Ascaris suum infective eggs upregulate IL-4, 5 and 10 in BALB/c mice

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Experimental animals such as BALB/c mice are invaluable models in the elucidation of immune responses in parasitic infections. The present study determined the effect of the infective eggs of *Ascaris suum* in BALB/c mice through the measurement of cytokine and immunoglobulin levels. Thirty BALB/c mice were randomly grouped into normal control group (NC) and Ascaris group (AS), divided into three trials (5 mice per group per trial). Those in the AS group were chronically infected with *Ascaris suum* (*As*) eggs, while those in the NC group were given placebos. At days 0, 36 and 72, stool samples were collected for fecalysis and formalin ether concentration technique (FECT) while blood samples were extracted and used for ELISA for the measurement of cytokines and *As*-specific antibodies. Liver, lungs and small intestines were harvested for histopathologic analysis. Chronic *Ascaris suum* infection in BALB/c mice significantly increased the levels of IL-4, IL-5, and IL-10. It did not significantly change the level of *As*-specific IgE but slightly increased the level of *As*-specific IgG. Stool specimens from all mice showed negative fecalysis and FECT results. Pathological conditions were observed from the liver, lungs and small intestines of infected mice. *Ascaris suum* is capable of infecting BALB/c mice, which indicates that it can cross species barrier. Immune response of mice was modulated through production of IL-4, IL-5 and IL-10 and *As*-specific IgG.

KEYWORDS

Parasitism, Ascaris, interleukins, immunomodulation, antibodies, cytokines

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INTRODUCTION

Parasitism is highly prevalent in developing countries compared to industrialized and developed countries (Matera et al. 2008; Yazdanbakhsh et al. 2001). In the Philippines, helminthiasis has long been regarded as one of the most common infections affecting school children (Baldo et al. 2004). The most common parasites infecting children are intestinal parasites such as *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms, with prevalences of 36.0%, 44.8%, and 7.0 %,

respectively (Baldo et al. 2004; Hotez 2000). Most parasites exhibit host specificity. For instance, *Trichuris trichiura* and *Ascaris lumbricoides* are specific for humans while *Trichuris suis* and *Ascaris suum* are specific for pigs. However, recent studies have shown the possibility of cross infections, i.e, *Ascaris lumbricoides* in humans and *Ascaris suum* in pigs have been shown to cross species barriers (Dold and Holland 2011a).

Parasitism may be categorized**,** based on the degree of infectivity**,** as acute or chronic infection. This degree of infectivity or intensity of infection is more important than the infection status because the intensity is the determinant of pathologic condition (Quinell 2003). Acute helminth infections follow a short period of exposure or even an infrequent exposure. This type of infection is associated with parasite-specific immune response, which is characterized by a combination of Th1 and Th2 responses, eosinophilia, and elevated IgE. Also, acute infection is associated with allergic syndromes because of the presence of intense eosinophilia. On the other hand, chronic helminth infections develop after long parasite exposure, is slanted to purely Th2 response and elevated levels of IL-10 and tumor growth factor beta (TGF-β), and to total IgE but less parasite specific IgE and high IgG4.

The high prevalence of parasitism necessitates the identification of alternative anti-helminthic drugs such as those derived from plant sources. However, in the course of the study of parasitism using experimental animal models, contrasting and inconclusive results have been found because of the lack of sufficient data on the pathological effect of parasites. Variability of results in animal models may be due to multiple factors such as differences in the stages of the parasite, life cycle of the parasite, immune response of the host and the potential presence of cross infection. For instance, a study on the different stages of *Schistosoma mansoni* and protection against allergy showed that only the worm stage of *S. mansoni* is capable of providing protection against airway inflammation in response to an allergen. The egg stage of the parasite and the egg plus worm stage, instead of protecting, rather exacerbate the airway hyperresponsiveness (Mangan et al. 2006). As with the life cycle of the parasite, one study mentioned that only parasites with pulmonary phase of larval migration can confer protection against allergic disorders (Cooper 2002). Furthermore, acute and chronic infection of parasitism may also contribute to differences in immune response in animal models (Quinell 2003). In this paper, cross infectivity of *Ascaris suum* in BALB/c mice is studied through detection of antigen-specific antibodies and interleukins. Chronic infection in humans is mimicked to induce parasitism in BALB/c mice.

MATERIALS AND METHODS

Ascaris suum Culture and Egg Counts

Adult female *Ascaris suum* worms were obtained from a slaughter house in La Loma, Quezon City, Philippines. Only female worms were used in this study. Ten female worms were dissected longitudinally as described previously (Akinboye et al. 2009). The uterus of each worm was isolated and placed in a mortar and pestle. Distilled water was added drop wise and the uterus was macerated into a paste-like consistency. A drop of this suspension was placed on a glass slide and viewed under the microscope (LPO, HPO) to check for the presence of *Ascaris suum* eggs. The suspension was then placed inside a petri dish

