

PRODUCTION OF FATAL BLACKHEAD IN TURKEYS BY
FEEDING EMBRYONATED EGGS OF HETERAKIS
PAPILLOSA.

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Since the protozoan agent of blackhead was described and its relation to the lesions of the ceca and liver defined by one of the authors¹ in 1895, very little concerning the mode of transmission of the agent or the conditions favoring its invasion and multiplication has come to light. In 1895 Moore² fed the liver and ceca of three diseased turkeys to two healthy ones. The days of feeding were Nov. 28, Dec. 3, and Jan. 3. On Jan. 11, 44 days after the first feeding and 8 days after the last feeding, one of the turkeys died of blackhead. In another experiment beginning Nov. 28, Moore penned four healthy turkeys with two diseased ones. During the night for a period of about 2 weeks the feces of the sick ones were collected and fed, mixed with the feed, to the well ones on the following day. One of the two sick turkeys died in 3 days and the other survived. Of the four which received the discharges and were also exposed during the day to the sick ones and their environment, three became diseased. Two died in 22 and 27 days respectively; the third bird, well when killed 46 days after the beginning of the experiment, showed extensive lesions of blackhead.

In Sept., 1913, one of the authors³ fed two incubator turkeys, hatched early in June of the same year and reared on virgin soil, the chopped up diseased ceca of two turkeys 2 to 3 months old. Both remained clinically well up to Dec. 24 when one was stolen. An autopsy on the other 3 days later showed it to be normal.

New Experiments in Feeding Feces.

During the years 1918 and 1919 certain attempts to induce blackhead by feeding feces of older turkeys which had successfully passed through exposure to disease in the preceding year were made. These

¹ Smith, T., *U. S. Dept. Agric., Bureau Animal Industry, Bull. 8*, 1895, 7.

² Moore, V. A., *U. S. Dept. Agric., Bureau Animal Industry, Circular 5*, 1896, 1.

³ Smith, T., *J. Med. Research*, 1915, xxxiii, 243.

attempts, although leading uniformly to negative results, are detailed below because of their bearing on subsequent more successful trials. It should be stated here that cohabitation in the same enclosure with the turkeys furnishing the feces produced blackhead in a certain number of young turkeys.

Experiment 1.—June 10, 1918. A male bird, 2 years old, was confined in an isolation unit after all soil had been washed from its feet. The feces were collected and fed to four young turkeys confined in a cage in the same unit. The turkeys were 30 days old. The feces were mixed with the food and fed on June 11, 12, 13, and 14. The experiment was closed on July 9 and the turkeys were released. At no time during a period of 28 days did they show symptoms of blackhead. These turkeys were used in other experiments during the season but none of them developed blackhead. One died on July 22 of a disease of the bones, resulting in all probability from confinement in the cage. The others were killed at the close of the year, one on Dec. 10 and the others on Dec. 20. The organs were uniformly normal.

Experiment 2.—Three turkeys, hatched on May 12, 1918, were fed feces of two old turkeys under the following conditions: The feces were collected on Sept. 2 and 3. A portion was fed on Sept. 2 with the food, morning, noon, and night. The remainder was placed in Petri dishes at room temperature until fed. The later feedings took place on Sept. 6, 9, and 13.

The young turkeys had been running with some chickens in a special enclosure and were kept in it except on the dates of the feeding. The feeding was done in a cage. They remained well and when two were killed late in Dec. no lesions were found. The older turkeys from which the feces were obtained belonged to an enclosure in which young had contracted blackhead. It will be noted that in this trial an incubation of the feces at room temperature for 3, 6, and 10 days respectively failed to make the test positive.

Experiment 3.—The feces of adult turkeys were fed to chickens.

