

Molecular Confirmation of *Ascaris suum*: Further Investigation into the Zoonotic Origin of Infection in an 8-Year-Old Boy with Loeffler Syndrome

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Abstract

An 8-year-old male from south Louisiana was diagnosed with Loeffler syndrome of suspected *Ascaris* origin. Further investigation of the farm recovered larvated, infective *Ascaris* eggs from the soil in drains surrounding pens on the family's small hog farm. Molecular analysis of the recovered eggs, in conjunction with *Ascaris*-specific IgE, inadequate farm management and sanitation, and behavioral risk factors indicate the patient had an *Ascaris suum* soil-transmitted infection.

Keywords: *Ascaris*, zoonoses, Loeffler syndrome, Louisiana

Introduction

ASCARIS LUMBRICOIDES AND ASCARIS SUUM are gastrointestinal nematodes that occur worldwide in humans and swine, respectively. *Ascaris* are transmitted through a direct fecal–oral cycle, and are closely linked with poverty and poor sanitation. Often asymptomatic, *Ascaris* can cause intestinal pain, malnutrition, and impaired cognitive and physical development (Bethony et al. 2006). In the lung migratory phase, larval *Ascaris* may cause severe pulmonary eosinophilic pneumonitis known as Loeffler syndrome. While *A. lumbricoides* is considered a human pathogen and *A. suum* a swine pathogen, *A. suum* has been reported in humans, *A. lumbricoides* has been reported in swine, and cross-hybridization between the two has been demonstrated as reviewed by Nejsum et al. (2012). While most prevalent in developing countries, human ascariasis is still sporadically found in developed countries, with most believed to originate from zoonotic *A. suum* swine cross-transmission.

Case Study

An 8-year-old male from south Louisiana was admitted to the Children's Hospital in New Orleans with acute respiratory insufficiency with tachypnea, cough, hypoxemia, and a week-long fever. Treatment with systemic corticosteroids resulted in rapid clinical improvement. Upon further evaluation, the child was diagnosed with Loeffler syndrome based

on peripheral eosinophilia (39%), pulmonary eosinophilia by bronchoalveolar lavage (86%), diffuse reticulonodular lung opacities, mixed obstructive and restrictive pulmonary function pattern, profoundly elevated serum *Ascaris*-specific IgE detected through the ImmunoCAP assay, performed by the ARUP National Reference Laboratory, and a fourfold increase in total serum IgE (3480–11,500 IU/mL) over a 4-day period (Gipson et al. 2016). *Strongyloides* was excluded using a *Strongyloides*-specific IgG ELISA also performed by the ARUP National Reference Laboratory and *Toxocara* infection was excluded through a Toxocariasis Enzyme Immunoassay performed by the Centers for Disease Control and Prevention (CDC). The CDC also tested for *Baylisascaris procyonis*-specific antibodies using an experimental immunoblot test, and the results were negative. Bacterial and fungal cultures and stains from a bronchoalveolar lavage, fungal serum markers, viral cultures, and a large viral PCR panel excluded several other medical disorders caused by bacteria and fungi, such as *Legionella*, tuberculosis, and histoplasmosis. There also were no new medications or exposures that could have caused an allergic reaction. One dose of albendazole 400 mg, as recommended by the CDC, was administered to treat the presumed *Ascaris* infection.

Patient history revealed he had never traveled outside of the United States. He lived on residential property in south Louisiana that had a small hog farm behind the main

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residence. His duties on the farm included feeding and watering the swine, as well as cleaning out the pens. Before the field visit, the two concrete pens had housed up to 30 pigs, but at the time of the visit only one 4-year-old, 204 kg intact male boar remained. A separate wooden pen had also housed a sow and piglets over 2 years before. When the operation previously had multiple younger pigs, the owner had dewormed them after noticing roundworms in the pig feces. No previous parasitological diagnostic testing outside of this study had been performed on the pigs.

