THE ANAEROBIC MICROFLORA OF *ASCARIDIA GALLI* (SCHRANK) AND OF THE CONTROL AND INFECTED HOST INTESTINE

by

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INTRODUCTION

Very little is known of the host-parasite relationship that exists between the chicken and its nematode intestinal parasite, Ascaridia galli (Schrank). Many investigators have studied the microflora of the infected and non-infected host intestine, but little is known of the gut microflora of A. galli. A review of the literature revealed that only two previous studies have been made of the intestinal microflora of the parasitic nematode. The first investigation (Li, 1933) revealed that only about 50 per cent of the various species of nematodes studied contained an intestinal microflora and that the predominant bacteria present belong to the "coli group". The second investigator (Baron et al., 1960) revealed that the predominant microflora of A. galli does not differ greatly from that of its host, at least in early tenure, and that the anaerobes are the predominating group in both the host intestine and in the gut of A. galli.

Prior to this investigation, little had been determined concerning the qualitative microflora of the host intestine and almost nothing of the qualitative anaerobic microflora of A. galli. It was therefore desirable to study, both qualitatively and quantitatively, the anaerobic microflora of A. galli in relationship to that of its host. Such information would be useful in attempted in vitro studies using anaerobic bacteria as a source of nutrients for A. galli. If such an attempt were successful it would be an invaluable tool in the in vitro study of the effects of vermifuges on A. galli.

The objectives of this study were: to determine quantitatively the anaerobic microflora of A. galli and of the infected and control host at

various periods after infection, and to isolate and determine the species of typical anaerobic bacteria found in the gut of A. galli and of the control and infected host intestine.

REVIEW OF LITERATURE

A review of the literature revealed few data on the study of anaerobes in the intestines of chickens or parasitic nematodes, therefore, data for aerobic and facultative microflora are also included.

The first study of the intestinal microflora of the chicken was performed by Kern (1897) who studied the intestinal flora of 24 birds and isolated 88 species of bacteria. Kern reported the following species to be obligate intestinal organisms: Bacterium coli commune, Bacillus vegatus, Bacillus vergatus, Bacillus defessus, Bacterium cavatum, Bacterium verruscosum, and Pseudomonas granulata. By means of direct microscopic preparations of stomach and intestinal contents, he concluded that many species are present which do not grow in ordinary culture media. He postulated that there were no definite species of bacteria present in the stomach, but that any species present were a consequence of diet and therefore variable.

Gage (1911) studied the intestinal contents of 45 healthy birds by direct microscopic preparations and cultural methods. He concluded from the direct microscopic smears that all organisms present do not grow when subjected to cultural methods and that this is probably due to the same agents which act in the human intestine. These agents were assumed to be the effect of nutrients being absorbed and the effect of bacterial antagonism. Examination of the smears also indicated that 60 per cent of the organisms present do not stain by Gram's method and that the

remaining 40 per cent are Gram negative and typical of the Escherichia coli group.

The anaerobic microflora was investigated by Wright's liquid method and it was concluded that "the absence of large numbers of obligate anaerobes of the Bacillus butrificus type would indicate that the intestinal conditions in the healthy domestic fowl are not inducive for anaerobiosis."

McCoy (1929) found that living bacteria constituted the food of hookworm larvae, Ancylostoma caninum, in developing to the infective stage. The following organisms yielded growth of the hookworm larvae: E. coli, Bacillus subtilis, Bacillus prodigiosus, Bacillus lactis aerogenes, Staphylococcus aureus, Staphylococcus rubrum, and Micrococcus citreus. Bacillus pyocyaneus and Sarcina lutea gave no growth. Growth was retarded using Bacillus cereus and Bacillus megaterium but growth occurred in a mixture of B. cereus and E. coli. McCoy did not attempt to isolate or identify any organisms from the intestine of the worm.

Emmel (1930) reported little difference in the flora of 10 chicks and 20 hens. Thirty-four species of organisms were isolated from the 10 chicks and the same number from 20 hens. E. coli appeared to constitute 60 per cent of the organisms isolated. There appeared to be more E. coli and E. coli communior in hens than in chicks at two weeks of age.

Only three species of anaerobes were isolated in nutrient broth and these were determined to be Clostridium sporogenes.

In his studies on intestinal roundworms, Li (1933) found that about 50 per cent of the samples, from seven species of nematodes, contained bacteria in their intestines. In these experiments Li used plain agar

and the plates were incubated under aerobic conditions. Using E. coli as a basis for comparison, Li concluded that the dominant species of bacteria isolated from nematodes belonged, like those of the host organism, to the coli group.

Johansson et al. (1948) demonstrated that the type of dietary carbohydrate influenced the intestinal microflora of the chicken. They reported that the pH of the alimentary tract also exerts an influence on the bacterial population. They also found that the bacterial population increased in numbers from the duodenum to the cecum.

Johansson and Sarles (1948) studied the cecal flora of 2-week-old chicks infected with the intestinal protozoan, Eimeria tinella. They reported that the numbers of lactobacilli and enterococci were reduced considerably by the fourth or fifth day following infection. Coliform numbers apparently remained unchanged in the ceca during the course of cecal coccidiosis. The growth of anaerobes resembling Clostridium perfringens was stimulated by coccidial infection of the cecum.

Shapiro and Sarles (1949) studied the intestinal tracts of normal chickens and reported large numbers (1.5×10^5/gm) of gas-forming bacteria to be present 21 hours after hatching. They reported that the flora increases rapidly after food and water are consumed and that the normal flora is established 40 hours after feeding. Lactobacilli were found to be the predominant group of organisms in all regions of the intestine except the colon. The coliform count was found to be highest in the cecal pouches. The principal anaerobe found was reported to be C. perfringens and attempts to isolate others were unsuccessful.

Shapiro et al. (1949) continued these studies and described the

lactobacilli present in the intestine of the normal fowl. They isolated and classified 125 species of lactobacilli on the basis of carbohydrate fermentation tests, but no species names were assigned.

Lev and Briggs (1956 a, b) studied the cecal flora of newly hatched chicks and found that a flora was established four hours after feeding and that this flora became balanced after 24 hours of feeding. These observations were based on the study of periodic plate counts of intestinal contents from the time of hatching until the chicks were 30-days-old. The principal flora was reported to consist mainly of the clostridia, and tests indicated that the organism isolated was Clostridium paraputrificum.

The influence of type of dietary carbohydrate on pH was studied by Wiseman et al. (1956). They found that the type of carbohydrate fed did not affect the pH of the intestine but the pH of the intestine affected the number of organisms present. The number of organisms present was found to increase as the pH of the intestine approaches neutrality, paralleling the increase in bacterial population from the gizzard to the cloaca.

Bhear (1957) conducted a quantitative study of the intestinal microflora of chickens parasitized with A. galli and uninfected control chickens. He studied six groups of organisms in four segments of the chicken intestine. Plate counts were made for aerobes, anaerobes, sporeforming aerobes, sporeforming anaerobes, lactic acid bacteria and coliforms in the four selected regions of the host intestine. He reported a general increase in numbers of bacteria from the duodenum to the cloaca and that counts were generally higher in the duodenum for infected birds than for noninfected birds. He also concluded that the lactic acid

bacteria constituted the major flora of the host intestine and that the lactobacilli were the most significant members of this group.

Few species determinations were made by Bhear, but he concluded that the following were probably the predominant organisms per group studied: coliform - E. coli; enterococci - Streptococcus faecalis; lactic acid bacteria - Lactobacillus spp; and obligate anaerobes - C. perfringens.

A study of the intestinal flora of A. galli was conducted by Baron et al. (1960) who found that the predominating coliform organisms isolated from the chicken intestine as well as that of the worm were E. coli. Streptococcus faecalis var. liquefaciens and Lactobacillus spp. were found to be the predominating enterococcus and lactic acid bacteria, respectively. Baron stated that "the anaerobes were the predominant group of bacteria for both the host and parasite followed by the lactics, aerobes, and coliforms, in that order."

Baron also showed that Roccal (a quaternary ammonium compound) in a dilution of 1:1000 is an effective agent for sterilization of the cuticle of A. galli. He also showed by chromatographic methods that Roccal, in germicidal concentrations, does not penetrate the cuticle of the worm. The pseudocoelomic cavity and the eggs of A. galli were reported to support no bacterial flora.

MATERIALS AND METHODS

Preparation of Animals

All chickens used in these experiments were non-sexed White Rocks obtained as day-old chicks from a commercial hatchery. The birds were immunized against Newcastle disease virus immediately after delivery and

placed in electric brooders.

Prior to infection with A. galli the chickens were banded and placed in wire cages. Thirty birds were selected at random and placed in a separate wire battery. These chicks were to be eventually infected with A. galli. The remaining chickens were to remain uninfected and to be used as controls.

The experiment was conducted using two series of birds. The first series of control and infected birds was kept on a non-antibiotic ration and the second series was fed the same ration with 20 grams of Aureomycin added per ton of feed.

The ration used for Series I chickens was "Page's 18 per cent chick starter". The analysis tag indicated that the feed contained: protein - not less than 18 per cent; crude fat - not less than 4 per cent; fiber - not more than 50 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, vitamin B-12 supplement, vitamin A acetate, D-activated animal sterols, riboflavin, calcium pantothenate, niacin, choline chloride, wheat protein hydrolysates, di-calcium phosphate, ground limestone, salt and the trace minerals: potassium iodide, manganese sulfate, iron sulfate, copper sulfate, and potassium sulfate.

The birds used for the second series of experiments were fed this same ration containing, in addition, 20 grams of Aureomycin per ton.

Preparation of A. galli Egg Cultures and Infection of the Chickens

A. galli to be used in the egg cultures were obtained from the small

intestine of chickens parasitized with A. galli by the hydraulic method of Ackert and Nolf (1929). The intestine from the gizzard to the yolk sac diverticulum was removed from the body cavity and attached to a small water hose and the intestinal contents flushed into one liter beakers by water pressure. The flushings were poured through a 20 mesh screen which retained the worms and allowed the intestinal debris to pass through. The mature female worms were separated from the males and immature females and saved for preparation of A. galli egg cultures.

