Ulcerative Enteritis in Quail

A. J. Durant and E. R. Doll

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INTRODUCTION

Diseases in game birds have recently become more prevalent, chiefly as a result of raising the birds in confinement for conservation and restocking purposes. This increase is a natural result of bringing together large numbers of birds in small areas, thus the concentration of causative agents of diseases, and the chance for spread of infection to other birds is enhanced. Food and water supplies are altered from those of natural conditions and, aside from nutritional disturbances which might occur, also favor spread of diseases through the medium of food and water.

Ulcerative enteritis is an epizootic affecting quail and other game birds. It is characterized by ulceration of the intestine with an associated high mortality. Shillinger and Morley state that, "Ulcerative enteritis, 'quail disease', is perhaps the disease best known and most feared by game breeders." The recent increasing demand for quail to be used by conservationists for restocking purposes has resulted in a larger number of game farms upon which a great number of birds are raised in comparatively small areas. Under such conditions, chances for the spread of ulcerative enteritis are greatly augmented and an epizootic frequently destroys every bird in a flock.

Epizootics have been observed by the authors in which flocks of fifty to sixty birds in batteries have all died within fifteen to eighteen days following the first loss from the disease. Flocks of young birds, ten days to two weeks of age, numbering from forty to sixty have been observed in which all birds succumbed in a week to ten days after the onset of the epizootic.

Propagation of game birds for restocking purposes is a costly enterprise; therefore, it is evident that an outbreak of ulcerative enteritis may cause great financial loss to game breeders. The loss of breeding stock which has become adapted to conditions of captivity may result in considerable difficulty when reestablishing a productive breeding flock.

In view of these conditions, the problem selected is one of timely importance, and one in need of investigation. Since the cause of this disease has not been definitely shown, the plan for research on the problem has been directed toward isolation of the etiological agent. A detailed study of the disease, history, symptoms, and histopathology is included.

*Superscript numerals refer to Bibliography page 27.
The disease has been reported in the common bobwhite, the California quail, the mountain quail, the sharptail grouse, the Gambel's quail, the European partridge, the chukar partridge, the wild turkey, and the domestic fowl. The number of game birds susceptible to spontaneous infection, and the high mortality associated with the disease make it a problem of considerable magnitude in conservation work.

REVIEW OF LITERATURE

Ulcerative enteritis apparently was first noted in grouse. A magazine article in 1817 called attention to a severe epidemic disease among grouse in England. Klein in writing a description of the disease, described features which indicate that the grouse disease and ulcerative enteritis in quail are closely related or identical infections.

Shillinger and Morley described the disease in acute form as killing quail without symptoms. Usually a diarrhea was present. In chronic forms, the birds were dull, listless, with ruffled feathers, and a progressive extreme emaciation, with loss of one-third to one-half the normal weight. Gross pathological changes were listed as being confined to intestines, lungs, and liver. The intestines were studded with ulcers, some perforating, others with caseous casts. The duodenum in some birds was enlarged and purplish-red in color. A few cases showed no change except a hemorrhagic enteritis. The lungs were regularly congested and occasionally showed consolidated areas. The liver did not always reveal gross changes but regularly showed congestion and degeneration on microscopic section. The disease was observed in wild turkeys and in young chicks. The infection was transferred to quail from chicks, but could not be passed on from such infected quail to healthy quail or chicks. Cultures from the turkeys and chicks were negative. The possibility of apparently healthy chicks acting as carriers was suggested.

These investigators failed to produce the disease in quail by feeding macerated liver, lungs, spleen, or heart and heart blood selected from infected birds that had no perforating ulcers. Blackhead lesions from quail would not produce ulcerative enteritis. Material (lesions or contents) infective for quail failed to produce the disease in pigeons, rabbits, guinea pigs, white rats, and mice. Protozoan organisms were not incriminated in ulcerative enteritis. Coccidia were found frequently in cases of ulcerative enteritis but were not associated with its etiology. No parasitic organisms were found in blood films. Attempts to isolate a bacterial agent causing ulcerative enteritis were without success. Both aerobic and anaerobic organisms failed
to produce the disease when given orally. On three occasions
the disease apparently was transmitted with mixed aerobic
cultures, but subsequent transfers from the mixed cultures failed
to produce the disease. Tissue extracts of liver and heart blood
passed through Berkefeld and Chamberland filters gave negative
results when fed and injected into quail, pigeons, chickens,
rabbits, guinea pigs, and rats. Similar results were obtained
by filtering intestinal contents; however, the residue of this
filtration was infective for quail.

Barger, Park, and Graham in writing on the “so-called
quail disease,” reported very high morbidity and mortality on
an Illinois game farm, with loss of more than 1,400 quail in a
flock of 2,000. Streptococci, diplococci, E. coli, and S. aertrycke
were isolated from dead birds. However, the description of
the gross pathology of the birds indicates that these investigators
were not dealing with ulcerative enteritis.

Le Dune reported observation of ulcerative enteritis in
ruffed grouse on an Illinois game farm. Symptoms and autopsy
findings described in the grouse were quite detailed and typical
of those seen in the bobwhite quail. Segregation, cleaning,
disinfection and the use of a fire gun were effective in checking
the losses.