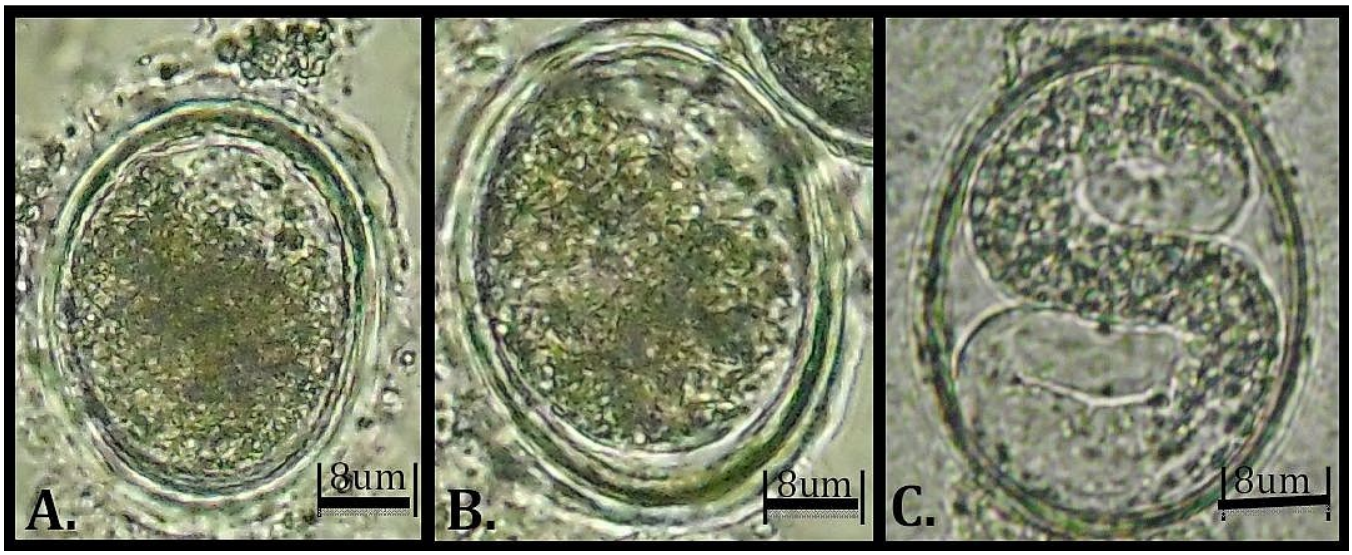
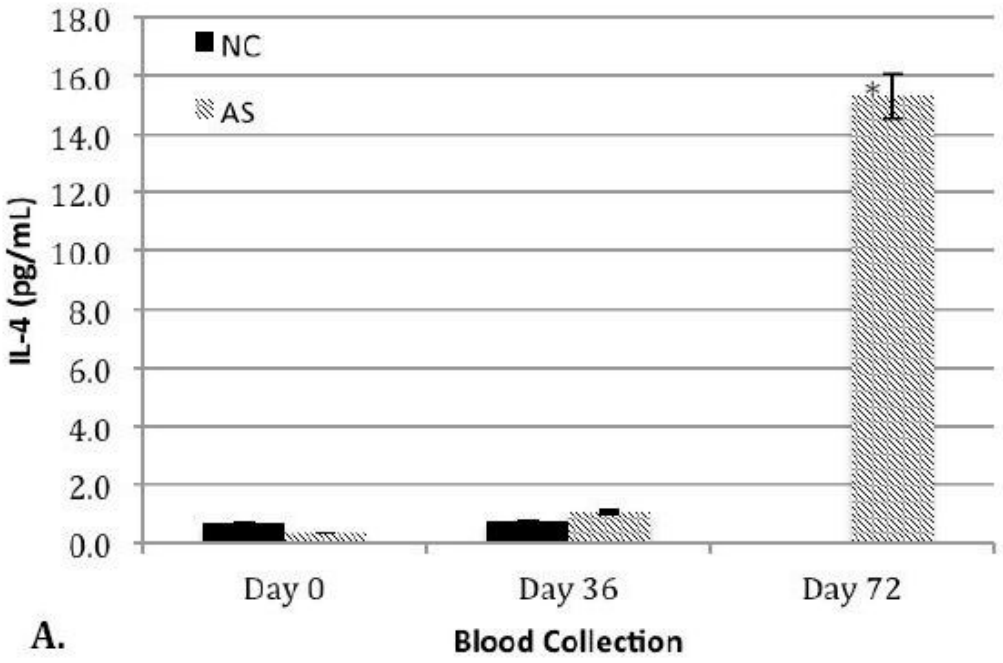


Figure 1. Micrographs of fertilized corticated *Ascaris suum* egg (A) with an outer albuminoid coat, which serves as an auxiliary barrier to permeability; fertilized decorticated *Ascaris suum* egg (B); and infective egg stage of *Ascaris suum* (C) with an S-shaped rhabditiform larva inside (LPO).



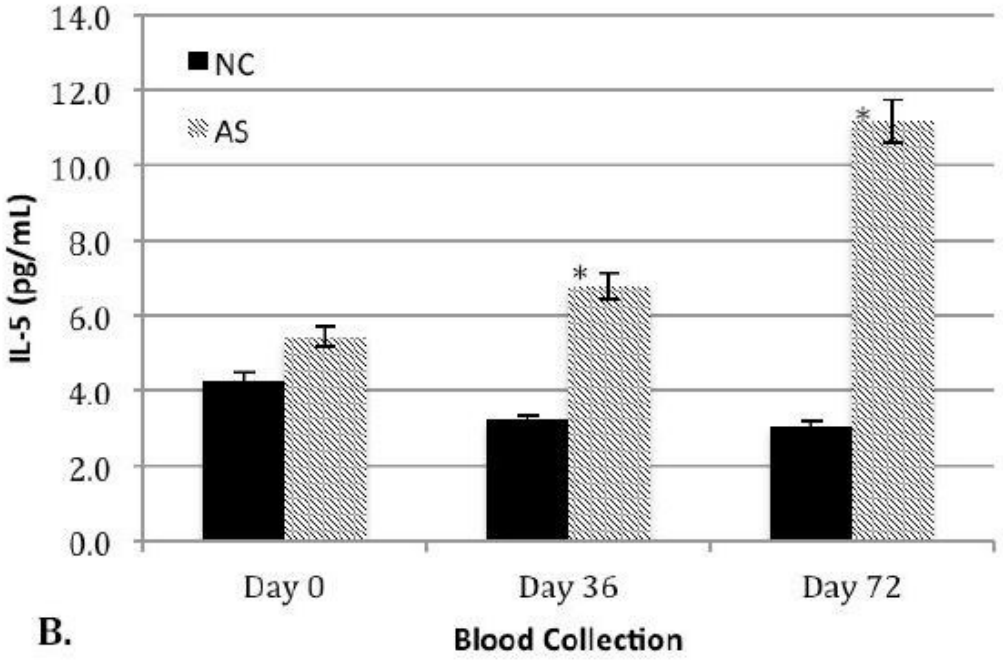


Figure 2. Comparison of the mean IL-4 (A) and IL-5 (B) levels in BALB/c mice infected with *Ascaris suum* eggs or placebo from day 0 to day 72. IL-4 and IL-5 were measured from blood sera using ELISA.

