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Experimental culture of *Ascaris* species and histopathological effects on visceral organs of rats (*Rattus*) and mice (*Microtus*)

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ABSTRACT

Ascaris lumbricoides is a cosmopolitan, gastrointestinal, parasitic nematode of man. Ascaris suum, infects pig and is similar to Ascaris lumbricoides. Studies of A. suum provides vital information about the biology of other zoonotic, ascarid nematodes. Eggs of A. suum were obtained from gravid females and incubated at 24^{o}C to obtain infective stage which was administered to rats, (Rattus species) and mice (Microtus species) and their organs later examined. Lung tissues of rats showed severe haemorrhage and mild hyperplasia of peribrochiolar lymphoid tissues. Heart tissues of rats and mice showed cellular infiltration, with infiltrating cells which included lymphocytes. The heart tissues of rats, in addition to cellular infiltration, showed mild centrilobular fatty (vacuolar) degeneration of hepatocytes. No liver tissues of rats showed significant lesion. Intestinal tissues of rats showed cellular infiltration and hyperplasia at 42 days post-infection, lungs tissues of rats and mice showed cellular infiltration. There were cellular casts with mucous materials in the lumens of the intestines of rats and mice. The pathology associated with ascariasis is mainly due to the histological changes in the hosts' visceral tissues. These changes are believed to be caused by the immunological responses elicited by the host. These are usually allergenic, leading to disease syndromes.

Key Words: *Ascaris Lumbricoides*, Cosmopolitan, Gastrointestinal, Parasitic Nematodes

INTRODUCTION

Ascaris are the best known parasite of man, apes, cattle, pigs and sheep, all over the world. They are the roundworms of phylum Nematoda, order Ascaridida (Smyth, 1996). *Ascaris lumbricoides* parasitize man, cattle, apes, sheep while *A. suum* parasitize pigs. These two species were thought to be cospecific and until recently both were referred to as *A. lumbricoides* (Richard, 2001).It is estimated that Ascaris lumbriccoides (human ascaris) infect 1.5 billion people, mainly in tropical and subtropical areas (Chan, 1997). Ascaris are responsible for significant morbidity in humans and economic loss in animals (Crompton, 2001).

Ascaris infection is often asymptomatic and its effects may contribute substantially to child morbidity when associated with malnutrition, pneumonia, enteric diseases and vitamin A deficiency (Lancet 1992). Most symptoms observed include malaise, fever, headaches, nausea, diarrhea, dyspnea, pneumonitis and pneumonic conditions (Khuroo, 1990). In patients with heavy worm loads, the adult worms cause abdominal pain, small bowel obstruction, biliary, colic, gallstone formation, cholecystitis, pyogenic cholangitis, liver abscess and pancreatitis (Khuroo, *et al.,* 1990).

Ascaris infection is associated with migrating larvae, particularly in the lungs. If large numbers of these larvae migrate through the lungs simultaneously, this may give give rise to a severe haemorrhagic pneumonia. The haemorrhages may lead to breathing difficulties, pneumonia and /or fever. Another complication may arise as proteins of the parasites give highly allergenic reaction. The presence of the migrating larvae in the lungs is often associated with allergic hypersensitivity reactions such as asthmatic attacks, pulmonary infiltration, urticaria and oedema of the lips. Pathology is associated with the adult parasite which consists of rather generalized digestive

disorders such as a vague abdominal discomfort, nausea and colic (Cam, 1998).

