play a role in the expression of parasite phenotypic characters (Perkins et al., 2011). It is unknown if the human or pig environment can differentially alter *Ascaris* protein expression, structure, or splicing; nonetheless, any such change could lead one to falsely conclude that a marker is diagnostic when in fact it was just an environmental alteration. In contrast, genetic markers, e.g., DNA sequence data, are not subject to the above mentioned caveat.

2.2. Genetic markers and genetic differentiation

As with the molecular phenotypic markers, no fixed differences between human and pig *Ascaris* have been observed with genetic markers. However, analyses of either genetic allele frequency data or sequence data have provided substantial insight into the genetic structure between human and pig *Ascaris*. For example, within two villages in Guatemala, restriction enzyme digests of mitochondrial DNA (mtDNA) revealed 29 haplotypes that fell into two mtDNA clades with about 3–4% divergence. The haplotypes of these two clades were distributed non-randomly between human and pig hosts (Anderson et al., 1993). However, there were a few identical worm haplotypes that were found infecting both host species. Using the same approach in two neighboring villages in China, Peng et al. (1998b) also found significant frequency differences between the two host-associated populations, with also a few identical haplotypes shared by both host species. Additional work that used sequence data of the mitochondrial ND1 (NADH dehydrogenase subunit 1) and CO1 (cytochrome *c* oxidase subunit 1) genes in China also revealed in each of six provinces, significant structure between human and pig *Ascaris* (Peng et al., 2005). Analyses with 23 autosomal microsatellite loci (Criscione et al., 2007a) confirm significant structure between humans and pig *Ascaris* on a local scale in Guatemala and China (Criscione et al., 2007b). Levels of genetic structure (when reported) in these studies tends to be high with F_{ST} (or related analogs; see de Meeûs et al., 2007 for *F*-statistics definitions) ranging from 0.18 to 0.65.

Hierarchical analyses have also been used to test for genetic structure between human and pig roundworms. Here, variation among geographic populations is taken into account before testing variation between the two host species (i.e., populations of a host species are nested within host species). Interestingly, results from regional analyses (i.e., among locations within a country/continent) contrast with global analyses (i.e., comparisons made across continents). For instance, between two Guatemalan villages Anderson and Jaenike (1997) found that G_{PH} (*F*-statistic analog of populations within a host species) for single copy nuclear loci was 0.024 and for mtDNA it was 0.04, whereas G_{HT} (*F*-statistic analog of between host species) was 0.193 for single copy nuclear loci and 0.547 for mtDNA. Thus, at a regional scale, there is more genetic structure between host species than among populations within a host species. Comparable results in China are obtained when the ND1 data are reanalyzed from Peng et al. (2005). Φ_{PH} (*F*-statistic analog of populations within a host) was 0.069 and Φ_{HT} (*F*-statistic analog of between host species) was 0.482. [Analysis of molecular variance (AMOVA) conducted with Arlequin v3.5.1 (Excoffier and Lischer, 2010). Both values are significantly >0 ($p < 0.01$) based on 10,000 permutations of haplotypes among populations within host species or populations between host species, respectively.] In contrast, the global hierarchal analyses of Anderson and Jaenike (1997) showed greater differentiation among populations within a host species than between host species at both single copy nuclear loci ($G_{PH} = 0.191$; $G_{HT} = 0.091$) and mtDNA ($G_{PH} = 0.366$; $G_{HT} = 0.150$). Similarly, Bayesian clustering analyses of multilocus microsatellite genotypes of individual worms showed genetic differentiation between populations which was primarily driven by global geography, with secondary differentiation resulting from host affiliation within locations (Criscione et al., 2007b). As a caveat, we note that classification into regional versus global is a qualitative assessment of geographic distance and that political and cultural boundaries do not equate to geographic boundaries. Nonetheless, the global analyses conducted thus far obviously incorporate a broader geographic area. The point we wish to illustrate is that the patterns of genetic differentiation between humans and pigs are reversed when comparing "regionally" versus "globally". This pattern is important as it suggests that there may not have been a single split resulting in a single human *Ascaris* lineage and a single pig *Ascaris* lineage (see Section 5).

The first internal transcribed spacer (ITS1) of the rDNA has also been examined at regional and global scales. Zhu et al. (1999) observed a nucleotide sequence difference of 1.3% in a ~300 bp region of ITS1 of the rDNA between human and pig *Ascaris*. However, the limited number of samples from widely separated geographical locations made the interpretation from this study guarded. Peng et al. (2003) analyzed six human–pig *Ascaris* populations (6 provinces in China) that covered a great diversity in geography, climate and transmission characters. They found 5 ITS1 genotypes (G1–G5) for human *Ascaris*, of which 3 (G1–G3) were also detected in pig *Ascaris*. G1 was more common in humans (63–74%) whereas G3 more frequent in pigs (79–86%), indicating a nonrandom distribution of ITS1 genotypes between human and pig hosts. The frequencies of the other three genotypes were substantially lower for each of the two host species (Peng et al., 2003). The predominant genotype G1 found in humans in China was also demonstrated to be the most prevalent genotypes in humans in Brazil although an additional genotype G6 was described (Leles et al., 2009). We note that ITS1 is a multicopy gene (~42 copies; Pecson et al., 2006) and recent findings of >2 alleles within individual worms (Leles et al., 2010) indicates that this marker should not be treated as a diploid locus in population genetic analyses. Nevertheless, ITS seems to have relatively useful diagnostic properties (e.g., Anderson, 1995), and it appears that ITS1 shows similar patterns of genetic structure to that of other genetic markers (e.g., compare Peng et al., 2003 to Peng et al., 2005).

3. Inference of transmission between and among humans and pigs

3.1. Contemporary cross-transmission between humans and pigs

Given the patterns of genetic differentiation discussed above, what can be inferred about transmission patterns between human and pig *Ascaris*? Anderson (2001) outlined three epidemiological scenarios: (I) a single source pool of infection shared by humans and pigs, (II) two separate transmission cycles where one cycles in humans and the other in pigs, (III) two host-associated transmission cycles, but limited cross-infection between the two. It is clear from local scale analyses in areas of endemic human and pig transmission that there is strong neutral genetic differentiation between host species (Anderson et al., 1993; Peng et al., 1998b, 2005; Criscione et al., 2007b). Thus, scenario I can be ruled out. However, the failure to find fixed allelic genetic (mtDNA or nuclear markers) differences in studies with sympatric sampling makes it difficult to determine between scenario II and III. For example, the same *Ascaris* mtDNA haplotype can be found in both humans and pigs within

