POPULATION STUDIES ON FIVE GENERA OF INTESTINAL BACTERIA OF SWINE

During Normal Conditions of Health

During Artificially Induced Hog Cholera

by

Mohan Singh Purohit

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Bacteriology and Public Health

1953

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AN ABSTRACT

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Approved W. J. Stapleton

Population studies of five genera of intestinal bacteria of swine during health and artificially induced hog cholera were made. During health, the lactobacilli were highest in number from six to seven weeks of age. The number of lactobacilli decreased in healthy pigs from eight weeks of age upward. There was a dramatic decrease or disappearance of lactobacilli from the intestinal tract once the animals had developed clinical symptoms of hog cholera.

The coliforms, which are usually regarded as harmless parasites, were least numerous during health irrespective of age differences. The average number of coliforms ranged between 13 to 37 millions per gram of fecal material. The number of coliforms increased many times once the animals under experiment became sick and developed diarrhea.

The number of enterococci was higher than that of the coliforms but lower than that of the lactobacilli up to seven weeks of age during health. In pigs from the age of eight weeks upward the enterococci were most numerous. The average number of enterococci was 42 to 84 millions per gram of fecal material. There were no significant differences between their numbers during infection. Salmonella choleraesuis was isolated from healthy as well as diseased swine. During the entire period of experiment S. choleraesuis was isolated seven times.

S. cubana was isolated from the intestinal tract of infected pigs for the first time (3 cases).

Among sixty-four cultures of enterococci isolated from fecal samples the predominant group was Streptococcus fecalis.

DEDICATED TO

Dr. H. J. Stafseth, an inspiring teacher and sincere friend, with an ideal philosophy for directing graduate studies.

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During Normal Conditions of Health

During Artificially Induced Hog Cholera

Outline of Studies

Major subject: Veterinary Bacteriology
Minor subjects: Surgery and Medicine, Animal Pathology

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INTRODUCTION

The importance of the bacterial flora of the intestines has long been recognized. It has been suggested that animal life would be impossible without the micro-organisms found in the digestive tract.

In order to gain an understanding of the role of the microflora of the gut, Schottelius (1), in 1899, raised normal and germ-free chickens on diets which today would be considered totally inadequate. The slow growth and abnormalities of the chicks were such that he concluded that normal chicks could not be raised germ-free. Further, he came to the conclusion that the function of the normal nonpathogenic flora was to aid in preparing ingesta for absorption, to stimulate the intestinal wall and to promote peristalsis, to oppose the establishment of the pathogens, and to aid in defense of the host.

Cohendy (2), in 1912, reported his results on germ-free work. His diets were protein free and vitamin deficient. He was unable to rear sterile chicks beyond five weeks. Neither Cohendy nor Schottelius was concerned with the systematic classification of the bacteria. In their studies quantitative assays were not made.

Creekmur (3), in 1922, studied the intestinal flora of rats on normal diets and on one deficient in vitamin A. The

feces of animals on deficient diet became dry and hard with distinct decrease in the total number of bacteria. The addition of vitamin A in the form of a few drops of cod liver oil daily produced normal conditions.

In germ-free studies the workers did not attempt to classify or enumerate the bacteria of the intestinal tract. Enrichment and selective media, by which these workers could have broken down these groups of intestinal bacteria, were not available.

As the intestinal bacteria play a significant role in health and disease, the writer took the opportunity to study these micro-organisms in swine under normal condition of health and in animals artificially challenged with hog cholera virus. The purpose of the study was to determine which of the intestinal organisms (coliforms, enteric streptococci, lactobacilli, or salmonellae) increase or decrease in numbers while the animal is suffering from hog cholera.

HISTORICAL REVIEW

Relation of bacteria to life. This has been a much debated question. Nuttal and Thierfelder (4), in 1895, (5) 1896, and (6) 1897, reared guinea pigs which had been delivered by Caesarian section under aseptic conditions. The animals increased in weight, but not as regularly as normal pigs. From these data the authors concluded that bacteria were not necessary for normal development.

Levin (7), in 1899, found that the intestines of most of the arctic animals were sterile. He drew no conclusions, but his work is of interest because normal growth took place without bacteria. Wollman (8) concluded from the experiment on flies that bacteria were not necessary for normal development.

Kianzine (9), in 1916, produced data concerning germ-free chicks which he thought weakened the argument of Cohendy. Kianzine believed that bacteria were of great aid in digestion; they carry on analytic and synthetic processes which are of much value to the host. He observed that when guinea pigs were made to breathe sterile air and eat sterile food they were greatly weakened, even after a few days. The bad results from the deprivation of bacteria were thought to be due to reduced oxidation and accumulation of leucomains in the

body. Loeb and Northrup (10) attempted to determine the ability of flies to synthesize their body proteins without the aid of micro-organisms. Larvae grown on sterile media (banana) did not do well, while those placed on sterile yeast culture developed normally. It was thought that yeast was essential for this species of fly.

Studies of different microflorae. The earliest studies of the flora of different animals and birds were not quantitative in the sense that there were no tallies of populations of different types; e.g., coliforms, enteric streptococci, aerobes and anaerobes. The enrichment and differential media available today had not been developed.

Gage (11), in 1911, studied the intestinal flora of chickens. He found that Nocardia asteroides, Bacillus mycoides, Bacillus subtilis, Micrococcus, pseudomonads, and Escherichia coli predominated, but there was variation due to environmental conditions and the age of the chickens. He also estimated that 60 percent of the bacteria in the intestinal tract of the chickens were gram negative in their staining characteristic. Some difficulty was encountered in distinguishing bacteria from debris, much of which was gram positive.

While working with rats, Hull and Rettger (12), in 1917, and Torry (13), in 1919, established beyond doubt a relationship between the type of flora predominating in the intestinal tract and the type of food ingested. Mens and

Rochlin (14), in 1929, while working with chickens, found the same bacterial species in all parts of the intestine, the difference being in quantity. This uniformity of the flora was explained on the basis of acid production which suppressed putrefactive bacteria and their associated decomposition processes. The flora producing lactic acid was: Streptococcus fecalis, Escherichia coli, and Lactobacillus plantarum, the latter being the most prominent anaerobe. Emmel (15), in 1930, found a decrease in the number of E. coli in 20 chickens suffering from enteritis. Salmonella pullorum was invariably in the intestines of the chickens suffering from pullorum disease, fowl typhoid, and paratyphoid.

In their work on the study of the influence of diet on the distribution of bacteria in the various parts of the gastrointestinal canal of rats fed on stock diet, Porter and Rettger (16), in 1940, showed that, whereas the normal bacterial flora of the young white rats is comparatively simple in the upper segments of the alimentary tract, it becomes increasingly more abundant in the lower sections of the small intestine.

Windblad (17), in 1941, and Mitchell and Isbell (18), in 1942, while working on rats, showed a relationship between the type of micro-organisms predominating in the intestinal tract and the type of diet given. During a study of the bacterial flora of the feces of rats, Miller (19), in 1945, observed that the number of organisms present varied from rat to

rat and in the same animal from day to day. McGinnis (20), in 1950, stated that growth acceleration in chicks, when antibiotics are fed, might be a result of either: 1) reduction in the total number of bacteria in the intestinal tract; 2) synthesis of vitamins by bacteria; 3) the inhibition of either pathogenic or toxin producing bacteria. Groschke (21), in 1950, suggested that antibiotics stimulate the growth in chicks by changing the intestinal microflora from undesirable to desirable ones. Couch et al. (22), in 1950, reported an increase in the total count of bacteria in the feces of chicks when penicillin was included in the ration. Wahlstorm et al. (23), in 1951, reported that penicillin had no significant effect on the number of coliform bacteria present in the feces of baby pigs fed a "synthetic" milk diet.

Coliform Group in Health and Disease

Escherichia and Aerobacter

Escherichia coli and Aerobacter aerogenes were isolated by Escherich from the feces of infants and described in 1886 (24). Since that time the organisms have been found in the intestinal tract of all vertebrates.

Hadley (25), in 1910, isolated B. coli from the liver and heart blood of sick chickens affected with a cholera-like disease. High temperature and yellowish diarrhea were the

symptoms observed upon examination; the liver was hyperemic and soft and the spleen enlarged and hemorrhagic. The inflammation extended through the entire intestine.

Johnes (26), in 1918, studied three cases of mastitis from two of which E. coli was isolated and from the third A. aerogenes was cultured. In two of the cases the milk contained numerous white floccules and in the third it had the appearance of hemolysed blood.

Gaiger and Dalling (27), in 1921, reported that lamb dysentery is caused by a B. coli type which invades the body from the intestines. Palmer and Baker (28), in 1922, found that Bacterium coli communis was the causative organism of an infectious enteritis of chickens, ducks, and turkeys in northern and central Delaware. Gaiger and Dalling (29), in 1923, reported that lamb dysentery is caused by an anaerobe of the Clostridium welchii type and a coli type conjointly.

Kinsley (30), in 1928, described B. coli communis, B. suipestifer, and other closely related bacteria commonly found in scours in pigs. He assumed that these enteric organisms had attained pathogenicity. Tunicliff et al. (31), in 1932, were able to produce fatal dysentery in lambs by feeding the cultures of several varieties of Escherichia, although several strains failed to produce dysentery. From this observation they came to the conclusion that "under favorable conditions certain strains of bacteria, principally Escherichia which are normally present in the intestines may produce disease".

Davis (32), in 1938, encountered colibacillosis in chickens in Maryland. Upon autopsy the livers were about 50 percent larger than normal, mottled, and contained numerous small white spots. The heart was slightly enlarged and the pericardial sac was markedly distended with amber colored fluid. Twisselmann (33), in 1939, reported that seventeen birds of a well isolated group of 250 pullets were attacked by a rapidly fatal disease of which he stated E. coli was the etiological agent. Bunyea and MacDonald (34), in 1941, studied the pathogenecity of E. coli for turkeys and considered it responsible for young poult mortality.

