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EFFECTS OF PARASITISM ON SELECTED PHYSIOLOGICAL MEASUREMENTS OF THE COTTONTAIL RABBIT[1]

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Abstract: Thirty-one penned cottontail rabbits (*Sylvilagus floridanus*) were used in a 2 x 2 factorial experiment to determine the effects of metazoan parasitism on selected physiological parameters of the host. The two treatments used were stomach tube administration of a broad spectrum anthelmintic and application of insecticide collars. Drug treatment caused significantly reduced numbers of nematodes, but had no noticeable effects on cestode or trematode numbers. No ectoparasites were recovered from insecticide collar-treated rabbits and only low numbers were recovered from the remaining experimental rabbits. Total serum proteins, serum globulin fractions, lymphocyte percentages, and monocyte percentages were significantly lower and neutrophil percentages were significantly higher in drug treated groups. Body weights, fat indices, selected organ weights, packed cell volumes, and basophil percentages were not significantly affected by the treatments employed.

INTRODUCTION

A sizable body of literature exists on the parasites of the cottontail rabbit. However, there are few reports on the effects of parasites on this host species. The more significant of these have associated parasitism with population decline,[16] mortality,[7,11,19] malnutrition,[6,7,13] increased white blood cell count,[7] damage to vital body organs,[2] and decreased reproduction.[18] The purpose of this investigation was to determine if metazoan parasites could be experimentally reduced in a semi-natural situation and, if so, to determine relationships between these parasites and selected physiological measurements of their host.

MATERIALS AND METHODS

Forty-seven cottontail rabbits were originally assigned to one of four treatment procedures in a 2 x 2 factorial experiment. Procedure number 1 consisted of the administration of the anthelmintic, 1-tetramisole hydrochloride (Tramisol, American Cyanamid) and insecticide collars containing 2, 2-dichlorovinyl, dimethyl phosphate (Sergeant's Sentry Dog Collar). The drug was administered in distilled water solution by syringe and stomach tube at a dosage of 8 mg per kg of body weight. Insecticide collars were applied as described by Jacobson and Kirkpatrick.[9] Procedure number 2 involved the application of insecticide collars and placebo treatments to nine males and three females. Placebo treatment consisted of administration of distilled water by syringe and stomach tube. The third procedure used was the administration of the drug and insecticide-free collars to nine males and three females. Insecticide-free collars were similar in size and shape to insecticide collars. The fourth procedure was the administration of placebo and insecticide-free collar treatments to eight males and three females.

Rabbits were trapped on the Radford Army Ammunition Plant, Montgomery

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[2] Department of Fisheries and Wildlife Sciences.

County, Virginia, between September 15, 1972, and October 14, 1972. Prior to treatment, they were weighed, sexed, grossly examined for ectoparasites, and tagged with metal ear tags. A heparinized tube of blood also was collected from a marginal ear vein puncture for packed cell volume determination. Rabbits were randomly assigned by sex to the four treatment procedures. Immediately after treatment, each rabbit was placed in one of four 0.1 hectare pens which had been randomly assigned to receive a particular treatment group. Commercial rabbit pellets and water were supplied *ad libitum* at several feeder stations placed within the pens. Ninety days after treatment and introduction of the first animal, all remaining rabbits were recovered by trapping.

Blood slides were made, and packed cell volumes were determined from blood collected by marginal ear vein puncture. Additional blood for serum protein determination was collected by cardiac puncture. Rabbits and their ectoparasites were killed with chloroform, and ectoparasites were then collected. Animals were next weighed and necropsied. Endoparasites free in the body cavity or attached to the mesenteries were recovered. The amounts of renal, abdominal and mesenteric fat were classified by describing these as heavy, medium, light, or none. Respective numerical values of 3, 2, 1, and 0 were assigned to each of these classes. Renal, abdominal and mesenteric fat values were then summed for each rabbit for statistical analysis. Adrenals, kidneys, spleen, liver, and eyes were collected and placed in 10% formalin. The stomach, small intestine, caecum, and large intestine were separated and examined for helminths by the gravity flotation technique of Clancy et al.[6] Only those helminths visible to the naked eye were recovered.

Packed cell volumes were determined by use of an Adams Readocrit centrifuge. Differential cell counts were made by counting 200 leukocytes under an oil immersion lens. Eosinophils, if encountered, were included in the neutrophil percentage. It is difficult to distinguish the eosinophil of the cottontail from the neutrophil since the rabbit neutrophil has eosinophil-like characteristics.[15] In addition, eosinophils described in the literature for the cottontail rabbit[17] differ from those which have been reported for the domestic rabbit.[15] As a result, we did not attempt to differentiate this cell type.

