THE BACTERIAL FLORA OF THE CECAL FECES OF HEALTHY TURKEYS[1]

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Infectious enterohepatitis or blackhead is a disease of turkeys that is of considerable economic importance. The parasite, *Histomonas meleagridis*, gains entrance to the bird's body via the alimentary tract in contaminated food and drink and localizes in the cecum. There it may produce ulcers of the cecal wall and as a result enter the portal circulatory system and be carried to the liver, where secondary foci are established. Because of damage done to the cecal wall by the protozoa, bacteria also may enter the circulatory system. The need for a study of the relationship of bacteria to protozoa has been apparent for some time.

One of the first steps in an inquiry into the microbiology of blackhead would be to obtain information about the normal flora of healthy turkeys. Very little has been reported on this subject. Menes and Rochlin (1929) stated that the intestinal flora of domestic birds (chickens, geese, and turkeys) is composed chiefly of *Escherichia acidi-lactici* and *Streptococcus faecalis*. Kucel (1934) reported finding 3 species of bacteria in the intestinal tract of 25 healthy turkeys: *Bacterium coli*, *Escherichia acidi-lactici*, and *Streptococcus faecalis*. No attempt was made to identify anaerobes.

Considerable data, however, have been compiled in regard to the flora of the chicken. King (1905) found the colon bacillus to be the predominating bacterial type in the intestinal mucosa of chickens. Rahner (1901) and Gage (1911) concluded from their experiments that *Bacterium coli* was the most numerous species of bacteria in the feces of chickens. Emmel (1930) studied the flora of the intestines of 20 normal hens. He plated samples of main bowel feces (no platings were made of cecal feces) and found that *Escherichia coli* and *Escherichia communior* were the predominating species, making up about 34 and 31 per cent of the total flora, respectively.

One cannot help wondering whether the reported predominance of coliforms in the feces of poultry is due to the ease with which these bacteria can be isolated and identified, especially since rather thorough studies on some mammals indicate that bacterial types other than coliforms are the most prevalent. Crecelius and Rettger (1943), for instance, have shown lactobacilli to be the predominating bacterial type in the intestine of guinea pigs, and Eggerth and Gagnon (1933) have shown obligate anaerobes of the genus *Bacteroides* to be the predominating bacterial type in human feces.

[1] This work was undertaken as part of a co-operative project dealing with a study of bacteria associated with *Histomonas meleagridis* in fecal matter and in blackhead lesions. The project was originated by H. M. DeVolt, Department of Animal Pathology, University of Maryland.

Recently, Johansson *et al.* (1948) observed the change in the intestinal flora of hens with an associated change of diet. The organisms were identified mainly as to bacterial type: coliforms, enterococci, lactobacilli, etc. Their data suggest that anaerobic lactobacilli exist in the intestine of chickens in considerable numbers.

The purpose of this paper is to report our findings on the bacterial flora of the ceca of turkeys. We have determined the predominance of the various bacterial types in the cecal feces of healthy turkeys and, when possible, have identified the bacteria as to species.

METHODS

Twelve cecal feces, each from a different turkey, have been studied. These 12 samples were taken from 3 different flocks. The turkeys of 2 of the flocks were grown in pens that were raised above the ground, and were fed grain and mash. The birds of the other flock were on range and therefore had access to insects and greens in addition to their grain and mash. All 12 of the birds were 5 months of age or older. The turkeys sampled were the Broad Breasted Bronze variety except several of the flock on range, which were White Hollands. Both sexes were represented.

Immediately after the feces were voided, a representative sample of them was taken to the laboratory and a 1-g portion was weighed, ground with sterile sand in a sterile mortar and pestle, and added to 99 ml of sterile 0.85 per cent sodium chloride in a dilution bottle. From the initial 10^{-2} dilution thus prepared, (1) a wet mount was made, mainly for the purpose of observing whether an abnormal fauna existed—this was done to ascertain that the turkey was not infected with pathogenic protozoa, since the purpose of the study is to determine the flora of healthy turkeys; (2) a gram stain was made for the purpose of obtaining an idea of the bacterial types to be encountered; and (3) serial dilutions of 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10} were made by transferring serially 1 ml of the diluted feces to other dilution bottles containing 99 ml of sterile saline.

Pour plates were prepared from these dilution bottles in the customary manner. The kinds of media to employ and the dilution range to use with each was determined in preliminary experiments. The following were finally decided upon: (1) Difco *Salmonella-Shigella* agar (SS) plated in dilutions of 10^{-2}, 10^{-3}, and 10^{-4} singly and incubated aerobically. (2) Difco eosin methylene blue agar (EMB) plated in dilutions of 10^{-4}, 10^{-5}, and 10^{-6} in duplicate and incubated aerobically. These SS and EMB plates were used for the isolation of gram-negative rods other than coliforms. (3) Urea ricinoleate agar of Zarett and Doetsch (1949) plated in dilutions of 10^{-2}, 10^{-3}, and 10^{-4} singly and incubated aerobically. This medium was used in an attempt to isolate *Proteus*. (4) Sodium azide agar of the following percentage composition: Difco tryptone, 0.5; Difco yeast extract, 0.5; glucose, 0.5; sodium azide, 0.04; and agar, 1.5; plated in dilutions of 10^{-4}, 10^{-5}, and 10^{-6} in duplicate for the isolation of "enterococci." Incubation was aerobic. This medium was modified from a formula developed by Winter and Sandholzer (1946). (5) Difco heart infusion agar plated in dilutions of 10^{-6}, 10^{-7}, and 10^{-8} in duplicate