Murphy (35), in 1943, described 70 cases of bovine mammary gland infection where coliform organisms were the chief invaders. For three years a study of milk and udders of a herd of about 120 Guernsey and Holstein cattle was made. The infections were observed from one day to twenty-two months and the degree of irritation ranged from negligible to severe acute mastitis. Sixty-nine of the infecting organisms were studied and the results were as follows: 41 (59.4 percent) were A. aerogenes type; 18 (26.1 percent) were intermediate types; and 10 (14.5 percent) were E. coli types.

Hjarre and Warmby (36), in 1945, described a specific granuloma-like condition in chickens which was found along the intestinal tract. The lesion was considered to be a typical form of tuberculosis, but a capsulated B. coli was the only organism isolated. The investigators subsequently found that

such an organism is the etiological agent. According to Udall (37), in 1947, it was recognized that various influences such as season, sanitation, and exposure contribute to the cause of diarrhea. Great importance was attached to the action of bacteria which are normally present in the lower intestine, especially B. coli. Osborne, Witter, and Hitchner (38), in 1947, studied chronic colibacillosis of fowls. The organism which they studied was proved to be very pathogenic for guinea pigs, rabbits, and chickens. Durant and MacDougle (39), in 1947, found E. coli in the blood of adult fowl affected with an occular form of fowl paralysis. When 1 ml amounts of the blood taken from the affected birds were injected into day old chicks, 84 of 85 chicks died.

Hagen (40), in 1951, stated that the organisms of the colibacillus type are generally regarded as causative agents of the disease commonly called calf scours. It is a known fact that the tissues of animals suffering from this disease are teeming with E. coli shortly before and after death. He further stated:

> Bacterium coli is a normal inhabitant of the lower bowels of warm blooded animals. It usually is absent from the intestines of fish and other cold blooded animals. Few or none are found in the stomach or anterior part of the bowel. Carnivora and omnivora usually harbor the organism in greater abundance than herbivora. The feces of cows and horses frequently show very few.

Enterococci in Health and Disease

The term enterococcus has long been used by French workers to designate a group of streptococci that normally occurs in the intestine of man. The most important of these organisms is Streptococcus fecalis. It is known that this organism occurs in the intestines of several domestic animals as well as of man. Enterococci occasionally have been found in pathogenic processes but generally are looked upon as harmless to man and animals. They are characterized by great hardiness.

Initial isolation. Since its use by Thiercelin (41) in 1899 and (42) in 1902, the term Enterococcus has had somewhat variable and hazy meanings. In some cases the name has been applied rather specifically as a synonym for Streptococcus fecalis. Thiercelin's findings led him to believe that enterococci are causal agents in certain diarrheal biliary infections and the cause of appendicitis. MacCullum and Hastings (43), in 1899, studied a case of acute endocarditis and isolated a new species which they named Micrococcus zymogenes and described it as follows:

> This micrococcus is very small, occurs mainly in pairs, sometimes in short chains, gram positive, liquefies gelatin slowly and is specially characterized by its behavior in milk which it acidifies, coagulates and liquefies. It produces milk curdling ferments, each of which is separable from the bacterial cells. It remains viable for months in old cultures and is tolerably resistant to the action of heat and antiseptics. The micrococcus is pathogenic for mice and rabbits causing abscesses or general infections.

Streptococcus fecalis was named by Andrews and Horder (44), in 1906, who gave an excellent description of the organism in view of the means for studying bacteria then at hand. The organism was described as nonhemolytic, having a strong reducing action on neutral red, coagulating milk and fermenting the dissacharides, salicin and mannitol, but not inulin and usually not raffinose. The fermentation of mannitol was considered very characteristic.

The work of Andrews and Horder on intestinal streptococci was verified by a number of subsequent workers. Winslow and Palmer (45), in 1910; Fuller and Armstrong (46), in 1913; and Broadhurst (47), in 1915.

According to Donaldson (48), in 1917, the French workers thought the enterococci to be constant inhabitants of normal intestines and commonly found in the small intestines of infants three days after birth. Further it was observed that the enterococcus was pathogenic for rabbits. Oppenheim (49), in 1920, studied the human fecal streptococci and observed that the mannite fermenting, nonhemolytic ones were the characteristic predominant types found in the feces of normal individuals. Hemolytic streptococci were found only occasionally in the stools of normal, healthy people.

Ayers and Johnson (50), in 1924, examined 33 human fecal cultures and found that the predominant species was Str. fecalis. Sherman and Wing (51), in 1935 and 1937, described a new species of hemolytic streptococcus which was tolerant

to heat and desiccation. On the basis of its physiological characteristics, Streptococcus durans was related by these workers to the enterococci, though it was not known to be of intestinal origin. Recent studies of the hemolytic streptococci of the human intestine by Sherman (52) have shown that this organism commonly occurs in human feces. Although a number of species of hemolytic streptococci may be isolated from human feces, so far as present information extends, Str. durans and Str. zymogenes are the only intestinal types which may be properly designated as "hemolytic enterococci".

Common Habitat of Enterococci

Brige (53), in 1905, isolated a micrococcus from laboratory animals corresponding morphologically and culturally to that isolated by MacCullum and Hastings in 1899, with the exception that this organism was nonpathogenic for laboratory animals. Andrews (54), in 1906, stated that streptococci cannot grow and multiply for any length of time outside the human body. Broadhurst (55), in 1915, was of the opinion that streptococci occurred less commonly in soils and water than most of the literature of that time implied. Alston (56), in 1928, studied 50 strains of streptococci from the alimentary tract of man, dog, and rat. Thirty-five percent of them were described as streptococci, oval in shape, and occurring in pairs or short chains, nonhemolytic and capable of fermenting manni-

tol. Smith and Sherman (57), in 1938, studied the hemolytic streptococci of human feces and found that the commonest hemolytic streptococcus of the human intestine is Str. zymogenes, with Str. durans following next. Porch (58), in 1941, demonstrated that the enterococci were the predominant group of streptococci recovered from the genitourinary tract. Winter and Sanhozer (59), in 1946, reported that, while streptococci were present in all samples of human and animal feces tested, these organisms were never found in virgin soils or soils from wooded areas. Mallmann and Litsky (60), in 1951, using dextrose azide broth as enrichment medium, could not isolate enterococci from soils which were not treated with sewage. They also stated that other than coliform organisms, the enterococci were the only organisms found in sewage that could be used as indicators of fecal pollution.

Salmonella Choleraesuis in Health and Disease

This organism was isolated and described by Salmon and Smith (61) in 1885 and was the first of the paratyphoid organisms to be recognized. It is because of this fact that these organisms are known under the generic name of Salmonella. The authors believed the organisms to be the cause of the destructive and prevalent disease known as hog cholera, but later work demonstrated that this is not the case, hog cholera being caused by a filtrable virus. Nevertheless, this organ-

ism plays an important role in porcine pathology, principally as a secondary invader in virus diseases.

Trawinski (62), in 1917, made bacteriologic examinations of the intestinal contents of 500 butchered hogs and isolated not less than 26 strains of microbes which apparently could be classified as colon-typhoid intermediates. Dorset (63), in 1921, stated that the presence of the virus of hog cholera vastly increases the susceptibility of hogs to suipestifer infection. Moussu (64), in 1924, believed that the causative microbes live in a given environment as saprophytes and as such may exist in the digestive tract of swine. Faulty sanitary conditions, indigestion, irritation of the digestive organs by feed of inferior quality, or even slight intoxications are apt to reduce the normal defensive powers of the body to such an extent that the infective agent begins to multiply in profusion and becomes progressively more virulent with an infectious enteritis as a result.

Glässer (65), in 1927, recognized damp, stuffy, dirty stables, faulty feeding, deficiency of minerals and protein in the ration, lack of paddocks and atmospheric influences, such as excessive humidity, as important predisposing factors for Salmonella infection.

That the hog cholera bacillus is not constantly associated with hog cholera was also shown by Uhlenhut and associates (66), in 1929, when they made careful bacteriological examination of 178 cases of hog cholera among which S. sui-

pestifer could be demonstrated in 76 animals. Birch et al. (67), in 1937, and Chick et al. (68), in 1938, did not believe that all swine enteritis is caused by the invasion of salmonellae, but the nutrition of the pig plays a very important part in the etiology of this disease. These workers found that severe deficiency could be produced in young pigs fed a low protein diet made up largely of corn, and that the deficiency could be prevented by the addition of niacin.

Dale (69), in 1942, stated that although normal hogs harbor this organism, it has been found in a sufficient number to indicate that there may be a carrier type in which the microbe is present without causing appreciable injury.

Stafseth, Lisa Neu, and Sholl (70), in 1944, isolated S. choleraesuis from a pup which had died. Cultures were made from the heart, liver, spleen, and kidneys. From all these sources almost pure cultures of a Salmonella-like organism were obtained. Studies of morphology, staining, cultural and biochemical characteristics of this organism indicated that it was S. choleraesuis, variety Kunzendorf. They were also able to isolate the same organism from the vaginal swab obtained from the mother of the pup.

Schofield (71), in 1944, stated:

> In making a diagnosis of salmonellosis based upon the constant isolation of salmonellae, it is important to remember that S. choleraesuis and other members of this group are not infrequently present in the intestine of normal swine; that such animals when attacked by a virus septicaemia, as in hog cholera, may suffer severe intestinal damage from activated and virulent

> salmonellae. It is quite possible, in fact already recognized by some investigators, that in other enteric diseases such as swine dysentery, salmonellae may appear either as secondary invaders or proliferate due to alteration in environment.

In hogs, necrotic enteritis was produced by Rasmussen, et al. (72), in 1944, by administering S. choleraesuis. A more severe reaction was secured when the culture was mixed in the feed than when given as a drench. Difference in virulence of the culture could have been responsible for the difference in reaction, but this reason is unlikely according to these authors. Many hogs died from the initial attack of the disease regardless of nutritional regimen, but during the recovery period nicotinic acid and some principle or principles present in liver, but not in sufficient quantities in yeast, were of benefit by promoting increased weight gains and decreasing the length of the recovery period. The authors believed that a composite of known B vitamins may give better response than nicotinic acid alone in the recovery phase of the disease.

