8-1967

The Effect Of High Temperature On The Infectivity Of Hymenolepis Diminuta Cysticercoids In Albino Rats

Laura Evelyn Petty
Prairie View Agricultural and Mechanical

THE EFFECT OF HIGH TEMPERATURE ON THE INFECTIVITY OF HYMENOLEPIS DIMINUTA CYSTICERCOIDS IN ALBINO RATS

PETTY

1967

THE EFFECT OF HIGH TEMPERATURE ON THE INFECTIVITY OF *HYMENOLEPIS DIMINUTA* CYSTICERCOIDS IN ALBINO RATS

By

Laura Evelyn Petty

Submitted in Partial Fulfillment
of the Requirements for the

Master of Science Degree
in the
Department of Biology
in the
School of Arts and Science

Prairie View Agricultural and Mechanical
College of Texas

August, 1967

This thesis for the Master of Science Degree
has been accepted and approved
by and for the Department of
Natural Science

J. E. Berry
Advisor

[redacted]
Head of Department

August 8, 1967
Date

ACKNOWLEDGEMENTS

The author wishes to thank Dr. J. E. Berry, Department of Biology, Prairie View Agricultural and Mechanical College, for his unwavering counseling throughout this investigation. She is grateful to the Store Room Manager, Mr. Mathis and his assistants for their cooperation during the experimental period.

L.E.P.

DEDICATION

With tender love and affection,
this paper is dedicated to my husband,
James E. Petty, Sr., and my three sons,
Edward, James and William.

L.E.P.

TABLE OF CONTENTS

STATEMENT OF PROBLEM

The purpose of this problem is to determine the effect of high temperature stress on the infectivity of *Hymenolepis diminuta* cysticercoids in Albino rats.

INTRODUCTION

Research on the effect of temperature on cysticercoid development of Hymenolepis diminuta was performed by Voge and Heyneman (1958). They found that infectivity varied with the length of exposure, sensitivity period, and temperature. The rate of infectivity was slightly lower for cysticercoids developed at 37°C for long periods of time. Approximately 60% of the cysticercoids exposed for six days at 37°C developed in the final host, while only about 15% of the cysticercoids exposed for eight days at the same temperature proved to be infective. All of the experiments with cysticercoids were performed with the larvae inside the intermediate host, Tribolium confusum.

Heyneman (1958) exposed cysticercoids of H. nana to temperatures between 10°C and 42°C. At 41°C and 42°C, development of the cysticercoids was completed in 7 days, but infection of the final host was not obtained. Cysticercoids matured at the maximum rate between 37°C

and 40°C. At these temperatures, the rate of infectivity is at the greatest level obtainable and required four days. A large increase in developmental time occurred at lower temperatures, varying from 11 days at 25°C to more than 50 days at 15°C. H. nana is well adapted to the higher temperatures studied and poorly adapted to the lower range in contrast to the development of the rat tapeworm, H. diminuta. In H. nana, development can occur at a temperature as high as 42°C, but with H. diminuta, completion of development is inhibited at 38.5°C. In H. diminuta, inhibition occurs early, but does not become apparent until the organism is further developed. When conditions are normal, H. diminuta develops into an infective cysticercoid in 8 days.

Studies by Voge and Heyneman (1958) have shown that growth under high temperatures could delay development of H. diminuta. Structural abnormalities occur in H. diminuta larvae when exposed to supraoptimal temperatures at 38.5°C and 40°C. Growth is completely inhibited at 40°C. The structural abnormalities are; fail-

ure of scolex withdrawal, asymmetry of the cysticercoid with abnormal distribution of some component parts and extreme fragility and transparency of the larva. When cysticercoids develop normally, the scolex is withdrawn into the cysticercoid cavity. They exhibit bilateral symmetry and the body is translucent. The outer membrane is well developed, and the distribution of the component parts is normal. However, Voge and Turner (1958) found that scolex withdrawal has been observed in all larvae grown at this temperature throughout the developmental period.

