



## Experimental Ascariasis Induced Changes in Haematological Parameters in WLH Chicks

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### 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 ascariasis 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. Ascariasis 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 ascariasis 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 Leucocyte 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 haemoglobinmeter (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

$$(f1) = \frac{PCV}{RBC\ count} \times 10$$

2. Mean corpuscular hemoglobin (MCH) in pico

$$\text{grams(pg)} = \frac{\text{Haemoglobin}}{\text{RBC count}} \times 10$$

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

## RESULT

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

### 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 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup>, 35<sup>th</sup> & 42<sup>nd</sup> day post infection. An overall fall from 4.21 million/mm<sup>3</sup> to 2.88 million/mm<sup>3</sup> 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 & the third week, ( $P < 0.02$ ) during fourths and fifth week of experiment, and over all decline was recorded from 4.21 million/mm<sup>3</sup> to 2.68 million /mm<sup>3</sup> in RBC counts during the experiment (table-3)

### 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 9.38gm/dl was recorded during the experiment. (table-1)

The chicks inoculated with 500 embryonated eggs, revealed, a note worthy 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 from 11.36gm/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 3<sup>rd</sup> 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 ascariasis, 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 fl to 91.53 fl 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 ascariasis 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, ( $P < 0.10$ ) 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 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup>, 28<sup>th</sup>, 35<sup>th</sup> and 42<sup>nd</sup> 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<sup>3</sup>/mm<sup>3</sup> to 23.79 10<sup>3</sup>/mm<sup>3</sup> was observed in subsequent weeks. (table-2)

**Table-1: Cellular haematological responses in blood of WLH chicks with experimental ascaridiasis**

Blood Parameters	Control	Infected with 25 embryonated eggs					
		7 days P.I.	14 days P.I.	21 days P.I.	28 days P.I.	35 days P.I.	42 days P.I.
Erythrocyte counts (million/mm <sup>3</sup> )	4.21 ± 0.0501	3.69* ±0.1008	3.73* ±0.0515	3.61* ±0.0205	2.76* ± 0.0957	3.16* ±0.0249	2.88* ±0.0148
Haemoglobin concentration (gm/dl)	11.36 ± 0.1190	11.12** ± 0.0499	10.28* ± 0.0960	9.87* ± 0.0655	9.20* ± 0.017	8.86* ± 0.0638	9.38* ± 0.0580
Packed cell volume (Percentage)	40.56 ± 0.1224	39.29* ±0.0886	35.28* ±0.2574	33.28* ±0.0594	36.13* ±0.1814	30.70* ±0.3917	27.87* ±2.5895
Mean Corpuscular volume MCV (fl)	95.02 ±0.5552	95.33 ±0.0354	93.46** ±0.0740	92.48* ±0.0402	92.34* ±0.06628	90.33* ±0.0525	92.53* ±0.0356
Mean Corpuscular Haemoglobin MCH (Pg)	28.48 ±1137	25.55* ±0.1002	23.55* ±0.5175	25.90 ±0.5507	26.30* ±0.2107	22.56* ±0.557	20.36* ±0.0619
Mean Corpuscular Haemoglobin Concentration MCHC (gm/dl)	30.58 ±0.0917	28.69* ±0.4283	27.13* ±0.2285	25.56* ±0.3029	26.42* ±0.0751	24.64* ±0.1387	25.31* ±0.360

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**

Blood Parameters	Control	Infected with 25 embryonated eggs					
		7 days P.I.	14 days P.I.	21 days P.I.	28 days P.I.	35 days P.I.	42 days P.I.
Total leucocyte count (10 <sup>3</sup> /mm <sup>3</sup> )	20.30 ± 0.0758	21.41* ±0.0401	22.33* ±0.0744	22.87* ± 0.0444	24.46* ± 0.1678	23.46* ±0.0783	23.79* ±0.0802
Neutrophils (in percent)	19.51 ± 0.1108	20.48* ± 0.0477	19.55 ± 0.0718	22.53* ± 0.1173	23.54* ± 0.0800	29.54* ± 0.0122	21.66* ± 0.0946
Lymphocytes (In percentage)	70.62 ± 0.1450	65.55* ±0.1147	69.50* ±0.0816	55.81* ±0.0686	50.40* ±0.1211	52.81* ±0.1066	55.34* ±0.1161
Eosinophils (in percentage)	4.27 ±0.0525	3.78* ±0.1013	3.53* ±0.0959	4.50* ±0.0902	3.34* ±0.0895	4.10* ±0.0356	2.30* ±0.0883
Monocytes (In percentage)	6.33 ±0.0760	5.38* ±0.1108	4.35* ±0.0763	4.18* ±0.0602	5.28* ±0.0703	3.33* ±0.0816	2.15* ±0.2717
Basophils (In percentage)	2.35 ±0.0619	1.13* ±0.494	1.27* ±0.0483	0.65* ±0.0918	1.23* ±0.0714	2.10* ±0.0577	1.40* ±0.0774

