SUSCEPTIBILITY OF FEMALE ALBINO MICE TO INFECTION WITH *HYMENOLEPIS NANA* USING EGGS DERIVED FROM DIFFERENT SOURCES

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1970

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1975

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ACKNOWLEDGEMENTS

The author expresses appreciation to Dr. Helen E. Jordan, his major adviser, for several constructive suggestions, guidance and assistance in editing the manuscript and for her criticism relevant to the experimental design, procedure and organization of data, and her concern in providing space for the completion of this study. Members of the committee, Dr. A. Kocan, Department of Veterinary Parasitology and Public Health; Dr. D. W. Howell, Department of Entomology; and Dr. R. Morrison, Department of Statistics, aided with their criticisms and encouragement. My thanks are also due to Mrs. Carolyn Hackett, Mrs. M. Jones, and Joyce Gazaway for typing and making grammatical corrections on parts of this paper and to Mrs. Sherri Kollar and Mrs. Kay Mayes for typing the manuscript. Special thanks are also due to my wife, Ruth, and children, Chepngeno and Chemutai, for their understanding, support and sacrifices. I would also like to thank several friends, particularly from Africa, and parents in Kenya, for their letters of support and encouragement.

Financial support and other assistance was provided by the African-American Institute and Oklahoma Agriculture Experiment Station, Project 1454, for which the author is very grateful.

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CHAPTER I

INTRODUCTION

Although H. nana utilizes both the direct and indirect mode of development, many investigators have used solely the direct route for their experiments. Even though the life cycle of this tapeworm has been known since 1887 (Grassi), numerous inconsistencies still exist in the literature pertaining to percent of adults that can be recovered at necropsy from a host (mouse) infected with a known dosage of eggs. Inconsistencies are also found in the egg output of this tapeworm in mice, and also in the prepatent period and the length of patency. The sex, age, source of the eggs used for infection or whether the eggs were fresh or had been kept for sometime in the external environment often were not specified by the reporting investigator(s). The diferences in sex of the host (Miller, 1965; Ehrenford, 1957; Ackert et al., 1950; Addis, 1946; Campbell, 1939; Campbell et al., 1940; and Mathis, 1954), age of the hosts (Africa, 1928; Fullebonn, 1928; Chaneller, 1931; Herrick, 1928; Miller, 1965; and Sanles, 1929) and length of time eggs are held in the external environment before exposure (Shorb, 1933; Larsh, 1943; and Schiller, 1958) have been shown to affect the susceptibility of the host to various species of parasites. The inconsistencies, in part, that are found in the literature could be attributed to the fact that investigators may have used different sources of eggs or hosts of different ages, or different or mixed sexes.

This study was designed to compare (1) the percent of adult Hymenolepia nana developing from eggs derived from different sources (from feces of infected mice and from gravid proglottids of unfixed H. nana) and as well as the effect of storage of eggs (fecal eggs for 48 hours at room temperature and proglottid eggs at 4^{o} C overnight) on 75-day-old white female mice and (2) to determine the effect of host age on the development of H. nana as measured by percent of infection dose recovered as adult worms, prepatent period, fecundity (determined as egg output per gm of fresh feces per day) and the longevity of patency.

CHAPTER II

LITERATURE REVIEW

Hymenolepis nana has been shown to complete its life cycle both by direct life cycle (Grassi, 1887; Grassi and Rovelli, 1892, Scott, 1924; Woodland, 1923, 1924) and the indirect one utilizing a variety of intermediate hosts including various fleas such as Ctenocephalides canis, C. cheopis, Pulex irritans and mealworm beetles, Tenebnio molitor and T. obscurus (Bacigallupo, 1931), flour beetle, Tribolium confusum (Schiller, 1959).

Although some investigators (Heyneman, 1962; Schiller, 1958, 1959) have studied the biology of H. nana utilizing the intermediate host, others (Short, 1933; Chandler, 1927) have used the direct mode for establishing infection of this tapeworm in the host. There are two sources of eggs that can be used for direct infection: (1) eggs can be obtained or recovered from feces, or (2) they can be obtained from the gravid proglottids of the recently recovered unfixed worms. Eggs from feces have been used more often than the eggs from the proglottid for the direct infection of rats or mice. Woodland (1924) adopted what he called "soup" method in which he fed mice with bread sprinkled with a homogenous fecal suspension containing the eggs. In other experiments, he forced fecal pellets from infected mice down the oesophagus of uninfected mice, making certain that it was swallowed. In this method of egg infection, it is difficult to estimate the size of dosage. The percentage,

therefore, of adult worms developing from these fecal eggs could not be determined. To overcome this obstacle, later investigators (Scott, 1923, 1924; Chandler, 1927; Shorb, 1933; Hunninen, 1935) modified Woodland's method of infecting mice with feces containing the eggs. These latter investigators recovered the eggs of H. nana by using differential floatation, centrifugation and sedimentation from feces recovered from infected mice. Eggs thus obtained could be counted before they were given to a host. Eggs from fresh feces (feces passed within a few hours before dosing) were preferred to old feces (feces stored for several days before dosing) by Scott (1923, 1924), Chandler (1927), Hunninen (1935), Shorb (1933) and Woodland (1924).

A less tedious method to obtain large quantities of Hymenolepis nana eggs than from the feces is to break open the gravid proglottid of the tapeworm in water, releasing the eggs from the uterus or to feed the entire proglottid containing the eggs directly to mice. Grassi (1887) and Grassi and Rovelli (1892) and also Shorb (1933) used fresh eggs from the proglottids to infect mice. Schiller (1958, 1959) and also Ghazal and Avery (1974) recovered eggs from the gravid proglottids that have been kept for about 24 hours at 4^{o} C. They did not, however, report any experimental evidence favouring the storage of eggs at 4^{o} C for 24 hours before using them.

Infection of a host by a parasite elicits a host-parasite interaction in which the success of the parasite in that host may depend on a number of factors such as acquired immunity, sex, stress, age and species of the host (Schiller, 1958, 1959). Several studies have shown that a host becomes increasingly refractory to parasite infection with advancing age (Africa, 1928; Fulleborn, 1928; Chandler, 1931; Herrick, 1928; Miller,

1965; Sarles, 1929). Shorb (1933) noted that mice, two one-half months old, were most susceptible to infection with Hymenolepis nana than were the mice one month or seven months old. Although Shorb (1933) did not specify the sex of mice he used to demonstrate the effect of age to infection with H. nana, several other investigators using other parasites on different hosts have repeatedly confirmed that susceptibility to a parasite infection is affected by sex of the host. Miller (1965) showed that female dogs develop resistance against Ancylostoma caninum at an earlier age than do males. Ehrenford (1957) demonstrated that sex influenced susceptibility with Toxocara canis in dogs. The greater refractoriness of female to parasitic infection has been found to be due to the female sex hormone (Ackert and Dewhirst, 1950; Addis, 1946; Campbell, 1939; Campbell and Melcher, 1940; and Mathies, 1954).

