Journal of Helminthology (2012) **86,** 148–155
doi:10.1017/S0022149X12000193

Assessing the zoonotic potential of *Ascaris suum* and *Trichuris suis*: looking to the future from an analysis of the past

P. Nejsum[1,2]*, M. Betson[3], R.P. Bendall[4], S.M. Thamsborg[1] and J.R. Stothard[3]

[1]Danish Centre for Experimental Parasitology, Department of Veterinary Disease Biology, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark: [2]Genetics and Bioinformatics, Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, Copenhagen University: [3]Disease Control Strategy Group, Liverpool School of Tropical Medicine, Liverpool, UK: [4]Royal Cornwall Hospital, Clinical Microbiology, Truro, UK

(Received 21 November 2011; Accepted 23 February 2012; First Published Online 19 March 2012)

Abstract

The two geohelminths, *Ascaris lumbricoides* and *Trichuris trichiura*, infect more than a billion people worldwide but are only reported sporadically in the developed part of the world. In contrast, the closely related species *A. suum* and *T. suis* in pigs have a truly global distribution, with infected pigs found in most production systems. In areas where pigs and humans live in close proximity or where pig manure is used as fertilizer on vegetables for human consumption, there is a potential risk of cross-infections. We therefore review this relationship between *Ascaris* and *Trichuris* in the human and pig host, with special focus on recent evidence concerning the zoonotic potential of these parasites, and identify some open questions for future research.

Introduction

Nematode worms of the genera *Ascaris* and *Trichuris* are the most prevalent helminth infections in humans and pigs worldwide (Holland & Boes, 2002). In both cases, the worms that infect pigs and humans are closely related and difficult to distinguish morphologically owing to a lack of discrete characters. The life cycle is direct and worms do not multiply directly within the definitive host. In order to complete the life cycle for both genera, oral ingestion of faecally excreted ova is required but eggs are only infectious after a period of environmental maturation. This life cycle makes it possible for worms of porcine origin to infect humans and (possibly) vice versa (Crompton, 2001).

*Fax: + 45 35282774
E-mail: pn@life.ku.dk

In order to implement appropriate control programmes for *Ascaris* and *Trichuris* in both hosts and to identify the source of the infection, a greater understanding of the transmission dynamics and host specificity of the parasites is needed. There has been an ongoing debate in the literature as to whether *Ascaris* and *Trichuris* are zoonotic infections and the precise levels of actual cross-over. Here, we review briefly the relationship between *Ascaris* and *Trichuris* in humans and pigs, with special focus on recent evidence concerning the zoonotic potential of these parasites.

Burden of infection

An estimated 1.2 billion humans are infected with *A. lumbricoides* and 600–800 million with *T. trichiura*, mainly in sub-Saharan Africa, Latin America and Asia, although sporadic cases are reported in developed countries (Chan, 1997; De Silva *et al.*, 2003; Hotez *et al.*, 2008). Human

infections are associated with diarrhoea, malnutrition, impaired growth and development, and can lead to death (De Silva *et al.*, 1997; Bethony *et al.*, 2006; Hall *et al.*, 2008; Dold & Holland, 2011). The burden of these two helminth infections has been estimated at around 17 million disability-adjusted life years (DALY), which is half the estimate for malaria (Chan, 1997), but there are substantial difficulties in generating DALY estimates due to the non-specific and insidious nature of much of the morbidity associated with intestinal helminth infections and uncertainty about the numbers of individuals infected (Brooker, 2010).

According to farming practices, *A. suum* and *T. suis* are present worldwide in pigs but their distributions are highly influenced by host environment (including management practices and hygiene) and geographical region (reviewed by Nansen & Roepstorff, 1999). In the Nordic countries, the mean prevalence of *A. suum* is 21.5% in fatteners and 11.3% in sows (Roepstorff *et al.*, 1998), whereas *T. suis* is found sporadically under these indoor conditions (Roepstorff & Jorsal, 1989; Roepstorff *et al.*, 1998). In contrast, free range or organic systems seem to favour higher prevalences as 9 out of 10 outdoor (organic) farms were positive for *T. suis* in Denmark and 37.5% were positive in The Netherlands (Carstensen *et al.*, 2002; Eijck & Borgsteede, 2005). Likewise, in rural communities in China and Uganda prevalences of around 16% were reported for *T. suis* and 40% for *A. suum* (Boes *et al.*, 2000; Nissen *et al.*, 2011).

Focus on ascariasis

Biology

Ingested infective *Ascaris* eggs hatch in the intestine, releasing L3 larvae which undertake a hepato-tracheal migration to end up in the small intestine where they mature. Adult males and females mate and eggs can be found in the faeces around 7 weeks after infection (e.g. Roepstorff *et al.*, 1997).

Ascaris has an enormous reproductive potential and it has been estimated that a single female may produce more than 1 million eggs per day (Kelley & Smith, 1956; Olsen *et al.*, 1958). This suggests that a single infected host may rapidly contaminate the environment, or that contamination might take place if faecal matter is used as fertilizer. The resistant nature of the eggs adds to this problem. *Ascaris* eggs have been shown to remain viable in the soil for at least 6 years (Muller, 1953) and up to 9 years (Krasnonos, 1978; Roepstorff *et al.*, 2011), resulting in a long-lasting health risk.