A. galli eggs used in the experiments were cultured using the methods of Hansen et al. (1954, 1956). The body contents of the adult female worms were stripped out and placed in a Petri dish containing a solution of 1.0 per cent pepsin and 0.5 per cent hydrochloric acid. When the uterine walls had been digested (5 to 10 minutes), tap water was added and after the eggs had settled to the bottom of the dish, the supernatant fluid was withdrawn. The digestive juice was removed in three to four additional washings with tap water. After the final washing 10 ml of fresh tap water was added to each Petri dish containing eggs. A drop of a 1:1000 merthiolate solution was added to each culture to prevent mold growth which would tend to make the eggs clump together causing irregular infection. All egg cultures were incubated at 30 to 33°C for 14 days. By the end of the incubation period the eggs were larvated and infective.

Each chicken to be infected was given 100 ± 10 A. galli eggs per os. This large number of eggs was administered because the process has been determined to be only 6 to 10 per cent efficient. A calibrated micropipette was used in feeding the eggs to the birds. A variation of the egg feeding technique of Hansen et al. (1954) was used.

All water was withdrawn from the Petri dish egg cultures 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 with a rubber policeman, the sugar-egg suspension was poured into a small bottle. A drop of the suspension was placed on a glass slide and the eggs were counted under a compound microscope. When it was necessary to dilute the suspension, additional 1.25 M sucrose solution was added and the eggs in several drops of the suspension were counted. The suspension was diluted until the micropipette would deliver 100 ± 10 eggs when filled to the calibration mark. The eggs were administered *per os* immediately after preparation. The chicks were one-month-old at the time of infection with the *A. galli* eggs.

Collection and Preparation of Samples for Enumeration

Five chickens were picked at random from the infected group and three from the control group at the following times after infection for Series I: 14, 28, 42, 56, 70, and 84 days. For Series II birds the following times were used: 14, 21, 28, 35, 41, and 48 days. It may be noted here that these particular days post-infection or the interval between collection of samples is relatively unimportant since it was desired only to obtain a total anaerobic count for the infected and control intestines and the intestine of the worm for comparison at the time of collection rather than to obtain a chronological record of total anaerobic counts at specific days after infection.

Each chicken was sacrificed quickly by breaking its neck and the body cavity opened. The intestine was tied off, at the point of attachment to the gizzard and just forward of the yolk sac diverticulum, and

snipped out. Each intestinal segment was placed in a separate sterile 250 ml beaker which was labeled according to its contents.

Immediately after collection of the samples the outside of each intestine was sterilized by immersion in a 1:1000 dilution of Roccal for 10 minutes followed by two or three rinses with sterile, distilled water. After sterilization each intestine was opened by snipping off one of the closed ends and the contents squeezed into a sterile Petri dish. The worms were separated from the fecal material of the infected birds and all worms from each sample were pooled and placed in separate Petri dishes.

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 using a keyboard analytical balance. The worms from each infected intestine were sterilized in a 1:1000 dilution of Roccal, rinsed well with sterile distilled water, blotted dry between sheets of sterile blotting paper, and weighed on a piece of sterile aluminum foil. It has been shown by Baron (1960) that Roccal cannot penetrate the cuticle of the worm or the intestinal wall of the chicken so this method of sterilization is quite satisfactory.

Each sample of feces was added to a 6-oz prescription bottle containing 99 ml of sterile distilled water and a few glass beads to aid in breaking up clumps. The bottles were shaken vigorously and refrigerated until all samples had been diluted. The 1:100 dilutions were then reshaken, diluted serially to $1:10^6$, and inoculated immediately into Petri dishes containing 10 ml of solidified medium. A spreader was used to spread each dilution over the surface of its respective plate. The plates were then overlayed with another 10 ml of medium.

Previous experiments had shown that this technique would give the most well-isolated colonies and prevent spreading of organisms between the agar and Petri dish bottom. It had been previously determined that a final dilution of $1:10^6$ was the maximum necessary for enumeration of anaerobic bacteria in the chicken and that a $1:10^5$ dilution was the maximum necessary for enumeration of anaerobes in the gut of A. galli.

The worms from each infected intestine were added to a sterile mortar and pestle containing sufficient sand to thoroughly macerate the worm and release its intestinal contents. Enough sterile distilled water was added to make a 1:10 dilution of the worms on a total weight basis. The worms were then ground until a homogeneous suspension was obtained. Serial dilutions of this suspension were made to $1:10^5$ and 1 ml aliquots were spread on the solid medium and overlayed in the same manner as were the intestinal samples from the chickens.

Cultural Procedures and Medium Used

For determination of total anaerobic counts, liver veal agar (Difco) was selected because it is a complete medium which will support a variety of organisms. Since it was desired to isolate typical species from the plates after enumeration, it was necessary to keep swarming by motile facultative anaerobes at a minimum. Sodium azide at a level of 0.01 per cent was found to inhibit swarming without reduction of bacterial numbers and was therefore added to the liver veal agar before sterilization. The final pH of the medium was adjusted to 7.2.

All plates were incubated at $37^{\circ}C$ in Brewer anaerobic jars containing an atomosphere of 100 per cent methane. A test tube containing

calcium oxide and one containing a reduced methylene blue-glucose mixture was also added to each jar (Brewer and Brown, 1938). The calcium oxide was added to remove part of the CO_2 formed by combustion of methane in the jars. The reduced methylene blue was added as an indicator of anaerobiosis. Reversion to the blue color of methylene blue would have indicated the presence of free oxygen. The jars were each evacuated with a vacuum pump for one minute and filled with methane. The jars were plugged into a 115 v AC electrical outlet for 30 to 45 minutes and any remaining oxygen was burned off.

Enumeration of Anaerobic Bacteria

An incubation period of 72 hours was allowed and after this period the plates were removed from the anaerobic jars. All plates containing 30 to 300 colonies were counted with the aid of a Quebec colony counter. Counts were calculated and recorded as total anaerobic bacteria per gram of the original sample.

Isolation of Typical Species of Anaerobic Bacteria

The plates used for enumeration were also used for isolation of typical species of anaerobic bacteria. Typical colonies were picked from these plates and streaked in duplicate on slants of AC medium (Difco) which had been modified by the addition of 14 grams agar agar per liter. One of these slants was incubated anaerobically and the other was incubated under aerobic conditions. Only those tubes showing obligate anaerobic growth upon primary isolation were saved for species identification. On the average, 15 organisms were picked from each of the 11 plates per group of samples. A total of 160 colonies was prepared in duplicate from

each group of samples, and the average yield of obligate anaerobes was approximately 30 per cent. The remaining organisms which exhibited both aerobic and anaerobic growth were assumed to be facultative organisms and were discarded.

The obligate anaerobes were obtained in pure culture by streaking them on AC medium. All plates were again incubated in Brewer anaerobic jars at 37°C. All isolates were purified twice by streaking before finally transferring them to be kept as stock cultures. All stock cultures were maintained in cooked meat medium until a generic name could be assigned to them. When the genus was determined it was sometimes necessary to maintain the stock cultures in some medium other than cooked meat medium.

Identification of Bacteria

The Gram reaction and morphology were first determined for each organism, followed by the heat test for endospores, optimum growth temperature, and oxygen relationship. The oxygen relation test was performed because many of the organisms which had originally been obligate anaerobes became microaerophilic to anaerobic after having been transferred several times on artificial media.

The genus Clostridium was separated on the basis of presence of spores, morphology, Gram reaction, and relationship to oxygen.

The genus Lactobacillus was separated on the basis of absence of spores, staining, relationship to oxygen and motility.

The genus Bacteroides was separated on the basis of Gram reaction, morphology and oxygen relationship.

All sporeforming anaerobes were stained using the Snyder modification of the Dorner method as given by Lord (1959).

After the initial tests for determination of genera had been performed each group of organisms was subjected to further biochemical tests as indicated in Appendix I. The ultimate species identification was based on comparison with descriptions recorded by Breed et al. (1957), Curran et al. (1933), Eggerth and Gagnon (1933), Reed (1942), Reed and Orr (1941), and Rogosa et al. (1953).

EXPERIMENTAL RESULTS

A total of 498 cultures was isolated from the samples of Series I (no antibiotic in feed) and Series II birds (feed containing Aureomycin). Of the total number of isolates, 143 cultures were obtained from control intestines, 221 cultures from intestines of chickens infected with A. galli, and 134 cultures from the gut of the worm. These cultures were purified by streaking on liver veal agar (Difco) and subjected to the various biochemical and morphological tests presented in Appendix I. The results are presented in Tables 1, 2 and 3. The results indicated in these tables were used in determining the species of each organism isolated.

Appendices III and IV show the species of bacteria isolated from each chicken, the number of days after infection with A. galli, the culture number, and the sample number. In these appendices "I" denotes infected intestine, "C" denotes control intestine, and "W" denotes the gut of A. galli.

Plate counts were made at various intervals after infection to determine the total anaerobic microflora of the control and infected host

intestine and of the gut of A. galli. These counts were made as two series: Series I counts are those for chickens fed a non-antibiotic ration and Series II counts are those for chickens fed the same ration, but with 20 grams of Aureomycin added per ton of feed. The results of these plate counts are presented in Appendix II and summarized in Plates I and II.

By examining Plates I and II it can be seen that the total anaerobic counts are generally higher for all groups of Series II birds than for Series I birds at corresponding periods of time.

It can also be seen that the plate counts for Series II indicate a more stable population of anaerobic bacteria than do those of Series I. This is indicated by the curves of Plates I and II which are generally more linear for Series II than for Series I chickens.