a village (Anderson et al., 1993; Peng et al., 1998b). This pattern could result from incomplete lineage sorting (retention of ancestral lineages in descendent taxa), current introgression (hybrid offspring resulting from cross-breeding between human and pig *Ascaris*), or cross-transmission, but no interbreeding (e.g., a worm is a first generation migrant from one host species to the other). If incomplete lineage sorting explained the genetic patterns, then one would infer scenario II. If human–pig *Ascaris* hybridization or first generation migrants caused the genetic patterns then scenario III would be inferred (Detwiler and Criscione, 2010). Unfortunately, most *Ascaris* studies have used a single genetic marker (ITS1 or mtDNA) or nuclear makers with low polymorphism (Anderson and Jaenike, 1997), and therefore lacked power to disentangle the latter processes in areas of sympatric human and pig infections. Nonetheless, molecular based studies in areas of non-endemic human transmission (USA, Denmark, and Japan) have provided evidence for cross-transmission from a pig source into humans (Anderson, 1995; Nejsum et al., 2005b; Arizono et al., 2010). For example, amplified length polymorphism (AFLP) markers showed an F_{ST} of 0.005 (not significantly different from 0) between human and pig samples in Denmark where the ITS1 helped verify a pig origin (Nejsum et al., 2005b). Indeed, Anderson (1995) noted that not only did the genetic data (ITS1) support pig *Ascaris* as the source of human infection in the USA, but also that the people with infections had recent contact with pigs (including the author himself!). Thus, in areas of non-endemic human transmission, there is support for cross-transmission. Does this cross-transmission lead to introgression between human and pig *Ascaris*? This latter question, which can only be addressed in areas of endemic human and pig transmission, was addressed by Criscione et al. (2007b). Multilocus microsatellites genotypes coupled with model-based Bayesian methods were employed to test the hypothesis of hybridization. In both sympatric samples from Guatemala and China, hybrid worms were detected (4% and 7%, respectively; Criscione et al., 2007b). These results indicate that there must have been contemporary interbreeding and thus, necessarily recent cross-transmission, between sympatric human and pig *Ascaris*, as the methods used by Criscione et al. (2007b) can only detect hybrids going back two generations (see Anderson and Thompson, 2002). Additional studies have confirmed cross transmission and hybridization in China (Zhou, 2011). Using the methods of Criscione et al. (2007b), 137 human and 121 pig *Ascaris* from six provinces in China were analyzed. Twenty individuals were identified as first generation migrants (19 were pig worms found in humans and 1 human worm in a pig) and of 20 worms identified as being of hybrid origin, 19 were from human hosts and 1 was from a pig host. These data, may suggest a greater tendency of pig *Ascaris* to infect humans than human *Ascaris* into pigs (Zhou, 2011). However, more data are needed to infer directionality in cross transmission. Nonetheless, these results indicate that pig *Ascaris* can serve as important source of human ascariasis in endemic area where human and pig *Ascaris* both exist. Current control strategies for human ascariasis in sympatric areas are usually developed without identifying pig *Ascaris* as an infection source for humans. Therefore, these studies highlight a need to reconsider and possibly, revise current control measures in China. In view of the current state of evidence, it appears that scenario III (host-associated transmission cycles, but limited cross-infection between the two) is the most plausible with regards to the epidemiology of human and pig *Ascaris*. Nevertheless, additional studies from sympatric populations and that use multilocus genotype data are warranted to determine if limited cross-transmission is a global theme especially in relation to different pig-raising, cultural, or economic conditions.

Because current evidence support contemporary cross-transmission, it will be important to establish the frequency of hybridization in additional sympatric populations. The latter data are critical for understanding the potential for gene introgression between the host-associated populations, especially in relation to the potential for the introgression of novel host infectivity genes or genes that may play a role in drug-resistance evolution (Criscione et al., 2007b; Zhou, 2011). It is interesting that despite the detected hybridization there exists strong genetic subdivision between sympatric human and pig *Ascaris* (Criscione et al., 2007b). This subdivision could be caused by ecological separation of host species and/or host-selective factors that result in decreased hybrid fitness (see McCoy, 2003). Given the likely continual exposure to eggs from pig *Ascaris* for those that raise pigs, ecological separation seems unlikely. In addition, preliminary studies by Peng et al. (2006) may suggest host selective factors are acting (discussed in Section 4). Still, more data are needed to ascertain the mechanisms for generating genetic subdivision in sympatry (see Section 4 for discussion of host affiliation).

3.2. Transmission among pigs

Different patterns of genetic structure among pig *Ascaris* infrapopulations (parasites within host individuals) have been observed at the local scale (among pigs in the same locality). Anderson et al. (1995) found significant structure among pigs within Guatemalan villages using mtDNA restriction enzyme data (for hosts within villages, $G_{HV} = 0.203$). With enzyme markers, Nadler et al. (1995) found F_{ST} values of 0.024 and 0.08 between two pairs of pigs from two farms in the USA. Significance was not tested, but these values suggest low levels of local differentiation. Similarly, AFLP markers revealed significant structure among pigs on farms in Denmark ($G_{ST} = 0.122$; Nejsum et al., 2005a). As Anderson et al. (1995) discussed, among-host structure suggests a non-random transmission process into hosts that was possibly caused by a clumped dispersal process of parasite offspring (see Criscione and Blouin, 2006 for how the transmission process can influence genetic structure among hosts). In contrast, the mtDNA restriction enzyme analysis by Peng et al. (1998b) did not reveal any significant genetic differentiation in *Ascaris* among pigs in two neighboring villages in China. Peng et al. (1998b) note they had low polymorphism (few restriction enzymes were used), which could have precluded detection of fine scale genetic structure. However, they also state that the agricultural practices of using human and pig excrement as fertilizer in China may have contributed to the homogenization of parasites among pigs and resulted in a random distribution of alleles within the parasite populations.

At a regional scale (among locations within a country/continent), mild but significant genetic structure appears to be a common theme. For example, Nejsum et al. (2005a) reported G_{ST} values (AFLP markers) between farms in a region or between regions in Denmark of 0.05 and 0.027 (confidence intervals do not overlap zero). In the USA, Nadler et al. (1995) noted an F_{ST} value of 0.062 between two states (significance not tested with the enzyme markers). One of three enzyme markers had significant differentiation ($p < 0.05$) between New Jersey and Iowa (USA) samples in the study by Leslie et al. (1982). [Data were reanalyzed in Arlequin using an exact test of population differentiation. Allele frequencies used as input are those reported in the publication.] One of three allozyme and three of five single copy nuclear loci showed significant ($p < 0.05$) allelic differentiation between two villages in Guatemala (Anderson et al., 1993; Anderson and Jaenike, 1997). [Data were reanalyzed in Arlequin using an exact test of population differentiation.] The mtDNA also revealed low differentiation between these two villages (village to the total, $G_{VT} = 0.106$; Anderson et al., 1995). In China, AMOVA analysis of the ND1 haplotypes estimated a Φ_{ST} of 0.054 ($p < 0.01$, reanalysis of data in Peng et al., 2005 conducted in Arlequin). Thus, at the regional scale, *Ascaris* populations in pigs are somewhat isolated and

do not represent a "panmictic" pool of parasites. Nonetheless, the low levels of differentiation likely reflect some gene flow, which is likely mediated by pigs being moved by humans, or only recent isolation at a regional scale.

Global scale analyses also reveal significant structure of *Ascaris* among pig populations. With the four single copy nuclear loci (*MYO*, *HEM*, *G9*, and *G12*) that Anderson and Jaenike (1997) used for both a regional analysis (two Guatemalan villages) and global analysis (samples from Peru, USA, Philippines, Scotland, and Switzerland), the mean F_{ST} was 0.087 (range, 0.019–0.189) at the regional level and 0.15 (range, 0.07–0.29) at the global scale. [Data were reanalyzed in Arlequin using the reported allele frequencies.] Similarly, Criscione et al. (2007b) report a multilocus (23 microsatellites) F_{ST} of 0.238 between Guatemalan and Chinese pig samples. Obviously, more data and better sampling designs are needed to verify the regional versus global levels of genetic differentiation. Nonetheless, the degree of differentiation at the global scale qualitatively appears to be higher than at the regional level. This pattern may reflect longer periods of isolation among global *Ascaris* populations in pigs, but could also be influenced by a history of multiple host-colonization events (see Section 5).