In writing on diseases of game birds, Shillinger and Coburn described symptoms and lesions of the disease. Methods of trans-
mission were briefly discussed, and control measures recom-

morley and wetmore claimed to have shown that ulcerative
enteritis is of bacterial etiology. They reported the isolation
of an organism in pure culture from the liver of a diseased quail,
and with this organism they produced typical lesions in twelve
out of fifteen test quail. This organism retained its virulence
through seven transfers. The results were repeated a number
of times, but the majority of strains rapidly lost their virulence.
The organism, tentatively named Corynebacterium perdicum,
was described as a Gram positive, pleomorphic, aerobic, non-
motile rod, similar in morphology to the Klebs-Loeffler bacillus.
The organism could be found in the liver, spleen, and intestines.
It could not be isolated on solid media, and when first isolated
grew poorly on fluid or semisolid media.

Bass in a paper concerning the etiology of the disease,
gave a description of the lesions of ulcerative enteritis with a
discussion of an anaerobic organism which he considered to be
the etiological agent. Chronic carriers were regarded as a factor
in maintaining and transmitting infection. Observations were
included to indicate that the disease may be transmitted to the
young through the egg. The causative agent, an anaerobic Gram
negative rod, was isolated in “pure culture seven times, five from
intestinal lesions, one from a similar, very small lesion in the liver, and one from the yolk sac of a baby quail.” The best growth was obtained in glucose agar (0.25% agar) with an added amount of aqueous extract of macerated quail intestine. Experimentally infected birds began to show symptoms and die in four to eight days. Small doses of infectious material produced lesions and a course similar to natural infections. Large doses of infectious material produced a fulminating form of disease in which there was much inflammation and a greater number of ulcers. One bird was dead 36 hours after inoculation. Birds infected by contamination of feed died over a period of four months, with 75% in the first two months, 15% in the third month, and 4% in the fourth month. Because of these latent deaths and those birds still living four months after being given infectious material, the possibility of carriers was proposed. Several “carriers” were known to have laid eggs. Bass suggested the possibility of egg contamination, either from feces, or from contamination in the peritoneal cavity of the yolk from ulcers adjacent to the ova. The disease was observed in quail of five, nine, and ten days of age. The “specific organism” was found in the yolk sacs of quail of fourteen and nineteen days of age, and was isolated in pure culture from the quail of fourteen days of age. Other quail were infected by feeding parts of the yolk sacs. The organism was not found in the eggs.

Pickens, De Volt, and Shillinger described an outbreak of ulcerative enteritis in bobwhite quail. Symptoms, lesions, and the results of bacteriological studies were described somewhat in detail. The organisms recovered from the diseased birds were described as belonging to the colon and paratyphoid groups. These cultures were pathogenic for quail and guinea pigs when injected, but failed to produce the characteristic ulcers in the intestines. Other visceral organs were described as, “appearing very much like those containing quail disease lesions.” None of the cultures were infective for quail when introduced by feeding. Filtrates of extracts of organs from diseased quail were given to guinea pigs without harmful effects. Saline extracts were pathogenic for guinea pigs. A lowered resistance in the quail and a combination of colon and paratyphoid organisms were suggested as the cause.

Morse described the disease in bobwhite quail, giving symptoms and post mortem findings. A brief investigation concerning the cause of the disease was given. The organism incriminated was one of the B. coli group. It was pathogenic for quail, mice, and guinea pigs when injected into the animals, producing “characteristic lesions.” These lesions were not described. Feeding tests with the cultures were negative.
Levine's reported an outbreak of ulcerative enteritis in ruffed grouse on a New York game farm. Lesions described were quite similar to the gross changes seen in quail. He was able to produce the disease in quail by feeding droppings from infected grouse. The disease was also produced in quail by feeding them flies which had been allowed to feed on infective material over night. After being allowed to dry and preen themselves, the flies were killed with ether vapor, and twenty were fed to each of six quail, all of which died in from three to five days.

Gallagher, as cited by Shillinger and Morley suggested the possibility of coccidia as a factor in the formation of the lesions. However, Shillinger and Morley could find no relation between the presence of coccidia and ulcerative enteritis.

**MATERIALS AND METHODS**

Early in this investigation the disease was perpetuated by serial passage from quail to quail using suspensions of the ulcerated intestine. The intestine containing lesions was ground in a mortar with sand, mixed with a small amount of water, and the product was then placed in the quail's crop. Serial passages of this nature were used to perpetuate the disease until it was found that the prepared suspensions could be stored in a refrigerator at 4°C. for long periods and retain their virulence.

Stock quail have been maintained free of the disease by being kept in separate rooms or in outside pens. Inoculated birds were usually kept in individual pens, except when group infections were made. Pens with raised wire floors and metal batteries have been used for confinement in nearly all instances.

In all cases, the means of transmitting infection experimentally has been by placing material in the crop with a medicine dropper. This was easily accomplished by opening the bird's mouth and guiding the medicine dropper along the roof of the mouth. As natural infection apparently occurs by ingestion of the causative agent, all bacteria, filtrates, and suspensions have been given per os.

Tissues for histological study have been taken from sick birds that were killed for examination or from birds that had been dead only a short time. All tissue was fixed in ten per cent formalin and imbedded in paraffin. The alcohol, chloroform-paraffin method was used. Sections were stained by Delafield's hematoxylin and eosin.

Culture media used early in the investigation were made according to standard nutrient broth and nutrient agar formulae. Beef infusion broth and agar were used when standard nutrient broth and agar failed to yield results. Infusion media were also made with the breast muscles of chickens and turkeys.
replacing the beef in the standard formula. In addition, infusion media were made from the muscles and visceral organs of quail by the same method.

