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Ascaris lumbricoides and *Ascaris suum* vary in their larval burden in a mouse model

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Abstract

Ascariasis is a neglected tropical disease, caused by *Ascaris lumbricoides*, affecting 800 million people worldwide. Studies focused on the early stage of parasite infection, occurring in the gut, liver and lungs, require the use of a mouse model. In these models, the porcine ascarid, *Ascaris suum*, is often used. The results obtained from these studies are then used to draw conclusions about *A. lumbricoides* infections in humans. In the present study, we sought to compare larval migration of *A. suum* and *A. lumbricoides* in mouse models. We used a previously developed mouse model of ascariasis, which consists of two mouse strains, where one mouse strain – C57BL/6J – is a model for relative susceptibility and the other – CBA/Ca – for relative resistance. Mice of both strains were infected with either *A. suum* or *A. lumbricoides*. The larval burden was assessed in two key organs, the liver and lungs, starting at 6 h post infection (p.i.) and ending on day 8 p.i. Additionally, we measured the larval size of each species (μm) at days 6, 7 and 8 p.i. in the lungs. We found that larval burden in the liver is significantly higher for *A. lumbricoides* than for *A. suum*. However, the inverse is true in the lungs. Additionally, our results showed a reduced larval size for *A. lumbricoides* compared to *A. suum*.

Introduction

Ascariasis is a neglected tropical disease widespread in Asia, Africa and South America (Jourdan *et al.*, 2018), with an estimated 800 million people infected with *Ascaris lumbricoides* worldwide (Pullan *et al.*, 2014). Children between the ages of five and 15 years suffer the majority of the worm burden (Jourdan *et al.*, 2018; Wright *et al.*, 2018). Ascariasis causes both chronic and acute symptoms. The latter are often more severe, but less common, and include intestinal obstruction and the so-called Loëffler syndrome, a characteristic allergic response due to larval migration (Loeffler, 1932, 1956; Ribeiro & Fisher, 2002). The chronic symptoms, which occur more frequently, include malnutrition and associated stunting (O'Lorcain & Holland, 2000; Deslyper & Holland, 2017). In addition, *Ascaris suum* is of considerable economic importance due to an increased feed-to-gain ratio and liver condemnation associated with porcine infection (Boes *et al.*, 2010).

Embryonated eggs, containing a third-stage larva (L3) with a second-stage larva (L2) cuticle, are orally ingested by the host animal (Murrell *et al.*, 1997; Geenen *et al.*, 1999). These eggs hatch in the intestines and migrate via the portal blood to the liver. Here, the larvae shed their L2 cuticle and increase in size (Roepstorff *et al.*, 1997). Subsequently, they migrate to the alveolar space in the lungs, where, again, they increase in size before moving to the pharynx. The larvae will be coughed up, swallowed again and migrate to the intestines where they mature into adult worms (Dold & Holland, 2011).

An important aspect of many macroparasites, is the aggregated distribution of worm burden whereby most hosts harbour few worms and a small proportion of hosts harbour heavy worm burdens (Crofton, 1971; Shaw & Dobson, 1995). Such aggregated distributions are observed in both humans and pigs infected with *Ascaris* (Crofton, 1971; Croll & Ghadirian, 1981; Holland *et al.*, 1989; Shaw & Dobson, 1995; Boes *et al.*, 1998b). The same people – and pigs – reacquire similar worm burdens upon reinfection, this is termed predisposition (Holland, 2009).

Both *A. suum* and *A. lumbricoides* are morphologically similar (Sprent, 1952a; Ansel & Thibaut, 1973; Maung, 1973). This has led to speculation about whether *A. suum* and *A. lumbricoides* are, in fact, two separate species. Cross transmission has been observed in non-endemic areas, where pig-to-human transmission can be verified (Anderson, 1995; Nejsum *et al.*, 2005; Arizono *et al.*, 2010; Betson *et al.*, 2014). However, human-to-pig transmission is more difficult to prove (Criscione *et al.*, 2007; Zhou *et al.*, 2012; Betson *et al.*, 2013). Fertile hybrids have been identified (Criscione *et al.*, 2007; Zhou *et al.*, 2012; Betson *et al.*, 2013), giving some weight to the one-species theory; however, the advancement of genetic technology has been able to shed new light on this question. The use of mitochondrial