for the enumeration of coliforms, micrococci, and other aerobic bacteria. Coliforms were enumerated on this medium rather than EMB agar because of the slight inhibition the latter has for these organisms. Incubation was aerobic. (6) Trypticase soy glucose agar (Baltimore Biological Laboratories) plated in dilutions of 10^{-8}, 10^{-9}, and 10^{-10} in duplicate and incubated anaerobically. Lactobacilli, anaerobic streptococci, and miscellaneous types of gram-positive, nonsporeforming rods were isolated from these plates. This medium was described by Pelczar and Vera (1949), who used it for the enumeration of bacteria in milk and milk products. It has the following percentage composition: trypticase, 1.5; phytone, 0.5; glucose, 0.5; sodium chloride, 0.4; sodium citrate, 0.1; sodium sulfite, 0.02; cystine, 0.02; and agar, 1.5. (7) Tomato juice glucose agar (Difco tomato juice agar to which were added 0.5 per cent glucose and 0.9 per cent additional agar) plated in dilutions of 10^{-8}, 10^{-9}, and 10^{-10} in duplicate and incubated anaerobically for the isolation of *Lactobacillus*.

Anaerobiosis was produced by the method of Weiss and Spaulding (1937). In every instance the pour plates were in the incubator within $2\frac{1}{2}$ hours after the feces had originally been voided by the turkey. The plates were incubated for 48 to 53 hours at 41 to 42 C. This temperature was chosen for two reasons: (1) Marsden and Martin (1939) have reported that the body (rectal) temperature of the adult turkey normally varies between 40.6 and 42.2 C, the average being 41.4 C. We believe that when the isolation of strains is made at this temperature the results will be more nearly correct than when isolation of strains is made at a lower temperature. (2) This relatively high incubation temperature should discourage the growth of some soil organisms that are naturally picked up by the turkey as a result of its feeding habits and that pass through the bird's intestine without actually proliferating therein.

After the incubation period the number of colonies on each of the plates was recorded. Colonies were picked and subcultured to trypticase soy glucose (TSG) agar slants and incubated for about 2 days. This medium was chosen for carrying stock cultures because it was found capable of supporting the growth of the widest variety of bacteria. Incidentally, in nearly every instance, the plate counts on this medium were the highest. The cultures isolated were carried and studied at the customary 37. C All slants made from the anaerobic plates were incubated anaerobically, whereas those from the aerobic plates were incubated aerobically or anaerobically depending upon the type and depth of the colony isolated. For instance, minute subsurface colonies from the aerobic heart infusion agar plates usually were found to be *Lactobacillus*; members of this genus produce better surface growth when cultivated under anaerobic conditions. Surface colonies from the aerobic plates and large subsurface colonies, which were considered probable aerobic or facultative anaerobic organisms, were subcultured under aerobic conditions. It should be mentioned here that either all the colonies on a plate were subcultured or a representative portion of the colonies on a plate were subcultured to the TSG agar slants.

After completion of the 2-day incubation period, the slants were observed as to type of growth, and gram stains were made. The anaerobic slants were sub-

cultured to new TSG slants for aerobic incubation in order to determine the oxygen tension preferred by the cultures. The parent anaerobic slants were again placed under anaerobic conditions so that any obligate anaerobes would not be lost.

After each culture had been tentatively identified by using the tests described in the *Manual of Methods for Pure Culture Study of Bacteria* (1945) and other tests to be mentioned later, representative cultures were kept in the icebox or were lyophilized. Later these were revived, restreaked, subcultured, and tested again en masse to insure proper identification. The occasional yeast and *Streptomyces* isolated were discarded, no attempt being made to classify organisms other than *Eubacteriales*.

RESULTS AND DISCUSSION

Wet mounts made from the initial 10^{-2} dilution of the sample were observed microscopically. An extremely large *Sarcina* (3 to 5 μ in diameter) was observed in about one-third of the feces samples (figure 1*A*). Although this organism was easily detected in cover slip preparations because of its characteristic size and shape, it was seen to exist in relatively small numbers, and we have not succeeded in isolating it from the pour plates. Streptococci, rods, and spirilla were easily seen in the wet mounts. The spirilla often appeared to be the only bacteria possessing motility; they also have not been isolated. By removing the cover slip from the wet mount and allowing the film thus formed to dry, stained preparations were made. Streptococci and various rod forms were observed in the stained films (figure 1*B*). Spirochetes of the genus *Borrelia* and spirilla were seen also (figure 1*C*). The spirochetes like the *Sarcina* and spirilla have not been isolated and grown in pure culture.

As has been mentioned previously, the anaerobic TSG plates gave the highest counts of all the media employed. The average plate count on this medium was about 13 billion colonies per gram of raw feces. The anaerobic tomato juice glucose agar plates also gave relatively high counts. Plate counts on the aerobic heart infusion agar averaged 30 million colonies per gram feces. EMB agar averaged about 2 million colonies per gram sample, and the sodium azide plates slightly less. Only a few colonies were ever found on the SS agar, these always being coliforms. Members of the genus *Proteus* were never recovered from the urea ricinoleate medium, or from any of the other media. The bacteria isolated from the pour plates will be discussed in the approximate order of increasing prevalence, as follows: (1) aerobic, sporeforming rods; (2) aerobic and facultative anaerobic micrococci; (3) facultative anaerobic streptococci; (4) gram-negative rods; (5) anaerobic streptococci; (6) gram-positive, nonsporeforming rods other than lactobacilli; and (7) lactobacilli.

