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SELECTED CLINICOPATHOLOGIC CHANGES ASSOCIATED WITH EXPERIMENTALLY INDUCED *Fascioloides magna* INFECTION IN WHITE-TAILED DEER [1]

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Abstract: Six white-tailed deer *(Odocoileus virginianus),* less than one year of age, were divided into two groups of three each and administered 50, or 500 metacercariae of *Fascioloides magna.* All six deer became infected. Three additional deer of the same age were uninoculated controls. All deer were monitored for up to 43 weeks after inoculation to investigate changes in weight, selected hematologic values, and blood chemistry values. Although clinical disease was not evident in the infected deer, a significant reduction ($p < .01$) in hemoglobin and packed cell volume was detected throughout the experiment. A significant elevation ($p < .01$) in the total serum protein level was detected in both infected groups from 0 to 5 months after inoculation. Increases were present in the beta and gamma globulin fractions. No differences ($p > .05$) were detected in the serum calcium, magnesium, or phosphorus levels, or in body weights between infected and uninfected control groups.

INTRODUCTION

The pathogenicity of the large American liver fluke, *Fascioloides magna,* is not well-known although this trematode is encountered in a variety of ungulates.[1,4-7,9,10,14] White-tailed deer *(Odocoileus virginianus)* and wapiti *(Cervus c. canadensis)* are considered to be normal definitive hosts and little pathology has been documented in infected animals.[8,13,20] Mature trematodes are enclosed within fibrous hepatic capsules in the hepatic parenchyma and perivascular inflammation generally is not detectable. Effects of hemorrhage and inflammation due to *F. magna* have not been thoroughly investigated.

Most reports of *F. magna* in white-tailed deer have been based on host surveys and postmortem examinations, but there are few reports of controlled experimental infections.[8] This paper presents the results of experimentally induced *F. magna* infection in white-tailed deer.

MATERIALS AND METHODS

Experimental Animals

The white-tailed deer were reared at the University of Wisconsin's Charmany and Reider Experimental Research Facilities and maintained on a diet of pelleted hay, grain and loose hay.

Nine white-tailed deer fawns, approximately 9 months of age, were monitored in three 6 m × 6 m isolation units, each containing three animals. All nine animals were treated with phenothiazine (300 mg/kg body weight) to eliminate nematodes; subsequent fecal examinations were negative for nematode eggs.

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Deer were bled weekly for 4 weeks to establish normal hematologic values before inoculation with infective metacercariae. For ease in weighing and bleeding, animals were immobilized with an intramuscular injection of succinylcholine chloride at the rate of 0.11 mg/kg. Immobilized deer were weighed to the nearest 0.5 kg. Twenty ml of blood was drawn from the jugular vein at each bleeding. Five ml of heparinized blood was used for hemoglobin (Hb) and packed cell volume (PCV) determinations and 15 ml used for serum. Hb concentration was determined by the cyanmethemoglobin technique.[2] PCV was determined by standard microhematocrit technique; total serum protein (TSP) was determined using a Bausch and Lomb Protein Meter.[3] Serum glutamic oxaloacetic transaminase (SGOT) levels were measured using the Sigma reagent system for colorimetric assay.[15] Serum calcium (Ca), magnesium (Mg), and phosphorus (PO_4) were determined using colorimetric procedures adapted for an autoanalyzer. Electrophoresis of serum was performed at room temperature on cellulose acetate strips,[4] which were stained with Gelman Ponceau S. General Protein stain for 8 min. and rinsed three times in 10% acetic acid. The strips were immersed in 100% methyl alcohol (4 min.) and 90% methyl alcohol-10% acetic acid mixture (1 min.) for clearing, air dried, and scanned.[5] The alpha (α), beta (β), gamma (γ), and albumin fractions were each calculated in grams per deciliter (g/dl).

Inoculum

One deer in each of the three isolation units (Group I deer) was orally inoculated with a total of 500 metacercariae in gelatin capsules in three approximately equal doses over a period of one week. The second deer in each of the three isolation units (Group II deer) was orally administered 50 metacercariae in a gelatin capsule as a single dose. The third deer in each of the units (Group III - Controls) was administered a gelatin capsule containing water. Animals were bled and weighed twice monthly until the experiment ended 44 weeks after the initial inoculation. Fecal samples were collected at each blood collection period and examined for trematode eggs.[8]

Seven hematologic and biochemical parameters, and weight gains between inoculation groups were compared by the Student's t test method.[19]

RESULTS

During the preconditioning period, hematologic values and weight gains were similar for all deer (Table 1 and 2). Nematode eggs were not detected in the feces after treatment.