Poor sanitation and use of night soil (human feaces) as fertilizer increased the prevalence of ascariasis in the community where such had been practiced (Fred Cox, 1999). Ascarids life cycle involves one host which becomes infected when it ingests ascarids egg (Fred Cox, 1999). The eggs are released to the surroundings by the hosts via faeces. Infection is spread by the feacal oral route (Fred Cox. 1999). Uninfected hosts usually man and pigs become infected when ascarids eggs are ingested if present in food or water. Some surveys have shown that in which helminth eggs, particularly those of *A. lumbricoides* were found in the following sites in addition to the soil and sewage, door handles, wash basins, furniture, dust (in house and public transport) fruit, vegetables, pickles, insects, fingers, chopping boards, boards, public baths, nasal discharge, underclothes, coins and school rooms. Each and all of these sites can lead to the swallowing of infective eggs and evidence show that the eggs may be inhaled and eventually swallowed as part of wind-borne dust particles (Crompton *et al.* 1989). A single host can harbour tremendous numerous of eggs daily. The female parasite lay an estimated 2 million eggs daily (Cam, 1998). Ascarid eggs are difficult to kill and remain viable in soil in the environment as bacteria (Fred Cox, 1999). Development is direct and there is no need of intermediate host. Eggs are passed out of the host animals through faeces and in suitable conditions, develop to coiled rhabditoid larvae, within 9-13 days, but do not hatch (except accidentally) until taken into the definitive host. Eggs can remain viable in the soil for up to six years in a cold dry atmosphere (Smyth, 1996). This is because the shell layers of the egg provide a remarkably resistant structure which can withstand many environmental factors and chemical agents. Good sanitary practices including careful hand-washing before eating, drinking of portable water, and careful handling of food can serve as preventive measures, minimizing infection if not completely eradicated.

The infection of Ascaris is established orally by infective third-stage larvae (L3) after the development from embryonated eggs (Grenen, *et al* 1999). The L3 invade the small intestine, where they develop into adult worms. Recent studies have revealed that *A. suum,* which is a parasite of pigs can infect and develop in humans, indicating its zoonotic importance (Anderson, *et al.,* 1993; Peng, *et al*, 1998). There is unequivocal evidence from experimental and accidental laboratory infections that *A.suum* infect man (Ly'sek, 1967) and *A. lumbricoides* can develop in pig. Nadler (1987) has discovered that A. Suum and A. lumbricoides are sibling species. *Ascaris suum*, was originally identified as a ubiquitous pathogenic parasite of pigs and has been biochemically and morphologically characterized (Soulsby, 1986). Studies of *A. suum* provide important information about the biology of other ascrid Nematodes that parasitize gastrointestinal tract and are widely distributed in both humans and animals worldwide. *Ascaris* wander through the liver, heart, lungs and intenstine of their hosts at various stages of development. They cause obstruction on these organs and the larvae of the worms burrow through the tissues of the organs, thereby damaging them. Consistent interference with the viscera of the hosts of these worms substantially affects the visceral cells and tissues of the hosts.

The objectives of this investigation are:

to culture and observe the development of *A.suum* from ova to larvae,

to determine the migratory behaviour of *A.suum* in experimental laboratory animals

to determine the organs affected by the migratory behaviour

to determine the histological states of the visceral tissues(lungs, heart, liver and intestine) of albino rats (*Rattus spp)* and albino mice (*Microtus spp)* infected with *A.suum.*

METHODOLOGY

Experimental animals were 12 rats (Rattus species) with 72g average weight and 12 mice (Microtus species) of 32g average weight. The animals were kept in different cages. Five females *Ascaris suum,* obtained from pigs were used.

Animal Treatment Before Experiment

Each of the rats and the mice were given lavimisole (Ketrax); a dewormer, orally to deworms in them, in case of any infection. The drug was administered using Pasteur pipette. They were kept in cages, each experimental animal separated form the other, and they were fed regularly with guinea feed cubes for four weeks before the commencement of the experiment in order to get the animals adjusted to the environment. Feacal samples of all the rats and mice were prepared and examined microscopically, to detect eggs of *Ascaris or* any other parasite.