Serum media were prepared from the above, making serum agar and broth by adding to the media ten per cent sterile serum of bovine or avian origin. The serum was sterilized by filtration through Berkefeld candles or Seitz discs. Centrifuging the serum for twenty to thirty minutes before filtering, shortened the time required for filtering.

Glucose was used in some of the media in 0.5 per cent concentration. Cystine was also used as an enriching agent in the strength of 0.1 per cent.

**Aerobic Cultural Methods**

Aerobic isolations were attempted on agar plates both by agar shakes poured in plates and by streaking. Isolation was also attempted by inoculating broth tubes and transferring to agar plates. All organisms selected from agar plates were transferred to slants or broth tubes incubated from 24 to 48 hours, and then given to quail per os in large doses, two cubic centimeters of broth culture or equivalent suspension from slants. Morphological and staining characteristics of all cultures were determined by the Gram method. Mixed cultures from first inoculation of broth tubes from diseased quail were also tested for infectivity. Also cultures selected from agar plates have been mixed before being given to the test quail.

Filtrates of diseased tissue were prepared by grinding ulcerated intestines in a mortar with sand, suspending in water, and allowing to sediment, or by centrifuging a short time before filtering. Berkefeld candles of N and V porosity and Seitz (E K) bactericidal discs were used for filtering. Sterility of the material was checked by inoculating broth tubes. Infectivity of the original suspension was checked by inoculating birds with excess unfiltered suspension or with residue washed from the filters. All filtrates were administered per os. Various colony selections from agar plates have also been given in conjunction with the filtrates by using the filtrate to wash the growth from agar slants.

Isolation from liver, heart blood, and spleen was attempted by streaking agar plates and by inoculating agar shakes and broth tubes.

Cultural work on ulcers from intestines was preceded by thorough washing and maceration of the lesions. Small, well circumscribed lesions of apparently new formation were selected. The intestine was opened and washed for several minutes in running tap water. Then portions of the intestine containing small lesions were selected and further washed two to three
times by shaking in sterile saline (100 cubic centimeters in Erlemeyer flasks). The ulcers were then dissected from the intestine with needles and were washed again by shaking in culture tubes containing ten cubic centimeters of sterile saline. A convenient method for macerating the lesions was found to be that of using a hollow ground slide with a few drops of water. The closed end of a ten millimeter test tube, which was used for grinding the material in the depression of the hollow ground slide, was roughened on a carborundum wheel. The tube and slide was sterilized in the autoclave or by boiling. Small amounts of material may be handled conveniently by this method for preparing suspensions to be used for inoculating culture media.

**Anaerobic Cultural Methods**

Among the anaerobic cultural methods employed were:

1. Buchner's method in which a large tube with a rubber stopper was used with pyrogallic acid and sodium hydroxide. The tubes of culture media were boiled; then cooled rapidly, inoculated, and placed inside the larger tube.

2. Covering tubes of culture media with sterile mineral oil and paraffin after boiling and rapid cooling immediately prior to inoculation.

3. Plate cultures, anaerobic conditions being established by the use of pyrogallic acid and sodium hydroxide in dessicators. Four plates were employed for each anaerobic culture made.

4. Anaerobic jars, Smillie, (A. H. Thomas & Co.). The hydrogen combustion method was not used. Oxygen was removed by partial exhaustion with a vacuum pump and by the subsequent use of pyrogallic acid and sodium hydroxide.

5. Anaerobic isolations, using glass tubing of three millimeter inside diameter with both ends drawn to capillary points. Nutrient agar was boiled to drive off the oxygen in solution, cooled rapidly to 45°C, inoculated, and then drawn into such capillary tubes, the ends of which were sealed in a flame. Colony selections were made by breaking the tube near the desired colony.

**SYMPTOMS**

In general, the diseased birds appeared dull and listless, with little or no disposition to move about. They usually remained apart from other birds, standing in a humped, droopy position, with head and neck retracted, and eyes partly closed. (Fig. 1). The feathers were ruffled, standing out from the body, appearing
dull, lusterless, and dirty with loss of natural gloss. A diarrhea, usually with stools of a watery or semi-fluid consistency and a light yellowish-brown in color, were a regular finding. Occasionally stools were seen which were very watery and flecked with urates.

Fig. 1.—Quail affected with ulcerative enteritis.

There was usually a marked emaciation, especially in birds which showed symptoms for six to eight days or longer. Such birds may lose from one-third to two-thirds of their original weight. There was a marked anorexia in the cases of longer standing, and the crops were found empty. A dermatoxerasia and tightness of the skin were frequently seen in the emaciated birds.

The symptoms given above are descriptive of cases seen under spontaneous infection. Deviations from these were seen among artificially infected quail. The more acute cases produced by artificial infection may die without showing definite symptoms. Artificially inoculated birds have died in 20 to 36 hours following inoculation. In these birds the only symptoms were a diarrhea which appeared shortly before death, and indisposition to move, ruffling of feathers, and a watery condition of the eyes. The crop and gizzard were filled with feed.

Birds dying in three to five days following inoculation, regularly showed a diarrhea, dullness, ruffling of feathers, and an unkempt appearance, but did not show marked emaciation. Such birds were usually quite active until 24 to 48 hours before death, and closely simulated symptoms of a group in which an acute infection spreads rapidly by natural means.

