A STUDY OF NUMBERS OF MICROORGANISMS IN THE INTESTINAL TRACT OF CHICKENS PARASITIZED WITH ASCARIDIA GALLI AND OF UNINFECTED CONTROL CHICKENS

by

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INTRODUCTION

The biology of the chicken merits comprehensive study because of the animal's great economic importance and wide use for nutritional studies.

Little is known about the intestinal microflora of the chicken parasitized with the round worm Ascaridia galli (Schrank), however, the normal microflora of many species of normal fowl had been subjected to studies before the end of the 19th century.

One quantitative study dealt with a protozoan parasite, Eimeria tenella (Railliet and Lucet), the agent of coccidiosis. This microorganism is a tissue invader of the intestinal type and opens the way for entrance of secondary bacterial invaders. It was found that this infection resulted in variance from the norm of particular types of bacteria of the intestinal microflora.

Because of the difference in numbers of bacteria in the intestine of normal chickens and chickens infected with E. tenella (Railliet and Lucet), it was thought that there might be found a similar relationship between chickens infected with A. galli (Schrank) and those left uninfected. However, the host-parasite situation is entirely different with the parasitic protozoan E. tenella (Railliet and Lucet) and the parasitic metazoan A. galli (Schrank) as A. galli (Schrank) resides either free in the lumen of the intestine or partly buried in the intestinal crypts, and as far as is known, at no time invades the tissue itself. Hence, there is no portal for bacterial infection set up due to the A. galli (Schrank) infection.

This study has been conducted mainly on a quantitative basis to determine the effect of parasitism by A. galli (Schrank) on the

numbers of the common inhabitants of four segments of the intestine of the chicken.

REVIEW OF THE LITERATURE

This study began with a search of the literature for data concerning the microflora of both the normal fowl and the parasitized fowl. Few data were discovered involving the effect of parasitism on numbers and kinds of microorganisms in the intestinal flora of birds, however, many studies have been conducted on the microflora of normal birds.

Kern (1897) was the first to study the intestinal microflora of fowl. He found 88 species of bacteria in 24 different species of birds. Direct microscopic preparations led him to suspect that large numbers of species do not grow when submitted to general cultural methods. He believed that degradation products from bacterial metabolism tended to kill the bacteria. He concluded that the following organisms were obligate intestinal forms: Bacterium coli (Escherichia coli)[1] Micrococcus nitidis, Bacillus vegatus, Bacillus defessus, Bacillus vergatus, Bacterium verruscosum, Bacterium cavatum, and Pseudomonas granulata. He found the colon bacillus the predominant microorganism. He also believed that the types of bacteria present were just a reflection of the food eaten, hence variable.

Rahner (1901) reported E. coli, gram positive cocci, molds, Bacillus megatherium, and lactic acid bacteria in the intestine of the

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[1] The names of the organisms mentioned in this thesis are those used by various authors cited in the literature. The designation according to Bergey's Manual of Determinative Bacteriology, 6th Edition, Baltimore: Williams and Wilkins, 1948, follows in parentheses each obsolete name.

hen. He thought that E. coli was a constant inhabitant and tended to crowd out the other organisms whose nature depended to a great extent upon the type of food eaten. Numbers of E. coli increased as the cloaca was approached.

King (1905) studied the bacterial flora of the intestinal mucosa and conjunctiva of the normal chicken. The conclusions that he reached were much the same as those of Rahner in that E. coli was the predominating organism and occurred in much greater abundance in the lower part of the intestinal canal and few if at all in the duodenum.

Gage (1911) in making differential counts of morphologically different bacteria found that the intestinal flora of 45 healthy birds varied to some extent with the conditions of environment and different stages of life. He reported that E. coli predominated, making up 60 per cent of the intestinal flora and that gram positive cocci formed 30 per cent of the flora. He indicated that he found gas in the cecas of newly hatched chicks but that he was unable to find anaerobes.

Menes and Rochlin (1929) reported from studies of hens, geese, and turkeys the finding of E. acidi-lactici (E. coli var. acidilactici), Streptococcus faecalis, and Lactobacillus beijerincki. He also concluded that the flora was identical at all levels of the tract.

Emmel (1930) found that E. coli and E. coli communior (E. coli var. communior) were the predominating organisms found in the feces of chicks and hens, constituting 60 per cent of the total organisms present. He found three obligate anaerobes in the flora of 30 chickens studied.

Johansson and Sarles (1948) studied the changes in the bacterial population due to infection with Eimeria tenella (Railliet and Lucet). They reported that on either a grain mash or synthetic diet, during

the course of infection, the numbers of lactobacilli and enterococci were reduced in the cecal flora. Coliforms remained unchanged during cecal coccidiosis, and the growth of anaerobes was stimulated by coccidiosis.

Shapiro and Sarles (1949) studied the microflora of the intestinal tract of normal chickens. They reported that newly hatched chicks harbor few organisms in their intestines. After feeding, the counts rose to a point where they leveled off at the 16-hour period after first feeding. The groups studied (coliforms, lactics, enterococci, anaerobic and aerobic sporeformers) were lowest in the duodenum, ileum, and colon, and highest in the cecal pouches. They reported lactobacilli species to be the most numerous group of bacteria in the tract. E. coli was the predominant coliform, Streptococcus faecalis the predominant enterococcus, and Clostridium perfringens the predominant anaerobic spore-former. They stated that anaerobic sporeformers were transient members of the intestinal flora. Shapiro et al. (1949) continued these studies with lactobacilli species and isolated and classified them according to their sugar fermentation reactions.

EXPERIMENTAL PROCEDURES

Preparation of Animals and Rations

The chickens used during the course of the experimentation were white Plymouth Rocks.