In view of Milks⁴ observations on the occurrence of blackhead in young chickens, it was believed that very young chickens would be suitable for feeding experiments, and such an experiment was undertaken in the spring of 1919, before young turkeys were available. This experiment was conducted in a brooder and was begun on Apr. 4, when the chicks were 8 days old. Six chicks were used, two of each of the following breeds: Rhode Island Red, White Leghorn, and Barred Plymouth Rock. The feces were collected as fresh as possible from the coops occupied by the old flock kept for breeding purposes. It consisted of eight birds, and in the main contained the same ones that were in it the previous year. Feces having different physical characters were collected. Those not fed fresh were kept in Petri dishes at room temperature. In all, from Apr. 4

⁴ Milks, H. J., *Louisiana Agric. Exp. Station, Bull. 108*, 1908, 1.

to 17, feces were fed on 12 days. They ranged in age, counting from the date of collection, from less than 1 to 5 days. The chicks at no time showed symptoms of blackhead. One showed lameness due in all probability to confinement.

The manner of feeding the feces was then modified to reproduce more nearly natural conditions. On Apr. 17 a galvanized iron tray was placed in the outer compartment of the brooder and filled with sterilized soil. Feces were placed from time to time on the soil and the feed was always placed there. The soil was kept moist. Fresh and 1 day old feces were placed on the tray on Apr. 17 and on Apr. 18, 19, 20, 22, 23, 24, and 25 fresh feces were placed there.

Aside from lameness the chicks showed no other symptoms. Three of them were chloroformed on May 2, and three on May 6, 28 and 32 days respectively after the beginning of the experiment, and all were found free from blackhead.

After it had been found that by placing young turkeys with the old flock, subsequent to removal to new soil, they contracted blackhead, it was decided to undertake still another experiment feeding the feces of the old flock to young turkeys.

Experiment 4.—The turkeys of the old infected flock were removed by twos and placed in an indoor unit to facilitate collecting feces of all alike. The feces not fed fresh were kept in Petri dishes at room temperature. Beginning on Aug. 7, four young turkeys 36 days old, two white and two bronze, were fed in a cage. The feeding was carried out daily, with the exception of Sunday, to Aug. 19. After the feeding they were still kept indoors, but in a pen to enable them to be more active. The feces from eight older birds were used. When fed they were either fresh or up to 8 days old. On Sept. 5 the young turkeys were removed to an outdoor enclosure. 23 days after the last feeding the experiment was closed. None had shown signs of disease in the meantime. Owing to a shortage of incubator turkeys these were used again in later experiments.

Feeding Experiments with Embryonated Eggs of Heterakis papillosa.

In experiments conducted in 1914,³ 1916,⁵ and since that time, relating to the mode of transmission of the disease, it appeared that young diseased turkeys which did not recover were incapable of transmitting blackhead to healthy ones penned with them. In addition to this, it became evident that soil recently occupied by old turkeys was infectious to young ones.⁶ These observations along with negative results in feeding feces suggested the existence of some

³ Smith, T., *J. Exp. Med.*, 1917, xxv, 405.

⁶ Smith, T., and Graybill, H. W., *J. Exp. Med.*, 1920, xxxi, 633.

additional factor or factors in the transmission of blackhead. Previous experience and experiments gave no direct clue as to the nature of such a factor. There were a number of hypotheses which might fit the conditions observed or at least not be contradicted by them. The protozoan parasite invading the walls of the ceca and the liver evidently multiplied very rapidly when once started. It was also evident that this multiplication came to an abrupt stop and that the parasites in the tissues perished rapidly owing to an acquired immunity of the host or some other factor operating against the invading parasite at the height of the invasion. It was assumed that this parasite might be aided by some lesion or injury of the mucosa of the ceca to enter the tissues and multiply there, since the invasion in itself did not seem to be a normal part of the life cycle of the parasite. There was no evidence of the formation of any resistant stages in the tissues or of the normal discharge of the parasite outward, as, for instance, in coccidiosis. If the parasite was not to be regarded as wholly aberrant, whatever normal cycle there was seemed to belong to the lumina of the ceca. In harmony with this hypothesis a nematode parasite, *Heterakis papillosa*, occurring in the ceca of turkeys, chickens, and some other birds, was brought into the experiment as the possible associated factor.