Technique of incubating Ascaris Eggs

The adult females *Ascaris suum* which averagely measured 33cm were used. Each of the worms was carefully dissected longitudinally. The uterus of each worm was carefully removed and put inside a mortar. Each of the uteri was mercerated into a paste, using pestle and mortar. Water was added to the paste and it was stirred. The paste from each of the worms was spread over moist tissue paper and placed in separate petri-dishes. Wet preparations of the pastes from the separate Petri-dishes were prepared and examined microscopically to check for the presence and nature of the eggs obtained from each of the uteri of the worms. The Petri-dishes containing the pastes were covered and left to incubate at room temperature for a week. The incubating eggs were often moistened with water, to prevent dryness. The average temperature of the room was 24^{o}C. Starting from day 8, a wet preparation was made from the incubating eggs, to examine the developmental stage of the eggs. This

exercise was repeatedly carried out everyday until day 15 of the incubation period, when the infective stage of the eggs was observed. The eggs were recovered by transferring the incubated eggs into a bijou bottle, containing 1.00 mls of distilled water. The eggs were concentrated by the process of centrifugation. (*Ascaris suum* can infect man, and therefore aseptic precautions should be taken while handling the eggs.)

Infection of Mice

Mice with the average weight of 32g were used. The infective eggs of *Ascaris suum,* from the culture, were administered orally, to eight mice, using a micro titre pipette to obtain 250 ul. The mice were labeled and kept in separate cages. The total number of mice was 12. They were fed regularly with guinea feed cubes, and given water.

Infection of Rats

Similarly, 12 rats of average weight of 72g were used. The infective eggs of *Ascaris suum,* from the culture were given to eight rats orally using a micro titre pipette to obtain 500 ul. The remaining four rats were not given *Ascaris* eggs. The rats were kept in cages, each rat separated from another. The total numbers of the rats used were 12. The rats were fed regularly with guinea feed cubes and were labeled as shown in table 3.

Examination of Feacal Samples

After 14 days post infection, feacal sample from each animal, including uninfected ones (controls) was prepared and examined microscopically. Five infected rats and five infected mice were sacrificed. Also two uninfected rats and mice were sacrificed. All the animals were dissected and their visceral, lungs, hearts, livers and intestines were removed for histological analysis. The feacal samples of the other animals were examined on day 21, day 28, day 35 and day 42 post-infection (until when the feacal samples of some of the infected rats showed few *Ascaris* eggs). After this, the remaining animals, both infected and the controls were sacrificed and dissected. Their viscera, lungs, hearts, livers and intestines were removed for histological analysis.

Histological Analysis

The technique used was sectioning technique.

(i) Samples from the lung, heart,
liver and intestine, from each animal were obtained.

(ii) Fixation: The tissues wereseparately fixed in Bouin's fluid for 24 hours.

(iii) Post fixation: The tissues were separately washed in 70% alcohol until the yellow colour from the tissues was removed, and stored in 70% alcohol.

(iv) Dehydration: The tissues were passed through 90%, 98% and absolute alcohol and were allowed to stay in each grades of alcohol for 3 hours.

(v) Clearing: The tissues were cleared in xylene for 30 minutes.

(vi) Embedding: each tissue was placed in a container with equal volume of xylene (clearing agent) and molten paraffin wax in an oven for 1 hour.

The tissue was placed in puremolten paraffin wax for 1 hour. This process was repeated twice by transferring the tissue in different pure molten paraffin wax 1 hour each. The purpose of changing the paraffin wax several times is to make sure that the clearing agent is completely removed.

(vii) Blocking: The procedures used for blocking were as follows:

- A paper was labeled to be affixed to the block, and the label was dipped in molten wax.
- L- shaped metal was smeared and glassbase was used to hold the metal in position with glycerol..
- The metal was arranged in position on the glassbase to form a rectangle.
- Molten paraffin wax from the oven was removed using concepts which were used to fill the mould.
- Air bubble was removed from the mould by running hot forceps through the middle of the molten paraffin wax.
- The tissues were removed using warm forceps in the infiltrating paraffin wax from the oven and placed in the mould.
- Warm forceps were used to orientate the tissues in the desired positions for sectioning and air bubbles entrapped were carefully removed.
- Prepared labels were placed at one edge of the paraffin surface.
- Air was blown on the surface until a thin film was formed.

