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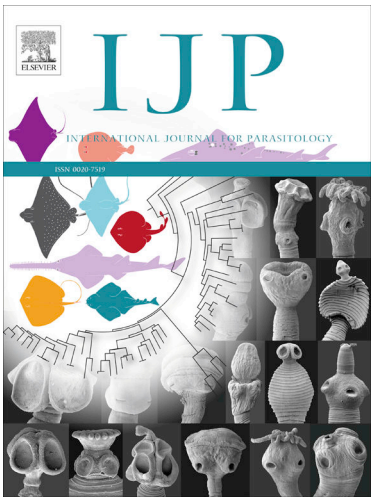

Current Opinion

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Current Opinion

The turkey ascarid, *Ascaridia dissimilis*, as a model genetic system

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Abstract

Parasitic nematodes cause significant effects on humans each year, with the most prevalent being *Ascaris lumbricoides*. Benzimidazoles (BZ) are the most widely used anthelmintic drug in humans, and although the biology of resistance to this drug class is understood in some species, resistance is poorly characterized in ascarids. Models such as *Caenorhabditis elegans* were essential in developing our current understanding of BZ resistance, but more closely related model nematodes are needed to understand resistance in ascarids. Here, we propose a new ascarid model species that infects turkeys, *Ascaridia dissimilis*, to develop a better understanding of BZ resistance.

Keywords: Ascarid, Model organism, Benzimidazole, Anthelmintic resistance

Parasitic nematodes cause significant deleterious effects to humans and livestock worldwide. In humans, an estimated 1.3 billion people are infected by at least one soil-transmitted helminth (STH) species, including hookworms, whipworms, and ascarids (Hotez et al., 2020). Among these STH species, ascarids are the most pervasive, with estimates as high as 1.2 billion people infected (Hotez et al., 2020). Ascarids cause the loss of 749,000 disability-adjusted life years (DALYs) per year (The World Health Organization, https://www.who.int/data/global-health-estimates) or approximately 40% of all DALYs caused by STHs. *Ascaris lumbricoides*, the primary ascarid of humans, follows a fecal-oral transmission route. After hatching in the intestine, larvae undergo hepato-tracheal migration, moving through the liver and lungs before making their way back to the small intestine. During their migration through the lungs, *A. lumbricoides* can cause verminous pneumonia, where inflammation and fluid accumulate, often causing significant respiratory distress (Spener et al., 2019). Infections (ascariasis) with adult parasites in the small intestine can cause stunted growth, malabsorption of nutrients, diarrhea, and intestinal blockage in humans that can be fatal if left untreated. Control of ascariasis in endemic regions is typically dependent on Mass Drug Administration (MDA) programs that deliver regular anthelmintic treatments to at-risk populations. The benzimidazole (BZ) class of anthelmintics is the treatment of choice for ascarids because of their ease of delivery and low costs. Historical efficacy of BZs, such as albendazole, against ascariasis has been high, with a typical cure rate of over 90% (Vercruysse et al., 2011). Despite this historically high efficacy, the development of resistance is of great concern.

Resistance to the BZ class is common in many veterinary parasites and has reached near ubiquity in species such as *Haemonchus contortus*, a strongylid parasite of small ruminants (Howell et al., 2008). Anthelmintic treatment is widespread in veterinary medicine, similar to MDA programs in humans, applying significant selection pressure on STH populations. Because veterinary parasite populations are large, they harbor high levels of genetic variation, creating ideal conditions to rapidly select for resistance and widespread failure of treatments (Silvestre et al., 2001). The mechanisms of action must be understood to properly study how resistance develops in natural populations.

In the 1970s, BZs were found to disrupt mitosis in the fungus *Aspergillus nidulans* (Davidse, 1973). It is thought that, in susceptible parasite populations, BZs disrupt the polymerization of microtubules, preventing normal development. Further research showed that, in nematodes such as *Caenorhabditis elegans* and *H.*

contortus, sensitivity to BZs is mediated by beta-tubulin genes (Driscoll et al., 1989) and that mutations in these genes at codons 167, 198 and 200 were correlated with resistance (Kwa et al., 1994a; Hodgkinson et al., 2008). Since this discovery, the study of BZ resistance has been focused on these codons, but differences among divergent nematode species mean that these same mutations might not play a role in BZ resistance in all nematode species (Roose et al., 2021).

