HYBRID ASCARIS SUUM/LUMBRICOIDES (ASCARIDIDAE) INFESTATION IN A PIG FARMER: A RARE CASE OF ZOONOTIC ASCARIASIS

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SUMMARY

We present a case of the 42 year old pig farmer from the province of Cuneo in Northwest Italy who was infected by the soil-transmitted nematode *Ascaris* sp.

In November 2010 the patient found one worm in his stool, subsequently identified as female specimen of *Ascaris* sp. After a first anthelmintic treatment, another worm was found in his stool, that was later identified as male *Ascaris* sp. Blood tests prescribed by the patient's family physician, as suggested by a parasitologist, found nothing abnormal. A chest x-ray was negative for Loeffler's syndrome and an ultrasound of the abdomen was normal with no evidence of hepatic problems. The nematode collected from the patient was genetically characterized using the ribosomal nuclear marker ITS. The PCR-RFLP analysis showed a hybrid genotype, intermediate between *A. suum/lumbricoides*.

It was subsequently ascertained that some pigs on the patient's farm had *A. suum* infection; no other family member was infected. A cross-infestation from the pigs as source was the likely way of transmission. This conclusion is further warranted by the fact, that the patient is a confirmed nail-biter, a habit which facilitates oral-fecal transmission of parasites and pathogens.

Key words: ascariasis, *Ascaris suum*, cross infection, pigs, zoonosis, hybrid genotype

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INTRODUCTION

Ascariasis is a parasitosis of the small intestine caused by one of two worm nematodes (family Ascarididae): *Ascaris lumbricoides Linnaeus*, 1758, which typically infects humans, and *A. suum Goeze*, 1782 (Fig. 1), which is generally found in pigs. The two species are morphologically very similar and both can cause cross infections in each of the hosts. Because of this there is an active discussion in the literature about the taxonomic validity of the two species (1).

The life cycle is direct and infestation occurs through ingestion of eggs with L_3 larva inside which hatch in the duodenum (2). The larvae then migrate toward the host's duodenum, the cecum and the proximal colon where they burrow through the mucous membrane, reaching the liver via the hepatic portal system, and continue on to the right side of the heart and the circulatory system. Once in the lungs, the larvae travel to the respiratory tract and, via the trachea, reach the esophagus where they are once again ingested. The L_3 larva then molts two more times in the small intestine, becoming L_5 (adult) (3). This maturation process takes 60–70 days; the life span of adults is approximately 12 months.

Pathogenicity, whether in animals or humans, is directly proportional to how many larvae have infected the host, and is contingent upon: tissue damage in the migration phase (larva) (4), obstruction (adults) and Type I hypersensitivity (eggs, larva and adults). As regards pigs the damage may have economic impact insofar as the infected animals do not gain weight despite the amounts of food being eaten (5).

The two species of nematode are both found throughout the world. *A. lumbricoides* is currently found mostly in tropical and subtropical countries lacking adequate hygiene (6, 7), while *A. suum* is widespread on pig farms (8).

In North America and North Europe human infection by *A. lumbricoides* is uncommon and it is mostly related to infections acquired during travels in highly endemic countries (9), the cross-infection from *A. suum* to human rarely occurs, although a study carried out in Denmark described the importance of *A. suum* in human cases of ascariasis (10, 11). Recently *A. suum* has been suggested as a source of human ascariasis also in endemic areas such as China (12).

MATERIALS AND METHODS

In the present study, a human case of ascariasis was clinically and genetically investigated. Clinical data and medical history were recorded: samples from the patient and from pigs of his farm were also collected.

Worms recovered were washed in saline and stored in 70% (v/v) ethanol until molecular characterization. DNA was isolated using the Wizard® Genomic DNA purification kit (Promega) according to the manufacturer's protocol. The female worm recovered in patient stool and six worms from pigs were geneti-

cally characterized using a PCR-RFLP approach on the nuclear ribosomal ITS region. The resulting patterns obtained using the endonuclease *Hae*III have been previously proved useful for the identification of human *A. lumbricoides*, pig *A. suum* and the hybrid genotype between the two species (10).

The ITS nuclear region was amplified using 5.0 µl of template DNA (20–40 ng), 10mM Tris-HCl (pH 8.3), 1.5mM MgCl2, 40mM of a nucleotide mix, 50 pmol/ml each of the forward primer NC5 (5'-GTAGGTGAACCTGCGGAAGGATCAT-3') and the reverse primer NC2 (5'-TTAGTTTCTTCCTCCGCT-3') described by Zhu et al. (13) and 1.0 U of BIOTAQ DNA Polymerase (Bioline) in a final volume of 50 µl. PCR was performed in a GenePro Eurocycler Dual Block (Bioer) using the following conditions: 10 min at 95ºC, 30 cycles of 30 sec at 95ºC, 40 sec at 52ºC, 75 sec at 72ºC, and a final elongation step of 7 min at 72ºC. A negative control (without genomic DNA) was included in each set of amplification reactions. Positive amplicons were subsequently digested with the restriction endonuclease *Hae*III.