The mould and paraffin wax were quickly transferred into cold water and left for 15-20 minutes. The mould was therefore removed.

(vii) Trimming of block: the excess waxes were taken off the sides of the blocks leaving enough wax round the tissues about 2mm in the side and front and 4mm at side to face the chuck (posterior size). The front side faced the microtone knife and they were parallel to each other.

Attachment of trimmed Block to Chuck:

a. Chips of trimmed wax were placed on the chuck and the wax melted with a heated handle of scapel.

b. The posterior side of the block was placed on the warm scapel on the chuck. The scapel was quickly removed and the block was pressed down firmly keeping it vertical.

c. The edges of the chuck were sealed up by putting chipped wax on the edges and melting it with hot scapel handle.

d. The chuck was plunged with attached block in cold water to solidify.

(viii) Section**:** The chuck carrying the block was attached on the block carrier of a microtone. The

return wheel of the microtone was pulled back. A sharp knife was placed on the knife holder (carrier) and screwed on. The advanced wheel was moved forward in so much that the block was closed to the knife. The gauge was set, and section was cut by moving the advanced wheel in a clockwise direction. The ribbons of the sections were laid on a wooden platform.

(ix) Fixing sections to slide: Some slides were cleaned using acid alcohol. The surface of each slide was rubbed to receive the section with liberal glycerine albumen. Few drops of distilled water were added on the albuminized side of the slide. Single ribbon from the arranged section was cut, and the ribbon was laid on slide using moistened camel hair brush. The slide was placed on the hot plate to set at 40^{o}c until the wax was properly stretched. Excess water was drained off and the section was arranged in the center of the slide. The slide was allowed to dry.

Staining Proceedure

(x) The section was dewaxed in xylol for 5 minutes, hydrated through 100%-90% and 70% alcohol for 2 minutes each and the section was stained with Herlicks haematoxylin for 15 minutes, and thereafter, excess stain was washed off in water, differentiated in 70% acid alcohol, blue in tap water for 10 minutes, counterstained in alcoholic eosin for ½ -1 minute, dehydrated through 70% 90%, 100%, alcohol concentrations, each for 5 minutes, cleared in Xylol and mounted in Canada balsam.

Precautions Taken During the Course of the Experiment

(i) Tha apparatus used such as
scapel, scissors and pins were decontaminated after use.

(ii) The gloves worm during the experiment
were burnt

(iii) The worms were carefully handled to
prevent infection

(iv) The containers used for sedimentation
of the eggs, the Petri-dishes, the mortar and pestle were boiled for 30 minutes after use.

RESULTS

Before the commencement of the experiment, after deworming, there were no ova or larvae of any parasite in the faecal samples of any of the rats or mice. All the experimental animals appeared healthy. A wet film sample prepared from the incubated eggs, on the first day showed many *Ascaris eggs.* A week after incubation the pastes appeared brownish in colour. Wet preparations on day 8 showed *Ascaris* eggs which resemble those of initial film preparation except that the eggs appeared distinct and some brownish patches were seen inside the eggs. The incubated eggs appeared very distinct on day 21. Distinctly coiled larvae were seen within the egg shells.

Appearance of the Experimental Animals After Infection

All the rats and mice appeared normal after infection from the first day up to the 7^{th} day. By day 14 post-infection, the infected rats and mice became sluggish, with the coat becoming ruffled and weighed less. The average weight then recorded was 68g in rats and 30g in mice. By the 28^{th} day, the lower parts of the abdomen and the legs of the remaining infected animals (i.e Rats 6, 7 & 8) that had not been sacrificed at this time appeared swollen. All the controls animals appeared normal, active and were feeding well.

