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Study of *Heligmosomoides polygyrus* larval infection in mice on various haematological parameters

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Abstract
The gastro-intestinal (GI) nematode *Heligmosomoides polygyrus* is an important experimental model in laboratory mice and a well-studied parasite of wood mice in the field.
Recent advances in basic immunology and biotechnology with the concomitant development of well-defined laboratory models of infection, immunoparasitologists, have more precisely analyzed and defined the different immune effector mechanisms during the infection resulting in great improvement in our current knowledge and understanding of protective immunity against gastrointestinal (GI) nematode parasites. Development of vaccines against gastrointestinal nematodes is essential to control the parasite infection. As of yet, no vaccine has been made commercially available to producers.
The present study deals with the role of eosinophils, lymphocytes and basophils in modulation of immune response during primary infection in control mice with *H. polygyrus* at different stages of infection, and their role in imparting protective immunity in vaccinated mice during challenge infections.

Keywords: *H. polygyrus,* eosinophils, vaccine

Introduction
Eosinophilia is caused by the effect of IL-5 synthesized from the Th2 cells. IL-5 is the most important cytokine in the transformation and development of eosinophils, and acts as an "eosinophil activator". One of the significant causes of the increase in the amount of eosinophils in blood is parasitic diseases. Toxiallergic effects of certain parasites on the host's organism lead to an increase especially in eosinophil numbers. Eosinophils are effectors against parasitic targets. Both sets of diseases are associated with a polarized Th2-type immune response, typified by reactive cell types (eosinophils and mast cells) (Yazdanbakhsh, *et al.,* 2001; Capron, *et al.,* 2002) [8, 2].
It is suggested therefore that the function of IL-5 and eosinophils is to protect against repeated exposure to gastrointestinal parasites. On the other hand the eosinophilia observed may represent an immunopathological rather than a protective response and may merely be a consequence of the generalized inflammation induced by the Th2 response following infection with parasites. Th2 response is essential for the expulsion of GI helminthes (Lawrence, 2003) [5].
Eosinophils are also responsible for considerable pathology in mammals because they are inevitably present in large numbers in inflammatory lesions associated with helminth infections or allergic conditions (Behm, *et al.,* 2000; Klion, *et al.,* 2004) [1, 4].

Materials and Methods
Experimental Animal
The Swiss albino mouse, *Mus musculus albinus* of either sex were selected as an experimental animal for the present investigation. The mice were obtained from the Institute of Nutrition (NIN) Hyderabad, India and were kept in the animal house under local conditions of light, temperature ventilation and food. Food and water were provided *ad libitum*. Male and female healthy mice of 6-8 weeks old and 15-20 gms in weight were used according to the need of the experimental design. Animal experimentations were conducted according to INSA ethical guidelines for the use of animals for scientific research purpose, after permission from the ethical committee.

The Parasite: *Heligmosomoides polygyrus*
Heligmosomoides polygyrus is a mouse intestinal nematode that establishes a chronic infection in the deodenum. It is a common nematode found in the duodenum and small intestine of woodmice and other rodents. They are 5-20 mm in length and bright red due to the pigmentation of their tissues. They are usually heavily coiled, with the female having 12-15 coils and the male 8-12. The male can be distinguished from the female by a prominent copulatory bursa and two long, thin spicules at the posterior end.

Experimental protocol
The mice were divided into following six groups –
1. Non Infected Non Vaccinated Control – 1
This group was utilized for collection of the blood to find out the differential counts of non-infected non-vaccinated mice (NINVC 1).
2. Infected Non –Vaccinated Control –2
300 viable infective L3 larvae were orally inoculated directly into stomach
of mice by 01 ml syringe having a blunt rubber cathedral. On day 1 and day 5 post infection (pi) the mice were sacrificed for larval recovery, at the same time collection of blood and separation of serum for the assessment of other test parameters was also done. On day 13 (pi), the next batch of mice were sacrificed and adult worms were counted. Collection of blood and separation of serum for assessment of other test parameters was also done. Intestine was removed for histology. This group of mice is also called as (INVC-2).
3. Infected Vaccinated with Larval Somatic Antigens
Mice were vaccinated on day 0 with L-3 larval somatic antigen containing 20,40,60,80,100 µg protein + FCA. At the same time control mice were given culture medium +

FCA. On day 5, again a booster dose of L-3 larval antigen containing the same amount of protein without FCA was given to experimental mice. The control mice were given only culture medium. On day 26 the mice were challenged with 300 L-3 larvae. On day 27 and 31, mice were sacrificed for larval recovery and assessment of the test parameters. On day 39 adult worms were recovered. Collection of blood and separation of serum for assessment of other test parameters were done. This group is also called as (IVLSAg).