Experiment 5.—This experiment consisted in feeding to young turkeys embryonated eggs of *Heterakis papillosa* and feces from adult turkeys. Worms were collected from the ceca of healthy chickens, killed for this purpose, by washing and sedimenting the contents and picking out the worms. These were cut up in a small quantity of normal salt solution to liberate the ova. The suspension was kept in Petri dishes at room temperature and distilled water added from time to time to make up for evaporation.

After the cultures had been kept for 17 days, examination showed that the ova contained living embryos. On Sept. 11 they were added to the feed of two turkeys which had been used in the preceding experiment. To ensure the complete ingestion of the mixture the turkeys were placed in a cage. On the same and the 2 following days feces from two older turkeys, collected on Sept. 10 and 11 were fed to the same birds. They were then placed with two other turkeys, also from the preceding experiment, which were to act as controls, in an outdoor enclosure.

Both infected turkeys became sick on Sept. 26; *i.e.*, 15 days after the feeding of ova. One died of blackhead after 3 days, the other after 6 days. The two control birds remained well and 43 days after the beginning of the experiment they were used in Experiment 7.

Experiment 6.—In this experiment the two factors, embryonated eggs of *Heterakis papillosa* and feces of adult turkeys, were kept distinct.

The young turkeys used in this experiment were from a group consisting of portions of three different broods of incubator turkeys, numbering in all about thirty individuals.⁶ They had been running together for some time in a large enclosure. In some unknown way the parasite of blackhead had been introduced into this enclosure and six out of eight turkeys in one brood died of this disease during June and the first few days of July before the broods were mingled. Following these cases one turkey died of blackhead on Aug. 13 and another on Oct. 20. *Heterakis papillosa* was known to be present from some of the autopsies made. The latter was the only case that appeared in the remainder of the flock after the birds chosen for this experiment had been removed. Of the turkeys used Nos. 308, 309, and 312 were hatched on May 12, 1919, Nos. 360, 361, and 362 were hatched on May 27, and Nos. 345, 346, 350, 351, 352, and 353 on July 2. The turkeys were separated into four groups on Oct. 16: Nos. 309, 312, and 352 (controls) were fed nothing. Nos. 346, 350, and 360 received embryonated eggs plus turkey feces. Nos. 345, 353, and 361 received embryonated eggs only. Nos. 308, 351, and 362 received turkey feces only.

The *Heterakis* eggs used in this experiment were obtained from worms collected from the ceca of four chickens on Oct. 1, and were prepared for culture and incubated as in Experiment 5. The feces were obtained from two older turkeys of the infected flock, the same ones that were used in the preceding experiment, and were less than 48 hours old when fed.

The three feedings of ova and feces, of ova, and of feces, respectively, were conducted in separate enclosures and the three groups of turkeys were brought together after the feeding in a new enclosure with the controls, care being taken to remove all traces of soil from the feet by washing them thoroughly before the groups were finally assembled.

The result of the experiment is briefly told. Of the group which was fed eggs plus feces, Nos. 346 and 360 showed symptoms on Oct. 28, and No. 350 on Oct. 29; *i.e.*, in 12, 12, and 13 days respectively. They were killed and autopsied a few days later and the diagnosis was confirmed by the lesions in ceca and liver. Of the group fed ova only, Nos. 345 and 361 showed symptoms on Oct. 27, and No. 353 on Oct. 29; *i.e.*, in 11, 11, and 13 days respectively. Several days later they were killed and the autopsy showed marked lesions of ceca and liver.

The three that were fed feces only showed no symptoms of disease and were killed and autopsied, two on Nov. 10, and one on Nov. 11; *i.e.*, about 2 weeks after all those fed eggs had become sick. No lesions were found in these birds. A careful search for *Heterakis* in the cecal contents showed the presence of one mature male in No. 308, eight mature males in No. 362, and four females, two males, and one larval nematode in No. 351.