Diagnosis

Microscopic detection of eggs in faecal samples remains the 'gold standard' for diagnosis, requiring specialized equipment and training, which is often lacking in developing countries. Most larvae are expelled from pig (and probably also human) hosts at an early stage, when they are too small to be observed by the naked eye (Roepstorff *et al.*, 1997; Nejsum *et al.*, 2009). Macroscopic *Ascaris* worms in faeces are seen only in a minority of cases. Thus diagnosis of infection is poor and prevalence is probably underestimated in medical and veterinary practice. A serological study has shown that 8% of Dutch primary schoolchildren and 7% of Swedish adults have antibodies to *Ascaris* even though ascariasis was rare (Van Knapen *et al.*, 1992). A similar prevalence (7%) has more recently been observed in 4-year-old children ($n = 629$) living in The Netherlands (Pinelli *et al.*, 2009), suggesting no change in *Ascaris* exposure over the years (Pinelli *et al.*, 2011). In a recent study from The Netherlands, 2838 serum samples were collected over a 12-year period from patients suspected to have visceral or ocular larva migrans and were analysed for the presence of *Ascaris* antibodies (Pinelli *et al.*, 2011). An average prevalence of 33% was found, with the lowest (19%) in the youngest age group (0–10 years) and the highest (50%) in the oldest age group (70 + years). Interestingly, in those patients suspected of visceral larva migrans the prevalence was four times higher for *Ascaris* than for *Toxocara*. Even though the enzyme-linked immunosorbent assay (ELISA) assay cannot discriminate between *A. suum* and *A. lumbricoides* infections, these studies suggest that *Ascaris* in humans is more common in the developed world than previously thought, and the high prevalence indicates that at least some cases might be of pig origin.

Zoonotic ascariasis

Ascaris infection is generally thought to be uncommon in humans in developed countries and associated with previous residence in, or travel to, endemic areas (Maguire, 2005) or due to foodborne infection (Räisänen *et al.*, 1985). However, sporadic cases of human ascariasis in areas with low incidence have been reported and have been explained by working with infective *A. suum* eggs or contact with pig manure (Jaskoski, 1961; Phillipson & Race, 1967; Crewe & Smith, 1971; Lord & Bullock, 1982). For example, in Denmark, pig manure was used as fertilizer in the vegetable gardens of a kindergarten and a small commune. In both cases, children subsequently expelled large adult *Ascaris* and analysis of soil samples revealed numerous infective eggs (Roepstorff *et al.*, 2011). These cases, together with the ELISA results discussed above (Van Knapen *et al.*, 1992; Pinelli *et al.*, 2009, 2011), suggest that pig *Ascaris* may cause zoonotic infections.

The taxonomic status and relationship between *Ascaris* in pigs and humans have been explored for decades. Both types of worms were shown to be capable of cross-infection (Takata, 1951; Galvin, 1968) but these studies also indicated that *Ascaris* is more adapted to its 'appropriate' host, implying that some speciation between pig and human worms has taken place. Morphological studies revealed subtle differences in denticle morphology and lip shape between *Ascaris* worms from the two hosts (Sprent, 1952; Ansel & Thibaut, 1973; Maung, 1973). Subsequently, immunological and biochemical methods were applied to the problem (Kennedy *et al.*, 1987; Nadler, 1987; Hawley & Peanasky, 1992). More recently Abebe *et al.* (2002b) used two-dimensional electrophoresis to identify species-specific proteins in extracts from adult worms from pig and human hosts. Using the same technique on lung-stage larvae, one major protein specific for each of the two parasites was detected (Abebe *et al.*, 2002a). However, it is

difficult to draw firm conclusions from these studies, as either the methods used did not have high-enough resolution to detect differences between *Ascaris* from the two hosts, or sympatric worms were not compared. Thus, the differences detected could be due to geographical and/or intrinsic variability in the *Ascaris* populations and not related to host specificity.

Molecular characterization of Ascaris

Sympatric areas in developing countries

Anderson *et al.* (1993) were the first to apply molecular methods (in this case polymerase chain reaction (PCR)-linked restriction fragment length polymorphism, PCR-RFLP) to sympatric *Ascaris* populations in order to explore transmission cycles/epidemiology in an area where humans and pigs lived in close proximity. This work was later supplemented with more worms ($n = 265$) and more detailed genetic analysis. It was concluded that *Ascaris* in humans and pigs in Guatemala represented two different reproductive populations with little or no gene flow between them, implying that *A. suum* is not a zoonosis in this region (Anderson *et al.*, 1993; Anderson & Jaenike, 1997). Application of a similar molecular approach to 115 sympatric worms obtained from humans and pigs in China (Peng *et al.*, 1998) led to an analogous conclusion. This was later confirmed by the use of single-strand conformation polymorphism (SSCP) to detect nucleotide variation in the internal transcribed spacer (ITS) region and mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) and NADH dehydrogenase subunit 1 (*nad*1) genes of 486 and 329 worms from humans and pigs, respectively, collected from six provinces in China (Peng *et al.*, 2003, 2005). Even though no fixed genetic differences were identified (i.e. diagnostic markers) between worms from the two hosts in any of the above-mentioned studies, the data suggest that *A. lumbricoides* and *A. suum* are different species based on the criterion of reproductive isolation in sympatry (Mayr, 1963).