The birds were purchased as baby chicks from a local hatchery at one day old and placed in wire battery cages. Thirty of the chicks were selected at random and were placed in a separate wire battery cage. These chicks were eventually to be infected with A. galli

(Schrank) and the remaining were to be left uninfected and kept as controls. Both groups were given the same rations throughout the experiments.

The rations used to feed the chicks through the work was "Page's 18 per cent Egg Mash". The label indicated that the ration contained: Protein - not less than 18.00 per cent, crude fat - not less than 4.00 per cent, fiber - not less than 50.00 per cent. The ration contained the following ingredients: Meat and bone scraps, soybean oil meal, wheat grey shorts, wheat bran and screenings, ground milo, dehydrated alfalfa meal, ground yellow corn, ground oats, distiller's dried grains with solubles, Vitamin B-12 and antibiotic feed supplement, vitamin A acetate (source of vitamin A), D-activated animal sterols (source of vitamin D-3), riboflavin, calcium pantothenate, niacin, choline chloride, wheat protein hydrolysates, di-calcium phosphate, ground lime-stone, salt, and the following trace minerals: potassium iodide, manganese sulfate, iron sulfate, copper sulfate, potassium sulfate.

Preparation of Ascaridia galli (Schrank) Eggs

The intestines from chickens parasitized with A. galli (Schrank) were removed and the intestinal contents flushed into a receptacle. Adult female worms were selected for preparation of the egg cultures. The cuticles were removed from the worms and the uteri separated from the other parts of the body cavity. These uteri were placed in a solution containing 1.0 per cent pepsin and 0.5 per cent HCL. The pepsin in an acid solution digested off the uterine walls freeing the eggs. The eggs were then washed with tap water to rid the eggs of

pepsin and HCL. At that time the eggs were placed in sterile dishes containing tap water and incubated at 30° C for 14 days. After 14 days the eggs were larvated and infective (Hansen et al., 1954).

Infecting the Chickens

When the eggs had developed to the infective stage, they were given, per os, to those chicks selected previously for growth of the adult worms. Each chick was given 100 ± 10 eggs. The large number of eggs was given because one can expect to obtain, on the average, only seven to 10 adult worms from each chicken, and this large number is necessary to insure infection. The remainder of the chickens were left uninfected as controls. At this time the chicks were approximately four weeks old.

Sample Preparation for Culturing the Intestinal Flora

Five chicks were picked at random from the infected group and three from the control group at the following times after infection; 0, 11, 21, 40, and 60 days. These times were selected to allow studies at the different stages the worm goes through during growth from the larval to mature state. Also, the worm is free in the intestine at the larvated egg stage and upon hatching it locates in the crypts of the intestine, and later is again free in the intestine until it reaches maturity.

Each chick was sacrificed quickly by breaking its neck and the intestinal tract from the gizzard to the cloaca was exposed. The particular segments to be examined were individually removed and placed in separate sterile petri dishes. These were placed in refrigeration

until all eight of the chicks to be sampled were used. The segments of the intestine examined were designated as parts A, B, C, and D. Part A was the duodenum. D was the part from the point of attachment of the cecal pouches to that point of the intestine one-half the distance between the cecal pouch attachments and the yolk sac diverticulum. Parts B and C were designated as the areas between A and D, divided into equal parts. B is the first part of that area and C the second part. (Fig. 1.)

The contents of each segment were squeezed under aseptic conditions into sterile petri dishes which were immediately refrigerated. The time required between autopsying the chicks and the refrigeration of all samples varied between 15 and 25 minutes.

After all samples had been obtained, each was thoroughly mixed in its petri dish and a one gram sample was weighed out on a piece of sterile aluminum foil. When the chicks were small, it was often difficult to obtain a one gram sample, in which case the amount available was weighed and used. The weighed sample was thoroughly mixed in a six-ounce dilution bottle containing 99 ml of sterile distilled water and a few small glass beads. The beads aided in breaking up the material to be examined. A small quantity of material was removed directly from the intestine and examined microscopically for the presence of larvae or macroscopically for adult A. galli (Schrank).

From the 1:100 dilutions, serial dilutions to $1:10^8$ were made and inoculations were made as rapidly as possible into the tubes and petri dishes required for the culturing procedures. The maximum dilution of $1:10^8$ was determined previously by culturing on the appropriate media for groups to be studied. Test ranges from 1:100 to $1:10^{11}$ were tried

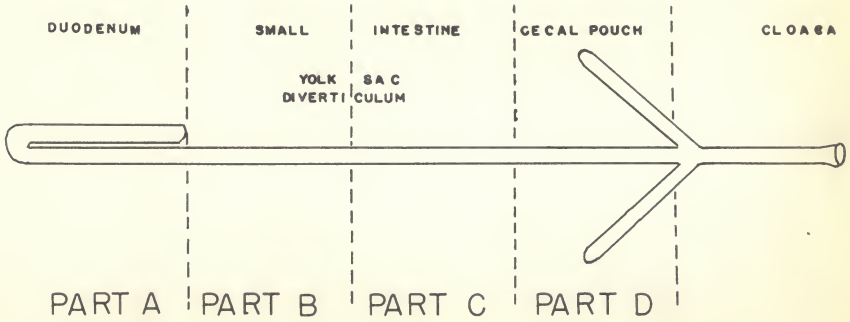

Fig. 1. The chicken intestine.

and range 10^8 determined as the maximum necessary.

Media and Cultural Procedures Used During the Experiments

(a). For aerobic agar plate counts the following medium was used: beef extract 0.3 per cent, peptone 0.5 per cent, agar-agar 1.5 per cent. The medium was adjusted to a pH of 6.8 to 7.0.