Total serum protein and albumin and globulin fractions were determined as described by Bausch and Lomb Incorporated.[4] This procedure uses the Biuret assay to determine total protein, and globulin/albumin fractions are determined by precipitating out the globulin. Eye lens weights were determined as described by Rongstad.[14] Analysis of variance testing was conducted through the use of a computerized program for least squares analysis of data with unequal subclass numbers by Walter R. Harney, Biometrical Services, USDA, Plant Industry Station, Beltsville, Maryland.

RESULTS

Thirty-one (23 males and 8 females) of the original 47 rabbits were recovered from the pens at the end of the treatment period. Ten rabbits died during the treatment period and six rabbits could not be accounted for. Six of the 10 deaths were known or suspected predator kills. One rabbit was badly decomposed when found and the cause of death was not determined. The three remaining deaths have been described.[10]

The mean number of nematodes and cestodes recovered from each group are given in Table 1. Treatment with l-tetramisole hydrochloride resulted in a significant ($P < 0.05$) reduction in the number of nematodes recovered from the stomachs and a highly significant ($P < 0.01$) reduction in the total number of nematodes recovered. There were no significant differences in numbers of cestodes recovered. Four species of Nematoda (*Obeliscoides cuniculi, Trichuris leporis, Trichostrongylus* sp., and *Dermatoxys* sp.) and two species of cestodes (*Cittotaenia* sp. and cysticerci of *Taenia pisiformis*) were identified. One species of Trematoda (*Hasstilesia tricolor*) was noted in every rabbit. Because of its

TABLE 1. Mean numbers of metazoan parasites recovered from 31 penned rabbits.

Treatment	Number of rabbits	Nematodes					Cestodes		Ectoparasites
		Stomach[a]	Small intestine	Large intestine	Caecum	Total[b]	Small intestine	Body cavity (cysticerci)	Total[c]
Insecticide collar + Drug	8	0.9	0.0	0.0	0.4	1.2	3.0	11.0	0.0
Insecticide collar + No drug	5	5.0	1.0	0.6	1.0	7.6	3.0	11.6	0.0
Insecticide-free collar + Drug	10	1.8	0.0	0.6	1.3	3.7	5.4	20.3	0.7
Insecticide-free collar + No drug	8	4.5	0.0	0.1	0.7	5.4	4.1	10.1	0.7

[a]Drug vs. No drug significantly different at $P < 0.05$

[b]Drug vs. No drug significantly different at $P < 0.01$

[c]Insecticide collar vs. Insecticide-free collar significantly different at $P < 0.01$

small size no attempt was made to recover or count numbers of this parasite. We have previously reported on effects of this parasite on its host.[10]

Ticks or fleas were noted on 34 of the 47 rabbits initially introduced into the pens. Actual prevalence rates may have been higher than this, since in order to provide minimum disturbance to these parasites, close examination of the skin and pelage was not attempted. At the termination of the experiment, no ectoparasites were recovered from the insecticide collar-treated groups. In contrast, 10 out of 18 of the insecticide-free collar-treated rabbits had ticks or fleas. However, few ticks and fleas were recovered from these rabbits (Table 1). Ectoparasites identified in the investigation included one dipteran (*Cuterebra buccata*), two Siphonaptera (*Cediopsylla simplex* and *Odontopsyllus multispinosus*) and two Acarina (*Ixodes dentatus* and *Haemaphysalis leporispalustris*). *Cuterebra* larvae were only noted during the initial collection of rabbits and the emergence scars of the parasite were well healed with no signs of secondary infection at the termination of the experiment. Fourteen of the 47 rabbits were infected with from one to three larvae at the time of introduction in the pens.

Mean hematological measurements collected are given in Table 2. Total serum proteins, serum globulin fractions, lymphocyte percentages, and monocyte percentages were significantly ($P < 0.05$) lower and neutrophil percentages were significantly ($P < 0.05$) higher in drug-treated groups. There were no significant differences between groups for mean eye lens weights, body weights, adrenal weights, kidney weights, spleen weights, liver weights, or body fat indices. Eye lens weights are used to indicate age of the cottontal rabbit[14] and were recorded to insure no age difference existed between the treatment groups.