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DNA (mtDNA) and the first internal transcribed spacer has shown that *A. suum* and *A. lumbricoides* are, in fact, two separate species (Anderson *et al.*, 1993; Zhu *et al.*, 1999). However, a comparison of whole mtDN (Liu *et al.*, 2012) showed high similarities and, again, pointed to one species. In short, 'based on the phonetic, phylogenetic, and evolutionary species concepts', *A. lumbricoides* and *A. suum* would be considered one species (Betson *et al.*, 2013). Conversely, when approaching this question from the biological concept of species, *A. lumbricoides* and *A. suum* would be considered two species, and it is this theory that currently has more support (Betson *et al.*, 2013).

Despite being abnormal hosts, and, therefore, having a truncated migratory path, mice have proven to be excellent model systems for *Ascaris* research (Holland *et al.*, 2013). Mice have some advantages with respect to the natural porcine host, because of size, husbandry and ethical constraints and a lack of inbred strains. Other potential model organisms, such as guinea pigs, rabbits and rats, all have lower larval recovery rates than mice (Douvres & Tromba, 1971; Roepstorff *et al.*, 1997). Our group has previously developed a mouse model of *A. suum* aggregation (Lewis *et al.*, 2006, 2007; Dold *et al.*, 2010). The migratory path is truncated in the mouse, allowing, therefore, only the study of early migration (Holland *et al.*, 2013). Two mouse strains were identified with contrasting phenotypes of resistance/susceptibility to *A. suum* infection, where one mouse strain – C57BL/6J – is a model for relative susceptibility and another mouse strain – CBA/Ca – for relative resistance (Lewis *et al.*, 2006). Using this model, our group was able to identify the liver stage during the parasite's life cycle as the period during which this difference in larval burden develops (Lewis *et al.*, 2007; Deslyper *et al.*, 2019a). This model has subsequently proven useful for the study of the liver proteome in order to help identify the underlying mechanisms of predisposition (Deslyper *et al.*, 2016; Deslyper *et al.*, 2019b).

Most mouse studies use *A. suum* as an infective agent when studying ascariasis, undoubtedly because this species is easier to obtain through abattoirs worldwide. However, the suitability of its use as a model for *A. lumbricoides* infection has, to our knowledge, never been thoroughly investigated.

In the present study, we used our previously developed mouse model of hepatic resistance (Lewis *et al.*, 2006, 2007) to compare, firstly, the migratory path, with a specific focus on the liver and lungs, and larval burden of *A. lumbricoides* and *A. suum* infection. Secondly we measured the larval sizes during the lung stage of infection.

Materials and methods

Ascaris eggs

The eggs from *A. lumbricoides* were obtained from dewormed children in Ile-Ife, Nigeria. Deworming was performed using pyrantel pamoate (Albendazole damages egg development; Boes *et al.*, 1998a). Female worms were transported on ice in 4% formalin. The worms were dissected, the uteri removed and mechanically broken up before being sieved (425 µm). The sieved eggs were placed in 0.05M H_2SO_4 (Aldrich, 32,050-1) in culture flasks with ventilated cap at 26°C and they were oxygenated twice per week.

Embryonated *A. suum* eggs were kindly donated by Dr Johnny Vlaminck (Ghent University, Belgium). These were shipped in a water solution, stored at 26°C in 0.05M H_2SO_4 and oxygenated twice per week.

Therefore, the eggs used for infection were, for both species, from a mixture of worms, mimicking the situation as it would occur naturally.

Infection experiment

Ninety (90) male C57BL/6J mice (Comparative Medicine Unit, Trinity College Dublin, Dublin, Ireland) and 90 male CBA/Ca mice (Envigo, the Netherlands) were infected with 1000 eggs each via oral gavage (FTP-20-38-50, Instech, Plymouth Meeting, PA, USA). In total, 45 mice of each strain were infected with *A. suum* and 45 were infected with *A. lumbricoides*. The mice were eight weeks old at the time of infection. Animals were provided *ad libitum* with sterilized water and feed.