with moistened filter paper and stored under light protection at room temperature (25°C). A total of 10 plates was used. The petri dishes were regularly moistened with distilled water to prevent desiccation. A wet preparation was made everyday starting on day 8 to monitor the developmental stage of the eggs until the infective stage of *Ascaris suum* was obtained (egg with larva inside). The culture was then transferred to an amber bottle with enough distilled water for storage until used. The number of eggs per 10 uL of the suspension was counted manually under the microscope. Counting was done ten times per aliquot and the mean count and SD were computed. The total number of eggs harvested was not counted.

Experimental Animals and Infection Protocol

BALB/c mice, 6 to 8 weeks old, weighing approximately 30 grams, were obtained from the University of the Philippines National Institute of Health (UP-NIH). Permit to handle and use animals was obtained from the University of Santo Tomas Institute of Animal Care and Use Committee (IACUC), with Permit No. AR-2011-15. All mice were handled following the guidelines and protocol of IACUC.

Thirty BALB/c mice were randomly grouped into a normal control group and an Ascaris group, separated into three trials (five mice per group per trial). Mice under the normal control (NC) group were given 250 uL of distilled water as placebo and mice under the Ascaris group (AS) were given 250 uL of *Ascaris*

suum egg suspension with approximately 25,700 eggs via oral gavage every three days until day 36 (1,028 eggs per 10-uL volume). Only a maximum of 250 uL of fluid volume is allowed for administration according to IACUC. Infection was based on the protocol of Schopf et al. (2005), with slight modifications.

Blood Collection

Blood was collected retro-orbitally from the mice before (day 0) and after infection (days 36 and 72). Approximately 400 uL of blood were collected and placed in a yellow microtainer tube with gel separator and clot activator (Beckton Dickinson). The tube was centrifuged at room temperature at 10,000 rpm for 5 minutes (Hettich) to separate the serum from the red cells. Sera were stored at -20°C until used (Sanyo® Biomedical Freezer).

Fecalysis and Formalin Ether Concentration Technique

Stool samples of mice were collected thrice at days 0, 36 and 72. The stool sample of each mouse was placed in a sterile plastic container and analyzed immediately. A portion of the stool was placed on a drop of NSS on a glass slide and covered with coverslip. Slides were viewed under LPO and HPO (Olympus). All negative fecalysis results were subjected to formalin ether concentration technique.

Approximately 1 to 1.5 grams of stool sample were mixed with 10 mL formalin in a centrifuge tube and stirred to form a suspension. The suspension was strained using two layers of wet surgical gauze directly into another centrifuge tube. A total volume of 10 mL was maintained by adding 10% formalin, and 3 mL ethyl acetate were then added to complete the suspension. The tube was covered with a rubber stopper to facilitate the mixing of the suspension for 10 seconds. The tube was centrifuged for 2 minutes at 500 rpm. After centrifugation, a suspension of four layers was achieved: ethyl acetate, fatty debris, formalin and the sediment. The sediment was placed on a glass slide and viewed under the microscope (Belizario and de Leon 2004).

Cytokine Assay

Serum samples were used to measure the cytokine levels by Sandwich ELISA according to the manufacturer's recommendations (Biolegend, Inc, USA). In brief, 50 uL of diluted capture antibody (anti-mouse IL-4, anti-mouse IL-5, and anti-mouse IL- 10) were placed in a 96-well plate and were incubated overnight at 4°C. Wells were washed three times with >200 uL/well wash buffer. To reduce background and block non-specific binding sites, 100 uL 1X assay diluent were added per well and incubated at room temperature for 1 hour. Samples and standards were added to appropriate wells after washing and incubated for two hours at room temperature. Incubation with 50 uL of detection antibody for 1 hour and diluted avidin-horse radish peroxidase (HRP) solution for 30 minutes followed. Plates were washed between steps. Freshly mixed tetramethyl benzidine (TMB) substrate solution (50 uL) was then added and incubated for 20 minutes. Reaction was stopped by adding 50 uL of stop solution to each well. Absorbance was read at 450 nm.

