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HAEMATOLOGICAL CHANGES IN CHICKENS, EXPERIMENTALLY INFECTED WITH BITING LICE (*PHTHIRAPTERA–INSECTA*)

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Summary

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The aim of the study was to determine the haematological changes in chickens, experimentally infected with biting lice from the following species: *Eomenacanthus stramineus* Nitzsch (1818), *Menacanthus cornutus* Schömmer (1913), *Menopon gallinae* L. (1758) and *Goniocotes gallinae* DeGeer (1778). The experiments were performed on 30 (20 experimental and 10 control) 2-month-old chickens (*Gallus gallus domesticus*).

The infection with biting lice resulted in reduction in red blood cell (RBC) counts and haemoglobin content, increase in the mean corpuscular haemoglobin (MCH), the mean corpuscular haemoglobin concentration (MCHC), the mean corpuscular volume (MCV) and the haemoglobin index (HbI), indicating the development of a hyperchromic anaemia. The white blood cell (WBC) counts were elevated as well as the percentages of eosinophils and monocytes following activation of the innate defense mechanisms. Lymphocyte percentages were lower. Both qualitative and quantitative haematological changes observed in chickens, infected with biting lice, were the highest during the period of most intensive invasion (September).

Key words: anaemia, biting lice, chickens, *Mallophaga*, *Phthiraptera*

INTRODUCTION

Using morphological and radiological methods, Derylo and Gogacz (1974), Lonc and Zlotorzycka (1984) and Trivedi et al. (1990) proved that *Mallophaga* representatives from the *Amblycera* suborder, ingest parts of feathers, desquamated epidermal cells as well as blood. According to Kettle (1990) and Arends (1997), *Eomenacanthus stramineus*, a species that infects chickens, ingests blood from the host via perforation of delicate feathers near to their base or destructs the superficial layers of the skin. Zlotorzycka et al. (1974) and Agarwal et al. (1983) reported a significant anaemia and blood loss in birds, infected with biting lice. Data about the type of anaemia and its correlation with the intensity of invasion are however insufficient.

Thus, our aim was to study the changes in some haematological parameters in chickens, experimentally infected by several *Mallophaga* species and to follow out the relationship of those changes with the intensity of invasion.

MATERIALS AND METHODS

Birds

The experiment was performed on 30 chickens (*Gallus gallus domesticus*) from the Israel breed Anak, at the age of 60 days. The chickens were in a good general condition and with normal appetite. The

parasitological examination using routine methods showed no invasion with endo- or ectoparasites. The birds were divided into 2 groups: experimental (n=20) and control (n=10). They were put into two separate premises, previously cleaned and disinfected with a warm 2% NaOH solution. The daily diet of all birds was 0.1 kg of a standard forage.

Blood samples

Blood was sampled using sterile needles and syringes from the ulnar cutaneous vein in all birds following aseptic preparation of sampling site. Haematological parameters were followed out monthly from May 2000 (baseline determination) to November 2000, after the infection.

Haematological parameters

Packed cell volume (PCV) was determined by the microhaematocrit method, and haemoglobin (Hb) concentrations - by a commercial kit (Human, GmbH, Wiesbaden, Germany). Red blood cell (RBC) counts, total and differential white blood cell (WBC) counts were determined according to Ibrishimov and Lalov (1984). The following RBC indexes were determined:

- Mean corpuscular haemoglobin (MCH)

$$MCH\ [pg] = Hb\ [g/l] / RBC\ [10^{12}/l]$$

- Mean corpuscular haemoglobin concentration (MCHC)

$$MCHC\ [g/l] = Hb\ [g/l] / PCV\ [l/l]$$

- Mean corpuscular volume (MCV)

$$MCV\ [fl] = PCV\ [l/l] \times 10^3 / RBC\ [10^{12}/l]$$

- Haemoglobin index (HbI)

$$HbI = \frac{RBC_c[10^{12}/l] \times Hb_e[g/l]}{RBC_e[10^{12}/l] \times Hb_c[g/l]}$$

where the index "c" = "control" and "e" = "experimental"

Morphological changes in RBC were assessed microscopically (Laboval 4, Zeiss, Austria).