Aerobic, sporeforming rods. These organisms were identified by the methods recommended by Smith, Gordon, and Clark (1946). The following were isolated once, each from a different sample: *Bacillus firmus*, *Bacillus megatherium*, *Bacillus pumilus*, and *Bacillus subtilis*. An unidentified *Bacillus* was isolated twice. In addition, *Bacillus cereus* was isolated thrice, each time from a different feces sample. Observations of wet mounts and stained films made from the initial 10^{-2} dilution

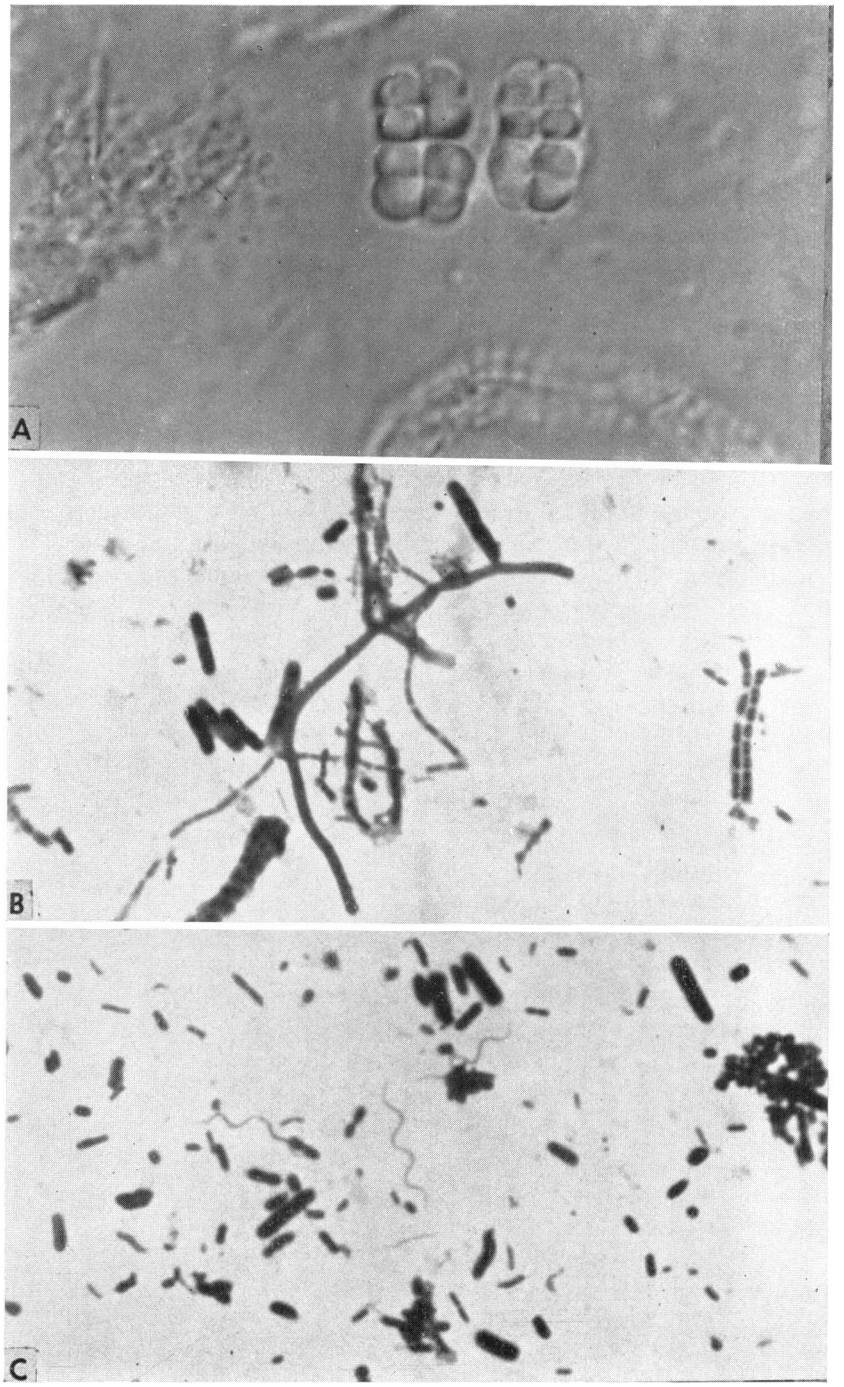

Figure 1. Bacterial types as seen in cecal feces of turkeys (× 1,400). *A*. A large *Sarcina* (wet mount). *B*. Various rod forms. *C*. Spirochetes of the genus *Borrelia* and spirilla (Stained with crystal violet).

of the feces never revealed spore-bearing rods, although occasionally a lone spore was seen. The 9 cultures of aerobic sporeformers were not found in a definite dilution range, a fact that suggests that members of the genus *Bacillus* are not indigenous to the ceca of turkeys.

Aerobic and facultative anaerobic micrococci. These cocci were isolated from the heart infusion, EMB, and TSG agars. Thirty-three cultures of the *Micrococcaceae* were isolated from 8 turkeys, all 3 flocks being represented. *Micrococcus epidermidis* and *Micrococcus pyogenes* var. *albus* were the species most frequently encountered. *Micrococcus aurantiacus* and *Micrococcus flavus* were isolated occasionally. Several strains have been isolated that will require additional study for identification; they do not fit into the breakdown of the genus listed in *Bergey's Manual* (Breed *et al.*, 1948).