Nematodes are divided into five clades (Blaxter and Koutsovoulos, 2015). Although BZ resistance is common in Clade V nematodes (*e.g.*, *C. elegans* and *H. contortus*), reports of resistance in Clade III nematodes (*e.g.*, ascarids) are more sporadic. Resistance has never been confirmed in *Ascaris* spp., but reduced efficacy has been observed (Krücken et al., 2017). A study of albendazole efficacy in Rwandan school children found reduced efficacy against *A. lumbricoides*. The authors looked across the beta-tubulin gene family but found no mutations at codons 167, 198, or 200, leading to suggestions that beta-tubulin genes and these codons might not be mediators of resistance (Krücken et al., 2017). Evidence of resistance has also been found in multiple veterinary ascarids (Table 1). A study of *Ascaridia dissimilis* in turkeys shows significantly reduced efficacy in a parasite population isolated from a commercial farm, and further studies confirmed anthelmintic resistance in this isolate (Collins et al., 2019). Multiple studies of horse ascarid species (*e.g.*, *Parascaris* spp.) have also shown definitive evidence of BZ resistance (Martin et al., 2021; Özben et al., 2022). However, it is important to note that the beta-tubulin mutations associated with BZ resistance have not been found in any of these species, suggesting that resistance mechanisms in ascarids might differ from Clade V nematodes. The sole focus on the codon 167, 198, or 200 beta-tubulin mutations has likely contributed to the failure to diagnose resistance in ascarids.

Long used as a model for all Clade V nematodes, *C. elegans* has informed much of what we know about the mechanisms of anthelmintic resistance (Enos and Coles, 1990). A cycle of discovery between *C. elegans* and *H. contortus* has provided important systems for anthelmintic discovery and anthelmintic resistance research (Wit et al., 2021), but these findings might not be applicable to all other nematode clades as shown by the apparent lack of beta-tubulin mutations in resistant ascarid species. *Caenorhabditis elegans* is a free-living nematode and is a highly scalable laboratory model. Clade III parasites, such as ascarids, have fundamental differences in life cycles when compared with Clade V nematodes, so a more closely related species should be

used as a model system to perform experiments in this clade. Historically, *Ascaris suum*, a swine ascarid and close relative of *A. lumbricoides*, has been the *de facto* model for ascarid research. *Ascaris suum* is also zoonotic, infecting humans, and is nearly genetically identical to *A. lumbricoides*, providing much debate if the two species are in fact the same (Leles et al., 2012). Swine are physiologically similar to humans, making them an important model for translational research. Biological compatibility of hosts and parasites makes *A. suum* in swine a useful system for studying the effects of ascariasis on the host and the effectiveness of treatments.

Although *A. suum* and swine are good models for translational research for humans, many caveats limit their usefulness for genetic studies and drug development. Models must be readily scalable, not cost-prohibitive, and easily manipulated. Swine are among the more expensive research hosts, making large-scale studies needed for genetics cost-prohibitive. Because of their large size and rapid growth, manipulation of the model is difficult, impacting the ability to execute controlled high-throughput studies. As demonstrated by discoveries made using *C. elegans*, a scalable and cost-effective model is necessary for quantitative and molecular genetics, and the ability of *A. suum* to be used in such studies is limited. A variety of other host species are available for use as a model for ascarid research such as dogs and horses, but these models are also cost-prohibitive and have their own problems with the perception of research on companion animals. Here, we propose a new ascarid model, using BZ-resistant ascarids of poultry (Fig. 1).