Three deer (one from each treatment group) died from causes not related to the experimental infections and were not included in the statistical results. One of the deer from Group I died 5 weeks postinoculation and two small immature trematodes were recovered from the liver. One deer from Group II died 15 weeks postinoculation and three immature trematodes were recovered from the liver. One deer from Group III died 13 weeks after administration of the water placebo; no trematodes were recovered. All experimentally-inoculated deer examined at necropsy contained trematodes. (Table 1). Clinical disease was not apparent in the infected animals.

[2] Spectronic 20, Bausch and Lomb Incorp., Rochester, New York.

[3] Bausch and Lomb Incorp., Rochester, New York.

[4] Sepraphore III, Gelman Instrument Co., Ann Arbor, Michigan.

[5] Gelman, Digiscreen Recorder and Gelman Digiscreen M Scanner, Gelman Instrument Co., Ann Arbor, Michigan.

TABLE 1. Weight gains, SGOT levels, and numbers of trematodes recovered from white-tailed deer experimentally infected with *Fascioloides magna*.

Parameter	Group Number* (No. Metacercariae)	Pre-Inoculation Values	Post-Inoculation Values	
			0-5 months	6-10 months
Weight	I (500)	.64 ± 0.9	.27 ± 2.1	.09 ± 2.7
Gains	II (50)	1.36 ± 0.9	.18 ± 1.9	.45 ± 1.3
(kg/month)	III (Controls)	.64 ± 0.9	.36 ± 2.4	.91 ± 1.5
	t-value**	.000	.190	.872
	Probability	n.s.	n.s.	n.s.
	I (500)	64.0 ± 31.3	50.4 ± 40.5	103.6 ± 78.0
SGOT	II (50)	52.8 ± 16.4	38.6 ± 36.0	82.0 ± 54.6
(SF units)	III (Controls)	50.1 ± 34.5	47.5 ± 35.4	86.3 ± 59.7
	t-value**	.727	.231	.621
	Probability	n.s.	n.s.	n.s.

	Weeks Post-inoculation	*Fascioloides magna* Immature	Mature	Recovered Total
	I 43	2	8	10
	(500) 34	10	6	16
Number	II 25	2	0	2
of	(50) 42	2	4	6
flukes	III 43	0	0	0
recovered	(Controls) 43	0	0	0

*two deer per group
**Group I vs. Group III

TABLE 2. Hematological findings in white-tailed deer experimentally infected with *Fascioloides magna*.

Parameter	Group Numbers* (No. Metacercariae)	Pre-Inoculation Values	Post-Inoculation Values	
			0-5 months	6-10 months
Hemoglobin (g/dl)	I (500)	19.0 ± 2.1	18.7 ± 1.8	16.2 ± 2.4
	II (50)	18.8 ± 4.4	18.9 ± 4.7	17.9 ± 4.1
	III (Controls)	19.5 ± 1.0	20.9 ± 1.8	19.5 ± 2.4
	t-value**	.701	4.240	4.102
	Probability	n.s.	<.01	<.01
Packed Cell Volume (%)	I (500)	48.7 ± 7.6	48.9 ± 4.5	43.2 ± 6.5
	II (50)	47.3 ± 10.0	48.0 ± 11.0	48.2 ± 8.4
	III (Controls)	48.9 ± 2.0	55.1 ± 6.5	52.0 ± 4.2
	t-value**	.177	4.135	4.209
	Probability	n.s.	<.01	<.01
Total Protein (g/dl)	I (500)	7.4 ± .3	8.3 ± .6	8.1 ± .5
	II (50)	7.7 ± .2	8.1 ± .5	7.7 ± .4
	III (Controls)	7.4 ± .2	7.5 ± .4	7.7 ± .4
	t-value**	.569	6.210	2.017
	Probability	n.s.	<.01	n.s.

*two deer per group
**Group I vs. Group III

The four deer remaining in Groups I and II appeared to be in good health at the end of the experiment, but had significantly lower Hb ($p < 0.01$) and PCV ($p < 0.01$) levels than the controls during postinoculation period (Table 2). Groups I and II also had significantly higher ($p < 0.01$) TSP levels 0-5 months after inoculation (Fig. 1, Table 2). Increases were present in the beta and gamma fractions (Fig. 1). No apparent differences were present in the albumin or alpha fractions. Weight gains and serum Ca, Mg, PO_4, and SGOT levels were not significantly different ($p > 0.05$) between groups during the experimental period (Table 1).

At necropsy, 10 and 16 *F. magna* were recovered from the two deer administered 500 metacercariae (Group I). Two and six *F. magna* were recovered from the two deer in Group II administered 50 metacercariae (Table 1).