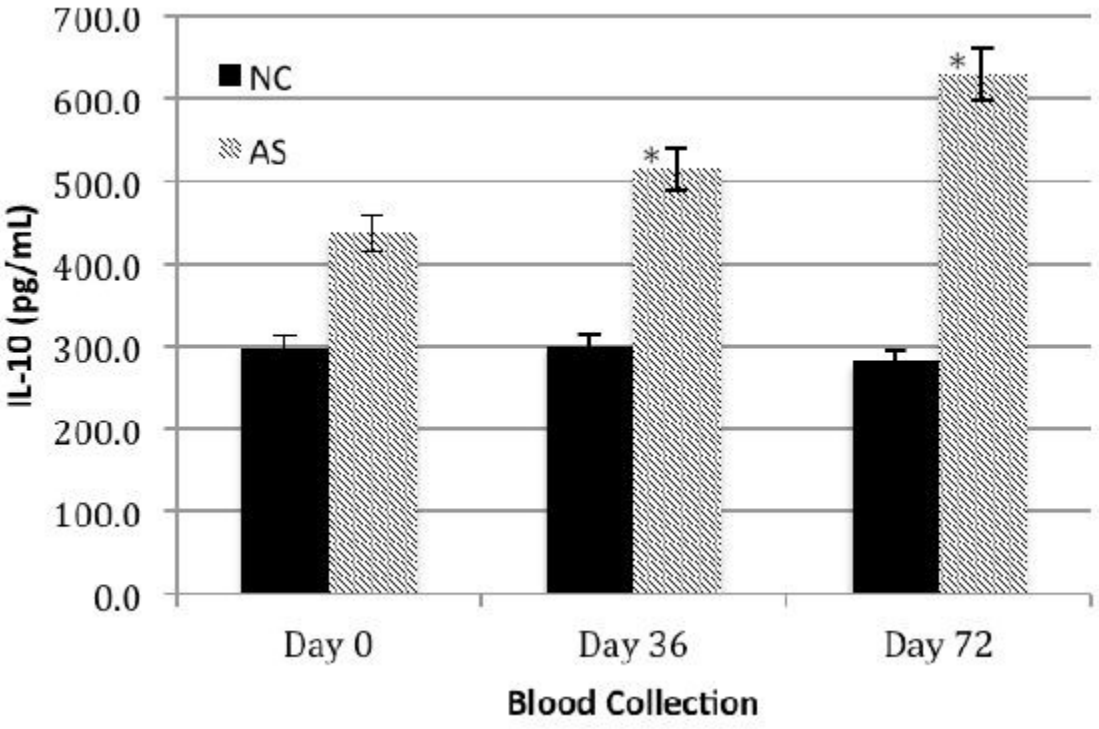


Figure 3. Mean IL-10 levels in BALB/c mice infected with *Ascaris suum* or placebo from day 0 to 72. IL-10 was measured from blood sera using ELISA.

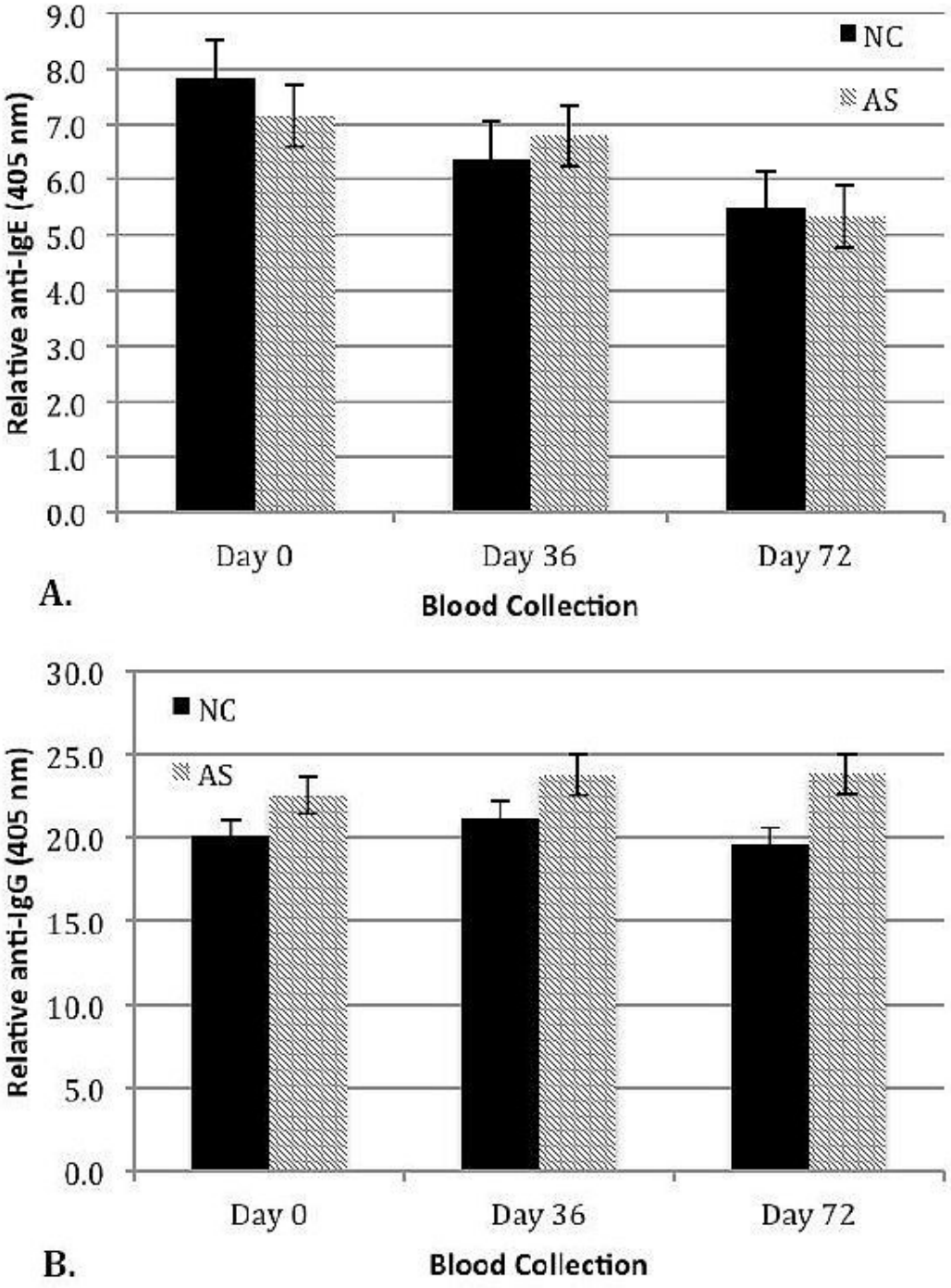


Figure 4. Mean relative Anti-*As* IgE (A) and Anti-*As* IgG (B) levels in BALB/c mice infected with *Ascaris suum* or placebo at day 0 to day 72. *Ascaris suum* specific antibodies were measured from blood sera using indirect ELISA.

