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SOME EFFECTS OF *Cuterebra emasculator* FITCH (DIPTERA: CUTEREBRIDAE) ON THE BLOOD AND ACTIVITY OF ITS HOST, THE EASTERN CHIPMUNK

GORDON F. BENNETT[1]

Abstract: Haematological values of the eastern chipmunk *(Tamias striatus)* infected with *Cuterebra emasculator* were compared to those of non-infected hosts. Parasitized animals were anaemic and had a leucocyte count approximately twice that of the parasite-free animals. The differential leucocyte count varied with the stage of development of the parasite. Activity of the host was little affected by the presence of the warble during its parasitic invasion of the host tissue. Subsequent to the parasite leaving the host, the lesions became purulent; the activity of the host at this time was severely reduced.

INTRODUCTION

Infction of various rodents by species of Cuterebridae are widely reported in North America.[5,6,7] Despite the frequency of parasitism, however, little factual evidence exists as to the effects of the parasites on the host or the host's reaction to them. This situation arises, in part, from the difficulty in rearing the parasite and/or the host and hence, the lack of controlled experiments. Previous studies[3] on *Cuterebra emasculator* enabled experimental studies to be undertaken on the effect of the parasite on the haematological values of the chipmunk. Reported herein are comparisons of the packed red cell volume, haemoglobin content, erythrocytes per mm,[3] leucocytes per mm,[3] and the differential leucocyte count between parasitized and non-parasitized animals. Miscellaneous observations on the effect of the parasite are also included.

MATERIAL AND METHODS

The field studies were conducted at the Wildlife Research Station in Algonquin Park, Ontario. Methods of obtaining and handling the chipmunks and the parasites were as described previously.[3] Blood was drawn from the femoral artery of chipmunks lightly anaesthetized with ether. The packed erythrocyte volume was determined by centrifuging blood samples for 8 minutes at 11,500 rpm in an International Hematocrit centrifuge. Haemoglobin values were obtained by using a Coleman photohaemoglobinometer and the number of erythrocytes and leucocytes per mm^3 were counted by standard haemocytometer methods. Differential counts of leucocytes were obtained from blood films fixed in 100% ethanol and stained with Giemsa's solution. The activity of the host was measured by the number of revolutions per day in a standard rat activity wheel of 114 cm circumference; the wheels were maintained in a little-used room to minimize disturbance to the experimental hosts. Larval transfer to uninfected chipmunks was by the method previously described.[3]

RESULTS AND DISCUSSION

HAEMATOLOGICAL VALUES

Non-parasitized chipmunks

The five blood values of non-parasitized chipmunks were determined as a basis of comparison with the similar values for parasitized animals. Chipmunks, obtained in the field, were considered to be

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"normal" if (i) they were free of *C. emasculator* infections and scars of previous infections and (ii) free from cuts, lesions, mite infestations or any other externally observable pathological condition. It was not known whether these animals harbored concurrent bacterial, viral or helminthic infections. However, in a sample of 50 animals from another study[5] examined for helminths, two-thirds of the animals harbored *Scaphiostomum* sp. infections in the pancreatic ducts, and the same proportion carried a trypanosome. It was assumed that a similar rate of parasitism occurred in the chipmunks used experimentally herein but post-experimental examinations could not be made as the chipmunks were required elsewhere. The experimental chipmunks were divided into four groups on the basis of age and sex.

The haematological values of normal chipmunks (Table 1) showed variation between the different age and sex groups. However, the variation between the averages of the different groups was not as extensive as that within a single group and the figures were combined to present an average picture for non-parasitized chipmunks. As the differential leucocyte counts also showed more variation within a group than between sex and age groups, all values were combined. In the 35 "normal" animals, lymphocytes (large and small) predominated, constituting 57% (30-88), neutrophils constituted 28% (8-40) and monocytes 14% (3-29); eosinophils and basophils were less than 1%.