Trematode eggs were detected in the feces of both deer in Group I and in the feces of one deer in Group II, approximately 32 to 34 weeks after exposure. Mature *F. magna* were recovered from all three deer at necropsy (Table 1).

The two control deer had similar hematologic values throughout the experiment. Trematodes were not recovered from the controls.

DISCUSSION

The preinoculation hematologic values for the deer in this study compared favorably with normal values for white-tailed deer.[12,22] Although the Hb and PCV were significantly reduced in the infected deer, indicating anemia, clinical disease was not apparent. *F. magna* has not been implicated previously as a factor in anemia. The acute and chronic anemias associated with *Fasciola*

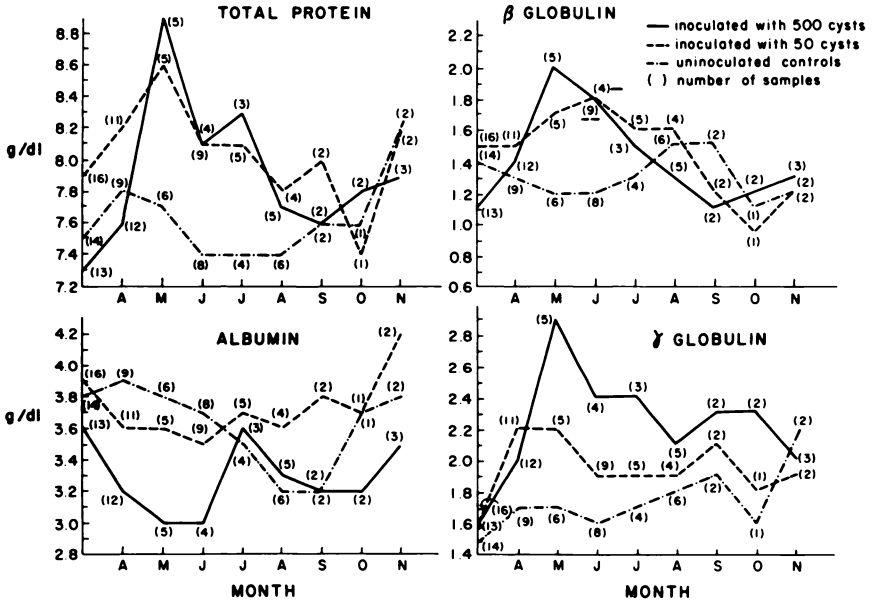

FIGURE 1. Total serum proteins, albumin, beta (β) and gamma (γ) globulins in white-tailed deer experimentally infected with *Fascioloides magna*.

hepatica infections in sheep are well documented and have been thoroughly investigated.[11,17,18,21]

In deer, both immature and mature *F. magna* inhabit the hepatic parenchyma.[8] Mature *F. hepatica* on the other hand, inhabit the biliary system of cattle and sheep, and chronic anemia can result by hemorrhage and blood loss via the bile ducts.[11] Symons and Boray[21] concluded that the anemia associated with migratory *F. hepatica* infection in sheep was primarily due to hemorrhage. In this study, it is presumed the anemia was caused by hemorrhage from immature *F. magna* migrating through and feeding upon the hepatic parenchyma. This migrating phase can persist for at least one year.[8]

Sinclair[17] reported a hypoalbuminemia and a rise in gamma globulin associated with *F. hepatica* infections in sheep. Hypoalbuminemia was attributed to impaired synthesis due to hepatic damage by migrating *F. hepatica*, and to increased blood loss to mature trematodes. It is possible the rise in beta and gamma globulin detected in this experiment with *F. magna* may be due to the presence of *F. magna* in the hepatic parenchyma and associated tissue destruction. However, the increase in gamma globulin also may be due to an immune response to *F. magna*.[16]

Nutrition also may affect the pathogenicity of *F. magna*. With *F. hepatica* infections in sheep, it has been reported that animals on a lower protein (6%) ration died earlier and developed more rapid anemia, hypoalbuminemia, and weight loss than infected animals on a higher protein (13%) ration.[2]

Environmental stresses could be important predisposing factors in fascioloidiasis. In New York, Cheatum[3] observed a higher number of *F. magna* in winter-killed deer than in apparently healthy deer collected from the same areas and suggested that with the stress of winter, the effects of the parasite may be more pronounced.

The sheltered and well-fed conditions under which the experimental animals were kept may have obscured some effects of *F. magna* infections in our deer. Similar experiments under field conditions will have to be conducted to prove this hypothesis.

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