Feacal Samples

All the feacal samples examined from both infected rats and mice and the controls on the 14^{th} day showed no presence of eggs. Also no eggs were observed in the feacal samples of the remaining rats and mice (both infected and uninfected) on days 21, 28, and 35. However, on day 42, the feacal samples of Rat_6 and Rat_8 were observed to contain few small *Ascaris* eggs. The eggs were covered with fragile looking shells which appeared dark. After sacrificing and dissecting the remaining rats and mice on day 42, worms (very small, about 5cm in rats and 3cm in mice) were recovered from the intestines of rat 6,7 and 8, and in mouse 8. Worms were not found in the intestines of mice 6 and 7, and in any of the uninfected animals.

Histological Changes of the Visceral Tissues

Observations showed that the tissues of the viscera, mainly lung, liver, heart and intestine, from infected rats and mice, significantly differ histologically, form those of uninfected rats and mice (Table 2 and 3).

All the lung tissues of the experimental rats showed pathological reactions. The lung tissues of Rat_1 Rat_2, Rat_4, Rat_6 and Rat_8 (all infected) showed only severe mononuclear cellular infiltration in the intersitium. Other area of intersitium appeared normal. Lung tissues of Rat_3 and Rat_5 (infected) in addition, to mononuclear cellular infiltration in the intersitium, also showed hemorrhage. Uninfected rats' lung tissues showed only a moderate congestion of interstitial capillaries in the lung parenchyma. No significant lesion was observed in Rat_7 (also infected). All the lung tissues of mice 1, 2, 3, 4 and 5, which were examined 14^{th} day post-infections, showed mild congestion of interstitial capillaries, alveolar and interstitial heamorrahage. There was also mild hyperplasia of peribronchiolar lymphoid tissues. The lung tissues of $Mouse_6$ which was examined 42 days post-infection showed hyperplasia without haemorrhage. The lung tissues of uninfected mice showed only diffuse, moderate congestion of interstitial capillaries, without haemorrhage or hyperplasia (Fig. 2 and 3).

The heart of Rat_1 Rat_2 and Rat_3 (infected) showed myocardial necrosis as well as linear areas of necrosis with cellular infiltration. The infiltrating cells include few lymphocytes and fibroblast. The heart tissues of Rat_5 and Rat_7 only showed cellular infiltration (Fig.3) No significant lesion was observed in Rat_4, (infected)

and Rat_6 and Rat_8 (infected) that were examined on day 42 post-infection. The myocardial capillaries of uninfected rats appeared normal with only moderate congestion (Fig. 4). In the case of mice, only $Mouse_4$ and $Mouse_5$ (infected) heart tissues showed mononuclear cellular tissues of the uninfected rats. All the intestinal tissues of infected mice except $Mouse_3$ showed cellular infiltration in the mucosal epithelium. Cellular casts with mucous materials were also observed in the lumen of the intestines of mice 6,7 and 8. No significant lesion was observed in the intestinal epithelium. The intestinal tissues of the uninfected mice showed no significant lesion and they all appeared normal.

DISCUSSION AND CONCLUSION

Female Ascaris lay unembryonated eggs which are transformed into embryonated ones after further development outside the body of the host. During the development, there is transformation of the eggs in the eggs that contain L2 larvae inside, which usually appear as coiled bodies inside the eggs. These are the infective stage of the eggs (Cam, 1998; Smyth, (1996); Crompton and Pawlowski, 1985; and Crompton et.al.1989). The incubated eggs reached infective stage, 15 days of the incubation. Smyth (1996) reported that between temperatures of (22-33)^{o}C, unembryonated *Ascaris* eggs gave rise to rhabditoid L2 larvae within 9-13 days. It requires temperature less than 30^{o}C, moisture and oxygen for unembryonated eggs to develop unto this infective stage of the eggs.