3.3. Transmission among humans

Local scale population genetic studies can greatly facilitate our understanding of the transmission process because they provide an indirect means to infer dispersal among host individuals. In Dhaka, Bangladesh, no genetic structure was found among *Ascaris* infrapopulations collected from eight children (Ibrahim et al., 1994). However, the low polymorphism at just three allozyme loci may have precluded detection of fine scale structure. Restriction enzyme data of the mtDNA have also been used to examine for local structure. Anderson et al. (1995) reported a non-random distribution of parasite haplotypes among individual people (for hosts within villages, $G_{HV} = 0.206$). With the caveats that mtDNA provides only a single marker and only reflects female dispersal, the data of Anderson et al. (1995) suggest a non-random recruitment of parasites into their hosts. In contrast, Peng et al. (1998b) observed no structure among people in the two villages. But as with the pig data of *Ascaris* in Peng et al. (1998b), the use of fewer restriction enzymes or cultural practices of using human and pig feces as fertilizer may explain the lack of structure.

Although population genetics studies yield data on the pattern of parasite transmission among hosts (e.g., random vs. non-random), incorporation of molecular data into a landscape genetics framework can provide detailed information on epidemiological correlates to the transmission process and potentially identify source pools of infection. The latter approach was used by Criscione et al. (2010) to examine the epidemiology of *Ascaris* in Jiri, Nepal. In this study, 23 autosomal microsatellites were analyzed with Bayesian clustering methods and spatial autocorrelation analyses. Analysis of 1094 worms from 320 people across 165 households revealed significant genetic structuring on a very small scale (14 km^2). The results of the population clustering analyses were subsequently incorporated into multivariate regression methods to elucidate spatial, geographical, or epidemiological features associated with the partitioning of genetic variation in the sampled worms. These analyses revealed three key insights into *Ascaris* transmission in Jiri. There were separate foci of transmission on this local scale, households and nearby houses shared genetically related parasites, and people reacquired their worms from the same source pool of infection over time. These results challenge the dogma that a single human community will correspond to a homogenous parasite population. Thus, in Jiri, multiple source pools of infection need to be considered when modeling parasite transmission, especially in relation to modeling drug treatment control strategies (Criscione et al., 2010).

In contrast to pigs, regional studies of *Ascaris* in humans do not always reveal significant structure. Although, mtDNA showed significant structure between two Guatemalan villages (village to the total, $G_{VT} = 0.09$; Anderson et al., 1995), three allozyme and five single copy nuclear markers do not show any significant differences (Anderson et al., 1993; Anderson and Jaenike, 1997). [Data were reanalyzed in Arlequin using the allele frequencies reported in the publications.] Microsatellite data did not reveal any significant structure among four villages of Unguja, Zanzibar (Betson et al., 2011) or villages in Uganda (Betson et al., 2012). In China (Peng et al., 2005), AMOVA analysis of the ND1 haplotypes returned a Φ_{ST} of 0.084 ($p < 0.01$, reanalysis of data conducted in Arlequin). Keep in mind, however, that the "regional" scale from the samples in China likely encompasses greater geographic distances, and thus may account for the difference to the other regional studies. With the current data, one can only speculate that human movement tends to disperse parasites at the regional scale.

As with the pig *Ascaris*, human *Ascaris* populations show strong structure among global populations. For example, Betson et al. (2011, 2012) found very high F_{ST} values (0.12–0.54) between Zanzibar villages and Uganda villages. Likewise, microsatellite data show very high F_{ST} values among samples from Guatemala, Nepal, and China (Criscione et al., 2007b) and the four single copy nuclear loci used by Anderson and Jaenike (1997) all showed significant ($p < 0.05$) allelic differentiation among global locations. [Data were reanalyzed in Arlequin using an exact test of population differentiation.] Again, global isolation and/or multiple host-colonization events may contribute to high structure at the global scale.

4. Inference of host affiliation and infection success

Knowledge of host affiliation and infection success into different host species is essential for understanding parasite transmission. Early experiments highlight both humans and pigs are possible hosts for each other's *Ascaris* parasite (Galvin, 1968; Takata, 1951). In addition, ascariasis was identified in dogs (Sharabi et al., 2010), squirrels, bears, primates and possible accidental infections in cattle and sheep (reviewed in Crompton, 1989b; Loreille and Bouchet, 2003). These findings raise questions about host affiliation and infection success of *Ascaris* in nature that are hard to address by traditional biological and epidemiological methods. As discussed in Sections 2 and 3 above, population genetics data have revealed clear host-associated populations. Nonetheless, the genetic differentiation observed between the natural *Ascaris* host associated populations may not reflect a genetic determinant that enables the parasites to differentially infect and reproduce within the different host species. For example, factors unrelated to the host such as the external environment, exposure opportunity, or manner of the process of infection may result in host affiliated parasite populations in nature (McCoy, 2003; Wakelin and Bradley, 2002). Therefore, it is necessary to use experimental infections to exclude environmental effects in order to determine if there is host selection for host-associated parasites.

Molecular markers have been useful in ascertaining infections success. Experimental infection of pigs and mice using *Ascaris* eggs of selected genotypes (e.g., ITS1 genotype G1 derived from humans and G3 derived from pigs) was conducted to test differential infection success of *Ascaris* originating from humans or pigs (Peng et al., 2006). Initial findings indicate that there is a significant difference in the ability of *Ascaris* eggs of genotype G1 and G3 to infect and establish as larvae in mice and as adults in pigs. The disparity in recovery rates from pigs (also from mice) could be explained as family variation in infection success as was observed in Nejsum

et al. (2009b) (discussed below). Nevertheless, the results are in the direction consistent with the very low prevalence of genotype G1 and very high prevalence of genotype G3 in naturally infected pigs in China, and may suggest host affiliation is driven by host-induced selection (Peng et al., 2003, 2007). It will be of interest to repeat this experiment where there is replication within genotype lines to account for among family variation (i.e., multiple families of G1 and G3 are tested). If the results remain the same, this will be a strong indication that host-selective forces may drive the local genetic differentiation patterns between human and pig associated populations of *Ascaris*. Note we are not saying that the ITS1 genotype itself is responsible for infection into different host species, rather the genotypes are simply markers of family lines and host origin.

Genetic markers have also been used to help characterize the infection success of *Ascaris* progeny of known maternal origin. Nejsum et al. (2009b) performed inoculation experiments on pigs using egg mixtures from 4 female worms. Infection success among these 4 families could be monitored due to the fact that each of the 4 female parents had a different mtDNA haplotype, which is maternally inherited. Results revealed significant differences in the abundance and distribution along the small intestine, as well as the size of worms among the 4 families within a pig (Nejsum et al., 2009b). Moreover, the pattern also varied among pigs. These results indicate that not only may there be "a competition mechanism" for the 4 strains of *Ascaris* within the same host individual, but that also host genetic variation may alter these interactions as family infection success varied among different pigs. Importantly, these data show that infection success can vary among worm families. The authors highlight that their results challenge the hypothesis of infection rates being determined by simple host contact rates.

