

ESTIMATION OF WORM BURDEN (Ascaridia galli)
IN CHICKENS, BY THE FECAL
EGG COUNT METHOD

by

CARL THEODORE TRAIN

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Approved by:

M. F. Hansen
Major Professor

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INTRODUCTION

There has been a need in basic research, in animal parasitology, to establish a fairly accurate method for determining worm burdens in laboratory animals. This need has become very apparent recently as much work has been done where it would be advantageous to know the number of worms present in the host animal. Several workers found, in basic research with radioactive materials on parasitic nematodes, that a good estimate of the worm burden would have been most helpful and would have prevented the wastage of time and expensive materials.

The fecal egg count method was chosen as a possible means for determining worm burden in lieu of other methods, because this method is practical in most laboratories and it is often used in human parasitology to determine degree of infection and egg productivity of the parasite. This method was also chosen due to the fact that Knapp (1963) attempted without success to use fluoroscopy and X-rays to determine Ascaridia galli worm burden in chickens.

A means of determining worm burden in laboratory animals has been the aim of the present study using the intestinal worm, Ascaridia galli, and its chicken host. This parasite and host are commonly used in laboratories for basic research studies on animal parasites. Statistical work was applied to the results to determine if a prediction equation could be developed to express unknown worm burden from a known egg count.

The reader should note that the data and the results were obtained under specific laboratory conditions and do not necessarily apply to other situations. The results obtained should be interpreted as being true under

these specific conditions but not necessarily so under other conditions.

REVIEW OF LITERATURE

Limited work has been done on the determination of parasitic worm burdens. However, some work has been done relative to the determination of parasitic worm egg productivity and degree of infectivity, particularly in respect to parasitic infections in humans. Here, the fecal egg count method has been used. Smillie (1921) stated that there was a definite relationship between the number of ova in the stools and the number of hookworms harbored by the host, when all cases were considered as a group. However, if hookworm cases were considered individually, there was only a trend toward such a relationship. Frequently, the number of ova in the stools gave a very untrustworthy index as to hookworm burden.

Darling (1922), working with Necator americanus and Ancylostoma duodenale in man, found little correlation between the number of ova in the stool and the number of female worms. He noted there was a great deal of variability or range in the number of ova expelled per female hookworm. He ascribed this to the fecundity of worms at different ages and to differences of physiological environment between human hosts.

Stoll (1923), working with the human hookworm, Necator americanus, developed a conversion factor to determine worm burdens from fecal egg counts. He based his conversion factors on the consistency of the fecal material; with a factor of 44 for formed stools, 25 for soft stools, and 12 for liquid stools. These factors are the average egg count per gram of excrement per female worm. Such factors were based on the fact that 51% of the worms were females and

that the average egg output per female worm per day was approximately 9,000 eggs.

Stoll (1924) stated that the egg output of N. americanus was conditioned by the daily variation in the size of the stool so that correlations should be based on a study of at least three consecutive daily egg outputs. He found that the average of three days of egg output removed most of the inequalities in determining the daily egg output of the female hookworm.

Sweet (1925) followed Stoll's work very closely in determining N. americanus worm burdens from egg counts. He used Stoll's conversion factors satisfactorily and noted that one egg count was not sufficient for a determination of worm burden, but could only be used as a rapid estimation of worm burden.

Hill (1926) reported that the average number of ova deposited by each female N. americanus per day was 2,693, with an average of 18.3 ova per gram of formed feces. He found that Stoll's factors would give too low an estimate of the worm burden. He also noted that egg output per female worm in the male host was one-half that in the female host and that more worms were harbored in the male host.

Soper (1927) studied the egg production of Necator americanus and Ancylostoma duodenale in four human cases in Paraguay. He noted a variation in the number of eggs produced, with total egg counts at times approximating but 5% of the estimated daily output. He stated that this variation in egg count may reflect either an irregularity in egg-laying capacity or a cycle in production of egg cells. He observed that the daily egg output per day of A. duodenale is between two and 2.5 times that of N. americanus, with the

average egg output of N. americanus being 10,000 and that of A. duodenale being 22,000.

Manalang (1927), using Stoll's method, found a perfect correlation between egg counts and hookworm burden. However, he noted individual variants in both egg and female worm counts. He ascribed these variations to volumetrical, biological, immunological, mechanical and chemical factors.

Keller (1934) examined 2,412 specimens for hookworm, using the Stoll egg-counting method and the direct smear method. Doing only a single examination by the two methods on each stool, he found 44% of the specimens were positive by the Stoll method and 39.4% were positive by direct smear. He noted that as the intensity of infection increased, the direct smear method became more accurate. He found that the lowest level of infection, at which the smear would be of value, was 1,200 eggs per gram of feces. The average egg count for those specimens which contained 1-5 hookworm eggs per smear was 1,690 eggs per gram (approximately 65 worms). The average intensity for the group showing 6-25 eggs per smear was 4,190 eggs per gram (approximately 160 worms). In that group of specimens containing 26-40 eggs per smear, the average egg count was 12,325 eggs per gram (approximately 492 worms). If 41 or more eggs per slide were found by the smear examination, the average egg count was 23,100 eggs per gram (approximately 924 worms).

Hurley (1959) used six daily fecal counts to estimate the number of eggs passed per day by four African subjects heavily infected with Necator americanus. The female worm load was calculated by arbitrarily dividing the total egg count by 10,000. The patients were then treated with an anthelmintic and all worms passed in the next 48 hours were collected, counted and sexed. The treatment was again administered in five days, to insure the recovery of all

of the worms. The recovery of female worms exceeded the calculated female load by 33% and the author suggested that the daily output of eggs per female worm be modified to 6,600. The female worms out-numbered male worms by almost 2 to 1. The author stated that a count of 5,000 eggs per gram of feces represented a female worm burden of 100.

Augustine et al. (1928) worked with 74 Egyptian hookworm cases and found that the female worm produces about 238 ova per cc of formed feces. They calculated that the factor 1.19 could be used to estimate the number of female worms present, when using the small drop, 0.075 cc, according to Stoll's method of egg counting. They also worked with 27 Ascaris lumbricoides cases and observed that the female worm produces about 2,700 ova per cc of formed feces. A factor of 13.5 was determined for computing the number of female worms harbored by the host, from the number of ova present in 0.075 cc of feces. They also noted that male and female worms occurred in equal numbers and that the total worm count could be determined by multiplying the estimated female worm count by two. Cram (1925), in work with the egg production of Ascaris lumbricoides, found that a female worm may contain as many as 27 million ova at one time.

Brown and Cort (1927) worked with two human cases infected with A. lumbricoides. In one case, the average egg production per female worm was 234,000 eggs, with the host harboring 43 female worms and 34 male worms. The average egg production, in the other case, was 735,000 eggs per female worm, where the host contained only one female worm. These two cases showed an average of 2,000 eggs per gram of feces per female worm and the authors gave this as a tentative egg-worm ratio.

Manalang (1928b) made egg counts for A. lumbricoides on 22 clinical and autopsy cases. He found, in normal cases, that the average egg count was 1,420 ova per gram per female worm and, in pathological cases, the average egg count was 1,460 ova per gram per female worm.

Brown (1927), in research with N. americanus, A. lumbricoides, and Trichuris trichiura, in humans, found that egg production of the parasite is a very constant phenomenon, while fecal passage by the host is not. He felt that egg count data could be used as an index of the number of worms harbored and that egg counts could serve as a valid measure of the degree of infection of the host. He stated that any variation in day-to-day egg output was a matter of irregularity of the host's functioning rather than irregularity of worm functioning.

Manalang (1928a) conducted work with Trichuris trichiura in four clinical cases and eighteen recently prepared cadavers. He found the T. trichiura egg factor to be around 310 ova per gram per female worm on a formed stool basis. He noted, however, that in cases with intestinal pathology the egg factor was 699. This, of course, would be difficult to diagnose on a living patient and hence is the cause of some of the extreme variation that he noted.

Burrows (1950), in his work with Trichuris trichiura, recovered 4,582 worms from nine patients and found that 54% of the worms were females. The male-female ratio of worms was 1:1.17. He observed that the average number of ova per gram of feces per female worm was about 215 and the average per worm was about 120. He reported a positive correlation between the size of the female worm and the number of ova per gram per female worm and a negative

correlation between the intensity of the infection and the number of ova per gram per female worm. The author stated that any estimation of the number of worms harbored would be incorrect due to the following factors: the age of the infection, the intensity of the infection, the size of the worms harbored, and the size of the normal stool. The estimation would be incorrect because these factors, in all probability, would be unknown to the investigator.

Hansen and Shivnani (1956) believed it was feasible to use larval counts as a means of estimating the nematode worm burden in young bovines. However, they did not attempt to relate egg and larval counts to worm burden.

Dewhirst and Hansen (1961) stated that egg counts have been used as a measure of the number of parasites infecting an animal; but usually egg counts are interpreted as the more eggs present, the more worms present. They attempted to estimate the number of parasites present in young bovines by a differential egg count and noted that because of the large variations inherent in EPG counts, it is apparent that large numbers of animals should be used to obtain exact results. They gave average egg-worm ratios for the following genera of nematodes: Haemonchus, Oesophagostomum, Trichostrongylus, Cooperia, Ostertagia, Nematodirus, Trichuris and Bunostomum. They found that the egg-worm ratios are not the same for all genera and that the ratio of one genus can vary from month to month. They noted a tendency for the number of worms represented by one egg to increase as the animal matured. They felt this increase could be due to three factors: a decrease in egg production, due to crowding, fecal dilution, and the quantity of roughage fed. The authors stated that one should consider the age and diet of the host animal

when interpreting egg counts. They stated that a worm burden estimate should be based on differential egg counts rather than on the usual and more easily-done total egg count, due to the different egg-worm ratios you find in different genera of nematodes.