GROSS PATHOLOGY

The primary lesions of ulcerative enteritis were confined to the intestinal tract. These lesions were seen on the mucosal surface as grayish yellow ulcers and were found from the
duodenum to the rectum, including the ceca. More frequently the ulcers were seen in the ileum, approximately ten centimeters anterior to and between the ceca.

Changes in the other visceral organs and peritoneum were regarded as secondary to ulcers which perforate the intestines, or as the result of hematogenic infections arising from the ulcerated areas.

Ulcers in the intestines may be multiple, involving nearly the whole of the intestine from the gizzard to the rectum. Usually the lesions were confined to the lower ileum; (Fig. 2) although cases have been observed where the only lesions found were located in the duodenum or the ceca. The size of the ulcers may vary from pin-point size to five millimeters in diameter. Ulceration was seen with greatest frequency in the ileum, ceca, duodenum, and jejunum, in the order named.

In a few cases, external examination of the intestines revealed no changes. However, the ulcerated areas can usually be seen through the serous surface. Most typically, the ulcers appeared as yellowish, rounded areas of necrosis beneath the serous surface or deeper in the intestinal wall. They were not raised above the serosal surface. Other ulcers that had extended

![Image of quail intestine with normal mucosal surface and ulcerative enteritis.](image-url)
through to involve the serosa appeared somewhat depressed in the centers, and in nearly all cases, there was a deposit of fibrin and purulent material over the surface. The coils of intestines in such areas were frequently bound in a firm mass by a fibrinous exudate and adhesions.

Frequently a discoloration of the intestines, particularly the duodenum was observed, consisting of a marked vascular engorgement, reddish-lavendar in color.

Occasionally stellate hemorrhagic areas were seen through the serosal surface of the intestine. These areas varied in size from small petechiae to three millimeters in diameter, and were bright red in color. Some of the larger hemorrhages showed brownish discoloration in their centers, and in others, the centers were yellowish in color so that there was seen the yellowish necrotic material of the ulcers surrounded with a ring of hemorrhage.

In the same intestine, there were seen through the serosa, ulcers without this encircling hemorrhagic area. The typical yellowish necrotic material was found in the mucosal surface of the intestine below the serosal hemorrhages.

With the intestine opened the lesions were seen as very small pin-point to three millimeter hemorrhages in the very early stage. The larger hemorrhages usually showed a necrotiz-
ing process which was seen as an area of grayish-yellow material in the centers. This necrosis was observed as becoming progressively greater until the whole hemorrhagic area was transformed into the typical ulcer.

The usual form of ulcer was seen to vary from scarcely visible to four or five millimeters in diameter. The ulcers may be rounded or oval in shape. Occasionally there was a fusion of ulcerated areas producing irregular forms. The surface of the ulcer was usually raised, rounded, and built up above the surface of the mucosa. Some large ulcers had a crateriform appearance with a hollowing out of their centers, and a brownish to black discoloration from the deposition of fecal material.

The smaller ulcers were usually firmly imbedded in the substance of the gut wall, or were attached in the villus processes and submucosa. The necrotic material making up the ulcer was of a somewhat pasty granular consistency and in the larger and older ulcers it became somewhat friable. Some very large patches of necrotic material seemed to be not imbedded deeply in the intestinal wall but built up on the tips of the villi. The edges of the ulcer were raised, and when the ulcer was stripped off, it left a granular, torn surface. The gross appearance of the ulcers did not differ greatly in various parts of the intestine. Perforations were more frequently seen in the lower intestine.

Ulcers in the ceca had a tendency to be larger, to have rolled edges, and to be more discolored from the fecal material while ulcers in the upper intestines, especially in the duodenum, tended to remain clean, with the usual grayish yellow color.

A localized peritonitis was frequently seen over ulcers that had involved the thickness of the wall of the intestine. Peritonitis may be confined over a loop of the gut, or may be seen as a larger area with fibrinous adhesions binding loops of the intestine together with their mesenteric folds.

Usually there was a hepatic and splenic involvement, but cases were seen in which the infection was well localized and there was no gross evidence of spread to the liver and spleen. Cases of generalized peritonitis were also frequently observed. A small number of cases from both artificial and spontaneous infections were seen in which there was a fulminating form of the disease. In these there was a great congestion of the visceral organs and a severe hemorrhagic enteritis which involved the whole length of the small intestine with a few or no ulcers. Three of the birds showed a catarrhal hemorrhagic inflammation of the glandular stomach (pars glandularis).

A variability of complicating lesions in the solid visceral organs was seen. Birds were frequently dead from ulcerative
enteritis when no changes could be detected in any of the organs, except the intestines. This condition was more frequently observed in birds which died six to eight days or longer following infection. Early deaths, under four to five days, nearly always showed a peritonitis and an involvement of other visceral organs.

HISTOPATHOLOGY

Sections from the intestines of birds with the acute form of ulcerative enteritis presented a variety of microscopic changes. In general, there was much desquamation of the epithelium, with considerable hemorrhage into the lumen of the gut.

![Quail intestine. Acute ulcerative enteritis. Note severe catarrhal inflammation. Cross section x-66.](image)

Sections from all parts of the intestine, taken from birds that died in two or three days were, except in the crypts, devoid of epithelium. The wall was edematous in all parts, especially in the submucosa. The blood vessels were greatly engorged. In the villi, the capillaries appeared devoid of all covering tissue, and were exposed to the lumen of the intestine. Some sections showed a large number of lymphocytes infiltrating the mucosa. The lumen of the intestine was filled with desquamated epithelium, blood cells, and fragments of the mucosa, (Fig. 4).