(b). For anaerobic agar plate counts, Brewer Anaerobic agar was used. Anaerobic conditions were obtained by incubating the plates in a Brewer Anaerobic jar containing a 100 per cent atmosphere of methane gas. A test-tube containing calcium carbonate and another of methylene blue-glucose mixture was also added to the jar (Van Reimsdijk, 1922). The calcium carbonate collected part of the carbon dioxide present in the jar and the methylene blue gave a visual indication of anaerobic conditions by becoming reduced and turning colorless. The Brewer Anaerobic jar was first evacuated with an electric vacuum pump for 10 minutes and then the jar was filled with methane. This procedure was again repeated to insure anaerobic conditions.

(c). Eosin methylene blue agar was used for coliform plate counts.

(d). Potato glucose agar acidified to pH 3.5 was used for yeast plate counts. (Standard Methods for the Examination of Water, Sewage, and Industrial Wastes, 1955).

(e). "SF" broth (Litsky et al., 1953) was used for dilution counts of enterococci.

(f). Orange serum agar was used to count lactobacilli.

(g). For spore counts the 1:100 dilution of each sample was heated in a water bath at 80° C $\pm$ 1° C for 11 minutes and then rapidly cooled. From this heat treated sample suitable dilutions were made

and aerobic agar plates of medium "a" were prepared. A series of plates of Brewer Anaerobic agar was prepared, inoculated and incubated anaerobically in an atmosphere of 100 per cent methane gas.

The plates in cultural procedures (a), (b), (c), (d), (f), and (g) were prepared singly by the usual "pour" plate method. The "SF" broth cultures in procedure (e) were prepared by using six dilutions, five tubes per dilution. The "SF" broth cultures and the orange serum agar cultures were incubated at room temperature (20 to 25° C) for five days. The remaining cultures were incubated for two days at 37° C.

Enumeration of Bacteria

After incubation, colony counts were made of suitable dilutions of all plates with the aid of a Quebec colony counter. The most probable numbers of bacteria in the "SF" broth cultures were determined by using the M.P.N. table in Standard Methods for the Examination of Water, Sewage, and Industrial Wastes, 1955. All counts were expressed on the wet weight basis.

EXPERIMENTAL RESULTS

At time of sacrificing, the chickens weighed approximately: 0 time, 138 grams; 11 days, 185 grams; 21 days, 450 grams; 40 days, 836 grams; 60 days, 1245.

The chicks were approximately four weeks old at the time of infection and their intestinal microflora was well established. No determinations of the bird's initial flora could be made.

To determine the groups and numbers of bacteria in the different parts of the chicken's intestine, samples were taken from the respective

parts of intestines of both infected and control chickens (five infected and three controls were used in each experiment) from time of infection to time of maturity of the worm (60 days).

Various media were used to determine the different groups present and the data for the eight counts made were plotted and are presented in Fig. 2.

During the entire course of the experimentation, no yeast colonies were found in dilutions as low as 1:100.

Part A of Infected Intestine

By examining the graph in Fig. 2, it can be seen that all groups of bacteria showed a decline during the 11 to 21 day period with the exception of the coliform group, which increased to a point where it leveled off at 21 days and remained fairly constant. The anaerobic counts were somewhat higher than the aerobic counts and the two counts did not parallel one another as was found in some other intestinal parts. The sporeforming anaerobic and sporeforming aerobic counts were not too far apart in the 21 to 60 day period. The lactic acid bacteria were the most numerous of the groups and the enterococci were the fewest, but this is probably of no significance.

Part A of Control Intestine

According to the graph in Fig. 3, there was an initial decline followed by an increase in enterococci, lactic acid bacteria, non-sporeforming aerobes, and sporeforming aerobes during the first 21 days. The anaerobes and coliforms increased in numbers while the sporeforming anaerobes remain constant throughout the period. The aerobic and anaerobic counts ran parallel following an initial fluctuation. In