The time interval between passage of eggs of H. nana from infected hosts and exposure of susceptible hosts seems to be an important factor that determines the viability and infectivity of the eggs. Shorb (1933) found, using fecal eggs, that infectivity declines with time. He observed that eggs kept in water for ten days at room temperature were no longer viable or infective to mice. Larsh (1943), however, performed an experiment to compare and to find the effect of storage of Hymenopelis nana eggs in feces and their infectivity to mice. He found that the eggs were most infective when the feces containing them were held for 48 to 72 hours at room temperature.

The development of Hymenolepis nana in mice has not shown a consistent recovery percentage of adult worms from fecal eggs. Larsh (1943) noted that H. nana obtained from mice of one locality had a different adult recovery in mice than were H. nana from another locality.

Even for mice obtained from the same locality, Larsh did not get consistent percent adult worms recoveries. Conclusions drawn by several other investigators have been extremely variable, a fact that can be ascribed to their different methods of obtaining the eggs from feces. Using H. nana eggs from one to 24 hours-old feces, Shorb (1933), Hunninen (1935), and Hearin (1941) reported that 0.1 to 1.0 percent, 1.0 to 8.0 percent and approximately 3.4 percent of infection dose recovered as adults, respectively, in mice. Larsh (1943) using eggs from feces that had been held at room temperature for 48 hours to 72 hours reported that 8.8 to 12.8 percent of the infection dose of H. nana developed to adults with one strain of the tapeworm obtained from one locality and 4.4 to 6.4 percent with another strain of the tapeworm. Hunninen (1962) reported a recovery of up to 9.0 percent using eggs recovered from feces that were collected over a period of 48 hours.

CHAPTER III

MATERIALS AND METHODS

Maintenancy of Mice

White female albino mice,[1] Cesarean Origin Pathogen Barrier Sustained (COBS), were used in both the maintenance of H. nana and in the experiments. As soon as they were received, a daily fecal smear was conducted on all the mice for the first ten days. This was a precautionary measure to assure tapeworm-free subjects. The selected mice were fed on commercial rat chow and water ad libidum. Except for periods when feces was collected, mice were maintained in cages lined with woodshavings or San-I-Cell[2] (a compressed cellulose absorbent product). These beddings were changed regularly, at least once a week. All the experimental mice were kept in the same room. The cages containing them were placed on an aluminum surface table along the wall in such a manner that all the mice were exposed to the same conditions of light, aeration, and approximately the same distance from the door.

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[1]The white albino mice were purchased from Charles River Breeding Laboratories.

[2]San-I-Cell material is manufactured by Southland Farm Store, Dallas, Texas.

Maintenance of Beetles

The beetle intermediate host, Tribolium confusum[3] was reared in large glass jars capped with porous lids and were fed on cracked whole wheat cereals. The beetles and the larvae were transferred to clean jars approximately every three months.

Maintenance of Hymenolepis nana

Hymenolepis nana[4] which was maintained in this laboratory for over a year prior to this study was routinely propagated either via the beetle intermediate host (Tribolium confusum) or by the direct route using the proglottid eggs.

In the direct route of propagation, infections were established by the method adopted by Schiller (1957, 1959) and Ghazal and Avery (1974). The proglottids were opened in water to release the eggs from the uterus. Excess water was decanted after a few minutes of settling. The eggs were then transferred to a 50 ml centrifuge tube containing between 20 and 30 ml of nutrient gelatin broth. The eggs were evenly mixed by drawing the suspension in and out of a 1.0 ml syringe. Immediately after the tenth stroke of mixing, a 0.01 ml aliquot of the egg suspension was removed by a 0.01 ml calibrated micropipette, and transferred to a glass slide. The eggs in this drop of suspension were carefully counted using 10x objective of the compound microscope. This procedure was repeated ten or more times with careful mixing between each count.

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[3]The original supply of beetles was supplied by Dr. R. C. Berberet, Oklahoma State University Department of Entomology.

[4]The original stock of H. nana was supplied by Dr. W. Friedberg, University of Oklahoma.

Calculation of eggs per ml of suspension was determined by averaging the number of eggs in 0.01 ml of suspension from all the drops of suspensions that were examined and multiplied by a factor of 100. To get a desired concentration of eggs in 1.0 ml of the suspension, more nutrient broth was added to lower the concentration of the eggs or if a higher concentration was needed, the tube containing the egg suspension was centrifuged for about three minutes at 1500 rpm and the nutrient broth from the upper layer was withdrawn. This was repeated until the desired eggs/ml of fluid was attained. Due to the small size of the mouse's intestine, exactly 0.5 ml of this standardized egg suspension was used to inoculate each mouse. Administration of eggs via stomach tube can be accomplished easily without anesthesia. The mouse's neck is held so that the entire back of the animal rests on the palm of the hand. The tube is then passed down the esophagus to the stomach and all the 0.5 ml of suspension was emptied by gently pushing in the syringe piston. The last third of the tip of the syringe needle was slightly bent to facilitate its passage to the stomach. After dosing each mouse, the tube was thoroughly cleaned and rinsed several times in water before it was used on the next mouse.

In the propagation utilizing the indirect life cycle of H. nana, the intermediate hosts, Tribolium confusum, were starved for five to ten days before being exposed to gravid proglottids. The gravid proglottids that were fed to these beetles were prepared in the same manner as those used for the direct infection except that the eggs were not released from the uterus. The beetles were allowed to feed on the segments of the proglottids placed on a moist filter paper in a petri dish. After feeding for a period of four to six hours, the beetles were removed to

clean petri dishes and put on their regular diet. The beetles were maintained for at least 23 days at room temperature (approximately 25° C). During winter, an infra-red lamp was installed to maintain this temperature. It was observed that beetles could carry the cysticercoids for life, approximately three months post infection. This was an advantage in insuring a stock of cysticercoids for a longer period of time than the stock of eggs from mice since worms were eliminated from the host intestine within three weeks from the time of infection.