The three control birds showed no symptoms of disease and were killed on Nov. 10 and 11. One of these (No. 352) had lesions of blackhead; the others were entirely normal. In the ceca of No. 309, two mature males and one female, and in

No. 312, one female *Heterakis* were found. In the affected control both ceca showed many elevated indurations up to 0.5 cm. in diameter. The largest had a central superficial slough or scab. In the liver only a few small whitish foci, 1 to 2 mm. in diameter, and one focus of congestion were found. In fresh preparations from the ceca a few *Heterakis* eggs and one larval worm were found. In the washed and sedimented feces two mature and two immature females of *Heterakis* and many larval worms with attenuated posterior extremity and a bulbous esophagus were found. The mouth was without buccal capsule and provided with four papillæ.

The lesions of this case suggest that the disease may have been produced by something that occurred after the beginning of the experiment. This bird may have ingested ova from the adult worms in the other birds or perhaps embryonated eggs passing through the intestines of the others soon after they had been fed. In any case, the causes bringing about disease in this instance will probably be cleared up in future experiments when the external sources of the two factors, *Amoeba meleagridis* and *Heterakis papillosa*, shall have been more definitely located.

Experiment 7.—This test may be regarded in a way as supplementary to Experiment 5. The two controls there surviving were fed 43 days after the beginning of Experiment 5 with embryonated eggs of *Heterakis papillosa*. The worms were collected from four chickens and the ova incubated as heretofore for 16 days, when they were fed mixed with the daily ration. The feeding was done on Oct. 24. One turkey became ill on Nov. 4, the other on Nov. 6; *i.e.*, 11 and 13 days after feeding. They were chloroformed and the diagnosis was confirmed by finding lesions of ceca and liver.

Experiment 8.—Owing to the lateness of the season, only one additional test was undertaken. This consisted in feeding the contents of the ceca of turkeys affected with blackhead which had died or had been chloroformed and the feces of older turkeys, both incubated for 15 days, but free from *Heterakis* eggs.

The contents of the ceca of four diseased turkeys were suspended in normal salt solution and passed through a No. 40 wire mesh. The strained suspension was placed in Petri dishes in shallow layers. The incubation began on Nov. 3. Careful examination of the fluid under a low power failed to show the presence of ova.

Nov. 3. The feces of older healthy turkeys were collected, suspended in water, and passed successively through a tea strainer and wire screens of Nos. 40, 60, 100, and 200 mesh respectively. The final sediment was washed twice in normal salt solution and distributed into four Petri dishes. Nov. 14. The incubated dishes were carefully examined under a low power. Two *Trichosoma* ova but no others were detected.

Nov. 18. Nine turkeys, taken from the group from which those in Experiment 6 came, were selected. Three were reserved as controls, three fed cultures from diseased turkeys, and three cultures from older turkeys. After the feeding the three groups were brought together in one outdoor enclosure. No symptoms of disease appeared, and the birds were killed and autopsied as shown in Table I.

It will be noted that the turkeys were killed 21 and 29 days after the experiment had been begun. In the collection of the worms the contents of the ceca were washed and sedimented. Lesions were uniformly absent.

TABLE I.

Turkey No.	Date killed.	No. of <i>Heterakis</i> individuals.
	1919	
310	Dec. 9	1 adult.
366	" 17	8 adults.
367	" 17	14 "
368	" 9	15 "
369	" 17	15 "
370	" 17	10 "
371	" 17	19 "
372	" 17	8 "
373	" 9	6 " and 2 young males.

DISCUSSION AND SUMMARY.

In four experiments, three with young incubator turkeys and one with young incubator chickens, in which the feces of old turkeys from an infectious flock, kept at room temperature up to 5, 8, and 10 days, were fed, no infection resulted.

In an experiment in which two of four young incubator turkeys used in one of the above experiments were fed embryonated eggs of *Heterakis papillosa* and feces of turkeys from an infectious flock both contracted blackhead. Two controls remained well. Later they were fed embryonated eggs of *Heterakis papillosa* and both contracted blackhead.