Schofield, loc. cit., in 1944, further came to the conclusion that S. choleraesuis is responsible for damage to the mucous membrane which later is frequently invaded by the normal inhabitant of the intestine, Actinomyces necrophorous. The pigs between the ages of eight to sixteen weeks appear to be particularly susceptible to this infection. Though defective nutrition may be a factor in predisposing to infection, poor sanitation and overcrowding are conducive to rapid spread of infection.

Doyle and Walkey (73), in 1946, proved experimentally that feeding of viscera from pigs affected with what was known as "necrotic enteritis" to cholera vaccinated shoats produced no clinical symptoms of disease. However, when parts of the same viscera were fed to cholera susceptible pigs, one-half of them showed lesions of hog cholera and enteritis. This would indicate that the virus of hog cholera produced enteritis. They also concluded that enteritis resulting from hog cholera was different from that produced by salmonellae. Lucke and associates (74), in 1947, and (75) in 1948, showed that the level of protein in the ration was directly related to the severity of the experimentally produced niacin deficiency. Pigs fed a ration containing only 15 percent protein developed severe deficiency symptoms, whereas those fed a ration containing 19 percent protein developed only mild symptoms of the disease. In much of the work involving the relationship of niacin to necrotic enteritis the procedure was to feed large doses of S. choleraesuis in culture broth to the pigs. It was observed that niacin had little effect in either preventing or curing the resulting enteritis.

Edwards and his associates (76), in 1948, discussed the finding of Salmonella types in swine and stated that "in the writer's experience salmonellae are much more likely to be isolated from cases of acute enteritis than from those with chronic necrotic enteritis". Lucke and his co-workers (77), in 1949, fed the pigs intestinal material from which Salmonella

newport and Salmonella typhimurium had been isolated. The animals showed no particular temperature rise nor diarrhea, while a pig in another group when fed S. choleraesuis showed temperature rise to 105° F for one day and failure to eat. The pig returned to normal after 72 hours.

Lactobacilli in Health and Disease

The genus Lactobacillus constitutes one of the most widely distributed groups of known micro-organisms. The group as a whole is primarily fermentive in character, and displays a marked degree of tolerance for acids. Members of this genus vary widely in their physiological activities and in their habitat. Some are characteristically associated with milk and dairy products; others are intimately associated with raw cereals and vegetables; and others are of oral and dental origin. Still others are primarily intestinal types. The intestinal types constitute the material for the present study. The genus Lactobacillus is grouped by Bergy and co-workers (78) in the family Lactobacteriaceae which includes the streptococci and the pneumococci. Bergey lists about 15 species, only two of which are parasitic, Lactobacillus acidophilus and Lactobacillus bifidus. The lactobacilli have not been successfully indicated as pathogens within the customary meaning of that term. On the contrary, they have been praised for beneficial effects in the vagina and intestine of human hosts.

Döderlein (79), in 1892, reported that vaginal secretion of many pregnant women contained a group of micro-organisms consisting of large numbers of bacilli and some yeasts. He also found that in women whose vaginal secretion was less acid the flora was more varied and there was a higher incidence of puerperal disease. He thought that lactic acid produced by vaginal microbes limited the growth of other micro-organisms in the vagina and thus protected the area against infection.

Moro (80), in 1900, isolated an organism from the feces of breast fed infants to which he gave the name Bacillus acidophilus. Moro's organism was, as he supposed, derived from the mother's breast and was found in the mouths and stomachs of infants as well as in the intestinal contents. He stated that it was not a single species but a group of closely related forms which preferred an acid medium for growth.

Weiss (81), in 1904, noted that when large quantities of milk were ingested a marked increase in organisms of the lactobacillus type was observed. Tissier (82), in 1908, in a study of the intestinal flora of infants observed that, at the age of about three days, breast fed babies developed an intestinal flora in which one organism was predominant. This organism had, in general, the staining reactions and morphological appearance of the lactobacilli. As Tissier noted the presence of many bifurcated forms, he called it Bacillus bifidus. There was a considerable controversy between Tissier and Moro as to the predominant organism in breast fed babies

and it was finally settled by Moro's admission that L. bifidus occurred in far greater numbers. However, both investigators concluded that L. acidophilus became predominant in the feces of babies whose chief food was cow's milk.

Rettger and associates (83) showed that the feeding of milk or lactose in large amounts to white rats and human subjects brings about the transformation of the intestinal flora to one in which the lactobacilli predominate. Morishita (84), in 1929, Curran et al. (85), in 1933, and Gillespie and Rettger (86), in 1938, suggested that oral lactobacilli are distinct from the intestinal ones, whereas Sherman et al. (87), in 1940, were unable to distinguish the oral lactobacilli from intestinal types.

Infection with lactobacilli. Howitt (88), in 1930, reported that in rabbits the intravenous injection of large doses of lactobacilli was followed by development of joint lesions with extensive mucopurulent exudate containing numerous polymorphonuclear leucocytes and macrophages, and occasional gram positive rods. Jay et al. (89), in 1932, noted the development of sterile abscesses following subcutaneous inoculations of lactobacillus vaccines in both human subjects and rabbits.

EXPERIMENTAL PROCEDURE

Media used. In the choice of media, it was thought desirable to have a set of media in which the determination could be made under identical conditions. Considering the large number of organisms to be estimated, a dilution count method was deemed most practical and was employed for nearly all organisms except salmonellae and lactobacilli. For salmonellae, different selective media were used to observe frequency of presence in the healthy and virus challenged pigs. For lactobacilli, the pour plate method was adopted. It was considered necessary that one medium give a total count of the viable bacteria, and that the selective media allow estimation of the relative numbers of each of the individual groups of bacteria.

The total count. The total count of aerobes was made by means of several media. Evenson (90), in 1946, studying the rodent flora, used a liver infusion broth with a chunk of liver settled at the bottom to permit the anaerobes to grow also. Dhanda (91), in 1947, and McClure (92), in 1949, while studying the intestinal flora of rats, used the same medium. Tryptose glucose yeast extract agar (T.G.E.) was employed by Shapiro and Sarles (93), in 1949. Elam and Gee (94), in 1951, used liver infusion broth for total bacterial (aerobic) count.

The writer found that tryptose glucose yeast extract agar with fresh liver extract added to it was more suitable. The liver extract enhanced the growth so the total number of organisms was higher.

Media for Coliform Organisms (*Escherichia coli* and *Aerobacter aerogenes*)

Coliform bacteria are of historic importance, particularly because they were reported by early workers as being the dominant flora in the gut and also because many animal nutrition studies suggested that the suppression of coliform bacteria was related to vitamin deficiencies. Bile or its salts were extensively used because of their marked selectivity for growth of coliform organisms. Jackson (95), in 1907, recommended the use of lactose broth containing bile for elimination of noncoliform gas producers. MacConkey (96), in 1908, used bile salt agar with neutral red, while Rector (97), in 1913, used dried whole bile. It was found, however, that the concentration necessary for the desired inhibition of gram positive organisms was somewhat inhibitory to the coliforms as well. Domnik and Lauter (98), in 1929, used a methylene blue-brom creosol purple combination as a confirmation for coliform organisms in water. Stark (99), in 1936, evaluated the use of formate ricinoleate broth for the detection of coliform organisms in milk. Mallmann and Darby (100) in

their studies on the detection of the coliform organisms in water used lauryl tryptose broth. They also found that eosin methylene blue agar and brilliant green bile broth could not be used if it was desired to confirm all positive presumptive tubes which contained coliforms. Evidence to support their statement came from the fact that they were able, in many cases, to isolate coliform organisms from lauryl tryptose positive presumptive tubes which they had failed to confirm by the use of eosin methylene blue agar or brilliant green bile broth.

By early workers, the E. coli medium of Hajna and Perry (101) was used as a selective medium for the coliform group. It has been reported that the bile salts in this medium inhibit the growth of fecal streptococci and spore formers. Faulkner (102) showed that the female hormones diethylstilbestrol, hetoestrol, and stilboestrol are much more selectively inhibitory for gram positive than for gram negative organisms. Chapman (103) discussed the remarkable selectivity of sodium alkyl sulfate (called Tergitol 7). He claimed that the only gram negative organisms inhibited by this substance were the members of the genus Proteus. According to Naghski, et al. (104), quercentin is inhibitory to certain gram positive organisms at a concentration of 0.075 to 0.1 mg per ml, the action being much less on gram negative group.

Rittenberg and Silliker (105), in testing penicillin against several organisms, found that coliform organisms could

withstand 50 units per ml, while Cl. welchii and enteric streptococci were inhibited at 30 units.

Vaughn et al. (106) described a buffered boric acid lactose medium for enrichment and identification of E. coli.

The writer used the lauryl tryptose broth prepared according to the formula of Mallmann and Darby, loc. cit., as a presumptive test medium to determine the presence of coliform organisms.

Medium for Enterococci

Bagger (107), in 1926, used sterile ox-bile with one percent peptone to grow fecal streptococci. Fleming (108), in 1932, reported that fecal streptococci will grow in a concentration of 1:15,000 of potassium tellurite which is inhibitory to gram negative bacteria. Mallmann (109), in 1940, reported on a medium containing sodium azide which was found useful in estimating the number of streptococci in sewage as it was found to support the growth of these bacteria while inhibiting the coliform group. Hajna and Perry, loc. cit., published the formula for another selective medium which was almost an exact duplicate of the medium suggested by Mallmann; but the former workers used an incubation temperature of 45° C for growth and the production of acid in this medium was stated to be almost complete evidence of the presence of Str. fecalis.

Chapman (110), in 1946, perfected a medium for the isolation of fecal streptococci. This is the mitis-salivarius agar. Enterococci produce a dark blue or black, slightly raised colony about 1 mm in diameter.

The writer chose azide-dextrose medium (Difco), which is primarily a result of the studies of Mallmann and Seligman (111) in 1950. For further confirmation, ethyl violet azide broth, developed by Mallmann and his associates (112) in 1952, was used. For a complete test for enterococci, brain heart infusion agar, brain heart infusion broth (incubated at 45° C), litmus milk, and tryptose phosphate broth with 6.5 percent Nacl were used.