The haematological values for *Tamias striatus* differ from the values reported for other rodents[1,10] such as woodchuck, ground squirrel, white mouse, etc., in several ways: (i) the packed cell volume and haemoglobin value is higher, (ii) the number of erythrocytes per mm^3 is generally higher, (iii) the number of leucocytes is generally lower and (iv) the differential leucocyte count differs in the virtual absence of eosinophils and basophils.

Parasitized chipmunks

Chipmunks infected with *C. emasculator* were initially divided into four groups on the basis of age and sex, and the values recorded (Table 1). Again, the variation within each group was larger than that between groups and hence all values were combined. In the overall totals, the haematological values af the parasitized chipmunks differed from the normal animals as follows: (i) the packed cell volume was slightly lower, (ii) the haemoglobin values were slightly lower, (iii) the number of erythrocytes per mm^3 was about twice as high. The latter was the most striking difference although the other values suggest that the parasitized animals are slightly anaemic.

The data used to obtain the values in Table 1 represent the pattern noted at an instant in the life of the host, without cognizance of either age of the infection or the number of parasites per host, or previous history of infection with *C. emasculator*. Presumably, the host response changes as the parasites mature and leave the host, and is dynamic rather than static.

Chipmunks infected with small *C. emasculator* larvae were maintained in captivity and the blood examined at weekly intervals until the parasites matured and the lesions in the host completely healed. To determine the effect of the number of cuterebrids per host on the blood values, the chipmunks were divided into two groups; one with 1-3, the other with 4-14 parasites. The initial counts were made when the chipmunks were first trapped, the parasites then being 4-7 days old. The minimum or maximum value (Table II) was the lowest or highest figure obtained during the series of examinations. The column "after larvae dropped" (Table II) represents the values 1-2 weeks after the warbles had left the host.

The initial packed cell volume of the chipmunks with 1-3 larvae (Table II) was not markedly abnormal. Even the minimum packed cell volume was not markedly lower. The final packed cell volume was the same as that for the normal chipmunks. A more striking difference was noted in the leucocyte counts where the initial reading was twice normal and the maximum average reading was three times the normal values. The leucocyte counts made after the larvae left the host averaged higher than normal

TABLE I Haematological values of infected and non-infected chipmunks of different sex and age.

		Percent erythrocytes	Haemoglobin	Erythrocytes/mm³ (millions)	Leucocytes/mm³	Mean no. larvae/host
Adult males	neg.	50.8(41-55)/ 7*	18.2(17 -19)/ 5	10.44(9.4-12.3)/ 4	7,185(3-10000)/14	—
	pos.	50.4(45-48)/ 5	18.5(-20)/ 4	9.51(8.0-10.2)/ 4	9,900(6-16000)/ 6	1.5
Juvenile males	neg.	49.0(40-55)/17	16.5(14-19.5)/ 9	8.36(6.0-10.2)/10	3,280(1100- 7600)/11	—
	pos.	43.6(26-50)/ 9	15.8(13-18.3)/ 7	7.61(6.2- 8.7)/ 7	10,000(7000-23000)/15	3.1
Adult females	neg.	49.9(43-57)/ 9	17.5(16.5-20)/ 6	9.84(8.2-10.4)/ 5	5,270(1500- 8400)/ 6	—
	pos.	49.1(48-50)/ 2	20.0	10.90	6,600 / 2	1.0
Juvenile females	neg.	47.5(37-55)/15	17.6(15-20)/13	8.78(7.6-10.4)/11	7,090(4300- 9000)/ 7	—
	pos.	44.6(27-50)/ 8	16.4(13-20)/ 6	8.45(7.1-11.1)/ 6	14,100(5600-25000)/12	2.2
Overall	neg.	49.0(37-57)/48	17.4(14-20)/33	9.30(6.0-11-1)/30	5,280(3000-10000)/28	—
	pos.	45.7(27-50)/24	16.7(13-20)/15	8.45(6.2-11.1)/18	11,200(5600-25000)/35	2.5 (1-14)

neg. = chipmunks without recent/current infections of *C. emasculator*.

pos. = chipmunks with current larval infections of *C. emasculator*.

* 50.8(41-55)/7 = mean of 50.8%, range of 41-55%, based on 7 observations.