In another experiment three incubator turkeys received embryonated eggs plus turkey feces from an infectious flock. All contracted blackhead. Three received embryonated eggs alone; all contracted blackhead. Three received turkey feces only; none contracted blackhead. Three controls received nothing; one showed blackhead lesions at the autopsy.

In a final experiment three turkeys were fed cultures of feces from the ceca of diseased turkeys, three were fed cultures of feces of old turkeys from an infected flock, and three controls were fed nothing. None contracted blackhead. The cultures of feces were prepared

precisely as were the earlier ones containing *Heterakis* eggs but without the latter.

From these experiments it becomes evident that blackhead may be produced in healthy incubator-raised turkeys, reared in the open in an environment where blackhead occurs, but out of direct contact with old turkeys and other poultry, by feeding cultures of embryonated eggs of *Heterakis papillosa*, prepared by cutting up the worms in isotonic salt solution and incubating the suspension at room temperature.

These very definite and clear-cut results outweigh any objections which may be raised against the use of turkeys which had been in earlier experiments and which came through such experiments without any signs of disease, or which came from control flocks in which spontaneous cases had occurred. The short time elapsing between feeding embryonated eggs and the first signs of disease made these experiments unusually impressive. It should be stated, furthermore, that from a precise individual record of all turkeys it was possible to select birds from control flocks in which the infection had either not appeared or was very low. All but two turkeys in flocks serving as sources of this material were killed at the close of the year. None at any time had shown symptoms of disease, and no scars or other abnormalities of ceca and liver were found. Furthermore, all other control birds and those in field experiments, with the exception of two reserved for breeding, were likewise killed. As a result of these autopsies, it was determined that of all birds in which symptoms of disease had not been recorded during life, none showed abnormalities or scars at autopsy. The protozoan factor in blackhead was probably disseminated when the first spontaneous cases occurred in the stock, unless it was present and made invasive by incubation in the cultures fed. This latter theory seems at present not acceptable because of the wholly negative outcome of Experiment 8.

The production of acute blackhead by feeding embryonated eggs to turkeys in whose ceca adults of *Heterakis papillosa* are already present seems incomprehensible at first thought. A tentative explanation to be offered is that the worms when invading the ceca in large numbers break down the resistance of the bird which is able to protect itself against a few. This may account for the very irregular

occurrence of cases in contact with older recovered birds on infected grounds. The rôle of *Heterakis* as a preliminary agent may also account for the continuing high mortality in turkeys in which the disease has been operating for so many generations to eliminate the most susceptible. It now seems highly probable that the turkey has become relatively resistant to the invasion of the protozoan parasite acting alone and that such invasion may require other agencies. Whether *Heterakis papillosa* is the only, or at any rate, the chief accessory agent or whether there are others, living or inert, which when ingested by the turkey assist in preparing the way for the destructive invasion of the walls of the ceca and the liver by *Amœba meleagridis* is a question now open to solution by experimentation.

The relation of common poultry to outbreaks of blackhead may be accounted for, at least in part, by the fact that they are hosts of *Heterakis papillosa*. How frequently they also carry *Amœba meleagridis* remains to be determined.

Since earlier communications have contained certain practical suggestions on the rearing of turkeys and the prevention of blackhead, it is not out of place here to point out that the additional information presented in this article simply emphasizes the suggestions already made. Turkeys should be raised in the incubator and brooder and kept away from older turkeys and poultry. The shelters should be moved from time to time to prevent a too concentrated infection of the soil with *Heterakis* ova. Inasmuch as the factors producing blackhead may be deposited by certain still undetermined birds on the wing, disease may be looked for at any time during the warm season. It is not, however, very readily transmitted, and in the experiments described elsewhere⁶ the mortality from spontaneous blackhead was low. The flock should be looked over as frequently as possible, and whenever a turkey begins to droop, it should be isolated and killed if the drooping continues over several days. If such turkeys are allowed to recover, they should not be returned to the young flock but kept with older, presumably infected birds. Such birds are entirely satisfactory as a source of eggs, since there is no evidence that the latter transmit the infection.