Description of a Case of Human Parasitosis

In early November 2010, the 42 year old male teacher and pig farmer from Cuneo, North Italy, found one worm in his stool and put it in a container. Medical history of the patient revealed a past diagnosis of teniasis (unidentified species) 4 months before; he made no trips to Europe in the last two years and never travelled to countries endemic for ascariasis. Upon advice from his practitioner, he began a treatment with niclosamide plus a laxative; parasitological stool examinations were performed over a 3-day period. The worm specimen was sent to a parasitology expert for identification and the stool sample was analyzed following standard laboratory procedure at the local hospital.

The worm was determined to be a stage L_5 *Ascaris* spp., and was fixed in 70% (v/v) ethanol to undergo genotypical analysis. Stool samples resulted negative for intestinal parasites. The patient was given a 3-day treatment of mebendazole. Eight days after the end of mebendazole treatment another *Ascaris* spp. specimen was expelled; morphological analysis determined it to be male.

Given the persistency of the parasitic infection, the patient's practitioner asked for a consultation with the parasitologist of the local hospital who suggested further treatment with mebendazole combined with an osmotic laxative; blood tests, chest x-ray (double view) and an ultrasound of the abdomen were also performed.

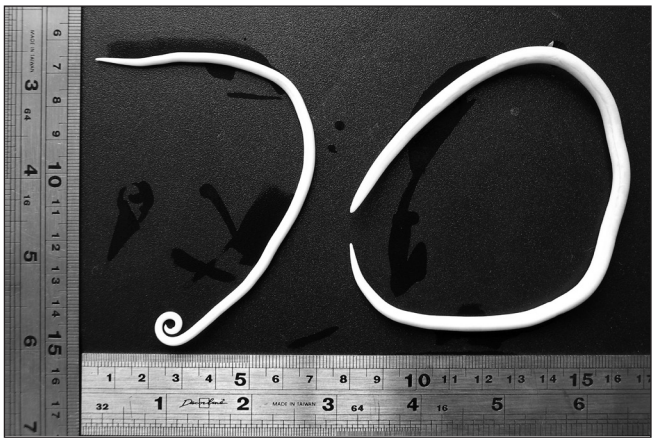

Fig. 1. *Male (left) and female (right) specimens of Ascaris suum (photo M. Dutto).*

Blood tests, including a complete blood count with leukocyte formula (eosinophil count 5.2%), were within the normal values as well as liver function tests, i.e. AST, ALT, gamma GT, alkaline phosphatase, and bilirubin.

Given the unusualness of the case, the parasitology expert visited the patient's home and farm. Parasitological stool exams in households were negative for parasites, thus ruling out a human-to-human transmission. Although there were neither sanitary nor hygiene anomalies, the patient was found to suffer from onychophagia and reported that in August and September had atypical bouts of coughing which cleared up spontaneously.

In agreement with the farm's veterinarian, it was decided that the pigs should be treated for infestation with ivermectin via oral delivery; few days later, several specimens of *Ascaris* spp. were retrieved from the pigsty and six nematodes were collected and fixed in ethanol (70% v/v) for genetic analysis.

One year later the patient did not report new findings of parasites in his stool; stool analysis for parasites resulted negative for intestinal parasites.

CONCLUSION

Molecular analysis of the nematodes from human patient and from pigs revealed that all six nematodes from pigs showed three bands of about 610bp, 230bp and 140bp, corresponding to the *A. suum* pattern, while the human isolate showed four bands of about 610bp, 370bp, 230bp and 140bp, typical of the *A. suum/lumbricoides* hybrid genotype. The human case was caused by a hybrid form of *Ascaris*, most probably transmitted by pigs. Also clinical investigation and infection dynamic (low number of worm expelled) suggest a zoonotic transmission, since almost 80% of zoonotic human cases described in literature show a low parasitic load and a single worm is generally expelled (9, 10, 14, 15).

The cause of infestation was probably the host's nail-biting habit and the practice of sucking the blood from minor wounds which often occur while castrating animals; even when gloves are employed, they are frequently not very resilient.

As far as diagnosis and therapy are concerned, it is of note that niclosamide is ineffective against ascariasis. Intestinal parasites, in particular those which cause little harm at fully mature stage, should always be treated only after accurate laboratory identification carried out by a specialist, since parasitology is wide and complex field.