Infection of birds

The experimental chickens were infected in May 2000 immediately following determination of baseline haematological values. The infection was performed with 4 adult donor birds with 5th degree of invasion (over 100 *Mallophaga* per bird, counted for a minute) with biting lice from the following species: *Eomenacanthus stramineus* Nitzsch (1818), *Menacanthus cornutus* Schömmer (1913), *Menopon gallinae* L. (1758) and *Goniocotes gallinae* DeGeer (1778). The intensity of invasion (II) of donors was detemined by the score system of Derylo (1974). In order to guarantee the direct contact, experimental birds were put together with donors for 120 min in wooden boxes throughout 3 consecutive days.

Differentiation of biting lice and detemination of the intensity of invasion

The differentiation of biting lice on experimental birds was done according to the manuals of Neveu-Lemaire (1938), Zlotorzycka et al. (1974) and Furman and Catts (1982). The lice were collected with a forceps from both feathers and skin of infected birds, conserved for 24 h in 70^o ethanol and embedded in Canada balsam for obtaining permanent microscopic preparations.

For determination of the intensity of invasion, each month (from June to November), the various species of biting lice were counted separately on previously chosen body surface fields, each of 10 cm^2: on head, neck, breast, abdomen, back, left and right wing, left and right inguinal region.

Statistics

The data were statistically processed using the one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Thirty days following the infection, a medium degree of mixed invasion was determined in all experimental birds using Derylo's classification (1974). The presence of the following species was evidenced: *Eomenacanthus stramineus* Nitzsch (1818), *Menacanthhus cornutus* Schömmer (1913), *Menopon gallinae* L. (1758) – all three belonging to the *Amblycera* suborder and *Goniocotes gallinae* DeGeer (1778) from the *Ischnocera* suborder of the *Phthiraptera* order.

The changes in the intensity of invasion during the experimental period are presented on Fig. 1. The highest intensity of invasion (115.75 insects per bird on the average) was observed in September and the lowest (21.75 insects per bird) – in November.

Haematological parameters obtained at baseline and at post infection months 1, 2, 3, 4, 5 and 6 are shown in Tables 1, 2 and 3. During the first post infection month, RBC counts in experimental birds were higher that those in controls. The next determinations, however, showed lower counts in experimental birds. This tendency was present until the end of the experiment and was the most apparent in September ($p<0.001$), when the intensity of invasion was the highest.

Haemoglobin values in the experimental group were lower than those in controls. The differences were statistically significant for post infection months 2–4 (July–September). By respect to baseline concentrations, Hb decreased up to the 3rd month and afterwards, increased significantly during post infections months 4 and 6.

The differences between PCV values of experimental and control groups were not statistically significant.

In infected birds, MCH decreased up to month 3 compared to controls. Afterwards, its values were higher than in controls with a peak at month 4 ($p<0.001$). MCHC was slightly decreased vs baseline for the first 3 months and then was elevated up to end of the experimental period. MCV increased from the 2nd

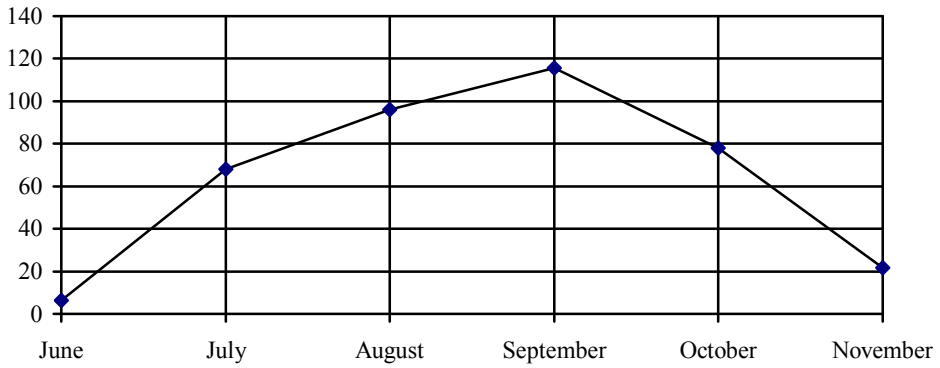

Fig. 1. Average number of insects, counted monthly on determined sites of chicken bodies (n=20) with an experimental mixed *Mallophaga* infection.