It is interesting to speculate if the variation in family infection success observed in pigs (Nejsum et al., 2009b) is a potential explanation for epidemiological patterns observed in human endemic ascariasis. For instance, Hall and Holland (2000) analysed geographical variation in fecundity (measured by EPG) of *A. lumbricoides*. They found that variation in fecundity was not due to competition for resources or a 'crowding effect' and, that for any given worm burden, there were relatively large differences in worm fecundity. Thus, is this variation in fecundity a result of different combinations of host–parasite genetic backgrounds? Similarly, Peng et al. (2003) studied *Ascaris* fecal egg profiles from humans. Eggs were classified into three profiles: a mixture of fertilized and unfertilized eggs (FUE), fertilized eggs only (FEO), and unfertilized eggs only (UEO). Results showed 71% FUE, 26% FEO, and 3% UEO from all adult *Ascaris* expelled after anthelmintic treatment (Peng et al., 2003). Given the evidence of contemporary cross-transmission and the finding of hybrid or first generation migrant worms into human hosts, it would be interesting to see if these unfertilized eggs are coming from worms that are of pig origin (or recent pig *Ascaris* ancestry). Such data may further highlight host-selection as a mechanism of the host-affiliation patterns (i.e., worms of pig or hybrid origin have reduced fitness in the human host).

If host selection is causing the host-affiliated *Ascaris* populations, then what might be some of the host–parasite interactions determining the infection outcome? Preliminary findings from experimental infections of mice with *Ascaris* of human origin (ITS1 G1 genotype) and pig origin (marked by the G3 genotype) showed a significant difference in host mean spleen weight (Table 1, Peng et al., 2006), and in the level of some cytokines (TNF-a, IFN-r, IL-2 and IL-5) (Zhang et al., 2008). Furthermore, distinct differences in egg hatching (the timing and location of hatching, and the numbers hatched), and in larvae migration and distribution (the means and constituent ratios, the time of peak recovery, and larvae reappearing in intestines) of the two different genetically marked lines were also observed (Qiu, 2007). These all call for future investigations on the relationship between *Ascaris* with known host-origin (which is followed via genetic markers) and host susceptibility/resistance and immune responses.

5. Inference of evolutionary history from genetic data

The evolutionary history and species status of human and pig *Ascaris* has been a bit more problematic to resolve. It is clear there is genetic divergence between sympatric human and pig *Ascaris*. Consequently, the question is, was there a single host-shift resulting in diverged host-affiliated populations (or species)? Anderson et al. (1993) first reported two divergent mtDNA clades (about 3–4%) and subsequent studies in China and Africa support the existence of these two clades (Peng et al., 1998b, 2005; Betson et al., 2011, 2012). At face value, it is tempting to infer that two such clades represent historical isolation among humans and pigs. However, there are four arguments that cloud this interpretation. First, from a genetic yardstick perspective, 3–4% mtDNA divergence is on the borderline of "cryptic species" recognition in many metazoan parasites (Blouin, 2002; Vilas et al., 2005). Therefore, maybe intermediate haplotypes representing intraspecific variation have just not yet been sampled. Second, under a population genetics model of continuous low-dispersal, deep phylogenetic lineages can emerge in the absence of historical barriers to gene flow (e.g., vicariance or host–race isolation) (Irwin, 2002; Kuo and Avise, 2005). Third, Anderson and Jaenike (1997) and Nejsum et al. (2010) both identified mtDNA haplotypes that fall out in a third clade that is about equally divergent from the other two known mtDNA clades. Thus, what led to the evolution of a third divergent clade? Fourth, genetic differentiation on a global scale is higher among populations of a host species relative to between host species for both mtDNA and nuclear markers (Anderson and Jaenike, 1997; Criscione et al., 2007b). In fact, the results of Criscione et al. (2007b) suggest that host-associated populations emerged after geographical isolation. Thus, geography and multiple host-colonization events may have also played an important role in the evolutionary history of human and pig *Ascaris*. Under a multiple host-colonization hypothesis, *Ascaris* (of pig or human origin) colonizes a new geographic location, but in a single host species. Next, a cross-transmission event leads to establishment in the other species in this new location. If conditions are suitable for transmission in both human and pigs, genetic differentiation progresses between the two host-associated populations in sympatry. The process would repeat upon colonizing a new geographic location. Considering reports of mature pig-derived parasites (i.e. gravid females) in humans from areas of non-endemic human transmission (Anderson, 1995; Nejsum et al., 2005b), multiple host colonization events seem plausible. Moreover, genetic data (AFLP, ITS1, and mtDNA) have shown that non-human primates (chimpanzees) have acquired *Ascaris* from pigs in a Denmark Zoo. These infections have persisted since 2003 and egg embryonation was confirmed (Nejsum et al., 2006, 2010). This example provides evidence of a successful host jump and colonization event.

Nadler and Hudspeth (2000) state "Addressing the species status of host-associated *Ascaris* taxa with sequence data requires population-level sampling". We agree with this argument, but to it add that a combined analysis with more global data from multiple sympatric human and pig samples and incorporating many sequenced loci (as such that might be obtained from next generation sequencing methods) will likely be needed to resolve the history of *Ascaris* between humans and pigs. Recent publications of the *A. suum* (Jex et al., 2011) draft genome and complete mtDNA genomes (one worm from humans and another from pigs; Liu et al.,

2012) should greatly facilitate future molecular based studies comparing human and pig *Ascaris*.

It is important to tease apart if multiple colonization events have occurred historically. If multiple colonization events have occurred then this suggests that even in areas of non-human transmission, there remains the possibility (likely depending on local economic and behavioral factors) of establishing a human transmission cycle if parasites persist in sympatric pig populations (Criscione et al., 2007b). Furthermore, a recurrent pattern of colonization means that one cannot infer local contemporary cross-transmission based on global allele frequency data. An allele common in one host species in one location may be rare in that same host species in another location. For example, Betson et al. (2011) found the CO1 haplotype H9-809 (a common *Ascaris* haplotype in humans in China) to be common in pigs from Uganda. Yet, H9-809 is rare in pigs in China (Peng et al., 2005). These authors (Betson et al., 2011) also note that the finding of "pig"-haplotypes (as found from Chinese samples; Peng et al., 2005) from human samples in Zanzibar was "perhaps surprising given the present rarity of these animals on Unguja". However, the result would not be so surprising under the multiple host-colonization hypothesis or a historical introgression event. Either way, these latter two historical scenarios also highlight the limitations of using single markers to interpret cross-transmission. Thus, determination of parasite allele frequencies (at multiple loci) in both local human and pig populations will be needed to test the hypothesis of local contemporary cross-transmission.