Kates (1947) found the average egg-worm ratio for all genera of ovine nematodes (Haemonchus, Trichostrongylus, Oesophagostomum, Nematodirus, Cooperia, and Ostertagia) was 1:1.4. This ratio was based on the number of eggs per pellet of feces. Since he obtained his results under certain specified conditions, he believed the ratios were of more value in illustrating the variation in egg productivity of the various nematode genera, than in serving as a basis for calculating nematode populations in sheep from egg count data obtained under different conditions. He also noted that egg production decreased as the total number of nematodes increased.

Andrews (1936) believed that egg production could be estimated on the basis of the number of egg-producing females found post mortem and the number of eggs these worms have produced daily during the period of infection. He daily infected 3 lambs, for a period of 126 days, with infective Cooperia curticei larvae. He made fecal egg counts and calculated the number of eggs passed during a 24-hour period. He noted a considerable difference in the number of eggs produced per female and noted that egg production per female decreased as the total number of parasites increased. In a lamb containing 4,137 female worms, the average daily egg production per female worm was 421 eggs. The average daily egg production per female worm in a lamb containing 3,822 female worms was 390 eggs. In a lamb containing 286 female worms, the average daily egg production per female worm was 937 eggs.

Knapp and Read (1961), in work with Haemonchus contortus, observed the total ova production of infected sheep, but did not attempt to relate fecal egg counts and worm burden. They observed a relationship between the amount of feces and the number of ova passed and that feed consumption was a conditioning factor.

Berger et al. (1961) infected male New Zealand White rabbits with embryonated A. suum eggs and observed the egg production of the female worms. Data from eight rabbits indicated that a mature female ascarid produced from 15,000 to 45,000 viable eggs per day.

Worley (1963) studied the egg production of Obeliscoides cuniculi, a stomach worm of the domestic rabbit. The ratio of eggs per gram of feces to worms in one rabbit was calculated to be 40 eggs per gram of feces per worm. This was in a six-month-old infection and the female to male worm ratio was about 3:1

Erhardt and Denecke (1939a) noted that seven days after infection of rats with filariform larvae of Strongyloides ratti, numerous eggs appear in the feces. The maximum number of eggs were passed between the thirteenth and fifteenth days post-infection, after which the number of eggs passed daily declined rapidly until about the thirtieth day. After the thirtieth day, the number of eggs passed daily remained quite constant and the number of eggs laid daily by a female worm, during this period of the "constant minimum", varied between 175 and 1,000 eggs.

Erhardt and Denecke (1939b) studied the egg laying of a new species of Strongyloides from a wild cat of Sumatra. They experimentally infected a laboratory kitten and the first eggs appeared in the feces on the seventh

day. There were two peaks of egg production, on the fifteenth day and the thirty-eighth day, with irregular numbers of eggs produced in between these days. They made a worm count at necropsy and found the egg production to be an average of 263 eggs per female worm per day.

Sarles (1929) conducted research with infections of Ancylostoma braziliense in dogs and cats. He found egg output increased more slowly and was always smaller for an equal number of larvae in adult animals than in juveniles. He found the average daily egg production per female worm was approximately the same in dogs and cats. He observed the average daily egg production to be 32 eggs at 21-22 days, 2,448 eggs at 43-45 days, and 4,244 eggs at 50-51 days. The total worm burden could be easily calculated, as male and female worms were found in approximately equal numbers.

Herrick (1928) conducted research on egg-worm ratios with Ancylostoma caninum in dogs. He found certain factors which definitely affected the egg-worm ratio; these factors were the age of the worms, the proportion of worms found in copulation, and the proportion of male and female worms. The average number of eggs produced per gram of feces per female worm was 440 and the average number of eggs produced per day per female worm was 10,000. He believed the average number of eggs produced per day per female worm was a better measure of an infection than eggs per gram per female worm because it was less variable and it more nearly estimated the number of worms found at necropsy.

Miller (1939) performed a controlled experiment with six dogs, experimentally infected with Trichuris vulpis. He made fecal egg counts on pooled three day total fecal output, over a period of at least eighteen days after the egg output had become relatively constant. The animals were then

sacrificed and the worms counted. He found the average daily egg output per female worm was 2,035 eggs or 1,350 eggs per worm in the host's bowel, since the ratio of female to male worms was 2:1.

Miller (1941) performed studies on ten juvenile dogs and eleven adult dogs experimentally infected with Trichuris vulpis and found no evidence of age resistance. He found the sex ratios and average daily egg production to be identical with his previous work. He observed that daily egg production was appreciably greater in less intense infections than in heavier infections.

There also has been some work done with the trematodes concerning egg production of the parasite and egg-worm correlations. Faust and Khaw (1941) noted that egg laying is continuous in Clonorchis sinensis. The variations in the number of eggs in the stool were related to irregularities in fecal output of the host, to differences in consistency of the stool, and to temporary lodgement of eggs in the bile ducts or gall bladder of the host. They experimentally infected various laboratory animals and found the daily egg production per worm to be 2,400 eggs in the cat, 1,600 eggs in the guinea pig and 1,000 eggs in the dog. They believed that calculations of the number of worms present in the host may be obtained from the average daily egg count, so recommended this means of calculation for use in human cases.

Stoll et al. (1927a, 1927b) studied a series of six individuals harboring Fasciolopsis buski, a large intestinal fluke of man. The egg output per day per fluke ranged from 14,623 to 48,125 eggs, with a mean output per fluke per day between 21,000 and 28,000 eggs. The overall average for all flukes was about 25,000 eggs per day. The authors calculated an eggs per gram per fluke factor and a close approximation to this was about one-hundredth

of the eggs per day. They found the dilution egg counting method to be superior to the smear method in estimating the daily egg production.

Scott (1931) attempted to determine the egg-worm correlations for Schistosoma mansoni and Schistosoma haematobium. The eggs were detected in the feces and there was very little variability in number of eggs found from day to day. He stated it was impossible to determine egg-worm correlations, but that it would be possible to estimate the relative size of infections.

There has been some work done on the relationship between the number of proglottids passed by the host and the number of tapeworms harbored by the host. Harwood (1938) infected a Plymouth Rock pullet with Raillietina cesticillus and observed the regularity of proglottid passage for the duration of the infection (18 months). He noted that proglottid elimination gradually declined over the study period. However, the decline was not regular, but was marked by periods of intense and then sparse proglottid elimination. He did not attempt to correlate the number of proglottids eliminated and the number of tapeworms harbored by the host.

Reid et al. (1938) did not attempt to find a correlation between tapeworm burdens and numbers of proglottids voided by chickens. They noted that most of the gravid proglottids were voided by the chickens in the afternoon and evening. They stated that this periodicity in shedding of proglottids was associated with feeding and digestion in the chickens and with absorption and assimilation in the tapeworm per se.

Abdou (1958) worked with Davainea proglottina, a tapeworm in the duodenal loop of the small intestine of the domestic fowl. He found that a variable number of proglottids were passed daily and that no relation could be established between the number of gravid proglottids passed in the droppings in 24 hours and the number of tapeworms parasitizing the bird.

Sawada (1960) observed the daily periodicity of segment discharge of birds infected with Raillietina kashiwarensis, R. cesticillus, and R.

echinobothrida. He counted the number of proglottids in the droppings at an interval of two hours from 6 A.M. to 6 P.M. He noted that the time of greatest segment discharge of birds infected with R. kashiwarensis and R. cesticillus was between 2 P.M. and 4 P.M., with no proglottids discharged during the night or early morning. The time of the greatest segment discharge for R. echinobothrida was between 4 P.M. and 6 P.M., with a small number of segments being passed during the night. He believed that the periodic shedding of gravid proglottids was controlled by physiological factors in the alimentary canal of the chicken rather than by internal factors within the tapeworm. He found that it was impossible to determine the number of tapeworms in a bird even if one divided proglottid output from a bird harboring several tapeworms by the proglottid output from a bird known to harbor only one tapeworm. He could not find any rhythmical cycles of segment production which mark increase or decrease of number of segments discharged each day. There also appeared to be a relationship between the beginning of moulting of a bird and a decrease in the segment discharge, but the relationship was obscure.

MATERIALS AND METHODS

One hundred and thirty-five straight-run White Rock chickens were used in the course of this investigation. Because 22 of these birds lost their infection during the experiment, only the results from 113 chickens were statistically analyzed. The chickens were purchased from a commercial hatchery in groups of 25, except for a pilot group of ten birds obtained from the Kansas State University Poultry Farm. The birds were raised in electric brooders, vaccinated internally against Newcastle disease and infectious bronchitis, fed a commercial ration, and watered ad libitum during the experimental period.

The birds were infected per os with 100 ± 10 embryonated Ascaridia galli eggs at the age of fourteen days. The eggs were cultured using an adaptation of the methods of Hansen, Olson and Ackert (1954), Hansen, Terhaar, and Turner (1956) and Larson (1957). A group of A. galli was placed in a mortar and pressure was applied with the pestle until the worms were thoroughly macerated. Artificial digestive juice (1.0% pepsin and 0.5% hydrochloric acid) was then poured on the macerated worms and the mixture was allowed to stand for four to six minutes. The mixture was then poured through an 80-mesh screen into a petri dish. The screen retained the worm cuticula and other debris. Tap water was added to the petri dish and after the eggs had settled to the bottom of the dish, the supernatant liquid was decanted. Three to four additional washings with tap water removed the artificial digestive juice and any remaining debris. The egg cultures were incubated at 30°C to 33°C , in petri dishes, for 14 days. A drop of 1:1000 merthiolate solution was added to 10 cc of water in each petri dish to prevent mold growth (Larson, 1957).