More recently, detailed genetic analysis (23 micro-satellite loci) identified 4 and 7% of worms from China and Guatemala, respectively, as being hybrids (Criscione *et al.*, 2007), suggesting that cross-infections and inter-breeding can take place between the pig and human worm populations in areas where humans and pigs live in close proximity. Even though this study only included a limited number of worms ($n = 129$), more research applying fine-scale genetic analysis to sympatric *Ascaris* populations is warranted in order to illuminate the zoonotic potential of *A. suum* in these settings. It is possible that previous studies might simply have lacked power to detect cross-transmission and hybridization due to the use of single/few markers.

Developed world

Molecular evidence for zoonotic *Ascaris* infections was first reported in North America by Anderson (1995), using PCR-RFLP on the ITS region, and identified ten worms from nine patients as pig worms. Nejsum *et al.* (2005) used amplified fragment length polymorphism (AFLP) to genetically compare worms obtained from humans in Denmark with *Ascaris* from the two hosts from worldwide locations. They concluded that all examined Danish human worms (32) were due to cross-infections from pig. This route of transmission has also been described in the UK using *cox*1 sequencing and PCR-RFLP (Bendall *et al.*, 2011). Here all 11 human UK worms were found to have genetic profiles resembling pig worms when compared with *Ascaris* obtained from humans ($n = 20$) and pigs ($n = 35$) from several geographical locations. The molecular results were supported by epidemiological evidence for both the UK and Denmark, since use of pig manure as a fertilizer in vegetable gardens, living in the countryside or close to pig farms, and being under 5 years of age were found to be risk factors for ascariasis (Nejsum *et al.*, 2005; Bendall *et al.*, 2011). These studies suggest that cross-infections from pigs are the most likely source of the infection in industrialized parts of the world. However, this may not always be the case since Arizono *et al.* (2010) found that humans in Japan most likely were infected with both *A. lumbricoides* and *A. suum*. Thus molecular methods supplemented with epidemiology are needed for proper identification of the source of infection.

The close phylogeny between *Ascaris* in humans and pigs may reflect the complex evolutionary history of *Ascaris* (Loreille & Bouchet, 2003) and may be due to multiple host colonization events, as suggested by Criscione *et al.* (2007) who found that worms assort first by geography (Nepal, China and Guatemala) and then by host origin. Sequence analysis of the *cox*1 gene (mitochondrial) indicates that *Ascaris* does not assort into two different monophyletic groups based on host origin, but instead splits into three or more groups, all but one of which include worms from both hosts (Nejsum *et al.*, 2010; Betson *et al.*, 2011; Zhou *et al.*, 2011). This likewise suggests a complex evolutionary history and might be the reason why a single diagnostic marker is presently hard or impossible to find, which is further supported by the recent published complete mitochondrial DNA (mtDNA) genomes of *A. suum* and *A. lumbricoides* (Liu *et al.*, 2012a).

Ascaris *infections in other animals*

Ascariasis has also been detected in chimpanzees in Copenhagen Zoo and a permanent transmission cycle appears to have been established there (Nejsum *et al.*, 2010). Interestingly, molecular analysis showed that the worms expelled by the chimpanzees mainly clustered phylogenetically with *Ascaris* from pigs rather than *Ascaris* from humans. The fact that *A. suum* is able to establish in primates may have implications for human infection and could serve as a model. Worms obtained from lambs that had been grazing on pastures that previously had been used for *A. suum* infection studies in pigs were AFLP genotyped and found to be of pig origin (Nejsum, unpublished data). Other studies also report on *Ascaris* in lambs with affected livers (Sauvageau & Frechette, 1980) and lungs (Clark *et al.*, 1989) due to migrating worms, all suspected to be of pig origin, and patent infections have been obtained after experimental *A. suum* infection (Pedersen *et al.*, 1992). Likewise, calves have repeatedly been reported to be infected with *Ascaris* from pigs (McCraw & Lautenslager, 1971; Roneus &

Christensson, 1977) and the above-mentioned studies provide further evidence that *A. suum* is not specific in its host preference.

Focus on trichuriasis

Biology

Infective *Trichuris* eggs hatch in the intestine releasing L1 larvae which enter the crypts of Lieberkühn in the caecum and upper part of colon (Beer, 1973). The larvae grow and undergo four moults and, as adults, the thick posterior ends are free in the lumen whereas the anterior part is attached and forms a tunnel within the epithelium (Tilney *et al.*, 2005). Eggs can be found in the faeces 7 weeks after infection (e.g. Kringel & Roepstorff, 2006). A *Trichuris* female may produce 2–20,000 eggs/day (Bundy & Cooper, 1989). In pigs, Pedersen & Saeed (2000) have estimated a ratio between faecal egg count (eggs/g) and number of worms to be around 10, where similar estimates are ~2–400 in humans (Bundy & Cooper, 1989). Once excreted *T. suis* ova remain viable for at least 11 years (Hill, 1957; Burden *et al.*, 1987).