Media for Isolation of Salmonella

For isolation of Salmonella, the following media were used: For the purpose of enrichment, the media most favored are sodium tetrathionate broth and selenite broth. The writer favored the use of sodium tetrathionate broth. The credit for discovering the usefulness of a tetrathionate broth for enriching typhoid and the paratyphoid organisms is ascribed to Mueller (113) who demonstrated clearly that it inhibited coliforms and permitted the paratyphoids to grow. Further demonstrations of the usefulness of this medium were made by Johnes (114), in 1936, and Ruys (115), in 1940. After 12-16 hours of incubation of the fecal sample in tetrathionate

broth, MacConkey's agar, and Shigella Salmonella agar (S. S. agar), plates were streaked.

S. S. agar. This medium was developed by Difco Laboratories to provide differentiation of the lactose fermenters from lactose nonfermenters and to give the maximum growth to coliform organisms without restriction of the growth of the pathogenic gram negative bacilli. Shigella, Salmonella, and other organisms not fermenting lactose form opaque, transparent and translucent uncolored colonies which generally are smooth. Lactose fermenters which are not inhibited are generally recognized by the formation of red color in the colony. Some coliforms do not show definite red color, being pink or nearly colorless. Some proteus and salmonella types produce black centered colonies.

Bismuth sulfite agar. The early history of the development of bismuth sulfite agar may be found in the reports of Wilson and Blair (116), in 1926. These workers clearly demonstrated the superiority of this type of medium over other media in the isolation of S. typhosa from feces, urine, and sewage. Cope and Kasper (117), in 1937, increased their findings of typhoid organisms from 1.2 to 16.8 percent among food handlers. Green and Beard (118), in 1938, used successfully bismuth sulfite agar in their studies on the survival of E. typhosa in sewage treatment processes. This medium so successfully inhibited sewage organisms that their interference was negligible.

Kligler's iron agar. Bacto-Kligler's iron agar is prepared with phenol red as an indicator of the production of acid, and ferrous sulfate as an indicator of H_2S production. The dextrose and lactose fermenters are also differentiated. The writer used this medium as one means of isolation of Salmonella in fecal samples.

Bacto-methyl red Voges Proskaur medium (M.R.V.P.). This medium was used for performance of the M.R.V.P. tests in differentiation of Salmonella from other organisms. Salmonellae are M.R. positive and V.P. negative. This medium was also used in differentiation of the coli-aerogenes group.

Bacto-urea broth concentrate. This medium was used to differentiate Salmonella and Proteus. Salmonellae are urea negative and could be checked by this medium as well as with M.R.V.P. and indol tests.

Bacto-tryptone. Bacto-tryptone in one percent concentration is specified in "Standard Methods for the Examination of Water and Sewage" (119), for the performance of the indol test. This medium was used along with the above mentioned media for isolation of salmonellae.

Media for Lactobacilli

A large group, not well defined as to the nutritional significance in the gut, is the lactic acid group which includes the enterococci, coliforms as well as lactobacilli. These organisms grow in anaerobic as well as aerobic media,

Tissier (120), in 1899, used glucose infusion agar in veillon tubes for the growth of lactobacilli. Torry (121), in 1917, employed a procedure in which he used a liver infusion glucose agar to which 10 percent rabbit blood was added. Mickle and Breed (122), in 1925, reported the use of tomato juice in culture media for lactobacilli. Later Kulp and White (123), in 1932, described a modification of the original medium which gave them relatively high quantitative counts. Tomato juice agar which has been used as a highly selective medium for lactobacilli has the disadvantage that it is not entirely selective for lactobacilli even at an acid reaction. Since yeasts, molds, streptococci, etc., may occur with varying frequency and this interferes with successful isolation and enumeration of lactobacilli. Evenson et al., loc. cit., used an acetic acid medium for growing lactobacilli, believing that a low pH would suppress the growth of other organisms. Dhanda, loc. cit., and McClure, loc. cit., also used this medium.

The writer used a selective medium developed by Morrison et al. (124) in 1951. This medium gave luxuriant growth of lactobacilli.

The Ingredients of Lactobacillus Medium

Trypticase	10 grams
Yeast extract	5 "
KH_2PO_4	6 "
Ammonium citrate	21 "
Glucose	20 "
Sorbitan mono-oleate	1 "
Sodium acetate hydrate	25 "
Acetic acid	1.32 ml
Salt solution	5 "
Agar	15 grams
Distilled water to	1 liter

Final pH: 5.4

Composition of Salt Solution

$MgSO_4$, $7H_2O$	11.5 grams
$MnSO_4$, $.2H_2O$	2.4 "
$FeSO_4$, $7H_2O$	0.68 "

Distilled water to 100 ml

Animals used. Six- to nineteen-week-old New Hampshire, Durock Jerseys, and Poland China pigs were used for this experiment. These pigs were shipped from the farms to the experimental station in a healthy condition. Their temperature was recorded before exposing them to hog cholera virus. At the same time fecal material was collected. The animals then were kept in lots in different experimental rooms with free access to fresh water and feed. During the period of the experiment all of the animals were on the same kind of diet.

Collection of fecal samples. The fecal material was collected in a sterile container by manipulating the anal area. Care was taken to avoid contamination. The samples were brought to the laboratory within an hour and were kept in a refrigerator until used.

Preparation of the fecal material. No common or standard procedures are given by previous workers for assaying the intestinal flora. Harrison and Hansen (125), in 1945, used sterile sand when grinding fecal material of rats. Shapiro et al. (126), in 1949, and Evenson et al., loc. cit., used glass beads in shaking bottles to disperse the fecal clumps. The glass beads were the writer's choice in preparing most of the fecal samples for serial dilutions. Under aseptic conditions, the writer weighed one gram of the fecal material and transferred it to a 99 ml water blank bottle containing a few glass beads to facilitate uniform suspension

following vigorous and thorough shaking (routinely 200 times). This suspension (1-100) of the fecal material was further diluted in decimal series to 10^{-11}. If the fecal material was hard, a one gram portion was placed in a sterile mortar and ground with sterile sand. This mixture was poured into a sterile dilution blank and the volume made up to 100 ml before subsequent tenfold dilutions were made. Portions of each dilution (starting from 1-one million to 1-one hundred billion) were introduced into various tubes containing selective and differential media.

Total count of aerobes. One ml of the fecal material from each of the serial dilution blanks was poured into a Petri dish and 15 to 20 ml of tryptose glucose yeast extract agar (T.G.E.) was poured to make pour plates. These Petri dishes, thus inoculated, were incubated for 24 to 48 hours and the colonies were counted by a Quebec colony counter. All along the experiment this method was followed for total aerobic bacterial count. In the beginning of the work plain T.G.E. agar was used, but later on when liver extract was added to the same medium the total bacterial count increased greatly, which might be due to the many growth stimulating factors which liver contains.

Coliform counts. Lauryl tryptose broth prepared according to the formula of Mallmann and Darby, cited before, was used as a presumptive test to determine the presence of coliform organisms. A tenfold serial dilution of fecal material

in sterile distilled water was made up to 10^{-11} and one ml of each dilution was added to 10 ml of broth. After 48 hours of incubation at 37° C, the highest dilution showing gas formation was used to indicate the number of coliform organisms present. To confirm the presence of the aerobacter group, eosin methylene blue agar plates were streaked from the tubes showing the growth of coliforms. I.M.V.I.C. tests were also performed for the confirmation of Aerobacter and Escherichia.

Enteric streptococci counts. One ml of each dilution of fecal material prepared as described for the coliforms was added to 10 ml of dextrose azide broth for the determination of streptococci. It was presumed that the positive tubes, after 24 hours incubation at 37° C, indicated the presence of streptococci. Further confirmation was made on ethyl violet azide broth. When tubes other than those showing turbidity at 24 hours were positive at 48 hours, transfers were also made into ethyl violet azide broth. It was necessary to use four standard loopfuls (diameter 5 mm) to accomplish the transfers. The highest dilution in which turbidity could be observed after 48 hours of incubation of ethyl violet azide at 37° C was used to estimate the number of enterococci present.

Isolation of single colonies of enteric streptococci. The method used for isolation of enteric streptococci was the general procedure described by Sherman, loc. cit. A small amount of culture material from the second highest positive ethyl violet azide dilution was used to streak a brain

heart infusion agar plate. After 24 hours of incubation at 37° C, a few typical single isolated colonies of enteric streptococci were transferred to brain heart infusion broth, tryptose phosphate broth with 6.5 percent salt, and litmus milk. All cultures were incubated at 37° C, except the brain heart infusion broth which was incubated at 45° C. Readings were made after 48 hours and 72 hours of incubation, and also after 96 hours in the case of litmus milk. Turbidity in the brain heart infusion broth and tryptose phosphate broth containing 6.5 percent salt was considered as a positive test for the presence of enteric streptococci independent of the reaction in milk. The reactions in litmus milk were recorded as partial reduction, or partial or complete coagulation for further study. The enteric streptococci isolated from fecal samples were separated in the following species: Str. fecalis, Str. liquefaciens, Str. zymogenes, and Str. durans. The identification was made according to the method proposed by Sherman, loc. cit., in 1937 (see Table 1) and Bergey's Manual, loc. cit., 1948. Resistance of enterococci to 6.5 percent sodium chloride was considered as a significant test for enterococci, hence all cultures growing in sodium chloride tryptose phosphate broth were further identified as follows:

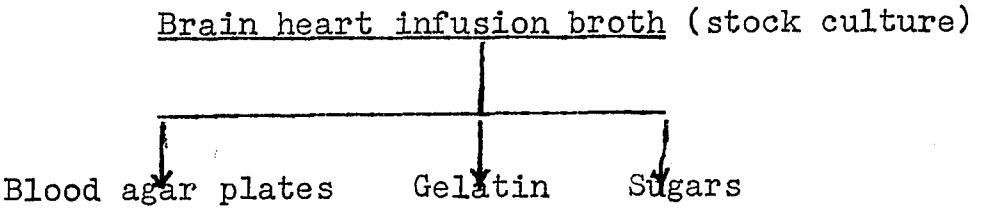

TABLE 1

Different Characteristics of Enterococci*

Species	Hemolysis	Gelatin liquefaction	Milk curdled	Acid production from						
				Arabinose	Maltose	Sucrose	Lactose	Glycerol	Mannitol	Sorbitol
Str. fecalis	-	-	≠	≠	≠	≠	≠	≠	≠	≠
Str. liquefaciens	-	≠	≠	≠	≠	≠	≠	≠	≠	≠
Str. zymogenes	≠	-	≠	≠	≠	≠	≠	≠	≠	≠
Str. durans	≠	-	≠	-	≠	-	≠	-	-	-

*Bacteriological Reviews, 1:80, 1937.