The chickens were infected by feeding the embryonated eggs to the birds with a calibrated micropipette. A variation of the egg administration technique of Hansen et al. (1956) was used. The water was poured off from the petri dish egg culture and 10 to 15 ml of a 1.25 M sucrose solution was poured into the dish. After the eggs had been scraped from the bottom of the dish, the sugar-egg suspension was poured into a small beaker. A drop of the suspension was placed on a glass slide and the eggs counted under a compound microscope. Several drops of suspension were counted to insure that a homogeneous suspension had been obtained. When it was necessary to dilute the suspension, additional 1.25 M sugar solution was added

and eggs in several drops of the new suspension were counted. The suspension was diluted until the micropipette would deliver 100 ± 10 eggs when filled to the appropriate calibration point.

The birds were kept in an uncrowded condition in cages until the infection was 60 days old. This period of time was chosen so as to insure the maturity of all worms. When the infection was 60 days old, the birds were sexed, tagged, and transferred to individual cages with individual feeders, water containers and dropping pans.

A 24-hour fecal sample was collected from each bird, weighed on a Harvard Trip Balance, then placed in glass pint jars and stored in a refrigerator until an EPG (eggs per gram of feces) count could be made. The egg count was made within two days after collection in order to reduce any variation in the egg count because of storage. Data were collected for each bird from eight 24-hour fecal samples. The eight fecal samples were collected at various times post-infection to investigate the effect of maturity of the worms on egg-worm correlation. However, the collections were not evenly spread over the period from 60 days post-infection to necropsy, so a complete picture of the effect of time on egg-worm correlations was not obtained. The time period between inception of fecal collection and necropsy was 18 days for Group A, 39 days for Group B, 33 days for Group C, 15 days for Group D, 43 days for Group E, and 16 days for Group F. However, each 24-hour sample was collected at the same time and day for each group.

Fecal egg counts were made according to the procedure of the modified Lane (1928) flotation technique of Dewhirst and Hansen (1961). With the use of this technique it was possible to determine the total egg count of each animal which was expressed as eggs per gram of feces (EPG).

From each fecal sample, a ten-gram sample, chosen as randomly as possible, was weighed into a 300 ml Erlenmeyer flask on a Harvard Trip Balance. Tap water was added to the 300 ml mark on the flask. The entire contents of the flask were poured into the standard 1000 ml glass container of a Waring blender. The samples were homogenized for 30 seconds in the blender. After homogenizing, approximately 50 ml of the homogenate were strained into a beaker. As quickly as possible following agitation of the beaker, two centrifuge tubes were filled to the 15 ml mark. This step is a very critical one and must be done quickly, because the eggs will settle rapidly in water. The tubes were centrifuged for 3 minutes at 1000 rpm (tachometer reading) in an International Model C50 Centrifuge. After allowing the centrifuge to stop without using the brake, the supernatant liquid was poured off, leaving the eggs and solid material packed at the bottom of the tube. The tubes were shaken by hand to loosen the packed material at the bottom of the tubes and enough aqueous sodium nitrate flotation solution (specific gravity 1.35-1.45) was added to fill the tubes and form a convex meniscus on top. An 18 mm square No. 2 cover glass was carefully placed on the top of each tube and the tubes were centrifuged again for 1 minute at 1000 rpm. A Walser Automatic Timer, Model 8066-B, which automatically stopped the centrifuge, was employed to help standardize the technique. After completion of the final centrifugation, the two cover slips were carefully removed and placed on 2 x 3 inch glass slides. The cover slips were systematically examined under a compound microscope equipped with a mechanical stage. All counts were made using the 10X ocular and 10X objective (low power). The number of eggs was recorded on a laboratory counter and

the number transferred to the data sheet. A technique employed to prevent the crystallization of the flotation solution of the second cover slip, while counting the first cover slip, was to place the slide on a damp towel and place a glass cover over it.

When eight 24-hour fecal samples had been collected and counted from each bird, the birds were necropsied. Worms were recovered from the birds by removing the small intestine from the gizzard to the yolk sac diverticulum and placing it in a flat pan of water. The small intestine was then split manually with a pair of scissors and the contents of the intestine were washed into a 20-mesh screen. In the event that the worms became fractionated, when the intestine was opened, the pieces were matched under a dissecting microscope, as completely as possible, according to body size and sex. The intestinal contents were flushed with water until only the worms remained on the screen. The worms were removed with a forceps, placed in water in jars and refrigerated for 3 days. The dead and relaxed worms were removed from the jars, counted, sexed, preserved in 10% formalin and stored in small vials. The number and sex of the worms were recorded with the appropriate bird and egg counts on the data sheet.

Group A was used as a pilot group to determine the number of cover slips necessary to recover all of the eggs in the centrifuge tube. Several workers have noted that the egg shell of A. galli is sticky. Therefore, the present author believed that this might affect the number of eggs adhering to the coverslip. Also, Farr and Luttermoser (1941) found that the highest percentage of recovery of Ascaridia galli and Heterakis gallinarum eggs was 25.8%. They conducted this experiment by placing known quantities of eggs

in fecal material and calculating the percentage of eggs recovered in an egg count. They used a sugar flotation solution with a specific gravity of 1.270.

A total of four coverslips was counted for each centrifuge tube. The number of eggs counted was recorded and the flotation solution and sediment were examined, under a compound microscope, for any eggs which failed to be floated. The four coverslips picked up approximately 100% of the eggs, as the number of eggs found in the flotation solution and sediment was either zero or few in number. This method of counting four coverslips for each tube was conducted on the eight 24-hour fecal samples collected for Group A.

The data from this four coverslip counting method was analyzed statistically to determine if a correction factor could be derived from this data. Whereby one coverslip could be counted and then, after application of the correction factor, this corrected one coverslip count would be a good estimate of the number of eggs in the tube. Statistical analyses showed that the correlation between the first coverslip and a total of four coverslips was .980592. Further analyses were performed and a correction factor of 1.4 was derived. When using this correction factor, the small egg counts would be well estimated and the large egg counts would be overestimated slightly, this overestimate would be six eggs per hundred.

EXPERIMENTAL RESULTS

A total of 135 straight-run White Rock chickens was used in the course of this investigation. Because 22 of the birds lost their infection during the experiment, only the results from 113 birds were statistically analyzed.

A total of 704 worms was recovered from the birds and the ratio of male to female worms was tested to determine if the sex ratio deviated significantly from a 1:1 male-female ratio. If the sex ratio deviated from a 1:1 ratio, this might have an effect on the prediction of the total number of worms. Table 1 shows the results of a Chi-square test on the sex ratio of the recovered worms. The sex ratio of the worms recovered from all the birds was tested as well as the sex ratio of the worms recovered from male and female birds, respectively. This was done to determine if the sex of the host had any effect on the sex ratio of the worms they harbored. The results showed that the sex ratio of the recovered worms did not deviate significantly from a 1:1 male-female ratio in the three groups of birds. The significant Chi-square value for all three tests was 3.84 and the experimental results were 0.68, 1.32, and 0.936 for total birds, males, and females, respectively.

Tables 2 through 7 indicate the relationship between egg count and worm burden. These tables are based on (1) the average EPG counts based on eight egg counts, (2) the average total egg count obtained by multiplying the average EPG count by the average 24-hour fecal weight, and (3) the average egg production per female worm obtained by dividing the average total egg count by the number of female worms. This method gave a somewhat inaccurate picture of the true relationship because several of the birds lost their female worms before the experiment was terminated. In such instances the EPG counts were zero on the last egg count, but fairly high EPG counts at the beginning of the experimental period made the average EPG count a poor indicator of the number of female worms present. For example, the average number of eggs per

Table 1. Sex ratio of recovered worms.

Sex	Birds		Worms				Chi-square Value	
	Total		Total	Female	Male	Expected Sex Ratio		Observed Sex Ratio
Both Sexes	113		704	341	363	1 : 1	1 : 1.06	0.68 ns*
Male	61		364	171	193	1 : 1	1 : 1.13	1.32 ns
Female	52		340	171	169	1 : 1	1 : 0.936	0.012 ns

* $p < .05 = 3.84$.

Table 2. Relationship between egg count and worm burden. Group A - Infected 78 days.

Bird Number	Sex	Worms		EPG	Eggs/Day (Avg.)	
		Total	Female		Total	Per Female
A-1	Male	1	1	12	1,616.4	1,616.4
A-2	Male	15	6	881	93,738.4	15,623.1
A-3	Male	4	2	371	40,847.1	20,423.5
A-4	Male	3	1	283	24,224.8	24,224.8
A-5	Male	9	4	1,939	143,292.1	35,823.0
A-6	Female	2	0	15	1,014.0	-
A-7	Female	16	9	2,181	171,426.6	19,047.4
A-8	Female	14	9	1,797	186,708.3	20,745.4
A-9	Female	4	3	1,505	88,494.0	29,498.0
A-10	Female	10	8	2,749	176,553.5	22,069.2

Table 3. Relationship between egg count and worm burden. Group B - Infected 99 days.

Bird Number	Sex	Worms		EPG	Eggs/Day (Avg.)		Per Female
		Total	Female		Total		
B-1	Female	4	3	592	78,795.2	26,265.1	
B-2	Male	1	1	3	482.4	482.4	
B-4	Male	5	4	794	132,443.6	33,110.9	
B-5	Male	1	0	5	748.5	-	
B-6	Female	4	2	479	90,215.7	45,107.8	
B-7	Male	6	4	591	105,375.3	26,343.8	
B-8	Male	3	1	88	18,779.2	18,779.2	
B-9	Female	3	2	519	59,892.6	29,946.3	
B-10	Female	8	2	455	61,834.5	30,917.2	
B-11	Female	8	5	654	103,135.8	20,627.1	
B-12	Female	34	18	3,596	656,270.0	36,459.4	
B-13	Male	2	1	298	55,457.8	55,457.8	
B-14	Male	3	2	400	102,080.0	51,040.0	
B-15	Male	12	6	716	134,966.0	22,494.3	
B-16	Male	1	0	21	4,132.8	-	
B-17	Female	4	2	332	45,583.6	22,791.8	
B-18	Male	4	2	106	27,634.2	13,817.1	
B-20	Female	1	1	2	273.8	273.8	
B-21	Female	3	1	162	29,403.0	29,403.0	
B-23	Female	11	8	2,123	313,567.1	39,195.9	

Table 4. Relationship between egg count and worm burden. Group C - Infected 93 days.