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

'P' value : \*<0.01 : \*\*<0-10 (Control vs infected groups of chicks)

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

Blood Parameters	Control	Infected with 500 embryonated eggs					
		7 days P.I.	14 days P.I.	21 days P.I.	28 days P.I.	35 days P.I.	42 days P.I.
Erythrocyte counts (million/mm <sup>3</sup> )	4.21 ± 0.0501	2.68*** ±0.7760	2.73* ±0.4303	3.22*** ± 0.5724	2.69** ± 0.6062	3.10** ±05006	3.30 ±0.8430
Haemoglobin concentration (gm/dl)	11.36 ± 0.1190	10.86 ± 0.6760	11.00 ± 0.8382	8.53* ± 0.8491	8.15*** ± 1.8123	7.06* ± 0.4104	9.55 ± 0.2789
Packed cell volume (Percentage)	40.56 ± 0.1224	35.48** ±1.2547	38.26* ±0.7657	33.35* ±0.9415	40.05*** ±0.2578	38.58* ±0.5022	33.30* ±0.8845
Mean Corpuscular volume MCV (fl)	95.02 ±0.5552	93.06** ±0.4923	93.05** ±0.4869	94.55 ±0.4745	95.35 ±0.6756	92.26* ±0.3419	91.53* ±0.1429
Mean Corpuscular Haemoglobin MCH (Pg)	26.48 ±0.1137	22.80* ±1.0308	25.53** ±0.7910	25.80** ±0.9916	27.53*** ±0.4310	24.43* ±0.0802	22.40* ±1.7007

Mean Corpuscular Haemoglobin Concentration MCHC (gm/dl)	30.58 ±0.0917	24.80* ±1.6072	21.83* ±0.2108	27.11* ±0.8654	22.30* ±0.7741	22.63* ±1.1284	26.73* 2.0642
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Result are mean S.E.(n=6) (Fisher's 't' test 1950)

'P' value : \*<0.01 : \*\*<0-10 (Control vs infected groups of chicks)

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

Blood Parameters	Control	Infected with 500 embryonated eggs					
		7 days P.I.	14 days P.I.	21 days P.I.	28 days P.I.	35 days P.I.	42 days P.I.
Total leucocyte count (10 <sup>3</sup> /mm <sup>3</sup> )	20.30 ± 0.0758	24.25** ±1.6296	27.06* ±0.8337	25.30* ± 0.3651	22.85* ± 0.7719	24.35* ±0.6849	25.95* ±0.5577
Neutrophils (in percent)	19.51 ± 0.1108	25.36* ± 0.3402	22.43* ± 0.5070	24.00* ± 0.76.37	19.18 ± 3.4419	21.60 ± 1.1108	20.38* ± 0.2638
Lymphocytes (In percentage)	70.62 ± 0.1450	62.36* ±0.65.20	64.80* ±1.0657	67.97 ±2.4494	65.30* ±1.0327	61.43* ±0.8608	63.68* ±0.3280
Eosinophils (in percentage)	4.27 ±0.0525	2.99** ±0.4699	3.16** ±0.2011	1.78* ±0.2442	3.23* ±0.0614	3.63* ±0.2940	2.40* ±0.3011
Monocytes (In percentage)	6.33 ±0.0760	3.40* ±0.4690	3.85* ±0.6864	5.13** ±0.4160	4.20* ±0.0577	2.31* ±0.0833	4.61*** ±1.1461
Basophils (In percentage)	2.35 ±0.0619	1.86*** ±0.2666	2.50* ±0.2113	2.00* ±0.3033	2.91* ±0.3807	1.34* ±0.0454	3.18 ±0.2242

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

'P' value : \*<0.01 : \*\*<0-10 (Control vs infected groups of chicks)

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<sup>3</sup>/mm<sup>3</sup> to 25.95 10<sup>3</sup>/mm<sup>3</sup> 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 ascariasis (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 3<sup>rd</sup> 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 7<sup>th</sup> and 14<sup>th</sup> 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. (Simaraks *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)

Hypohaemoglobinemia 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 hypohaemoglobinaemia 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.

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**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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