Preparation of Eggs for the Experiments

Eggs recovered from feces, approximately 48 hour old eggs (OFE) and eggs recovered from fresh feces, approximately four hours old (FFE) were obtained as follows: Mice having eggs in their feces, as determined by direct saline smear, were segregated into cages containing raised screens. Pans under the screens were lined with moist paper and feces passed was collected after the desired time span. For OFE, overnight fecal specimen were collected and stored at room temperature for 48 hours. Collection of feces for FFE was accomplished by confining mice to raised screens for not more than six hours. Sufficient fresh feces could be collected from ten mice in less than four hours. The two types of feces were transferred into labelled beakers. Water, approximately ten times the volume of feces, was added to each beaker of egg laden feces. Using a glass rod and tongue depressor, the feces was broken and stirred to make a homogenous mixture. The rods were rinsed into their respective beakers. These fecal suspensions were poured into labelled 50 ml centrifuge tubes and centrifuged for two minutes at 1500 rpm. The upper aqueous layer was aspirated off and the sediment of eggs and debris was

repeated until the aqueous layer was clear. After the last aspiration of the upper layer of water, the egg laden sediment was resuspended in a saturated sodium chloride solution. The tubes with these suspensions were centrifuged at approximately 1500 rpm for two minutes to increase settling of the heavier fecal particles. Following centrifugation the centrifuge tubes were allowed to stand undisturbed for an additional two to four minutes. Then the upper layer of the levitation solution was removed by a pasteur pipette and transferred to clean labelled centrifuge tubes. These tubes were then filled with tap water and recentrifuged when the eggs settled to the bottom of the tubes and the supernate was aspirated off. To remove excess salt, the eggs were washed three times. The final egg sediment was suspended in a gelatin nutrient broth and a standardized dose was determined according to the preceding procedure outlined under maintenance of H. nana.

Eggs recovered from proglottids were obtained as described for those used in the maintenance of H. nana. For the old proglottid eggs (OPE), the gravid segments were kept in water at 4^{o} C for 12 hours before removing the eggs. For fresh proglottid eggs (FPE), however, the gravid proglottids were used as soon as they were removed from the mouse's intestine. Eggs freed from both fresh and old proglottids were put into labelled test tubes. Since eggs settle in water without centrifugation, the centrifuge tubes containing the suspension of these proglottid eggs were allowed to stand undisturbed for 30 minutes. The upper aqueous layer was then aspirated off to concentrate the eggs. The final egg sediment was suspended in a gelatin nutrient broth and standardized as described previously in the maintenance of H. nana.

CHAPTER IV

EXPERIMENT I: COMPARISON OF *HYMENOLEPIS NANA* DEVELOPING FROM EGGS DERIVED FROM OPE, OFE, FPE, AND FFE

Procedure

Four sources of eggs, OFE, OPE, FFE and FPE respectively were used to infect 75 day old female white mice (COBS). The mice were randomly selected for inoculation with the above egg materials. A mouse, after exposure with a particular egg source was separated from others and placed in a cage that had previously been labelled to correspond to the egg source. Four mice were exposed individually to the four egg sources and this exposure procedure was systematically repeated until ten mice were exposed with each egg source. After dosing each mouse, the inoculation tube was thoroughly cleaned, as previously described, before it was used to dose the next mouse. Approximately 300 eggs were administered orally to each mouse. To reduce coprophagy, all the infected mice were kept in cages containing raised screens. The pans collecting the feces at the bottom of the screen were emptied and washed with soap (borateem) every other day. Fifteen days after they were infected, all the mice were killed and necropsied. The number of worms in each mouse was recorded by counting the scolices. Worms were considered relaxed at room temperature when they no longer showed any contraction. The smallest and largest worms were placed on a glass plate and their lengths were

measured as wet mounts.

Results

All the adult worm count, range of lengths and percent of infection dose recovered as adults in 75-day-old female mice from FFE, OFE, FPE and OPE sources of eggs are recorded respectively in Table I, and graphically plotted on Figure I. From the table and particularly from Figure I, there is a significant difference (significant at 5% level of confidence) between the percent of adults derived from proglottid and fecal eggs. Irrespective of source, old eggs yielded a higher percent adults than fresh eggs. However, this was not a statistically significant difference.

Discussion

Fecal eggs about 48 hours old were found to be more infective to female mice. This agrees with Larsh's (1943) findings for infectiveness of eggs derived from feces. Although no comparative study has been made to study variation in infectivity of fecal eggs and proglottid eggs, this experiment showed that infectivity of fecal eggs is much greater than that of proglottid eggs. The fecal eggs developed at the rate of 3.8 percent and 5.1 percent for FFE and OFE, respectively, while that of proglottid eggs was about 1.0 percent and 1.66 percent for FPE and OPE, respectively.

The percentage of development of H. nana from OFE and FFE sources of eggs was not as high as that observed by Larsh (1943). A possible explanation for the variation between this study and Larsh's is that there was a strain difference between either the mice he used or the

TABLE I

DEVELOPMENT OF HYMENOLEPIS NANA IN MICE INFECTED WITH EGGS FROM DIFFERENT SOURCES:

OFE source Dosage 305 eggs			FFE Source Dosage 320 eggs			OPE Source Dosage 385 eggs			FPE Source Dosage 300 eggs		
No. of worms	% Adults Recovered	Approx lengths in cm	No. of worms	% Adults Recovered	Approx. lengths in cm	No. of worms	% Adults Recovered	Approx. lengths in cm	No. of worms	% Adults Recovered	Approx. lengths in cm
12	4.0	5-7	9	2.8	8-9	7	1.8	7-10	3	1.0	10-12
14	4.6	5-7	15	4.7	6-7	8	2.1	8-10	2	0.7	10-12
16	7.5	6-7	8	2.5	9-12	9	2.3	9-11	3	1.0	10-12
18	6.0	5-6	19	6.0	6-7	7	1.8	8-12	3	1.0	10-12
12	4.0	6-8	16	5.0	4-7	4	1.0	9-10	5	1.7	9-12
18	6.0	6-7	15	4.7	6-7	6	1.6	9-11	3	1.0	9-12
15	5.0	7-8	3	0.9	8-12	10	2.6	9-11	1	0.3	10-12
9	3.0	8-10	14	4.4	6-7	5	1.3	9-10	2	.67	10-12
22	7.2	3-5	9	2.8	7-8	4	1.0	9-12	5	1.7	10-11
11	3.8	6-8	15	4.7	6-7	4	1.0	7-12	2	.67	10-12
Average = 5.11			Average = 3.8			Average = 1.65			Average = 0.97		
Std. dev. = 1.284 N.S.*			Std. dev. = 1.533 N.S.			Std. dev. = 0.57 N.S.			Std. dev. = 0.44 N.S.		
Range = 4.26			Range = 5.1			Range = 1.6			Range = 1.4		