In contrast to *Ascaris* the relationship between *Trichuris* in humans and pigs has not been given much attention, suggesting that their taxonomic status is settled (i.e. *T. trichiura* in humans and *T. suis* in pigs). This, together with close similarity in egg morphology, which means that cases of cross-infections are undetected by standard methods, might be the reason why the zoonotic potential of *T. suis* has been ignored to date. In contrast, the larger eggs of the dog whipworm, *T. vulpis* are more easily identified and several cases of humans cross-infected by *T. vulpis* have been described, even though most of these cases have not been properly evaluated (reviewed by Traversa, 2011). Recently Areekul *et al.* (2010) used molecular methods and found that 11% of the *Trichuris*-positive children in Thailand had *T. vulpis* eggs in their faeces, suggesting that this parasite should be considered a zoonosis in this area. *Trichuris suis* is a more obvious candidate than *T. vulpis* since it is phylogenetically more closely related to *T. trichiura* (e.g. Areekul *et al.*, 2010); yet it has received very little attention to date. Below we highlight some of the studies on the relationship between *Trichuris* in humans and pigs in order to shed light on the taxonomic status of these parasites and the zoonotic potential of *T. suis*.

It has proven difficult or impossible to discriminate between *Trichuris* from human or pig hosts by morphology, whether examining adult worms, larvae or eggs (Beer, 1976; Soulsby, 1982). Even though a range of different morphometric characters has been measured, most show overlapping ranges (Beer, 1976; Ooi *et al.*, 1993; Spakulova, 1994; Cutillas *et al.*, 2009). Although spicule length seemed to hold the most promise as a discriminating characteristic, contrasting results have been reported (Spakulova, 1994; Cutillas *et al.*, 2009). This suggests that morphometric measures should be interpreted with care, since the phenotype of the worm may be shaped by host species, e.g. as a reflection of differences in host physiology. In this way, Cutillas *et al.* (2009) found the opposite trend with respect to worm size and spicule length to Spakulova (1994) and Nissen *et al*. (2012). Likewise, Knight (1984) found that anterior length of *T. ovis* was influenced by its development in lambs, goats or calves. Of course, if sympatric material is not included, differences might simply reflect geographic variation and not be related to host species.

Beer (1976) has reported on two patent *T. suis* infections in humans, with egg excretion 40 and 60 days postinfection, respectively. These eggs were subsequently embryonated and used to produce patent infections in pigs. In a human receiving *T. suis* egg therapy, worms have also been observed (Kradin *et al.*, 2006) but other means of infection could not be ruled out. Even though adult worms might develop only exceptionally in these cases, the beneficial immuno-modulatory effects with this kind of egg therapy in patients with inflammatory bowel disease or other immune-related disorders suggest that *T. suis* worms can establish, at least temporarily, in humans (e.g. Summers *et al.*, 2005). These findings may not, however, be applicable in 'natural' settings. *Trichuris trichiura* can be established in pigs but, so far, most of the worms do not persist (Beer, 1976). Overall, these reports show that humans can become cross-infected with *T. suis* under experimental conditions, but also indicate that *T. suis* and *T. trichiura* are more adapted to their 'appropriate' hosts.

Molecular characterization of Trichuris

Cuttilas *et al*. (2009) have performed sequence analysis of the ITS-2 region of *Trichuris* eggs from non-human primates (*Colobus guereza kikuyensis* and *Nomascus gabriellae*) and worms from pigs in Spain. These data suggested that *Trichuris* in the two hosts belonged to two different species, but it is not clear whether the monkeys in the zoo were infected with *T. trichiura* or another trichurid. Using a similar sequence analysis of the ITS-2 region followed by PCR-RFLP on sympatric worm material from Uganda, Nissen *et al*. (2012) showed that worms from the two hosts primarily belonged to two different populations (i.e. are two different species) but also suggested that 3 out of 29 (10%) human worms were of pig origin. This study examined a limited number of worms and further studies on the transmission dynamics of *Trichuris* are needed to unravel the zoonotic potential of *T. suis* and to further develop molecular tools that can be used to trace the source of the infection.

Some future perspectives

For both *Ascaris* and *Trichuris* the extent to which pig-associated and human-associated worms represent different species is difficult to assess because conventional morphological or biometric criteria does not seem to be useful for differentiation between worms in the two hosts. Only molecular methods have shown the ability to discriminate between these closely related parasites and, even here, great caution should be exercised not to reach premature conclusions. Ideally, both nuclear and mitochondrial markers should be used in molecular characterization (Anderson, 2001) or at least several multilocus markers such as microsatellites, also with a general 'global' appraisal of the diversity in such markers

before more focused application and interpretation in selected epidemiological settings. As the biodiversity of worms may not be evenly partitioned across the transmission landscape, according to phylogeographic processes, and the monophyly of 'species' as we recognize them today is contentious, some recourse to inspection of genetic variation in other related Ascaridid worms is needed. It may well be that either species is a local chimera of polyphyletic origins. The recent publication of the *A. suum* genome may lead to identification of further markers that can be used to distinguish between pig-associated and human-associated worms (Jex *et al.*, 2011).

It seems that *A. suum* is a zoonotic infection in developed countries, since expelled worms from humans in this area of the world are mainly of pig origin, based on genetic characterization of the worms as reviewed above, although some cases of ascariasis in this region of the world might be 'imported' once in a while (i.e. *A. lumbricoides*). In contrast, in developing countries most studies suggest that cross-infections are a very rare event, except for recent work by Criscione *et al.* (2007). This discrepancy could reflect different transmission routes in different populations, but is more likely related to the methodology used, or more subtle local geographical structuring of parasite populations. The use of 23 microsatellite markers by Criscione *et al.* (2007) allows more fine-scale genetic mapping, and future molecular studies on sympatric *Ascaris* should include the use of microsatellite markers.