The lack of sanitary farm conditions likely played a role in the zoonotic *Ascaris* infection. For example, a water hose, used in the daily care of the swine and to wash pig manure out of the pens, was allowed to lie on the concrete between the two concrete pens. Additionally, an open drainage ditch, where fecal runoff drained into a septic pit, was located at the entrance of the pens. No protective clothing and gloves were used, nor a hand sanitation station provided near the enclosures. The patient also displayed severe onychophagia (fingernail biting), and combined with the lack of proper farm sanitation, lead to the suspicion that infection was established through hand-to-oral contact while working, and the infective agent was zoonotic *A. suum* acquired from either the sewage runoff or the surrounding infected soil.

To confirm the suspected etiology and zoonotic swine origin of the boy's infection, a laboratory investigation to the patient's family farm was initiated by the Louisiana Animal Disease Diagnostic Laboratory (LADDL).

Fecal samples were obtained from the patient 4 months after albendazole treatment, the patient's sibling, and the boar. These were analyzed using a double-spin centrifugal flotation method (Smith et al. 2007) with ZnSO₄ and Sheather's sugar flotation media for the human samples and MgSO₄ for the swine sample. The boar sample revealed one *Ascaris* egg. No *Ascaris* eggs were found in either human sample, although *Enterobius vermicularis* eggs were found in both.

To determine if infective *Ascaris* eggs of swine origin were present in the environment, soil sample transects were taken from drains surrounding the holding pens and an unfiltered septic tank drainage ditch adjacent to the holding pens. Soil samples were processed using a modified egg extraction technique (Dunsmore et al. 1984). The extraction produced a large number of larvated, infective-stage *Ascaris* eggs. The recovered eggs were then extracted for PCR analysis.

PCR analysis of the *Ascaris* nuclear ribosomal DNA sequences spanning the first internal transcribed spacer (ITS-1) (accession no. AJ000896) (Zhu et al. 1999), was used for speciation. This segment contains polymorphic *Hae*III restriction sites (GGCC) and has been used to differentiate *A. lumbricoides* sequences, which have been shown to contain one restriction site, from *A. suum* sequences, which generally contain two sites.

Forward (5'-TGTAATAGCAGTCGGCGGTT-3') and the reverse (5'-AACCCGATGGCGCAATGT-3') primers were created to amplify a 356 bp segment containing these restriction sites (LA_Soil-1). While not an absolute diagnostic marker (Leles et al. 2012), *Hae*III polymorphisms are the best single biomarker to aid in differentiating the two species (Arizono et al. 2010). Analysis of the LA_Soil-1 (accession no. MG012802) PCR product revealed two *Hae*III restriction sites, indicating that the soil eggs were *A. suum* (Fig. 1).