Bird Number	Sex	Worms		EPG	Eggs/Day (Avg.)		Per Female
		Total	Female		Total		
C-2	Female	3	3	.1	8.7	2.9	
C-3	Female	7	1	390	26,637.0	26,637.0	26,637.0
C-4	Male	8	4	4,161	129,823.2	32,455.8	32,455.8
C-5	Male	3	2	337	40,376.7	20,188.3	20,188.3
C-6	Male	2	2	7	789.6	394.8	394.8
C-7	Male	4	1	700	53,340.0	53,340.0	53,340.0
C-8	Female	3	3	386	37,673.6	12,557.0	12,557.0
C-9	Female	8	4	1,298	156,149.4	39,037.3	39,037.3
C-10	Male	14	6	1,741	227,548.7	37,924.8	37,924.8
C-11	Male	4	1	526	57,649.6	57,649.6	57,649.6
C-12	Female	4	1	77	9,147.6	9,147.6	9,147.6
C-13	Male	3	2	530	55,862.0	27,931.0	27,931.0
C-14	Male	6	2	197	32,000.4	16,000.2	16,000.2
C-15	Male	1	1	35	4,854.5	4,854.5	4,854.5
C-16	Female	2	1	573	46,928.7	46,928.7	46,928.7
C-17	Male	2	0	3	150.5	-	-
C-18	Male	1	1	.9	24.8	24.8	24.8
C-19	Female	2	2	23	1,313.3	656.6	656.6
C-21	Male	8	1	101	12,766.4	12,766.4	12,766.4
C-22	Male	9	3	320	50,784.0	16,928.0	16,928.0

Table 5. Relationship between egg count and worm burden. Group D - Infected 75 days.

Bird Number	Sex	Worms		EPG	Eggs/Day (Avg.)		Per Female
		Total	Female		Total		
D-1	Female	9	1	207	20,420.9	20,420.9	20,420.9
D-3	Male	14	7	1,947	152,450.1	152,450.1	21,778.6
D-4	Male	2	1	545	40,548.0	40,548.0	40,548.0
D-5	Male	3	1	377	29,368.3	29,368.3	29,368.3
D-6	Female	3	1	690	47,548.0	47,548.0	47,548.0
D-8	Female	3	0	1	75.2	75.2	-
D-9	Female	8	2	973	61,007.1	61,007.1	30,503.5
D-10	Female	3	1	141	13,098.9	13,098.9	13,098.9
D-12	Female	6	0	1	52.1	52.1	-
D-13	Male	13	3	573	67,843.2	67,843.2	22,614.4
D-15	Male	23	5	523	73,481.5	73,481.5	14,696.3
D-16	Male	49	25	6,063	769,394.7	769,394.7	30,775.8
D-18	Female	8	1	17	1,824.1	1,824.1	1,824.1
D-19	Male	19	11	492	88,756.8	88,756.8	8,068.8
D-23	Female	13	7	886	123,862.8	123,862.8	17,694.7
D-24	Male	7	2	270	46,278.0	46,278.0	23,139.0
D-25	Female	8	3	909	84,264.3	84,264.3	28,088.1
D-26	Female	25	9	2,802	190,536.0	190,536.0	21,170.7

Table 6. Relationship between egg count and worm burden. Group E - Infected 103 days.

Number	Bird		Total	Worms		EPG	Eggs/Day (Avg.)		Per Female
	Sex	Sex		Total	Female		Total	Female	
E-1	Male		1			18		5,441.4	5,441.4
E-2	Female		1	1		395	1	117,038.5	117,038.5
E-3	Female		3			206	3	32,980.6	10,993.5
E-4	Male		1	1		3	1	623.1	623.1
E-6	Female		11	4		1,555	4	194,841.5	48,710.4
E-7	Male		1	0		21	0	4,111.8	-
E-8	Male		3	2		336	2	117,902.4	58,951.2
E-10	Female		5	3		1,030	3	201,777.0	67,259.0
E-11	Female		1	0		94	0	23,283.8	-
E-12	Female		3	2		990	2	166,221.0	83,110.5
E-13	Female		1	0		79	0	13,327.3	-
E-14	Male		8	2		395	2	121,225.5	60,612.7
E-16	Male		5	0		45	0	10,030.5	-
E-17	Male		3	1		346	1	93,593.0	93,593.0
E-18	Female		15	8		4,274	8	774,021.4	96,752.7
E-19	Male		2	0		3	0	762.6	-
E-20	Male		5	3		925	3	208,680.0	69,560.0
E-22	Male		2	0		347	0	57,116.2	-
E-23	Female		5	2		861	2	111,757.8	55,878.9
E-24	Male		2	0		133	0	33,396.3	-

Table 7. Relationship between egg count and worm burden. Group F - Infected 76 days.

Bird Number	Sex	Worms		EPG	Eggs/Day (Avg.)		Per Female
		Total	Female		Total		
F-1	Male	4	2	254	57,099.2	28,549.6	
F-2	Female	7	6	596	126,292.4	21,048.7	
F-3	Female	14	9	2,479	453,144.4	50,571.6	
F-4	Male	2	2	104	22,640.8	11,320.4	
F-5	Male	6	5	671	200,293.5	40,058.7	
F-6	Female	4	2	481	98,797.4	49,398.7	
F-7	Female	4	2	344	62,332.8	31,166.4	
F-8	Female	4	2	1,068	154,539.6	77,269.8	
F-9	Female	1	0	2	253.4	-	
F-10	Female	1	0	.1	11.6	-	
F-11	Male	5	1	238	58,928.8	58,928.8	
F-12	Male	3	3	4	770.0	293.3	
F-13	Male	7	3	554	72,407.8	24,135.9	
F-14	Female	6	4	900	124,020.0	31,005.0	
F-15	Male	1	1	1	189.1	189.1	
F-17	Male	11	9	1,302	267,148.7	29,772.4	
F-18	Male	16	10	3,041	671,148.7	67,114.8	
F-19	Female	1	1	369	55,977.3	55,977.3	
F-20	Male	3	2	557	141,756.5	70,878.2	
F-21	Female	8	7	1,418	324,438.4	46,348.3	
F-22	Male	1	1	7	1,436.4	1,436.4	
F-23	Female	4	2	471	93,163.8	46,581.9	
F-24	Male	2	2	2	383.4	191.7	
F-26	Male	3	3	543	100,292.1	33,430.7	
F-27	Male	2	0	2	489.0	-	

female worm varied from 273 to 117,038. Even though this bias was present, the tables nevertheless show that there was a relationship between egg counts and worm burden.

These tables also show the relationship of time to egg counts and worm burden. The data indicate that the longer the time of infection, the more variable the average egg count per female worm and the greater the spontaneous elimination of female worms. Therefore, an average egg count became an unreliable indicator of worm burden as the length of time of the infections increased. Group A, containing 10 birds, was killed 78 days post-infection; Group B, containing 20 birds, was killed 99 days post-infection; Group C, containing 20 birds, was killed 93 days post-infection; Group D, containing 18 birds, was killed 75 days post-infection; Group E, containing 20 birds, was killed 103 days post-infection; and Group F, containing 25 birds, was killed 76 days post-infection.

A graphical example of variations in egg counts with time for birds in Group A is shown in Plate I. Six different birds and eight egg counts for each bird are illustrated. This figure indicates that there is more variance in the egg counts of birds with greater worm burdens than among those with lesser worm burdens. Bird A-1 harbored one worm (one female worm) and the egg counts varied from 26 to one, Bird A-3 harbored four worms (two female worms) and the egg counts varied from 438 to 162, Bird A-4 harbored three worms (one female worm) and the egg counts varied from 387 to 162, Bird A-5 harbored nine worms (four female worms) and the egg counts varied from 3361 to 947, Bird A-7 harbored 16 worms (nine female worms) and the egg varied from 3106 to 749, and Bird A-9 harbored four worms (three female worms) and the egg counts varied from 1527 to 658.

EXPLANATION OF PLATE I

Relationship between egg counts (EPG)
and time in Group A.