DISCUSSION

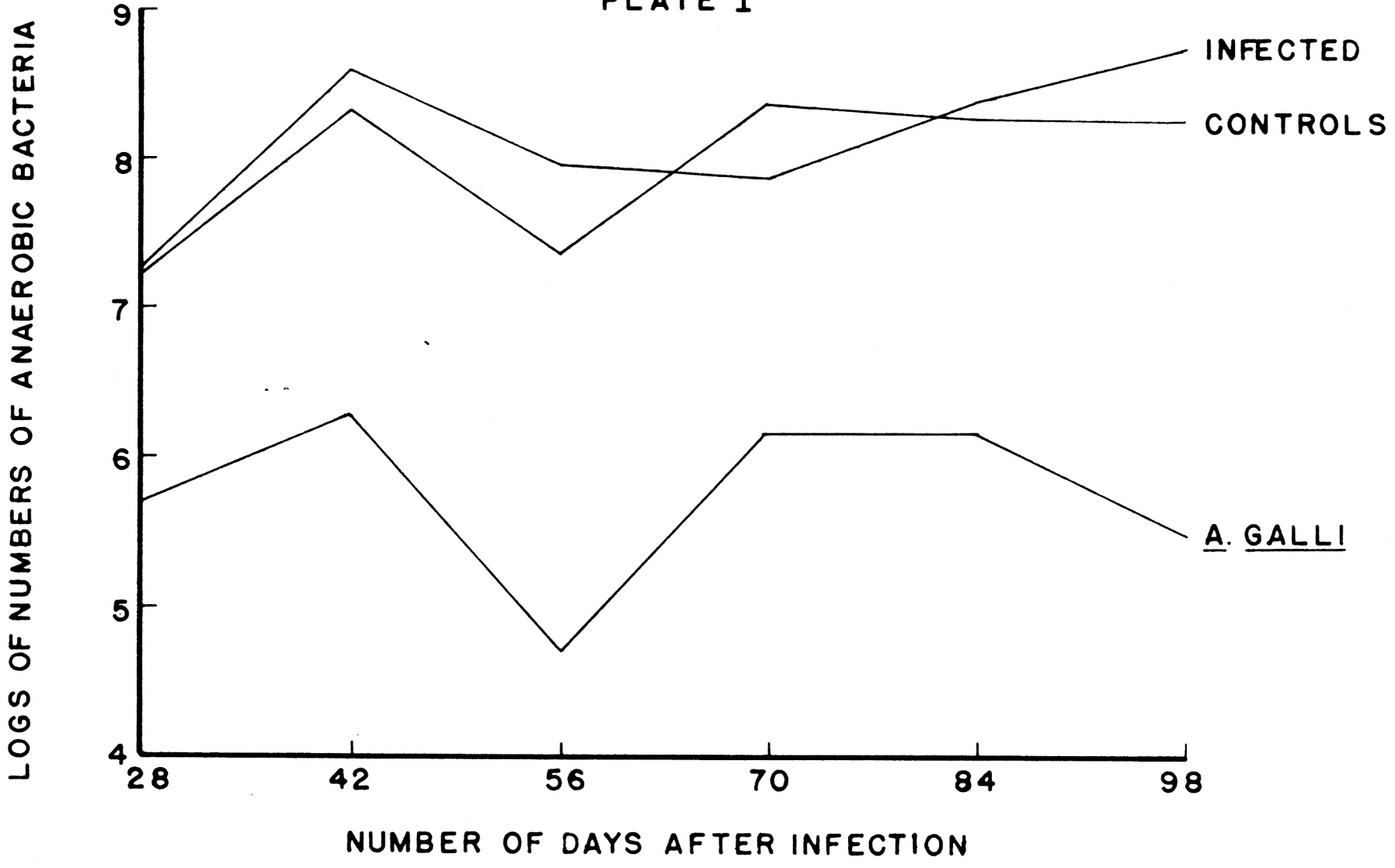
Quantitative Studies on the Total Anaerobic Microflora of the Host and Parasite

Examination of the data on quantitative studies of the anaerobic microflora (Appendix II) at various intervals of time indicated a relatively stable anaerobic population within each group (control group, infected group, and A. galli from infected group) for both Series I and Series II chickens. However, plate counts were generally higher for all groups of Series II chickens than for Series I chickens. Since Series II chickens were fed a ration containing 20 grams of Aureomycin per ton and Series I chickens were fed the same ration with no antibiotic, the generally higher counts for Series II chickens and worms may be assumed to be due to elimination of antagonizing organisms in the intestine by

EXPLANATION OF PLATE I

Graph showing total anaerobic counts for Series I chickens at various days post infection. The chickens were fed a non-antibiotic ration.

PLATE I


LOGS OF NUMBERS OF ANAEROBIC BACTERIA
9
8
7
6
5
4
28
42
56
70
84
98
NUMBER OF DAYS AFTER INFECTION
INFECTED
CONTROLS
A. GALLI

EXPLANATION OF PLATE II

Graph showing total anaerobic counts for Series II chickens of various days post infection. The chickens were fed a ration containing antibiotic.

PLATE II

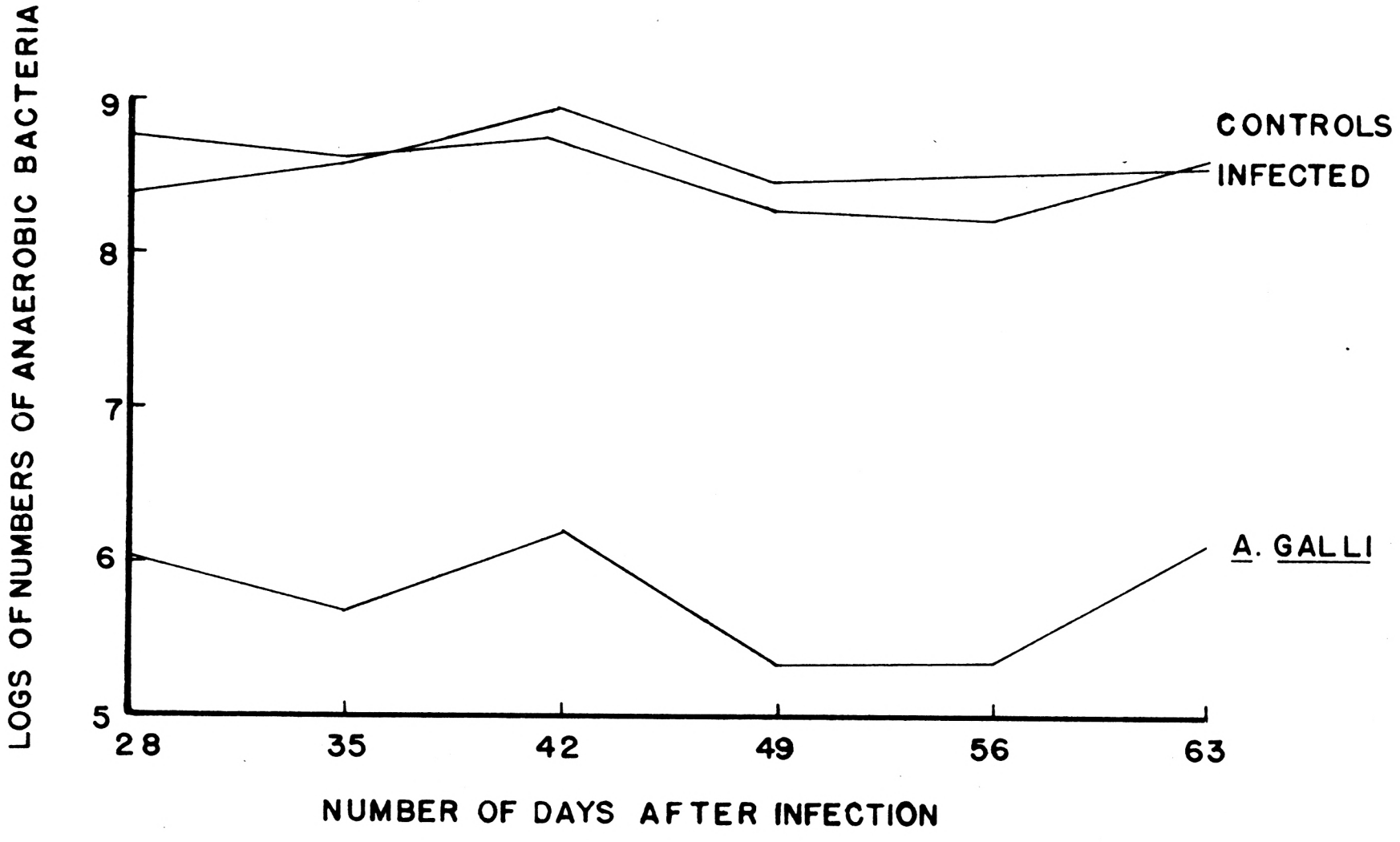

LOGS OF NUMBERS OF ANAEROBIC BACTERIA
9
8
7
6
5
28
35
42
49
56
63
CONTROLS
INFECTED
A. GALLI
NUMBER OF DAYS AFTER INFECTION

Table 1. Characteristics of Bacteroides species Isolated

Organism	Number of strains isolated	Morphology and staining	Oxygen relationship	Optimum temperature	Motility	Gas from peptone	Hemolysis	Litmus milk	Cellulose hydrolysis	Gelatin hydrolysis	Hydrogen sulfide test	Nitrate reduction test	Starch hydrolysis	Arabinose	Cellobiose	Dulcitol	Fructose	Glucose	Glycerol	Inositol	Inulin	Lactose	Maltose	Melibiose	Melezitose	Raffinose	Rhamnose	Salicin	Sorbitol	Sorbose	Sucrose	Trehalose	Xylose	Indole
Bacteroides convexus	11	Gram - rods	Anaerobic	37°C	-	-	-	Acid coagulation	-	+	+	-	+	-	+	+	+	+	-	-	+	+	+	+	-	+	-	-	-	-	+	-	+	-
Bacteroides exiguus	12	Gram - rods	Anaerobic	37°C	-	-	-	Acid	-	+	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	-	-	-	±	+	-	+	-
Bacteroides ovatus	8	Gram - rods	Anaerobic	37°C	-	-	-	Acid coagulation	-	-	+	-	+	-	+	+	+	+	-	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+
Bacteroides uniformis	11	Gram - rods	Anaerobic	37°C	-	-	-	Acid coagulation	-	-	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+	+	-	+	-	±	+	+	+	-
Bacteroides variegatus	1	Gram - rods	Anaerobic	37°C	+	-	-	Acid coagulation	-	-	-	-	-	+	-	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+
Bacteroides sp.	1	Gram - rods	Micro. to anaerobic	37°C	±	-	-	Acid coagulation	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-
Bacteroides sp.	1	Gram - rods	Anaerobic	37°C	-	-	-	No change	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
Bacteroides sp.	1	Gram - rods	Anaerobic	37°C	-	-	-	Acid coagulation	-	-	-	-	-	+	+	-	+	+	-	-	-	+	+	+	-	+	+	+	-	+	+	+	+	-