*N.S. = Not significant

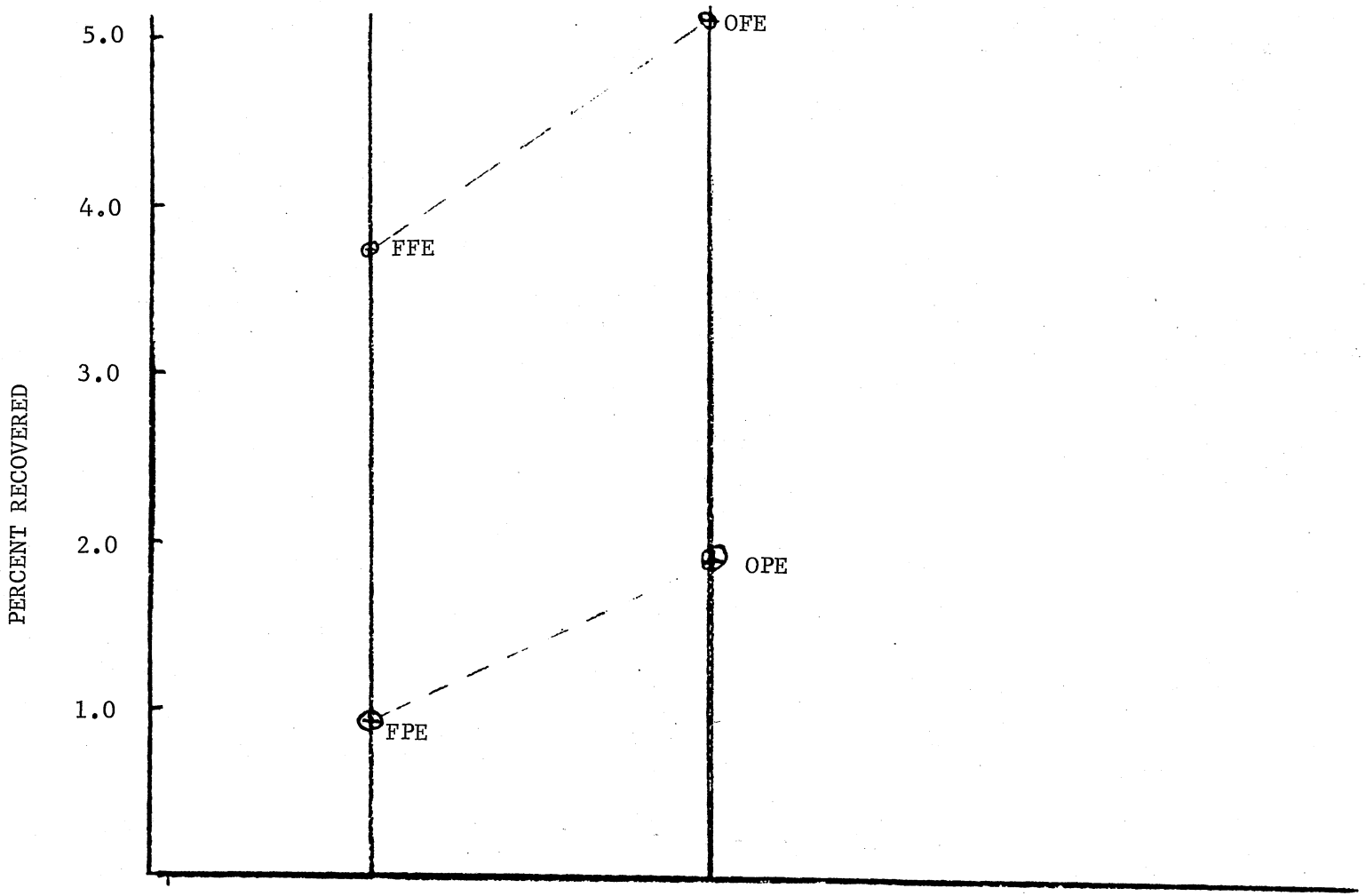

Figure 1. % of Adult H. nana Recovered at Necropsy from Mice Infected with Eggs from Different Sources: OFE, OPE, FFE, and FPE.

tapeworm itself from which he obtained the eggs. Another factor that could account for these variations could be that the age and/or sex of mice he used were different from those that were used in this study. Age and sex have been demonstrated to influence susceptibility of the host and the percent of exposure to develop.

The factor of storage of proglottid eggs to increase infectiveness is supported by the experimental evidence in the study; however, such a comparison has not been reported although Schiller (1957, 1959) preferred to use proglottid eggs that had been kept overnight in the refrigerator (4^{o} C), with no experimental explanation offered.

The storage of eggs either contained within or freed from proglottids, at 4^{o} C overnight markedly increase the percent of adult worms to develop for a given exposure dose of eggs. The fresh proglottid eggs that developed to adult worms in this study was less than 1.0 percent of the dosage whereas that from stored proglottids was about 1.66 percent. The analyses of data from Table I show the differences in percent of tapeworms developing from fresh and old eggs from feces is about the same as the differences between fresh and old proglottid eggs. Figure 1 shows that there is quite a large difference due to fecal eggs and proglottid eggs regardless if fresh or stored. This difference between fecal and proglottid eggs is 4.33% - 1.31% (3.02%). There is also a significantly larger percent of worms produced by using old eggs from either source (Ave. % from proglottid eggs -Ave. % from fecal eggs, i.e., 3.38 - 2.38 = 1.0%). These results indicate that female mice are more susceptible to infection with old eggs originating from either source (proglottids or feces) and also more susceptible from fecal than from proglottid eggs.

The role of the environment on altering the viability and infectivity of H. nana eggs is difficult to determine. Although this was not part of the study in this paper, the environment could influence infectivity by providing the eggs with physiological development such as brief aerobic metabolism. This in turn could account for a higher infection rate for stored eggs over that of fresh ones obtained either from feces or proglottids. However, metabolism alone may not explain the increased infectivity of proglottid eggs kept at low temperature of the refigirator. Lower temperature physiologically hinders or slows down metabolic or chemical processes that may be necessary for development. Since bacterial activity causes fast deterioration of proglottid eggs, the effect of room temperature on eggs was not studied.

Although fecal eggs do yield more adult H. nana than the proglottid eggs of the same dose, it would appear that for practical purposes, eggs from refrigerated proglottids would still be preferable to use for infecting laboratory animals. Proglottid eggs are fast to prepare for use, completely free from debris, and like fecal eggs, as shown in this experiment, the animals inoculated with proglottid eggs were 100 percent infected.

CHAPTER V

EXPERIMENT II: EFFECT OF AGE OF HOST (MICE) ON THE PREPATENT PERIOD FOR *HYMENOLEPIS NANA*

Procedure

Fecal eggs (OFE) were used in this experiment because it was determined in the previous experiment to yield a higher worm burden than other egg sources in 75 day-old female mice. After mice were confirmed to be tapeworm-free, three groups of ten mice each aged 30, 75, and over 300 days old were given per os approximately 300 eggs from the above source. The eggs had previously been recovered and standardized according to the procedure already outlined in materials and methods. As in Experiment I, all the infected animals were maintained in raised screens to reduce coprophagy.

After the seventh day post infection, the mice were checked for eggs by using floatation technique. Examination of the mice feces continued until all the mice were positive. The prepatent period (time that elapsed between exposure of the host to eggs and detection of the parasite eggs in the feces) was noted for each group of mice.

Results

The 30 and the 300 day old mice were the first to show the eggs of *H. nana* in their feces. Two mice aged 30 and three aged 300 days shed

the eggs of this tapeworm ten days after infection. On the eleventh day, there were three mice that were positive from each of the above groups. Eight 30 day and six 300 day-old mice were positive on the twelfth day while only two of the 75-day-old mice were positive for the first time and 15 days elapsed before all mice were shedding eggs in their feces. The number of mice that were positive each day after the ninth day post infection is recorded in Table V.