PLATE I

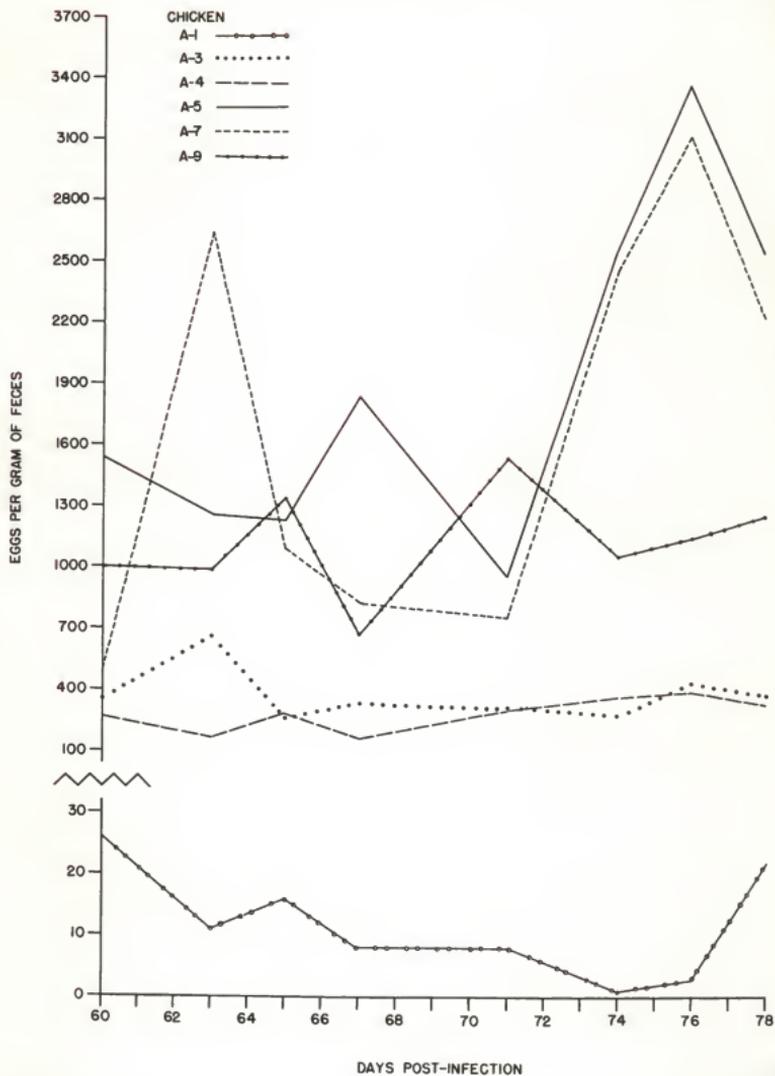


Table 8 shows the linear correlation coefficients between the following variables: the sex of the host, the egg count (EPG), the fecal weight, the days to necropsy, and the number of female worms. All correlations were significant at the $P < .05$ level. The first two correlations were calculated with the male birds equal to one and the female birds equal to zero. Thus, correlation $-.098114$ means with respect to variables 1 vs 2 that the male birds had a significantly lower egg count than did the female birds. Likewise, the correlation $-.072838$ shows that the male birds had significantly fewer female worms. These correlations and their meaning will be discussed later. In correlations 2 vs 3 and 2 vs 4, the egg count decreased as the fecal output and the days to necropsy increased. Correlation $.765752$ between variables 2 vs 5 shows that the egg counts increased as the number of female worms increased. Correlation $.285710$ between variables 3 vs 4 shows that the fecal output increased as the days to necropsy increased. A negative correlation of $-.197233$ between variables 4 vs 5 indicates that the number of female worms decreased as the days to necropsy increased.

If the sex of the bird, fecal weight, and days to necropsy, together with the egg count, were included, a multiple regression coefficient of $.786998$ was obtained between these variables and the number of female worms. The difference between this multiple regression coefficient and the simple regression coefficient ($.765752$) between egg count and the number of female worms was statistically significant, but one doesn't gain enough of a change in the coefficient to warrant the usage of the multiple regression coefficient, since its computation is more difficult and involved. Simple linear regression was used in calculating correlation coefficients reported in Tables 8 and 9.

Table 8. Test of linear correlation coefficients for host-parasite variables.

Coefficient	:	Variables
-.098114	:	1 vs 2
-.072838	:	1 vs 5
-.149191	:	2 vs 3
-.094260	:	2 vs 4
.765752	:	2 vs 5
.285710	:	3 vs 4
-.197233	:	4 vs 5

$P < .05$, significance level = .062.

- 1 = sex of host
- 2 = egg count
- 3 = fecal weight
- 4 = days to necropsy
- 5 = number of female worms

Table 9. Test of linear correlation coefficients for host-parasite variables.

Coefficient	:	Variables
-.098114	:	1 vs 2
-.041730 ns	:	1 vs 5
-.151190	:	2 vs 3
-.097190	:	2 vs 4
.732039	:	2 vs 5
.281646	:	3 vs 4
-.225021	:	4 vs 5

$P < .05$, significance level = .062.

ns = not significant

- 1 = sex of host
- 2 = egg count
- 3 = fecal weight
- 4 = days to necropsy
- 5 = total number of worms

Table 9 gives the linear correlation coefficients between the following variables: the sex of the host, the egg count (EPG), the fecal weight, the days to necropsy, and the total number of worms. These correlations were all significant ($P < .05$) except 1 vs 5. The first two correlations were calculated in the same manner as were the first two correlations in Table 8. Correlation $-.098114$ means that with respect to variables 1 vs 2, the male birds had a significantly lower egg count than did the female birds. The nonsignificant correlation $-.041730$ shows that there was no difference in total worm burden between male and female birds. In correlations 2 vs 3 and 2 vs 4, the egg count decreased as the fecal output and days to necropsy increased. Correlation $.732039$ between variables 2 vs 5 shows that the egg counts increased as the total number of worms increased. Correlation $.281646$ between variables 3 vs 4 shows that the fecal weight increased as the days to necropsy increased. A negative correlation of $-.225021$ between variables 4 vs 5 indicates that the total number of worms decreased as the days to necropsy increased.

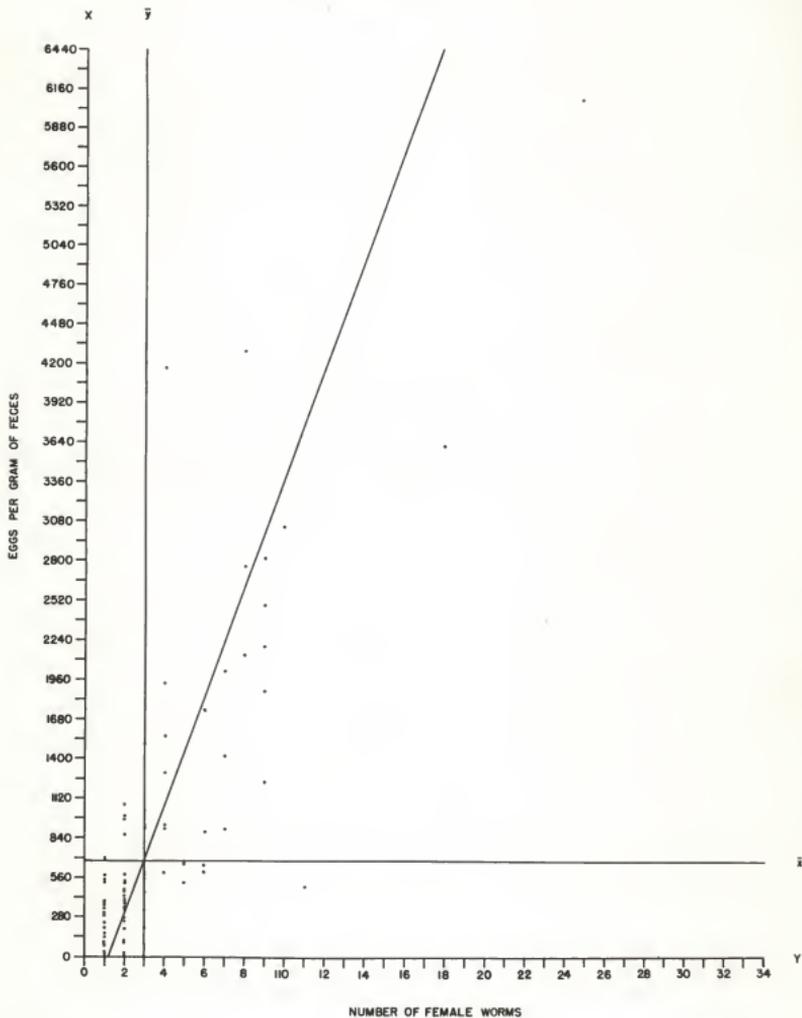
If one included the sex of the bird, the fecal weight, and the days to necropsy, together with egg count, a multiple regression coefficient of $.75007$ was obtained between these variables and the total number of worms. The difference between this multiple regression coefficient and the simple regression coefficient of $.732039$ (egg count vs total number of worms) was statistically significant. Again, the change in size of the coefficient was not large enough to warrant the usage of the multiple regression coefficient.

Plate II is a scatter diagram showing the relationship between the number of eggs per gram of feces and the number of female worms. The X axis is

EXPLANATION OF PLATE II

Scatter diagram showing the relationship between egg counts (EPG) and the number of female worms.

PLATE II



the EPG count and the \bar{x} line is the average EPG count (682.8). The Y axis is the number of female worms and the \bar{y} line is the average number of female worms (3.04). The diagonal line in the figure is the sample regression line, which was computed as follows:

$$\hat{Y}_Q - \bar{y} = b(X - \bar{x})$$

$$\hat{Y}_Q - 3.04 = .0026 (X - 682)$$

\hat{Y}_Q = the estimated number of female worms for a given egg count

\bar{y} = the average number of female worms

b = the slope of the regression line, the change in Y for each unit change in X

X = the number of eggs per gram of feces

\bar{x} = the average number of eggs per gram of feces

Example: What is the estimated number of female A. galli when a bird has an EPG count of 50?

$$\hat{Y}_Q - 3.04 = .0026 (50 - 682)$$

$$\hat{Y}_Q = 3.04 + .0026 (50 - 682)$$

$$\hat{Y}_Q = 1.27 + .0026 (50)$$

$$\hat{Y}_Q = 1.40$$

Several points were computed using different egg counts in the same manner as was the point representing 50 EPG. A line, representing the sample regression line, was drawn connecting these points. This sample regression

line indicates the relationship between X and Y and is the best estimate of Y for a given value of X. This line must be determined before you can compute the prediction equation, for it is from this regression line that you determine the value of "a", the value where the regression line intersects the Y axis when X is zero. The constant, "a", is used in the computation of the prediction equation.