TABLE II Effect of different numbers of **C. emasculator** at different times during the course of the infection on the packed red cell volume and leucocyte counts of chipmunks.

	Packed red cell vol. %			Leucocytes/mm³			
	Initial count	Minimum count	Larvae dropped	Initial count	Maximum count	After larvae dropped	Mean No. larvae
18 *Tamias* with 1-3 larvae	47.3 (35-56)	41.7 (27-49)	50.6 (45-59)	11,460 (8,000-22,000)	18,800 (9,000-44,000)	8,950 (3,000-22,000)	1.6
7 *Tamias* with 4-14 larvae	39.0 (26-50)	26.0 (12-34)	48.4 (46-52)	20,650 (11,000-27,000)	26,600 (17,000-41,000)	12,500 (6,000-20,000)	7.0
48 Uninfected *Tamias*	49.0 (37-57)	—	—	5,280 (3,000-10,000)	—	—	—

and probably reflected, in part, the purulent phase which usually occurred in the lesion.[3,6,7] The possible role of bacteria in this purulent phase is unknown. However, since the fibrotic capsule surrounding the warble is open to the exterior for the life of the parasite, and remains open for 1-2 days following the departure of the mature third instar larva, one assumes that bacteria may be secondary invaders. Why the lesions do not become highly suppurative during the period that the larva inhabits them is an enigma, for Landi[9] has shown that the excreta of larval *C. emasculator* is neither bacteriostatic nor bacteriocidal. In some way the presence of the larva may inhibit bacterial growth.

Benjamini and Feingold,[2] in summarizing immunity to myiasis-producing Diptera, have shown that the host animals develop antibodies to the excreta and haemocoel fluids of second and third stage larvae but not to first instar forms. This antibody formation could be the explanation of the greatly increased white blood cell count noted (Table II). No study was carried out to determine if such antibody formation occurred. However, it has been the general experience [3,5,6,7,8] that natural hosts of cuterebrids do not develop a strong immunity to the parasite, for the same individual host can be repeatedly re-infected, all the parasites apparently developing normally in the usual developmental time period. Thus, if antibody formation does occur, it may be of little protective value to the host.

The haematological values of chipmunks harboring four or more parasites showed marked differences from the normal. The initial packed cell volume (Table II) was considerably lower, the average minimum level approximately one-half that of normal. Two animals, which subsequently died, had packed cell volumes of one-quarter the normal values. It was clear that infections of four or more larvae could result in severe anaemia. Surviving chipmunks, however, quickly recovered from this anaemia and the final packed cell volume was approximately normal. Compared to the striking differences noted in the red cell values of chpimunks with four or more larvae, the presence of many warbles did not noticeably influence the leucocyte count. Although the averages were higher, the ranges were similar. The reasons for the marked anaemia displayed by chipmunks harboring four or more larvae is unknown. The larvae of several species of *Cuterebra,* including *C. emasculator,* are not known to feed on blood,[3,6,7,8] but significantly lowered haemoglobin concentrations and marked anaemias were also reported by Sealander[11] for *Peromyscus* infected with *Cuterebra.*

Changes in the differential leucocyte count were determined by examination of smears taken at weekly intervals. Although a greater frequency would have been desirable, it was early discovered that sampling blood at bi- or tri-weekly intervals led rapidly to acute anaemias and hence to experimental distortion of the haematological values. As the hosts harbored different numbers of larvae at different stages of development, the results were treated on an individual basis and a generalized pattern was subsequently obtained. The data for six typical hosts are presented in Table III.

The types of leucocytes vary in proportion throughout the period that the parasite is present in the host (Table III). Generally, when the chipmunk was first obtained and the parasites were small, lymphocytes were in the majority, but their proportion decreased as the parasites matured. After the larvae left the host, the lmphocytes again increased and became the most numerous white cell. In early infections, neutrophils were low in numbers but midway through the developmental period of the parasite, they increased sharply, then decreased as the larvae matured. Conversely, the monocytes increased during the second half of the infection period. Some of the chipmunks (Table III) were obtained either at or just after, the peak numbers of neutrophils. The maximum number of leucocytes per mm^3 was correlated with this peak. After the larva had left the host and the lesion became purulent, the leucocyte count did not increase to the level observed during the early stages of infection.