± = variable

Table 2. Characteristics of Clostridium species Isolated

Organism	Number of strains isolated	Morphology and staining	Location of spores	Shape of spores	Motility	Oxygen relationship	Optimum temperature	Hemolysis	Reduced iron milk	Gelatin hydrolysis	Coagulated serum	Coagulated egg albumin	Hydrogen sulfide test	Nitrate reduction test	Cellulose hydrolysis	Dulcitol	Fructose	Galactose	Glucose	Glycerol	Inositol	Lactose	Maltose	Mannitol	Mannose	Salicin	Sucrose	Soluble starch	Potato starch	Indole
Clostridium aerofoetidam	7	Gram + rods	Subterminal	Ovoid	+	Anaerobic	37°C	No hem.	Acid, gas coagulation	+	+	-	+	+	-	-	+*	+	+	-	+	+	+	-	+	+	-	+	+	-
Clostridium butyricum	11	Gram V rods	Subterminal	Ovoid	+	Anaerobic	37°C	No hem.	Acid, gas coagulation	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+	+	+	+	-
Clostridium cochlearium	3	Gram + rods	Terminal	Ovoid	+	Anaerobic	37°C	Beta hem.	No change	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clostridium haemolyticum	4	Gram + rods	Subterminal	Ovoid to cylindrical	+	Anaerobic	37°C	Beta hem.	Acid coagulation	+	-	-	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Clostridium hastiforme	3	Gram + rods	Subterminal	Ovoid	+	Anaerobic	37°C	No hem.	Coagulation & digestion	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clostridium lentoputrescens	2	Gram V rods	Terminal	Spherical	+	Anaerobic	37°C	Beta hem.	Coagulation, some digestion	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Clostridium multifermentans	17	Gram - rods	Central to subterminal	Ovoid	+	Anaerobic	37°C	Beta hem.	Acid, gas coagulation	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-	+	+	+	+	-
Clostridium perfringens	89	Gram + rods	Central to subterminal	Ovoid	-	Anaerobic	37°C	Beta hem.	Acid, gas coagulation	+	-	-		+	-	-	+	+	+	V	+	+	+	-	+	-	+	+	+	-
Clostridium sporogenes	33	Gram + rods	Subterminal	Ovoid	+	Anaerobic	37°C	Beta hem.	Peptonization	+	+	+	+	- (gas)	-	-	+	+	+	-	-	-	+	-	+	-	-	+	+	-
Clostridium tertium	9	Gram + rods	Terminal	Ovoid	+	Anaerobic to aerotolerant	37°C	Beta hem.	Acid, gas coagulation	-	-	-	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	-
Clostridium sp.	2	Gram V rods	Subterminal	Ovoid	+	Anaerobic	37°C	Beta hem.	Acid, gas coagulation	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Clostridium sp.	3	Gram + rods	Terminal	Ovoid	+	Anaerobic	37°C	Beta hem.	Acid, gas coagulation	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

* A (+) reaction for carbohydrates, polyhydroxy alcohols or glucosides signifies an acid-gas reaction.

Table 3. Characteristics of Lactobacillus species Isolated

Organism	Number of strains isolated	Morphology and staining	Oxygen relationship	Optimum temperature	Motility	Hemolysis	Litmus milk	Cellulose hydrolysis	Gelatin hydrolysis	Hydrogen sulfide test	Nitrate reduction test	Starch hydrolysis	Arabinose	Cellobiose	Dulcitol	Fructose	Glucose	Glycerol	Inositol	Inulin	Lactose	Maltose	Melibiose	Malezitose	Raffinose	Rhamnose	Salicin	Sorbitol	Sorbose	Sucrose	Trehalose	Xylose	Indole
Lactobacillus acidophilus	66	Gram + rods	Micro-aerophilic	37°C	-	-	Acid coagulation	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	±	-	-	-	-	+	±	-	-
Lactobacillus bifidus	27	Gram + rods	Anaerobic	37°C	-	-	Acid	-	-	-	-	+	+	+	-	+	+	-	-	+	+	+	+	-	+	+	±	-	±	+	+	±	-
Lactobacillus bulgaricus	38	Gram + rods	Micro-aerophilic	45°C	-	-	Acid coagulation	-	-	-	-	-	-	-	-	+	+	-	-	-	+	+	+	-	+	-	-	±	-	+	-	-	-
Lactobacillus delbrueckii	12	Gram + rods	Micro-aerophilic	45°C	-	-	No change	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-
Lactobacillus plantarum	92	Gram + rods	Anaerobic	28°C to 37°C	-	-	Acid coagulation	-	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	±	+	+	+	-
Lactobacillus sp.	3	Gram + rods	Micro-aerophilic	45°C	-	-	Acid coagulation	-	-	-	-	+	+	-	-	-	+	+	-	-	+	+	+	-	+	-	-	-	-	+	-	-	-
Lactobacillus sp.	2	Gram + rods	Micro-aerophilic	37°C	-	-	Acid coagulation	-	-	-	-	-	-	+	-	-	+	-	-	-	+	+	-	-	+	+	+	-	-	+	-	+	-
Lactobacillus sp.	2	Gram + rods	Micro. to anaerobic	37°C	-	-	Acid coagulation	-	-	-	-	-	-	+	-	+	+	-	-	-	+	+	+	-	+	-	+	-	+	-	+	-	-
Lactobacillus sp.	1	Gram + rods	Micro. to anaerobic	37°C	-	-	Acid coagulation	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	-	-
Lactobacillus sp.	2	Gram + rods	Micro. to anaerobic	37°C to 45°C	-	Alpha hem.	Acid coagulation	-	-	-	-	-	+	-	-	+	+	-	-	-	+	+	+	-	+	-	-	-	-	+	+	-	-
Lactobacillus sp.	1	Gram + rods	Anaerobic	37°C	-	-	No change	-	-	-	-	-	+	+	-	+	+	+	-	-	+	+	+	-	+	+	-	+	+	+	+	-	-
Lactobacillus sp.	1	Gram + rods	Micro. to anaerobic	37°C	-	Beta hem.	Unchanged	-	-	-	-	-	+	+	-	+	+	-	-	-	+	+	+	-	+	+	-	+	+	+	+	+	-

± = variable

Table 3. Characteristics of Lactobacillus species Isolated (concl.)

Organism	Number of strains isolated	Morphology and staining	Oxygen relationship	Optimum temperature	Motility	Hemolysis	Litmus milk	Cellulose hydrolysis	Gelatin hydrolysis	Hydrogen sulfide test	Nitrate reduction test	Starch hydrolysis	Arabinose	Cellobiose	Dulcit	Fructose	Glucose	Glycerol	Inositol	Inulin	Lactose	Maltose	Melibiose	Melezitose	Raffinose	Rhamnose	Salicin	Sorbitol	Sorbose	Sucrose	Trehalose	Xylose	Indole
Lactobacillus sp.	1	Gram + rods	Anaerobic	37°C	-	-	Acid coagulation	-	-	-	-	-	-	+	-	+	+	-	-	+	+	-	-	-	+	-	-	-	-	+	-	+	-
Lactobacillus sp.	3	Gram + rods	Micro. to anaerobic	37°C	-	Alpha hem.	No change	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-	+	+	-

Aureomycin. If this be true, the counts for Series II chickens would be higher and more stable than those of Series I chickens. Examination of the data presented in Appendix II and Plates I and II tend to support this assumption, but in order to prove this, plate counts would have to be made for other groups of organisms such as the coliforms, aerobes, enterococci, and sporeforming aerobes. Because of limitations in equipment and time, these additional counts were not performed.

Qualitative Studies on the Anaerobic Microflora of the Host and Parasite

A total of 498 anaerobes were obtained in pure culture from the plates used in determining total anaerobic counts. Of these, 143 were from the intestines of control chickens, 221 were from infected chickens, and 134 were from the gut of A. galli. These organisms were subjected to the various biochemical and cultural tests as indicated in Tables 1, 2 and 3. The results of these test were used to determine the species of each organism.

All organisms were found to belong to one of the following genera: Bacteroides, Clostridium or Lactobacillus. Of the total, 254 species of Lactobacillus, 183 species of Clostridium and 51 species of Bacteroides were found constituting 51.0, 36.8 and 10.2 per cent of the obligate anaerobic population, respectively.

Results of species determination can best be summarized by Tables 4, 5 and 6.

These data indicate the principal anaerobic bacteria to be found in the majority of samples were Clostridium perfringens, Clostridium sporogenes, Lactobacillus plantarum, Lactobacillus acidophilus, and Lactobacillus

Table 4. Species of Bacteroides isolated

Organism	No. of strains isolated	Percentage of total
Bacteroides convexus	11	2.2
Bacteroides exiguus	12	2.4
Bacteroides ovatus	8	1.6
Bacteroides uniformis	16	3.2
Bacteroides variegatus	1	0.2
Bacteroides spp.	3	0.6

Table 5. Species of Clostridium isolated

Organism	No. of strains isolated	Percentage of total
Clostridium aerofoetidum	7	1.4
Clostridium butyricum	11	2.2
Clostridium cochlearium	3	0.6
Clostridium haemolyticum	4	0.8
Clostridium hastiforme	3	0.6
Clostridium lentoputrescens	2	0.4
Clostridium multifermentans	17	3.4
Clostridium perfringens	89	18.0
Clostridium sporogenes	33	6.6
Clostridium tertium	9	1.8
Clostridium spp.	5	1.0

Table 6. Species of Lactobacillus isolated

Organism	No. of strains isolated	Percentage of total
Lactobacillus acidophilus	66	13.3
Lactobacillus bifidus	28	5.6
Lactobacillus bulgaricus	40	8.0
Lactobacillus delbrueckii	12	2.4
Lactobacillus plantarum	92	18.5
Lactobacillus spp.	16	3.2

bulgaricus. The remaining species isolated were found in lower numbers and were not present in the majority of samples taken. These species should be considered as transient members of the intestinal anaerobic

microflora.

No significant difference was found between the species of bacteria isolated from Series I chickens and those of Series II.

SUMMARY

Total anaerobic counts indicated that the numbers of intestinal anaerobes of the chicken and of A. galli are higher and more stable when the antibiotic Aureomycin was included in the diet.

Species of Lactobacillus which were strictly anaerobic on primary isolation (51.0 per cent of total isolates) were found to be the predominant anaerobes in the intestine of both the chicken and A. galli. The remaining flora appears to consist of species of Clostridium and Bacteroides which constituted 36.8 per cent and 10.2 per cent of the organisms isolated, respectively.

The predominant species were found to be Lactobacillus plantarum (18.5 per cent), Clostridium perfringens (18.0 per cent), Lactobacillus acidophilus (13.3 per cent), Lactobacillus bulgaricus (8.0 per cent), and Clostridium sporogenes (6.6 per cent), in that order. The remaining species isolated were assumed to be transient members of the flora of both the host and parasite.

No selective change in the species of organisms comprising the microflora was noted when Aureomycin was included in the diet.

The anaerobic microflora of the host intestine should be considered as a possible source of nutrients for A. galli. An attempt should be made to grow this parasite in vitro using typical anaerobic species as a sole source of nutrients. Success in this would be of great value to the parasitologist.