Larval recovery and enumeration

Five mice from each group were sacrificed daily via cervical dislocation, starting at 6 h post infection (p.i.) until and including eight days p.i. Subsequently, the mice were dissected, livers and lungs removed and larvae were recovered using the modified Baermann method (Lewis *et al.*, 2006). For ease of use, the lungs were split into the left and right lung. The resulting saline solution, containing the larvae, was spun at 1389 g for 5 min. The supernatant was decanted to a level of 10 ml and then 10 ml of 70% ethanol was added. Preceding the larval counts, the samples were spun at 805 g for 5 min and 15 ml of the supernatant was decanted.

The pellet was resuspended and larval counts were performed on the remaining 5 ml. For the lungs, larvae in 1 ml of each sample was counted on a nematode counting chamber (Chalex Corporation, Park City, UT, USA). The number obtained for the left lung and the right lung were added to provide the total number of larvae recovered from the lungs from each mouse. For the liver, 100 µl aliquots were screened for the presence of larvae, and this was repeated five times per sample.

Measuring of larval lengths (µm)

Larval lengths (µm) from the lungs were measured at days 6, 7 and 8 p.i. The larvae were photographed (Olympus digital camera C-5050, Shinjuku, Tokyo, Japan) and their length (µm) from anterior to posterior calculated using ImageJ, version 1.52a (Lewis *et al.*, 2006).

Statistical models

General linear models were run in R, version 3.5.1 (R Core Team, 2018) for the numbers of liver and lung larvae, separately. All models used a combination of the three categorical independent variables, *Ascaris* species, mouse strain and day p.i. The data for larvae recovered from the liver and lungs were overdispersed. The MASS package (Venables & Ripley, 2002) and pscl package (Zeileis *et al.*, 2008) were used to build the negative binomial (log link) and zero-inflated negative binomial (logit link) models, respectively. Model selection was based on a combination of Akaike Information Criterion (AIC), log-likelihood values and number of expected zeroes.

For the liver, the total number of zeroes in the data was 59, meaning the zero-inflated models were a better fit. Model number 1 and 3 were very similar (supplementary table S1). The likelihood ratio test did not reveal statistical significant differences

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between the two (Pr(>Chisq) = 0.4996). The interaction was not significant in model number 3. Using the parsimony principle, the simpler of the two models, number 1, was chosen.

The data for the lungs were not zero-inflated, so a negative binomial model was preferred. Model 1 and 2 had the best AIC scores (supplementary table S2) and the likelihood ratio test (Pr(>Chisq) = 0.9343) did not reveal statistical significant differences between the two. As the interaction between strain and day was not significant, the simpler model – number 1 – was chosen.

Statistical analysis of larval length

Larval lengths were analysed at day 8 p.i. Other time points did not yield enough larvae/data points for statistical analysis. We used a two-way analysis of variance (ANOVA) with *Ascaris* species and mouse strain as factors.

Results

Higher A. lumbricoides counts in the liver

The number of larvae recovered in the liver was fairly constant over the observed time period for both mouse strains and ascarid species. The number of larvae recovered from the CBA/Ca strain was consistently lower than that of C57BL/6J. The C57BL/6J mouse strain had, for both ascarid species, much higher mean larval numbers than the CBA/Ca mouse strain. Our results show that C57BL/6J had a peak at day 6 p.i. of 208 ± standard error of mean (SEM) 71.7 larvae for mice infected with *A. lumbricoides*. As for the same mouse strain, but with *A. suum* infection, the peak was found earlier at day 3 p.i. with 56 ± SEM 26.8 larvae. For the relatively resistant strain, CBA/Ca, the peak for *A. lumbricoides* and *A. suum*, respectively, was at day 1 p.i. with 82 ± SEM 18.8 larvae and day 2 p.i. with 46 ± SEM 26.2 larvae.

Overall, higher counts of *A. lumbricoides* than *A. suum* larvae (see fig. 1a, b) were observed in the liver. This was true for nearly all time points, except on days 2 and 3 in CBA/Ca mice. However, the difference in mean larval burden on those days was found to be quite low. CBA/Ca mice, on days 2 and 3, infected with *A. lumbricoides* had a mean of 34 ± SEM 10.3 and 20 ± SEM 6.3 respectively, whereas *A. suum*-infected mice had a mean of 46 ± SEM 26.2 and 24 ± SEM 11.2 for those days, respectively.