4. Infected Vaccinated with Adult Somatic Antigens

First dose of homogenate, whose protein contents were preassessed containing 20,40,60,80,100 µg protein of mature worms with FCA was administered on day 0 to experimental mice (IVASAg). Culture medium + FCA was given to control mice. Again on day 5, a booster dose of vaccine in five grades without FCA was administered to experimental mice and only culture medium to control mice. On day 26 the mice were challenged with 300 L3 larvae.

On day 27 and 31 (pi), mice were sacrificed for larval recovery, collection of blood and separation of serum for assessment of other test parameters. On day 39 adult worms were recovered, collection of blood and separation of serum was done for assessment of other test parameters.

5. Infected Vaccinated with Larval ES Antigens

Different concentrations of ES antigens from L-3 larvae stage + FCA were administered on day 0. On day 5, booster dose without FCA was vaccinated. On day 26, each mice was challenged with 300 viable L-3 larvae. Mice were sacrificed on 27 and 31 day for the recovery of L-3 larvae and collection of blood and separation of serum for assessment of other test parameters. On day 39 adult worms were recovered. Collection of blood and separation of serum was done for assessment of other test parameters. On day 39 adult worms were recovered and test parameters were assessed. This group is called as (IVLESAg).

6. Infected Vaccinated with Adult ES Antigens

Different concentration of ES antigens from adult worm stage + FCA were administrated on day 0. On day 5, booster dose without FCA was vaccinated. On 26 day each group of mice was challenged with 300 viable L-3 larvae. Mice were sacrificed on day 27 and 31, for the recovery of L-3 larvae. Collection of blood and separation of serum was done for assessment of other test parameters. This group of mice is also called as (IVAESAg).

Maintenance of the strain of *H. polygyrus* in mice

Heligmosomoides polygyrus strain was originally obtained from the Experimental Parasitology unit, Department of Zoology, S.V. Veterinary University, Tirupati. *H. polygyrus* was maintained in the Helminthology–Immunology laboratory by infecting fresh batches of 25 young mice with 300 larvae/mice after every three months. Third stage infective filariform larvae were obtained by the petridish method of Van Zandit (1961) [7]. Faecal pallets from infected mice were collected on a damp filter paper. Two pieces of Whatmann filter paper No. 40 were placed in sterilized culture dishes with the faecal pallets placed in the centre and covered with thoroughly washed activated charcoal. The mixture was kept moist by 0.5% saline, aerated during the entire culture period daily for 30 minutes and incubated at 21-300C for about 6 days by the end of which larvae were flushed out into a jar containing distilled water. They were allowed to settle for sometime, the supernatant was discarded and the larvae re-suspended in distilled water and stored in cold.

Preparation of inoculums for Infection

A larval suspension of about 100 ml was prepared in a glass stoppered measuring cylinder of 100 ml capacity. The numbers of actively motile larvae were counted by the dilution method of Scott (1928) [6]. After vigorous shaking, 1 ml of the suspension was pipette out, transferred onto several glass slides with squares already made on their reverse with a glass marking pencil and the larvae in all squares were carefully counted under a suitable dissecting microscope. Three such counts were repeated and the average count in 1 ml was multiplied by the total volume to get the total number of the larvae. An inoculum containing the desired number of actively motile larvae was adjusted in 0.2-0.3 ml to be given to each mouse. Each mouse was orally inoculated directly into stomach with the desired number of larvae (300) by 1 ml syringe having a blunt 18 gauge-feeding needle. After inoculation, mice were kept in cages in groups of five and labeled according to the design of experiments and were fed routinely with the same standard diet.