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APPENDIX I

Biochemical Tests and Stains Employed

Carbohydrate fermentation test. For the genus Clostridium, thioglycollate medium (Difco) containing brom thymol blue indicator was prepared. To this fermentation base was added 0.5 per cent of the desired fermentable carbohydrate. A Durham tube was added to each tube of fermentation medium and all tubes were autoclaved at 121°C for 15 minutes. Those sugars which are decomposed by autoclaving were sterilized separately by filtration and added to the sterilized base after autoclaving.

The tubes were inoculated by introducing a few drops of an 18- to 24-hour broth culture into the bottom of the tube by means of a Pasteur pipette. The tubes were incubated at the optimum temperature for each organism and observed after the 1st, 2nd, 4th, 7th and 10th days of incubation. Fresh indicator was added to the tubes which showed reduction of the indicator at the time of observation.

For the genus Lactobacillus, brom cresol purple yeast extract fermentation base (Lord, 1959) was used. The tubes were prepared, inoculated, and read in the same manner as described above. All fermentation tubes were observed for the presence of acid, gas, or alkalinity.

Cellulose utilization test. Cellulose salts medium (Lord, 1959) containing a strip of filter paper was inoculated and incubated at the optimum temperature for 21 days. Visable damage to the filter paper strip was considered a positive test.

Coagulated albumin test. This test was performed using nutrient broth containing a small strip of boiled egg white. The tubes were inoculated and incubated in a Brewer anaerobic jar for 10 days. The

test was considered positive if damage to the albumin could be detected at the end of the incubation period.

Coagulated serum test. Loeffler's serum slants were prepared by mixing 3 parts of calf serum and one part of nutrient broth containing 0.5 per cent glucose. The mixture was tubed in tightly capped screw cap tubes, placed in the autoclave in a slanted position, and coagulated by autoclaving at 121°C for 15 minutes. The tubes were inoculated by streaking and stabbing and incubated in a Brewer anaerobic jar for 10 days. A positive test was indicated by digestion of the coagulated serum.

Colony and agar streak characteristics. Nutrient agar plates and slants containing 0.5 per cent glucose were streaked and incubated anaerobically for 48 hours. The colony and agar streak characteristics were observed and recorded at the end of the incubation period.

Gelatin hydrolysis test. Modified Frazier's gelatin agar (Lord, 1959) was streaked and incubated anaerobically for 48 hours. When growth was established, the plates were flooded with Frazier's gelatin developer. Hydrolysis of gelatin was indicated by a clear zone surrounding the bacterial colonies.

Fifteen per cent nutrient gelatin tubes were also inoculated by stabbing and incubated for 48 hours. If the incubation temperature resulted in liquefying the control tube, the tubes were placed in the refrigerator and read for liquefaction after sufficient time was allowed for the solidification of negative tubes.

Heat test for endospores. Organisms from an old stroke culture were suspended in 3 ml of sterile distilled water and heated for 15

minutes by immersion in a hot water bath at 85°C. The contents of each tube was aseptically poured into a sterile Petri dish and liver veal agar was added. When the plates had cooled, they were placed in Brewer anaerobic jars and incubated for 48 hours. Growth at the end of this period indicated a positive test for endospores.

Hemolysis of red blood cells. Blood agar plates were prepared using tryptose blood agar base (Difco) plus 5 per cent sterile bovine blood. The plates were incubated for 48 hours under anaerobic conditions. The plates were observed and recorded as green, clear, or no hemolysis.

Hydrogen sulfide test. Hydrogen sulfide was detected by use of a semisolid nutrient medium (Reed and Orr, 1941) containing 0.05 per cent ferric ammonium citrate. After anaerobic growth was obtained, the cultures were checked for hydrogen sulfide production which was indicated by a blackening of the medium.

Indole test. Tryptone broth containing 0.1 per cent sodium thioglycollate (Reed and Orr, 1941) was inoculated and incubated at the optimum temperature for 48 hours. At the end of the incubation period the tubes were checked for indole by placing several drops of Ehrlich's Indole Test reagents (Lord, 1959) on the cotton plug which was then pushed down to within 1 inch of the medium. The tubes were then heated in a boiling water bath for 15 minutes. A red color developing on the cotton plug was recorded as a positive test for indole. A known culture of Escherichia coli was run with each series of tubes for use as a positive control.

Litmus milk test. Litmus milk was inoculated with the culture to be determined by means of a Pasteur pipette. After a 48-hour incubation period the tubes were observed for acid curd, rennet curd, peptonization,

reduction of litmus, and gas production. The tubes were reincubated after the initial reading and observed thereafter at 2-day intervals for 12 days.

Motility test. Each organism to be determined for motility was stabbed into motility test medium (Difco). The tubes were observed for motility after 8, 16, 24, and 48 hours of incubation. Any organism giving a negative test or failing to grow in motility test medium was observed, microscopically, for motility by means of a hanging drop slide.

Neutral red reduction and fluorescence test. This test was performed according to the method of Kaufman and Weaver (1960). It is based on the fact that species of Clostridium fluoresce under ultraviolet light when grown in a medium containing 0.004 per cent neutral red. The test has been shown to be specific for the genus Clostridium and the fluorescence obtained is easily distinguishable from the pale yellow color obtained by simple alkaline reduction of the medium.

The test was performed by streaking the organism to be determined over the surface of liver veal agar containing 0.004 per cent neutral red. The plates were overlayed with more of the same medium and incubated in a Brewer anaerobic jar for 48 hours. At the end of the incubation period the plates were observed under ultraviolet light and cultures which were surrounded by a golden yellow zone of fluorescence were considered positive for neutral red fluorescence. Any cultures surrounded by a pale yellow zone as seen by artificial light were considered positive for neutral red reduction.

Nitrate reduction test. This test was performed according to the method of Reed (1942). Tryptone broth containing 0.1 per cent potassium

nitrate and 0.1 per cent agar was prepared and inoculated with the organism to be determined. A vial was added to each tube for the collection of any gas which might be produced from nitrate. The tubes were tested for nitrite at 1, 2 and 5 days after incubation by adding 0.5 ml of sulfanilic acid reagent and 0.5 ml of dimethyl-alpha-napthylamine reagent to 5 ml of the broth culture. A red color indicates the presence of nitrite. If no color resulted, a pinch of zinc dust was added to detect the presence of nitrate. If no nitrate was present after the addition of zinc dust, the nitrate was assumed to have been reduced beyond nitrite. The vials were also observed for the presence of gas.

Optimum temperature determination. Four tubes of 0.1 per cent glucose yeast extract agar, per organism, were melted and cooled to 46°C. "Shake" cultures were prepared by inoculating each of these tubes with a straight needle and immediately solidifying them by cooling in an ice bath. The tubes were incubated at 20, 28, 37 and 45°C for 48 hours. At the end of the incubation period the tubes were observed and the lowest temperature at which the most growth occurred was recorded as the optimum temperature for the organism.

Oxygen relationship determination. Oxygen relationship was determined by using the tube selected in the optimum temperature determination. This tube was observed for the position of growth and the oxygen relationship was recorded as microaerophilic to anaerobic or anaerobic.

Reduced iron milk. The medium was prepared by tubing 10 ml amounts of fresh, unhomogenized whole milk. A pinch of powdered iron was added to each tube before sterilization. The tubes were inoculated in the lower portion by means of a Pasteur pipette and incubated at the optimum

temperature. The tubes were read at 1, 2, 4, 7 and 10 days and recorded as acid, gas, blackening, clot, digestion, or a combination of these.

Spore stain. Spore stains were prepared according to the Snyder modification of the Dorner method as follows: A smear of the organism was prepared, dried, and covered with a piece of blotting paper. The blotting paper was saturated with Ziehl's carbol fuchsin and steamed for 10 minutes. The slide was then decolorized without using 95 per cent ethanol. The slide was then rinsed with water, a drop of saturated aqueous nigrosin was added, spread with a loop, and allowed to dry. The slides were observed microscopically for size, shape, and position of the endospores in the sporangium.

Starch hydrolysis test. Starch agar (Difco) was prepared and plates were streaked with the organisms to be determined. The plates were incubated for 48 hours in a Brewer anaerobic jar. When growth was established, the plates were flooded with Gram's iodine solution. Those organisms which exhibited a clear zone around the colonies were considered as positive for starch hydrolysis.

All liquid or semisolid media used in these experiments was steamed and rapidly cooled immediately before inoculation.

Except where noted, all media and methods used in the determination of species are listed in Determinative Bacteriology Laboratory Manual (Lord, 1959) or The Manual of Microbiological Methods (Society of American Bacteriologists, 1957).

APPENDIX II

Table 1. Results of plate counts for Series I chickens

Sample number	Total anaerobic count	Average no. anaerobes per gram
Group I: 28 days after infection		
1155C	1.2×10^7	
1158C	3.8×10^7	
1161C	3.8×10^7	
1162C	1.2×10^7	
1169C	1.1×10^7	2.2×10^7
1177I	1.8×10^7	
1182I	1.5×10^7	
1186I	3.0×10^7	
1188I	3.9×10^7	
1211I	1.6×10^7	2.4×10^7
1182W	6.8×10^5	
1188W	7.2×10^5	7.0×10^5
Group II: 42 days after infection		
1130C	1.0×10^8	
1135C	2.9×10^7	
1146C	1.1×10^9	
1147C	3.0×10^8	
1149C	6.7×10^6	3.1×10^8
1174I	6.5×10^8	
1184I	4.2×10^8	
1190I	5.8×10^8	
1193I	1.1×10^9	
1207I	2.2×10^8	5.9×10^8
1174W	3.5×10^4	
1184W	2.1×10^6	
1190W	1.2×10^6	
1193W	6.5×10^6	2.9×10^6
Group III: 56 days after infection		
1133C	3.6×10^7	
1142C	2.6×10^7	
1151C	3.3×10^7	
1166C	2.5×10^7	
1167C	5.0×10^7	3.6×10^7
1175I	2.8×10^6	
1180I	7.3×10^7	
1189I	3.2×10^8	
1192I	3.4×10^7	
1202I	5.0×10^7	9.6×10^7
1175W	4.8×10^4	
1189W	3.7×10^4	
1192W	1.2×10^5	
1202W	7.5×10^4	7.0×10^4
Group IV: 70 days after infection		
1132C	4.5×10^7	
1137C	4.0×10^8	
1153C	1.0×10^9	
1154C	8.9×10^7	
1170C	3.4×10^8	3.7×10^8
1172I	7.7×10^7	
1179I	8.6×10^7	
1194I	8.5×10^7	
1201I	7.5×10^7	
1208I	9.0×10^7	8.7×10^7
1172W	2.9×10^6	
1179W	1.2×10^6	
1194W	8.3×10^5	1.6×10^6
Group V: 84 days after infection		
1127C	4.4×10^8	
1129C	1.6×10^8	
1135C	1.7×10^8	2.6×10^8
1152I	6.6×10^8	
1173I	4.2×10^8	
1185I	6.9×10^7	
1196I	2.5×10^7	
1205I	7.6×10^8	3.9×10^8
1173W	9.9×10^4	
1185W	4.1×10^6	
1196W	9.7×10^5	1.7×10^6
Group VI: 98 days after infection		
1126C	3.9×10^8	
1139C	2.9×10^8	
1157C	1.1×10^8	2.6×10^8
1143I	1.2×10^9	
1176I	6.2×10^8	
1178I	1.6×10^8	
1183I	1.5×10^9	
1195I	1.5×10^8	7.3×10^8
1178W	3.1×10^5	
1195W	6.6×10^5	4.9×10^5