Discussion

Prepatent period of H. nana was variable for all age groups of infected mice. To use an average prepatent period as was done by Shorb (1933) does not offer an explanation as to the cause of the variation of prepatency. The existence of this inconsistent prepatent period may be due to a variety of reasons. It is recognized that in any given infection, no two infected individuals have the exact same susceptibility. Individual variation of the hosts seems to be an important mechanism that induces differences in the prepatent period. Another explanation is that worms have inherent variabilities thus do not mature at the same rate. Mice that were killed as soon as patency was demonstrated contain both sexually mature adults containing eggs in the gravid proglottid as well as immature worms. This difference in the stage of maturity of the worms at necropsy substantiates the observation that the development of the prepatent period varies from worm to worm, which may be due either inherent to the worm or host influence. These two variations are difficult to control due to the fact that they are unidentified properties of individual hosts or worms.

TABLE II

PREPATENT PERIOD OF *HYMENOLEPIS NANA* IN FEMALE ALBINO MICE AGED 30, 75, AND 300 DAYS OLD, RESPECTIVELY

Number of Mice Passing Eggs of *H. nana* After the Ninth Day Post Infection

Age of Mice (15 Mice in Each Group)	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
30	0	2	3	8	15	15	15
75	0	0	0	2	8	11	15
300	0	3	3	6	15	15	15

Analyses of Variance

Age in days	Days to Infection															
30	10,	10,	11,	12,	12,	12,	12,	12,	15,	15,	15,	15,	15,	15,	15 = 196	Ave.= 13.07
75	12,	12,	13,	13,	13,	13,	13,	13,	14,	14,	14,	15,	15,	15,	15 = 204	Ave = 13.6
300	10,	10,	10,	12,	12,	12,	13,	13,	13,	13,	13,	13,	13,	13,	13 = 183	Ave = 12.2

Source	df	ss	ms	f
Total	4.4	105.91		
Age	2	14.98	7.49	
Linear	1	0.73	0.73	N.S

Source	df	ss	ms	f
Quad	1	14.25	14.25	6.58**
Within	42	90.93	2.165	

c.v. = $\frac{2.165}{12.95}$ x 100 = 11.35

xx good c.v.

CHAPTER VI

EXPERIMENT III: EFFECT OF AGE ON THE PERCENT OF INFECTION DOSE RECOVERED AS ADULTS OF HYMENOLEPIS NANA IN FEMALE MICE INFECTED WITH OFE SOURCE OF EGGS

Procedure

Three groups of ten tapeworm-free mice aged 30, 75 and 300 days old were infected with about 300 eggs each.

All the 30 mice were killed and necropsied on the fifteenth day after infection. Worms were carefully removed from each mouse and put into a petri dish of water. The total number of scolices was used as an index of adult worms present per mouse. An average worm count for one group of mice was made and compared to the average for the other groups. Using the average count, expressed as a percent of infection dose recovered as adult worms, the development of Hymenolepis nana to adults in mice of different age groups was evaluated.

Results

Mice aged 75 days old had the greatest percent of infection dose recovered as adult H. nana. The percent of adult worms recovered ranged from 3.3 to 8.3 percent while the percent of adults recovered in 30 and

300 day old mice was 1.3 to 4.0 and 0 to 2.6, respectively. The results are summarized in Table VI and are shown graphically in Figure 1.

TABLE III

PERCENTAGE OF INFECTION DOSE RECOVERED AS ADULTS OF HYMENOLEPIS NANA IN MICE OF DIFFERENT AGES INFECTED WITH 48-HOUR-OLD FECES*

Mouse Number	30 Day Old		73 Day Old		300 Day Old	
	Worms	Percent	Worms	Percent	Worms	Percent
1	8	2.6	25	8.3	0	0
2	5	1.6	24	8.0	2	0.7
3	12	4.0	11	3.7	2	0.7
4	9	3.0	16	5.3	8	2.6
5	9	3.0	14	4.7	8	2.6
6	8	2.6	18	6.0	4	1.3
7	4	1.3	15	5.0	8	2.6
8	5	1.6	21	7.0	2	0.7
9	7	2.3	10	3.3	0	0
10	6	2.0	16	5.3	3	1.0
Average	7.3	2.7	17	5.7	3.5	0.8
Standard Deviation		2.45	--	5.04	--	3.09
Range	8	2.7	15	5	8	2.6

*Dosage: 305 eggs.