Since expulsion of adult worms is a very poor indicator of the actual *A. suum* exposure in the developed part of the world, the analysis of serum samples, as conducted in The Netherlands (Pinelli *et al.*, 2009), is needed in order to estimate how many are exposed to infective eggs. However, identification of specific molecules that can be used in an ELISA for unequivocal discrimination of *Ascaris* and *Toxocara* infections is needed. Here the use of recombinant antigens may be very useful and, with the advancement of transcriptomics, this technology could help us to identify molecules that would ideally allow us to differentiate not only between *Ascaris* and *Toxocara* but also between *T. canis* and *T. cati*, with the latter most often ignored as a zoonosis (Fisher, 2003; Lee *et al.*, 2010), and at some stage it might even be possible to differentiate between *A. suum* and *A. lumbricoides* on the basis of antigenicity.

The migratory pattern of *A. suum* in humans is, for obvious reasons, poorly understood. For example, does *A. suum* typically cause visceral larva migrans, i.e. migration into organs other than the liver or lungs, as is the case for *Toxocara*? It has been described to cause encephalopathy (Inatomi *et al.*, 1999) but is this the exception rather than the rule? It is possible that *A. suum* migrates normally in the human host but that the main difference in comparison with *A. lumbricoides* is reduced ability to establish in the small intestine, so it is expelled, as is the fate for most larvae after the hepato-tracheal migration in the pig host (Roepstorff *et al.*, 1997). More work is required to determine the range of pathological effects caused by *A. suum* infection in humans.

Since it is most likely that expelled *T. suis* from humans in developed parts of the world will pass undetected, an estimation of the prevalence is only possible by detecting specific antibodies in serum. But as the prevalence of *T. suis* in most conventional pig production systems is much lower compared to that of *A. suum,* the potential zoonotic problem is not so big for this parasite in this region of the world. It could, however, be very interesting to explore, and might be relevant due to the beneficial immuno-modulating property of *T. suis* in humans, as has been the case in The Netherlands where Pinelli *et al.* (2009) tested for an association between human seropositivity for *Ascaris* antibodies and allergy.

In sympatric areas there is an urgent need to investigate the transmission dynamics of *Trichuris* in humans and pigs and to explore the zoonotic potential of *T. suis.* However, this might not be an easy task as it is notoriously difficult to expel and collect *T. trichiura* from humans (Olsen *et al.*, 2009). Alternatively, eggs from faeces can be used for genotyping. If this approach is used, at least two things need to be borne in mind. First, eggs passed in faeces do not necessarily correspond to worms in the host but might be ingested eggs just passively passing through the host. Second, even if a human is cross-infected with *T. suis*, this might not lead to eggs, due to a mating barrier or the possibility that eggs of *T. suis* might be 'outnumbered' by *T. trichiura* and therefore not detected by the method. The impending publication of the human and pig *Trichuris* mitochondrial genome sequences could provide important insights into the relationship between *T. trichiura* and *T. suis* (Liu *et al.*, 2012b).

Conclusion

Though the debate still continues as to whether *Ascaris* in pigs and humans represents the same or two distinct species, it is clear that *A. suum* is indeed a zoonosis in developed countries, and maybe also to some degree in developing countries. This implies that in communities where access to pig manure or bedding material is common, or pig manure is used as fertilizer, simple public health measures should be encouraged, including thorough handwashing with soap (particularly for children) and rinsing and cooking of vegetables. The data on the zoonotic potential of *Trichuris* is far sparser and further research is required to determine whether *T. suis* presents a risk to human health in the developed and developing world. With greater disease surveillance and molecular epidemiological investigations the extent of zoonotic transmission could soon be quantified.

Acknowledgements

The authors would like to thank the British Society for Parasitology for sponsoring this short symposium on zoonotic infections. We especially thank Professor John Lewis for assistance in redaction of this manuscript.

References

Abebe, W., Tsuji, N., Kasuga-Aoki, H., Miyoshi, T., Isobe, T., Arakawa, T., Matsumoto, Y. & Yoshihara, S. (2002a) Lung-stage protein profile and antigenic

relationship between *Ascaris lumbricoides* and *Ascaris suum*. *Journal for Parasitology* **88**, 826–828.

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

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 in Parasitology* **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, 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.

Ansel, M. & Thibaut, M. (1973) Value of the specific distinction between *Ascaris lumbricoides* Linne 1758 and *Ascaris suum* Goeze 1782. *International Journal for Parasitology* **3**, 317–319.

Areekul, P., Putaporntip, C., Pattanawong, U., Sitthicharoenchai, P. & Jongwutiwes, S. (2010) *Trichuris vulpis* and *T. trichiura* infections among schoolchildren of a rural community in northwestern Thailand: the possible role of dogs in disease transmission. *Asian Biomedicine* **4**, 49–60.

Arizono, N., Yoshimura, Y., Tohzaka, N., Yamada, M., Tegoshi, T., Onishi, K. & Uchikawa, R. (2010) Ascariasis in Japan: is pig-derived *Ascaris* infecting humans? *Japanese Journal of Infectious Diseases* **63**, 447–448.