The model revealed that the difference in larval burden between the two mouse strains and the two species was statistically significant ($P < 0.01$). The theta value of the model was 1.3873, or an alpha value of 0.7208. The coefficients of the zero-inflated negative binomial model (table 1) indicated that, for the counts portion of the model, the difference in the logs of expected larval counts decreased with 0.52 units if the mouse was infected with *A. suum* compared to *A. lumbricoides*. As for the CBA/Ca mouse strain, a decrease of 0.53 units was observed compared to C57BL/6J. Both these values were statistically significant, with observed lower larval counts for *A. suum* infection, compared to *A. lumbricoides* infection. The same was true for the CBA/Ca mouse strain, indicating this mouse strain had statistically significantly lower larval numbers than the C57BL/6J mouse strain.

In the logistic portion of the model, or the zero-inflated portion, the difference in the logs of expected zeroes increased with 2.69 units for *A. suum*-infected mice compared to *A. lumbricoides*-infected mice. The model predicted an increase of 1.20 units for every difference in the logs of the larval counts if it was a CBA/Ca mouse rather than a C57BL/6J mouse. Again, these values were statistically significant, meaning that a higher number of zeroes was expected in mice infected with *A. suum* or of the CBA/Ca species.

According to the model, the time point of 6 h p.i. and seven days p.i. showed a decrease of 1.46 and 0.86 units, respectively, for every log increase of larval counts compared to day 1 p.i. This was the case for the counts portion of the model. When looking at the logistic portion of the model the story changes, where 6 h p.i. and eight days p.i. were expected to increase logs of expected larval counts by 3.21 and 1.97 units, respectively, compared to day 1 p.i.

A drop of A. lumbricoides counts in the lung

In the lungs, there was a consistently higher larval count for *A. suum* compared to *A. lumbricoides* (see fig. 1c, d). Larval counts were much lower overall for both mouse strains compared to the liver. Additionally, the CBA/Ca strain had consistently lower larval numbers compared to the C57BL/6J strain. Additionally, both *A. suum*- and *A. lumbricoides*-infected mice had higher larval burdens in C57BL/6J compared to CBA/Ca, with a mean peak for C57BL/6J mice infected with *A. lumbricoides* of 47 ± SEM 29.1 larvae at day 8 p.i. and 86 ± SEM 37.2 larvae for *A. suum*-infected mice at the same time point. These peaks were much lower for the CBA/Ca mouse strain, with this mouse strain also showing a mean peak for both ascarid species at day 8 p.i. of 5 ± SEM 4.1 and 3 ± SEM 1.8 larvae for *A. lumbricoides* and *A. suum*, respectively.

The difference between the two species was found to be statistically significant ($P < 0.01$). Additionally, there was a statistically significant difference between the two mouse strains ($P < 0.01$). The binomial model for the lungs predicted (table 1) that the difference in the logs of larval counts increased with 1.18 units if the mouse was infected with *A. suum*. If the mouse strain was CBA/Ca, the difference in the logs of expected larval counts decreased with 2.14 units compared to C57BL/6J. However, no statistical difference was found regarding the different time points.

Lung larval length differs between Ascaris species and mouse strain

In total, 559 larvae from the lungs were measured for days 6, 7 and 8 p.i. (table 2). These data showed larval growth over the course of the measured days for all studied groups. Overall, the mean length of *A. suum* was greater than for *A. lumbricoides*. This was true for both mouse strains. Additionally, *A. lumbricoides* had a lower larval length in the relatively resistant strain at day 8 p.i., when compared to the relatively susceptible strain. As for *A. suum*, this difference was not found at day 8 p.i.; however, it was observed in the earlier days, where the larvae had lower length in the relatively susceptible strain when compared to the relatively resistant strain.

Due to insufficient number of larvae available in the samples, analysis (two-way ANOVA) was only performed on the results from day 8 p.i. This analysis revealed that both mouse strains ($F_{1401} = 8.37$, $P = 0.004$) and *Ascaris* species ($F_{1401} = 71.15$, $P < 0.0001$) had a statistically significant effect on larval length for this day. The interaction between the two factors was found not to be statistically significant.