Voge and Turner (1956) reported that cysticercoid development of H. diminuta may proceed normally at temperatures as high as 37°C, but the optimal temperature is 30°C. They observed that scolex withdrawal has occured in some developing cysticercoids grown for an 8 day period at 37°C. Scolex withdrawal is highest when exposure occurs for a 24 hour period on the third and fourth day of an eight day developing period, but the infectivity of the larvae is reduced. If exposed for 24 hours between the fourth and fifth day, larval structures are affected only to a

slight degree but infectivity to the final host is still inhibited.

Voge (1959) stated that eggs of H. diminuta develop into infective cysticercoids in 8 days and that the major amount of growth and development of the larva occurs between the second and sixth day. If growing larvae are exposed for 6 hours at 40°C at any state of their development, apparently no deleterious changes are produced. If during the first and second day of an eight day developmental period, the larvae are exposed to 38.5°C or 40°C for a 24 hour period, infectivity is not appreciably affected. Voge also determined the effect of high temperature on fully developed normal cysticercoids by first exposing beetles containing larvae developed at the optimal temperature of 30°C for an eight day period, and then reexposing them to 40°C for a four day period. The cysticercoids were then dissected out in the usual manner, examined microscopically and fed to rats. Not only were all of the larvae structurally normal, but as seen from an autopsy of rats 20 days later, they were also as infective

as the untreated controls. This clearly demonstrates that the influence of high temperature which is deleterious during certain critical stages of development may be harmless for the fully developed organism.

Research has also been conducted on the effect of temperature on invertebrate instars. Precht, et. al., (1955), summarized the general effects of high temperature on the development of some insects and stated that adverse effects are greatest when exposure occurs during periods of extensive development and growth. It was also stressed that the magnitude of effects differs at different stages during the life of the organism.

Henkle, et. al., (1941) observed in their studies with Drosophila that total time of development is increased by exposure to high temperature. Henke also established the presence of sensitive periods and the effect that increased temperature has on the development of the fruit fly when applied during this time.

A thorough search of the literature revealed that work has not been done on direct exposure of

H. diminuta cysticercoids to high temperatures.

MATERIALS AND METHODS

Tenebrio molitor, infected with cysticercoids of Hymenolepis diminuta, was secured from Carolina Biological Company. The head, legs and wings of the beetles were removed and discarded. The remainder of the organism was placed in 0.9% saline solution and the thin dorsal integument removed. The entire contents of the body cavity were then scraped into a petri dish and examined for larvae with a dissecting microscope.

The cysticercoids were divided into experimental and control groups. Group 1 was exposed to a temperature of 37°C; group 2, 39°C; group 3, 41°C; group 4, 43°C; group 5, 45°C; and group 6, the controls, 30°C which was room temperature. The cysticercoids were kept at the above temperatures in incubators for a 24 hour period.

Thirty albino Wistar rats were obtained from Texas Agricultural and Mechanical University. The rats were weighed, sexed and grouped according to the respective cysticercoids they received.

The animals were distributed according to sex as evenly as possible in experimental and control groups. Each group consisted of 5 rats, and each rat was inoculated with 10 cysticercoids. Experimental and control animals were maintained on water and Purina laboratory chow throughout the experimental period. At the end of three weeks, the rats were sacrificed and autopsied. The intestines of the rats were removed and examined for H. diminuta. The worms were counted, measured, observed for abnormalities and then preserved in 10% formalin solution.

RESULTS

Rats inoculated with cysticercoids of *Hymenolepis diminuta* yielded a small percentage of infectivity. Group 1 received larvae kept at a temperature of 37°C. Of the five inoculated rats, four worms were recovered from three of them. One of the rats was negative and another one died (Table I). The average percentage of infection for group 1 was 10% (Table II). Group 2 received larvae kept at a temperature of 39°C and yielded three adult worms (Table I). Two of the rats were negative and one died (Table I). This represented an average percentage of infection of 7.5% (Table II). Groups 3,4, and 5 were negative.

The control received larvae maintained at 30°C which was room temperature (Table I). Four adult worms were recovered from two of them, two were negative, and one died. The average percentage of infection was 10%. Statistical analysis revealed no significant difference in the number of tapeworms recovered from the experimental and con-

trol groups.