Lactobacilli counts. One ml of each dilution of fecal material prepared as described for coliforms and enterococci was placed in Petri dishes and 15-20 ml lactobacillus medium was poured into each plate. A uniform mixture of fecal material and the medium was obtained by gentle rotation of the plates. These plates were incubated at 37° C for 48 to 72 hours. The colonies were counted, using a Quebec colony counter.

Isolation of salmonellae. To freshly prepared tetrathionate broth 0.25 ml iodine solution was added (6 grams of iodine, 5 grams of potassium iodide in 20 ml of water) per 10 ml of the medium. Nearly one gram of fecal material was placed in this medium and it was then incubated for 12-16 hours at 37° C. After this incubation period the following procedure was adopted:

Tetrathionate broth

a loopfull of material streaked on SS agar
MacConkey plates (incubate 24 hours 37° C

colorless colonies picked

Kligler's iron agar
(H_2S+, alkaline slant, acid butt)

Indol, M.R.V.P. and urea tests

Sugars

When these tests were completed and the organism in question was suspected to be Salmonella, the culture was sent to the Michigan Department of Health laboratories for final confirmation and typing.

Bismuth sulfite agar plates were also inoculated with fecal material for isolation of salmonellae, but later on this medium was discontinued as the writer was quite satisfied with the media and methods mentioned before.

Microscopic Examination

Throughout the experimental period, smears were prepared from the cultures from time to time and gram staining was done to confirm the microscopic characteristics of the organisms under study.

RESULTS AND DISCUSSION

Comparison of Total Aerobic Plate Counts, Coliforms, Enterococci, and Lactobacilli During Health

As expected, the total counts of the bacteria were always higher than those of the different species during health and disease, irrespective of age differences (Tables 2-22). This can be observed in Figures 1, 2, 3, and 4. Evenson and co-workers, loc. cit., while working on the intestinal flora of white rats, also noticed the same results. Since the animals were exposed to hog cholera virus soon after the first fecal cample was taken, no further samples could be obtained for comparison of day to day variations. By an analysis of variance, it was shown that there were no significant differences between arithmetic averages of these counts (Table 2).

The coliforms, which are usually regarded as harmless parasites, were least numerous during healthy conditions irrespective of age differences or conditions under which these pigs had been raised.

Table 22 shows that the average coliform counts ranged between 13 to 37 millions per gram of fecal material. Such differences in bacterial counts were also observed by White (127), in 1942, and Driesens (128), in 1952. By analysis of

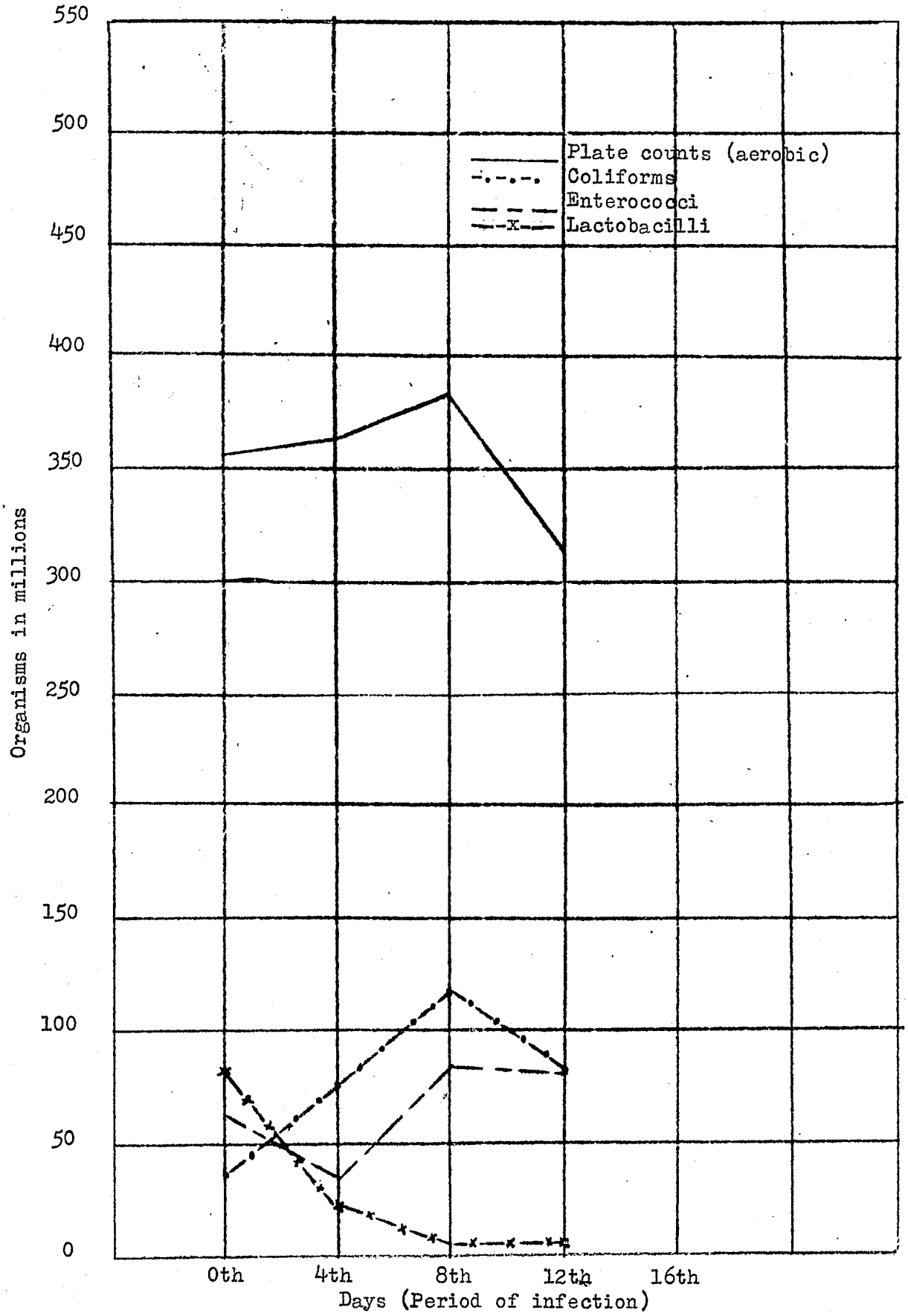

Fig. 1 Variations in intestinal bacterial counts in six weeks old pigs after infection with hog cholera virus.

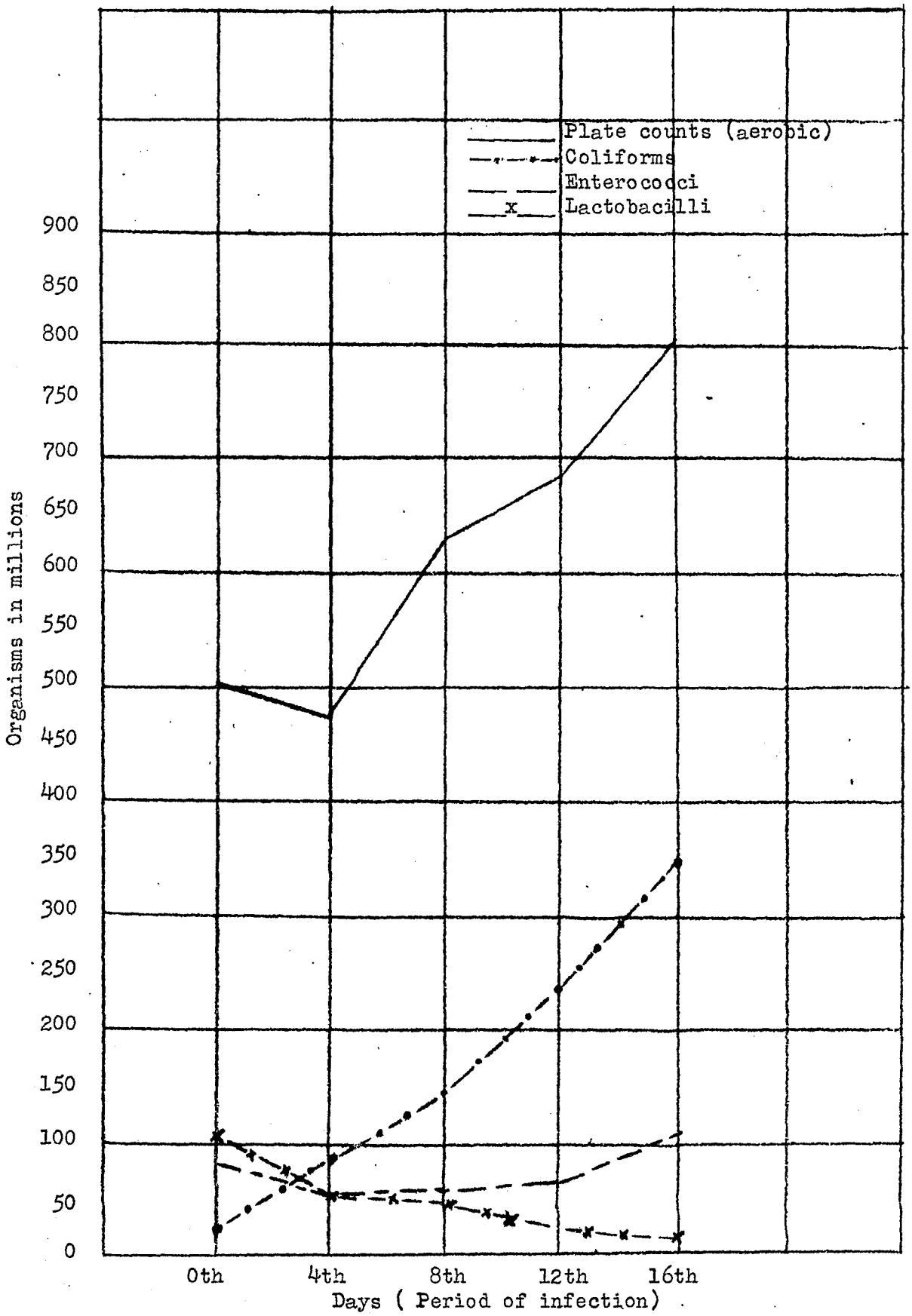

Fig. 2 Variations in intestinal bacterial counts in seven weeks old pigs after infection with hog cholera virus.