Discussion

In a mouse model of ascariasis, *A. suum* has tended to be the species of choice, with *A. lumbricoides* much less commonly used

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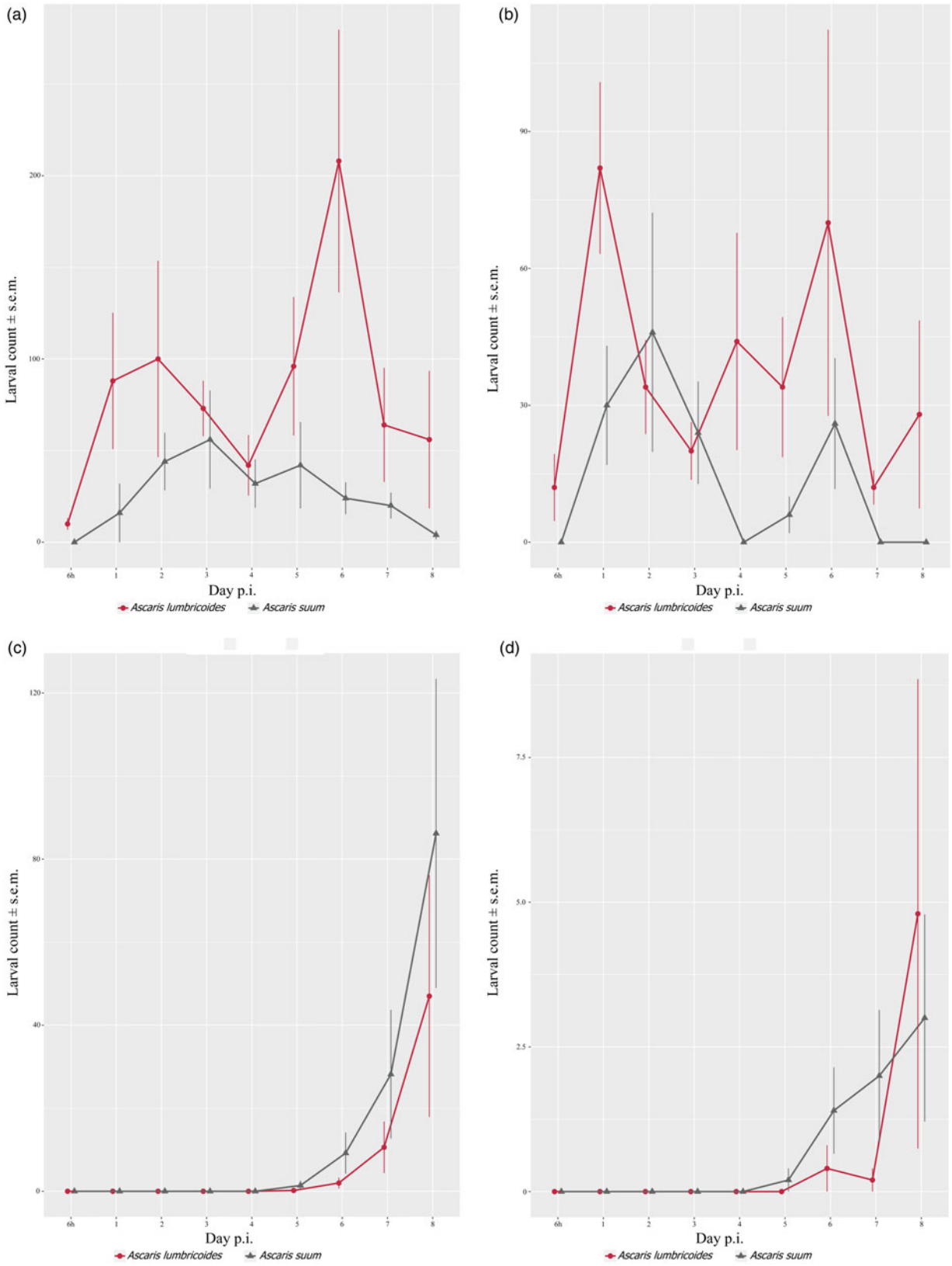

Fig. 1. Mean (±SEM) larval counts of *A. lumbricoides* and *A. suum* in C57BL/6J mice – a relatively susceptible strain – and CBA/Ca mice – a relatively resistant strain – for both the liver and the lungs. The red lines are the counts for *A. lumbricoides* and the grey lines are the counts for *A. suum*. (a) Liver counts of CBA/Ca; (b) liver counts of C57BL/6J; (c) lung counts of CBA/Ca; (d) lung counts of C57BL/6J.