In addition, a number of cultures of cocci have been isolated that are facultative anaerobes, growing along the entire depth of an agar stab. Only thin growth is produced on aerobic slants, which resembles the type growth produced by the streptococci. Chain formation never occurs, however; the organisms occur singly, in pairs, or more often (especially in milk) as tetrads. The cells average 0.6 to 0.8 μ in diameter. Nitrate is not reduced, gelatin not liquefied, and $NH_4H_2PO_4$ not utilized as the sole source of nitrogen. All produce some acid in litmus milk, but not sufficient for curdling. No reduction of the litmus occurs. Cultures 4–43 and 24–17 produce no hemolysis when streaked on beef blood agar, but cultures 4–27 and 24–4 produce a slight hazy zone, or what is often referred to as an α' reaction. Two cultures, 4–27 and 4–43, are able to survive when heated to 61 C for 30 minutes in litmus milk. It is difficult to classify these strains from the information available with any degree of finality. They resemble *Gaffkya* morphologically, but closer examination shows that the similarity is superficial, e.g., they do not produce capsules under any circumstances, they are not pathogenic to mice when inoculated intraperitoneally, and they do not produce gas. We prefer not to include them in the genus *Micrococcus* because they appear to be a different entity; they are more difficult to grow than the typical *Micrococcus*, and produce only scant surface growth. They are not obligate anaerobes, and have little in common with the anaerobic species of *Micrococcus* listed in *Bergey's Manual* (Breed *et al.*, 1948). Three of the strains isolated (4–27, 4–43, and 24–17) are catalase-negative, the other (24–4) very weakly catalase-positive. We have tentatively placed them in the genus *Pediococcus*. This genus, as listed in *Bergey's Manual* (Breed *et al.*, 1948) includes cocci of an intermediate type, between the *Streptococcus* on the one hand and the *Micrococcus* and *Sarcina* on the other. Fermentation reactions of 3 strains of pediococci are listed in table 1. Our strains agree well in fermentation characters with the *Pediococcus* types listed by Orla-Jensen (1943).

Facultative anaerobic streptococci. A total of 32 cultures were isolated from the sodium azide, EMB, and heart infusion agar plates. Thirty of these are members of the "enterococcus" group; the remaining 2 are members of the "viridans" group.

The "enterococci" were isolated from 8 different turkeys, all 3 flocks being

represented. These streptococci were able to grow at 10 C, and all except 1 strain (12–7) were able to grow at 45 C. They were able to survive a temperature of 61 to 62 C for 30 minutes, were able to grow in the presence of 0.1 per cent methylene blue, 6.5 per cent sodium chloride, and in broth adjusted to a pH of 9.6. They reduced litmus milk prior to curd formation. Two species were identified: *Streptococcus liquefaciens*, and *Streptococcus faecalis*.

Ten cultures of *S. liquefaciens* were isolated from 3 turkeys. These cocci liquefy gelatin, peptonize milk, and are not *beta*-hemolytic on horse blood agar pour plates. Paralleling this organism's ability to liquefy gelatin and peptonize milk is its ability to attack the alcohols: glycerol, sorbitol, and mannitol. The latter two it ferments quite vigorously. The final pH produced in various substrates by 3 representative cultures of this species (2–6, 4–4, and 24–13) is tabulated in table 1.

Twenty cultures of *S. faecalis* were isolated from 5 turkeys. They do not liquefy gelatin or peptonize milk. Sufficient acid is produced in skim milk by most cultures to cause curdling of this substrate. One of our cultures (29–24) differs from the others in that it ferments glycerol and not sucrose (table 1). Sherman (1937) recognizes strains of *S. faecalis* that may, or may not, ferment glycerol and sucrose. Orla-Jensen (1919), on the other hand, differentiates between streptococci of this group that attack glycerol and those that do not ferment this alcohol. The former he calls *Streptococcus glycerinaceus*, the latter *Streptococcus faecium*. (Other fermentation characteristics are also considered, as weak glycerol fermentation may occur in his *S. faecium* group.) Culture 29–24 resembles the *S. glycerinaceus* of Orla-Jensen in that it ferments glycerol and not melibiose, but differs in that it does not ferment sorbitol or melezitose. In this work we have considered *S. faecalis* to include strains of varied fermentative powers, as do Sherman, Mauer, and Stark (1937); hence we consider 29–24 to be *S. faecalis*. Cultures 12–7 and 21–2 differ from the other cultures in regard to fermentation, attacking fewer carbohydrates than the typical *S. faecalis*. Because of this fact it might be suggested that they should be considered varieties of *Streptococcus durans*. However, they are not *beta*-hemolytic, and they produce a strong reduction of litmus milk prior to curdling; they have therefore been tentatively classified as varieties of *S. faecalis*. (Sherman and Wing, 1937, consider that *beta*-hemolysis and lack of reduction of litmus milk before curdling are the most important characteristics for differentiating *S. durans* from the other "enterococci.") Before ending our discussion concerning the *S. faecalis* of turkeys, it should be mentioned that our cultures do not ferment sorbitol, and in this respect they differ from the typical *S. faecalis* described by Sherman (1937) in his monograph. Orla-Jensen (1919), however, has described strains of *S. faecium* that, like ours, are sorbitol-negative. Sherman, Mauer, and Stark (1937) have pointed out that fermentation reactions within this species are so diverse that only minor taxonomic importance can be attached to them.