Table 2. Results of plate counts for Series II chickens

Group I: 28 days after infection		
Sample number	Total anaerobic count	Average no. anaerobes per gram
1C	1.3×10^9	
2C	7.0×10^8	
3C	2.6×10^8	7.5×10^8
1195I	2.5×10^8	
1216I	8.9×10^7	
1221I	1.1×10^9	
1227I	2.3×10^8	
1390I	3.5×10^8	4.0×10^8
1195W	1.4×10^6	
1390W	1.9×10^6	1.2×10^6

Group II: 35 days after infection		
Sample number	Total anaerobic count	Average no. anaerobes per gram
4C	1.7×10^9	
5C	9.2×10^7	
6C	8.2×10^7	6.2×10^8
1217I	8.9×10^8	
1223I	5.5×10^8	
1224I	9.1×10^8	
1230I	3.6×10^6	
1391I	6.5×10^8	6.0×10^8
1217W	1.9×10^5	
1224W	1.8×10^6	
1391W	7.8×10^4	6.9×10^5

Group III: 42 days after infection		
7C	1.4×10^9	
8C	6.2×10^8	
9C	1.9×10^8	7.4×10^8
1219I	2.1×10^8	
1388I	4.4×10^8	
1392I	1.3×10^9	
1393I	2.5×10^9	
1394I	2.0×10^8	9.5×10^8
1392W	6.0×10^6	
1393W	1.9×10^5	
1394W	5.2×10^4	2.1×10^6

Group IV: 49 days after infection		
10C	3.5×10^8	
11C	2.4×10^8	
12C	2.5×10^8	2.8×10^8
1218I	5.9×10^8	
1220I	6.6×10^7	
1225I	3.6×10^8	
1229I	7.1×10^8	
1232I	6.1×10^8	4.7×10^8
1220W	3.2×10^5	
1225W	4.0×10^5	3.6×10^5

Group V: 56 days after infection		
13C	2.1×10^8	
14C	2.8×10^8	
15C	2.6×10^8	2.3×10^8
1226I	7.0×10^8	
1233I	5.6×10^8	
1397I	3.0×10^8	
1398I	6.1×10^8	
1399I	4.8×10^8	5.3×10^8
1226W	5.8×10^5	
1233W	2.7×10^5	
1397W	4.6×10^5	
1398W	1.5×10^5	

Group VI: 63 days after infection		
16C	1.4×10^7	
17C	1.7×10^9	
18C	1.5×10^8	6.2×10^8
1228I	8.0×10^8	
1231I	7.6×10^8	
1389I	4.9×10^8	
1396I	1.9×10^8	5.6×10^8
1228W	9.3×10^4	
1231W	2.3×10^6	1.2×10^6

Table 3. A summary of the average total anaerobic bacteria per gram of intestinal contents at various times after infection.

Series I	Number of days after infection					
	28	42	56	70	84	98
Control group	2.2×10^7	3.1×10^8	3.6×10^7	3.7×10^8	2.6×10^8	2.6×10^8
Infected group	2.4×10^7	5.9×10^8	9.6×10^7	8.7×10^7	3.9×10^8	7.3×10^8
A. galli from infected group	7.0×10^5	2.9×10^6	7.0×10^4	1.6×10^6	1.7×10^6	4.9×10^5

Series II	Number of days after infection					
	28	35	42	49	56	63
Control group	7.5×10^8	6.2×10^8	7.4×10^8	2.8×10^8	2.3×10^8	6.2×10^8
Infected group	4.0×10^8	6.0×10^8	9.5×10^8	4.7×10^8	5.3×10^8	5.6×10^8
A. galli from infected group	1.2×10^6	6.9×10^5	2.1×10^6	3.6×10^5	3.7×10^5	1.2×10^6

APPENDIX III

APPENDIX III

Organisms isolated from each intestine of Series I chickens (non-antibiotic ration).

No. of days post-infection	Culture number	Sample number	Organisms Isolated
28	1	1155C	Clostridium aerofoetidum
28	2	1182W	Lactobacillus sp.
28	3	1182W	Lactobacillus delbrueckii
28	4	1188W	Clostridium perfringens
28	5	1162C	Lactobacillus bifidus
28	6	1169C	Lactobacillus acidophilus
28	7	1169C	Lactobacillus bulgaricus
28	8	1188I	Clostridium sporogenes
28	9	1186I	Clostridium perfringens
28	10	1188I	Lactobacillus sp.
28	11	1161C	Bacteroides uniformis
28	12	1186I	Clostridium aerofoetidum
28	13	1186I	Lactobacillus plantarum
42	14	1190W	Clostridium perfringens
42	15	1190W	Clostridium perfringens
42	16	1190W	Clostridium tertium
42	17	1190W	Clostridium perfringens
42	18	1190W	Clostridium haemolyticum
42	19	1190W	Clostridium multifermentans
42	20	1174W	Clostridium perfringens
42	21	1174W	Clostridium perfringens
42	22	1174W	Clostridium butyricum
42	23	1174W	Clostridium perfringens
42	24	1174W	Lactobacillus acidophilus
42	25	1174W	Lactobacillus plantarum
42	26	1193W	Lactobacillus bulgaricus
42	27	1193W	Clostridium multifermentans
42	28	1184W	Clostridium perfringens
42	29	1184W	Clostridium perfringens
42	30	1207I	Lactobacillus bifidus
42	31	1207I	Clostridium perfringens
42	32	1146C	Clostridium perfringens
42	33	1146C	Lactobacillus acidophilus
42	34	1130C	Clostridium sporogenes
42	35	1130C	Lactobacillus delbrueckii
42	36	1135C	Clostridium sporogenes
42	37	1135C	Bacteroides ovatus
42	38	1147C	Lactobacillus acidophilus
42	39	1147C	Lactobacillus bifidus
42	40	1174I	Lactobacillus bulgaricus
42	41	1174I	Bacteroides exiguus
42	42	1184I	Clostridium haemolyticum
42	43	1184I	Clostridium lentoputrescens

Appendix III. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
42	44	1184I	Lactobacillus bulgaricus
42	45	1184I	Lactobacillus acidophilus
42	46	1184I	Lactobacillus plantarum
42	47	1193I	Clostridium cochlearium
42	48	1193I	Clostridium perfringens
42	49	1193I	Clostridium perfringens
42	50	1190I	Clostridium sporogenes
42	51	1190I	Bacteroides uniformis
56	52	1167C	Clostridium multifermentans
56	53	1167C	Bacteroides uniformis
56	54	1175I	Lactobacillus acidophilus
56	55	1202W	Lactobacillus sp.
56	56	1202W	Clostridium multifermentans
56	57	1175I	Clostridium sporogenes
56	58	1189I	Lactobacillus bifidus
56	59	1180I	Lactobacillus bifidus
56	60	1180I	Lactobacillus acidophilus
56	61	1133C	Clostridium perfringens
56	62	1192W	Lactobacillus plantarum
56	63	1189I	Bacteroides convexus
56	64	1142C	Clostridium sporogenes
56	65	1151C	Clostridium perfringens
56	66	1192I	Clostridium perfringens
56	67	1192I	Lactobacillus bulgaricus
56	68	1192I	Lactobacillus sp.
56	69	1202I	Clostridium sporogenes
56	70	1166C	Clostridium sporogenes
56	71	1175I	Lactobacillus bifidus
70	72	1132C	Clostridium perfringens
70	73	1132C	Bacteroides ovatus
70	74	1132C	Lactobacillus acidophilus
70	75	1132C	Bacteroides exiguus
70	76	1137C	Bacteroides uniformis
70	77	1137C	Clostridium tertium
70	78	1137C	Lactobacillus plantarum
70	79	1153C	Lactobacillus acidophilus
70	80	1153C	Bacteroides sp.
70	81	1153C	Lactobacillus plantarum
70	82	1154C	Lactobacillus plantarum
70	83	1154C	Bacteroides uniformis
70	84	1154C	Lactobacillus acidophilus
70	85	1154C	Lactobacillus plantarum
70	86	1154C	Clostridium perfringens
70	87	1170C	Clostridium sporogenes
70	88	1170C	Lactobacillus plantarum
70	89	1170C	Clostridium perfringens
70	90	1172I	Lactobacillus acidophilus