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Table 1. The coefficients from the models for both liver and lungs. Ascarid species is *A. suum* compared to *A. lumbricoides*. Mouse strain is CBA/Ca compared to C57BL/6J, and time points are compared to day 1 p.i.

	Liver – zero-inflated negative binomial				Lungs – negative binomial	
	Counts portion of model		Logistic portion of model			
	Coefficient	Standard error	Coefficient	Standard error	Coefficient	Standard error
Intercept	4.66**	0.25	−3.95**	0.85	−33.87	2.852e + 06
Ascarid species (*A. suum*)	−0.52**	0.18	2.69**	0.51	1.18**	0.38
Mouse strain (CBA/Ca)	−0.53**	0.17	1.20**	0.43	−2.41**	0.38
6 h p.i.	−1.46**	0.42	3.21**	0.93	4.28e−03	4.034e + 06
2 days p.i.	0.05	0.30	−0.98	1.04	4.28e−03	4.034e + 06
3 days p.i.	−0.31	0.31	−0.44	0.96	4.28e−03	4.034e + 06
4 days p.i.	−0.32	0.34	1.67	0.87	4.28e−03	4.034e + 06
5 days p.i.	−0.28	0.31	0.36	0.89	34.48	2.852e + 06
6 days p.i.	0.32	0.31	0.37	0.89	35.06	2.852e + 06
7 days p.i.	−0.86*	0.33	1.02	0.87	35.90	2.852e + 06
8 days p.i.	−0.66	0.35	1.97*	0.87	37.40	2.852e + 06

**Pr(>|z|) < 0.01.
*Pr(>|z|) < 0.05.

(Stewart, 1917, 1918; Sprent, 1952b; Bhowmick, 1964; Cho, 1967; Buske & Engelbrecht, 1968; Kumar & Singh, 1968; Maung, 1978; Massara *et al*., 1990, 1991; Peng *et al*., 2012; Gazzinelli-Guimarães *et al*., 2013). To the best of our knowledge, no previous study has provided a detailed comparison of the larval burden of the two ascarid species derived from the two most important organs in early migration, the liver and the lungs. Additionally, we measured the larval length in the lungs at the later time points of day 6, 7 and 8 p.i., considering this parameter as a measure of larval fitness. Using our mouse model of hepatic resistance, and thus examining two mouse strains, we were able to observe whether any differences were strain specific or not.

Larval counts

The larval counts differed significantly between the two *Ascaris* species in both the lungs and liver as well between the two mouse strains. In the liver, *A. lumbricoides* was found to have consistently higher larval numbers. In the lungs, however, the inverse was observed, with *A. suum* counts being consistently higher than *A. lumbricoides* counts. Taken together, these could indicate a delayed, but more pronounced immune defence against *A. lumbricoides*. The inversion of the larval recoveries is quite interesting. It confirms our previous findings, indicative of a role of the liver in larval attrition (Lewis *et al*., 2007; Dold *et al*., 2010; Deslyper *et al*., 2016, 2019b; Nogueira *et al*., 2016). Our results indicate that *A. lumbricoides* larvae have a higher infectivity rate, potentially associated with higher antigenicity, and, therefore, reach the liver in higher numbers. However, upon reaching the liver, this presumed higher infectivity and antigenicity becomes a burden as the immune system ramps up and targets *A. lumbricoides* larvae at a higher proportion than *A. suum* larvae. Based on those findings, we conclude that *A. suum* infection in a mouse model is not a perfect substitute for *A. lumbricoides* infection and results obtained with *A. suum* eggs should be interpreted carefully.

We also observed that the relatively susceptible strain, C57BL/6J, had consistently higher larval counts than the relatively resistant strain, CBA/Ca. This was true for both Ascarid species. We can, therefore, conclude that our mouse model of hepatic resistance can also be used for *A. lumbricoides* infection.

