

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

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## 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).

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

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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; Nejsun *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 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) 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 interbreeding 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 *cox1* 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 *cox1* 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*

Cutillas *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.

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