Cuterebra emasculator is in the first and second instars during the early part

TABLE III Haematological values of host during course of infection with **C. emasculator.** (Hosts naturally infected, parasites at different stages of development at start of counts).

Counts on day	Parasites	% packed red cell volume	Leucocytes/mm³	Percent Leucocytes*		
				Monocytes	Lymphocytes	Neutrophils
0	1(2nd instar)	50.0	13280	37	42	21
7	1	49.0	21640	29	33	38
14	sup.	55.0	11720	30	44	26
21	sup.	58.0	8520	46	42	12
0	1(3rd instar)	47.5	9740	47	45	8
7	sup.	50.5	7680	42	40	18
14	sup.	51.5	7080	33	32	35
21	sup.	52.0	6040	42	27	29
28	healed	48.0	7440	37	37	26
35	healed	49.5	7000	15	69	16
0	2(2nd instar)	52.5	7920	26	59	15
7	2	50.0	9200	22	27	51
14	2	42.5	7760	15	30	55
21	sup.	56.0	2320	15	59	26
35	sup.	52.0	3280	8	56	36
42	healed	55.0	4000	8	82	10
0	3(3rd instar)	36.0	22920	40	35	25
7	1 + sup.	52.5	17160	40	46	14
14	1 + sup.	53.0	11440	36	39	25
21	sup.	59.0	9480	44	45	11
28	healed	57.0	5240	44	45	11
0	5(3rd instar)	46.5	25600	25	32	43
7	5	44.5	27600	51	24	25
14	1 + sup.	20.0	21600	40	51	9
21	sup.	36.0	15840			
28	healed	44.5	15660	20	70	10
35	healed	48.5	14080	24	58	18
42	healed	50.0	10800	26	56	18
0	6(3rd instar)	25.5	25240	33	37	30
7	4	31.5	18040	23	45	33
14	sup.	39.5	14600	31	34	35
21	sup.	46.0	7120	41	40	19
28	healed	47.5	8000	20	60	20
Average for 37 infected chipmunks		45.5	11200	27	38	34
Average for 48 non-infected chipmunks		49.0	5280	14	58	28

* eosinophils and basophils less than 1% of differential.
sup. = suppurating warble lesions.
healed = healed warble lesions.

of the infection. The first instar is probably of short duration,[3] but the second requires 8-10 days for development. Most of the blood changes noted seemed to take place during this first 8-10 days. Whether or not the second instar is directly responsible for such changes awaits further study.

EFFECT OF THE PARASITE ON HOST ACTIVITY

Previously it was reported[3] that infections of three to four *C. emasculator* larvae did not seriously hamper the chipmunk's activity. This conclusion was based on direct observations on animals turning somersaults in their cages. These observations were further tested by comparing the activity of chipmunks before or after infection in standard rat activity wheels. Activity of these diurnal animals was evaluated by the number of revolutions of the wheel per hour during daylight hours (approximately 10-11 hours per day). The activity of each animal, either naturally or experimentally infected, was noted for a period either before infection or after the larvae had left, to obtain a "normal" activity for that individual. Four chipmunks were tested, each for at least 28 days. The activity of the first three days was discounted as this represented the period during which the animal adjusted to captivity and/or to the wheel.

Chipmunks #1 and #2 (Fig. 1) were naturally infected and carried second instar larvae at the initiation of the test. During the growth of the second stage and early third instar, the activity of both animals was high. At the time the larvae left the host, however, the activity dropped markedly. This low level of activity was