6. Use of genetic markers to study mating dynamics, diagnostics and paleoparasitology

Parasite mating and reproductive success are important epidemiological parameters as they determine a parasite's fitness and ultimately, control population growth of the parasite. Thus, it is critical to understand how the host–environment affects parasite fecundity, mating opportunities, or infection success of parasite larvae. Unfortunately, because *Ascaris* is an endoparasite, mating interactions cannot be directly observed. Furthermore, following the infection success of parasite offspring of a given parent would be next to impossible in the field. However, genetic markers can be employed to address these latter two situations under experimental conditions. For example, Zhou et al. (2011) used microsatellites and paternity analyses to show that there is polyandry in pig *Ascaris*. Whereas previous studies were limited to assessing female reproductive success (e.g., Walker et al., 2010), paternity analyses provide a means to determine male reproductive success. It is also interesting to note that several epidemiological studies on human *Ascaris* have found a male to female ratio less than 1 (Cabrera, 1984; Elkins and Haswell-Elkins, 1989; Monzon, 1991; Peng et al., 2002; Seo, 1990). Paternity analyses from field data could help confirm if there are really fewer males in the host or if some males that were successful at mating were just lost from the host prior to sampling. Understanding male and female reproductive skew in the field will also be important for assessing what controls *Ascaris* effective population size (an important population genetics parameter that affects overall levels of genetic diversity; Criscione and Blouin, 2005).

Several molecular diagnostic approaches have been developed to aid in epidemiological studies of *Ascaris* because sometimes only egg or larval stages are available for study. For example, Carlsgart et al. (2009) successfully sequenced ITS1 and mtDNA from a multiplex PCR of individual eggs. Such a method may be helpful in screening patients from fecal samples (e.g., Leles et al., 2009). Egg or larval stages are also often morphologically indistinguishable among closely related parasite species, but genetic markers can help identify species (Criscione et al., 2005). For example, because experimental infections have shown that chickens can possibly serve as a paratenic host to *Ascaris*, Ishiwata et al. (2004) undertook a study to identify tissue-embedded ascarid larvae in naturally infected turkeys. In this case, the molecular identification revealed larvae of *Toxocara canis* and not *Ascaris*. Such methods may prove useful in confirming suspected human cases of visceral larva migrans caused by *Ascaris* (e.g., Maruyama et al., 1996). Another useful application was the recent development of a real-time PCR method of the ITS1 rDNA to quantify *Ascaris* egg viability (Pecson et al., 2006). As the authors note, further development of this method may provide a faster and more accurate measurement of *Ascaris* eggs after treatment of wastewaters.

Ascaris molecular epidemiology has also made its way into the field of paleoparasitology. Loreille et al. (2001) were the first to successfully amplify small fragments of *Ascaris* DNA (176 bp of 18s rDNA and 98 bp of CytB mtDNA) from eggs found in 600 year old coprolites in Namur, Belgium. Subsequent studies have used the same DNA regions to verify *Ascaris* identifications from coprolite or mummified samples from pre-Columbian South American sites (as far back as 8000 years before present; Leles et al., 2008) and a medieval tomb in Seocheon, Korea (Oh et al., 2010). As with all fossil data, it should be recognized that the lack of finding particular genetic variants in paleosamples is not evidence that such variants never existed in that region as the fossil record is likely incomplete. Nonetheless, as suggested by Loreille and Bouchet (2003), molecular paleoparasitological data on *Ascaris* has the potential to provide insight into the evolutionary history of *Ascaris*. For example, if the three divergent mtDNA clades that are currently seen in modern samples (see Section 5) can be found in samples that pre-date pig domestication, then the currently observed mtDNA divergence would unlikely be due to mechanisms arising from modern day human–pig interactions. Also, pre-Columbian samples may shed light on genetic diversity in *Ascaris* of humans, but without the presence of pigs as pigs were not yet introduced into the Americas.

Acknowledgement

The work supported by the National Natural Science Foundation of China (W.P.) (Grant No. 30972580).

References

Abebe, W., Tsuji, N., Kasuga-Aoki, H., Miyoshi, T., Isobe, T., Arakawa, T., Matsumoto, Y., Yoshihara, S., 2002a. Species-specific proteins identified in *Ascaris lumbricoides* and *Ascaris suum* using two-dimensional electrophoresis. Parasitol. Res. 88, 868–871.

Abebe, W., Tsuji, N., Kasuga-Aoki, H., Miyoshi, T., Isobe, T., Arakawa, T., Matsumoto, Y., Yoshihara, S., 2002b. Lung-stage protein profile and antigenic relationship between *Ascaris lumbricoides* and *Ascaris suum*. J. Parasitol. 88, 826–828.

Alba, J.E., Comia, M.N., Oyong, G., Claveria, F., 2009. *Ascaris lumbricoides* and *Ascaris suum*: a comparison of electrophoretic banding patterns of protein extracts from the reproductive organs and body wall. Vet. Arh. 79, 281–291.

Anderson, T.J.C., 1995. *Ascaris* infections in humans from North America: molecular evidence for cross infection. Parasitology 110, 215–219.

Anderson, T.J.C., 2001. The dangers of using single locus markers in parasite epidemiology: *Ascaris* as a case study. Trends Parasitol. 17, 183–188.

Anderson, T.J.C., Jaenike, J., 1997. Host specificity, evolutionary relationships and macrogeographic differentiation among *Ascaris* populations from humans and pigs. Parasitology 115, 325–342.

Anderson, E.C., Thompson, E.A., 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160, 1217–1229.

Anderson, T.J.C., Romero-Abal, M.E., Jaenike, J., 1993. Genetic structure and epidemiology of *Ascaris* populations: patterns of host affiliation in Guatemala. Parasitology 107, 319–334.

Anderson, T.J.C., Romero-Abal, M.E., Jaenike, J., 1995. Mitochondrial DNA and *Ascaris* microepidemiology: the composition of parasite populations from individual hosts, families and villages. Parasitology 110, 221–229.

Arizono, N., Yoshimura, Y., Tohzaka, N., Yamada, M., Tegoshi, T., Onishi, K., Uchikawa, R., 2010. Ascariasis in Japan: is pig-derived *Ascaris* infecting humans? Jpn. J. Infect. Dis. 63, 447–448.

Betson, M., Halstead, F.D., Nejsum, P., Imison, E., Khamis, I.S., Sousa-Figueiredo, J.C., Rollinson, D., Stothard, J.R., 2011. A molecular epidemiological investigation of