Table 1. Dynamics of changes in haemoglobin (Hb), red blood cell counts (RBC), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentrations (MCHC) and the mean corpusular volume (MCV) in control chickens (n=10) and chickens with a mixed experimental *Mallophaga* infection (n=20). Data are presented as mean ± SEM

Intervals (months)	Hb (g/l)	RBC (x 10^{12}/l)	PCV (l/l)	MCH (pg)	MCHC (g/l)	MCV (fl)
Control birds						
Baseline	81.63 ± 2.21	2.01 ± 0.06	0.36 ± 0.02	40.88 ± 1.87	227.0 ± 6.2	181.1 ± 10
Month 1	84.25 ± 1.92	1.55 ± 0.08[3]	0.34 ± 0.01	54.6 ± 2.62[3]	253.4 ± 11.3[1]	220.5 ± 13.8[1]
Month 2	89.25 ± 2.02[1]	1.86 ± 0.09	0.36 ± 0.004	48.91 ± 3.06[1]	247.8 ± 4[1]	196.8 ± 10.3
Month 3	86.79 ± 3.39	2.15 ± 0.11	0.36 ± 0.01	40.75 ± 1.61	238.8 ± 10.5	172.4 ± 8.9
Month 4	98.72 ± 3.1[3]	2.18 ± 0.1	0.38 ± 0.02	45.97 ± 2.3	261.9 ± 8.03[2]	177.3 ± 12.3
Month 5	97.80 ± 3.92[2]	2.06 ± 0.1	0.38 ± 0.02	47.83 ± 1.97[1]	256.1 ± 7.3[2]	188.4 ± 11.2
Month 6	90.05 ± 4.83	2.14 ± 0.09	0.43 ± 0.03	41.23 ± 1.7	212.0 ± 6.4	200.0 ± 8.9
Experimental birds						
Baseline	81.63 ± 2.21	2.01 ± 0.06	0.36 ± 0.02	40.88 ± 1.87	227.0 ± 6.2	181.1 ± 10
Month 1	79.75 ± 1.61	1.73 ± 0.09[2]	0.36 ± 0.02	47.05 ± 2.43[a1]	224.5 ± 12.1	214.6 ± 17
Month 2	78.00 ± 2.64[b]	1.69 ± 0.04[3]	0.38 ± 0.02	46.35 ±1.73[1]	214.0 ± 7[c]	228.2 ± 10.7[a2]
Month 3	79.24 ± 1.98[a]	2.01 ± 0.1	0.39 ± 0.02	39.39 ± 1.71	207.2 ± 13.6	198.7 ± 15.2
Month 4	89.29 ± 1.22[b2]	1.49 ± 0.02[c3]	0.37 ± 0.01	60.10 ±1.05 [c3]	236.1 ± 12.2	246.0 ± 7.5 [c3]
Month 5	91.03 ± 2.18[2]	1.85 ± 0.1	0.37 ± 0.01	50.11 ±2.58[2]	245.2 ± 6.4[1]	206.0 ± 13.5
Month 6	90.44 ± 1.03[2]	1.86 ± 0.1	0.39 ± 0.02	49.57 ± 2.59[b2]	236.0 ± 11.5	215.7 ± 20.8

[a] - $p < 0.05$; [b] - $p < 0.01$; [c] - $p < 0.001$ between groups at each interval; [1] - $p < 0.05$; [2] - $p < 0.01$; [3] - $p < 0.001$ vs baseline.

month onward till the end of the study. The highest (statistically significant) difference was observed at month 4 ($p<0.001$).

The HbI values in infected birds (Table 2) varied within the normal range (0.85-1.0) up to the 3rd month of the study. Then they increased and reached a peak at post infection month 4 (September).

Table 2. Dynamics of changes in haemoglobin index (HbI) in experimental chickens (n=20) with a mixed experimental *Mallophaga* infection

Intervals (months)	HbI
baseline	1.00
1 month	0.85
2 month	0.95
3 month	0.98
4 month	1.32
5 month	1.04
6 month	1.16

During the whole experimental period, there were no morphological deviations in RBC in control birds. Prior to the infection and by the 4th month, such changes were not observed in experimental chickens too. From September to November however, an obvious anisocytosis and poikilocytosis were present.

The changes in thrombocyte counts in experimental birds (Table 3) was characterized by a slight, but statistically insignificant elevation vs controls during the whole period of the study.

Total WBC counts were also increased after the infection and were statistically significantly higher compared to those in controls from the 3rd month (August) ($p<0.05$) onward until the end of the experimental period ($p<0.01$).