Some of the early ulcers were seen as hemorrhagic necrotizing areas of small size, involving the villi, and extending into the mucosa and submucosa. There was a destruction of the
tissue in the lesions, with an intermingling of blood cells and tissue remnants. Cells in the immediate area showed degenerative changes and coagulation necrosis with lysis and rhexis of the nuclei. Small masses of bacteria were seen in some of the crypts in the affected area and in the necrotic tissue. There were a few lymphocytes in the area; polymorphonuclears with rods were very few in number.

The larger grayish yellow ulcers appeared as thick, piled up masses of granular acidophilic coagulated material mixed with cellular detritus and bacteria. Nearly all of the structure of the mucosa was destroyed, except that at borders of the ulcers where it was seen in a coagulated necrotizing condition, (Figs. 3 and 5).

![Fig. 5.—Quail intestine. Ulcerative enteritis. From a bird dying six days following exposure. Note the well defined line of demarcation (1), below the necrotic material of the ulcer (2), and clumps of bacteria (3). The epithelium is intact except in the ulcer. Cross-section x-66.](image)

The ulcers extended into and through the submucosa and into the muscularis. The cells bordering the ulcers were seen in a coagulated necrosing condition with the presence of nuclear fragments and hemorrhage. Reactive cells in the area were lymphocytes, polymorphonuclears with rods, and large round cells. The endothelium of the vascular channels was quite swollen in many sections. A number of the granular leucocytes and large round cells was seen in the edematous areas, but the reactive cells were not a conspicuous part of the changes.
In the generally observed acute cases these changes as described were seen in conjunction with a general catarrhal inflammation of the intestine, along with the generalized vascular changes and lymphocyte activity.

Masses of bacteria were a common finding in the necrotic material making up the ulcer, and were frequently seen in crypts contiguous to the ulcers. In some sections, the bacteria were invading the tissue with evidences of cell destruction. In other areas, the changes were not marked. In the submucosa and muscularis small blood vessels near the ulcers were sometimes found completely occluded by bacteria. In some sections, the intravascular masses of bacteria were quite removed from the site of the ulcer.

Ulcers of the perforating type were similar in appearance to those previously described (Fig. 6). There was the typical necrotizing change with a hemorrhagic and a hyperemic zone and reactive cell zone demarcating the ulcer. This line of demarcation may be seen at any depth in the intestinal wall. In the perforating ulcers there was a destruction of the entire thickness of the wall, including the visceral peritoneum, with a fibrino-purulent deposit on the serosal surface. Masses of bacteria were seen at any depth in the ulcer, frequently near the serosal surface, or in the vascular channels draining the area.

Fig. 6.—Quail intestine—acute ulcerative enteritis. Perforating form of ulcer. Necrotic material with bacteria (1), line of demarcation (2), coagulation necrosis of the muscularis (3), bacterial occlusion of blood vessel (4). Longitudinal section x-66.
The ulcerated areas presented much the same appearance when seen in very acute or in less acute cases. However, in the more chronic cases in which ulcers were well separated, the intestinal epithelium was intact except in the ulcerated areas. In sections of the intestine that were well removed from the ulcers, the entire section appeared normal. Undermining ulcers were not found.

Some sections showed ulcerated areas involving only the tips of the villi. The villi immediately under the granular acidophilic material of the ulcer were devoid of epithelium and had the appearance of coagulation necrosis. In one section the surface epithelium at the edge of the ulcer was intact, seeming to have grown beneath the edge of the necrotic material of the ulcer.

In sections from one bird which died seven days after the appearance of symptoms, there was evidence of a reparative process. Ulceration in these sections had extended through the submucosa, involving the muscularis. The normal structure of the muscularis was destroyed, the area being filled with early granulation tissue. Large numbers of polymorphonuclears with rods and round cells were present, especially in the area demarking the ulcer from the granulation tissue. Had the bird lived, the healing process would probably have been completed by forming a constricting scar.

Close examination of the nuclei and cytoplasm of cells from the various organs, liver, spleen, kidney, heart, lungs, and intestines failed to reveal the presence of inclusion bodies of any kind.

**BACTERIOLOGY**

As the etiological agent of ulcerative enteritis has not been definitely determined, much of the work of this investigation has been directed toward the isolation of a causative factor. Aerobic and anaerobic cultural methods, reduced oxygen tensions, and filtration of material for filterable viruses were included in the study.

The Corynebacterium perdicum isolated by Morley and Wetmore* produced inconsistent results when they fed cultures to susceptible birds. An anaerobic organism as described by Bass* has not been found. Shillinger* states that pure cultures isolated from lesions have not produced the disease with sufficient regularity to establish them as the causative agent.

In this investigation, all selections of organisms from aerobic cultures have regularly failed to produce the disease. Various media have been used in streak and pour methods for isolation. In each attempt to isolate an organism, colonies were selected to represent all growths on the agar plates and transferred to
broth or solid media. These cultures were incubated 24 to 43 hours and were then given to susceptible quail. A number of enriched media have failed to grow an aerobic organism pathogenic for quail when given per os.

