Experimental Ascaridiasis Induced Changes in Haematological Parameters in WLH Chicks

Charu Tyagi*, Anju Panwar and Yougesh Kumar

Zoology Department, DAV College, Muzaffarnagar
*Corresponding author: charu2052@gmail.com

ABSTRACT

The Species Ascaridia galli is a common and serious helminth parasite of poultry. Present study was conducted to determine its effects on the poultry. The impact of experimental ascaridiasis was studied on WLH chicks in terms of blood parameters. The result obtained is analyzed statistically and are presented here.

Keywords: Ascaridia galli, WLH chicks, TLC, Lymphocytes, PCV, MCV.

INTRODUCTION

Helminthes are common gastrointestinal parasites of commercial poultry. They interfere with the host metabolism in more than one way. It leads to highest degree of pathogenicity. Sazikova (1975) found the weight gain in infected chicks was much lower than that of uninfected one, with reduced egg laying capacity and more over the eggs were not fully developed. Ascaridiasis leads to malnutrition in chicks, which results in decreased return of products derived from the poultry (WHO, 1967). A.galli infection also alters nutrition, utilization & absorption negatively resulting in suppressed growth rate. This study is part of doctoral work of first author. The objective of this study was to determine the impact of experimental ascaridiasis on haematological parameters.

MATERIALS & METHODS

For haematological studies 78 WLH chicks were divided into three groups. Chicks of first group considered as control were not inoculated with any embryonated eggs of A.galli. Each chick from second group was challenged with 25 embryonated eggs of A.galli & those from third group were inoculated with 500 embryonated eggs of A.galli each.

The control group were sacrificed on day zero of infection. Six birds of second & third group each were sacrificed after 7,14,21,28,35 & 42 days of infection for collection of blood. The blood collected was used for different haematological studies

Cellular haematological investigations in the blood of control and infected groups of chicks were carried out according to the method described by Archer and Jeffcott (1977), Wintrobe (1976), Dennington and Lucas (1955) and Oser (1976).

Total Leucoyte counts- using brilliant cresyl blue stain (Dennington and Lucas, 1955).

Differential Leucocyte counts – Using Leishman stain (Archer and Jeffcot, 1977)

Packed cell volume- Standard technique (Archer & Jeffcott 1977)

Erythrocyte counts- Dilution and Neubauer’s chamber technique (Wintrobe, 1976.)

Haemoglobin Concentration- Using Sahli’s haemoglobinimeter (Oser, 1976).

The other three standard ratios (MCH, MCV and MCHC) from the obtained data were calculated according to the following formulae (Wintrobe, 1976).

1. Mean corpuscular volume (MCV) in femtoliters

   \(\text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC count}} \times 10\)

2. Mean corpuscular hemoglobin (MCH) in pico
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grams(pg) = \( \frac{\text{Haemoglobin}}{\text{RBC count}} \times 10 \)

3. Mean corpuscular hemoglobin (MCHC) in percentage = \( \frac{\text{Haemoglobin}}{\text{PCV}} \times 100 \)

RESULT

Significant alterations were observed in haematological parameters of WLH chicks as compared to control during experimental ascaridiasis.

Erythrocyte counts

The RBC count revealed a noteworthy fall in second group of chicks infected with (25 embryonated eggs). Statistically highly significant (P<0.001) fall was found in erythrocyte counts between control and infected groups of birds during 7th, 14th, 21st, 28th, 35th & 42nd day post infection. An overall fall from 4.21 million/mm\(^3\) to 2.88 million/mm\(^3\) was observed during the investigation. (table-1)

A significant fall (P<0.01) was observed during second week of infection when treated with 500 embryonated eggs of A.galli and further, a significant fall (P<0.10) was observed during first & third week, (P<0.02) during fourths and fifth week of experiment, and over all decline was recorded from 4.21 million/mm\(^3\) to 2.68 million/mm\(^3\) in RBC counts during the experiment (table-1)

Haemoglobin concentration

After 25 embryonated eggs inoculation a significant (P<0.02) fall in haemoglobin concentration was recorded during first week, statistically highly significant (P<0.01) fall was noticed between infected and control groups during second week of infection and subsequent weeks. An overall decline from 11.36 gm/dl to 9.38 gm/dl was recorded during the experiment. (table-1)

The chicks inoculated with 500 embryonated eggs, revealed, a noteworthy fall during the experiment in hemoglobin concentration. Statistical analysis revealed highly significant (P<0.01) fall at fifth and sixth week of experiment. An overall deviation was found from 11.36 gm/dl to 7.06 gm/dl was revealed during the experiment (Table-3).