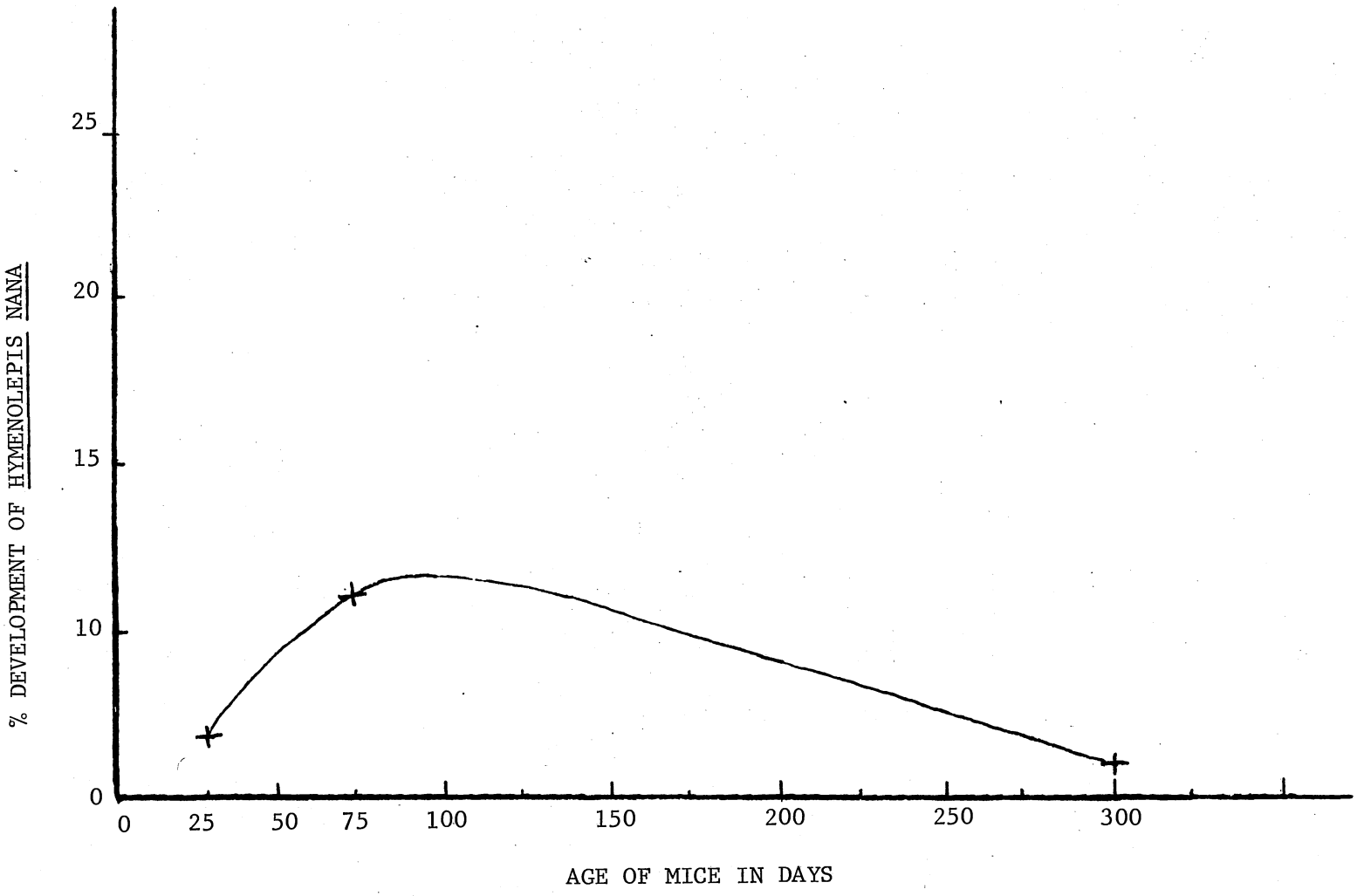

Figure 2. A Curve of % Development of H. nana in Mice Against the Age of Mice (Hosts)

Discussion

In this experiment mice aged 30, 75, and 300 days old showed differences in susceptibility to infection with Hymenolepis nana. This study agreed with those observations by Shorb (1933), that the youngest and the oldest mice were more refractory to infection than were the 75 day old. Heyneman (1962) in immunity experiments with H. nana reported a higher infection with mice over one year than in mice six months or younger. Shorb (1933), Larsh (1943) and Heyneman (1962) emphasized that differences in susceptibility of mice to Hymenolepis nana was due to strains of both the host and the parasite. However, if the age of the host is taken as a sole variable, each strain of the host should respond to the infection in a predictable fashion. The differences, for example, between the observations of Shorb and Heyneman and the results obtained in this experiment may be due to other factors in addition to host age. These workers did not, for example, define the sex of mice they used while all variable factors investigated in this study were conducted solely with the female mice and a single host strain source (COBS) to reduce uncontrolled variables.

Larsh (1943) associated and verified that the low infection of young mice with H. nana was due to the smaller size of intestines in these animals than in the older ones. Lack of or low susceptibility in the very old animals cannot, of course, be explained by the small size of the intestine. Small size of intestine suggested a crowding effect. Age only may be a natural factor that influences the susceptibility rather than or in addition to the size of the intestine (Larsh, 1943). The host age has been demonstrated to be a factor influencing development for

other parasites. From the observations of Shorb (1933) on the effect of crowding, infection was higher with increasing dosage. Other workers have observed this same phenomena for H. nana. Therefore, the higher percent of development obtained by Shorb with increasing dosage cannot be accounted for by the size of the intestine.

In this experiment, only three sets of age groups of female mice were selected for the comparison study. A better range in the development of H. nana as a factor of age of the host would be provided by selecting more age groups of mice with about 30 days in between. A curve of age and percent of development would provide the age of mice most susceptible to infection. If this is repeated also for the male mice, a means of evaluating the significance of age in the epidemiology of this parasite could be obtained.

CHAPTER VII

EXPERIMENT IV: DAILY EGG RECOVERY FROM 30, 75 AND 300 DAY OLD FEMALE MICE INOCULATED WITH APPROXIMATELY 300 EGGS OF HYMENOLEPIS NANA

Procedure

Three groups of ten tapeworm-free female mice (COBS) aged 30, 75 and 300 days old respectively were given orally approximately 300 eggs (OFE source) of Hymenolepis nana. As soon as a patent infection was established, about the tenth day of post exposure, approximately 0.5 gm of fresh feces collected for each group was weighed and transferred into a 50 ml calibrated centrifuge tube. Gelatin broth in the amount of 20 to 30 ml was poured into this centrifuge tube and a fecal suspension in this fluid was made. A 1.0 ml syringe without the needle was then inserted into the suspension until its tip was halfway in the fluid. By pulling the plunger in an out for about three minutes, the contents of the tube became uniform and without large fecal debris. At this point, like the egg standardization described earlier, exactly 0.5 ml of the egg suspension was transferred to a glass slide, a cover slip was applied, and all the eggs in that aliquot were counted by systematically scanning the cover slip at low power of the compound microscope. Ten or more drops of 0.05 ml of suspension was scanned after the suspension was well

mixed each time. From three repeated counts, an average of the egg per 0.05 ml was determined for each group of mice. This procedure was repeated every day at the same time of the day (11:00 a.m. to 12:30 p.m.) until no more eggs could be recovered. The time in days between prepatent period and the disappearance of eggs in feces from the mice (i.e., patency) was also noted for each group of mice in this experiment.

The number of eggs in each 0.05 ml drop of suspension was converted to number per gram of fresh feces by the following mathematical formula:

$$y = \frac{n}{0.05} \times \frac{V}{W}$$

where:

y = egg/gm of fresh feces

n = number of eggs in the 0.05 ml drop

V = total volume of fecal suspension before the sampling began

W = weight in grams of the fresh feces contained in the "V" volume of suspension

Results

Determination of eggs/gm of fresh feces/day from each of the three age groups of mice is recorded in Table VII, and graphically presented in Figure 1.

The number of eggs per gram of feces fluctuated daily from the beginning of the prepatent period up to the time eggs were no longer present in feces. For example, the 30-day-old mice passed 13.6×10^3 eggs and 97.4×10^3 eggs per gram of feces on days four and five, respectively. On these two day, however, strobila with 73 and 102 proglottids per worm, respectively, were recovered in the suspension on day

TABLE IV

DAILY EGG OUTPUT BY HYMENOLEPIS NANA IN FEMALE MICE AGED 30, 75 AND 300 DAYS, RESPECTIVELY

Number of Eggs/gm of Feces/Day From Each Group of Mice x 1000

	Days After Prepatent Period														
Mice Age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
30	6.4	44.8	10.3	13.6	97.4	72.7	43.9	12.5	29.1	13.9	27.9	10.9	10.2	12.7	10.3
75	2.2	5.0	5.9	7.6	7.12	97.5	12.5	9.5	16.3	19.3	7.4	22.9	8.9	6.7	9.5
300	1.5	6.5	7.8	21.8	15.1	14.7	24.5	32.6	11.2	14.2	33.5	16.1	15.0	51.0	28.0
	Days After Prepatent Period														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
30	17.2	17.9	16.2	14.3	4.1	8.6	10.0	12.5	8.0	14.2	37.4	74.4	28.0	2.8	0
75	11.7	36.3	8.8	39.5	8.9	6.3	5.1	7.7	20.0	12.5	32.3	20.0	1.4	4.2	5.5
300	21.7	7.1	3.2	4.0	2.3	3.3	2.2	13.1	1.3	1.1	0.3	0	0	0	0
	Days After Prepatent Period														
	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
75	1.9	3.6	5.8	0	0	0	0	0	0	0	0	0	0	0	0
300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