Ascaris on Unguja, Zanzibar using isoenyzme analysis, DNA barcoding and microsatellite DNA profiling. Trans. R. Soc. Trop. Med. Hyg. 105, 370–379.
Betson, M., Nejsum, P., Llewellyn-Hughes, J., Griffin, C., Atuhaire, A., Arinaitwe, M., Adriko, M., Ruggiana, A., Turyakira, G., Kabatereine, N.B., Stothard, J.R., 2012. Genetic diversity of *Ascaris* in southwestern Uganda. Trans. R. Soc. Trop. Med. Hyg. 106, 75–83.
Blouin, M.S., 2002. Molecular prospecting for cryptic species of nematodes: mitochondrial DNA versus internal transcribed spacer. Int. J. Parasitol. 32, 527–531.
Boes, J., Willingham 3rd, A.L., Fuhui, S., Xuguang, H., Eriksen, L., Nansen, P., Stewart, T.B., 2000. Prevalence and distribution of pig helminths in the Dongting Lake Region (Hunan Province) of the People's Republic of China. J. Helminthol. 74, 45–52.
Bundy, D.A.P., Guyatt, H. 1996. Schools for health: focus on health, education and the school-age child. Parasitol. Today 12 (Insert), 1–16.
Cabrera, B.D., 1984. Reinfection and infection rates of ascariasis in relation to seasonal variation in the Philippines, Southeast Asian. J. Trop. Med. Public Health 15, 394–401.
Carlsgart, J., Roepstorff, A., Nejsum, P., 2009. Multiplex PCR on single unembryonated *Ascaris* (roundworm) eggs. Parasitol. Res. 104, 939–943.
Chan, M.S., Medley, G.F., Jamison, D., Bundy, D.A.P., 1994. The evaluation of potential global morbidity attributable to intestinal nematode infections. Parasitology 109, 373–387.
Copeman, D.B., Gaafar, S.M., 1972. Sequential development of hepatic lesions of ascaridosis in colostrum-deprived pigs. Aust. Vet. J. 48, 263–268.
Criscione, C.D., Blouin, M.S., 2005. Effective sizes of macroparasite populations: a conceptual model. Trends Parasitol. 21, 212–217.
Criscione, C.D., Blouin, M.S., 2006. Minimal selfing, few clones, and no among-host genetic structure in a hermaphroditic parasite with asexual larval propagation. Evolution 60, 553–562.
Criscione, C.D., Poulin, R., Blouin, M.S., 2005. Mol. Ecol. of parasites: elucidating ecological and microevolutionary processes. Mol. Ecol. 14, 2247–2257.
Criscione, C.D., Anderson, J.D., Raby, K., Sudimack, D., Subedi, J., Rai, D.R., Upadhayay, R.P., Jha, B., Williams-Blangero, S., Anderson, T.J.C., 2007a. Microsatellite markers for the human nematode parasite *Ascaris lumbricoides*: development and assessment of utility. J. Parasitol. 93, 704–708.
Criscione, C.D., Anderson, J.D., Sudimack, D., Peng, W., Jha, B., Williams-Blangero, S., Anderson, T.J.C., 2007b. Disentangling hybridization and host colonization in parasitic roundworms of humans and pigs. Proc. R. Soc. Biol. Sci. Ser. B 274, 2669–2677.
Criscione, C.D., Anderson, J.D., Sudimack, D., Subedi, J., Upadhayay, R.P., Jha, B., Williams, K.D., Williams-Blangero, S., Anderson, T.J.C., 2010. Landscape genetics reveals focal transmission of a human macroparasite. PLoS Neg. Trop. Dis. 4, e665.
Crompton, D.W.T., 1989a. Biology of *Ascaris lumbricoides*. In: Crompton, D.W.T., Nesheim, M.C., Pawlowski, Z.S. (Eds.), Ascariasis and its Prevention and Control. Taylor and Francis Ltd., London and Philadelphia, pp. 9–44.
Crompton, D.W.T., 1989b. Prevalence of ascariasis. In: Crompton, D.W.T., Nesheim, M.C., Pawlowski, Z.S. (Eds.), Ascariasis and its Prevention and Control. Taylor and Francis Ltd., London and Philadelphia, pp. 45–69.
Crompton, D.W.T., 2001. *Ascaris* and ascariasis. Adv. Parasitol. 48, 285–375.
Crompton, D.W., Nesheim, M.C., 2002. Nutritional impact of intestinal helminthiasis during the human life cycle. Annu. Rev. Nutr. 22, 35–59.
de Meeûs, T., McCoy, K.D., Prugnolle, F., Chevillon, C., Durand, P., Hurtrez-Bousses, S., Renaud, F., 2007. Population genetics and molecular epidemiology or how to "debusquer la bete". Infect. Genet. Evol. 7, 308–332.
de Silva, N.R., Brooker, S., Hotez, P.J., Montresor, A., Engels, D., Savioli, L., 2003. Soil-transmitted helminth infections: updating the global picture. Trends Parasitol. 19, 547–551.
Detwiler, J.T., Criscione, C.D., 2010. An infectious topic in reticulate evolution: introgression and hybridization in animal parasites. Genes 1, 102–123.
Dold, C., Holland, C.V., 2011a. *Ascaris* and ascariasis. Microb. Infect. 13, 632–637.
Dold, C., Holland, C.V., 2011b. Investigating the underlying mechanism of resistance to *Ascaris* infection. Microb. Infect. 13, 624–631.
Elkins, D.B., Haswell-Elkins, M., 1989. The weight/length profiles of *Ascaris lumbricoides* within a human community before mass treatment and following reinfection. Parasitology 99, 293–299.
Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10, 564–567.
Forsum, E., Göranzon, H., Rundgren, M., Thilén, M., Hambraeus, L., 1981. Protein evaluation of mixed diets. Comparative study in man and in the pig and rat of vegetable-animal and vegetable protein diets. Ann. Nutr. Metab. 25, 137–150.
Galvin, T.J., 1968. Development of human and pig *Ascaris* in the pig and rabbit. J. Parasitol. 54, 1085–1091.
Hall, A., Holland, C., 2000. Geographical variation in *Ascaris lumbricoides* fecundity and its implications to helminth control. Parasit. Today 16, 540–544.
Holland, C.V., 2009. Predisposition to ascariasis: patterns, mechanisms and implications. Parasitology 136, 1537–1547.
Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., Jacobson, J., 2008. Helminth infections: the great neglected tropical diseases. J. Clin. Invest. 118, 1311–1321.
Ibrahim, A.P., Conway, D.J., Hall, A., Bundy, D.A.P., 1994. Enzyme polymorphisms in *Ascaris lumbricoides* in Bangladesh. Trans. R. Soc. Trop. Med. Hyg. 88, 600–603.
Irwin, D.E., 2002. Phylogeographic breaks without geographic barriers to gene flow. Evolution 56, 2383–2394.
Ishiwata, K., Shinohara, A., Yagi, K., Horii, Y., Tsuchiya, K., Nawa, Y., 2004. Identification of tissue-embedded ascarid larvae by ribosomal DNA sequencing. Parasitol. Res. 92, 50–52.