Packed cell volume (PCV)

The PCV also decreased during both experiments. Highly significant fall (P<0.01) was observed during all days of experiment, and an overall fall from 40.56 percent to 27.89 percent was recorded in PCV in the chicks infected with (25 embryonated egg). (table-1)

PVC was found to decrease during the experiment. Statistically highly significant fall was (P<0.01) after 7 days, (P<0.02) after 14 days, (P<0.01) after 21 days, (P<0.10) after 28 day, (P<0.01) after 35 and 42 days of post infection. An overall declination was recorded from 40.56 percent to 33.30 percent in PCV during the present investigation (table-3)

Mean corpuscular volume (MCV)-

The mean corpuscular volume revealed a slight rise during the first & second week of experiment. But highly significant (P<0.01) fall was recorded during 3rd week of experiment & subsequent weeks, when treated with 25 embryonated eggs of A.galli. (table-1)

The mean corpuscular volume was observed to decrease during the experimental ascaridiasis, chicks treated with 500 embryonated eggs. A significant fall was recorded (P<0.02) during first and second week post infection. A further highly significant fall was recorded (P<0.01) during fifth and sixth week post infection. An overall declination from 95.02 f1 to 91.53 f1 in MCV was observed during the experiment. (Table-3)

Mean corpuscular hemoglobin (MCH)-

MCH revealed a highly significant fall during first week and subsequent weeks, (P<0.01). An overall deviation was found from 28.48 pg to 20.36 pg during experimental ascardiasis when treated with 25 embryonated eggs. (table-1)

A highly significant fall (P<0.01) was observed during first week, (P<0.02) during second & third week, (P<0.01) during fourth week (P<0.01) during fifth and sixth week of experiment treated with 500 embryonated eggs of A.galli.

An overall declination was found to be from 28.48pg to 22.40 pg during experiment. (Table-3)

Mean corpuscular hemoglobin concentration (MCHC)

The mean corpuscular hemoglobin concentration also revealed a highly significant fall during 7th, 14th, 21st, 28th, 35th and 42nd day of experiment, when treated with 25 embryonated eggs of A.galli. An overall declination was observed from 30.58gm/dl to 25.31gm/dl during this investigation. (table-1)

A highly significant fall (P<0.01) also observed in between control & infected group of chicks (500 embryonated eggs of A.galli) during all days of experiment. (Table-3)

Total leucocyte counts

The A.galli infection in chicks revealed a rise in total leucocyte count during both experiments (25 & 500 embryonated eggs). A highly significant elevation from 20.30 10\(^3\)/mm\(^3\) to 23.79 10\(^3\)/mm\(^3\) was observed in subsequent weeks. (table-2)
Table 1: Cellular haematological responses in blood of WLH chicks with experimental ascaridiasis

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>Control</th>
<th>Infected with 25 embryonated eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte counts (million/mm³)</td>
<td>4.21</td>
<td>3.69* ±0.0108 3.73* ±0.0515 3.61* ±0.0205 2.76* ±0.0957 3.16* ±0.0249 2.88* ±0.0148</td>
</tr>
<tr>
<td>Haemoglobin concentration (gm/dl)</td>
<td>11.36</td>
<td>11.12** ±0.0499 10.28* ±0.0960 9.87* ±0.0655 9.20* ±0.017 8.86* ±0.0638 9.38* ±0.0580</td>
</tr>
<tr>
<td>Packed cell volume (Percentage)</td>
<td>40.56</td>
<td>39.29* ±0.0886 35.28* ±0.2574 33.28* ±0.0594 36.13* ±0.1814 30.70* ±0.3917 27.87* ±2.5895</td>
</tr>
<tr>
<td>Mean Corpuscular volume MCV (fl)</td>
<td>95.02</td>
<td>95.33 ±0.0354 93.46** ±0.0740 92.48* ±0.0402 92.34* ±0.06628 90.33* ±0.0525 92.53* ±0.0356</td>
</tr>
<tr>
<td>Mean Corpuscular MCH (Pg)</td>
<td>28.48</td>
<td>25.55* ±0.0102 23.55* ±0.5175 25.90 ±0.5507 26.30* ±0.2107 22.56* ±0.557 20.36* ±0.0619</td>
</tr>
<tr>
<td>Haemoglobin Concentration MCH (gm/dl)</td>
<td>30.58</td>
<td>28.69* ±0.0118 27.13* ±0.0118 25.56* ±0.0477 24.62* ±0.0886 24.64* ±0.0309 25.31* ±0.0187</td>
</tr>
</tbody>
</table>