Two cultures of *Streptococcus equinus* ("viridans" group streptococci) were isolated. They were found in the feces of 2 turkeys. These cocci were able to grow at 45 C, but not at 10 C. They could not survive when heated to 61 to 62 C for

TABLE 1

Determinations of the final pH in broth cultures of representative strains of Streptococcus and Pediococcus when grown in the presence of various substrates after 14 days' incubation at 37 C

STRAIN NUMBER	GLYCEROL	XYLOSE	ARABINOSE	RHAMNOSE	SORBITOL	MANNITOL	INOSITOL	FRUCTOSE	GLUCOSE	MANNOSE	GALACTOSE	SUCROSE	TREHALOSE	MALTOSE	MELIBIOSE	LACTOSE	MELEZITOSE	RAFFINOSE	DEXTRIN	STARCH	SALICIN	SKIM MILK	NO SUBSTRATE	SPECIES
Control	7.1	6.9	6.9	6.7	7.1	7.1	7.1	6.5	6.6	6.6	6.6	7.2	7.2	7.2	7.1	7.0	7.1	7.2	7.1	7.1	7.2	6.3	7.2	
2–6	5.8	6.9	6.9	6.9	5.1	5.0	6.8	4.4	4.4	4.4	5.6	6.7	4.8	4.5	7.1	5.3	4.8	7.3	5.1	7.2	4.5	5.2	7.2	
4–4	6.1	6.9	6.9	6.5	5.2	5.1	6.6	4.3	4.4	4.6	5.5	5.7	4.8	4.7	7.1	5.9	4.8	7.3	5.2	7.0	4.5	5.2	7.2	*Streptococcus liquefaciens*
24–13	5.6	6.9	6.8	6.4	5.0	4.9	6.9	4.3	4.3	4.3	5.2	4.4	4.9	4.5	7.2	5.2	4.8	7.2	5.1	7.1	4.6	5.3	7.4	
1–37	7.2	7.0	6.4	6.9	7.3	5.6	7.4	4.1	4.1	4.3	4.8	4.6	5.0	4.3	7.1	4.8	7.4	7.2	5.3	7.4	5.2	4.8	7.2	
12–6	7.1	6.9	7.0	6.8	7.1	5.5	7.3	4.0	4.1	4.1	4.9	4.4	5.1	4.6	6.0	4.8	7.3	6.6	5.1	7.2	5.1	4.8	7.2	
12–7	7.1	6.9	7.0	6.7	7.2	7.0	7.2	4.3	4.3	4.3	4.6	7.2	4.6	7.2	7.1	4.7	7.2	7.2	7.0	7.2	4.8	5.5	7.2	
21–2	7.0	7.1	7.1	6.9	7.2	6.8	7.4	4.2	4.1	4.5	4.7	7.3	4.8	4.5	5.5	5.1	7.5	7.5	6.0	7.4	4.5	5.3	7.2	
28–2	7.0	7.0	7.1	6.9	7.3	7.2	7.4	4.5	4.4	4.5	4.9	5.8	4.8	4.5	7.3	5.7	7.5	7.5	5.1	7.4	4.9	5.2	7.2	
28–9	7.2	7.1	6.7	7.0	7.3	5.2	7.4	4.2	4.2	4.2	4.7	4.3	5.0	4.5	5.3	4.9	7.5	7.5	5.2	7.4	4.9	4.9	7.2	*Streptococcus faecalis*
28–13	7.2	6.9	7.1	6.7	7.0	4.6	7.3	4.2	4.1	4.2	4.7	4.5	5.0	4.6	6.4	4.8	7.3	7.1	4.9	7.1	5.0	5.0	7.2	
29–18	7.0	7.1	6.8	7.0	7.3	5.3	7.4	4.1	4.1	4.2	4.6	4.4	4.8	4.6	7.1	4.8	7.4	7.3	5.6	7.4	4.9	4.7	7.2	
29–21	7.1	6.9	7.0	6.8	7.0	5.2	7.3	4.1	4.1	4.2	4.8	4.5	5.1	4.6	7.1	4.8	7.3	7.0	5.2	7.2	4.8	4.7	7.2	
29–23	7.0	7.0	6.6	7.0	7.1	5.6	7.4	4.3	4.2	4.3	4.8	4.7	5.6	4.6	5.9	4.8	7.4	7.1	6.1	7.3	4.8	4.7	7.2	
29–24	5.8	6.9	6.6	6.9	7.1	4.7	7.3	4.0	4.1	4.2	4.7	7.3	4.8	4.3	7.1	4.8	7.3	7.3	5.0	7.3	4.5	4.9	7.2	
24–21	7.1	6.9	6.9	6.7	7.2	6.4	7.1	4.9	4.7	5.0	5.6	4.9	5.2	4.9	6.8	6.9	7.1	4.8	6.5	7.1	5.2	6.2	7.2	*Streptococcus equinus*
4–27	6.6	5.4	5.6	6.9	7.1	7.1	7.2	4.1	4.0	4.0	4.4	7.1	7.2	3.9	7.2	5.4	7.3	7.2	6.9	7.3	6.9	5.9	7.2	
4–43	6.8	6.9	5.2	6.9	7.3	7.1	7.2	4.2	4.1	4.0	4.6	7.3	4.6	7.2	7.2	5.8	7.3	7.2	7.1	7.3	6.4	6.2	7.2	*Pediococcus* sp.
24–17	7.1	6.8	6.8	6.7	7.1	7.0	7.0	6.0	5.5	5.8	6.7	7.2	7.1	6.2	7.0	6.9	7.0	7.0	6.6	7.0	7.1	6.2	7.2	

30 minutes, and could not grow in the presence of 0.1 per cent methylene blue or 6.5 per cent sodium chloride. Some growth occurred in broth adjusted to a pH of 9.6. They are not *beta*-hemolytic, do not liquefy gelatin, and do not reduce litmus milk prior to curdling. Their fermentation reactions are characteristic of the species.