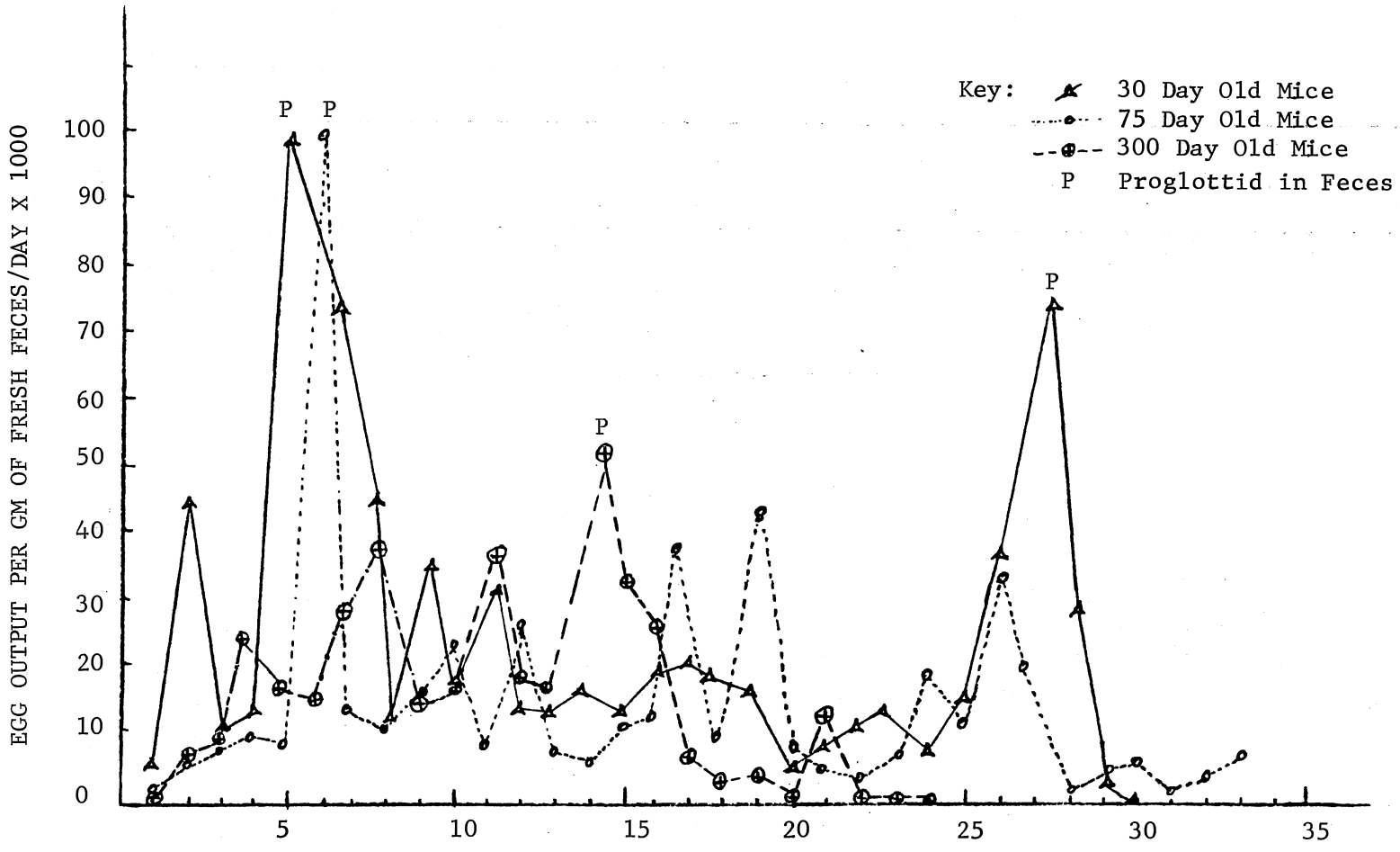

Figure 3. Egg Output by H. nana in female Mice Aged 30, 75, and 300 Days, Respectively Infected with 300 Eggs of H. nana.

five while proglottids were absent on day four. Presence of proglottids were also found on days 6, 7, and 27 and on each occasion, the egg count was markedly higher than on the perceding day when no proglottids were found. Similar observations were noted on mice aged 75 and 300 days old.

Thirty-day-old mice showed complete absence of eggs of H. nana in their feces on day 30 after prepatent period (i.e., 40 to 42 days post inoculum). Feces of the 300-day-old mice were negative for eggs on day 27 (37 to 39 days post inoculum). The 75-day-old mice, however, were all negative on day 34 (44 to 46 days post infection).

Discussion

The amount of eggs/gram of fresh feces per day varied from day to day and the pattern of this variation was similar to that observed by Shorb (1933). Shorb (1933), however, reported a much lower egg output per day compared to the egg output per gram of fresh feces per day as reported in this experiment. The cause of this difference is difficult to determine. However, the variability in technique of counting the eggs is certainly a factor to consider. Whereas Shorb (1933) was counting the eggs on the cover slip from a floatation vial, total eggs suspended in a viscous media was adopted in the current study. He probably lost a lost of eggs during the centrifugation, washings and floatation processes which were avoided in this experiment by mixing and homogenizing the fecal material in one vial, the 50 ml centrifuge tube.

Shorb (1933) attributed the fluctuations in the egg count to repeated infections. Since prepatent periods of Hymenolepis nana in his infection experiments and also in the results reported in this paper were about 11 days, it would be expected that fluctuations due to reinfection

would occur some 10 to 11 days after the prepatent period rather than daily as soon as the prepatent period was reached. Variations such as this may be the result of dissimilar rates of proglottid disintegration, water content of the feces (results in Appendix) and different rates at which Hymenolepis nana mature in mice. Whole proglottids were noted on several occasions marked "P" on the graph. The number of the proglottids was quite variables, ranging from two segments in one to 63 segments in another. It would seem that the variation in the egg count is dependent on the size of the strobila that is voided out in the feces.