There is a paucity of comparative data on the differences in larval burden between *A. suum* and *A. lumbricoides* in a mouse model. However, a previous study reported that 5–6-week-old male (non-pathogen-free white) mice were infected with what was described as '*A. lumbricoides* from man' or '*A. lumbricoides* from pig' (Sprent, 1952b). The author found that both species had a similar migratory path. However, it was observed that the 'human strain appeared to have about twice the infectivity' (Sprent, 1952b). These data, therefore, showed a similar pattern to our own observations – specifically, the higher number of larvae in the liver of the human ascarids. However, the author observed a higher number of larvae of this human ascarid in the lungs as well, the exact opposite of our own findings.

A more recent study (Peng *et al*., 2012) infected C57BL/6 mice and pigs with either an *Ascaris* genotype which mainly infects humans or one that mainly infects pigs. The findings were similar to results obtained in this study, with a higher larval count in the liver of the genotype, mainly associated with infecting humans compared to the genotype mainly infecting pigs. Conversely, Peng *et al*. (2012) also found this to be true in the lungs where, in contrast to our results, they identified a higher larval burden for *A. suum* in the lungs. However, their lung larval counts did not show the typical slow increase in larval counts that we observed; rather, they observed several peaks, with larvae detected in this organ as early as 8 h p.i.

Another study that investigated how the age of an *Ascaris* egg culture influences infectivity, briefly touches on the question of the use of *A. lumbricoides* in a mouse model (Gazzinelli-Guimarães *et al*., 2013). The authors found no statistically significant differences between *A. suum* and *A. lumbricoides* larval

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Table 2. Larval length of *A. suum* and *A. lumbricoides* in C57BL/6j and CBA/Ca mouse strains on day 6, 7 and 8 p.i. in µm of lung samples.

		Day 6	Day 7	Day 8
C57BL/6J with *A. suum*	Mean ± SD	810 ± 124	809 ± 207	1122 ± 253
	Median	815	764	1116
	Range	395	936	1476
	Sample size	28	83	253
C57BL/6J with *A. lumbricoides*	Mean ± SD	527 ± 91	717 ± 146	932 ± 184
	Median	503	747	938
	Range	188	548	1018
	Sample size	3	28	131
CBA/Ca with *A. suum*	Mean ± SD	719 ± 191	754 ± 201	1104 ± 141
	Median	676	703	1124
	Range	424	538	343
	Sample size	4	6	8
CBA/Ca with *A. lumbricoides*	Mean ± SD	–	681	767 ± 162
	Median	–	–	769
	Range	–	–	563
	Sample size	0	1	11

SD, standard deviation.

counts in male BALB/c mice; however, larval counts were only compared from lung samples derived from day 8 p.i.

In short, our study showed similar results to previous studies regarding larval counts in the liver, where the human ascarid has a higher larval count than the porcine ascarid. However, our study differs from these studies regarding the lung. Where we found higher *A. suum* larval counts for this organ, other studies found higher *A. lumbricoides* burdens.

Larval length

When measuring the larval length in the lungs, we found, interestingly, that the mean larval length for *A. suum* was longer in both mouse strains. Taken together with the data from the larval counts, it appears that *A. lumbricoides* may provoke a stronger immune response, which, therefore, results in smaller larvae in the lungs.

One study immunized eight-week-old male C57BL/6 mice with *A. suum* via oral infection, followed by a challenge infection (Johnstone *et al.*, 1978). The larvae recovered from these mice were compared to mice which had received one single dose of eggs. The authors found that for the liver the larval counts were quite similar; however, the difference in larval counts was quite substantial in the lungs, with the non-immunized having a much higher larval count compared the immunized animals. The authors, therefore, confirm the idea that 'the mechanism of immunity against *A. suum* operates primarily in the liver rather than in the gastrointestinal tract'. Interestingly, between days 5 and 9 p.i., the difference in larval lengths in the liver was significantly lower for the immunized animals compared to non-immunized animals. So, despite there being no significant difference in larval burden in the liver, the larvae are already smaller at this point in time. This trend continues in the lungs, where a statistically significant difference in larval lengths is observed.