The final pH produced in various substrates by representative cultures of facultative anaerobic streptococci isolated from the cecal feces of turkeys is recorded in table 1. The basal medium employed had the following percentage composition: Difco tryptone, 2.0; K_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.01. Sufficient substrate was added to produce 1 per cent substrate. All were sterilized by autoclaving at 15 pounds for 15 minutes except xylose, arabinose, maltose, and melibiose, which were sterilized by filtration and added aseptically to the autoclaved

TABLE 2

Biochemical reactions of the coliforms isolated from the cecal feces of turkeys

SPECIES OR VARIETY	NUMBER OF CULTURES ISOLATED AND STUDIED	LACTOSE	SUCROSE	SALICIN	I	M	VI	C
Escherichia coli (atypical cultures)	1	++	−	−	+	−	−	−
	4	++	−	−	−	+	−	−
Escherichia coli var. *acidi-lactici*	45	++	−	−	+	+	−	−
Escherichia coli		++	−	++	+	+	−	−
Escherichia coli var. *communior*		++	++	−	+	+	−	−
Escherichia coli var. *neapolitana*	47	++	++	++	+	+	−	−
Escherichia intermedium	1	++	−	−	+	+	−	+
Paracolobactrum coliforme	2	++	−	−	+	+	−	−

−, negative reaction; +, positive reaction; ++, acid and gas.

All cultures failed to produce H_2S in Kligler iron agar butts. *Escherichia* cultures produced acid and gas in lactose broth within 18 hours, whereas *Paracolobactrum coliforme* required 4 days for the fermentation of this disaccharide.

base. No indicator was added to the medium; the pH was determined by means of the glass electrode.

Gram-negative rods. Coliforms were isolated from the heart infusion agar and also from the EMB and SS agar pour plates. *Escherichia coli* was encountered in every feces. A total of 92 cultures of this bacterium were isolated and studied. Fermenters and nonfermenters of sucrose were found to occur in almost equal numbers. King (1905) observed this same relationship in the case of *E. coli* isolated from the intestinal mucosa of chickens. We isolated *Escherichia intermedium* once and *Paracolobactrum coliforme* twice. These 3 species were the only gram-negative rods isolated from the 12 cecal feces studied. The biochemical reactions of the coliforms have been summarized in table 2; the names given were arrived at through the use of the key in *Bergey's Manual* (Breed *et al.*, 1948). It is interesting that *Aerobacter* and *Proteus* were never encountered.

Anaerobic streptococci. These organisms were isolated from the TSG plates. They can be divided into 3 types: those that do not ferment carbohydrates,

those that produce acid, and those that produce acid and gas in media containing fermentable carbohydrate. The streptococci of the last 2 types usually ferment glucose, lactose, maltose, and trehalose. Some also ferment arabinose, sucrose, and mannitol. They form long chains; the cells are often quite pleomorphic, being

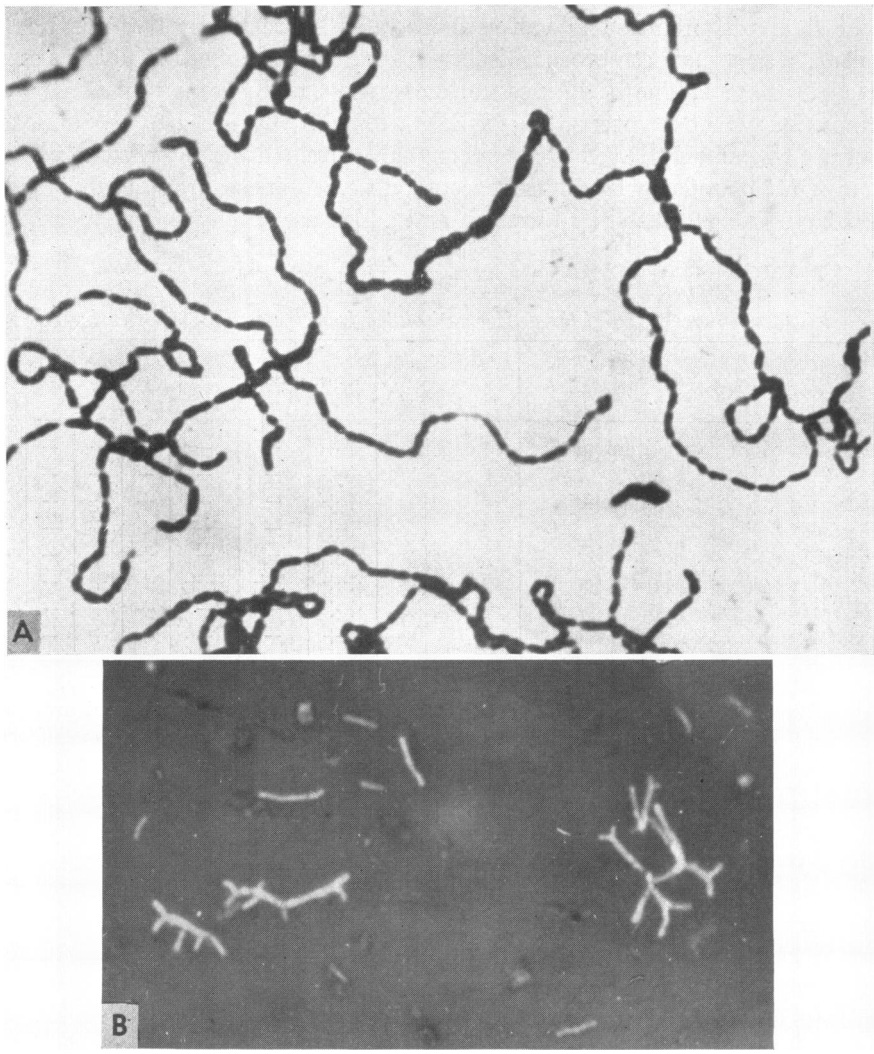

Figure 2. Anaerobic bacteria isolated from the cecal feces of turkeys (× 1,400). *A*. Pleomorphic streptococci, 21–29 (37 C, 3-day broth culture stained with crystal violet). *B*. *L. bifidus*, 21–39 (37 C, 6-day broth culture counterstained with India ink).

elongate and swollen. Culture 21–29 (figure 2*A*) is typical. These streptococci have not been identified. In fact, we use the term streptococci here in its descriptive sense; these organisms may not be members of the genus *Streptococcus*.