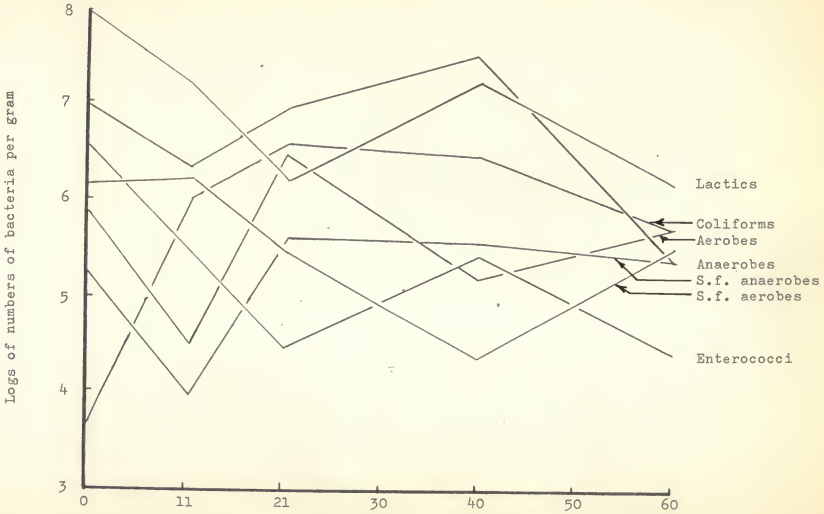

Fig. 2. Part A of the infected intestine

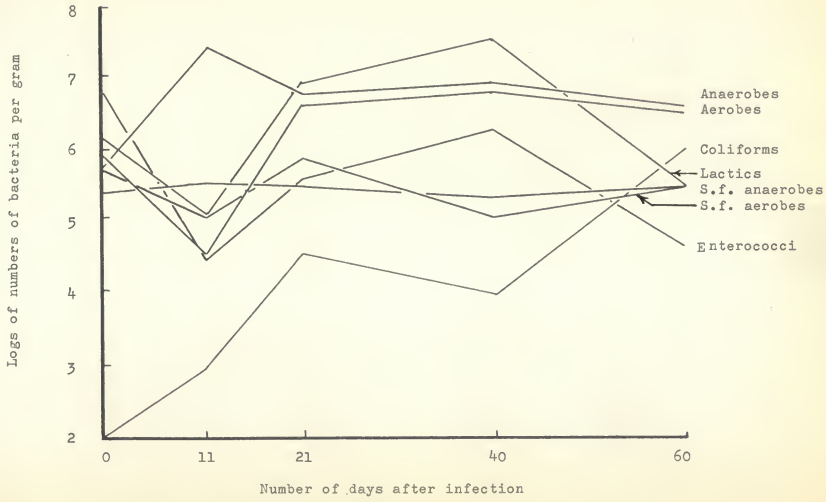

Fig. 3. Part A of control intestine

this part of the intestine the anaerobes and lactic acid bacteria were the most numerous while the coliforms were the fewest throughout the 40 day period.

Part B of Infected Intestine

The graph in Fig. 4 shows an initial decline followed by a rise in lactic acid bacteria, enterococci, anaerobes and aerobes. There does not seem to be the parallel relationship between aerobic and anaerobic counts in this case, but the counts on sporeforming aerobes and sporeforming anaerobes do parallel each other. The lactic acid bacteria were the highest in number, while there was no definite low group during the entire 60 days as these groups were within the same range.

Part B of the Control Intestine

Figure 5 shows an initial decline with subsequent rise up to 21 day period occurred in lactic acid bacteria, anaerobes, aerobes, and enterococci groups. Sporeforming aerobes and sporeforming anaerobes increased initially to 11 days where they leveled off and ran parallel during the rest of the experiment. The aerobes and anaerobes paralleled each other, while the coliforms and lactic acid bacteria were the lowest and the highest of the groups, respectively.

Part C of the Infected Intestine

Figure 6 shows that an initial decline and rise to 21 days occurred in the coliform, aerobic, anaerobic, and enterococci groups, while a continuous increase in the lactic acid bacteria took place during the first 21 days. The aerobic and anaerobic groups paralleled each other during the course of the experiment as did the sporeforming aerobes