The detection limits of the assays were 1pg/mL, 4 pg/mL, and 16 pg/mL for IL-4, IL-5, and IL-10, respectively.

Preparation of *Ascaris suum* antigen

Live adult *Ascaris suum* worms were placed in a solution of borate-buffered saline at pH 8.0 and homogenized in an Ultra Turrax apparatus and stirred overnight at 4°C. The mixture was centrifuged and dialyzed against distilled water for 24 hours at 4°C. Lyophilized extract was suspended in 5% sodium chloride and subjected to sonication for 10 minutes on water at 100 Hz-1 and 6 pulses/sec (Enobe et al. 2006). Protein content of this *Ascaris suum* antigen was quantified using Bradford's Technique Protein (BioRad, CA, USA). Briefly, in appropriate wells, 160 uL of diluted standard (BSA) and samples were pipetted and 40 uL of dye reagent concentrate were added. Sample and reagent were mixed thoroughly and incubated for at least 5 minutes. Absorbance was read at 595 nm (Ramos et al. 2003).

Enzyme Linked-Immuno-sorbent Assay

Serum samples were likewise used for anti-*Ascaris suum* IgE and IgG level measurement. ELISA plates were coated with 50 uL of 5 ug/mL *Ascaris suum* extract antigen in 0.1 M $NaHCO_3$, pH 8.4 and incubated overnight at 4°C. After overnight incubation, the plates were washed three times with 200 uL/well of PBS-T. The wells were then blocked with 100 uL of 1% BSA in PBS per

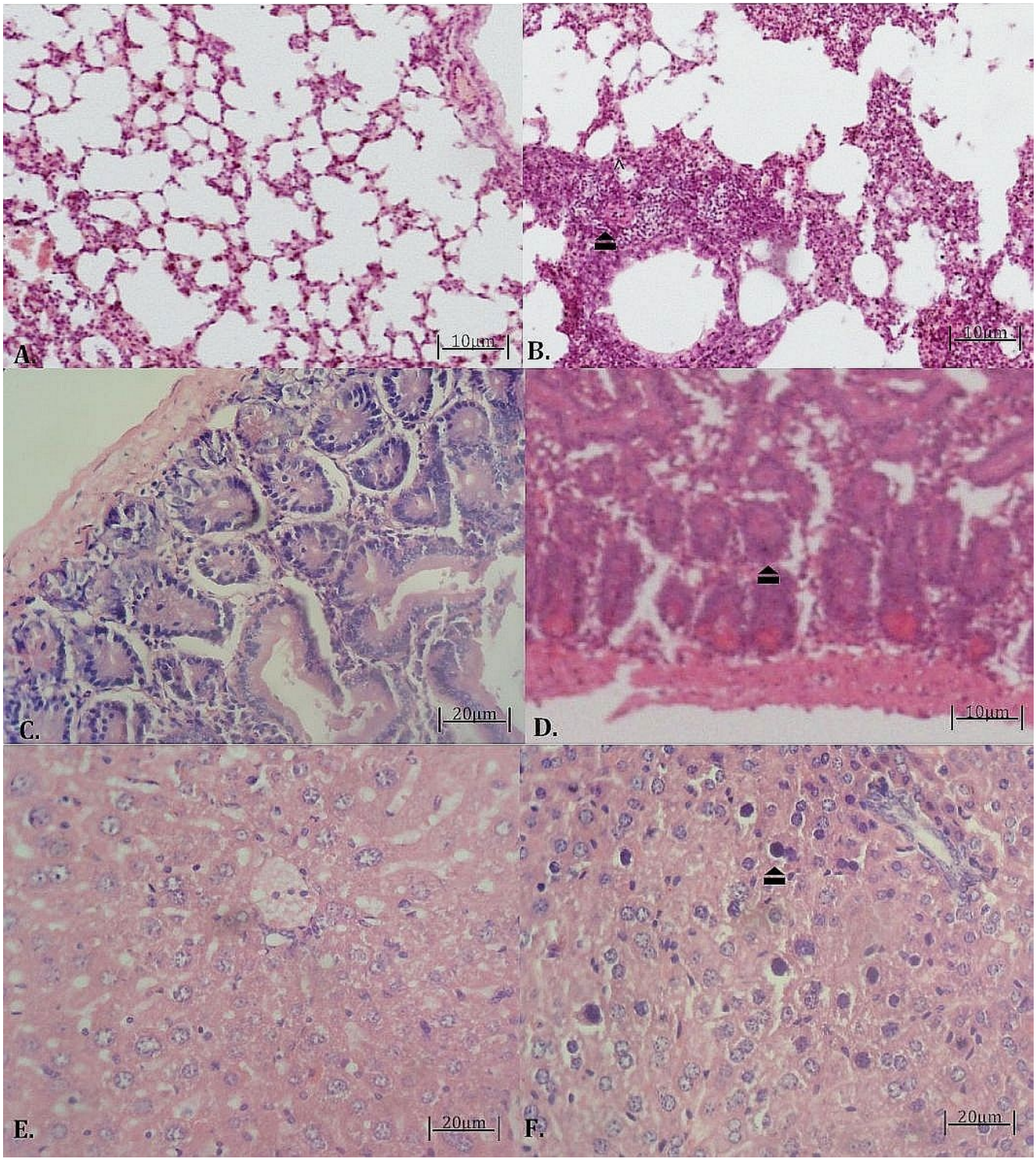


Figure 5. Histopathologic analysis of lung, small intestines and liver tissues of BALB/c mice. Lung, small intestines and liver of uninfected BALB/c mice showed no pathological abnormality (A, C, E). Lung tissue of AS group (B) showed pathological conditions described as mild to moderate interstitial edema (⏏) with neutrophilic infiltrates (^). Focal hyperplastic changes (⏏) were observed in the small intestines of mice in AS group (D). While presence of lymphocytic infiltrates (⏏) were noted in the liver tissues of mice in AS group (F).