Gram-positive, nonsporeforming rods other than lactobacilli. This is a heterogene-

ous group of organisms. All are nonsporeforming rods that are gram-positive or, as in a few instances, gram-variable. Nearly all are aerobes or facultative anaerobes, and they may be divided into 3 types on the basis of motility, catalase-production, and nitrate reduction. Those that possess motility also possess catalase, but may or may not reduce nitrate. The nonmotile rods do not produce catalase but do reduce nitrate. The few obligate anaerobes comprise a fourth type; they are nonmotile, do not produce catalase, and do not reduce nitrate. These are extremely large rods, averaging 1 to 2 μ by 8 to 15 μ, which ferment arabinose or xylose, or both, rapidly.

Some of the aerobic and facultative anaerobic, gram-positive, nonsporeforming rods have the morphology characteristic of *Corynebacterium* and have been placed in this genus. The others have been tentatively classified as *Bacterium*, but it is probable that additional study would indicate some of these to be members of the *Lactobacilleae*.

Lactobacilli. Gram-positive, nonsporeforming, nonmotile, non-nitrate-reducing, catalase-negative rods are the predominating bacterial type found in the cecal feces of turkeys. These rods can be divided into 2 types on the basis of their oxygen requirements. The majority encountered cannot multiply in the presence of oxygen; no growth occurs on agar slants incubated aerobically. About 85 cultures have been isolated and studied. We have identified them as *Lactobacillus bifidus*. (They resemble closely the strains described by Orla-Jensen *et al.*, 1936.) The morphology is characteristic of this species, with branched, "antlerlike" cells (figure 2*B*). Our cultures ferment lactose, maltose, sucrose, raffinose, and melibiose; most ferment trehalose and arabinose; a few attack xylose; but mannitol, sorbitol, and rhamnose are not fermented. Studies on several cultures have shown that about 50 per cent of the glucose fermented by these organisms is converted to volatile acid. The nonvolatile acid produced is dextro-rotatory lactic acid. *L. bifidus* was isolated from the TSG and tomato juice glucose agar pour plates. Obligate anaerobes of the *L. bifidus* type were the most numerous bacteria found in the turkeys tested, averaging (geometric mean) about 4.1 billion per gram of cecal feces.

The second group consists of facultative anaerobic lactobacilli. They will multiply on aerobic agar slants, but do so poorly, producing only a thin growth. The growth becomes relatively heavy, however, if the cultures are incubated under a reduced oxygen tension or under complete anaerobiosis. These organisms are more hardy than the obligate anaerobes mentioned above, which sometimes fail to grow after the second or third subculture. The facultative anaerobic lactobacilli are not so numerous as *L. bifidus*, the average (geometric mean) plate count being 21 million per gram. About 40 cultures have been isolated and studied. These include both homofermentative and heterofermentative species. The identity of these lactobacilli, some of which deviate from the types described in the literature, has been made the object of a special study to be reported in a subsequent paper.

The plate counts of the different bacterial types are listed in table 3. The last 9 samples of cecal feces studied are tabulated. The counts (as billions per gram

of raw feces) were determined by multiplying the number of the bacterial type in question with the reciprocal of the dilution of the plate from which it was isolated. It is apparent that some types, for instance the micrococci, vary greatly in numbers. Thirty billion per gram were encountered on one feces, but none were isolated from 4 of the samples. Likewise, anaerobic streptococci gave extremely high counts in some samples, whereas none were isolated from several turkeys. On the other hand, anaerobic lactobacilli were isolated from every feces, and in amounts that do not vary appreciably from one sample to another. The gram-negative rods, although not so numerous, are also fairly constant as regards plate counts.

The anaerobic lactobacilli are the predominant bacterial type found, making up, on the average, about 50 per cent of the total cultivatable flora. Gram-positive, nonsporeforming rods other than lactobacilli are the next in predominance, comprising, on the average, 19 per cent of the flora. The coliforms and "enterococci" are at the other end of the scale; except for 1 sample, each made up less than 1 per cent of the cecal flora of the turkeys tested.

TABLE 3

Plate counts of the different bacterial types found in the cecal feces of healthy turkeys
(Counts are in billions of organisms per gram sample)

FLOCK NUMBER	SAMPLE NUMBER	AEROBIC AND FACULTATIVE ANAEROBIC MICROCOCCI	FACULTATIVE ANAEROBIC STREPTOCOCCI	GRAM-NEGATIVE RODS	FACULTATIVE ANAEROBIC LACTOBACILLI	ANAEROBIC STREPTOCOCCI	GRAM-POS., NONSPOREFORMING RODS OTHER THAN LACTOBACILLI	ANAEROBIC LACTOBACILLI
1	4	2	30	0.02	0.5	10	1	10
	7	0.001	0.02	0.1	0.2	10	2	10
	12	0 (<0.001)	0.001	0.001	0.1	1	2	2
2	20	0.1	0 (<0.00001)	0.001	0.03	2	1	4
	21	30	0.0002	0.01	0.07	3	10	10
	24	0 (<0.001)	0.002	0.001	0.0002	0 (<0.1)	1	4
3	28	0.001	0.0002	0.003	0.004	0 (<0.1)	1	3
	29	0 (<0.001)	0.0006	0.002	0.008	1	2	5
	30	0 (<0.001)	0 (<0.00001)	0.026	0.005	0.4	0.2	0.7

SUMMARY

Cecal feces have been studied in some detail in order to determine the bacterial flora of the ceca of healthy turkeys. Twelve fecal samples, each from a different bird, have been evaluated, 3 flocks being represented. The flora of birds of different flocks was found to vary in no greater degree than the flora of birds of the same flock.