Currently, resistance has been found in two closely related (Fig. 2A) species of ascarids in poultry: *Ascaridia dissimilis,* a turkey ascarid (Collins et al., 2019), and *Heterakis gallinarum* (Collins et al., 2022), a cecal ascarid of gallinaceous birds. Secondary infection caused by *Histomonas meleagridis* harbored by *H. gallinarum* reduces its usefulness as a model (Daş et al., 2021), making *A. dissimilis* a better choice*. Ascaridia dissimilis* has an atypical ascarid life cycle (Fig. 2B) without the need for hepato-tracheal migration found in other ascarids, although hepato-tracheal migration has been observed in rare cases (Ackert, 1931). *Ascaridia dissimilis* infects the host by the consumption of fully developed embryos from the environment. Third-stage larvae hatch in the crop and proventriculus, and are then carried to the small intestine where they complete their development (Pankavich et al., 1974). The development from initial infection to sexually mature adults typically takes 31 - 35 days (Pankavich et al., 1974). After maturity, adult females are 8 - 10 cm in length and produce approximately 2,000 embryos per day (Permin et al., 1997). Depending on environmental conditions, embryos will take 2 - 3

weeks to develop to the infective stage and can persist for years in the environment until consumed by the host. Typically, infections with *A. dissimilis* are asymptomatic but can cause lethargy, intestinal distension, and intestinal blockage in severe cases. Biological similarity to other ascarids and the lack of significant pathology in infections make this ascarid a useful parasite model.

BZ resistance in *A. dissimilis* was first suspected in a 2013 farm study (Yazwinski et al., 2013) and more recently has been documented in a controlled efficacy study (Collins et al., 2019). Briefly, four isolates of *A. dissimilis* were collected from commercial turkey farms, three with suspected resistance and one from an organic farm, although benzimidazoles were used previously on that farm. Birds were infected with one of four isolates, and 28 days p.i., half of the birds were treated with fenbendazole for 5 days. One week after treatment, birds underwent necropsy and parasites were recovered. Fenbendazole was found to be >99% effective against three isolates but only 63.7% efficacious against the isolate from the organic farm. These findings are, to our knowledge, the first documentation of BZ resistance in a controlled laboratory ascarid study and were validated further in subsequent studies using this isolate (Collins et al., 2021). With confirmed resistant isolates, *A. dissimilis* represents a highly tractable ascarid model for studying the genes that mediate anthelmintic resistance in Clade III nematodes.

Animal costs for studies of *A. dissimilis* are 5 - 10% of the cost of the current ascarid model in swine. Turkeys are less than 10% of the size of swine, greatly improving handling as well as reducing the amount of space needed. An adult pig needs over four square meters of space, but an adult turkey needs a sixth of that space, greatly increasing the replication that can be done in the same space. Ease of handling and the potential for greatly increased replication make turkeys a higher throughput host to study ascarid biology and genetics compared with swine hosts.

Ascaridia dissimilis provides many improvements over *A. suum*, but limitations impact its overall usefulness. Swine will be the gold standard with respect to host immune responses because of the similarity to human hosts. Additionally, *A. suum*, similar to *A. lumbricoides* in humans, undergoes obligatory hepato-tracheal migration, unlike ascarids in poultry, making it possible to study the hepatic and respiratory effects of infection. Studies of host physiology or parasite migration will likely always depend on a swine parasite model. The lack of mutations at codons 167,198, and 200 of beta-tubulin in BZ-resistant ascarids highlights the need for a genetic

model of resistance in these species. Further work in a tractable model ascarid, such as *A. dissimilis*, is imperative to understand the genetics of resistance to other anthelmintic classes as well. Resistance in ascarids, as an emerging problem, is poorly understood, and *A. dissimilis* provides a cost-effective and scalable means of building the *in vivo*, *in vitro*, and molecular toolkits found in other important nematode parasite species.