well and incubated for two hours at room temperature. Diluted serum samples (50 uL) were added in appropriate wells and incubated overnight at 4°C prior to incubation with 50 uL of biotin anti-mouse IgE or anti-mouse IgG for two hours at room temperature, and ExtraAvidin Alkaline phosphatase incubation for 1 hour at room temperature with constant shaking. The plates were washed thrice with PBS-T between steps. After incubation, 50 uL of nitrophenyl phosphate were added and plates were incubated in the dark at room temperature for 15 minutes. Absorbance was read at 405 nm at 60 minutes using an ELISA reader (Biotek ELX 800) (Fernandes et al. 2010).

Histopathologic Analysis

At day 72, liver, lungs and small intestines of randomly selected mice, three per group, were harvested for histopathologic analysis. Tissues were suspended in 10% formalin for fixation and processed using routine histopathology procedures. Processed specimens were embedded in paraffin, sectioned and stained with hematoxylin and eosin solution (Akinboye et al. 2009). Two sections of each organ per slide were prepared. Slides were viewed by a pathologist under the microscope using high power objective, with 400X magnification (Olympus).

Statistical Analyses

Mixed Model Analysis of Variance was used to determine the significant difference between the two groups (NC and AS), and the different trials (trial 1, trial 2 and trial 3). The groups were considered as fixed factors and the trials as random factors. Repeated Measures Analysis of Variance (RMANOVA) was used to determine if there is a significant change in the means from the baseline, with Bonferroni correction to compare the baseline with the third extraction. All statistical tests were performed under 5% level of significance using SPSS 17.0 for Windows.

RESULTS

***Ascaris suum* egg culture**

Wet mount preparations done at day 0 of culture (during dissection) showed fertilized *Ascaris suum* eggs. Most of these fertilized eggs were decorticated and some were corticated. The eggs were broadly oval, measuring about 40 to 70 by 30 to 50 µm. The outer covering of the corticated eggs is an albuminoid coat and appears brownish in color. Decorticated eggs have no albuminoid coat but with a thick inner shell (Fig. 1A and 1B). On day 10, the infective stage (eggs with larva inside) of the parasite was observed in six out of 10 plates. The eggs appeared oval with an S-shaped structure inside, which is the larva (Fig. 1C). On day 11, the infective stage of *As* was also observed in the remaining four plates. The suspension was harvested and transferred to an amber bottle. Egg count of the homogeneous suspension was identified as 1,028 eggs per 10-uL suspension with 1.42 mg/mL protein concentration.

BALB/ c Mice *Ascaris suum* Infection Models

All mice appeared normal after two weeks of acclimatization. Mice in the NC group maintained a normal morphological appearance and were active throughout the experiment. Mice in the AS group became sluggish, thinner and with ruffled skin post infection with *Ascaris suum* infective larval stage. Negative fecalysis results were observed in the stool samples collected from all mice, before and after infection protocol. No eggs were seen in the stool specimens. These negative results were confirmed by negative FECT results.

***Ascaris suum* upregulates production of IL-4, IL-5 and IL-10**

All BALB/c mice produced similar levels of IL-4 and IL-5 at day 0 ($p=0.190$). Mice in the NC group maintained these levels of IL-4 and IL-5 throughout the experiment from day 0 to 36 to 72 ($p=0.511$). Mice in the AS group, on the other hand, showed a significant increase in IL-4 and IL-5 at day 72 ($p=0.010$). Thus, the BALB/c mice were capable of responding to an infectious agent through production of IL-4 and IL-5.

Levels of IL-10 were also measured at days 0, 36 and 72 (Fig. 4). Mice in the NC group maintained a constant level of IL-10 throughout the experiment ($p=0.819$). The IL-10 level in mice of the AS group significantly increased from day 0 to 36 and from day 36 to 72 ($p=0.040$). Thus, *Ascaris suum* infective egg suspension increased the level of IL-10 in BALB/c mice.

Immunoglobulin Response of *As*-Infected BALB/c Mice

Antibody specific to *Ascaris suum* was measured at days 0, 36 and 72 (Fig. 4A). Anti-*As* IgE did not significantly change in mice in either the NC and AS group at days 0, 36 and 72 ($p=0.236$ and $p=0.647$). Also, there was no significant difference between the levels of anti-*As* IgE of both NC and AS groups ($p=0.166$).

Anti-*As* IgG in normal mice (NC) decreased slightly but not significantly from day 0 to 72 ($p=0.880$) (Fig. 4B). The level of anti-*As* IgG in both the NC group and the AS group at day 0 did not differ significantly ($p=0.166$). The anti-*As* IgG of the AS group increased slightly from day 0 to 36 ($p=0.136$). Thus, the BALB/c mice were able to respond to the infectious agent through production of anti-*As* IgG.

***As* Infection Affected the Lungs, Liver and Small Intestines of BALB/c mice**

Histological analysis of lungs, small intestines and liver of normal mice showed no pathological abnormality and can be described as normal tissues (Fig. 5A, C, and E). Lung tissues of

infected mice (Fig. 5B) showed pathological conditions with mild to moderate edema and congestion accompanied by moderate interstitial neutrophilic infiltrates. Small intestines of infected mice (Fig. 5D) showed focal hyperplastic changes. Liver tissues of infected mice (Fig. 5F) showed mild perivascular lymphocytic infiltrates. Slides of those organs from every group of mice exhibited similar pathologic changes, except for the liver of mice 2-AS, which had normal histological appearance (not shown).