Haematological Studies

Total leucocyte count was measured by using Neubauer's Chamber (Dacie and Lewis, 1982) [3].

Observations and Results

Heligmosomoides polygyrus is a natural intestinal helminth of mice. It inhibits in the duodenal region of small intestine. In the present investigation, albino mice were colonized with 300 larvae each time by placing larvae directly in the stomach through gastric lavage. The larvae migrated to duodenum, to house in the submucosa where they matured and then emerged as adult worms migrating in the intestinal lumen by 13 days of time.

The present study was carried out to investigate the immunological responses and possibility of vaccine development.

Haematological studies

Effect of *Heligmosomoides polygyrus* larval (300 larvae dose) infection in mice on various haematological parameters was studied after 1, 5 and 13 days of infection and after different vaccinations and the results are shown in graphs No. 1-4.

1. Differential Leucocyte Count (DLC) from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of L-3 larval somatic antigens

Total leucocyte count was significantly increased after infection upto 5 days and then slight decrease in leucocyte increase was observed after 13 days of infection. After 13 days of infection, the rise in leucocyte count was 222.18%. However, in vaccinated (IVLSA) mice the increase in total leucocyte count was just 203.39%. At all the durations, the increase in total leucocyte count in vaccinated mice was slightly less than those in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited more rise in total leucocyte count

(95.43%) than that in non-vaccinated infected mice (58.42%).

Neutrophil count was found tobe highly increased after 5 days of infection and there after the increase in neutrophil count was found tobe declined when counted after 13 days. The increase in neutrophil count was 480.63% after 5 days of infection and after 13 days it was 299.67%.

However, in vaccinated (IVLSA) mice after 13 days of vaccination, the increase in number of neutrophil was just 58.02%. At all the durations, the increase in neutrophil count in vaccinated mice was very less than those in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in neutrophil count (26.42%) than that in non-vaccinated mice (60.70%).

Lymphocyte count was significantly increased after 5 days of infection and then slight decrease in lymphocyte increase was observed after 13 days of infection.

After 13 days of infection, the lymphocyte count was 203.77%. However, in vaccinated (IVLSA) mice the increase in lymphocyte count was 262.26%. At all the durations the increase in lymphocyte count in vaccinated mice was slightly more than those in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited more rise in lymphocyte count (128.69%) than that in non-vaccinated infected mice (53.96%).

Monocyte count was significantly increased after infection upto 5 days and then decrease in increase rate of increase of monocytes was observed after 13 days of infection.

After 13 days of infection, the monocyte count was 296.83%. However,in vaccinated (IVLSA) mice the increase in monocyte count was 200.00%. At all the durations the increase in monocyte count in vaccinated mice was slightly less than that in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited more increase in monocytes (98.00%) than that in non-vaccinated infected mice (90.48%).

Number of basophils was significantly increased after *H. polygyrus* infection. After 13 days of infection the rise in number was 820.00%. However, in vaccinated (IVLSA) mice the increase in basophil count was 350.00%. After 1, 5 and 13 days of infection and vaccination the increase in basophil number in vaccinated mice was slightly less than that in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited fewer rise in total basophil (34.00%) than that in non-vaccinated infected mice (60.00%). Number of eosinophils was significantly increased after *H. polygyrus* infection. After 13 days of infection, the rise in number was 1702.00%. Correspondingly, in vaccinated (IVLSA) mice the increase in eosinophil count was 1038.00%. At all the durations the increase in eosinophil number in vaccinated mice was less than those in non-vaccinted infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in total eosinophils (114.00%) than that in non-vaccinated infected mice (400.00%).

2. Differential Leucocyte Count (DLC) from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of adult somatic antigens:

Total leucocyte count was significantly increased after infection upto 5 days and then slight decrease was observed in increased number of leucocyte after 13 days of infection. After 13 days of infection, the rise was 247.92%. However, in vaccinated (IVASA) mice the increase in total leucocyte count was only 199.23%. At all the durations, the increase in total leucocyte count in vaccinated mice was slightly less than those in non-vaccinated mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited more rise in total leucocyte count 79.93%) than that in non-vaccinated infected mice (58.42%).