Beer, R.J.S. (1973) Studies on the biology of the life-cycle of *Trichuris suis* Schrank, 1788. *Parasitology* **67**, 253–262.

Beer, R.J.S. (1976) Relationship between *Trichuris trichiura* (Linnaeus 1758) of man and *Trichuris suis* (Schrank 1788) of pig. *Research in Veterinary Science* **20**, 47–54.

Bendall, R.P., Barlow, M., Betson, M., Stothard, J.R. & Nejsum, P. (2011) Zoonotic ascariasis. *United Kingdom. Emerging Infectious Diseases* **17**, 1964–1966.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D. & Hotez, P.J. (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* **367**, 1521–1532.

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. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **105**, 370–379.

Boes, J., Willingham, A.L., Fuhui, S., Hu, X.G., 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. *Journal of Helminthology* **74**, 45–52.

Brooker, S. (2010) Estimating the global distribution and disease burden of intestinal nematode infections: Adding up the numbers - a review. *International Journal for Parasitology* **40**, 1137–1144.

Bundy, D.A.P. & Cooper, E.S. (1989) *Trichuris* and Trichuriasis in humans. *Advances in Parasitology* **28**, 107–173.

Burden, D.J., Hammet, N.C. & Brookes, P.A. (1987) Field observations on the longevity of *Trichuris suis* ova. *Veterinary Record* **121**, 43.

Carstensen, L., Vaarst, M. & Roepstorff, A. (2002) Helminth infections in Danish organic swine herds. *Veterinary Parasitology* **106**, 253–264.

Chan, M.S. (1997) The global burden of intestinal nematode infections – fifty years on. *Parasitology Today* **13**, 438–443.

Clark, E.G., Vondewitz, A. & Acompanado, G. (1989) Spurious *Ascaris suum* Infection in lambs. *Canadian Veterinary Journal* **30**, 903.

Crewe, W. & Smith, D.H. (1971) Human infection with pig *Ascaris* (*A. suum*). *Annals of Tropical Medicine and Parasitology* **65**, 85.

Criscione, C.D., Anderson, J.D., Sudimack, D., Peng, W., Jha, B., Williams-Blangero, S. & Anderson, T.J.C. (2007) Disentangling hybridization and host colonization in parasitic roundworms of humans and pigs. *Proceedings of the Royal Society Biological Sciences* **274**, 2669–2677.

Crompton, D.W. (2001) *Ascaris* and ascariasis. *Advances in Parasitology* **48**, 285–375.

Cutillas, C., Callejon, R., de Rojas, M., Tewes, B., Ubeda, J.M., Ariza, C. & Guevara, D.C. (2009) *Trichuris suis* and *Trichuris trichiura* are different nematode species. *Acta Tropica* **111**, 299–307.

De Silva, N.R., Chan, M.S. & Bundy, D.A.P. (1997) Morbidity and mortality due to ascariasis: re-estimation and sensitivity analysis of global numbers at risk. *Tropical Medicine & International Health* **2**, 519–528.

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 in Parasitology* **19**, 547–551.

Dold, C. & Holland, C.V. (2011) *Ascaris* and ascariasis. *Microbes Infections* **13**, 632–637.

Eijck, I.A.J.M. & Borgsteede, F.H.M. (2005) A survey of gastrointestinal pig parasites on free-range, organic and conventional pig farms in The Netherlands. *Veterinary Research Communications* **29**, 407–414.

Fisher, M. (2003) *Toxocara cati*: an underestimated zoonotic agent. *Trends in Parasitology* **19**, 167–170.

Galvin, T.J. (1968) Development of human and pig *Ascaris* in the pig and rabbit. *Journal for Parasitology* **54**, 1085–1091.

Hall, A., Hewitt, G., Tuffrey, V. & de Silva, N. (2008) A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Maternal and Child Nutrition* **4** (Suppl. 1), 118–236.

Hawley, J.H. & Peanasky, R.J. (1992) *Ascaris suum*: are trypsin inhibitors involved in species specificity of Ascarid nematodes? *Experimental Parasitology* **75**, 112–118.

Hill, C.H. (1957) The survival of swine whipworm eggs in hog lots. *Journal of Parasitology* **43**, 104.

Holland, C. & Boes, J. (2002) Distribution and predisposition: people and pigs. pp. 1–24 *in* Holland, C.V. &

Kennedy, M.W. (*Eds*) *The geohelminths: Ascaris, Trichuris and hookworm. World class parasite*, vol. 2. Boston, Kluwer Academic Publishers.

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. *Journal of Clinical Investigation* **118**, 1311–1321.

Inatomi, Y., Murakami, T., Tokunaga, M., Ishiwata, K., Nawa, Y. & Uchino, M. (1999) Encephalopathy caused by visceral larva migrans due to *Ascaris suum*. *Journal of the Neurological Sciences* **164**, 195–199.

Jaskoski, B.J. (1961) An apparent swine *Ascaris* infection of man. *Journal of the American Veterinary Association* **138**, 504–505.

Jex, A.R., Liu, S., Li, B., Young, N.D., Hall, R.S., Li, Y., Yang, L., Zeng, N., Xu, X., Xiong, Z., Chen, F., Wu, X., Zhang, G., Fang, X., 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., Wang, J., Wang, J. & Gasser, R.B. (2011) *Ascaris suum* draft genome. *Nature* **479**, 529–533.