DISCUSSION

Ascaris suum is a parasitic nematode (Family Ascarididae) causing infections specifically in pigs. Although Ascaris spp. are usually host specific, cases of cross infectivity to humans have been detected (Dold and Holland 2011a). On the other hand, the human roundworm, *Ascaris lumbricoides,* has also been detected in pigs. *A. suum* and *A. lumbricoides* are morphologically indistinguishable nematodes described as large white or pink worms ranging from 10 to 31 cm for males and 22 to 35 cm for females. They are smooth, with finely striated cuticle, conical anterior and posterior extremities and with terminal mouth with three oval lips with sensory papillae (Belizario and de Leon 2004). The only difference between the two species was reported in their genome sequence where they differ by six nucleotides (1.3%) in the first internal transcribed spacer (ITS-1) and by 3-4% in the mitochondrial genome (mtDNA) sequence (Dold and Holland 2011a). Because of the close resemblance of these two Ascaris spp, *Ascaris suum,* which is more commonly available and easily harvested from pigs, has been commonly used as a model for *Ascaris lumbricoides* infection. In this present study, Ascaris worms obtained from pigs were used and identified as *Ascaris suum.* Although studies have mentioned that *Ascaris suum* and *Ascaris lumbricoides* are two distinct species of Ascaris, a recent study concluded that *Ascaris suum* and *Ascaris lumbricoides* are a single species and, since *A. lumbricoides* has a taxonomic priority, *Ascaris lumbricoides* Linnaeus 1758 will be used as its taxonomic name and *Ascaris suum* is a synonym (Leles et al. 2012). This recent finding still needs further investigation. Studies of Slotved et al. (1998) focused on the biology of *Ascaris suum* in mice models. They confirmed that the migratory pattern of *A. suum* is similar in murine and porcine hosts, thus mice is an appropriate model for Ascaris infection. In this present study, BALB/c mice were used to investigate the immunomodulatory effect of cross infection of Ascaris in mice. BALB/c mice are among the most commonly used inbred strains of mice particularly in the field of immunology because of their normal response against antigens (www.informatics.jax.org). However, some studies found that Ascaris in mice represents an abnormal host-parasite relationship involving only the larval stages of the lifecycle and worms do not mature in the small intestines (Dold and Holland 2011b). Another study found that mice are semipermissive hosts of *A. suum* with normal migration from the cecum to the liver, lungs, and small intestines but not farther (Schopf et al. 2005). The inability of the worms to migrate back to and go beyond the small intestines results in the absence of eggs in the stool of mice. The negative fecalysis and FECT results in this study may indicate that the worms did not return to the small intestine for maturation and no eggs were expelled within the period of infection. Histopathologic analysis of liver, lungs and small intestines was done to determine the extent of migration and infection. Liver, lungs and small intestines of infected mice showed pathological conditions indicating that the ingested infective ova with larva inside may have hatched in the small intestines, migrated to the liver and to the lungs. Aside from histopathologic changes, antibody production indicates the development of the injected *Ascaris suum* in the animals. As expected, *As*-specific IgE production was not enhanced because the mice were chronically infected with *As.* Chronic infection in mice enhanced production of *As*-specific IgG, although in this study only a slight increase was observed.

Studies on parasites show a wide range of immune reactions. The immunomodulatory activity of parasites varies on the type of parasite, as well as on the portion or stage of the parasite. Different parasites induce different immune responses in experimental models. For instance, BALB/c mice exposed to *Schistosoma japonicum* eggs produced more IL-10 but little IL-4 (Yang et al. 2007). While mice chronically infected with *Schistosoma mansoni* have downregulated levels of IL-4, IL-10, IL-13 and IFN-γ (Smits and Yazdanbakhsh 2007). Moreover, in another study, infection with *Schistosoma haematobium* induced significant production of IL-5 and IL-10 (van den Biggelaar et al. 2000). In humans, parasites can exist in different developmental stages such as eggs, larvae or adult forms. The egg stage may still be differentiated further into different forms in some parasites. In the case of Ascaris, eggs may be fertilized or unfertilized and corticated or decorticated. Ascaris spp. have been widely used in research and different components have been used to induce infection. In one study, extracts of *Ascaris suum* from male and female worms with stored eggs were used to investigate the immunosuppressive properties of the parasite in DBA/2 mice (Souza et al. 2002). DBA/2 mice showed suppression of IL-2 and IFN-γ and enhanced production of IL-4 and IL-10. Another study used recombinant *Ascaris suum* 16-kilodalton protein to determine the capability of this preparation to render the BALB/c mice immune to Ascaris infection (Tsuji et al. 2001). Different components of *Ascaris suum* have been studied and a variety of immune responses has been observed depending on the component or part of the parasite used. In this study, the immunomodulatory activity of the infective egg stage of *Ascaris suum* in BALB/c mice was studied in terms of IL-4, IL-5 and IL-10, and production of antigen specific antibodies. IL-4 and IL-5 are cytokines produced by Th2 cells with pro-inflammatory functions and are important in IgE responses and eosinophilia, while IL-10 has anti-inflammatory functions and may inhibit Th1 cytokine production. Helminth infections are known to induce Th2 responses and enhance production of cytokines such as IL-4 and IL-5. They can also interact with the hosts' adaptive immune response and induce anti-inflammatory cytokines like IL-10 (van Riet et al. 2007). The contrasting

function of these three cytokines is a good subject of comparison in cross infection and immunomodulation studies.