No significant interactions were present between treatments for any of the variables measured. Because the number of females recovered did not allow sufficient degrees of freedom to analyze the data by sex, only the combined data for both sexes has been presented. However, a separate analysis was conducted on the males alone which revealed results almost identical to those reported above.

DISCUSSION

The two treatments employed were effective in reducing parasite numbers. Drug treatment significantly reduced nematode numbers and insecticide collars completely eliminated ectoparasites on treated rabbits.

Since we recovered only those helminths which were visible to the naked eye, the effectiveness of the drug in reducing numbers of small or larval nematodes was not determined. That the drug was effective against adult stomach worms (*O. cuniculi*) can be seen by the significant reduction that occurred in the numbers of these (Table 1). Adult nematodes recovered in the large intestine and caecum (*T. leporis* and *Dermatoxys* sp.) were not significantly reduced by drug treatment. The intestinal worm (*Trichostrongylus* sp.) was recovered only from two of the placebo-treated rabbits. Because of the small size of this worm, its presence in other rabbits could possibly have been overlooked.

The low infestations of ectoparasites found on insecticide-free collar-treated rabbits were unexpected. Ten wild rabbits collected in Montgomery County, Virginia, at the same time that the penned rabbits were sacrificed, had mean tick or flea infestations of 21.2 (Jacobson, unpublished data). This compares to a mean of 0.7 per rabbit for the 18 insecticide-free collar-treated rabbits and leads us to believe that the environment of the pens or the proximity of insecticide collar-treated rabbits in adjacent pens may have limited infestations of the insecticide-free collar-treated rabbits.

The significant differences in hematological measurements of the drug treated rabbits may be due to the response of humoral and cellular immune systems. Drug treatment alone would not account for the differences, since l-tetramisole hydrochloride is metabolized and eleminated at a rapid rate and residues are

TABLE 2. Mean hematological measurements from 31 penned cottontail rabbits.

Treatment	Number of rabbits	Packed cell volume (percent) initial	Packed cell volume (percent) final	Serum protein (gm/100 ml) total[a]	Serum protein (gm/100 ml) alb.	Serum protein (gm/100 ml) glob.[a]	Differential cell count (percent) neut.[a]	Differential cell count (percent) lymp.[a]	Differential cell count (percent) mono.[a]	Differential cell count (percent) baso.
Insecticide collar + Drug	8	44.3	44.4	6.5[b]	4.0[b]	2.5[b]	57.9	31.3	9.2	2.0
Insecticide collar + No drug	5	44.2	44.8	8.3	4.9	3.4	37.5	48.6	12.3	1.7
Insecticide-free collar + Drug	10	43.1	45.8	5.4	3.4	2.0	54.0	34.7	8.4	3.4
Insecticide-free collar + No drug	8	44.5	46.2[b]	7.8[b]	4.0[b]	3.8[b]	41.2[b]	45.1[b]	12.6[b]	1.8[b]

[a]Drug vs. No drug significantly different at $P < 0.05$

[b]One less observation than shown

TABLE 3. Mean eye lens weights, body weights, organ weights, and fat indices of 31 penned cottontail rabbits.

Treatment	Number of rabbits	Eye lens (mg)	Body initial (g)	Body final (g)	Paired adrenals (mg)	Paired kidneys (g)	Spleen (mg)	Liver (g)	Body fat[a] index
Insecticide collar + Drug	8	238	1223	1152	242	9.0	968	32.3	3.75
Insecticide collar + No drug	5	221	1158	1154	250	9.2	756	35.3	6.60
Insecticide-free collar + Drug	10	229	1207	1213	253	9.0	939	34.5	5.50
Insecticide-free collar + No drug	8	260	1317	1218	270	9.1	909	35.2	4.25

[a]Fat index for each rabbit was calculated by assigning a value of 0 - 3 to each of 3 fat categories (kidney, loin, and mesentary) and these values were then totaled.

nondetectable after a 3 day period.[1] The role of serum globulins and lymphocytes in immunological response is well described.[8] Increased serum globulins due to metazoan parasitism have been reported[3,12] and lymphocytosis and neutropenia are two of the chief effects of parasitic nematodes.[5] Because of this, we feel the significantly higher serum globulin fractions and lymphocyte percentages of placebo-treated rabbits reflect an immunological response to the significantly higher nematode infections which they harbored. The significantly lower neutrophil percentages of these same rabbits may indicate a relative neutropenia due to the higher percentages of lymphocytes and monocytes. The significantly higher monocyte percentages of the placebo-treated rabbits may indicate the need for macrophages to remove dead worms and necrotic tissue.