Jex, A.R., Liu, S.P., Li, B., Young, N.D., Hall, R.S., Li, Y.R., Yang, L.F., Zeng, N., Xu, X., Xiong, Z.J., Chen, F.Y., Wu, X., Zhang, G.J., Fang, X.D., Kang, Y., Anderson, G.A., Harris, T.W., Campbell, B.E., Vlaminck, J., Wang, T., Cantacessi, C., Schwarz, E.M., Ranganathan, S., Geldhof, P., Nejsum, P., Sternberg, P.W., Yang, H.M., Wang, J., Gasser, R.B., 2011. *Ascaris suum* draft genome. Nature 479, 529–533.
Kennedy, M.W., Qureshi, F., Haswellelkins, M., Elkins, D.B., 1987. Homology and heterology between the secreted antigens of the parasitic larval stages of *Ascaris lumbricoides* and *Ascaris suum*. Clin. Exp. Immunol. 67, 20–30.
Kuo, C.H., Avise, J., 2005. Phylogeographic breaks in low-dispersal species: the emergence of concordance across gene trees. Genetica 124, 179–186.
Kurimoto, H., 1974. Morphological, biochemical and immunological studies on the differences between *Ascaris lumbricoides* Linneaus, 1758 and *Ascaris suum* Goez, 1782. Jpn. J. Parasitol. 23, 251–267.
Leles, D., Araujo, A., Ferreira, L.F., Vicente, A.C.P., Iniguez, A.M., 2008. Molecular paleoparasitological diagnosis of *Ascaris* sp from coprolites: new scenery of ascariasis in pre-Columbian South America times. Mem. Inst. Oswaldo. Cruz. 103, 106–108.
Leles, D., Araujo, A., Vicente, A.C.P., Iniguez, A.M., 2009. Molecular diagnosis of ascariasis from human feces and description of a new *Ascaris* sp genotype in Brazil. Vet. Parasitol. 163, 167–170.
Leles, D., Araujo, A., Vicente, A.C.P., Iniguez, A.M., 2010. ITS1 intra-individual variability of *Ascaris* isolates from Brazil. Parasitol. Int. 59, 93–96.
Leslie, J.F., Cain, G.D., Meffe, G.K., Vrijenhoek, R.C., 1982. Enzyme polymorphism in *Ascaris suum* (Nematoda). J. Parasitol. 68, 576–587.
Liu, G.-H., Wu, C.-Y., Song, H.-Q., Wei, S.-J., Xu, M.-J., Lin, R.-Q., Zhao, G.-H., Huang, S.-Y., Zhu, X.Q., 2012. Comparative analyses of the complete mitochondrial genomes of *Ascaris lumbricoides* and *Ascaris suum* from humans and pigs. Gene 492, 110–116.
Loreille, O., Bouchet, F., 2003. Evolution of ascariasis in humans and pigs: a multi-disciplinary approach. Mem. Inst. Oswaldo. Cruz. 98, 39–46.
Loreille, O., Roumat, E., Verneau, O., Bouchet, F., Hanni, C., 2001. Ancient DNA from *Ascaris*: extraction amplification and sequences from eggs collected in coprolites. Int. J. Parasitol. 31, 1101–1106.
Macko, J.K., Dubinsky, P., 1997. Taxonomic deliberations on human and pig ascarids. Helminthologia 34, 167–171.
Maruyama, H., Nawa, Y., Noda, S., Mimori, T., Choi, W.Y., 1996. An outbreak of visceral larva migrans due to *Ascaris suum* in Kyushu, Japan. Lancet 347, 1766–1767.
McCoy, K.D., 2003. Sympatric speciation in parasites - what is sympatry? Trends Parasitol. 19, 400–404.
Monzon, R.B., 1991. Replacement patterns of *Ascaris lumbricoides* populations in Filipino children. Southeast Asian J. Trop. Med. Pub. Health 22, 605–610.
Nadler, S.A., 1987. Biochemical and immunological systematics of some ascaridoid nematodes: genetic divergence between congeners. J. Parasitol. 73, 811–816.
Nadler, S.A., Hudspeth, D.S.S., 2000. Phylogeny of the ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: hypotheses of structural and sequence evolution. J. Parasitol. 86, 380–393.
Nadler, S.A., Lindquist, R.L., Near, T.J., 1995. Genetic structure of midwestern *Ascaris suum* populations: a comparison of isoenzyme and RAPD markers. J. Parasitol. 81, 385–394.
Nansen, P., Roepstorff, A., 1999. Parasitic helminths of the pig: factors influencing transmission and infection levels. Int. J. Parasitol. 29, 877–891.
Nascetti, G., Grappelli, C., Bullini, L., Montalenti, S.G., 1979. Ricerche sul differenziamento genetico di *Ascaris lumbricoides* e *Ascaris suum*. Atti della Accademia Nazionale dei Lincei Rendiconti Classe di Scienze Fisiche Matematiche e Naturali 67, 457–465.
Nejsum, P., Frydenberg, J., Roepstorff, A., Parker, E.D.J., 2005a. Population structure in *Ascaris suum* (Nematoda) among domestic swine in Denmark as measured by whole genome DNA fingerprinting. Hereditas 142, 7–14.
Nejsum, P., Parker, E.D., Frydenberg, J., Roepstorff, A., Boes, J., Haque, R., Astrup, I., Prag, J., Sorensen, U.B.S., 2005b. Ascariasis is a zoonosis in Denmark. J. Clin. Microbiol. 43, 1142–1148.
Nejsum, P., Grondahl, C., Murrell, K.D., 2006. Molecular evidence for the infection of zoo chimpanzees by pig *Ascaris*. Vet. Parasitol. 139, 203–210.
Nejsum, P., Roepstorff, A., Jorgensen, C.B., Fredholm, M., Goring, H.H.H., Anderson, T.J.C., Thamsborg, S.M., 2009a. High heritability for *Ascaris* and *Trichuris* infection levels in pigs. Heredity 102, 357–364.
Nejsum, P., Roepstorff, A., Anderson, T.J.C., Jorgensen, C., Fredholm, M., Thamsborg, S.M., 2009b. The dynamics of genetically marked *Ascaris suum* infections in pigs. Parasitology 136, 193–201.
Nejsum, P., Bertelsen, M.F., Betson, M., Stothard, J.R., Murrell, K.D., 2010. Molecular evidence for sustained transmission of zoonotic *Ascaris suum* among zoo chimpanzees (*Pan troglodytes*). Vet. Parasitol. 171, 273–276.
Nganga, C.J., Karanja, D.N., Mutune, M.N., 2008. The prevalence of gastrointestinal helminth infections in pigs in Kenya. Trop. Anim. Health Prod. 40, 331–334.
Oh, C.S., Seo, M., Lim, N.J., Lee, S.J., Lee, E.J., Lee, S.D., Shin, D.H., 2010. Paleoparasitological report on *Ascaris* aDNA from an ancient East Asian sample. Mem. Inst. Oswaldo. Cruz. 105, 225–228.
O'Lorcain, P., Holland, C.V., 2000. The public health importance of *Acaris lumbricoides*. Parasitology 121, S51–S71.
Pecson, B.M., Barrios, J.A., Johnson, D.R., Nelson, K.L., 2006. A real-time PCR method for quantifying viable *Ascaris* eggs using the first internally transcribed spacer region of ribosomal DNA. Appl. Environ. Microbiol. 72, 7864–7872.