On day 14 post-infection of the experimental animals, the animals became inactive, ruffled and weighed less as a result of shortage or diminish in the nutrients available to be utilized by the host cells due to the presence of *Ascaris* larvae among the various tissues in their body. This is because after the establishment of the worms in the experimental animals, they started competing with the hosts for the nutrients available in the hosts' systems. The worms fed on the nutrients of the hosts, thus this resulted in the decrease in the weight of the hosts as it had been observed on the day 14 post-infection. The inactivity and the ruff skin and hairs of the mice and rats must have been due to the reactions of host systems toward the pathology caused by the larvae after infection.

The changes in the histological states of the hosts' visceral tissues, were probably due to the interference of the *Ascaris* larvae ad the adult worm with these visceral tissues, and also the immunological response elicited by host immune system toward the presence of the worms.

The haemorrhage observed in the lung interstitial of both infected rats and mice were probably due to the *Ascaris* larvae migrating into these tissues. This is in agreement with the findings of Cam (1998) who reported that ascarids larvae migrated into the lungs where they caused severe haemorrhagic pneumonia. The haemorrhages were small but might lead to breathing difficulties, pneumonia and/or fever. Vince (1991) reported that migration of larvae through the lungs caused blood vessels of the lungs of haemorrahage. Hyperplasia is an abnormal increase in the number of cells in a tissue. The mild hyperplasia formed in the peribronchiolar lymphoid tissues of the infected mice was as a result of immunological response elicited by the host immune system toward the presence of *Ascaris* larvae in the site. The accumulated cells in the lung were mainly immune cells from the blood to fight against the infectious agents. They were not necessarily the cells of the lung itself. Cam (1998) reported that the parasite produced antigens that were allergenic and these could induce immunological responses that were hypersensitive in nature. The antigens associate with allergic hypersensistivity and oedema of the lips.

The severe mononuclear cellular infiltration in the intersitium in the lung, of the infected rats was probably due to immunological response elicited by the hosts. There was accumulation of cells in the tissues which caused obstruction on the tissues and this might lead to inflammation. These accumulated cells were mainly immune cells. The cellular infiltration was probably due to the reaction of host immune cells toward the *Ascaris* larvae that migrated unto the lung tissues. Solano et.al (2001) reported that migrating *Ascaris* larvae could cause pulmonary infiltration. The reason for observing some differences in the histological states in the lung tissues of infected rats and mice could be points of interest.

The necrosis observed in the heart tissues of the infected rats was probably due to the action of the migrating larvae on the tissues. The linear areas of necrosis observed wad the pathway of a larva moving along the heart tissues. Necrosis is the deadness of cells. The cellular infiltration observed in the heart tissues of the infected rat and mice due to immunological response induced by the host immune system against the infection. The infiltrating cells included lymphocytes and fibroblasts; good examples of immune cells from the blood tissues.

The centrilobular fatty (vacuolar) degeneration of hepatocytes observed in the liver tissues of the infected mice was probably due to *Ascaris* infection. The accumulation of lipids (occurring as globular materials) in the tissues could cause the liver cells (hepatocytes) not to function normally. The cells would not be able to metabolize easily. This might have been the reason why *Ascaris* infection could lead to liver abscess. Javaid *et al.,* (2001) reported that in patients with a heavy worm load, the adult worms could cause abdominal pain, small bowel obstruction, biliary colic, gallstone formation, cholecystitis, pyogenic cholangitis, liver abscess and pancreatitis. The reasons for liver tissues of all the infected rats examined not to show any significant lesions were not fully understood.

The severe hyperplasia of the submucosal lymphoid tissues and a mild infiltration of the mucosal epithelium by lympoctes were due to immunological response elicited by the host as a result of the presence

of *Ascaris* larvae which burrowed into the intestinal lining tissues. Smyth (1996) reported that after *Ascaris* eggs hatched into larvae in the intestine, the larvae burrowed into the mucosa and penetrated blood vessels to appeared as second-stage larvae.