Result are mean ± S.E. (n=6) (Fisher’s ‘t’ test 1950)

‘P’ value : *<0.01; **<0.02; ***<0.10 (Control vs infected groups of chicks)

Table 2: Cellular haematological responses in blood of WLH chicks with experimental ascaridiasis

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>Control</th>
<th>Infected with 25 embryonated eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leucocyte count (10³/mm³)</td>
<td>20.30</td>
<td>21.41* ±0.0401 22.33* ±0.0744 22.87* ±0.0444 24.46* ±0.1678 23.46* ±0.0783 23.79* ±0.0802</td>
</tr>
<tr>
<td>Neutrophils (in percent)</td>
<td>19.51</td>
<td>20.48* ±0.0477 19.55 ±0.0718 22.53* ±0.1173 23.54* ±0.0800 29.54* ±0.0122 21.66* ±0.0946</td>
</tr>
<tr>
<td>Lymphocytes (In percentage)</td>
<td>70.62</td>
<td>65.55* ±0.1147 69.50* ±0.0816 55.81* ±0.0686 50.40* ±0.1211 52.81* ±0.1066 55.34* ±0.1161</td>
</tr>
<tr>
<td>Eosinophils (in percentage)</td>
<td>4.27</td>
<td>3.78* ±0.0103 3.53* ±0.0959 4.50* ±0.0902 3.34* ±0.0895 4.10* ±0.0356 2.30* ±0.0883</td>
</tr>
<tr>
<td>Monocytes (in percentage)</td>
<td>6.33</td>
<td>5.38* ±0.0110 4.35* ±0.0763 4.18* ±0.0602 5.28* ±0.0703 3.33* ±0.0816 2.15* ±0.2717</td>
</tr>
<tr>
<td>Basophils (In percentage)</td>
<td>2.35</td>
<td>1.13* ±0.0494 1.27* ±0.0483 0.65* ±0.0918 1.23* ±0.0714 2.10* ±0.0577 1.40* ±0.0774</td>
</tr>
</tbody>
</table>

Result are mean S.E. (n=6) (Fisher’s ‘t’ test 1950)

‘P’ value : *<0.01; **<0.02; ***<0.10 (Control vs infected groups of chicks)

Table 3: Cellular haematological responses in blood of WLH chicks with experimental ascaridiasis

<table>
<thead>
<tr>
<th>Blood Parameters</th>
<th>Control</th>
<th>Infected with 500 embryonated eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte counts (million/mm³)</td>
<td>4.21</td>
<td>2.68*** ±0.7760 2.73* ±0.4303 3.22*** ±0.5724 2.69** ±0.6062 3.10** ±0.5006 3.30 ±0.8430</td>
</tr>
<tr>
<td>Haemoglobin concentration (gm/dl)</td>
<td>11.36</td>
<td>10.86 ±0.6760 11.00 ±0.8382 8.53* ±0.8491 8.15*** ±1.8123 7.06* ±0.4104 9.55 ±0.2789</td>
</tr>
<tr>
<td>Packed cell volume (Percentage)</td>
<td>40.56</td>
<td>35.48** ±1.2547 38.26* ±0.7657 33.35* ±0.9415 40.05*** ±0.2578 38.58* ±0.5022 33.30* ±0.8845</td>
</tr>
<tr>
<td>Mean Corpuscular volume MCV (fl)</td>
<td>95.02</td>
<td>93.06** ±0.4923 93.05** ±0.4869 94.55 ±0.4745 95.35 ±0.6756 92.26* ±0.3419 91.53* ±0.1429</td>
</tr>
<tr>
<td>Mean Corpuscular MCH (Pg)</td>
<td>26.48</td>
<td>22.80* ±0.10308 25.53** ±0.7910 25.80** ±0.9916 27.53*** ±0.4310 24.43* ±0.0802 22.40* ±1.7007</td>
</tr>
</tbody>
</table>
Experimental Ascaridiasis Induced Changes in Haematological Parameters in WLH Chicks