The various organisms selected have been given in mixtures to susceptible birds to determine if the disease producing factor was of a symbiotic nature. Among the organisms selected from the cultures were Gram positive cocci, a small micrococcus, and a variety of Gram negative rods. The small micrococcus has been found with great regularity. Large Gram positive rods have also been isolated. Identification of these organisms was not attempted as none of them either singly or in mixture would produce ulcerative enteritis in quail. The birds receiving the cultures were proved to be susceptible to ulcerative enteritis by giving infective intestinal suspensions two or three weeks after they received the cultures. In all cases, the birds succumbed in three to five days to the infection with suspensions of ulcerated intestines.

In one instance a bird receiving a culture of a Gram negative bacillus died six days later with ulcerative enteritis. Subsequent transfers and inoculations from this culture failed to produce any symptoms in quail.

There was a total of forty selections of colonies from plates incubated under aerobic conditions, none of which could be associated with the disease.

**Filtrates Negative**

Attempts to produce the infection with filtrates of suspensions of ulcerated intestines have given negative results. Bacteriologically sterile filtrates from Berkefeld candles of N and V porosity and Seitz (E K) discs have failed to produce the disease. In all cases the residue from the filtration was infective for susceptible quail. Filtrates were used to suspend various selections of bacteria before giving them to quail, but no infection was produced. The filtrates were administered per oris in quantities of four to eight cubic centimeters.

Anaerobic cultural methods yielded negative results in all cases. The culture media were for anaerobic cultures and were the same as that used in aerobic methods. In all cases the media was boiled then cooled rapidly before inoculating. Colony selections made from pour plates and glass tubes were transferred to agar slants or to broth tubes and incubated 48 hours before inoculations were made. Thirty anaerobic colony selections were made but none were pathogenic for quail when given per oris. Mixed anaerobic broth cultures made from washed macerated lesions dissected from the intestines were noninfective.
The infectious agent in the form of suspensions of ulcerated intestine was stored in a mechanical refrigerator at 4°C for long periods. Two suspensions, similarly prepared by macerating the diseased tissue in tap water and storing in a refrigerator were found to be infectious for quail when given per oris at 163 days and 169 days. These suspensions were stored in glass vials with cork or rubber stoppers. Attempts to isolate an organism from these suspensions, which would produce ulcerative enteritis, failed.

Exposure of the infectious principle in soil has shown it to remain viable 47 days. Soil in a working condition was pulverized and then made into a pasty condition with an infectious suspension of ulcerated intestine. This wet soil was allowed to dry to working condition and then was pulverized again. Following this procedure petri dishes were filled with the material; one was stored in a refrigerator at 4°C. and another buried one inch deep on the north side of a building with atmospheric temperature ranging from 10°C. to 22°C. Both groups of contaminated soil were found to be infectious 47 days following preparation.

**RESISTS DRYING**

Infected intestinal contents placed on a glass plate and allowed to dry in a desk drawer at room temperature for 47 days when resuspended in water were found infectious for quail. However, a suspension of ulcers from the same bird when subjected to similar exposure was found to be non-infectious at 47 days.

It would appear from this test that decomposition probably destroyed the infectious agent in the suspension, whereas the agent was preserved 47 days by drying the intestinal content.

Heating of suspensions of infective material at 60°C. for ten minutes destroyed the disease-producing agent.

**MISCELLANEOUS OBSERVATIONS**

Of the more than one hundred birds artificially exposed there were four which did not show symptoms. Two of these birds were exposed twice with massive doses of infectious material. Following this observation, tests were made to ascertain whether or not serum from these two birds would protect other birds against infection.

Two healthy quail were given 0.7 cubic centimeters and 0.3 cubic centimeters of serum taken from the resistant birds. Two days later each bird was given approximately one cubic centimeter of an infectious suspension. These serum treated birds died from ulcerative enteritis with no difference in incuba-
tion period, acuteness of symptoms or lesions from those of the controls.

A number of attempts have been made to transmit the disease to domestic fowl, domestic turkey, and chukar partridge. Material used in these attempts was suspensions of ulcerated intestine from quail. A dose of 0.5 cubic centimeters of the material was regularly infective and lethal for quail. Doses for the other birds have been large, ranging from four to eight cubic centimeters.

Turkey poults varying in age from two to five weeks have been exposed to large doses (four to eight cubic centimeters) of infectious suspensions by way of the mouth. A total of fifteen poults were exposed at different times without producing symptoms or death in any of the birds. Control quail receiving the same suspensions regularly succumbed to the infection. Young chicks, four to five weeks of age, have been exposed to infectious material from quail. Dosage with intestinal suspensions similar in amount to that given the turkey poults failed to produce symptoms or death in fourteen chicks.

CHUKAR PARTRIDGE HIGHLY RESISTANT

A small number of cases of ulcerative enteritis have been observed in the chukar partridge. These cases were complicated by a marked infection of coccidiosis. Large numbers of oocysts could be found in direct smears from the ceca. Three cases as described were received from a game farm. The intestinal contents and ulcers from one bird produced typical cases of ulcerative enteritis in a young chukar and in two quail. However, material taken from quail which died of ulcerative enteritis has proved noninfectious to eleven chukar partridges. With the exception of two adult birds these partridges had been on raised wire floors since hatching, with no known previous exposure to ulcerative enteritis. Ages of the exposed birds varied from one to five months.