Treatment of adult patients should be based on piperazine, pyrantel pamoate, levamisole or mebendazole (16). However, in Italy it is possible to use only mebendazole administered 100 mg twice a day for 3 days; a single dose has shown to be ineffective (17). An osmotic laxative is recommended at the end of the treatment. In cases involving parasitic bronchopneumonia, only the symptoms should be treated.

Ascaris suum is a widespread parasite found on pig farms and spreads via oral-fecal transmission (5, 8). In animals older than 5 months it is nearly asymptomatic due to immunization procedures which, however, do not rule out the possibility of being infested.

Environmental spread can also be aided through mechanical means, for example *Musca domestica* (18) which can result in contaminated food, animals and terrain.

Additionally, our case sheds light on the important problem of workplace safety, considering the ease with which farmers can contract ascariasis through ingestion or inhalation of eggs. It is therefore necessary to recognize ascariasis as a zoonotic occupational disease, which is probably more common than indicated in the literature.

Prevention includes the correct use of personal protective equipment (PPE), in this case gloves and masks, in addition to applying stringent personal and workplace hygiene measures: heightened control over pests such as flies, rodents and cockroaches as well as periodic treatment of floors and stalls in the barns with strong oxidizers (sodium hypochlorite). These procedures should be carried out throughout the entire pig farm during the periods in which these premises are empty.

REFERENCES

1. Loreille O, Bouchet F. Evolution of ascariasis in humans and pigs: a multi-disciplinary approach. Mem Inst Oswaldo Cruz. 2003;98 Suppl 1:39-46.
2. Geenen PL, Bresciani J, Boes J, Pedersen A, Eriksen L, Fagerholm HP, et al. The morphogenesis of Ascaris suum to the infective third-stage larvae within the egg. J Parasitol. 1999 Aug;85(4):616-22.
3. Dold C, Holland CV. Ascaris and ascariasis. Microbes Infect. 2011 Jul;13(7):632-7.
4. Kakihara D, Yoshimitsu K, Ishigami K, Irie H, Aibe H, Tajima T, et al. Liver lesions of visceral larva migrans due to Ascaris suum infection: CT findings. Abdom Imaging. 2004 Sep-Oct;29(5):598-602.
5. Dottori M. The helminth infestations in pigs. Atti della Società Italiana di Patologia ed Allevamento dei Suini. 2008:23-27. (In Italian.)
6. Chan MS, Medley GF, Jamison D, Bundy DA. The evaluation of potential global morbidity attributable to intestinal nematode infections. Parasitology. 1994 Sep;109(Pt 3):373-87.
7. Crompton DW. How much human helminthiasis is there in the world? J Parasitol. 1999 Jun;85(3):397-403.
8. Olson ME, Guselle N. Are pig parasites a human health risk? Adv Pork Prod. 2000;11:153-62.
9. Nejsum P, Parker ED Jr, Frydenberg J, Roepstorff A, Boes J, Haque R, et al. Ascariasis is a zoonosis in Denmark. J Clin Microbiol. 2005 Mar;43(3):1142-8.
10. Anderson TJC. Ascaris infections in humans from North America: molecular evidence for cross-infection. Parasitology. 1995 Feb;110(2):215-9.
11. Anderson TJ, Jaenike J. Host specificity, evolutionary relationships and macrogeographic differentiation among Ascaris populations from humans and pigs. Parasitology. 1997 Sep;115 (Pt 3):325-42.
12. Zhou C, Li M, Yuan K, Deng S, Peng W. Pig Ascaris: an important source of human ascariasis in China. Infect Genet Evol. 2012 Aug;12(6):1172-7.
13. Zhu X, Chilton NB, Jacobs DE, Boes J, Gasser RB. Characterisation of Ascaris from human and pig hosts by nuclear ribosomal DNA sequences. Int J Parasitol. 1999 Mar;29(3):469-78.
14. Takata I. Experimental infection of man with Ascaris of man and the pig. Kitasato Arch Exp Med. 1951 Mar;23(4):151-9; English transl, 49-59.
15. Koino S. Experimental infections on human body with ascarides. Jpn Med World 1922; 15(2): 317-20.
16. Brawley RL, Van Meter Q. Mebendazole and Ascaris migration. West J Med. 1986 Oct;145(4):514-5.
17. Tankhiwale SR, Kukade AL, Sarmah HC, Salunkhe DS, Kulkarni AS. Single dose therapy of ascariasis - a randomized comparison of mebendazole and pyrantel. J Commun Dis. 1989 Mar;21(1):71-4.
18. Förster M, Klimpel S, Sievert K. The house fly (Musca domestica) as a potential vector of metazoan parasites caught in a pig-pen in Germany. Vet Parasitol. 2009 Mar 9;160(1-2):163-7.

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