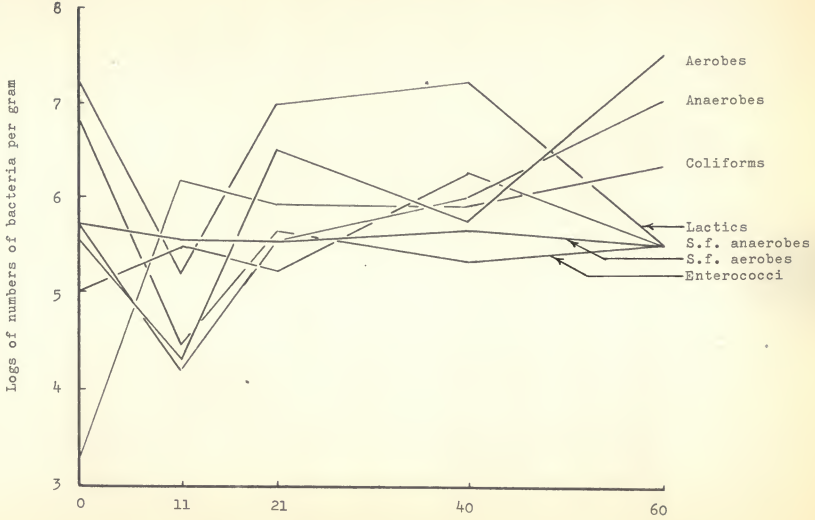

Fig. 4. Part B of infected intestine

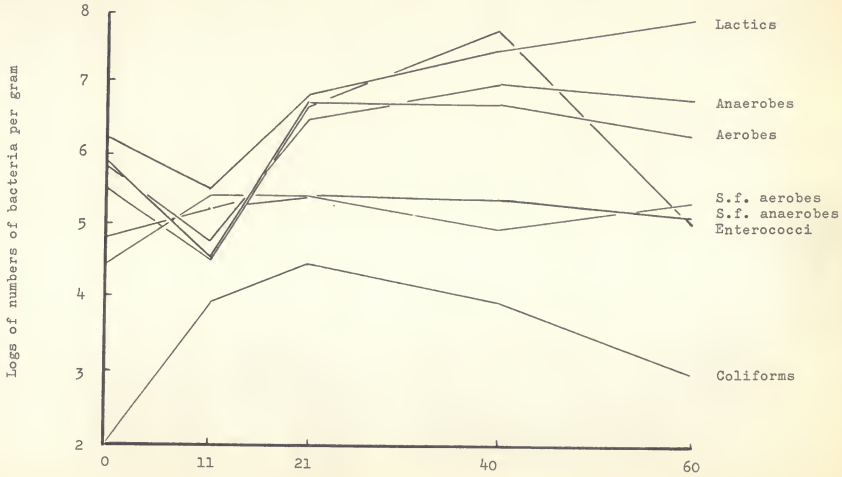

Fig. 5. Part B of control intestin

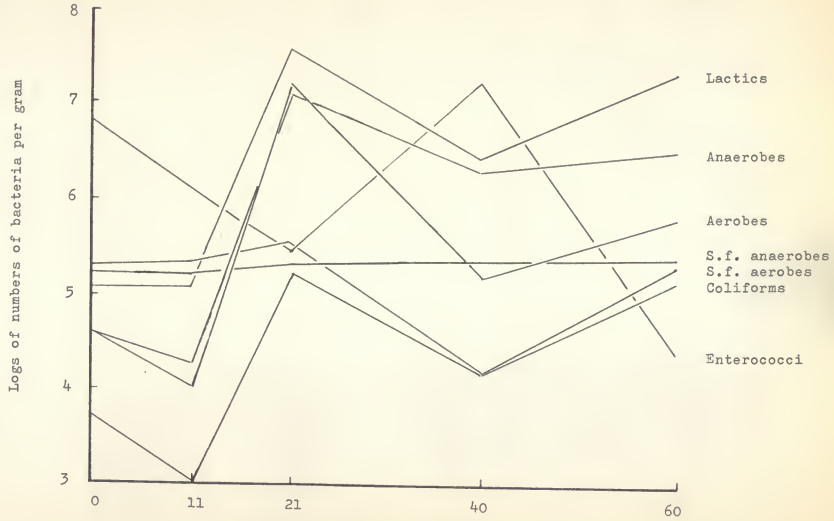

Fig. 6. Part C of infected intestine

and sporeforming anaerobes. Lactic acid bacteria counts were the highest and coliform counts the lowest.

Part C of the Control Intestine

Figure 7 shows no initial decline and rise in bacterial numbers as in the other intestinal parts; however, the aerobes and anaerobes paralleled each other and the numbers of sporeforming aerobes and sporeforming anaerobes were close together even though they did not run parallel. Lactic acid bacteria counts were again the highest and coliforms the lowest.

Part D of the Infected Intestine

Figure 8 shows the sporeforming aerobes were lowest while the coliforms were higher than in any other part of the intestine studied. The lactic acid bacteria were again the most numerous and a parallelism is revealed between the aerobes and anaerobes. Part D exhibited wide variance in numbers of sporeforming aerobes and sporeforming anaerobes.

Part D of the Control Intestine

Figure 9 reveals that sporeformers, anaerobic sporeformers, and coliforms paralleled each other during the entire period. The aerobes, anaerobes and lactic acid bacteria exhibited the highest counts in this case, while the enterococci gave the lowest counts, but this is probably of no significance.