A naturally occurring epizootic was observed where sixteen quail and twenty chukar partridges were cohabitating. All of the quail were dead from ulcerative enteritis eight days following the first death. No symptoms were noted in the chukar partridges at any time while the quail were dying from the disease. The chukars were held in the same pen for two months without exhibiting symptoms or having death loss from ulcerative enteritis.

MORTALITY RATE IN QUAIL

Considerable difference in mortality rate and in acuteness of the disease has been observed in different forms of exposure. In three groups of birds receiving exposure in different ways there was a marked difference in the time required for deaths
<table>
<thead>
<tr>
<th>GROUP I</th>
<th>Twenty-five healthy quail exposed to a pen in which three birds had died three days previously from ulcerative enteritis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of Exposure</td>
<td>1 to 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22</td>
</tr>
<tr>
<td>Number of deaths from Ulcerative Enteritis</td>
<td>0 1 0 1 2 1 4 3 2 5 0 0 1 2 3</td>
</tr>
<tr>
<td>GROUP II</td>
<td>Twenty-nine healthy birds exposed in a pen in which other birds were sick and dying from ulcerative enteritis</td>
</tr>
<tr>
<td>Days of Exposure</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12</td>
</tr>
<tr>
<td>Number of deaths from Ulcerative Enteritis</td>
<td>0 7 2 4 4 4 1 1 2 4</td>
</tr>
<tr>
<td>GROUP III</td>
<td>Fifty-two birds inoculated per oris with suspensions of ulcerated intestines from dead birds</td>
</tr>
<tr>
<td>Days following Inoculation</td>
<td>1 2 3 4 5 6 7 8 9 10 11</td>
</tr>
<tr>
<td>Number of deaths from Ulcerative Enteritis</td>
<td>4 17 8 9 6 5 3</td>
</tr>
</tbody>
</table>
to occur and in the mortality rates. Table 1 and Fig. 7 show the number of deaths occurring by consecutive days following inoculation with infectious material and exposure to contaminated quarters.

The birds in group one, Table 1, were placed in a pen in which three infected birds were held until they died from ulcerated enteritis. The birds in this group were all dead from ulcerative enteritis in twenty-two days. The earliest death occurred in nine days with twenty per cent of the birds dead within 13 days. Fifty-six per cent died between the fourteenth and seventeenth day while twenty-four per cent succumbed between the eighteenth and twenty-second day.

![Chart of curves showing the mortality rate by days of quail affected with ulcerative enteritis when exposed to infection by different methods as described in Table 1.](image)

Fig. 7.—Chart of curves showing the mortality rate by days of quail affected with ulcerative enteritis when exposed to infection by different methods as described in Table 1.

Group I—25 birds. Healthy quail exposed to infection in pen in which 3 birds had died three days previously.

Group II—29 birds exposed in a pen in which other birds were sick and dying.

Group III—52 birds inoculated per oris with suspension of ulcerated intestine.

In group two, all of the birds were dead in ten days. These twenty-nine birds were exposed in a pen in which other birds were sick and dying from ulcerative enteritis. The deaths in this group occurred much earlier than in group one. Seven of the birds were dead on the second day of exposure with 63% dead at five days while 35% died between the fifth and tenth day. All birds were dead at ten days.

Group three represents birds that were infected artificially to maintain a source of infectious material or to be used for cultural purposes; all fifty-two birds in this group died from the infection. The maximum time until death was seven days,
with only three birds living until the seventh day. Deaths occurred much earlier than in the naturally exposed groups, with four deaths on the first day and seventeen on the second. Mortality by the fifth day was 84% with 16% dying on the sixth and seventh days.

Table 2, shows the relative incubation periods for the three groups. For groups one, two, and three, the average incubation period was sixteen, six and three and one-half days respectively. In groups one and two, the time from potential exposure until death was reduced approximately two-thirds by increasing the amount of contamination in the pens. These figures are calculated from the time of potential exposure since the time of actual contraction of the disease could not be determined.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to Infected Cages</td>
<td>9</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Infected Cages &amp; Sick Birds</td>
<td>22</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Oral Inoculation</td>
<td>16</td>
<td>6</td>
<td>3.5</td>
</tr>
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</table>

Observations on young quail of one to two weeks of age indicate that severe losses may occur from ulcerative enteritis without typical ulceration of the intestines being manifested. Numerous young quail have been autopsied by the authors in which the only change noted was a severe hemorrhagic enteritis. No protozoan organisms could be found in the intestinal contents or droppings. Changes in the visceral organs are variable or may be absent. From a number of such young birds, the intestines and contents were ground and given to adult quail, producing ulcerative enteritis in its typical form.
DISCUSSION

Ulcerative enteritis in quail produces a uniform aggregate of symptoms and characteristic lesions in the intestines. The ulcers in the intestine are typical and pathognomonic for this disease. The symptoms, however, may be simulated by other diseases, especially those of a chronic debilitating nature in which the sick birds look and act much like those affected with chronic ulcerative enteritis.

Birds may be found dead presumably having died from ulcerative enteritis in which no ulceration of the intestine is found. This phenomenon was observed in birds which died in one to two days following infection and particularly in young birds one to two weeks of age. Lesions of note are an acute catarrhal and hemorrhagic enteritis with much blood in the intestinal contents. Intestinal contents from both young and adult birds which present this fulminating form of the disease will transmit the infection to healthy susceptible adults, producing typical lesions in the intestines. A very rapid and high mortality rate is associated with this disease in young birds.