Differential WBC counts revealed that the percentage of heterophils (the equivalent of the neutrophils in mammals) decreased in both groups, but less obviously in infected birds. Eosinophil percentages in controls changed a little, being statistically lower than baseline values by the 3rd month ($p<0.01$). In infected birds, eosinophils were statistically higher vs controls during all experimental periods. Similar changes were observed in monocytes as well. The differences by the 2nd , 3rd , 4th and the 5th months were statistically significant vs controls, the greatest difference being registered at month 2 ($p<0.001$). In controls, lymphocytes increased from the 1st to the 6th month, whereas in the experimental group the changes vs baseline were biphasic. Initially they tended to decrease up to month 3 (statistically significantly by month 2) and within post infections months 4–6, the tendency was towards elevation ($p<0.001$ by month 6 vs baseline). In infected birds, lymphocyte percentages were significantly lower vs controls from months 1 to 6. In controls, the initial values of basophils increased. The maximum values were at months 3 and 4. In infected birds, there was also an increase in basophil percentages, but less apparent – with a peak at month 3 and variations with ups and downs until the end of the experiment. The differences in basophils between both groups were not statistically significant.

DISCUSSION

The changes in the intensity of invasion showed that it was the highest in September and the lowest – in November (the last month of the study). Similar data for the seasonal dynamics of *Mallophaga* infections in chickens and turkeys are repoted by El-Kifl et al. (1973) and Sa'-idu et al. (1994).

The decreased RBC counts and Hb concentrations in infected birds, compared to controls, evidenced that the infection

Table 3. Dynamics of changes in total white blood cell (WBC) counts, basophils (Ba), eosinophils (Eo), heterophils (He), lymphocytes (Ly), monocytes (Mo) and thrombocytes (Thr) in control chickens (n=10) and chickens with a mixed experimental *Mallophaga* infection (n=20)

Intervals (months)	Leu (x 10^9/l)	Ba (%)	Eo (%)	He (%)	Ly (%)	Mo (%)	Thr (x 10^9/l)
Control birds							
Baseline	35.03 ± 2.61	0.13 ± 0.1	2.75 ± 0.41	30.25 ± 1.58	64.13 ± 2.62	2.70 ± 0.98	83.63 ± 2.92
Month 1	31.13 ± 2.79	1.30 ± 0.3^{2}	2.75 ± 0.73	18.88 ± 1.62^{3}	74.50 ± 2.37^{2}	2.75 ± 0.67	48.25 ± 5.36
Month 2	36.38 ± 1.94	2.13 ± 0.6^{2}	2.00 ± 0.19	19.25 ± 2.26^{2}	72.50 ± 2.11^{1}	4.13 ± 0.67	57.25 ± 12.98
Month 3	31.75 ± 2.31	2.00 ± 0.27^{3}	0.88 ± 0.4^{2}	15.00 ± 1.81^{3}	77.88 ± 1.95^{3}	4.25 ± 0.7	70.38 ± 16.84
Month 4	31.13 ± 2.04	2.00 ± 0.38^{3}	1.75 ± 0.31	17.50 ± 1.07^{3}	75.50 ± 1.59^{2}	3.25 ± 0.49	69.00 ± 7.94
Month 5	32.50 ± 2.03	1.25 ± 0.37^{1}	2.25 ± 0.65	13.63 ± 1.19^{3}	79.50 ± 0.87^{3}	3.38 ± 0.46	67.88 ± 7.28
Month 6	29.13 ± 1.66	1.25 ± 0.37^{2}	2.00 ± 0.38	10.25 ± 0.7^{3}	83.00 ± 0.78^{3}	3.50 ± 0.65	30.25 ± 2.25
Experimental birds							
Baseline	35.03 ± 2.61	0.13 ± 0.1	2.75 ± 0.41	30.25 ± 1.58	64.13 ± 2.62	2.70 ± 0.98	83.63 ± 2.92
Month 1	37.75 ± 3.22	1.75 ± 0.4^{2}	5.00 ± 0.68^{a1}	28.00 ± 5.24	60.00 ± 6.34^{a}	5.25 ± 0.9^{a}	70.75 ± 18.85
Month 2	40.63 ± 5.12	1.25 ± 0.45^{2}	4.75 ± 1.37^{a}	27.50 ± 3.77	54.88 ± 3.52^{c1}	11.66 ± 1.03^{c3}	70.75 ± 15.36
Month 3	52.00 ± 8.92^{a}	3.50 ± 1.3^{2}	8.88 ± 3.07^{a1}	19.13 ± 3.03^{2}	62.50 ± 3.96^{b}	6.00 ± 1.84	107.88 ± 36.34
Month 4	44.75 ± 5.24^{b}	1.88 ± 0.35^{3}	4.63 ± 0.96^{b}	18.38 ± 1.75^{3}	68.38 ± 2.1^{b}	6.75 ± 1.01^{b1}	97.13 ± 34.55
Month 5	45.38 ± 3.35^{b1}	0.88 ± 0.35^{1}	5.63 ± 1.35^{a1}	18.25 ± 1.52^{a3}	68.25 ± 2.32^{c}	7.00 ± 1.39^{b1}	97.63 ± 24.58
Month 6	37.88 ± 2.5^{b}	2.25 ± 0.7^{2}	4.63 ± 0.63^{b1}	11.37 ± 1.16^{3}	75.88 ± 3.01^{a3}	5.88± 1.14^{1}	36.00 ± 4.8