The surface proteins of the worms are antigentic, this can induce immunological responses. The immune cells produced were mainly lymphocytes which are antibodies mediated immune response. The entrocytes (intenstinal cells lining) of infected rats and mice examined on day 42 post-infection produced mucous materials due to irritation caused by the adult worms to form cellular casts that were seen in the intestinal lumen of the rats and the mice. *Ascaris* infection causes abdominal pain (Khuroo, 1996). The abdominal pain must have been partly due to the obstruction inflicted by the adult worms on the intestinal tissues and partly due to immunological responses of the submucosal lymphoid tissues of the intestine toward the presence of the adult worms in the intestine.

The alteration in the histological states of the visceral tissues of the mice and rats infected with *Ascaris suum* showed evidences that the worms produced a lot of antigens in which some were allergenic. This confirms the report of Coles (1988), and cam (1998) that the proteins of the parasite were highly allergenic. The histological alterations of the tissues were mainly due to immunological responses elicited by the host immune system toward the antigens produced by the worms. Due to the observations in the controls, the histological alterations observed in the infected experimental animals were due to the presence of *Ascaris infection.*

Ascaris infections caused a lot of pathological reactions in the host animals. The larvae inflict a lot pathological injuries on the lungs, heart and liver and the intestine was mostly affected by the worms. The histological effects of the visceral tissues of the hosts, such as liver, lungs, heart and intestine were the main causes of pathology in ascariasis.

The transmission of the disease is mainly linked to unhygienic habits. Water and foods are easily contaminated by infected individuals through carelessness. The diseases, which can easily be controlled or eradicated through environmental modifications and health education, still remains as a prevalent disease in African countries, including Nigeria. If the pathology caused by *Ascaris* infection is considered, which was a result of histological damaging of the visceral tissues, the role of *Ascaris* in the health cycle should be emphasized.

Table 1: Labeling of the Experimental Animals

Infected Rats	Uninfected Rats	Infected Mice	Uninfected Mice
Rat_1	Rat c_1	$Mouse_1$	Mouse c_1
Rat_2	Rat c_2	$Mouse_2$	Mouse c_2
Rat_3		$Mouse_3$	
Rat_4		$Mouse_4$	
Rat_5		$Mouse_5$	
Rat_6	Rat c_3	$Mouse_6$	Mouse c_3
Rat_7	Rat c_4	$Mouse_7$	Mouse c_4
Rat_8		$Mouse_8$	

Rats (1-5), Rats (C1 & C2), Mice (1-5), and Mice (C1 & C2) were sacrificed on day 14 post-infection. Rat (6-8), Rat (C3 & C4), Mice (6-8) and Mice (C3 & C4) were sacrificed on day 42 post infection.

Table 2: Histological results of visceral tissues from rats and mice, 14th day post-Infection

Animals	Lung Tissues	Heart Tissues	Liver Tissues	Intestinal Tissues
Rat1	+	+	-	-
Rat2	+	+	-	+
Rat3	+	+	-	+
Rat4	+	-	-	+
Rat5	+	-	-	+
Rat_{c1}	-	-	-	-
Rat_{c2}	-	-	-	-
$Mouse_1$	+	-	-	-
$Mouse_2$	+	-	+	+
$Mouse_3$	+	-	-	-
$Mouse_4$	+	+	+	+
$Mouse_5$	+	+	+	+
$Mouse_{c1}$	-	-	-	-
$Mouse_{c2}$	-	-	-	-

Pathological reactions (+); Non pathological reactions (-)

Table 3: Histological results of visceral tissues from rats and mice, 42nd day post-Infection

Animals	Lung Tissues	Heart Tissues	Liver Tissues	Intestinal Tissues
Rat_6	+	-	-	+
Rat_7	-	+	-	+
Rat_8	+	-	-	+
Rat_{c3}	-	-	-	-
Rat_{c4}	-	-	-	-
$Mouse_6$	+	-	+	+
$Mouse_7$	+	-	+	+
$Mouse_8$	+	-	+	+
$Mouse_{c3}$	-	-	-	-
$Mouse_{c4}$	-	-	-	-

Pathological reactions (+); No pathological reactions (-)

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