Genetic research has improved our understanding of mechanisms involved in anthelmintic resistance. Bulk-segregant genetic mapping has been used in *H. contortus*, made by crossing resistant and susceptible populations, to discover putative resistance genes (Doyle et al., 2019; Doyle and Cotton, 2019). Males from a susceptible and females from a resistant population were transplanted into a naive animal, and then underwent multiple generations of backcrossing to the susceptible population while maintaining anthelmintic selective pressure every other generation. The progeny from multiple generations of anthelmintic selection maintained resistance alleles and linked loci in an otherwise susceptible genetic background. These animals were subjected to whole-genome sequencing and those sequences were compared with the susceptible parent's genetic background. Variants that were statistically enriched in the resistant population compared with the susceptible parent background were then identified and candidate resistance genes were proposed (Doyle et al., 2019, 2022; Laing et al., 2022). No genetic crossing system has been established for an ascarid species. Tractable ascarid models with established resistant isolates are necessary to replicate the successes of the *H. contortus* model. *Ascaridia dissimilis* is a good candidate for this system because resistant isolates are already known. This species is relatively quick to grow and easy to collect for genetic crosses. The lack of pathology in low-level infections is also ideal, as it removes potential confounding effects in transplantation attempts. Non-surgical means of transplantation of parasites in poultry have previously been explored (Permin et al., 2003), and even with low rates of success, the scalability of the poultry model makes this technique a more cost- and resource-efficient option than surgical means in a less scalable model such as swine. Beyond an efficient and cost-effective means of performing genetic crosses, a high-quality genome is essential for genetic mapping. Refinement of the *H. contortus* genome was critical for genetic mapping. Current ascarid genomes lack the quality needed to replicate this success. However, work is ongoing to create a high-quality *A. dissimilis* genome. Once these resources are generated, *A. dissimilis* can be used as a genetic model for ascarids, including

investigating the genetics of BZ resistance in ascarids. A poultry ascarid model could provide many improvements over the swine model, but other potential model systems should also be considered.

Rodent models have long been used in a wide range of biological studies, and the genetics and biology of the various populations of rodents are well understood. *Heterakis spumosa*, an ascarid of rodents, lives in the upper colon of infected hosts but is not known to cause pathology. Similar to other *Heterakis* spp., adults are approximately 1 cm in length. Unlike *Heterakis gallinarum*, *Heterakis spumosa* is not a known vector of any pathogens. Laboratory model systems using a well understood and easily manipulated host could make rodents a powerful ascarid model for anthelmintic resistance studies if drug-resistant isolates of *H. spumosa* can be identified. Although costs might exceed the poultry model, the ease of handling and well understood host genetics makes rodents a possible ascarid model as well.

The emergence of drug resistance in multiple species of ascarids and evidence of reduced efficacy in ascarid infections of humans highlights the immediate need for scalable models for ascarids. Research on resistance in Clade V nematodes has been dependent on *C. elegans* as a highly tractable model, but evidence indicates that it is less translatable to Clade III nematodes such as ascarids. Although a great model for human physiology, the classic swine model is cost-prohibitive for drug development and genetic studies of anthelmintic resistance. Thus, a more affordable and tractable model is needed. With resistant isolates already established in a laboratory setting, *A. dissimilis* infections of turkeys offer a powerful model to pursue. Inexpensive hosts that are relatively easy to handle enable a wide range of higher throughput studies to better understand ascarid biology, the genetics of resistance in ascarids, and the discovery and development of new anthelmintics. As research funding becomes tighter, the need for inexpensive models with quality genetic resources will continue to increase. The poultry ascarid models represent a powerful system for inexpensive, large-scale studies, especially as anthelmintic resistance in these species continues to emerge.

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Figure legends

Fig. 1. Comparison of *Ascaris suum* and *Ascaridia dissimilis* as a model ascarid.

Fig. 2. Phylogenetic relationship and life-cycle of *Ascaridia dissimilis*. **(**A) Relationship of selected parasitic and free-living helminths. Sequences of the internal transcribed spacer 2 (ITS2) region from multiple ascarid species and the Clade V nematodes were used to generate a phylogenetic tree. The *A. dissimilis* sequence was sourced from unpublished data and all other sequence data are available from GenBank (KR872308, JN636101, MF358962, AJ007452, MF358961, LC592776). (B) Life cycle of *A. dissimilis*. Infective stage embryos are consumed by the host and hatch within the crop and proventriculus. Larvae are carried to the small intestine and develop to the adult stage 31 - 35 days after ingestion. After mating, adult females produce embryos that are excreted into the environment. Embryos develop to the infective stage in 3 - 4 weeks.

Table 1. Studies that have documented reduced efficacy or confirmed resistance to benzimidazoles in ascarid parasites.