^{a} - $p < 0.05$; ^{b} - $p < 0.01$; ^{c} - $p < 0.001$ between groups at each interval; ^{1} - $p < 0.05$; ^{2} - $p < 0.01$; ^{3} - $p < 0.001$ vs baseline.

with biting lice resulted in the development of anaemia. The latter was reported in chickens with mallophagosis also by Zlotorzycka et al. (1974) and Agarwal et al. (1983). Our results agree with the statements of Kalamarz (1963 a, b), Derylo (1970), Derylo and Gogacz (1974), Lancaster and Meisch (1986), Trivedi et al. (1990) about the haematophagosis of the biting lice from the *Amblycera* suborder, infecting chickens. They damage the skin and furthermore, ingest blood; thus, their pathogenicity is more pronounced compared to that of *Ischnocera* (Ribbeck, 1982). In the experimental mixed infection, performed by us, 3 out of the 4 biting lice species belonged to the *Amblycera* suborder and only one – to the *Ischnocera* suborder, hence explaining the occurrence of anaemia in experimental birds.

The increase in red cell indexes MCH and MCHC (from months 4 to 6) as well as MCV (from the 2nd month on) could be due to the development of anaemia in chickens infected with biting lice. This anaemia was accompanied by an increased HbI, that was the highest at month 4 and suggested a hyperchromic anaemia. This state could be possibly due to the deficiency of vitamin B_{12}, typical for parasitic infections (Baldy, 1997). The marked anisocytosis and poikilocytosis, observed in all infected birds after the post infection month 4, also confirmed the presence of anaemia.

The changes in the parameters of the red blood picture were the most significant in September (month 4) and coincided with the most extensive invasion. This showed a good correlation between the severity of anaemia and the intensity of invasion with biting lice.

Infected chickens demostrated a tendency towards increase in counts of thrombocytes compared to controls. This is probably due to their participating in phagocytosis. The data of Awadhiya et al. (1980) suggesting that avian thrombocytes are very active phagocytes are supporting our hypothesis.

The increase in WBC counts especially after the 3rd post infection month and the higher eosinophil and monocyte percentages in experimental chickens could be explained by the involvement of those elements in the innate mechanisms of defense, triggered after the infection. It is known that monocytes and particularly eosinophils, have a protective role against parasites (Maxwell, 1993). The decreased percentage of lymphocytes in invaded birds could be probably due to a toxin, released by biting lice that has an immunosuppressing effect. This assumption, however, requires further studies.

The increase in lymphocytes in controls could be related to some activation of immune system. The changes in basophils could hardly be interpreted because in birds, these cells are scarcely studied.

CONCLUSIONS

The experimental mixed infection of chickens with biting lice from the following species: *Eomenacanthus stramineus* Nitzsch (1818), *Menacanthus cornutus* Schömmer (1913), *Menopon gallinae* L. (1758) and *Goniocotes gallinae* DeGeer (1778) resulted in decreased RBC counts and Hb content, increased MCH, MCHC, MCV and HbI as well as morphological changes in RBC indicating the development of a hyperchromic anaemia.

The infection of chickens with biting lice increased the RBC counts, eosinophil and monocyte percentages (suggesting activation of the innate mechanisms of defense) and decreased lymphocyte percentages.

The qualitative and quantitative changes in haematological parameters in chickens with mallophagosis were the most significant during the period of the most intensive invasion.

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