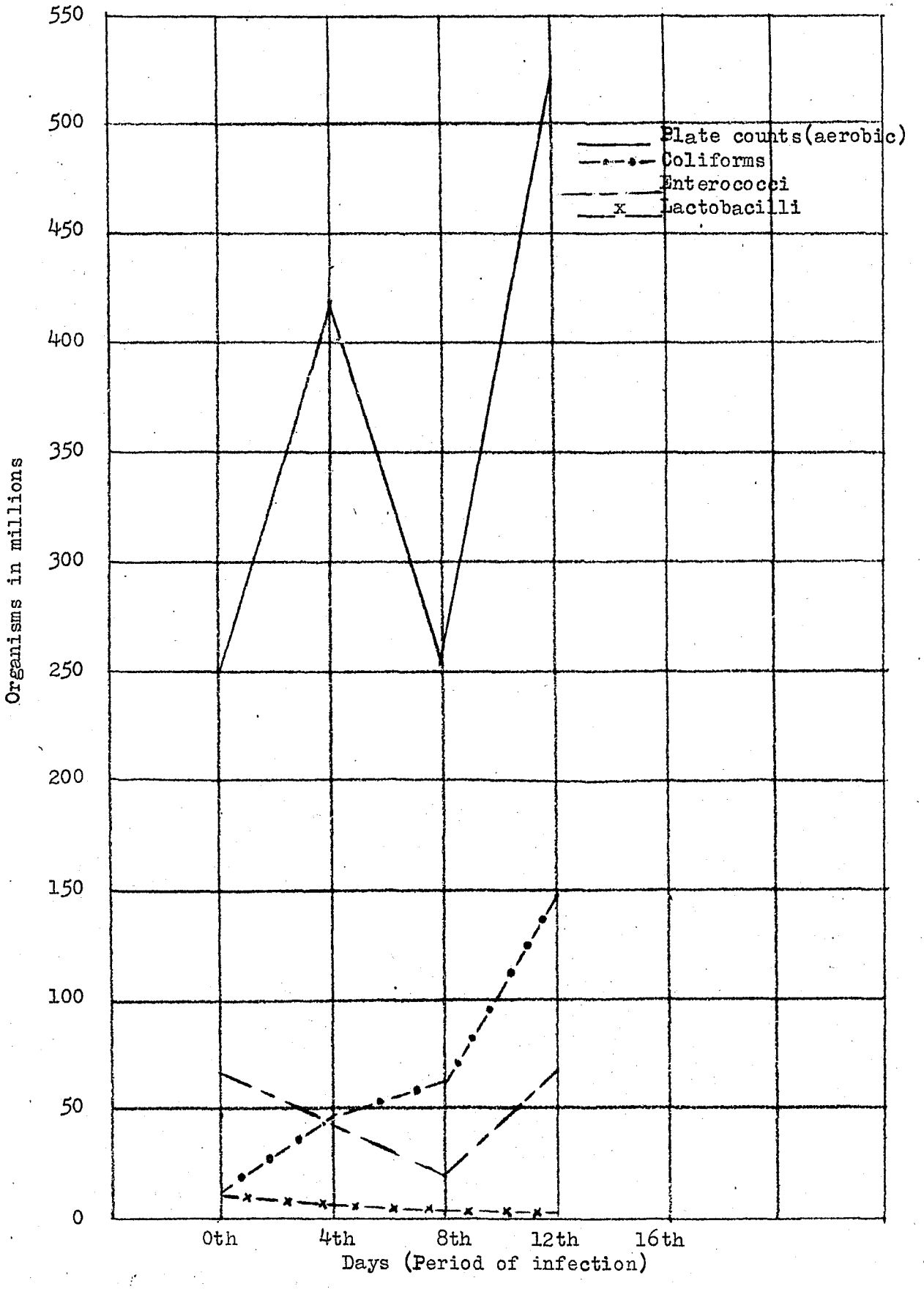

Fig. 3 Variations in intestinal bacterial counts in sixteen weeks old pigs after infection with hog cholera virus.

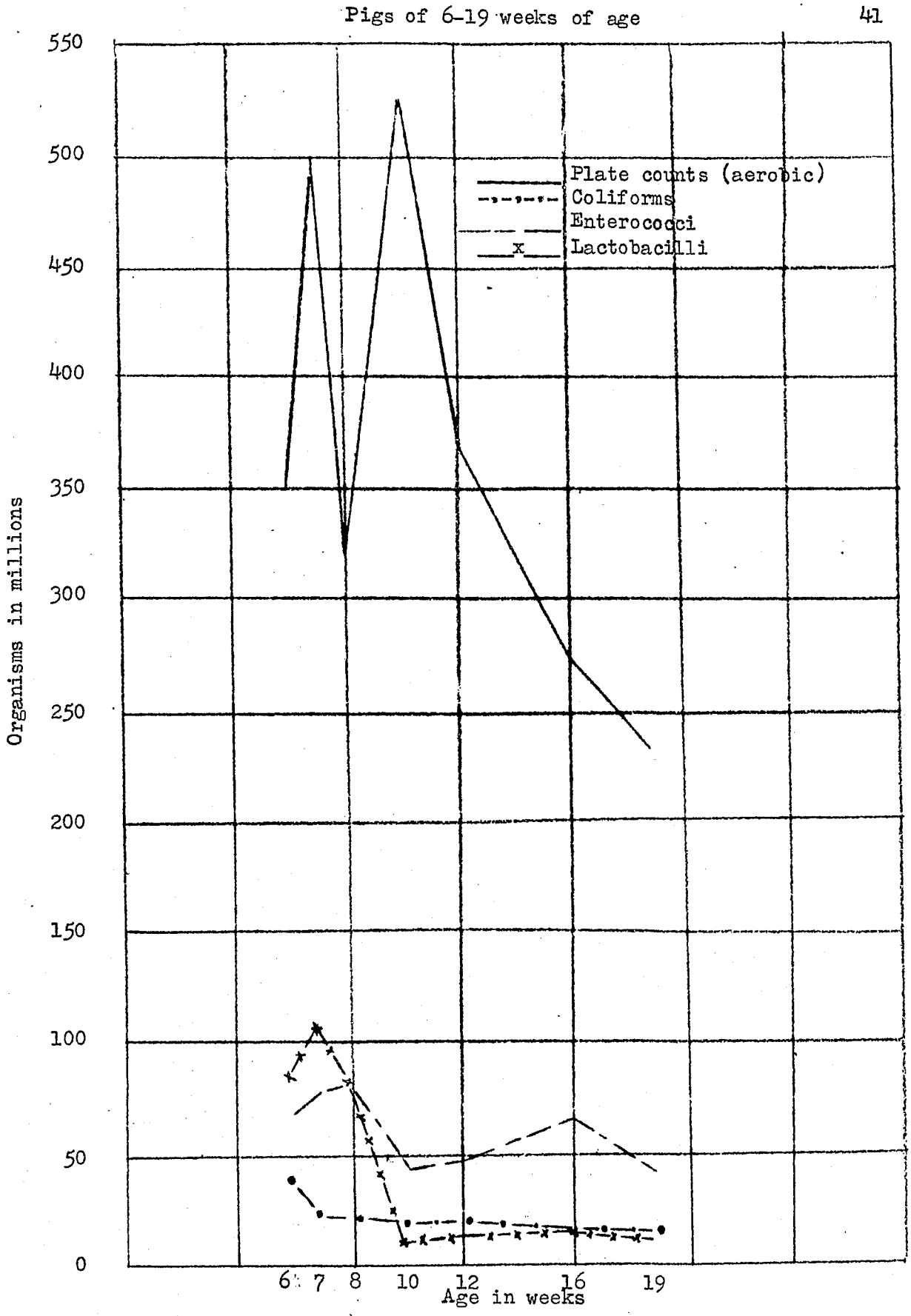

Fig. 4 Variations in intestinal bacterial counts in healthy pigs from six to nineteen weeks of age.

variance, it was found that there was no significant variation due to difference in age.

The number of enterococci was higher than that of the coliforms but lower than that of the lactobacilli up to seven weeks of age during good health. From eight weeks onward the enterococci were most numerous, the coliforms were next, and the lactobacilli were least numerous. The average number of enterococci was 42-84 millions per gram of the fecal material. An analysis of variance showed that the number of enterococci (68-84 millions) was significantly higher in pigs six to eight weeks of age than in pigs ten, twelve, sixteen, and nineteen weeks of age (42-66 millions). Radison (129), in 1952, while working with calves, found higher enterococci counts in the first few weeks after birth. It may be presumed that since the enterococci are lactose fermenters they could grow luxuriantly in the milk diet with high lactose content and acid medium. Although the total number of enterococci had decreased after the pigs had reached the age of eight weeks, they did not decrease to the level of lactobacilli, due to age differences, but remained near the same level.

The number of lactobacilli was highest at six to seven weeks of age so far as individual genera were concerned. However, there was no significant difference among the average counts of the pigs six to eight weeks of age, nor among those of the ten to nineteen weeks old pigs. This was confirmed

by an analysis of variance. The high lactobacilli counts observed in the pigs while nursing their mothers was to be expected. The milk with its high lactose content has been known since its early studies by Hull et al. (130), in 1917, and Cruickshank (131), in 1938, to favor the development of an aciduric flora and depress the growth of coliforms. Figures 1, 2, 3, and 4 and Table 22 show a decrease in the numbers of lactobacilli in healthy pigs from eight weeks of age and upward. This could have been due to the fact that the composition of the stock diet did not offer readily available energy for the growth of lactobacilli. In other words, a large amount of indigestible material and high protein present in the stock diet may have had its effect in this respect.