Mean Corpuscular Haemoglobin Concentration (MCHC) (gm/dl)

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E. (n=6)</td>
<td>7 days P.I.</td>
</tr>
<tr>
<td>Total leucocyte count (10^3/mm^3)</td>
<td>20.30 ± 0.0758</td>
<td>24.25**</td>
</tr>
<tr>
<td>Neutrophils (in percent)</td>
<td>19.51 ± 0.1108</td>
<td>25.36*</td>
</tr>
<tr>
<td>Lymphocytes (In percentage)</td>
<td>70.62 ± 0.1450</td>
<td>62.36*</td>
</tr>
<tr>
<td>Eosinophils (in percentage)</td>
<td>4.27 ± 0.0525</td>
<td>2.99**</td>
</tr>
<tr>
<td>Monocytes (In percentage)</td>
<td>6.33 ± 0.0760</td>
<td>3.40*</td>
</tr>
<tr>
<td>Basophils (In percentage)</td>
<td>2.35 ± 0.0619</td>
<td>1.86***</td>
</tr>
</tbody>
</table>

Result are mean S.E. (n=6) (Fisher’s ‘t’ test 1950)

Table-4: Cellular haematological responses in blood of WLH chicks with experimental ascaridiasis

<table>
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<tbody>
<tr>
<td></td>
<td>7 days P.I.</td>
<td>14 days P.I.</td>
</tr>
<tr>
<td>Total leucocyte count (10^3/mm^3)</td>
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<td>2.35</td>
<td>1.86***</td>
</tr>
</tbody>
</table>

Result are mean S.E. (n=6) (Fisher’s ‘t’ test 1950)

On the other hand statistically significant rise (P<0.02) was observed during first week of post infection in leucocyte counts. A further highly significant fall (P<0.01) was recorded during second week and subsequent weeks of experiment.

An overall elevation from 20.30 10^3/mm^3 to 25.95 10^3/mm^3 in WBC was observed during the present investigation. (table-4)

**Differential leucocyte counts**

**Neutrophils**
A highly significant rise (P<0.01) was recorded in neutrophils during first week post infection when treated with 25 embryonated eggs of *A.galli* insignificant rise was observed during second week. A further highly significant rise (P<0.01) was recorded after third week & subsequent weeks of experiment. An overall rise from 19.51 percent to 29.54 percent was observed during this investigation. (table-2)

A highly significant rise (P<0.01) was observed in neutrophils during first, second & third week, (P<0.10) during fifth week, (P<0.01) during sixth week when treated with 500 embryonated eggs of *A.galli* (Table-4)

**Lymphocytes**
Statistical analysis showed highly significant change (a fall) in lymphocyte counts (P<0.01) in between control and infected groups of chicks. An overall fall was observed from 70.62 percent to 50.40 percent over all days of experiment during ascaridiasis (25 embryonated eggs). (table-2)

Statistically highly significant fall was also observed in lymphocyte between control and infected chicks with 500 embryonated eggs, during all weeks except 3rd week post infection. An overall decline was recorded from 70.62 percent to 61.43 percent during this investigation. (table-4)

**Eosinophils**
Eosinophils revealed a fall in 25 embryonated eggs treated groups of chicks. Statistically, highly significant (P<0.01) fall was observed during first week and subsequent weeks. An overall change from 4.27 percent to 2.30 percent was recorded during the investigations. (table-2)

A significant fall (P<0.02) was also observed in eosinophils of 500 embryonated eggs inoculated group of chicks after 7th and 14th days, (P<0.01) after 21 and 28 days, (P<0.02) after 35 days, and (P<0.01) after 42 days post infection (Table-4)

**Monocytes**
Highly significant fall (P<0.01) was observed in all days of experiment. An overall fall was recorded from 6.33 percent to 2.15 percent in monocytes count during experimental ascaridiasis, treated with 25 embryonated eggs of *A.galli*. (table-2)