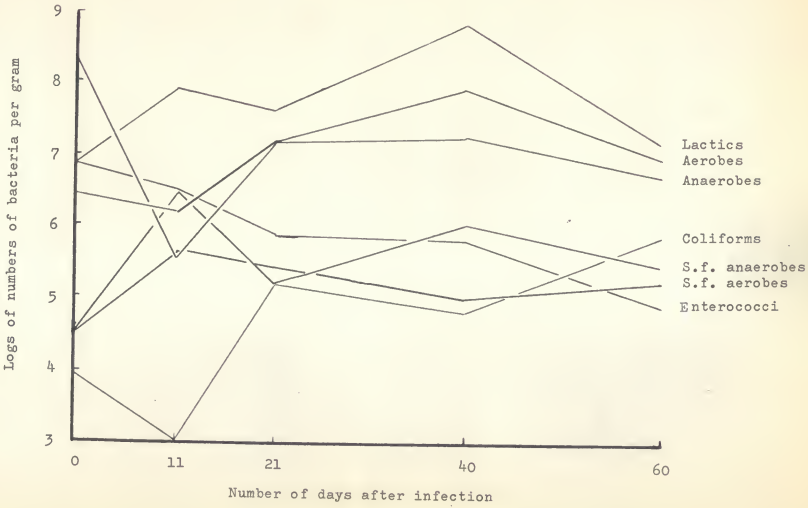

Fig. 7. Part C of control intestine

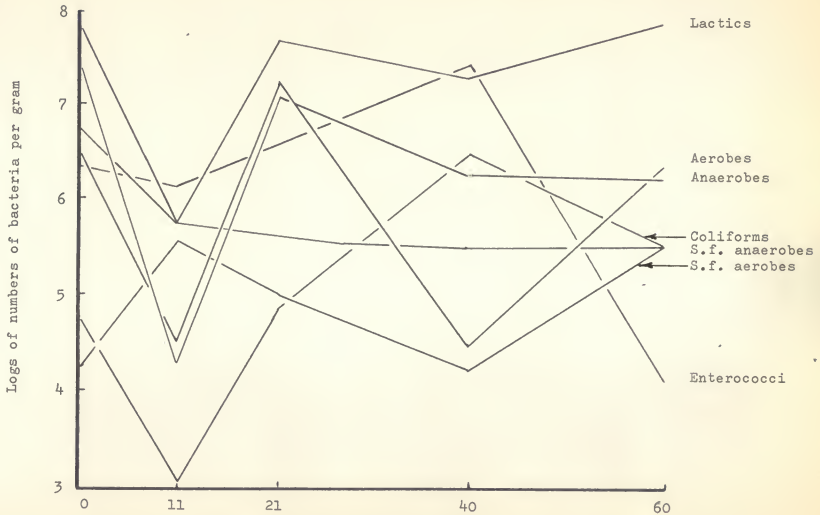

Fig. 8. Part D of infected intestine

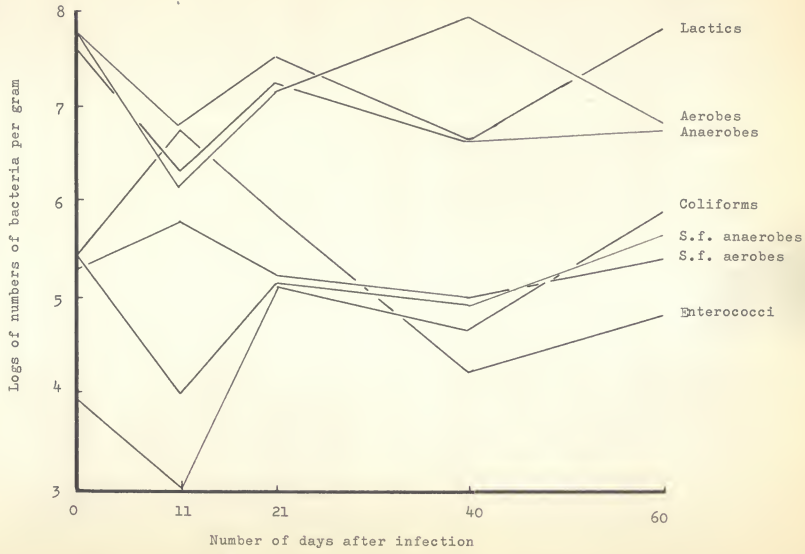

Fig. 9. Part D of control intestine

DISCUSSION

Studies conducted on a quantitative basis of the microorganisms in the intestinal tracts of both normal and parasitized chickens revealed, for most of the groups studied, a general, slight decline in counts at the 11-day sampling period and a rise at the 21-day period. At the 21-day sampling period, the groups tended to continue to rise or level off and in a very few cases to decline. In most of the cases studied, the coliform counts started rather low at initial infection with Ascaridia galli (Schrank) and rose quite rapidly as the chicks grew older.

By examination of the tables in Appendix I, one can see that with the control chickens the total aerobic counts were higher in the lower part of the small intestine (parts C and D) than in the upper part of the gut (parts A and B), as also were the anaerobic counts in the control chickens. The situation is different in the infected birds where the aerobic counts were similar in all parts of the intestine. The anaerobic counts were higher in the extreme parts of the intestine and were lower in the two central parts examined. These differences, if significant, could be explained perhaps by the fact that the worm has a regulatory effect on the flora by ingesting quantities of bacteria present in the intestine and also has to be considered.

Another possibility explaining this phenomenon is suggested by the work of Bushnell and Erwin (1949). They found that A. galli (Schrank) contains an enzyme antitrypsin in its tissue which probably protects it from the action of endogenous proteolytic enzymes. A primary method by which bacteria obtain nutrients from the gut is through action of the enzyme trypsin that they produce. If this trypsin is destroyed by

the antitrypsin produced by the worm, then the bacteria could die from starvation.

Both control and infected chickens showed that numbers of sporeforming anaerobes were the same throughout, and control chicks showed the sporeforming aerobes to be of similar numbers in all parts of the intestine. In the infected chicken the number of sporeforming aerobes was slightly higher in two parts of the intestine than in the other two parts. Results showed that the higher numbers were found in the duodenum rather than the lower parts of the intestine and only varied by 1000 per gram. If one attaches little significance to this increase in the duodenum, the results agree with those obtained by Shapiro and Sarles (1949), and support the contention that spores are transient forms which passed through the intestinal tract of the chicken.

The numbers of lactic acid bacteria and coliforms were greater in the lower parts of the intestine than in the duodenum in the control chickens, but results obtained with the infected birds gave opposite conclusions as the coliforms and lactic acid bacteria were more numerous in the duodenum than the lower parts of the intestine. This is difficult to account for logically and may be due to daily variance in numbers that one would naturally encounter.

Enterococci counts were significantly higher in the lower parts of the intestine than in the duodenum of the infected chicken, but were higher by 1000 per gram in the duodenum than in the lower parts of the intestine. This is probably insignificant and could be due to a day to day variance in numbers.

Qualitative studies of the microflora would be of considerable value to complement the quantitative studies conducted. It would be

of particular interest to study the factors which allow the lactic acid bacteria to become established as the most numerous species of bacteria found in the intestine of both parasitized and normal chickens. Also further work could be done to determine why, numerically, the coliforms comprise the smallest group of bacteria in the small intestine of the chicken.

Since the experiment was not set up on a qualitative basis, few determinations were made of the predominant types of bacteria in each group. However, Clostridium perfringens was probably the principle obligate anaerobe present, various species of lactobacilli the predominant enterococcus, and Escherichia coli the predominant coliform.

The experiment could be improved by making daily counts of all groups of bacteria and by using duplicate plate counting techniques. However, this was not practical during this experimentation because of the expense of quartering the birds, of hiring additional personnel, and of the equipment and materials necessary to conduct the work on such a large scale.

SUMMARY

Bacterial group counts from four levels of intestinal contents of chickens parasitized with A. galli (Schrank) indicated that, in general, numbers of bacteria increase from the gizzard toward the cloaca. This also held true for the uninfected control group of chickens.

Species of lactic acid bacteria were the most numerous of the groups studied (anaerobes, aerobes, lactic acid bacteria, coliforms,

enterococci, and aerobic and anaerobic sporeformers). Correspondingly, coliform counts were the lowest.

The probable predominating bacteria per groups studied were: coliforms - E. coli; enterococci - Streptococcus faecalis; lactic acid bacteria - lactobacillus species, and obligate anaerobes - Clostridium perfringens.

Counts of aerobic and anaerobic sporeformers were at a nearly constant level throughout the intestinal tract and probably represent transient members of the chicken's intestinal tract.

Daily variations in counts should be taken into consideration to establish the validity of those counts obtained and practical techniques sought to reduce personnel, equipment, materials and expenses needed to conduct such experiments.