Domestic fowl and turkeys used in this investigation failed to show any symptoms from massive doses of known infectious material. Control quail receiving one-fourth to one-eighth of the amount which was given to chicks and pouls died from ulcerative enteritis. Chukar partridges, however, have been observed with typical lesions of ulcerative enteritis.

Transmission from chukar to chukar was accomplished in only one instance. This case and others observed in chukar partridges have been complicated with coccidiosis. The degree of the infection with coccidia, as judged by the number of oocysts present in smears of cecal contents, has varied from light to severe. It is considered possible that the coccidia could act as a predisposing agent by lowering the general resistance of the chukar, or that damage to the intestinal epithelium offers a point of entry for the causative agent. Healthy chukar partridges proved refractory to artificial exposure with large doses of infectious material taken from quail. Chukar partridges have also proven refractory to infection when cohabitating with diseased quail.

Ulcerative enteritis is a highly infectious and contagious disease. This is shown by the group infections. In group one, (Table 1) in which the birds were allowed to acquire infection from quarters which were contaminated three days before their confinement, there was a longer lapse of time before death occurred. Also there was a rise to a peak in mortality rate and then a drop in mortality before all birds died. In group two, (Table 1) healthy birds were exposed in pens in which other
birds were sick and had died of ulcerative enteritis. These birds died much earlier following exposure. Seven were dead on the second day and all were dead from ulcerative enteritis by the tenth day as compared to the ninth day for the first death in group one.

That there was a much greater concentration of the virulent agent in pen two is very evident. This means that birds would have the opportunity through the presence of more contamination to contract the disease sooner; hence the higher and more rapidly mortality rate. The mortality and incubation period in group two simulated the condition in the artificially exposed group. These groups of birds predict the course of an epornitic under conditions where quail are held in flocks.

Attempts to isolate the agent causing ulcerative enteritis have met with failure. Filtrates prepared from suspensions of lesions have failed consistently to produce the disease. That the causative agent is not filter passing is proven by the regular infectivity of the residue or supernatant fluid. Massive doses of filtrates were used in comparison to the small amount of residue required to produce the disease. Histological studies also show no inclusion bodies, intranuclear or cytoplasmic, as produced in some virus diseases.

A bacterial agent as the cause of the disease was not found. Various organisms selected on different culture media and by selected cultural methods have failed to yield results. Seventy colony selections from aerobic and anaerobic cultures from lesions in the intestines were given to healthy susceptible birds. These organisms have not produced the disease either singly or in mixtures.

Protozoan organisms have not been demonstrated in the lesions or intestinal contents of the quail. Apparently there is no association of this disease with protozoan parasites.

Since filtrates will not produce the disease, and no protozoa were present, a bacterial cause is suspected. This is supported by the length of time that the causative agent will remain viable in storage in the refrigerator at 4°C., in soil, and in dried intestinal contents. Its destruction by heat (60°C. in ten minutes) indicates that the cause is probably a non-spore-forming organism.

Since the natural infection is apparently contracted by ingestion of the causative agent, all cultures, filtrates, and suspensions have been given per oris. Injection of suspensions of the ulcers into the blood stream produced a septicemic disease comparable to changes seen in the solid viscera in cases with perforating ulcers. No intestinal lesions have been produced by injection of infectious material. The same material when
given per os produces typical intestinal lesions. Contamination of feed and water also produces the disease in typical form.

Bacterial emboli found in kidneys, spleens, lungs, and heart are regarded as agonal showers since very little or no inflammatory changes were associated with them.

SUMMARY

Ulcerative enteritis is an infectious contagious epizootic of undetermined etiology, primarily affecting quail. It is characterized by a high morbidity and mortality in all ages, with the formation of rounded to oval, raised, grayish yellow ulcers in the intestine, usually the lower ileum. Death usually results from a terminal systemic infection arising from the ulcers.

The etiological factor in this disease has not been determined. Exhaustive use of Berkefeld and Seitz filtrates prepared from ulcers has shown the causative agent is not a filter-passing virus. The supernatant fluid or residue from the filtration has in all cases proven infective for quail.

No protozoan organism has been associated with the etiology of ulcerative enteritis in quail. Findings in the intestinal contents and smears from ulcers have been negative. A large number of histologic sections have not shown protozoan organisms. However, limited observations on this disease in chukar partridges indicated that coccidia may act as a predisposing agent.

Although no organism was isolated which would produce the disease, the etiological factor is thought to be of bacterial nature. This idea is supported by the infectivity of residues from filtration, and the ease with which the infectious material may be maintained outside the host. In the form of macerated lesions the etiological agent has remained viable 168 days in the refrigerator at 4°C, 47 days in moist soil, and 47 days in dried intestinal contents. The infectious agent is also destroyed by heating at 60°C for ten minutes. The contagious nature of the disease, and the failure to incriminate a filterable virus or protozoan organism also indicate an etiological factor of bacterial nature.

Quail have been found to offer very little resistance to this disease. Approximately 250 birds were observed during this investigation, and no bird which showed symptoms following exposure or inoculation per os with known infectious material has recovered. From more than one hundred birds which were given infectious suspensions only four failed to show symptoms of the disease. Two of these birds were subjected to repeated inoculations without producing symptoms. Small amounts of
serum from two of these resistant birds failed to protect healthy birds against the disease.

Chukar partridges have been markedly resistant to ulcerative enteritis, but cases have been observed in conjunction with coccidiosis. Domestic chicks and poults have been found refractory to this disease.

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