Neutrophil count was found tobe highly increased after 5 days of infection and there after the increase in neutrophil count was found tobe declined when counted after 13 days. The increase in neutrophil count was 480.96% after 5 days of infection and after 13 days it was 299.68%.

Whereas, in vaccinated (IVASA) mice after 13 days of vaccination,the increase in neutrophil was 82.33%. At all the durations, the increase in netrophil count in vaccinated mice was very less than those in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in nutrophil count (29.66%) than that in non- vaccinated mice (60.70%).

Lymphocyte count was significantly increased after 5 days of infection and then slight decrease in lymphocytes increase was observed after 13 days of infection.

After 13 days of infection, the lymphocyte count was 204.53%. However, in vaccinated (IVASA) mice the increase in lymphocyte count was 245.28%. At all the time of infection the increase in lymphocyte count in vaccinated mice was slightly more than those in non-vaccinated infected mice (INVC-2). However, after one of infection, the vaccinated mice exhibited more rise in lymphocyte count (103.77%) than that in non-vaccinated infected mice (53.96%).

Monocyte count was significantly increased after infection upto 5 days and then decrease in increased number of monocyte was found after 13 days of infection.

After 13 days of infection, the monocyte count was 301.59%. However, in vaccinated (IVASA) mice the increase in monocyte count was 130.16%. At all the durations the increase in monocyte count in vaccinated mice was slightly less than that in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in monocyte (53.97%) than that in non-vaccinated infected mice (90.48%).

Basophil count was significantly increased from 1, 5 upto 13 days of infection. After 13 days of infection the rise in number was 820.00%. However, in vaccinated (IVASA) mice, the increase in basophil count was 372.00%. After 1, 5 and 13 days of infection and vaccination the increase in basophil number was slightly less than that in infected non-vaccinated mice (INVC-2). However, after one day of infection the vaccinated mice exhibited little decrease in total basophil count (44.00%) than that in non-vaccinated infected mice (60.00%).

Significant increase was observed in eosiniphil number from day 1 to 13 days of infection. After 13 days of infection, the rise in eosinophil number was 1702.00%. Likewise, in vaccinated (IVASA) mice increase eosinophil count was 1044.00%. At all the durations the increase in eosinophil number in vaccinated mice was less than that of non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in total eosinophils (136.00%) than that in infected non-vaccinated mice (400.00%).

3. Differential Leucocyte Count (DLC) from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of L-3 larval ES antigens:

Total leucocyte count was significantly increased after infection upto 5 days and then slight decrease in rate of leucocyte increase was observed after 13 days of infection. After 13 days of infection, the rise in leucocyte count was 247.92%. However, in vaccinated (IVLESA) mice increase in total leucocyte count was 214.93%. At all the durations, the increase in total leucocyte count in vaccinated mice was slightly less than that of non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited more rise in total leucocyte count (115.86%) than that in non-vaccinated infected mice (58.42%).

Significant increase number of neutrophil count was found after 5 days of infection and then increase in neutrophil count was found tobe declined when counted after 13 days of infection. The increase in neutrophil count was 480.96% after 5 days of infection and after 13 days it was 299.67%.

However, in vaccinated (IVLESA) mice after 13 days of vaccination, the increase in neutrophil count was 19.61%. At all the durations, the increase in neutrophil count in vaccinated mice was very much less than that in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in neutrophil count (13.74%) than that in non-vaccinted mice (60.70%).

Lymphosite count was significantly increase upto 5 days of infection and then slight decrease in increased number was found after 13 days of infection.

After 13 days of infection, the lymphocyte count was 204.53%. However, in vaccinated (IVLSA) mice the increase in lymphocyte count was 300.68%. At all the durations the increase in lymphocyte count in vaccinated mice was slightly more than those in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited enormous increase in lymphocyte count (166.11%) than that in non-vaccinated infected mice (53.96%). Monocyte count was significantly increased after 5 days of infection and then decrease in increase number of monocyte count was observed after 13 days of infection.