Host Species	Ascarid Species	Drug Used	Study
Turkey	*Ascaridia dissimilis*	Fenbendazole	Yazwinski et al., 2012
Human	*Ascaris lumbricoides*	Albendazole	Krucken et al., 2017
Turkey	*Ascaridia dissimilis*	Fenbendazole	Collins et al., 2019
Horse	*Parascaris univalens*	Fenbendazole	Martin et al., 2021
Chicken	*Heterakis gallinarum*	Fenbendazole	Collins et al., 2022

Graphical Abstract (for review)

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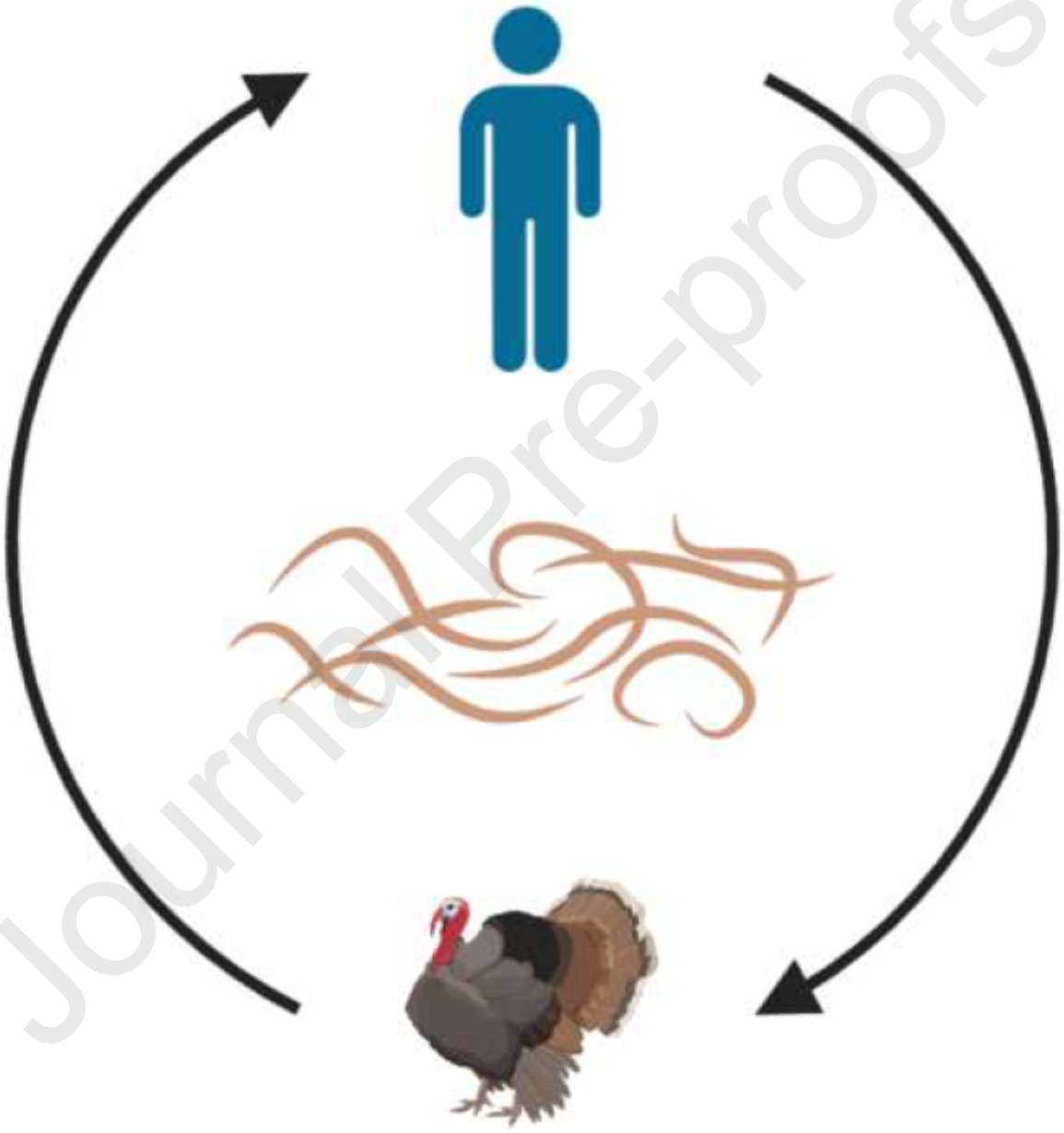

Highlights

- Benzimidazole resistance is an emerging problem in ascarids.
- Swine ascarid models are expensive, and poultry offers a more tractable alternative.
- Resistant isolates are established for use in the poultry model.
- Studies using ascarids of poultry as a model will provide insights into the biology of resistance in ascarids.