CHAPTER VIII

EXPERIMENT V: DETERMINATION OF LONGEVITY OF *HYMENOLEPIS NANA* PATENCY IN 30, 75, AND 300 DAY OLD FEMALE MICE

Procedure

Ten mice in each group of 30, 75 and 300 day old mice were randomly inoculated with about 300 OFE source of *H. nana* eggs. As soon as the prepatent period was reached, individual mice in each group were examined every other day to insure that they were all infected. Beginning on day seven after the prepatent period, one mouse from each group of mice was placed individually into clean plastic clean plastic cages and allowed to remain there for about ten minutes. The feces passed during this time was put in a fecal paper cup and tested for *H. nana* eggs by a floatation test using saturated sodium nitrate solution ($NaNO_3$). This was repeated for every individual mouse. The presence, absence, or loss of eggs in the feces was used to determine the contribution of each individual mouse to the variation of egg recovery in Experiment IV. The number, therefore, of mice from each group that gave negative results and the time in days during which they had been positive was recorded. All the mice used in this experiment were the same ones that were used for egg count in Experiment IV

Results

The 30 day old mice were the first to show negative eggs in their feces. Three individuals were negative to the floatation test on the twenty-first day after prepatent period (about 31 days post inoculum) and all the ten mice were negative on the twenty-seventh day. The 300 day old mice were next with two mice negative to the test on the twenty-third day and all the mice were no longer positive on the thirty-first day after prepatent period. The 75 day old mice were the last to become negative to H. nana by floatation technique. One mouse was negative to the test on day 27 and all were no longer showing evidence of the tapeworm infection on day 35 after prepatent period. The number of mice from each group that was negative to the test every other day are recorded and tabulated in Table VIII.

Discussion

The duration that mice shed Hymenolepis nana eggs in the feces was found to be influenced by age of the host. The maximum time for 30 day old mice to shed eggs (duration of infection) was 26 days, for the 300 day old mice was 30 days, and the 75 day old mice was 36 days. Woodland (1924) reported that the mice were free of infection after 35 days from exposure or after evidence of infection. Shorb (1933), however, did not indicate the age of the animals in his experiment, but he demonstrated for sex that mice lose the infection after 16 days. Inconsistencies that appear in the literature, relating to infectivity, patency interval and egg output might be decreased if some important known factors that contribute to the variability (e.g., sex and strains of mice) were specified and controlled. Results from this experiment indicated that the

TABLE V

THE NUMBER OF MICE NEGATIVE TO THE FLOATATION TEST AFTER THE PREPATENT PERIOD*

Age of Mice	Days 7------19	Number of Mice Negative to the Floatation Test								
		21	23	25	27	29	31	33	35	37
30	All Mice Were	3	7	7	10	10	10	10	10	10
75	Positive to	0	0	0	1	2	5	7	9	10
300	Floatation Test	0	2	3	6	8	10	10	10	10

*10 mice per group.

variation in longevity of parasite patency may be due in part to age of the host mice.

CHAPTER IX

CONCLUSION

Age of the host has been determined to definitely influence the development of H. nana in female albino mice. The 75-day-old mice were the most susceptible and the youngest (30 days old) and the oldest (300 days old) appeared to be more refractory to infection.

The course of infection is characterized by a prepatent period ranging from 11 to 15 days and the egg output which varies from day to day (ranging from less than 6,000 to over 90,000 per gm of feces). The longevity of patency was also variable, ranging from 21 to 27 for 30-day-old mice, 23 to 31 in 300-day-old mice and 27 to 37 for 75-day-old mice.

The source of eggs (proglottid or fecal) definitely influences susceptibility. Fecal eggs are more infective than proglottid eggs. Age of eggs in the external environment also increases susceptibility.

Differences between observations made in this paper and those obtained in the literature are ascribed to some variations in the latter that were not controlled such as the age, strain and sex of animals used.

Effect of the external environment, and age of host as noted in this study, may be helpful and applicable to other estode studies such as Monieza expansa of cattle.

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APPENDIX

DETERMINATION OF THE EFFECT OF MOISTURE CONTENT OF FECES ON EGG COUNT AS NOTED ON EXPERIMENT

From the calculations of egg output per gram of feces in experiment IV, it was observed that the fecal material freshly voided by infected as well as uninfected mice varied from hard pellets to soft and occasionally a very moist and watery matter. Variation of water content could alter greatly the eggs/gram of feces if not standardized. This experiment was then made to determine the variations of fecal moisture daily for eight consecutive days beginning on the tenth day after prepatent period. The feces were weighed as described in experiment IV but instead of counting the eggs immediately, the feces were left to dry in the fecal disposable cups. The feces and the cups were finally reweighed 24 hours later and the change in weight was associated with water loss.

Results

Water content loss of feces varied from 17 percent to 66 percent in a 24 hour period as shown in Table IX.

Discussion

It would be expected that on days in which there was a high water content there would also be a corresponding low egg content. Because the

water content determination was started ten days following the egg count, the results of water content matched with the egg output beginning on the tenth day. This verifies that the water composition of feces affected the count.

TABLE VI

DAILY VARIATION IN THE WATER CONTENTS OF FECES FROM THE INFECTED MICE

Day	Age of Mice	Weights of Feces Fresh and Dry			
		Fresh Feces	Dry Feces	Wt. of Water	% of Water
1	30	0.50 gm	0.37 gm	0.13	26.0
	75	0.47 gm	0.32 gm	0.15	31.9
	300	0.85 gm	0.41 gm	0.44	51.8
2	30	0.52 gm	0.43 gm	0.09	18.0
	75	0.58 gm	0.26 gm	0.32	53.5
	300	0.65 gm	0.41 gm	0.144	24.0
3	30	0.64 gm	0.38 gm	0.26	40.1
	75	0.62 gm	0.51 gm	0.11	17.7
	300	0.70 gm	0.32 gm	0.38 gm	54.8
4	30	0.87 gm	0.42 gm	0.45	51.7
	75	0.74 gm	0.56 gm	0.18	24.3
	300	1.03 gm	0.64 gm	0.39	37.8
5	30	0.38 gm	0.21 gm	0.17	42.1
	75	0.70 gm	0.31 gm	0.39	55.7
	300	0.55 gm	0.28 gm	0.27	49.1
6	30	0.62 gm	0.25 gm	0.37	59.1
	75	0.49 gm	0.19 gm	0.30	61.2
	300	0.61 gm	0.37 gm	0.24	39.3

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Master of Science

Thesis: SUSCEPTIBILITY OF FEMALE ALBINO MICE TO INFECTION WITH HYMENOLEPIS NANA USING EGGS DERIVED FROM DIFFERENT SOURCES

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Education: Graduated with Cambridge School Certificate at Kericho High School, Kenya in 1964; and with Cambridge Higher School Certificate at Friends School, Kamusinga, Kenya in 1966. Attended the University of East Africa in Nairobi in September, 1967 and received the Degree of Bachelor of Science in June, 1970; attended the Oklahoma State University in January, 1972 and completed the requirements for the degree of Master of Science in May, 1975; currently attending Oklahoma State University to complete the requirements for the Doctor of Philosophy.

Professional Experience: Assistant manager, Quality Control, Edible Oil Processing Plant in Kenya, 1970 to 1972; Laboratory Assistant, Zoology Department, University of Nairobi (during vacations). Member of Kenya Amateur Athletic Association.

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