The group average length for tapeworms recovered in group 1 was 28.4 cm. (Table III). The range in length was 24.3 cm. to 33.1 cm. Group 2 exhibited a group average length of 28.16 cm., resulting from a range of 25.3 cm. to 30.2 cm. The group average length of the control was 34.6 cm. The range in length was 32.5 cm. to 36.6 cm. (Table III). Statistical analysis revealed that this variance in length of the worms in the experimental and control groups was of no significant difference.

The rats were weighed before and after the experimental period. The control showed a markedly increased average net gain over the experimental animals. In the experimental rats, group 1 had an average net weight gain of 55.73 g.; group 2, 57.33 g.; group 3, 48.14 g.; group 4, 44.40 g.; group 5, 19.56 g.; and the control, 76.81 g. (Table IV).

The tapeworms recovered showed no morphological abnormalities.

DISCUSSION

While numerous studies have been conducted on the influence of high temperature upon cysticercoids of Hymenolepis diminuta inside the intermediate host, no experiments have dealt with the effect of high temperatures on cysticercoids on the outside of the intermediate host.

In this investigation, there was a 7.5% establishment of infectivity at 39°C, 10% establishment at 37°C, and no infectivity at 41°C. The control yielded a 10% tapeworm recovery. It appears that fully developed cysticercoids may remain infective up to a temperature of 40°C. Since there was no significant difference in the number of tapeworms recovered from experimental and control groups, this study supports the fact that normal development may proceed up to 39°C. The results obtained in this investigation coincide with the findings of Voge (1959) in that fully developed normal cysticercoids are not affected by temperature as

high as 40°C.

In this investigation there was no recovery of tapeworms from rats who received cysticercoids exposed to temperatures of 41°C, 43°C and 45°C. It therefore seems that the subsequent infectivity in the final host is completely inhibited at these temperatures. Heyneman (1958) observed that the development of H. diminuta is not well adapted to high temperatures.

The average length of the worms in the control was greater than that of the experimental groups. However, statistical analysis revealed that this disparity in average length was not of significant difference.

This study showed that the net weight gain of the experimental groups was less than that of the control. In the experimental rats, group 1 had an average net weight gain of 55.73g.; group 2, 57.33 g.; group 3, 48.14 g.; group 4, 44.40 g.; group 5, 19.56 g.; and the control 76.81 g. A through search of the literature revealed no comparison of the gain in weight of rats with

reference to developing cysticercoids. This therefore warrants further study and investigation to determine if there is any relation between the gain in weight of rats and the degree of parasite infectivity.

In this investigation, cysticercoids were directly exposed to high temperatures, while those reviewed in the literature were subjected to temperatures within the intermediate host. Voge (1959) reported that adult helminths were recovered at 40°C, but did not indicate the number. Therefore it is impossible to compare the results.

SUMMARY

Experimental cysticercoids of Hymenolepis diminuta were directly exposed to temperatures of 37°C, 39°C, 41°C, 43°C and 45°C. The control was directly exposed to 30°C which was room temperature. Each experimental group and the control consisted of five rats. Twenty-four hours after temperature exposures, ten cysticercoids of H. diminuta were fed to each rat. The incidence of infection and worm size were determined.

Temperatures above 39°C prevented the establishment of tapeworms. 37°C and 39°C apparently did not affect the establishment of cysticercoids since there was no difference in the number of recovered worms in the control and experimental groups.

High temperatures have no influence on the length of the worms recovered since statistical analysis revealed no significant difference in the length of worms for the experimental and control. Rats infected with cysticercoids exhibited a markedly increased weight.

BIBLIOGRAPHY

Henkle, K., E. V. Fink. 1941. Uber sensible perioden fur die auslosung von Helzemodifikationen bie Drosphila und die beziehunger zwischen modifikationen und mutation. Z. indukt Abstamm. 79(3): 267-316.

Heyneman, D. 1952. Biology of the eggs of members of the genus Hymenolepis. Masters Thesis, Library of the Rice Institute, Houston, Texas.

_____, 1957. The effect of temperature on the rate of development and viability of the cestode Hymenolepis nana in its intermediate host. Exptl. Parasitol. 7(3): 374-382.