Seven different media were employed for the isolation of cultures. Trypticase soy glucose agar plates incubated anaerobically gave the highest counts of the various media used. The plate counts on this medium averaged about 13 billion per gram sample.

Members of the following 12 genera have been identified: *Bacillus*, *Bacterium*, *Borrelia*, *Corynebacterium*, *Escherichia*, *Lactobacillus*, *Micrococcus*, *Paracolobactrum*, *Pediococcus*, *Sarcina*, *Spirillum* and *Streptococcus*. More than 20 species have been isolated. The great bulk of the bacterial population, however, is composed of but few genera and species.

Anaerobic lactobacilli (the *Lactobacillus bifidus* type) are the most numerous bacteria in the cecal feces of the turkeys tested, making up about 50 per cent of the total cultivable flora. (Facultative anaerobic lactobacilli are also present, but these exist in relatively small numbers.) Gram-positive, nonsporeforming rods other than lactobacilli and anaerobic streptococci are the next in predominance. Members of the genus *Micrococcus* were found to be the most variable; some feces contained them in large numbers whereas they were never recovered from many of the turkeys. Coliforms and "enterococci" exist in relatively small numbers, each usually comprising less than 1 per cent of the flora. It is apparent from the above that in the cecal flora anaerobic bacteria are in the majority, and that almost the entire flora is composed of gram-positive organisms.

REFERENCES

BREED, R. S., MURRAY, E. G. D., AND HITCHENS, A. P. 1948 Bergey's manual of determinative bacteriology. 6th ed. Williams & Wilkins Co., Baltimore, Md.

CRECELIUS, H. G., AND RETTGER, L. F. 1943 Intestinal flora of the guinea pig. J. Bact., **46**, 1–13.

EGGERTH, A. H., AND GAGNON, B. H. 1933 The *Bacteroides* of human feces. J. Bact., **25**, 389–413.

EMMEL, M. W. 1930 Bacterial flora in feces of the normal fowl. J. Infectious Diseases, **46**, 293–297.

GAGE, G. E. 1911 A study showing bacteria and animal organisms determined in the feces and intestinal mucosa of healthy chickens. Maryland Agr. Expt. Sta., Bull. **153**, 201–226.

JOHANSSON, K. R., SARLES, W. B., AND SHAPIRO, S. K. 1948 The intestinal microflora of hens as influenced by various carbohydrates in a biotin-deficient ration. J. Bact., **56**, 619–634.

KING, W. E. 1905 The bacterial flora of the intestinal mucosa and conjunctiva of the normal chicken. Am. Med., **10**, 400–404.

KUCEL, J. 1934 Aerobni mikroorganizmi u crijevu zdravih pura. Veterinarski Archiv (Zagreb), **4**, 335–341.

Manual of methods for pure culture study of bacteria. 1945 Committee on bacteriological technique of the Society of American Bacteriologists. Biotech Pub., Geneva, N. Y.

MARSDEN, S. J., AND MARTIN, J. H. 1939 Turkey management. 1st ed. Interstate Press, Danville, Ill.

MENES, E., AND ROCHLIN, E. 1929 Darmmikroflora des Hausgeflügels. Zentr. Bakt. Parasitenk., I, Orig., **113**, 321–324.

ORLA-JENSEN, S. 1919 The lactic acid bacteria. Mém. acad. roy. sci. Danemark, Sect. sci., 8 ser., **5**, 81–197.

ORLA-JENSEN, S. 1943 The lactic acid bacteria. Ergänzungsband. Mém acad. roy. sci. Danemark, Sect. sci. biol., **2**, (3), 1–145.

ORLA-JENSEN, S., ORLA-JENSEN, A. D., AND WINTHER, O. 1936 *Bacterium bifidum* and *Thermobacterium intestinale*. Zentr. Bakt. Parasitenk., II, **93**, 321–343.

PELCZAR, M. J., JR., AND VERA, H. D. 1949 An improved medium for the enumeration of bacteria in milk and milk products. Milk Plant Monthly, **38**, 30–33.

RAHNER, R. 1901 Bakteriologische Mitteilungen über die Darmbakterien der Hühner. Zentr. Bakt. Parasitenk., I, Orig., **30**, 239–244.

SHERMAN, J. M. 1937 The streptococci. Bact. Revs., **1**, 3–97.

SHERMAN, J. M., MAUER, J. C., AND STARK, P. 1937 *Streptococcus fecalis*. J. Bact., **33**, 275–282.

SHERMAN, J. M., AND WING, H. U. 1937 *Streptococcus durans* n. sp. J. Dairy Sci., **20**, 165–167.

SMITH, N. R., GORDON, R. E., AND CLARK, F. E. 1946 Aerobic mesophilic sporeforming bacteria. U. S. Dept. Agr., Misc. Pub. 559.

WEISS, J. E., AND SPAULDING, E. H. 1937 A simple method for obtaining effective anaerobiosis. J. Lab. Clin. Med., **22**, 726–728.

WINTER, C. E., AND SANDHOLZER, L. A. 1946 Recommended procedure for detecting the presence of enterococci. U. S. Dept. Interior, Fish and Wildlife Service, Commerical Fisheries TL 2.

ZARETT, A. J., AND DOETSCH, R. N. 1949 A new selective medium for the quantitative determination of members of the genus *Proteus* in milk. J. Bact., **57**, 266.