A later study, using a reinfection experiment, developed a simpler version of the above experiment (Song *et al.*, 1985). The authors compared reinfected mice with primary infected mice and found that the larval length in both liver and lungs was lower for the reinfected mice compared to the primary infected mice. As for the liver specifically, the difference in larval length was relatively small during early infection; however, it increased over time. As for the lungs, the initial differences were quite big, but the difference actually decreased. The authors concluded that the 'development of larvae in the liver of immune mice were probably repressed by the immune mechanisms being rised [sic] in the livers' (Song *et al.*, 1985).

Lewis *et al.* (2006) identified that infection dose influenced the length of the larvae, with a higher egg dose resulting in higher larval burden in the lungs, but their length was reduced. They explain that increased larval count could be due to increased tissue damage allowing for more larvae to migrate to the lungs, and reduced size could occur due to a greater innate response hampering larval growth in the case of an increased infective dose. This could explain our own observations, where the decreased mean larval size of *A. lumbricoides* in both mouse strains could be related to the observed higher larval burden in the liver and the potentially associated pronounced immune response.

In addition to using a mouse model, some research groups have used the natural host, the pig, to study *A. suum* adult worm length. Pigs were immunized with *A. suum*, through repeat infection, with some pigs receiving fenbendazole treatment after each infection (Stewart *et al.*, 1985). After an *A. suum* challenge infection, the adult worms were measured and counted. The authors found that fewer and smaller adult worms were recovered from the groups that received the anthelmintic after each vaccination infection, compared to those that were immunized but did not receive any anthelmintic. The authors concluded that the fenbendazole treatment probably heightened the immune response against the parasite.

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Another group infected pigs with different *A. suum* haplotypes and found that there was a consistent statistically significant difference in the worm length between the haplotypes, with one haplotype consistently being larger than the others (Nejsum *et al.*, 2009). Furthermore, the effect of the different hosts – that is, different pigs – on worm length was statistically significant. This, therefore, implies that not only the genetics of the specific haplotype but also the interaction with the host has an effect on worm size. The authors subsequently found that worm length was highly correlated to the length of the female worm from which the eggs were taken. The authors suggested, assuming all eggs from one female are fertilized by one male, that 18–46% of the worm length can be explained by heritability.

We see similar results in our CBA/Ca mouse strain, where mice infected with *A. lumbricoides* had an earlier larval peak in the lungs – at day 1 p.i. – compared to *A. suum*-infected mice – at day 2 p.i. Additionally, we found that larval length and burden is lower for *A. lumbricoides* in the lungs. This would indicate that there is a more pronounced immune response against this species compared to *A. suum*, much like the case for the immunized or reinfected animals in the previous experiments. One could argue that the observed reduction in larval size of *A. lumbricoides* in the lungs is a consequence of density-dependent growth retardation. However, we could argue that evidence from the reinfection experiments (Johnstone *et al.*, 1978; Song *et al.*, 1985) points towards a more pronounced immune response against *A. lumbricoides*. We postulate that the antigenicity and infectivity of *A. lumbricoides* is higher in this mouse model, with higher larval counts in the liver than *A. suum*, followed by lower larval counts in the lungs. However, *A. lumbricoides* may evoke a much stronger immune response than *A. suum*, leading to a reduced larval count and length in the lungs compared to *A. suum*. These results, therefore, indicate that more research is necessary to compare the early immune response to *A. suum* and *A. lumbricoides* in a mouse model.

In conclusion, we observed a difference in larval burden and length between the two ascarid species both in the liver and the lungs. Our results, therefore, indicate a potentially different host response towards *A. suum* compared to *A. lumbricoides*. This has previously been investigated by proteomics, using an *A. suum* infection in the mouse model (Deslyper *et al.*, 2016; Deslyper *et al.*, 2019b). However, in order to fully understand the mechanisms behind this difference we will need to understand the immune response in the liver against *Ascaris*. We are currently undertaking an experiment using flow cytometry to determine which immune cells are activated in the liver during *Ascaris* infection. We are investigating if there is a different immune response between the two mouse strains of the mouse model and if there is a different immune response against *A. suum* and *A. lumbricoides*.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X20000127

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The mouse samples used in this study were approved by the TCD Animal Research Ethics Committee and the Health Products Regulatory Authority, the Irish regulator for scientific animal research in Ireland, under Directive 2010/63/EU and its Irish transposition, SI no. 543 of 2012 (project authorization ID: AE19136/P078 ID; case reference 7026410).

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