Precht, H., J. Christophersen and H. Hensel. 1955. Temperatur und leber. Springer Verlag, Berlin. 15(4): 514-525.

Voge, M. (1959). The sensitivity of developing Hymenolepis diminuta to high temperature stress. Jour. Parasitol. 45(2): 175-181.

_____, and D. Heyneman. 1942. The effect of high temperature on larval development of Hymenolepis diminuta. Cestode-Cyclophyllidea. Jour. Parasitol. 44(3): 249-261.

_____, 1957. The development of Hymenolepis diminuta in the intermediate host, Tribolium confusum. University of California Publication - Zoology. 59(9): 549-580.

_____, and J. A. Turner. 1956. The effect of temperature on larval development of the cestode Hymenolepis diminuta. Exptl. Parasitol. 5(3): 374-382.

TABLE I

Number of *Hymenolepis diminuta* Found in Albino Rats Infected with Larvae Subjected to High Temperature Stress*

Rat Classification Number	Number of Worms Recovered Per Rat					
	Experimental Groups					Control
	1	2	3	4	5	
1	1	1	0	0	0	-
2	1	2	0	0	0	0
3	2	-	0	0	0	3
4	0	0	0	0	0	0
5	-	0	0	0	-	1
TOTAL	4	3	0	0	0	4

Group temperatures
1-37°C
2-39°C
3-41°C
4-43°C
5-45°C
Control-30°C

(-) rats died

TABLE II

Percent of *Hymenolepis diminuta* Found in Albino Rats Infected with Larvae Subjected to High Temperature Stress

Rat Classification Number	Percent of Worms Recovered Per Rat					
	Experimental Groups					Control
	1	2	3	4	5	
1	10	0	0	0	0	-
2	10	30	0	0	0	0
3	20	-	0	0	0	30
4	0	0	0	0	0	0
5	-	10	0	0	-	10
Average Percentage	10	7.5	0	0	0	10

(-) rats died

TABLE III

Average Length of Parasites in Albino Rats Infected With *Hymenolepis diminuta* Cysticercoids Subjected to High Temperature Stress

Group	Length of Each Worm (cm.)	Average Length Per Group (cm.)	Range in Length (cm.)
1	30.0 28.2 33.1 24.3	28.40	24.30-33.10
2	29.0 30.2 25.3	28.16	25.30-30.20
Control	35.8 32.5 36.6 33.7	34.65	32.50-36.60

TABLE IV

Weight of Albino Rats Before and After Inoculation of Hymenolepis diminuta

Group	Sex	Rat Classification Number	Weight Of Rats Before Inoculation	Weight Of Rats 3 Weeks Later (g)	Net Weight Gain (g)	Average Net Weight Gain Per Group (g)
1	M	1	53.45	110.00	56.55	55.73
	M	2	60.85	123.60	62.75	
	M	3	88.35	142.30	53.95	
	M	4	46.85	96.50	49.65	
	M	5	34.00	-	-	
2	F	1	43.50	67.95	24.45	57.33
	M	2	57.95	179.01	121.06	
	M	3	40.95	-	-	
	M	4	54.64	84.60	29.96	
	M	5	57.05	110.91	53.86	
3	M	1	59.35	104.30	44.95	48.14
	M	2	52.45	92.51	40.06	
	F	3	70.45	115.65	45.20	
	M	4	77.90	122.85	44.95	
	M	5	80.55	146.11	65.56	
4	M	1	58.10	100.00	41.90	44.40
	F	2	56.60	95.50	38.90	
	M	3	62.50	112.35	49.85	
	M	4	71.75	121.40	49.65	
	M	5	59.35	101.05	41.70	
5	F	1	79.25	94.70	15.45	19.56
	M	2	64.45	89.25	24.80	
	M	3	99.85	111.95	12.10	
	M	4	81.05	107.04	25.99	
	M	5	54.55	-	-	
Control	M	1	51.25	-	-	76.81
	M	2	32.07	87.75	55.68	
	M	3	67.70	158.85	91.15	
	M	4	51.10	129.00	77.90	
	F	5	52.95	135.45	82.50	

(-) Rat died