Chronic infection protocol has been found to be effective in inducing interleukin production. Oral administration of approximately 25,700 eggs every three days for 36 days mimics chronic infection in humans (Schopf et al. 2005). IL-4 and IL-5 levels have been markedly elevated in infected mice on day 72 but not in normal mice. Increase in the levels of these cytokines indicates that BALB/c mice are capable of responding to the infectious agent and that an inflammatory process is occurring. The presence of neutrophilic infiltrates in the lungs of infected mice, as well as congestion, is also an indication of inflammation. The inflammatory process is a mechanism that is dependent on the production of IL-4 and IL-5, which have pro-inflammatory functions (Dourado et al. 2010). Chronic helminth infections are known to skew immune responses towards Th2. Th2 immune response is characterized by elevated levels of IL-4, IL-5, and IL-13 (van Riet et al. 2007). IL-13 was not measured in this study. IL-4 and IL-5 induce B-lymphocytes to switch to IgE antibody production.

IL-10 levels were significantly higher in the infected group than in the normal group. Several studies have revealed the protective role of IL-10 in the host's response against infection. *A. suum* infection in mice upregulates the production of IL-10. IL-10 production is part of the host's adaptive immune response in chronic-phase infection (van Riet et al. 2007). According to one study, IL-10 is a regulatory response that is induced to hinder potentially damaging inflammatory responses of the host against infection (Dold and Holland 2011b). It promotes tolerance and suppresses inflammatory responses such as production of IL-4 and IL-5. IL-10, as an anti-inflammatory cytokine, is produced to limit tissue damage that results from continuous administration of infectious agents (Yazdanbakhsh et al. 2001).

No significant increase in the relative level of IgE specific to *Ascaris suum* was detected in infected mice. IgE, according to studies, is related to protection rather than to exposure to infection and is usually produced in cases of acute infection (Gause et al. 2003; MacDonald et al. 2002; Weiss 2000). In cases of acute infection, IgE is produced and binds to the helminthic parasite, while eosinophil binds to the IgE. Eosinophil then degranulates against the parasite. Parasites are not phagocytized but rather are killed by the toxic granules of the eosinophil. This is how IgE and eosinophil act as the host's protective mechanism against parasites (Winter et al. 2000; Acevedo and Caraballo 2011). Production of parasite-specific IgE is indicative of the host immunity against the parasite and is associated with resistance to infection or re-infection (Acevedo and Caraballo 2011; Turner et al. 2005). In mice which had been chronically infected with *Ascaris suum*, the chronic infection induces a modified Th2 response characterized by the absence of IgE specific to parasite antigens and the production of IgG (Fitzsimmons and Dunne 2009). Low levels of parasite-specific IgE among Ascaris-infected subjects have also been reported in another study and this was attributed to helminth-released compounds, as well as cytokines, that control IgE secretion (Matera et al. 2008). In yet another study, it was reported that children continually infected with the parasite have low levels of anti-parasite IgE antibodies (Weiss 2000). As discussed above, production of IL-4, IL-5, and IL-10 is enhanced in infected mice as compared to normal mice. Production of these interleukins may have exerted an effect on the production of antibodies. IL-4 is a known cytokine that enhances antibody switching to IgE, while IL-10 enhances antibody switching to IgG (Chaplin 2010). Since both IL-4 and IL-10 were markedly elevated in infected mice, the effect of one cytokine counteracts the other, so that antibody switching is modulated. The presence of IL-10 can differentially regulate B-cells stimulated by IL-4 to produce IgG instead of IgE (Liu and Szeffler 2003; Sereda et al. 2008). Another possible reason for this is the ability of IgG to block Fc receptors of other antibodies such as IgE (Mekhaiel et al. 2011; Wilson and Maizels 2006). In this study, no significant change in the level of IgE was observed, but there was a slight increase in the level of IgG. IgG, specifically IgG2 and IgG4, can block other effective antibodies such as IgE and IgA (Mekhaiel et al. 2011). Moreover, a significant increase in the level of IL-10 and normal to elevated levels of IL-4 in response to parasitic infection, result in relatively little IgE and elevated IgG levels (Maizels 2005). Interestingly, a study conducted in the central province of Cameroon, where *Ascaris lumbricoides* is hyperendemic, showed that individuals infected with the parasite had measurable levels of IgG4 and only 30% of them had detectable IgE levels. Moreover, individuals with detectable levels of IgE, but not IgG4, had the lowest average infection, whereas those with significant levels of IgG4, but not IgE, had higher average levels of infection. Thus, IgG4 has been linked, and positively correlated, to infection level Turner et al. 2005).

We showed that the infective stage of *Ascaris suum* is capable of infecting BALB/c mice. The mice responded with upregulation of IL-4, IL-5 and IL-10, which have pro-inflammatory or anti-inflammatory functions. Chronic *Ascaris suum* infection increased *As*-specific IgG but not *As*-specific IgE. The modified effect of chronic *Ascaris suum* infection in BALB/c mice in terms of immunoglobulin production is attributed to the presence of both IL-4 and IL-5, and IL-10. These cytokines apparently modulated the antibody production in the mice. Since IgE antibody is also associated with allergy, chronic parasitism that inhibits production of IgE is a potential candidate regimen against allergy. The pattern of migration of *A. suum* includes passage to the small intestines, liver and lungs. Fecalysis and FECT results were negative and suggest that the parasite did not return to the small intestines to complete its typical migratory pattern. From these findings, extensive study on the immunomodulatory activity of *Ascaris suum* on different host/strain of animal models should be conducted. The potential helpful effects of parasite antigens in the prevention and/or protection against allergic disorders must also be evaluated. The migratory pattern of *Ascaris suum* and the immunomodulatory

activity of other parasites on animal models should also be explored.

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CONTRIBUTIONS OF INDIVIDUAL AUTHORS

MP and JR contributed equally to the research and have read and approved the final manuscript.

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