The following prediction equation was developed to estimate the number of female worms from a known egg count. This prediction equation was developed from a simple regression statistical method as indicated above.

$$\hat{Y}_{\phi} = a + bX$$

$$\hat{Y}_{\phi} = 1.2352 + .0026X$$

$$\hat{Y}_{\phi} = \text{the predicted number of female worms}$$

a = the "Y intercept", the value where the estimating equation intersects the Y axis when X is zero, the value of \hat{Y} when X is zero

b = the slope of the estimating equation, indicating by its value the amount of change in the Y series for each unit change of the X series

X = the number of eggs per gram of feces

Example: What is the estimated number of female A. galli in a bird having an EPG count of 50?

$$\hat{Y}_{\phi} = 1.2352 + .0026 (50)$$

$$\hat{Y}_{\phi} = 1.2352 + .1300$$

$$\hat{Y}_{\phi} = 1.3652$$

This prediction equation would explain 58.7% of the variability in female worm count from bird to bird. The percentage of variability was obtained by squaring the correlation coefficient (.765752), egg count vs number of female worms. The squared product was then expressed as a percentage.

r = correlation coefficient between the egg count and the number of female worms.

$$r = .765752$$

$$r^2 = (.765752)^2$$

$$r^2 = .587$$

expressed as a percentage = 58.7%.

If one included the sex of the bird, the 24-hour fecal output and the days to necropsy, the resulting equation would explain 61.9% of the variability in female worm counts, between birds. Again, the percentage of variability was obtained by squaring the multiple regression coefficient. This difference between the simple regression technique and the multiple regression technique in explaining the variability was statistically significant, but the multiple regression method was not used in this work for reasons previously stated.

Confidence intervals can be placed on the prediction equation. This confidence interval is based on the variance of the female worm counts. The confidence interval was computed as follows:

$$\hat{Y}_{\phi} \pm t_{.05} \sqrt{s^2_Y (1 - r^2)}$$

\hat{Y}_{ϕ} = the estimated number of female worms

$t_{.05}$ = the t value at the .05 probability level and at an infinite number of degrees of freedom

s^2_Y = the variance of the female worm counts

r^2 = the square of the correlation between egg counts and the number of female worms

$$\hat{Y}_{\phi} \pm 1.96 \sqrt{12.85(1 - .587)}$$

$$\hat{Y}_{\phi} \pm 1.96 \sqrt{12.85(.413)}$$

$$\hat{Y}_{\phi} \pm 1.96 (2.3037)$$

$$\hat{Y}_{\phi} \pm 4.515$$

Therefore, at a given egg count, you can be 95% confident that the estimated number of female worms will be within the interval of ± 4.515 . Confidence intervals at different levels of confidence can also be computed by substituting t values of different probability levels into the equation. For example, 80% confidence interval is $\hat{Y}_{\phi} \pm 2.949$ and the 60% confidence interval is $\hat{Y}_{\phi} \pm 1.935$.

Plate III is a scatter diagram showing the relationship between the EPG counts and the total number of worms. The X axis is the EPG count and the \bar{x} line is the average EPG count (682.8). The Y axis is the total number of worms and the \bar{y} line is the average number of worms (6.2). The diagonal line in the figure is the sample regression line and was computed in the following manner:

$$\hat{Y}_T - \bar{y} = b(X - \bar{x})$$

$$\hat{Y}_T - 6.2 = .0048(X - 682)$$

\hat{Y}_T = the estimated number of worms for a given egg count.

\bar{y} = the average number of worms.

b = the slope of the regression line, the change in Y for each unit change in X.

X = the number of eggs per gram of feces.

\bar{x} = the average number of eggs per gram of feces.

Example: What is the estimated total A. galli burden in a bird having an EPG count of 50?

$$\hat{Y}_T - 6.2 = .0048(50 - 682)$$

$$\hat{Y}_T = 6.2 + .0048(50 - 682)$$

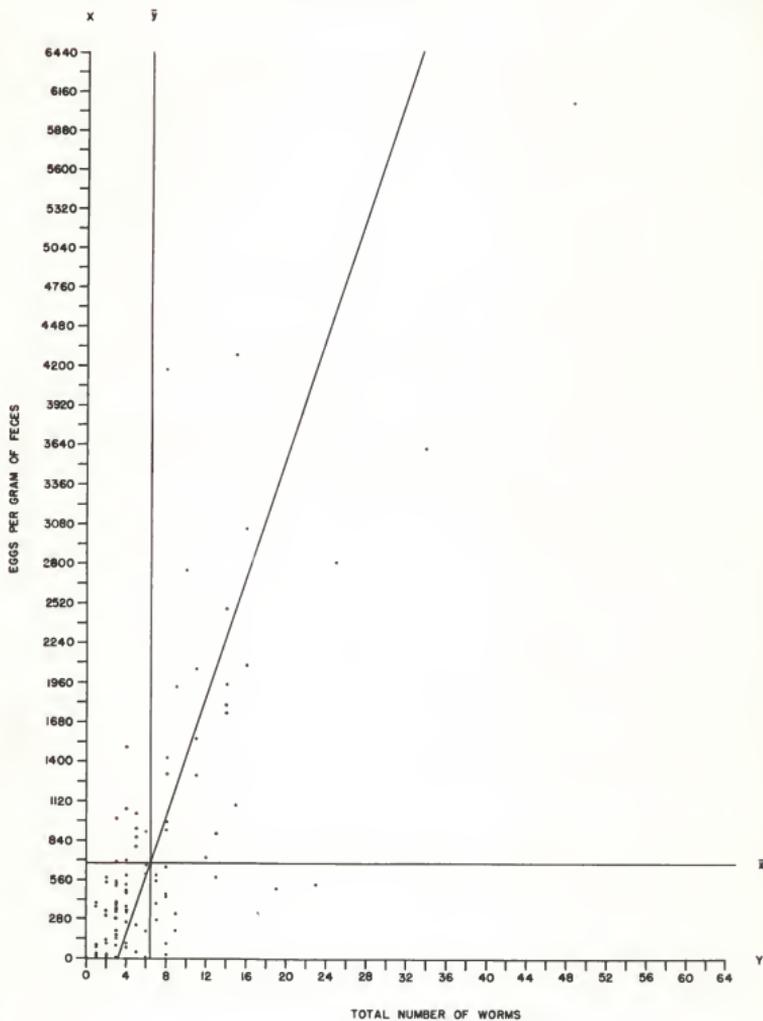
$$\hat{Y}_T = 2.93 + .0048(50)$$

$$\hat{Y}_T = 3.17$$

EXPLANATION OF PLATE III

Scatter diagram showing the relationship between egg counts (EPG) and the total number of worms.

PLATE III



Several points were computed for different egg counts, just as was done for Plate II. These points were then connected to give the sample regression line in order to determine the value of the constant "a" relative to the total number of worms.

The prediction equation for the total number of worms was also developed from a simple regression statistical method and was based on the fecal egg count. The prediction equation is as follows:

$$\hat{Y}_T = a + bX$$

$$\hat{Y}_T = 2.9374 + .0048X$$

$$\hat{Y}_T = \text{the predicted total number of worms}$$

a = the "Y intercept", the value where the estimating equation intersects the Y axis when X is zero, the value of \hat{Y} when X is zero.

b = the slope of the estimating equation, indicating by its value the amount of change in the Y series for each unit change of the X series.

X = the number of eggs per gram of feces.

Example: What is the estimated total A. galli burden in a bird having an EPG count of 50?

$$\hat{Y}_T = 2.9374 + .0048 (50)$$

$$\hat{Y}_T = 2.9374 + .2400$$

$$\hat{Y}_T = 3.1774$$

This equation will explain 53.6% of the variability in total worm count between birds. This percentage was obtained by a squaring of the correlation coefficient between the egg count and the total number of worms. The squared product is then expressed as a percentage.

r = correlation coefficient between the egg count and the total number of worms.

$$r = .732039$$

$$r^2 = (.732039)^2$$

$$r^2 = .536$$

expressed as a percentage = 53.6%.

If one included the sex of the bird, the 24-hour fecal output, and the days to necropsy, the resulting equation would explain 56.3% of the variability in total worm count between birds. This percentage was obtained similarly as the one above, by squaring the multiple regression coefficient. This difference in explanation of variability between the simple regression technique and the multiple regression technique was statistically significant, but did not justify using the more difficult to calculate multiple regression technique.

Confidence intervals can be placed on the prediction equation for total worm burden. This confidence interval is based on the variance of the total worm counts. The confidence interval was computed as follows:

$$\hat{Y}_T \pm t_{.05} \sqrt{s^2 Y(1 - r^2)}$$

\hat{Y}_T = the estimated total number of worms.

$t_{.05}$ = the t value at the .05 probability level and at an infinite number of degrees of freedom.

s^2_Y = the variance of the total worm counts.

r^2 = the square of the correlation between egg counts and the total number of worms.

$$\hat{Y}_T \pm 1.96 \sqrt{46.6(1 - .536)}$$

$$\hat{Y}_T \pm 1.96 \sqrt{46.6(.464)}$$

$$\hat{Y}_T \pm 1.96(4.65)$$

$$\hat{Y}_T \pm 9.114$$

Therefore, at a given egg count you can be 95% confident that the estimated number of worms will be within the interval of ± 9.114 . Confidence intervals at different levels of confidence can also be computed by substituting t values of different probability levels into the equation. For example, the 80% confidence interval is $\hat{Y}_T \pm 5.952$ and the 60% confidence interval is $\hat{Y}_T \pm 3.906$.

DISCUSSION

The sex ratio of the recovered worms did not deviate significantly from a 1:1 female-male ratio and the sex of the host did not influence this ratio. This was determined because if the sex ratio was not 1:1, this might affect the prediction equation for the total number of worms. Stoll (1923), Sweet (1925), Hill (1926), Augustine et al. (1928), Sarles (1929), in work with human hookworm, and Burrows (1950), with Trichuris trichiura, reported a

sex ratio of 1:1. Therefore, they needed to multiply the predicted female worm count by two to determine the total number of worms. However, Miller (1939 and 1940), working with Trichuria vulpis, Hurley (1959), with Necator americanus, and Worley (1963), with Obeliscoides cuniculi, found the female-male ratio varied from 2:1 to 3:1.