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APPENDIX

Bacteria per Gram in Contents of Intestinal Parts at Various Times after Infection

Part A of Infected Intestine:

Kinds of bacteria	Number of days after infection				
	0	11	21	40	60
Total aerobes	$8x10^5$	$5x10^4$	$4x10^6$	$3x10^5$	$7.2x10^5$
Total anaerobes	$9x10^6$	$4x10^6$	$9x10^6$	$5x10^7$	$4x10^5$
Sporeforming aerobes	$2x10^6$	$3x10^6$	$4x10^5$	$4x10^4$	$6x10^5$
Sporsforming anaerobes	$3x10^5$	$1x10^4$	$6x10^5$	$5x10^5$	$4x10^5$
Coliforms	$6x10^3$	$1x10^6$	$6x10^6$	$5x10^6$	$7.3x10^5$
Lactic acid bacteria	$1x10^8$	$3x10^7$	$2x10^6$	$4x10^7$	$2x10^6$
Enterococci	$6.4x10^6$	$4.9x10^5$	$4.9x10^4$	$4x10^5$	$4x10^4$

Part B of Infected Intestine:

Total aerobes	$5x10^5$	$3.1x10^4$	$4x10^6$	$7x10^5$	$4.3x10^7$
Total anaerobes	$7.1x10^5$	$2x10^4$	$5x10^5$	$1x10^6$	$1.2x10^7$
Sporeforming aerobes	$7x10^5$	$5.4x10^5$	$5x10^5$	$7.3x10^5$	$5x10^5$
Sporeforming anaerobes	$1x10^5$	$4x10^5$	$3.2x10^5$	$3x10^6$	$5x10^5$
Coliforms	$2x10^3$	$2x10^6$	$9x10^5$	$8x10^5$	$4x10^6$
Lactic acid bacteria	$2.4x10^7$	$2x10^5$	$1x10^7$	$3.8x10^7$	$5x10^5$
Enterococci	$7.9x10^6$	$4.3x10^4$	$5.8x10^5$	$4.3x10^5$	$5x10^3$

Bacteria per Gram in Contents of Intestinal Parts at Various Times after Infection

Part C of Infected Intestine:

Kinds of bacteria	Number of days after infection				
	0	11	21	40	60
Total aerobes	$6x10^4$	$1x10^4$	$3x10^7$	$2x10^5$	$7.5x10^5$
Total anaerobes	$6x10^4$	$3x10^4$	$1.5x10^7$	$3.5x10^6$	$4x10^6$
Sporeforming aerobes	$3.4x10^5$	$4x10^5$	$5.2x10^5$	$2.2x10^5$	$3x10^5$
Sporeforming anaerobes	$3x10^5$	$2.5x10^5$	$3.8x10^5$	$4x10^5$	$3.5x10^5$
Coliforms	$6.9x10^3$	$9x10^2$	$3x10^5$	$2x10^4$	$5x10^5$
Lactic acid bacteria	$1x10^5$	$1.4x10^5$	$5x10^7$	$4x10^6$	$3.9x10^7$
Enterococci	$7.9x10^6$	$1.3x10^6$	$4.3x10^5$	$3.1x10^7$	$4x10^4$

Part D of Infected Intestine:

Total aerobes	$5x10^6$	$4x10^4$	$3.2x10^7$	$4x10^4$	$3.2x10^6$
Total anaerobes	$4x10^7$	$2.3x10^4$	$1.2x10^7$	$3x10^6$	$2x10^6$
Sporeforming aerobes	$2x10^4$	$6.2x10^5$	$1x10^5$	$2x10^4$	$5x10^5$
Sporeforming anaerobes	$6.9x10^6$	$7x10^5$	$6x10^5$	$5x10^5$	$6.2x10^5$
Coliforms	$7x10^4$	$2x10^3$	$8x10^4$	$4x10^6$	$5x10^5$
Lactic acid bacteria	$7x10^7$	$7x10^5$	$6x10^7$	$3x10^7$	$8x10^7$
Enterococci	$4.3x10^6$	$1.3x10^6$	$4.9x10^6$	$4.3x10^7$	$1.1x10^4$

Bacteria per Gram in Contents of Intestinal Parts at Various Times after Infection

Part A of Control Intestine:

Kinds of bacteria	Number of days after infection				
	0	11	21	40	60
Total aerobes	$9x10^5$	$5x10^4$	$5x10^6$	$7x10^6$	$4x10^6$
Total anaerobes	$6x10^5$	$4x10^7$	$7.8x10^6$	$8.9x10^6$	$5x10^6$
Sporeforming aerobes	$6.3x10^5$	$9x10^4$	$8x10^5$	$1x10^5$	$4x10^5$
Sporeforming anaerobes	$3x10^5$	$5x10^5$	$5x10^5$	$3.2x10^5$	$4.1x10^5$
Coliforms	$1x10^2$	$1x10^3$	$5x10^4$	$1x10^4$	$9x10^5$
Lactic acid bacteria	$2x10^6$	$1x10^5$	$8x10^6$	$6x10^7$	$5x10^6$
Enterococci	$7.6x10^6$	$4.1x10^4$	$5.8x10^5$	$3.1x10^6$	$5.8x10^4$

Part B of Control Intestine:

Total aerobes	$9x10^5$	$6x10^4$	$7x10^6$	$7.2x10^6$	$3.8x10^6$
Total anaerobes	$8.1x10^5$	$7x10^4$	$5x10^6$	$1x10^7$	$7.5x10^6$
Sporeforming aerobes	$4x10^4$	$4x10^5$	$4x10^5$	$1x10^5$	$3x10^5$
Sporeforming anaerobes	$8x10^4$	$3.2x10^5$	$4x10^5$	$3x10^5$	$2.1x10^5$
Coliforms	$1x10^2$	$9x10^2$	$5x10^4$	$1x10^4$	$1x10^3$
Lactic acid bacteria	$2x10^6$	$5x10^5$	$8x10^6$	$5x10^7$	$1x10^8$
Enterococci	$4.9x10^5$	$6.2x10^5$	$7x10^6$	$7.9x10^7$	$1.1x10^5$