After 13 days of infection, the monocyte count was 301.59% and in that of vaccinated (IVLESA) mice increase in monocyte was 14.29%. At all the durations the increase in monocyte count in vaccinated mice was slightly less than that in non-vaccinated infected mice (INVC-2). However, after one day of *H. polygyrus* infection, the vaccinated mice exhibited less rise in monocyte (20.63%) than that in non-vaccinated infected mice (90.48%).

Significant increase was found in the number of basophils after infection. After 13 days of infection the rise in number was 820.00%. However, in vaccinated (IVLESA) mice the increase in basophil count was 314.00%.

After 1, 5 and 13 days of infection and vaccination the increase in basophil number in vaccinated mice was slightly less than that of non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited very less rise in total basophil count (16.00%) than that in non-vaccinated infected mice (60.00%).

Number of eosinophils was significantly increased after 1, 5 and 13 days of infection. After 13 days of infection, the increase in number of eosinophil was 1702.00%. Correspondingly, in vaccinated (IVLESA) mice the increase in number was 964.00%. After 1, 5 and 13 days of infection, the increase in eosinophil number in vaccinated mice was less than those in infected non-vaccinated mice (INVC-2). However, the vaccinated mice exhibited less rise in total eosinophils (80.00%) than that in non-vaccinated infected mice 400.00% after one day of infection [Table – 4.7, Fig. 4.7.1-4.7.7].

4. Differential Leucocyte Count (DLC) from infected mice (with 300 larvae of *H. polygyrus*) after 1, 5 and 13th day of administration of adult ES antigens

Total leucocyte count was significantly increased after infection upto 5 days and then slight decrease in number was observed after 13 days of infection. After 13 days of infection, the rise in leucocyte count was 247.92%, and the increase number of leucocyte in vaccinated (IVAESA) mice was just 211.51%.

After 1, 5 and 13 days of infection, the increase in total leucocyte count in vaccinated mice was slightly less than that in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in total leucocyte count (112.24%) than that in infected non – vaccinated mice (58.42%).

Neutrophil count was found tobe highly increased after 5 days of infection and there after the increase in neutrophil count was found tobe declined when measured after 13 days. The increase in neutrophil count after 5 and 13 days of infection was 480.96% and 299.68% respectively. However, in vaccinated (IVAESA) mice after 13 days of vaccination the increase in number of neutrophil was just 32.09%. At all the durations, the increase in neutrophil count in vaccinated mice was very much less than that of non-vaccinated infected mice (INVC-2). Less increase in neutrophil count (19.12%) was observed when counted after one day of infection than that in non-vaccinated mice (60.70%).

Lymphocyte count was significantly increased after 5 days of infection and then slight decrease in increased number of lymphocyte was observed after 13 days of infection.

After 13 days of infection, the lymphocyte count was 204.53%. However, in vaccinated (IVAESA) mice the increase in lymphocyte count was 287.51%. After, 1, 5 and 13 days of infection the increase in lymphocyte count in vaccinated mice was slightly more than those in non – vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited more rise in lymphocyte count (157.58%) than that in infected non-vaccinated mice (53.96%).

Monocyte count was significantly increased after 1, 5 and 13 days of infection and decreased monocyte count was observed after 13 days of infection.

After 13 days of infection, the monocyte count was 301.59%. However, in vaccinated (IVAESA) mice the increased number of monocyte was 87.30%. At all the durations the increase in monocyte count in vaccinated mice was slightly less than that in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in monocyte (52.38%) than that in non-vaccinated infected mice (90.48%).

Number of basophil was significantly increased after 1, 5 and 13 days of *H. polygyrus* infection. After 13 days of infection the increase in number of basophil was 820.00%. However, in vaccinated (IVAESA) mice the increase in

basophil count was 434.00%. At all the durations the increase in basophil number in vaccinated mice was less than that in non-vaccinated mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited less rise in total basophils (22.00%) than that in infected non-vaccinated mice (60.00%).

Number of eosinophil was significantly increased after 1, 5 and 13 days of infection. After 13 days of infection, the rise in number of eosinophil was 1702.00%. Correspondingly, in vaccinated (IVAESA) mice the increase in eosinophil count was 990.00%. At all the durations the increase in eosinophil number in vaccinated mice was less than that in non-vaccinated infected mice (INVC-2). However, after one day of infection, the vaccinated mice exhibited very less rise in total eosinophils (98.00%) than that in infected non-vaccinated mice (400.00%).