RATION FED TO THE PIGS DURING EXPERIMENT

Ground corn	60 Parts
Ground oats	13 "
Meat scrap	10 "
Soybean oilmeal	14 "
Mineral	3 "
Irradiated yeast	120,000 units
(Vitamin D_2)	Per 100 pounds

Comparison of Total Aerobic Plate Counts, Coliforms, Enterococci and Lactobacilli in Swine Challenged with Hog Cholera Virus

After taking the first fecal sample, the pigs were injected intracranially or intramuscularly with hog cholera virus. The animals were kept in different experimental pens. During the assay of the fecal sample after every fourth day, it was noticed that there was no significant difference in the average of the total aerobic plate counts. The numbers remained high during the entire period of infection (Tables 2, 3, 10, and 17). At the same time the growth curves during the infected stage corresponded to the trend of the curve of the coliforms, as shown in Figures 1, 2, and 3.

The number of the coliforms increased many times when the animals under experiment became sick and developed diarrhea. This may be observed in Tables 4, 5, 11, and 18. In most of the cases the coliform index remained high during the entire period of infection, and there was little tendency to decrease. There were significant differences between the daily average counts and between the weekly average counts at the one percent level. The increase of coliforms during the period of infection and diarrhea might be due to the decrease of lactobacilli and, therefore, less competition for nutriments; there might be some unknown condition favoring their multiplication.

As for enterococci, there were no significant differences between the averages of the total numbers caused by infection, diarrhea, or constipation, as shown in Tables 6, 7, 12, and 19. It may be presumed that this was due to their ability to survive in adverse environment, as shown by the work of Sherman, loc. cit., Table 23.

The decrease or disappearance of the gram positive aciduric organisms from the intestinal tract, once the pig had developed the clinical symptoms, as shown in Tables 8, 13, and 20 and Figures 1, 2, and 3, was probably due to decreased food intake during the period of infection or due to the absence of utilizable carbohydrates, or the alkaline reaction of the medium. While working on the intestinal bacteria of rats, Porter et al. (132), 1940, also observed similar results. According to these workers, fasting the rats for a period of two days brought about only slight change in the character of the bacterial flora of the intestinal tract. However, when the food was withheld for five days, a very marked transformation took place. Aciduric organisms of the L. acidophilus type were no longer present in the different sections of the alimentary canal, while the coliform bacteria, which ordinarily were observed to be restricted to the ileum and cecum, appeared throughout the small intestine and constituted a large portion of the flora. In this respect it is interesting to note that Rougentzoff (133) observed that when rabbits were allowed to fast there was an absence of an acidophilic

flora in the intestines but an increase in the number of anaerobes. Also, Poppens (134), in 1921, found that E. coli increased in the duodenum of dogs when the animals were allowed to fast.

Presence of Salmonellae in the Intestinal Tract of Swine During Health and Disease

Isolation of S. choleraesuis. The writer was interested to know how often S. choleraesuis could be isolated from the healthy as well as diseased swine. S. choleraesuis, which is regarded as a normal inhabitant of the intestinal tract, according to Runnells (135), Van Es (136), and Dale, loc. cit., was isolated infrequently. During the entire period of the experiment, S. choleraesuis was isolated only seven times. S. choleraesuis may have been present in other animals, but isolation was not possible because it may have been outnumbered by other intestinal organisms, such as enterococci, coliforms, lactobacilli, Pasteurella suiseptica, etc. So, when one wants to isolate S. choleraesuis, even on a selective medium, the chances of isolating it from fecal material become meager. On the other hand, when the animals had developed infection this organism became a secondary invader and entered the general circulation. In the opinion of the writer, it was because of this that early workers, such as Dorset et al. (137), in 1904, frequently were able to isolate S. choleraesuis from the blood of infected pigs but not

from the intestinal contents. As this study was not concerned with S. choleraesuis in the general circulation, no attempt was made to isolate it from the internal organs.

The presence of S. choleraesuis may produce enteritis in swine, but when both the virus and S. choleraesuis are present it is still not proved which one is the real causative agent of necrotic enteritis. On this problem further work needs to be carried out. Healthy germ-free pigs should be made available and infected with virus and S. choleraesuis separately. It also may be presumed that in hog cholera infection there is a disturbance in host-parasite balance, so that when the natural resistance of the host has declined, S. choleraesuis may invade the tissues through the necrotic areas present in the intestinal tract.

Isolation of S. cubana. S. cubana was isolated from pigs (numbers 659, 666, and 667) for the first time. According to Seligman, et al. (138), this organism was first isolated by Curbelo in 1945 in Havana during an outbreak of diarrhea among chicks. The organism was found in feces and in some instances in the heart blood of affected chicks. These chicks were imported from Florida in good health. No case of human infection has been reported. No symptoms developed as a result of feeding the culture, but the mice died within 20 hours when the culture was injected subcutaneously.

It was quite interesting to observe that pigs (numbers 665, 666, 667, 668, 690, and 692) from which salmonellae were

isolated, showed no marked degree of pathological changes in the digestive tract after the period of infection with the hog cholera virus. However, on the other hand, pigs 659, 646, 647, and 689 which were harboring salmonellae had developed severe hemorrhagic gastritis and enteritis with a few necrotic foci. Their submaxillary lymph nodes also revealed peripheral hemorrhage.

E. Coli in the Blood Stream

During the experimental period, E. coli was isolated from the internal organs (heart blood, liver, kidneys, and spleen) on MacConkey agar plates, eosin methylene blue agar plates, and in lauryl tryptose broth. The isolation of E. coli was correlated with the changes in the digestive tract. On post-mortem examination these animals revealed either necrotic gastroenteritis or petechiae. So far as the pathogenicity is concerned, the writer is unable to say whether E. coli plays any significant role. But this much can be said, most of these pigs had gastritis or necrotic enteritis. As there was a break in the surface epithelium of the intestinal tract, E. coli had made its way into the general circulation and thus was found in the heart blood, liver, kidney and spleen.

McBride (139), in 1934, while working on an acute enteritis in young pigs due to infection with the colon group,

isolated E. coli from the internal organs in every case in which enteritis had developed. Hagen, loc. cit., is of the opinion that E. coli escapes from the digestive tract into the blood stream and is disseminated to all parts of the body about the time of death.

Predominant Subgroups of Enterococci in Feces

Sixty-four cultures of enterococci isolated from fecal samples taken from healthy pigs, on brain heart infusion agar, were classified by acid production in different sugars, liquefaction of gelatin and production of hemolysis on blood agar and reduction of litmus milk in 24-48 hours. Most of the organisms of this group were Str. fecalis, as shown in Table 24. The writer's results agree with those of Ayers and Johnson (140) who examined 39 human fecal cultures of enterococci and found that the predominant species was Str. fecalis. They also concluded that Str. fecalis was similar to, if not identical with, Str. lactis.

Ritter and Treece (141), in 1948, isolated 79 strains of streptococci from swimming pools. Fifty-two (65.8 percent) were classified as Str. fecalis. These were confirmed by the Lancefield technique and were further classified as type D.

TABLE 2

Average Plate Counts (Aerobic) in Millions

Days	Age of pigs in weeks		
	6	7	16
1	356	502.6	249.5
4	364	473.5	424.5
8	465	631.6	251.8
12	312	685.0	522.5

TABLE 3

Plate Counts (Aerobic) in Millions

Serial no.	Pig no.	6 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	494	280	760	470	170	-
2	495	342	242	320	600	-
3	496	400	980	890	310	✗
4	497	1000	160	900	0	266
5	513	170	250	270	280	-
6	514	225	800	1000	200	-
7	515	940	210	✗		
8	525	115	215	313	-	
9	526	186	306	318	-	
10	528	276	528	260	✗	
11	512	115	200	250	-	
12	527	225	400	130	-	
Average		356	364	465	312	
Median		250	246	318	280	

- = Destroyed

✗ = Dead

TABLE 4

Average Coliforms in Millions

Days	Age of pigs in weeks		
	6	7	16
1	37.5	21.6	13.1
4	75.	90.7	49.4
8	118	149	65
12	89.5	240	147.5

TABLE 5

Coliform Counts in Millions
(Most probable numbers)

Serial no.	Pig no.	6 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	494	140	14	140	45	-
2	495	2.5	140	45	25	-
3	496	110	45	140	145	≠
4	497	9.5	45	95	45	95
5	513	25	45	150	160	-
6	514	45	140	160	117	-
7	515	20	110	≠		
8	525	11	140	250	-	
9	526	14	110	14	-	
10	528	45	2	16	≠	
11	512	14	40	180	-	
12	527	11	70	110	-	
	Average	37.5	75	118	89.5	
	Median	17	57	140	81.0	

- = Destroyed

≠ = Dead

TABLE 6

Average Enterococci in Millions

Days	Age of pigs in weeks		
	6	7	16
1	68.2	78.7	65.6
4	34.3	56.2	42.9
8	83.1	59.2	19.6
12	79	67.5	67.5

TABLE 7

Enterococci Counts in Millions
(Most probable numbers)

Serial no.	Pig no.	6 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	494	110	15	140	140	-
2	495	2.5	45	45	140	-
3	496	95	95	40	4	≠
4	497	75	45	45	45	6
5	513	120	20	110	15	-
6	514	25	15	140	130	-
7	515	16	2	≠		
8	525	25	25	15	-	
9	526	140	20	45	-	
10	528	140	4	140	≠	
11	512	45	110	84	-	
12	527	25	15	110	-	
Average		68.2	34.3	83.1	79	
Median		60	22	84	87	

- = Destroyed

≠ = Dead

TABLE 8

Average Lactobacilli Counts in Millions

Days	Age of pigs in weeks		
	6	7	16
1	85	106	13.4
4	23	69	6.2
8	3	46	3.1
12	3.2	21	0.2