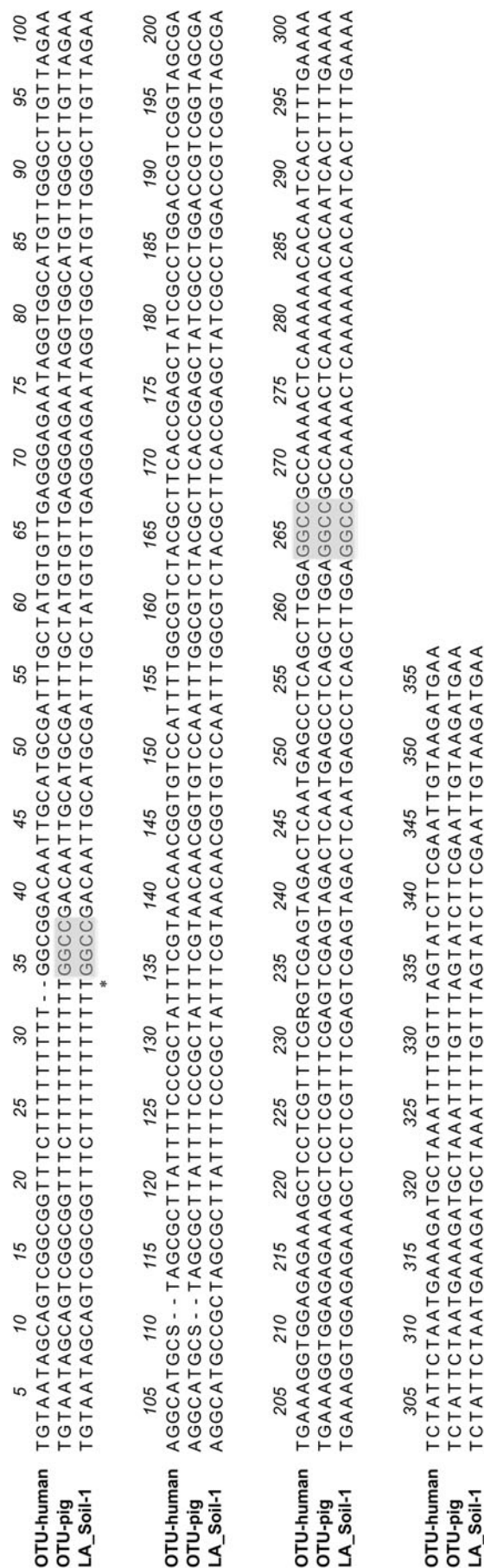


FIG. 1. Partial *Ascaris* ITS-1 alignments comparing OTU-Pig, OTU-Human, [AJ000895, AJ000896 (Zhu et al. 1999)], and our LA_Soil-1 (MG12802). The *Hae*III-binding sites are highlighted. *Indicates T deletion found in LA_Soil-2 (MG12803).

LA_Soil-1 also revealed two additional bps, G and C, at positions 110 and 111, respectively, compared with the Zhu sequences (Zhu et al. 1999). A second replicate, LA_Soil-2 (accession no. MG012803), displayed a T deletion at position 33, but was otherwise identical to LA_Soil-1.

Conclusions

The patient's *Ascaris*-specific IgE, his duties working with the swine, the lack of protective equipment and sanitary farm operation, the patient's onychophagia, demonstration of infective eggs in the soil, and the subsequent DNA analysis all indicate that the patient had an *A. suum* soil-transmitted infection. Human *Ascaris* and other soil-transmitted helminth infections were once endemic throughout southern and Appalachian regions of the United States (Starr and Montgomery 2011). Improvements in education, sanitation, and hygiene practices have greatly reduced infections, but they still occasionally occur in the United States and other developed countries (Nejsum et al. 2005, 2012, Arizono et al. 2010, Miller et al. 2015, McKenna et al. 2017).

Young children in rural settings and those with direct or indirect exposure to swine and swine manure are at increased risk for zoonotic *A. suum* infections (Nejsum et al. 2005, Miller et al. 2015). We posit that many zoonotic *Ascaris* infections in the United States remain undetected in these higher-risk groups. A recent report (Miller et al. 2015) about human ascariasis on small-scale Maine farms demonstrates the risk of zoonotic *Ascaris* infections in small farm environments. This risk is compounded with improper farm management and unsanitary conditions, as was observed in the current study.

Another factor that may increase risk of ascariasis in the southern United States is the explosive increase in feral hog populations. Feral hogs in Louisiana harbor heavy parasite burdens, including *Ascaris*, and are considered a pest animal in southern states (McClure et al. 2015). Work conducted in the LADDL Parasitology Section found a 31.5% *Ascaris* prevalence rate in feral hogs hunted on Louisiana public lands from the years 2014–2015 (B. Delcambre, unpublished data). Feral swine may interact with their domestic counterparts, exposing previously treated swine as well as humans to *Ascaris* and other parasites. In this study, the farm owner noted that feral swine were previously sighted passing through the farm site.

This case provides evidence that zoonotic *A. suum*, while rare, continues to occur within the United States, particularly in small-scale hog farming operations. Lack of proper protocols to limit zoonotic transmission in small farm operations, coupled with the explosive increase in feral swine populations in the southern United States, increases the risk of *A. suum* infections in both humans and domestic swine in the future.

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Author Disclosure Statement

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