A highly significant fall first & second week, (P<0.01) was also recorded during first & second week, (P<0.02) during third week, (P<0.01) during fourth and fifth weeks and (P<0.10) during sixth week. When treated with 500 embryonated eggs of *A.galli* (Table-4)

**Basophils**

Highly significant (P<0.01) fall was recorded in basophils also, during the experiment. An overall fall from 2.35 percent to 0.65 was found after 7, 14, 21, 28, 35, and 42 days of experimental ascaridiasis treated with 25 embryonated eggs. (table-2)

Statistically significant (P<0.01) fall in basophils during first week and (P<0.01) fifth week during experimental ascaridiasis (500 embryonated eggs) An overall decline was recorded from 2.35 percent to 1.34 percent in basophils during present investigation (Table-4)

**DISCUSSION**

In general, blood serum parameters are reliable indicators of health status and reflect any physiological, nutritional or even pathological changes that occur in the organism. (Simarak et al., 2004).

Haematological parameters have been discussed in relation to present experiment. Helminth infection especially those of nematode parasites cause blood alteration in their respective hosts (Backer and Douglas, 1966). Investigations of physiological changes produced in the hosts by parasites are essentials for an understanding of pathogen city (Sadun and Williams, 1966)

Hypoaemoglobinemia followed by a fall in total erythrocyte counts and hemoglobin content at all the time intervals during the present investigation has been observed

Depression in erythrocyte counts was observed during experimental ascaridiasis (Sadun, 1950). Muraveva (1977) has also reported decrease in haemoglobin concentration and erythrocyte counts during trichuriasis in adults and children.

The erythrocytopenia and hypoaemoglobinaemia as observed in the present investigations might be due to the increased rate of erythrocytic sediments. This is in accordance with the findings of Matta and Ahluwalia (1982) in fowls during *A.galli* infection & Deka & Borah (2008) in quails & chickens during *A.galli* infection.

Suppression in the packed cell volume, as observed in the present investigations, may well be attributed to the fall in erythrocyte counts. A high degree of positive correlation between erythrocyte counts and the values of packed cell volume apparently indicated the fall in packed cell volume (PCV) was due to depression of erythrocyte counts. A decrease in the values of PCV has been reported by Paciejewski (1980) in pigs during larval ascaridiasis.

A significant fall in MCH and MCHC values at all time intervals was observed during present investigations. Whereas The MCV value were found to be slightly increased in initial stage but significant fall was found at all the time intervals during the experimental ascaridiasis.

Rani (1986) reported a mean decrease in the MCV, MCH and MCHC values at every stage of post infection in chicks infected with high and low doses of *A.galli* embryonated eggs.

Rao (1991) also reported; the chicks infected with *A.galli* revealed a fall in MCH and MCHC values at all time intervals post infection. But the MCV values were found to decrease by eighth week post infection.

A significant rise in total leucocyte counts was observed during the investigations. A high rise in total leucocyte counts, heterophils and eosinophils in chicks with *A.galli* infection reported by Sadun (1950), Kaushik and Sen (1978), who also reported increased values of total leucocyte counts in chicks with *A.galli* infection. Contradictory, to this Lal and Kumar (1983) observed a fall in total leucocyte counts in experimental bunostomiasis

The differential leucocyte counts revealed a significant rise in neutrophils with a fall in lymphocytes, eosinophils, monocytes and basophils. Sadun (1950) also reported a high rise in eosinophils in chicks during experimental ascaridiasis. Kaushik and Sen (1978) observed a high percent of eosinophils heterophils and monocytes with no significant changes in lymphocyte counts in chicks with *A.galli* infection. Increase in eosinophils has also been reported by Paciejewski (1980) in pigs during larval ascaridiasis.

A statistically significant fall is observed in the mean values of lymphocytes during the investigations. Lymphocytes play a definite role in the development of immunity and in phagocytising the antigen antibody complexes. This was in accordance with the fact that, there always exists a definite correlation between the suppression of immunity and fall in the numbers of lymphocytes.

The fall in the number of lymphocytes, eosinophils, monocytes and basophils was obviously due to the suppression of immune response against low and high doses of *A.galli* infection in WLH chicks.
ACKNOWLEDGEMENTS:
Authors are thankful to the Principal of our college for providing laboratory facilities to us.

Declaration: We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.

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