Figure 1

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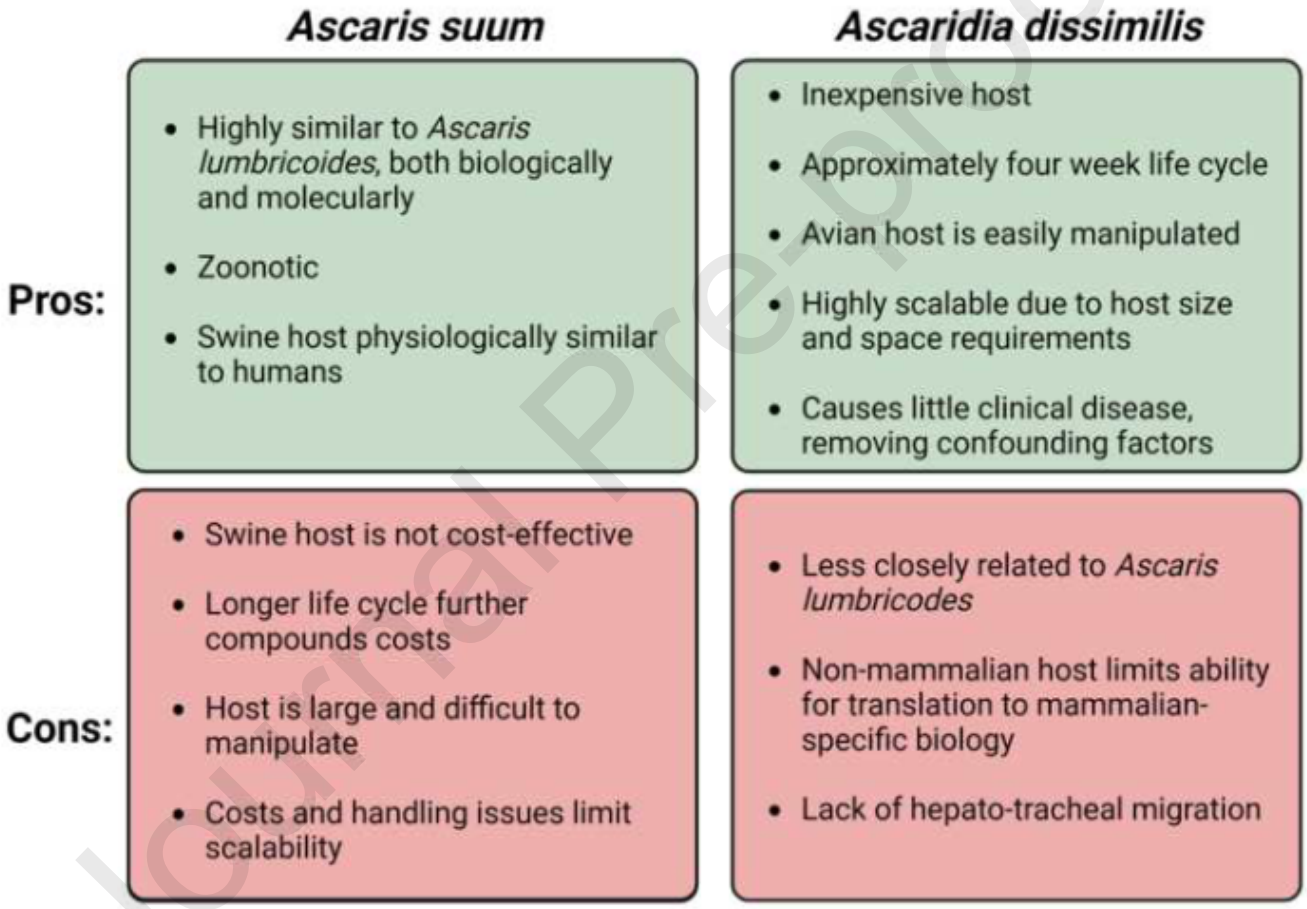

Figure 2

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