It was found, on the basis of Tables 2 through 7, that an average egg count can be a very untrustworthy indicator of the number of worms harbored by the host. Darling (1922) stated that variations in egg counts were due to differing degrees of fecundity in female hookworms. Smillie (1921) found that a single egg count often gave a very unreliable estimate of the number of hookworms harbored. Soper (1927) also noted a variation in egg counts of human hookworms and Zawadowsky and Zvjaguintzev (1932) found a seasonal variation in the number of Nematodirus eggs in the feces. Andrews (1936) noted a considerable difference in egg production between female Cooperia curticei. Spedding (1952) found the egg content of the feces of sheep varied considerably, from day to day, in every animal tested.

An average egg count was used in the present study as a broad base of evaluation because an average egg count would remove many of the effects of the daily variations inherent in individual egg counts. Other workers have also stated that an average egg count was the best basis for egg-worm correlations. Stoll (1924) stated that an average of at least three days of egg output removed most of the variations in egg counts of human hookworms. Sweet (1925) also stated that several egg counts should be made and that the average egg count obtained from these was the best estimate of human hookworm burden. He observed that a single egg count could only be used for a rapid estimation of degree of infection.

Plate I showed the variation which existed in egg counts and also their relationship to time. This figure showed that the higher egg counts apparently have much more variation in the number of eggs in the stools than those stools with lower egg counts. Birds A-1, A-3, and A-4 showed very little variation in their egg counts. Their egg counts, when plotted, formed a comparatively straight line. Bird A-9, even though there was some variation, showed a fairly even or stable egg production. However, Birds A-5 and A-7 showed an extreme variation in their egg counts. This variation, in part, explains the wide confidence intervals and the large variability unexplained by the prediction equations. Other workers have also noted variations between egg counts.

It was found, upon computing the correlation coefficients between the different variables in the present study, that even though most of the coefficients were statistically significant, they were not large enough to have a great influence in the development of the prediction equation. However, the coefficient indicating the relationship between the egg count and the number of female worms was quite high (.765732) which one would expect. The more female worms present in the host, the more eggs one would expect to find in the feces. Researchers such as Smillie (1921), Stoll (1924), Manalang (1927), and Andrews (1936) reported similar findings with other nematodes. A negative correlation was observed in the present study between the sex of the host and the egg count. This correlation was not too high (-.098114) and it should probably be ignored because it could have occurred as a coincidence in computation of the data. However, Hill (1926) found that egg output per female Necator americanus in the male host was one-half that in the female host.

It was noted that male birds harbored fewer female worms. However, this correlation coefficient was small ($-.072838$) and probably should be considered as a coincidence in computation. Hill (1926), however, noted that the male host harbored more Necator americanus than the female host. A negative correlation ($-.149191$) was also found between the egg count and the 24-hour fecal output. This would be expected, because as the fecal output increased with the age of the bird, the egg counts would become smaller because of fecal dilution.

A negative correlation of $-.094260$ was also observed between the egg count and the number of days to necropsy. This correlation was small; nevertheless, one would expect this trend as the worms reached the end of their egg production, died, and passed out of the birds. Sarles (1929) found that the egg output of Ancylostoma braziliense increased to a maximum peak and then slowly decreased. However, Herrick (1928), observed that the number of eggs produced by each female Ancylostoma caninum increased considerably with age. The small negative correlation obtained could have been caused by an interaction between this increase in egg production in some worms and the completion of egg production and death of other worms.

A positive correlation ($.235710$) was also found between the fecal weight and the days to necropsy. This would be expected considering the physiology of the host. As the host increased in age, it grew, ingested more food, and more feces would be voided by the host.

A negative correlation ($-.197233$) was noted between the days to necropsy and the number of female worms. This would be expected because worms would be eliminated or have their egg production curtailed as they became senile.

Also, the hosts may have developed an age resistance as Kerr (1954) found in work with Ascaridia galli.

It was interesting to note in the present work that one could obtain a good estimate of the female worm burden without taking such factors as the sex of the bird, the 24-hour fecal output, and the days to necropsy into consideration. These factors are generally considered by other workers as having an important effect on worm burden estimations. In fact, it was stated by Burrows (1950), in work with Trichuris trichiura, that such factors as the age of the infection, the intensity of the infection, the size of the worms harbored, and the size of the normal stool would be of extreme importance in estimation of the number of worms harbored by the host.

Similar simple regression correlation coefficients were found between the variables mentioned previously and the total number of worms. The only difference that was noted was a nonsignificant negative correlation of $-.041730$ between the sex of the host and the total number of worms. The sex of the host, therefore, did not influence the size of the total worm burden. However, the sex of the host could have an influence on the sex of the worms as shown by the significant correlation between the sex of the host and the number of female worms.

The prediction equation used to estimate the number of female worms was developed from a simple regression technique. This was done because one does not gain enough in predicting value, by using all of the other factors, (sex of host, fecal output, time), to warrant the use of the more difficult to compute multiple regression. However, with the use of this equation, one could not explain 41.3% of the variability in female worm counts between birds. If the multiple regression method would have been used, one still

could not explain 38.1% of the variability. Even with the factors of egg count, sex of the host, the 24-hour fecal output and the days to necropsy taken into consideration, this large unexplained variability still remained. The explanation of this remaining variability is something that should be investigated. Manalang (1927) stated that volumetrical, biological, immunological, mechanical, and chemical factors caused variations in human hookworm egg counts. It may be some aspects of host physiology, it could be some unrecognized environmental effect, or it could be some aspects of parasite physiology that caused this variation. These unexplained factors could be the basis for future research work and they are factors which should be investigated.

A number of workers have stated that certain aspects of host physiology caused the variability in egg counts. Brown (1927) stated that any variation in egg output was due to an irregularity in the host's functioning, rather than to an irregularity of nematode functioning. Manalang (1928a) found that pathological conditions in the host's digestive tract influenced egg output of Trichuris trichiura. He also stated that egg destruction by the host's body could not be a factor because of the resistant nature of the egg shell. Burrows (1950) observed that such factors as the size of the host's normal stool could have an effect on the egg output of Trichuris trichiura. Stoll (1923), Sweet (1925), Levine et al. (1956), and Rodriguez (1958) emphasized the importance of taking into account the fecal consistency when making nematode egg counts. However, Scott and Headlee (1938), Riek, et al. (1958), and Peters and Lelper (1940) stated that there was little value in making adjustments for fecal consistency when interpreting egg counts.

Dewhirst and Hansen (1961) observed that the diet and age of the host animal should be considered when egg counts are interpreted. Risk et al. (1958) stated that adjustments should be made in fecal egg counts to account for changes in fecal output associated with the age and body weight of cattle. Spedding (1952) stated that variation in nematode egg counts could be due to a rhythm in intestinal activity of the sheep host or to some type of fecal rhythm. Hill (1926) noted that the sex of the host had an effect on the egg output of Necator americanus and also on the number of worms harbored by the host. Zawadowsky and Zvjagintzev (1932) noted a seasonal variation in the number of ova passed by llamas infected with a species of Nematodirus. They attributed this variation to the temperature of the air. Taylor (1935) and Fudalewicz-Niemczyk and Lenkiewicz (1960) noted a seasonal variation in egg counts of sheep infected with various genera of nematodes.

Other workers have stated that variation in egg counts were due to various aspects of parasite physiology. Many workers have noted that the egg production of the individual worm decreased as the total number of worms increased (Andrews, 1936; Kates, 1947; and Dewhirst and Hansen, 1961). Soper (1927), in work with human hookworms, stated that variation in egg counts was due either to an irregularity in egg laying by the parasite or to a cycle in the production of egg cells by the parasite. Taylor (1935) believed that fluctuations in trichostrongylid egg counts were due to variations in the rate of egg production of the adult worms. Cushnie and White (1948) stated that variations in egg counts during the year were due to greater egg laying activity by nematodes at certain times of the year.

Darling (1922) stated that variation in egg count was evidently an expression of the fecundity of female hookworms of different ages in different individuals. Burrows (1950), studying Trichuris trichiura, found a positive correlation between the size of the female worm and the number of eggs per gram of feces per female worm.

These factors would also be important in the determination of the total worm burden. The prediction equation used to estimate the total number of worms was also developed from a simple regression technique, using fecal egg counts only, rather than a multiple regression technique. This prediction equation explained only 53.6% of the variability and a prediction equation developed from a multiple regression technique would still leave 43.7% of the variability unexplained. The aspects of variability mentioned before, such as physiology of the host, environment, and physiology of the parasite, would play just as important a role in explaining this variability as it would in explaining it when determining the female worm burden. These are factors which need to be investigated further.

The confidence intervals computed for these prediction equations enables one to predict the number of females and total number of worms within a specific range. These confidence intervals were computed from the variance of the female worm counts and the total worm counts and the large amount of unexplained variability accounts for the large confidence intervals. The 95% confidence interval for the female worm prediction was ± 4.515 . Therefore, one would be 95% confident that the actual number of female worms would be within ± 4.515 of the predicted number of female worms. The 80% confidence interval of ± 2.949 and the 60% confidence interval of ± 1.935 could be used in the same manner. The confidence intervals for the prediction

of the total number of worms were larger than the confidence intervals for the prediction of the number of female worms because of the much larger variance of the total worm counts. This would be expected because irrespective of the overall worm sex ratio of 1:1, one would find some individual worm burdens were not in a 1:1 sex ratio. This would cause the variance to be large; therefore, the confidence interval would be large. For example, the 95% confidence interval for total worm count was ± 9.114 , the 80% confidence interval was ± 5.952 , and, at the 60% level, the confidence interval was ± 3.906 . These confidence intervals cannot be used without regard for the number of eggs found in the feces. For example, if one predicted a female worm burden of two, it must be realized that the bird would not contain minus two female worms, a number that could be achieved if the 95% confidence interval were interpreted too literally. Obviously a bird passing eggs in the feces has some female worms.