Kelley, G.W. & Smith, L.J. (1956) The daily egg production of *Ascaris suum* and the inability of low levels of aureomycin to affect egg production and embryonation. *Journal of Parasitology* **42**, 587.

Kennedy, M.W., Qureshi, F., Haswell-Elkins, M. & Elkins, D.B. (1987) Homology and heterology between the secreted antigens of the parasitic larval stages of *Ascaris lumbricoides* and *Ascaris suum*. *Clinical and Experimental Immunology* **67**, 20–30.

Knight, R.A. (1984) Morphological differences in *Trichuris ovis* associated with different host species. *Journal of Parasitology* **70**, 842–843.

Kradin, R.L., Badizadegan, K., Auluck, P., Korzenik, J. & Lauwers, G.Y. (2006) Iatrogenic *Trichuris suis* infection in a patient with Crohn disease. *Archives of Pathology and Laboratory Medicine* **130**, 718–720.

Krasnonos, L. (1978) Prolonged survival of *Ascaris lumbricoides* L.,1758 ova in the soil in Samarkand. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **47**, 103–105 (in Russian).

Kringel, H. & Roepstorff, A. (2006) *Trichuris suis* population dynamics following a primary experimental infection. *Veterinary Parasitology* **139**, 132–139.

Lee, A.C., Schantz, P.M., Kazacos, K.R., Montgomery, S.P. & Bowman, D.D. (2010) Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends in Parasitology* **26**, 155–161.

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. (2012a) Comparative analyses of the complete mitochondrial genomes of *Ascaris lumbricoides* and *Ascaris suum* from humans and pigs. *Gene* **15**, 110–116.

Liu, G.H., Gasser, R.B., Su, A., Nejsum, P., Wei, S.J., Peng, L., Lin, R.Q., Li, M.W. & Zhu, X.Q. (2012b) Clear genetic distinctiveness between human- and pig-derived *Trichuris* based on analyses of mitochondrial datasets. *PLoS Neglected Tropical Diseases* **6**, e1539.

Lord, W.D. & Bullock, W.L. (1982) Swine *Ascaris* in humans. *New England Journal of Medicine* **306**, 1113.

Loreille, O. & Bouchet, F. (2003) Evolution of ascariasis in humans and pigs: a multi-disciplinary approach. *Memorias do Instituto Oswaldo Cruz* **98**, 39–46.

Maguire, J. (2005) Intestinal roundworms. pp. 3260–3267 *in* Mandell, G.L., Bennett, J.E. & Dolin, R. (*Eds*) *Principles and practice of infectious diseases*. 6th edn. Philadelphia, Elsevier.

Maung, M. (1973) *Ascaris lumbricoides* Linne, 1758 and *Ascaris suum* Goeze, 1782: morphological differences between specimens obtained from man and pig. *Southeast Asian Journal of Tropical Medicine and Public Health* **4**, 41–45.

Mayr, E. (1963) *Animal species and evolution*. Cambridge, Belknap Press.

McCraw, B.M. & Lautenslager, J.P. (1971) Pneumonia in calves associated with migrating *Ascaris suum* larvae. *Canadian Veterinary Journal* **12**, 87–90.

Muller, G. (1953) Untersuchungen über die Lebensdauer von Askarideneiern in Gartenerde. *Zentrallblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* **159**, 377–379 (in German).

Nadler, S.A. (1987) Biochemical and immunological systematics of some ascaridoid nematodes: genetic divergence between congeners. *Journal for Parasitology* **73**, 811–816.

Nansen, P. & Roepstorff, A. (1999) Parasitic helminths of the pig: factors influencing transmission and infection levels. *International Journal for Parasitology* **29**, 877–891.

Nejsum, P., Parker, E.D., Frydenberg, J., Roepstorff, A., Boes, J., Haque, R., Astrup, I., Prag, J. & Sørensen, U.B.S. (2005) Ascariasis is a zoonosis in Denmark. *Journal of Clinical Microbiology* **43**, 1142–1148.

Nejsum, P., Thamsborg, S.M., Petersen, H.H., Kringel, H., Fredholm, M. & Roepstorff, A. (2009) Population dynamics of *Ascaris suum* in trickle-infected pigs. *Journal of Parasitology* **95**, 1048–1053.

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*). *Veterinary Parasitolology* **171**, 273–276.

Nissen, S., Poulsen, I.H., Nejsum, P., Olsen, A., Roepstorff, A., Rubaire-Akiiki, C. & Thamsborg, S.M. (2011) Prevalence of gastrointestinal nematodes in growing pigs in Kabale District in Uganda. *Tropical Animal Health and Production* **43**, 567–572.

Nissen, S., Al-Jubury, A., Hansen, T.V.A., Olsen, A., Christensen, H., Thamsborg, S.M. & Nejsum, P. (2012) Genetic analysis of Trichuris recovered from human and pigs in a sympatric setting in Uganda. *Veterinary Parasitology* accepted.

Olsen, A., Namwanje, H., Nejsum, P., Roepstorff, A. & Thamsborg, S.M. (2009) Albendazole and mebendazole have low efficacy against *Trichuris trichiura* in school-age children in Kabale District, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **103**, 443–446.