Appendix III. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
70	91	1172I	Clostridium perfringens
70	92	1172I	Bacteroides exiguus
70	93	1172I	Lactobacillus sp.
70	94	1179I	Bacteroides convexus
70	95	1179I	Lactobacillus bifidus
70	96	1179I	Bacteroides convexus
70	97	1179I	Lactobacillus acidophilus
70	98	1194I	Lactobacillus delbrueckii
70	99	1194I	Lactobacillus acidophilus
70	100	1194I	Lactobacillus bulgaricus
70	101	1194I	Lactobacillus plantarum
70	102	1201I	Lactobacillus bulgaricus
70	103	1201I	Bacteroides uniformis
70	104	1201I	Clostridium perfringens
70	105	1201I	Lactobacillus acidophilus
70	106	1201I	Lactobacillus plantarum
70	107	1208I	Clostridium butyricum
70	108	1208I	Lactobacillus bulgaricus
70	109	1208I	Lactobacillus plantarum
70	110	1208I	Clostridium perfringens
70	111	1208I	Clostridium sporogenes
70	112	1179W	Lactobacillus plantarum
70	113	1179W	Lactobacillus bifidus
70	114	1179W	Lactobacillus sp.
70	115	1172W	Lactobacillus bifidus
70	116	1172W	Lactobacillus bulgaricus
70	117	1172W	Lactobacillus bifidus
70	118	1172W	Bacteroides sp.
70	119	1172W	Clostridium sporogenes
70	120	1172W	Lactobacillus plantarum
70	121	1194W	Lactobacillus acidophilus
70	122	1194W	Lactobacillus bulgaricus
70	123	1194W	Lactobacillus plantarum
70	124	1194W	Clostridium perfringens
70	125	1194W	Lactobacillus bifidus
70	126	1194W	Lactobacillus acidophilus
70	127	1194W	Clostridium butyricum
70	128	1194W	Clostridium haemolyticum
70	129	1194W	Lactobacillus bifidus
70	130	1179W	Lactobacillus plantarum
70	131	1179W	Clostridium sporogenes
84	132	1173I	Lactobacillus acidophilus
84	133	1173I	Bacteroides exiguus
84	134	1173I	Clostridium multifermentans
84	135	1173I	Lactobacillus bifidus
84	136	1135C	Lactobacillus plantarum

Appendix III. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
84	137	1135C	Lactobacillus bulgaricus
84	138	1135C	Lactobacillus acidophilus
84	139	1135C	Clostridium perfringens
84	140	1135C	Clostridium multifermentans
84	141	1135C	Lactobacillus bifidus
84	142	1152I	Lactobacillus bulgaricus
84	143	1152I	Bacteroides ovatus
84	144	1152I	Lactobacillus acidophilus
84	145	1152I	Lactobacillus bifidus
84	146	1152I	Lactobacillus plantarum
84	147	1152I	Lactobacillus plantarum
84	148	1185I	Clostridium perfringens
84	149	1185I	Clostridium multifermentans
84	150	1185I	Bacteroides uniformis
84	151	1129C	Lactobacillus acidophilus
84	152	1129C	Clostridium perfringens
84	153	1129C	Clostridium sporogenes
84	154	1129C	Clostridium tertium
84	155	1129C	Lactobacillus plantarum
84	156	1129C	Bacteroides ovatus
84	157	1129C	Lactobacillus bulgaricus
84	158	1196I	Clostridium perfringens
84	159	1196I	Lactobacillus bulgaricus
84	160	1196I	Clostridium aerofoetidum
84	161	1127C	Lactobacillus plantarum
84	162	1127C	Clostridium perfringens
84	163	1127C	Clostridium perfringens
84	164	1205I	Clostridium tertium
84	165	1205I	Clostridium perfringens
84	166	1173W	Lactobacillus bulgaricus
84	167	1173W	Clostridium multifermentans
84	168	1173W	Clostridium perfringens
84	169	1173W	Lactobacillus acidophilus
84	170	1185W	Clostridium butyricum
84	171	1185W	Clostridium sp.
84	172	1185W	Clostridium multifermentans
84	173	1185W	Bacteroides exiguus
84	174	1196W	Clostridium perfringens
84	175	1196W	Clostridium sporogenes
84	176	1196W	Lactobacillus plantarum
84	177	1196W	Clostridium perfringens
98	178	1195W	Clostridium perfringens
98	179	1178W	Clostridium sporogenes
98	180	1178W	Clostridium perfringens
98	181	1178W	Clostridium sporogenes
98	182	1178W	Lactobacillus acidophilus

Appendix III. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
98	183	1178W	Bacteroides uniformis
98	184	1195W	Clostridium cochlearium
98	185	1195W	Lactobacillus plantarum
98	186	1195W	Discarded
98	187	1195W	Bacteroides exiguus
98	188	1195W	Bacteroides ovatus
98	189	1195W	Clostridium perfringens
98	190	1195W	Lactobacillus plantarum
98	191	1176I	Lactobacillus bulgaricus
98	192	1176I	Lactobacillus acidophilus
98	193	1176I	Clostridium perfringens
98	194	1176I	Clostridium multifermentans
98	195	1176I	Lactobacillus bifidus
98	196	1176I	Clostridium sporogenes
98	197	1176I	Lactobacillus plantarum
98	198	1139C	Discarded
98	199	1139C	Lactobacillus acidophilus
98	200	1139C	Lactobacillus delbrueckii
98	201	1139C	Lactobacillus plantarum
98	202	1195I	Lactobacillus acidophilus
98	203	1126C	Clostridium multifermentans
98	204	1195I	Lactobacillus delbrueckii
98	205	1195I	Lactobacillus acidophilus
98	206	1195I	Lactobacillus plantarum
98	207	1195I	Lactobacillus bulgaricus
98	208	1195I	Lactobacillus bifidus
98	209	1195I	Clostridium perfringens
98	210	1195I	Lactobacillus plantarum
98	211	1185I	Lactobacillus bifidus
98	212	1185I	Clostridium perfringens
98	213	1185I	Lactobacillus delbrueckii
98	214	1183I	Lactobacillus bulgaricus
98	215	1183I	Lactobacillus sp.
98	216	1183I	Lactobacillus acidophilus
98	217	1183I	Bacteroides convexus
98	218	1157C	Lactobacillus plantarum
98	219	1157C	Clostridium sp.
98	220	1157C	Bacteroides uniformis
98	221	1157C	Lactobacillus bulgaricus
98	222	1143I	Clostridium multifermentans
98	223	1143I	Clostridium perfringens
98	224	1143I	Lactobacillus plantarum
98	225	1143I	Lactobacillus acidophilus
98	226	1143I	Clostridium perfringens
98	227	1178I	Lactobacillus plantarum
98	228	1178I	Lactobacillus delbrueckii

Appendix III. (concl.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
98	229	1178I	*Lactobacillus acidophilus*
98	230	1178I	*Lactobacillus* sp.
98	231	1178I	*Lactobacillus bulgaricus*
98	232	1178I	*Lactobacillus plantarum*

APPENDIX IV

APPENDIX IV

Organisms isolated from each intestine of Series II chickens (antibiotic ration).

No. of days post-infection	Culture number	Sample number	Organisms Isolated
28	233	1C	Clostridium sporogenes
28	234	1C	Lactobacillus acidophilus
28	235	1C	Lactobacillus plantarum
28	236	1C	Bacteroides convexus
28	237	1C	Clostridium tertium
28	238	1C	Bacteroides exiguus
28	239	1C	Lactobacillus acidophilus
28	240	2C	Lactobacillus bifidus
28	241	2C	Lactobacillus delbrueckii
28	242	2C	Lactobacillus plantarum
28	243	2C	Lactobacillus delbrueckii
28	244	3C	Lactobacillus plantarum
28	245	3C	Lactobacillus plantarum
28	246	3C	Clostridium perfringens
28	247	3C	Clostridium aerofoetidum
28	248	1195I	Bacteroides uniformis
28	249	1195I	Clostridium perfringens
28	250	1195I	Clostridium sporogenes
28	251	1195I	Lactobacillus plantarum
28	252	1216I	Clostridium butyricum
28	253	1216I	Clostridium perfringens
28	254	1216I	Clostridium sporogenes
28	255	1221I	Lactobacillus acidophilus
28	256	1221I	Lactobacillus delbrueckii
28	257	1221I	Lactobacillus plantarum
28	258	1221I	Lactobacillus bifidus
28	259	1221I	Lactobacillus bulgaricus
28	260	1221I	Clostridium perfringens
28	261	1227I	Lactobacillus acidophilus
28	262	1227I	Clostridium sporogenes
28	263	1227I	Lactobacillus plantarum
28	264	1227I	Clostridium perfringens
28	265	1227I	Bacteroides exiguus
28	266	1227I	Lactobacillus bulgaricus
28	267	1227I	Clostridium perfringens
28	268	1390I	Clostridium perfringens
28	269	1390I	Discarded
28	270	1390I	Bacteroides uniformis
28	271	1390I	Lactobacillus plantarum
28	272	1390I	Clostridium sporogenes
28	273	1390I	Lactobacillus acidophilus
28	274	1195W	Lactobacillus acidophilus
28	275	1195W	Bacteroides exiguus
28	276	1195W	Bacteroides convexus
28	277	1195W	Lactobacillus plantarum
28	278	1195W	Lactobacillus sp.

Appendix IV. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
35	279	4C	Clostridium perfringens
35	280	4C	Discarded
35	281	4C	Lactobacillus delbrueckii
35	282	4C	Lactobacillus bulgaricus
35	283	4C	Lactobacillus acidophilus
35	284	4C	Lactobacillus plantarum
35	285	5C	Discarded
35	286	5C	Clostridium perfringens
35	287	5C	Clostridium hastiforme
35	288	5C	Lactobacillus delbrueckii
35	289	5C	Lactobacillus plantarum
35	290	6C	Clostridium perfringens
35	291	6C	Lactobacillus acidophilus
35	292	6C	Lactobacillus bifidus
35	293	6C	Lactobacillus bulgaricus
35	294	6C	Lactobacillus plantarum
35	295	6C	Clostridium perfringens
35	296	1217I	Lactobacillus plantarum
35	297	1217I	Clostridium perfringens
35	298	1217I	Bacteroides uniformis
35	299	1217I	Lactobacillus acidophilus
35	300	1223I	Clostridium aerofoetidum
35	301	1223I	Lactobacillus bifidus
35	302	1223I	Lactobacillus sp.
35	303	1223I	Lactobacillus plantarum
35	304	1223I	Bacteroides convexus
35	305	1223I	Lactobacillus bulgaricus
35	306	1224I	Lactobacillus acidophilus
35	307	1224I	Clostridium perfringens
35	308	1224I	Clostridium sporogenes
35	309	1224I	Lactobacillus plantarum
35	310	1224I	Bacteroides exiguus
35	311	1230I	Clostridium perfringens
35	312	1230I	Lactobacillus plantarum
35	313	1391I	Lactobacillus bulgaricus
35	314	1391I	Discarded
35	315	1391I	Lactobacillus acidophilus
35	316	1391I	Clostridium perfringens
35	317	1391I	Lactobacillus plantarum
35	318	1391I	Lactobacillus bulgaricus
35	319	1217I	Lactobacillus plantarum
35	320	1217I	Clostridium perfringens
35	321	1217I	Lactobacillus acidophilus
35	322	1224W	Lactobacillus plantarum
35	323	1224W	Lactobacillus acidophilus
35	324	1224W	Lactobacillus bulgaricus
35	325	1224W	Clostridium aerofoetidum