The present author found that the number of eggs in the feces did indicate the number of female worms. The prediction equation developed gave an indication of the number of female worms harbored and confidence intervals can be placed on this prediction. The total number of worms can also be estimated on the basis of fecal egg counts. However, more variability was present in the prediction of total worm count, thus the confidence intervals were larger. Nevertheless, a large part of the variability remained to be explained and this explanation will be attempted in future research. There are many other workers who have views similar to the present author (Smillie, 1921; Stoll, 1924; Manalang, 1927; and Spedding, 1953). However, other workers stated that there wasn't any way to determine egg-worm correlations. Herrick (1928) stated that factors, such as the age of the worms, the proportion of the worms found in copulation, and the

proportion of male and female worms, would prevent accurate determination of the egg-worm correlations of Ancylostoma caninum. Scott (1931) stated that it was impossible to determine egg-worm correlations of schistosomes and Burrows (1950) stated that any estimation of the number of Trichuris trichiura harbored would be incorrect due to the following factors: the age of the infection, the intensity of the infection, the size of the worms harbored, and the size of the normal stool.

This work can be considered as a preliminary work, for many things remain to be answered. However, it has been learned that the number of female worms and the total number of worms can be predicted and that egg counts can be used in this prediction. Therefore, future work should be based upon this investigation. Some questions that parasitologists have desired to answer for a long time are: why is it that a majority of birds in groups given the same dosage of A. galli eggs harbor only a few worms, whereas, a few birds have a large worm burden? Is it an aspect of host physiology or is it concerned with parasite physiology? These questions remain to be answered but we cannot find the answers without knowing which bird harbors a few worms and which one has a large worm burden. This must be answered by a fairly accurate estimate of the worm burden. In order to obtain a more accurate prediction of the worm burden, we must find out what some of the variables are that caused 38.1% and 43.7% of the variability to be unexplained. It is planned to determine if the consistency or moisture content of the feces play a part in variability, if the age and size of the worms play a part, if large worm burdens cause an erratic egg count, and if the entire egg production is steady or erratic throughout the patency period of the infection. It is also planned to check the accuracy of the prediction

equation by using one egg count as the indicator of worm burden and also using the average of two, three, four, etc. egg counts as the indicator. It is planned to determine what effect spontaneous elimination of the worms has on the egg count by checking the feces for passed worms and noting the effect of this elimination on the egg count. The usefulness of the prediction equation will also be tested in determining worm burden during short, intermediate, and long periods of patency of infection. This method can also be used to determine the equation's accuracy in predicting the size of both laboratory and natural infections. The effect of the sex of the host and fecal output on egg-worm correlations should be investigated further. It seems that these factors should play an important role in explaining variability. It should also be determined if such factors as fasting and the rate of peristalsis of the host have an effect on egg counts. The results of this study have given direction to future research in resolving the problem of egg-worm correlations. The author is continuing this study for his doctorate thesis.

SUMMARY

A total of 135 straight-run White Rock chickens were used in the course of this investigation. Because 22 birds lost their infection during the course of this investigation, results from only 113 birds were statistically analyzed. The birds were experimentally infected at 14 days of age with 100 ± 10 embryonated Ascaridia galli eggs. When the infection was 60 days old, the birds were transferred to individual cages, eight 24-hour fecal samples were collected for each bird, and egg counts (EPG) were made on these samples. When eight egg counts were made, the birds were necropsied

and the worms recovered, counted, sexed, and preserved. The results, including the corrected egg counts, the number and the sex of the worms, the fecal weight, the sex of the host, and the number of days to necropsy, were statistically analyzed.

A total of 704 worms was recovered from the birds and these worms occurred in a male-female sex ratio of 1:1.

It was found that average egg counts often gave a very untrustworthy index as to the number of worms (female and total) harbored by the host. However, these averages did show the trend of egg-worm correlations and that they can be used for this purpose. Average egg counts also helped to alleviate some of the extreme variation noted in individual egg counts.

Statistical analysis showed that the different variables in the experiment were correlated in various ways. A positive correlation (.765752) between the egg count and the number of female worms means that the more female worms in the host, the more eggs one would find in the feces. The correlation between the egg count and the total number of worms (.732039) also indicated that more worms in the host would give a higher egg count. Positive correlations of .285710 and .281646, between the fecal weight and the days to necropsy mean that older birds would void more feces. A negative correlation (-.098114) indicated that the male birds had a lower fecal egg count than female birds. A negative correlation of -.072838 showed that male birds had fewer female worms than female birds; however, the sex of the host did not influence the total worm burden as shown by a nonsignificant correlation of -.041730. Negative correlations of -.149191 and -.151190, between the egg count and the fecal weight, mean that egg counts get smaller as the fecal weights become larger, due to fecal dilution. Correlations of -.094260 and -.097190 were noted between the egg count and the days to

necropsy. This means that the egg counts became smaller as the number of days to necropsy became larger. This would be expected as worms would be eliminated or have their egg production curtailed as they became senile. It was found that not enough was gained in explanation of the variability in total and female worm counts between birds, by including the sex of the bird, the fecal weight, and the days to necropsy, along with egg count, to warrant use of the multiple regression technique over the simple regression technique.

A prediction equation was developed to estimate the number of female worms from the EPG count. The X in the equation stands for the number of eggs per gram of feces. This prediction equation was:

$$\hat{Y}_F = 1.2352 + .0026X$$

A prediction equation was also developed to estimate the total number of worms from the EPG count. The X in the equation stands for the number of eggs per gram of feces. This prediction equation was:

$$\hat{Y}_T = 2.9374 + .0048X$$

Confidence intervals were also developed from the variances of the female worm counts and the total worm counts. These confidence intervals were quite wide, but this was because of the large variance that was present. However, these confidence intervals should be very useful when interpreting the prediction equations. Confidence intervals for predicting the female worm burden were as follows:

$$CI_{.95} = \hat{Y}_F \pm 4.515$$

$$CI_{.80} = \hat{Y}_F \pm 2.949$$

$$CI_{.60} = \hat{Y}_F \pm 1.935$$

Confidence intervals for predicting the total worm burdens were as follows:

$$CI_{.95} = \hat{Y}_T \pm 9.114$$

$$CI_{.80} = \hat{Y}_T \pm 5.952$$

$$CI_{.60} = \hat{Y}_T \pm 3.906$$

These prediction equations still leave 41.3% of the variability in female worm counts between birds and 46.4% of the variability in total worm counts between birds unexplained. This variability could be the result of some aspect of host physiology, environment or parasite physiology as yet undetermined or it could be an interaction of two or more of these factors. Additional investigations are needed to more accurately determine the causes of this variability.

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ESTIMATION OF WORM BURDEN (Ascaridia galli)
IN CHICKENS, BY THE FECAL
EGG COUNT METHOD

by

CARL THEODORE TRAIN

B. S., Bethany College, Lindsborg, Kansas, 1961

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Department of Zoology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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There has been a need in basic research, in animal parasitology, to establish a fairly accurate method for determining worm burden in laboratory animals. This need has become very apparent recently as much work has been done where it would be advantageous to know the number of worms harbored by the host. The fecal egg count method was chosen in the present study as a possible means of determining worm burden, because it can be done in most laboratories and has been used with some success in human parasitology.

The results from 113 White Rock chickens were statistically analyzed. Chickens were infected at 14 days of age with 100 ± 10 embryonated Ascaridia galli eggs. When the infection was 60 days old, the birds were sexed and transferred to individual cages. A total of eight 24-hour fecal samples were collected for each bird and a fecal egg count (EPG) was made on each sample. When the eight egg counts had been made, the birds were necropsied and the recovered worms were counted, sexed and preserved. The compiled data were then statistically analyzed.

The recovered worms were found in a 1:1 sex ratio. Average egg counts often gave an untrustworthy index of worm burden. However, these average egg counts did show the trend of egg-worm correlations and helped to reduce some of the extreme variability noted in individual egg counts. Various correlations were found between the factors involved in the experiment. Most of the correlations were fairly small, but the correlations between the egg counts and the number of female worms and the total number of worms were .765752 and .732039, respectively. The more female worms present in the host, the more eggs one would expect to find in the feces.

Prediction equations were developed whereby the female worm burden or the total worm burden could be predicted from the egg count. These prediction equations were based on the egg count alone and computed with the use of a simple regression technique. A multiple regression technique, which used the factors of the sex of the host, the fecal weight, and the days to necropsy, together with egg count, offered no advantage over the simple regression technique. The prediction equation for the number of female worms was $\hat{Y}_Q = 1.2352 + .0026X$, where X equaled the number of eggs per gram of feces. The prediction equation for the total number of worms was $\hat{Y}_T = 2.9374 + .0048X$, where X equaled the number of eggs per gram of feces.

Confidence intervals were developed for these prediction equations, based on the variance of the worm counts. The confidence intervals for the female worm predictions were $CI_{.95} \hat{Y}_Q \pm 4.515$; $CI_{.80} \hat{Y}_Q \pm 2.949$; and $CI_{.60} \hat{Y}_Q \pm 1.935$. The confidence intervals for the total worm predictions were $CI_{.95} \hat{Y}_T \pm 9.114$, $CI_{.80} \hat{Y}_T \pm 5.952$, and $CI_{.60} \hat{Y}_T \pm 3.906$.

A great deal of variation was noted between egg counts and worm burdens in this study and a large portion of this variation still is unexplained. Other workers have also noted and attempted to explain this large variation between egg counts and worm burdens. They have related this problem to host physiology, environment, parasite physiology or interactions of these aspects.