Peng, W., Zhou, X., Cui, X., Crompton, D.W., Whitehead, R.R., Xiong, J., Wu, H., Peng, J., Yang, Y., Wu, X., Xu, K., Yan, Y., 1996. *Ascaris*, people and pigs in a rural community of Jiangxi Province, China. Parasitology 113, 545–557.

Peng, W., Zhou, X., Crompton, D.W.T., 1998a. Ascariasis in China. Adv. Parasitol. 41, 109–148.

Peng, W., Anderson, T.J.C., Zhou, X., Kennedy, M.W., 1998b. Genetic variation in sympatric *Ascaris* populations from humans and pigs in China. Parasitology 117, 355–361.

Peng, W., Cui, X., Zhou, X., 2002. Comparison of the structures of natural and re-established populations of *Ascaris* in humans in a rural community of Jiangxi. China. Parasitology. 124, 641–647.

Peng, W., Yuan, K., Zhou, X.M., Hu, M., El-Osta, Y.G.A., Gasser, R.B., 2003. Molecular epidemiological investigation of *Ascaris* genotypes in China based on single-strand conformation polymorphism analysis of ribosomal DNA. Electrophoresis 24, 2308–2315.

Peng, W., Yuan, K., Hu, M., Zhou, X.M., Gasser, R.B., 2005. Mutation scanning-coupled analysis of haplotypic variability in mitochondrial DNA regions reveals low gene flow between human and porcine *Ascaris* in endemic regions of China. Electrophoresis 26, 4317–4326.

Peng, W., Yuan, K., Hu, M., Peng, G., Zhou, X., Hu, N., Gasser, R.B., 2006. Experimental infections of pigs and mice with selected genotypes of *Ascaris*. Parasitology 133, 651–657.

Peng, W., Yuan, K., Hu, M., Gasser, R.B., 2007. Recent insights into the epidemiology and genetics of *Ascaris* in China using molecular tools. Parasitology 134, 325–330.

Pérez, J., García, P.M., Mozos, E., Bautista, M.J., Carrasco, L., 2001. Immunohistochemical characterization of hepatic lesions associated with migrating larvae of *Ascaris suum* in pigs. J. Comp. Pathol. 124, 200–206.

Perkins, S.L., Martinsen, E.S., Falk, B.G., 2011. Do molecules matter more than morphology? Promises and pitfalls in parasites. Parasitology 138, 1664–1674.

Qiu, L., 2007. Migration and distribution of *Ascaris* representing different genotypes in C57BL/6 mice. Master's Thesis, Nanchang University, China (in Chinese with English summery). Available from: <http://www.epub.cnki.net/grid2008/detail.aspx?filename=2008041426.nh&dbname=CMFD2008>.

Roepstorff, A. Nansen, P., 1998. Epidemiology, diagnosis and control of helminth parasites of swine. In: FAO Animal Health Manual No. 3, Food and Agricultural Organization of the United Nations, Rome.

Roepstorff, A., Nilsson, O., O'Callaghan, C.J., Oksanen, A., Gjerde, B., Richter, S.H., Ortenberg, E.O., Christensson, D., Nansen, P., Eriksen, L., Medley, G.F., 1999. Intestinal parasites in swine in the Nordic countries: multilevel modeling of *Ascaris suum* infections in relation to production factors. Parasitology 119, 521–534.

Schwab, A.E., Churcher, T.S., Schwab, A.J., Basanez, M.G., Prichard, R.K., 2006. Population genetics of concurrent selection with albendazole and ivermectin or diethylcarbamazine on the possible spread of albendazole resistance in *Wuchereria bancrofti*. Parasitology 133, 589–601.

Seo, B.S., 1990. Epidemiology and control of ascariasis in Korea. Korean J. Parasitol. 28 (Suppl.), S49–S61.

Sharabi, H.A., Abdel-Shafy, S., Derbala, A.A., 2010. The role of dogs in transmission of *Ascaris lumbricoides* for humans. Parasitol. Res. 106, 1021–1026.

Stephenson, L.S., 1987. The Impact of Helminth Infection on Human Nutrition. Taylor and Francis Ltd., London, Philadelphia.

Stephenson, L.S., Latham, M.C., Ottesen, E.A., 2000. Malnutrition and parasitic helminth infections. Parasitology 121, S23–S38.

Stewart, T.B., Hale, O.M., 1988. Losses to internal parasites in swine production. J. Ani. Sci. 66, 1548–1554.

Sun, F.H., Shen, M.X., Xu, X.Z., Cao, H.J., Jiang, W.C., Ji, Z.H., 2008. Investigation on human ascaris infection in China. J. Path. Biol. 3, 936–939.

Takata, I., 1951. Experimental infection of man with *Ascaris* of man and pig. Kitasato Arch. Exp. Med. 23, 49–59.

Thein-Hlaing, 1993. Ascariasis and childhood malnutrition. Parasitology 107, S125–S136.

Vilas, R., Criscione, C.D., Blouin, M.S., 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology 131, 839–846.

Wakelin, D., Bradley, J.E., 2002. Parasite strain diversity and host immune responses. In: Holland, C.V., Kennedy, M.W. (Eds.), World Class Parasites, The Geohelminths: *Ascaris*, Trichuris and Hookworm, vol. 2. Kluwer Academic Publishers, Boston, pp. 199–218.

Walker, M., Hall, A., Basanez, M.G., 2010. Trickle or clumped infection process? An analysis of aggregation in the weights of the parasitic roundworm of humans, *Ascaris lumbricoides*. Int. J. Parasitol. 40, 1373–1380.

Walker, M., Hall, A., Basanez, M.G., 2011. Individual predisposition, household clustering and risk factors for human infection with *Ascaris lumbricoides*: new epidemiological insights. PLoS Neg. Trop. Dis. 5, e1047.

Weng, Y.B., Hu, Y.J., Li, Y., Li, B.S., Lin, R.Q., Xie, D.H., Gasser, R.B., Zhu, X.Q., 2005. Survey of intestinal parasites in pigs from intensive farms in Guangdong Province, People's Republic of China. Vet. Parasitol. 127, 333–336.

WHO, 1987. Prevention and Control of Intestinal Parasitic Infections. Technical Report Series, 749. World Health Organization. Geneva.

WHO, 2006. Preventative chemotherapy in human helminthiases: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme Managers. World Health Organization, Geneva.

Williams-Blangero, S., Subedi, J., Upadhayay, R.P., Manral, D.B., Rai, D.R., Jha, B., Robinson, E.S., Blangero, J., 1999. Genetic analysis of susceptibility to infection with *Ascaris lumbricoides*. Am. J. Trop. Med. Hyg. 60, 921–926.

Wu, S.Q., Lin, X.M., Zhu, X.Q., 2010. Identification and functional analysis of differentially expressed genes of *Ascaris suum* Goeze, 1782 from *Ascaris lumbricoides* Linnaeus, 1758. Agric. Sci. China 9, 903–910.

Zhang, S.K., Yuan, K., Yuan, F., Dai, Z.F., Hu, Y., Huang, Y.Q., Peng, W., 2008. Influence on host immunoregulation of *Ascaris* of selected genotypes. In: Proceedings of the Sixth National Immunology Academic Conference of China, p. 276. Available from: http://www.cpfd.cnki.com.cn/Article/CPFDTOTAL-IGMD200810001396. htm>.

Zhou, C.H., 2011. Further investigations of molecular genetics on *Ascaris* populations from humans and pigs from China. Doctoral dissertation, Nanchang University, China (in Chinese with English summery).

Zhou, X., Peng, W., Crompton, D.W.T., Xiong, J., 1999. Treatment of biliary ascariasis in China. Trans. Roy. Soc. Trop. Med. Hyg. 93, 561–564.

Zhou, C.H., Yuan, K., Tang, X., Hu, N., Peng, W., 2011. Molecular genetic evidence for polyandry in *Ascaris suum*. Parasitol. Res. 108, 703–708.

Zhu, X.Q., Chilton, N.B., Jacobs, D.E., Boes, J., Gasser, R.B., 1999. Characterisation of *Ascaris* from human and pig hosts by nuclear ribosomal DNA sequences. Int. J. Parasitol. 29, 469–478.