Appendix IV. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
35	326	1224W	Clostridium sporogenes
35	327	1224W	Clostridium sp.
35	328	1391W	Lactobacillus plantarum
35	329	1391W	Lactobacillus acidophilus
35	330	1391W	Clostridium perfringens
42	331	1393W	Lactobacillus bulgaricus
42	332	1393W	Bacteroides uniformis
42	333	1393W	Lactobacillus acidophilus
42	334	1393W	Clostridium perfringens
42	335	1393W	Lactobacillus plantarum
42	336	1388I	Lactobacillus bulgaricus
42	337	1388I	Lactobacillus bifidus
42	338	1388I	Discarded
42	339	1388I	Lactobacillus plantarum
42	340	1388I	Clostridium perfringens
42	341	1388I	Lactobacillus acidophilus
42	342	1394W	Lactobacillus plantarum
42	343	1394W	Lactobacillus acidophilus
42	344	1394W	Clostridium perfringens
42	345	1394W	Lactobacillus plantarum
42	346	7C	Lactobacillus acidophilus
42	347	7C	Clostridium sporogenes
42	348	7C	Discarded
42	349	7C	Lactobacillus plantarum
42	350	7C	Lactobacillus acidophilus
42	351	7C	Clostridium perfringens
42	352	7C	Lactobacillus bulgaricus
42	353	9C	Clostridium hastiforme
42	354	9C	Clostridium tertium
42	355	9C	Clostridium butyricum
42	356	9C	Clostridium perfringens
42	357	9C	Clostridium haemolyticum
42	358	9C	Lactobacillus plantarum
42	359	9C	Bacteroides convexus
42	360	8C	Clostridium butyricum
42	361	8C	Clostridium aerofoetidum
42	362	1219I	Bacteroides convexus
42	363	1219I	Clostridium perfringens
42	364	1219I	Bacteroides ovatus
42	365	1219I	Clostridium sporogenes
42	366	1219I	Clostridium multifermentans
42	367	1393I	Lactobacillus plantarum
42	368	1393I	Lactobacillus bifidus
42	369	1393I	Clostridium tertium
42	370	1393I	Clostridium perfringens
42	371	1393I	Lactobacillus acidophilus
42	372	1393I	Clostridium multifermentans

Appendix IV. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
42	373	1392W	Bacteroides convexus
42	374	1392W	Clostridium perfringens
42	375	1392W	Lactobacillus plantarum
42	376	1392W	Bacteroides uniformis
42	377	1392W	Discarded
42	378	1392W	Lactobacillus acidophilus
42	379	1394I	Lactobacillus acidophilus
42	380	1394I	Bacteroides ovatus
42	381	1394I	Lactobacillus plantarum
42	382	1394I	Clostridium perfringens
42	383	1392I	Lactobacillus bulgaricus
42	384	1392I	Clostridium butyricum
42	385	1392I	Lactobacillus plantarum
42	386	1392I	Bacteroides ovatus
49	387	1220W	Lactobacillus plantarum
49	388	1220W	Bacteroides variegatus
49	389	11C	Lactobacillus bifidus
49	390	11C	Lactobacillus plantarum
49	391	11C	Discarded
49	392	11C	Lactobacillus sp.
49	393	11C	Clostridium sp.
49	394	11C	Lactobacillus plantarum
49	395	11C	Lactobacillus acidophilus
49	396	10C	Lactobacillus bifidus
49	397	10C	Lactobacillus bulgaricus
49	398	12C	Bacteroides convexus
49	399	12C	Clostridium multifermentans
49	400	12C	Lactobacillus plantarum
49	401	1229I	Lactobacillus plantarum
49	402	1229I	Lactobacillus acidophilus
49	403	1229I	Lactobacillus plantarum
49	404	1229I	Clostridium perfringens
49	405	1229I	Lactobacillus bulgaricus
49	406	1229I	Clostridium sporogenes
49	407	1225I	Lactobacillus acidophilus
49	408	1225I	Clostridium perfringens
49	409	1225I	Lactobacillus bulgaricus
49	410	1225I	Lactobacillus plantarum
49	411	1225I	Lactobacillus sp.
49	412	1232I	Clostridium perfringens
49	413	1232I	Lactobacillus plantarum
49	414	1232I	Clostridium perfringens
49	415	1218I	Lactobacillus plantarum
49	416	1218I	Lactobacillus acidophilus
49	417	1220I	Lactobacillus plantarum
49	418	1220I	Lactobacillus acidophilus

Appendix IV. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
49	419	1220I	Bacteroides uniformis
49	420	1225W	Lactobacillus bulgaricus
49	421	1225W	Clostridium perfringens
49	422	1225W	Clostridium perfringens
49	423	1225W	Clostridium perfringens
56	424	13C	Clostridium tertium
56	425	13C	Lactobacillus plantarum
56	426	13C	Clostridium sporogenes
56	427	13C	Lactobacillus acidophilus
56	428	14C	Bacteroides exiguus
56	429	14C	Clostridium perfringens
56	430	14C	Lactobacillus bulgaricus
56	431	15C	Lactobacillus plantarum
56	432	15C	Clostridium butyricum
56	433	1226I	Lactobacillus plantarum
56	434	1226I	Lactobacillus bulgaricus
56	435	1233I	Lactobacillus plantarum
56	436	1233I	Lactobacillus acidophilus
56	437	1233I	Clostridium sporogenes
56	438	1397I	Clostridium hastiforme
56	439	1397I	Lactobacillus bulgaricus
56	440	1397I	Lactobacillus acidophilus
56	441	1398I	Clostridium perfringens
56	442	1398I	Bacteroides sp.
56	443	1398I	Clostridium cochlearium
56	444	1398I	Lactobacillus plantarum
56	445	1398I	Lactobacillus bulgaricus
56	446	1399I	Lactobacillus acidophilus
56	447	1399I	Clostridium butyricum
56	448	1399I	Lactobacillus plantarum
56	449	1399I	Lactobacillus plantarum
56	450	1226W	Clostridium multifermentans
56	451	1226W	Lactobacillus plantarum
56	452	1226W	Clostridium perfringens
56	453	1233W	Clostridium multifermentans
56	454	1233W	Clostridium perfringens
56	455	1233W	Lactobacillus sp.
56	456	1397W	Lactobacillus acidophilus
56	457	1397W	Lactobacillus plantarum
56	458	1398W	Lactobacillus plantarum
56	459	1398W	Clostridium perfringens
56	460	1398W	Clostridium perfringens
63	461	16C	Clostridium perfringens
63	462	16C	Lactobacillus sp.
63	463	17C	Clostridium sp.
63	464	17C	Bacteroides uniformis

Appendix IV. (cont.)

No. of days post-infection	Culture number	Sample number	Organisms Isolated
63	465	17C	Lactobacillus plantarum
63	466	17C	Clostridium sporogenes
63	467	17C	Lactobacillus acidophilus
63	468	17C	Clostridium perfringens
63	469	18C	Clostridium lentoputrescens
63	470	18C	Clostridium perfringens
63	471	18C	Lactobacillus plantarum
63	472	18C	Clostridium sporogenes
63	473	1228I	Lactobacillus plantarum
63	474	1228I	Lactobacillus acidophilus
63	475	1228I	Bacteroides ovatus
63	476	1228I	Lactobacillus sp.
63	477	1231I	Lactobacillus plantarum
63	478	1231I	Clostridium tertium
63	479	1231I	Lactobacillus acidophilus
63	480	1389I	Lactobacillus plantarum
63	481	1389I	Lactobacillus bifidus
63	482	1389I	Lactobacillus bifidus
63	483	1396I	Bacteroides exiguus
63	484	1396I	Lactobacillus plantarum
63	485	1228W	Clostridium perfringens
63	486	1228W	Lactobacillus acidophilus
63	487	1228W	Lactobacillus plantarum
63	488	1228W	Lactobacillus sp.
63	489	1228W	Bacteroides convexus
63	490	1228W	Clostridium perfringens
63	491	1228W	Clostridium sporogenes
63	492	1228W	Lactobacillus plantarum
63	493	1228W	Clostridium butyricum
63	494	1231W	Clostridium perfringens
63	495	1231W	Lactobacillus plantarum
63	496	1231W	Clostridium sporogenes
63	497	1231W	Lactobacillus acidophilus
63	498	1231W	Clostridium perfringens

THE ANAEROBIC MICROFLORA OF *ASCARIDIA GALLI* (SCHRANK) AND OF THE CONTROL AND INFECTED HOST INTESTINE

by

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B. S., Kansas State University, 1960

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Little is known of the host-parasite relationship that exists between the chicken and its nematode intestinal parasite, Ascaridia galli (Schrank); however, the host intestine has been studied intensively by various investigators.

A review of the literature revealed only two previous studies of the intestinal flora of A. galli, but these studies revealed little concerning the qualitative microflora of this parasitic nematode. Also little had been determined concerning the anaerobic microflora of the host intestine. For these reasons, it was decided to study both quantitatively and qualitatively, the anaerobic microflora of the host and parasite intestines to determine any similarities or differences which may exist between the two.

The chickens used in these experiments were infected with 100 ± 10 larvated eggs of A. galli. Two series of birds were used, one of which was fed an antibiotic ration while the other was fed identically but without antibiotics.

At various intervals after infection, groups of chickens were sacrificed, their intestines removed, and the contents of the host and parasite intestines were cultured anaerobically for qualitative and quantitative studies.

Quantitative results showed the anaerobic population to be higher for both the host and parasite when the chickens were fed an Aureomycin-containing ration than when fed without Aureomycin. This was attributed to elimination of antagonizing organisms by Aureomycin, but a study of other groups of organisms would have to be made to definitely establish this assumption.

Qualitative results using 498 obligate anaerobes isolated from control and infected intestines and from the gut of A. galli showed no significant difference in species isolated regardless of whether or not Aureomycin was included in the diet.

Species of Lactobacillus which were strictly anaerobic on primary isolation were found to be the predominant intestinal organisms in both the host and parasite. The remaining species isolated belonged to the genera Clostridium and Bacteroides in that order of importance.

The predominant anaerobic species of both the host and parasite were found to be Lactobacillus plantarum, Clostridium perfringens, Lactobacillus acidophilus, Lactobacillus bulgaricus, and Clostridium sporogenes in that order. The remaining species isolated were assumed to be transient members of the anaerobic microflora of both the host and parasite.

The possible role of the anaerobic intestinal microflora of the host in the nutrition of the parasite was discussed.