Summary and Conclusion

Heligmosomoides polygyrus is a natural intestinal helminth of mice. It inhibits in the duodenal region of small intestine. In the present investigation, albino mice were colonized with 300 larvae each time by placing larvae directly in the stomach through gastric lavage. The larvae migrated to duodenum, to house in the submucosa where they matured and then emerged as adult worms, migrating in the intestinal lumen by 13 days of time. The present study was carried out to investigate the immunological responses and possibility of vaccine development.

In the present investigation the total leucocyte count in control mice was 7938 cells/cmm and in infected mice the total WBC count was 37302 cells/cmm i.e. 4.7 times increase after 5 days of infection and 27575 cells/cmm i.e. 3.5 times increase after 13 days of infection.

Blood eosinophilia is typically seen during *H. polygyrus* infection. The increase in eosinophil count was 1702% after 13 days of infection, however, after 13 days of vaccination by larval somatic antigens, adult somatic antigens, larval excretory- secretory antigens and adult excretory- secretory antigens separately, the eosinophil count was declined to 1038%, 1044%, 964% and 990% respectively indicating that larval excretory- secretory antigens are more potent than rest of the three others. These findings also indicate that eosinophils are antiparasite effector cells.

Neutrophil number was increased in all the infected mice by 480.63% and 299.67% after 5 and 13 days of infection respectively indicating their recruitment to sites of infection during tissue invasion by the *H. polygyrus*. The early neutrophilia observed during present investigation was probably associated with the damage caused by developing larvae in the intestinal mucosa. That is why number of neutrophils were more after 1st and 5th days of infection and it decreased gradually after 13th days of infection.

In the present investigation, basophil count was also increased in all the parasitized mice after 1, 5 and 13 days of infection.

Immediately after one day of parasitization and challenge infection, the total leucocyte count further increased to about two times and this rise was because of increase in lymphocyte number indicating initial recruitment of other blood cells at the site of infection and at the same time lymphocytes start differentiating and proliferating in the blood and most probably in lymph nodes – a preparatory stage during immune response. The recruitment of eosinophils at the site of infection is probably the 1st, as eosinophils are multifunctional cells.

Eosinophils normally account for only 0.5 to 2 percent of peripheral total blood leucocytes. In the present investigation the eosinophils are 3.26% of the total leucocyte count after 13 days of infection. These results clearly indicate eosinophilia.

Thus, the results of the present investigation and the earlier work of parasitologist and immunologists clearly indicate that infection of *H. polygyrus* results into eosinophilia which inturn activates the immune responses through T and B cells probable initiating a cascade of diverse biochemical reactions in the host- for protection. These protective responses can be initiated by administrating the Excretory-Secretory and somatic antigens of the larval and adult stages of the parasite. In the present investigation, the larval Excretory-Secretory antigens are proved to produce better protection to the host and hence it is inferred that larval ES antigens can be used as potential vaccine against the helminth infection.

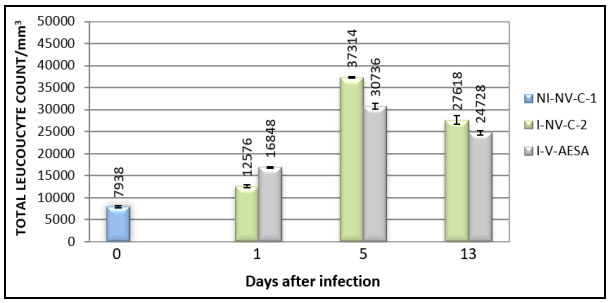

Fig 1: Total Leucocyte Count (TLC) from infected mice after 1, 5 and 13 days of administration of adult ES antigens