TABLE 9

Lactobacilli Counts in Millions

Serial no.	Pig no.	6 weeks old pigs				
		Before infection: 1st day	After infection: 4th day	8th day	12th day	16th day
1	494	55	2	1.5	1.0	-
2	495	43	6	1.0	12.6	-
3	496	56	9.5	8.2	3.5	✗
4	497	1.0	1	2.7	0.7	.01
5	513	13	6	3	1.7	-
6	514	105	4.5	3	.018	-
7	515	80	20	✗		
8	525	31.8	.073	.013	-	
9	526	312	.020	.015	-	
10	528	200	23.	13	✗	
11	512	98	85	5	-	
12	527	32	127	.018	-	
	Average	85	23	3	3.2	
	Median	68	4	3	1.3	

- = Destroyed

✗ = Dead

TABLE 10

Plate Counts (Aerobic) in Millions

Serial no.	Pig no.	7 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	610	1380	2060	3850	3900	3950
2	613	350	215	307	312	200
3	614	1180	2150	2060	✗	
4	659	267	235	277	155	-
5	660	238	133	283	✗	
6	661	265	238	333	-	
7	662	313	380	650	-	
8	663	211	210	500	317	-
9	664	270	219	184	208	500
10	665	363	315	720	257	330
11	666	363	328	311	470	306
12	667	236	213	330	295	300
13	668	246	305	217	211	630
14	501	1460	215	82	✗	
15	503	1110	146	132	-	
16	612	168	213	190	-	
Average		502.6	473.5	631.6	685.0	802
Median		291	236	216	295	330

- = Destroyed

✗ = Dead

TABLE 11

Coliform Counts in Millions
(Most probable numbers)

Serial no.	Pig no.	7 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	610	15	35	30	140	250
2	613	20	30	140	250	450
3	614	115	140	20	≠	
4	659	4	140	300	325	-
5	660	4	200	300	≠	
6	661	11	15	112	-	
7	662	8	150	160	-	
8	663	45	110	160	140	-
9	664	12	110	140	120	200
10	665	45	30	110	140	150
11	666	45	45	110	300	450
12	667	7.5	250	450	450	300
13	668	15	20	140	300	450
14	501	25	25	45	≠	
15	503	15	11	140	≠	
16	612	30	140	34	≠	
Average		21.6	90.7	149	240	300
Median		15.0	77.5	140	250	227

- = Destroyed

≠ = Dead

TABLE 12

Enterococci Counts in Millions
(Most probable numbers)

Serial no.	Pig no.	7 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	610	140	160	25	30	14
2	613	45	4	16	7.5	20
3	614	110	45	9.5	≠	
4	659	30	40	140	11.5	-
5	660	25	16	45	≠	
6	661	140	16	45	-	
7	662	20	11	14	-	
8	663	140	110	45	30	-
9	664	30	4	110	45	45
10	665	9.5	20	25	30	16
11	666	110	140	150	140	250
12	667	110	120	160	110	160
13	668	140	15	30	200	150
14	501	140	40	110	≠	
15	503	25	14	45	-	
16	612	45	20	10	-	
Average		78.7	56.2	59.2	67.5	105
Median		77.5	20	45	30	45

- = Destroyed

≠ = Dead

TABLE 13

Lactobacilli Count in Millions

Serial no.	Pig no.	7 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	610	16	.08	.09	.07	.07
2	613	18	.036	.06	.03	.04
3	614	72	.03	.04	✗	
4	659	68	53	38	7	-
5	660	212	31	93	✗	
6	661	28	18	7	-	
7	662	68	3	.08	-	
8	663	276	13	8	10	-
9	664	232	240	133	27	7
10	665	66	218	23	25	7
11	666	180	182	193	12	45
12	667	215	209	206	86	3
13	668	178	55	18	20	0.82
14	501	6	14	10	✗	
15	503	26	64	8	-	
16	612	48	0.07	.065	-	
Average		106	69	46	21	10
Median		68	24	9	10	3

- = Destroyed

✗ = Dead

TABLE 14

Total Bacterial Population (in Millions)
(8 weeks old pigs before inoculation)

Serial no.	Pig no.	Plate counts (aerobic)	Coliforms	Enterococci	Lactobacilli
1	459	165	11.5	45	6
2	463	163	40	140	43
3	516	1110	15	45	122
4	517	240	45	110	98
5	518	308	14	160	58
6	646	293	15	140	50
7	647	314	20	110	158
8	650	205	25	7.5	17
9	692	158	15	20	50
10	693	227	3.5	15	88
11	695	367	16	140	150
	Average	322	20	84	76
	Median	227	15	110	58

TABLE 15

Total Bacterial Population (in Millions)
(10 weeks old pigs before inoculation)

Serial no.	Pig no.	Plate counts (aerobic)	Coliforms	Enterococci	Lactobacilli
1	464	980	15	15	3.5
2	465	643	4	9	3.0
3	466	251	20	15	21.6
4	467	250	25	140	12.0
Average		521	16	45	10
Median		447	17.5	15	7.5

TABLE 16

Total Bacterial Population (in Millions)
(12 weeks old pigs before inoculation)

Serial no.	Pig no.	Plate counts (aerobic)	Coliforms	Enterococci	Lactobacilli
1	417	225	4	25	16
2	418	303	5	20	9
3	422	455	4	40	8
4	423	450	16	14	9
5	424	136	40	40	29
6	425	670	45	140	7
Average		371	19	46	13
Median		376	10	32	9

TABLE 17

Plate Counts (Aerobic) in Millions

Serial no.	Pig no.	16 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	599	253	308	312	315	-
2	603	520	1870	✗		
3	604	248	300	311	✗	
4	600	248	200	-		
5	602	67	198	153	730	✗
6	605	138	140	300	-	
7	606	250	130	137	-	
8	608	275	250	298	Constipated No sample	✗
Average		249.5	424.5	251.8	522.5	
Median		249	225	299	522.5	

- = Destroyed

✗ = Dead

TABLE 18

Coliform Counts in Millions
(Most probable numbers)

Serial no.	Pig no.	16 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	599	15	17	20	45	-
2	603	5	15	≠		
3	604	25	140	150	≠	
4	600	10	15	-		
5	602	16	140	160	250	≠
6	605	4	9.5	14	-	
7	606	15	14	12	-	
8	608	15	45	35	Constipated No sample	≠
Average		13.1	49.4	65	147.5	
Median		15	16	27	147.5	

- = Destroyed

≠ = Dead

TABLE 19

Enterococci Counts in Millions
(Most probable numbers)

Serial no.	Pig no.	16 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	599	140	45	25	110	-
2	603	110	20	≠		
3	604	110	25	30	≠	
4	600	25	140	-		
5	602	45	25	20	25	≠
6	605	20	30	20	-	
7	606	45	45	15	-	
8	608	30	13	7.5	Constipated No sample	≠
Average		65.6	42.9	19.6	67.5	
Median		45	27	20	67.5	

- = Destroyed

≠ = Dead

TABLE 20

Lactobacilli Counts in Millions

Serial no.	Pig no.	16 weeks old pigs				
		Before infection	After infection			
		1st day	4th day	8th day	12th day	16th day
1	599	8.6	3.8	4.5	0.3	-
2	603	3	18	≠		
3	604	45	5	2	≠	
4	600	6	5	-		
5	602	0.8	0.6	0.3	0.1	≠
6	605	7.8	6.	0.4	-	
7	606	22.	15	18.	-	
8	608	14	0.8	0.16	Constipated No sample	≠
Average		13.4	6.2	3.1	0.2	
Median		8	5	1.2	0.2	

- = Destroyed

≠ = Dead

TABLE 21

Total Bacterial Population (in Millions)
(19 weeks old pigs before inoculation)

Serial no.	Pig no.	Plate counts (aerobic)	Coliforms	Enterococci	Lactobacilli
1	448	149	7.5	30	5
2	449	132	25.0	120	2
3	450	400	15	16	8
4	452	200	8	16	10
5	454	158	11.5	30	7
Average		208	13	42	6.4
Median		158	11.5	16	7

TABLE 22

Mean of the Counts of the Micro-organisms (in Millions) at Different Ages of Pigs on the First Day

(Before infection)

Total counts	Coliforms	Enterococci	Lactobacilli
	6 weeks age		
356	37	68	85.6
	7 weeks age		
502.6	21.6	78.6	106.8
	8 weeks age		
322	20	84	76.4
	10 weeks age		
521	16	45	10
	12 weeks age		
371	19	46	13
	16 weeks age		
249	13	66	13.4
	19 weeks age		
208	13	42	6.4

TABLE 23

Classification Chart Showing
Growth of Streptococci in Different Conditions*

Division	Group of species	Growth at		Growth in presence of			Survival at 60° C, 30 min.
		10° C	45° C	6.5% NaCl	pH 9.6	0.1% methylene blue	
Enterococcus	S. fecalis	≠	≠	≠	≠	≠	≠
	S. liquefaciens	≠	≠	≠	≠	≠	≠
	S. zymogenes	≠	≠	≠	≠	≠	≠
	S. durans	≠	≠	≠	≠	≠	≠

*Bact. Rev., 1:16. 1937.

TABLE 24

Percentage Distribution of Enterococci Subgroups

Name of organism	Number of cultures	Percent
Str. fecalis	52	81.25
Str. durans	8	12.5
Str. liquefaciens	3	4.68
Str. zymogenes	1	1.2

SUMMARY AND CONCLUSIONS

In the study of the intestinal flora of swine during health at the ages of six to seven weeks, the highest count was found to be that of lactobacilli, followed by enterococci, with the coliforms being the least numerous. The total counts always exceeded the sum of the group counts.

In the pigs ten to nineteen weeks of age, the lactobacilli counts decreased dramatically even during health. Although enterococci counts were quite low after eight weeks of age, they did not become insignificant, and were higher than those of the coliforms and lactobacilli.

There was a great increase (15-20 times) in coliforms during the period of infection and diarrhea. However, the lactobacilli drcreased in numbers when the coliform index increased. Infection showed little effect on the counts of enterococci.

S. choleraesuis was present in the intestines of healthy as well as diseased pigs.

S. cubana was isolated for the first time from the intestines of swine.

E. coli was isolated from the internal organs of the pigs affected with necrotic and hemorrhagic enteritis.

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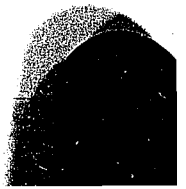

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