Olsen, L.S., Kelley, G.W. & Sen, H.G. (1958) Longevity and egg-production of *Ascaris suum*. *Transactions of the American Microscopy Society* **77**, 380–383.

Ooi, H.K., Tenora, F., Itoh, K. & Kamiya, M. (1993) Comparative study of *Trichuris trichiura* from

non-human primates and from man, and their difference with *T. suis*. *Journal of Veterinary Medical Science* **55**, 363–366.

Pedersen, K., Monrad, J., Henriksen, S.A., Bindseil, B., Nielsen, J.S., Jensen, E. & Knold, P. (1992) Infektion med svinets spolorm (*Ascaris suum*) hos lam. *Dansk Veterinærtidsskrift* **75**, 170–174.

Pedersen, S. & Saeed, I. (2000) Experimental infection of pigs with three dose levels of *Trichuris suis*. *Parasite* **7**, 275–281.

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

Peng, W.D., 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.D., 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.

Phillipson, R.F. & Race, J.W. (1967) Human infection with porcine *Ascaris*. *British Medical Journal* **3**, 865.

Pinelli, E., Willers, S.M., Hoek, D., Smit, H.A., Kortbeek, L.M., Hoekstra, M., de Jongste, J., van Knapen, F., Postma, D., Kerkhof, M., Aalberse, R., van der Giessen, J.W. & Brunekreef, B. (2009) Prevalence of antibodies against *Ascaris suum* and its association with allergic manifestations in 4-year-old children in The Netherlands: the PIAMA birth cohort study. *European Journal of Clinical Microbiology and Infectious Diseases* **28**, 1327–1334.

Pinelli, E., Herremans, T., Harms, M.G., Hoek, D. & Kortbeek, L.M. (2011) *Toxocara* and *Ascaris* seropositivity among patients suspected of visceral and ocular larva migrans in the Netherlands: trends from 1998 to 2009. *European Journal of Clinical Microbiology and Infectious Diseases* **30**, 873–879.

Räisänen, S., Ruuskanen, L. & Nyman, S. (1985) Epidemic ascariasis – evidence of transmission by imported vegetables. *Scandinavian Journal of Primary Health Care* **3**, 189–191.

Roepstorff, A. & Jorsal, S.E. (1989) Prevalence of helminth infections in swine in Denmark. *Veterinary Parasitology* **33**, 231–239.

Roepstorff, A., Eriksen, L., Slotved, H.C. & Nansen, P. (1997) Experimental *Ascaris suum* infection in the pig: worm population kinetics following single inoculations with three doses of infective eggs. *Parasitology* **115**, 443–452.

Roepstorff, A., Nilsson, O., Oksanen, A., Gjerde, B., Richter, S.H., Ortenberg, E., Christensson, D., Martinsson, K.B., Bartlett, P.C., Nansen, P., Eriksen, L., Helle, O., Nikander, S. & Larsen, K. (1998) Intestinal parasites in swine in the Nordic countries: prevalence and geographical distribution. *Veterinary Parasitology* **76**, 305–319.

Roepstorff, A., Mejer, H., Nejsum, P. & Thamsborg, S.M. (2011) Helminth parasites in pigs: new challenges in pig production and current research highlights. *Veterinary Parasitology* **180**, 72–81.

Roneus, O. & Christensson, D. (1977) Mature *Ascaris suum* in naturally infected calves. *Veterinary Parasitology* **3**, 371–375.

Sauvageau, R. & Frechette, J.L. (1980) Hepatic ascariasis in a lamb. *Canadian Veterinary Journal* **21**, 66 (in French).

Soulsby, E.J.L. (1982) *Helminths, arthropods and protozoa of domesticated animals*. 7th edn. London, Baillière Tindall.

Spakulova, M. (1994) Discriminant-analysis as a method for the numerical evaluation of taxonomic characters in male trichurid nematodes. *Systematic Parasitology* **29**, 113–119.

Sprent, J.F.A. (1952) Anatomical distinction between human and pig strains of *Ascaris*. *Nature* **170**, 627–628.

Summers, R.W., Elliott, D.E., Urban, J.F. Jr, Thompson, R.A. & Weinstock, J.V. (2005) *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology* **128**, 825–832.

Takata, I. (1951) Experimental infection of man with *Ascaris* of man and the pig. *Kitasato Archives of Experimental Medicine* **23**, 49–59.

Tilney, L.G., Connelly, P.S., Guild, G.M., Vranich, K.A. & Artis, D. (2005) Adaptation of a nematode parasite to living within the mammalian epithelium. *Journal of Experimental Zoology. Part A, Comparative Experimental Biology* **303**, 927–945.

Traversa, D. (2011) Are we paying too much attention to cardio-pulmonary nematodes and neglecting old-fashioned worms like *Trichuris vulpis*? *Parasites and Vectors* **4**, 32.

Van Knapen, F., Buijs, J., Kortbeek, L.M. & Ljungstrom, I. (1992) Larva migrans syndrome: *Toxocara, Ascaris,* or both? *Lancet* **340**, 550–551.

Zhou, C., Li, M., Yuan, K., Hu, N. & Peng, W. (2011) Phylogeography of *Ascaris lumbricoides* and *A. suum* from China. *Parasitology Research* **109**, 329–338.