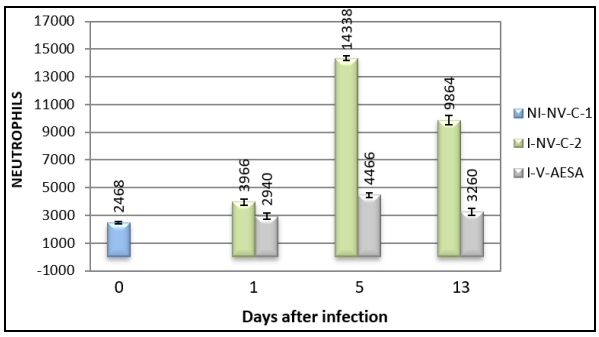

Fig 2: Alterations in Neutrophil count from infected mice after 1, 5 and 13 days of administration of adult ES antigens

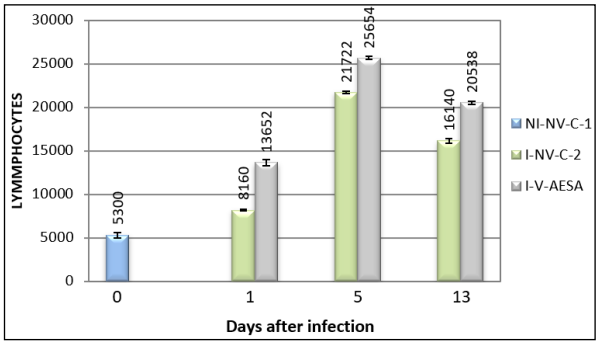

Fig 3: Alterations in Lymphocyte count from infected mice = after 1, 5 and 13 day s of administration of adult ES antigens

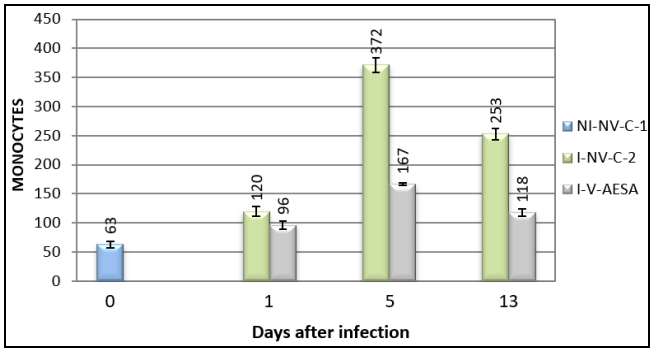

Fig 4: Alterations in Monocyte count from infected mice after 1, 5 and 13 days of administration of adult ES antigens

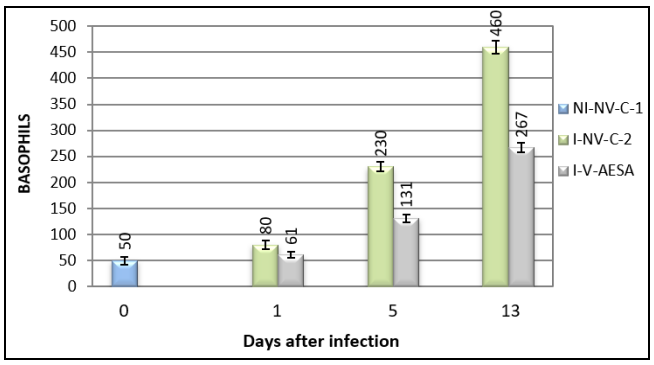

Fig 5: Alterations in Basophil count from infected mice after 1, 5 and 13 days of administration of adult ES antigens

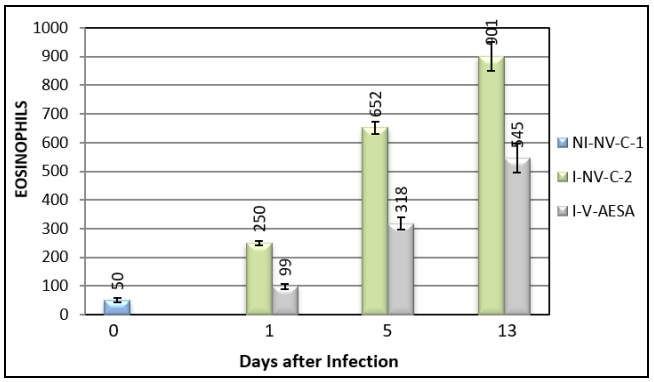

Fig 6: Alteration in Eosinophils count from infected mice after 1, 5 and 13 -days of administration of adult ES antigens

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