Although inclusion of eosinophils in the neutrophil count may have biased the neutrophil percentages obtained, bias of this nature might have reduced the actual statistical significance between neutrophil percentages of the drug and placebo-treated groups. Eosinophilia occurs commonly in some nematode infections,[5,8] and therefore it might be expected that the neutrophil percentages (as determined in this investigation) for the placebo-treated rabbits would be higher as a result. In addition, under normal conditions eosinophils constitute a small percentage of the leukocytes in peripheral circulation[15] and we would expect little alteration of neutrophil percentages if eosinophils were included in the neutrophil count.

The significant differences in total serum protein of the placebo and drug-treated rabbits appear to be due to the differences in serum globulin fractions of these rabbits. This statement is supported by the fact that serum albumin fractions were not significantly affected by the treatments given (Table 2).

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LITERATURE CITED

1. AMERICAN CYANAMID COMPANY. 1971. Ripercol L. injectable and soluable powder anthelmintic for cattle and sheep. Princeton, N.J.
2. ANDREWS, C. L. 1969. Parasitism and other disease entities among selected populations of cottontail rabbits (*Sylvilagus floridanus*). Ph.D. Dissertation, Univ. of Georgia, Athens.
3. ALJEBOORI, T. I. and M. H. IVEY. 1970. *Toxocaria canis* infection in baboons, antibody, white-cell, and serum-protein responses following infection. Am. J. Trop. Med. & Hyg. 19: 249-254.
4. BAUSCH AND LOMB INCORPORATED. 1965. Clinical methods manual, Spectronic 20. Rochester, N.Y.
5. BELDING, D. L. 1965. Textbook of parasitology. Appleton-Century-Crafts, N.Y., N.Y.
6. CLANCY, C. F., E. JUNGHERR and P. R. SINE. 1940. Internal parasites of cottontail rabbits in Connecticut. J. Wildl. Manage. 4: 152-168.
7. GEIS, A. D. 1957. Incidence and effect of warbles on southern Michigan cottontails. J. Wildl. Mgmt. 21: 94-95.

8. GUYTON, A. C. 1971. Textbook of medical physiology. W. B. Saunders Co., Philadelphia.

9. JACOBSON, H. A. and R. L. KIRKPATRICK. 1973. The use of insecticide-generating collars for the investigation of parasitic disease in wildlife populations. Proc. S. E. Assoc. Game and Fish Comm. 27 (In Press)

10. ————, ———— and R. B. HOLLIMAN. 1974. Emaciation and enteritis of cottontail rabbits infected with *Hastilesia tricolor* and observations on a fluke to fluke attachment phenomenon. J. Wildl. Dis. 10: 111-114.

11. McGINNES, B. S. 1964. Parasites of cottontail rabbits in southwestern Virginia. Wildl. Dis. 35 (on microcard).

12. PAYNE, J. A., P. B. DUNAWAY, G. D. MARTIN and J. D. STORY. 1965. Effects of *Cuterebra angustifrons* on plasma proteins of *Peromyscus leucopus*. J. Parasit. 51: 1004-1008.

13. PELTON, M. R. 1968. A contribution to the biology and management of the cottontail rabbit (*Sylvilagus floridanus mallurus*) in Georgia. Ph.D. Dissertation. Univ. of Georgia, Athens.

14. RONGSTAD, O. J. 1966. A cottontail rabbit lens-growth curve from southern Wisconsin. J. Wildl. Mgmt. 30: 114-121.

15. SCHALM, O. W. 1965. *Veterinary Hematology*. Lea and Febiger, Philadelphia.

16. SMITH, R. H. and E. L. CHEATUM. 1944. The role of ticks in the decline of an insular cottontail population. J. Wildl. Mgmt. 8: 311-377.

17. VANDE VUSSE, F. J. 1964. Cell numbers and hemoglobin content of the blood of cottontails in Iowa. M.S. Thesis. Iowa State Univ. Ames.

18. YUILL, T. M. 1964. Effects of gastrointestinal parasites on cottontails. J. Wildl. Mgmt. 28: 20-26.

19. ———— and J. L. ESCHLE. 1963. Myiasis of penned nestling cottontails. J. Wildl. Mgmt. 27: 477-480.

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