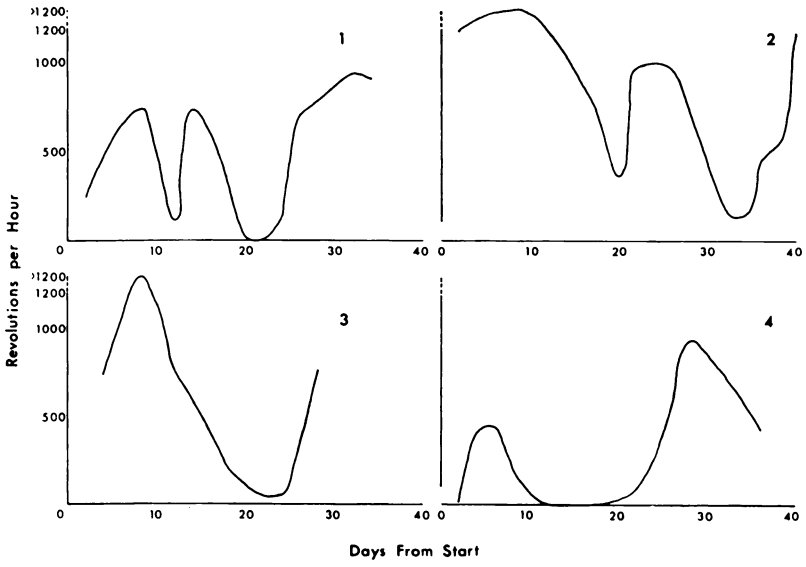

Figure 1. Patterns of activity shown by four chipmunks infected with **C. emasculator.**

Chipmunk No. 1. Naturally infected with two 2nd instar larvae at start of test.

Chipmunk No. 2. Naturally infected with five 2nd instar larvae at start of test.

Chipmunk No. 3. Naturally infected with three 3rd instar larvae, experimentally infected with two 2nd instar larvae. Total: 5 larvae.

Chipmunk No. 4. Experimentally infected with six 2nd instar larvae.

maintained during the period that the lesions were purulent. When the lesions were healing, the activity returned to a high level. The period of inactivity is particularly marked in chipmunk #2.

Chipmunks #3 and #4 were experimentally infected with second instar larvae. The lesions of these animals did not become purulent after the larvae left. Activity of chipmunk #3 was similar in pattern to that of #1 and #2, but not as pronounced. The activity of chipmunk #4 was markedly lower following transfer of larvae,[3] presumably due to post-operative shock. The activity of this chipmunk was also lower during the period that the larvae were leaving it but this individual showed the least response of the four.

Other observations of a qualitative nature were made on caged animals and support the previous observations. Generally, animals with four or more larvae showed marked reduction of activity during the time the parasites were leaving the host. Two animals with 9 and 14 larvae respectively (natural infection) died at the time the first two larvae to mature were leaving. The inactivity of these heavily infected animals was accompanied by reduction in food intake and increased consumption of water. This was particularly prominent in the two animals cited above. These chipmunks, for the week prior to their death, were drinking 50-100 ml of water per day, about 10 times the intake of non-infected animals. At the same time, these two animals virtually stopped feeding; at death they weighed one-half to two-thirds their original weight when captured.

The results showed that the response of chipmunks to infections with *C. emasculator* was variable. However, the results indicated that the major decrease in activity occurred at the time the larvae were leaving the host and the lesions became purulent, not during the initial development of the parasites. Purulent lesions were normal in naturally infected animals, and it might be expected that infected chipmunks in the wild population would show some reduction in activity at this time. The degree of reduction in activity and the period through which it lasted undoubtedly varied both with the individual and with the extent of the infection.

Observations on the activity and feeding of infected chipmunks indicated that heavily infected animals suffered severely although animals with one to three warbles were relatively unaffected. The effect of heavy infections of *C. emasculator* on a chipmunk population are potentially severe. Some animals harboring numerous warbles may be killed. Less heavily infected animals might be less active and this could result in increased vulnerability to predation. Both factors operating in conjunction could reduce the chipmunk population. Such reduction, however, would not be uniform over the chipmunk population in a region, for it has been shown[4] that chipmunks in specific habitats, such as second-growth forests, harbor 2-3 times as many warbles as those in habitats such as a mature coniferous forest. It would be anticipated, therefore, that chipmunk populations in forest habitats favored by the warble would be most prone to adverse effects from *C. emasculator*.

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