Bacteria per Gram in Contents of Intestinal Parts at Various Times after Infection

Part C of Control Intestine:

Kinds of bacteria	Number of days after infection				
	0	11	21	40	60
Total aerobes	$4x10^6$	$2.5x10^6$	$2x10^7$	$8.6x10^7$	$8x10^6$
Total anaerobes	$3.2x10^8$	$5x10^5$	$2x10^7$	$2x10^7$	$7.5x10^6$
Sporeforming aerobes	$5x10^4$	$6x10^5$	$4x10^5$	$1x10^5$	$2.1x10^6$
Sporeforming anaerobes	$5x10^4$	$4x10^6$	$2x10^5$	$1x10^6$	$4x10^5$
Coliforms	$9x10^3$	$1x10^3$	$2.9x10^5$	$7x10^4$	$8x10^5$
Lactic acid bacteria	$8x10^6$	$8x10^7$	$5x10^7$	$8x10^8$	$2x10^7$
Enterococci	$7.9x10^6$	$4x10^6$	$7.9x10^5$	$6.9x10^5$	$9.5x10^4$

Part D of Control Intestine:

Total aerobes	$7x10^7$	$2x10^6$	$2x10^7$	$9x10^7$	$8x10^6$
Total anaerobes	$6.5x10^7$	$3x10^6$	$3.2x10^7$	$6x10^6$	$7.1x10^6$
Sporeforming aerobes	$3x10^5$	$7.6x10^5$	$3x10^5$	$1x10^5$	$5x10^4$
Sporeforming anaerobes	$4x10^5$	$1x10^4$	$2.6x10^6$	$9.3x10^4$	$5.1x10^5$
Coliforms	$9x10^3$	$1x10^3$	$2x10^5$	$7x10^4$	$8x10^5$
Lactic acid bacteria	$7.2x10^7$	$6.9x10^6$	$5x10^7$	$6x10^6$	$2x10^7$
Enterococci	$4.3x10^5$	$5.9x10^6$	$7.9x10^5$	$3.1x10^4$	$7.9x10^4$

A STUDY OF NUMBERS OF MICROORGANISMS IN THE INTESTINAL TRACT OF CHICKENS PARASITIZED WITH ASCARIDIA GALLI AND OF UNINFECTED CONTROL CHICKENS

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Little is known about the intestinal microflora of the chicken parasitized with the round worm Ascaridia galli (Schrank), however, the normal microflora of many species of normal fowl had been subjected to studies before the end of the 19th century.

A search of the literature revealed that several investigators isolated much the same types of bacteria from the intestine of normal chickens. Numbers of bacteria differed in parasitized and control chickens, in the case of parasitism with Eimeria tenella (Railliet and Lucet).

The white Plymouth Rocks used during the experiments were infected with 100 ± 10 larvated eggs of A. galli (Schrank) and materials from four segments of the small intestine were cultured in various media to select the different bacterial groups present. Bacterial cultures of the intestinal flora were made at the following times after infection with A. galli (Schrank); 0, 11, 21, 40, and 60 days.

Numbers of most of the bacterial groups studied (aerobes, anaerobes, sporeforming aerobes, sporeforming anaerobes, coliforms, lactic acid bacteria, and enterococci) declined slightly at the 11-day sampling period and rose at the 21-day sampling period. At the 21-day sampling period, the groups tended to continue to rise or level off and in a very few cases to decline. This general trend indicated that as the chicken becomes older, bacterial counts go up.

Results from examinations of four levels of the intestine showed that, in general, numbers of bacteria increased from the gizzard toward the cloaca.

The aerobic counts were higher in the lower parts of the small intestine than in the upper parts of the gut for the control chickens,

as also were the anaerobic counts in the control chickens. The situation is different in the infected birds where the aerobic counts were similar in all parts of the intestine. The anaerobic counts were higher in the extreme parts of the intestine and were lower in the two center parts examined.

Both control and infected chickens showed that numbers of sporeforming anaerobes were the same throughout, and control chicks showed the sporeforming aerobes to be of similar numbers in all parts of the intestine. In the infected chicken, the number of sporeforming aerobes was slightly higher in two parts of the intestine than in the other two parts. Results showed that the higher numbers were found in the duodenum rather than the lower parts of the intestine and only varied by 1000 per gram.

The numbers of lactic acid bacteria and coliforms were greater in the lower parts of the intestine than in the duodenum in the control chickens, but results obtained with the infected birds gave opposite conclusions as the coliforms and lactic acid bacteria were more numerous in the duodenum than the lower parts of the intestine. This difference may have been due to daily variance in numbers that one encounters.

Enterococci counts were significantly higher in the lower parts of the intestine than in the duodenum of the infected chicken, but were higher by 1000 per gram in the duodenum than in the lower parts of the intestine. This difference might be due to daily variance.

Few determinations were made of the predominant types of bacteria in each group since the experiment was not set up on a qualitative basis. However, Clostridium perfringens was probably the principle

obligate anaerobe present, various species of lactobacilli the predominant lactic acid bacteria, Streptococcus faecalis the predominant enterococcus, and Escherichia coli the predominant coliform.

The experiment could be improved by making daily counts of all groups of bacteria and by using duplicate plate counting techniques. Neither was practical in this case.