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**AN INVESTIGATION CONCERNING THE INCIDENCE AND
PATHOGENICITY OF PENTATRICHOMONAS GALLINARUM
AND ITS RELATIONSHIP TO HISTOMONAS MELEAGRIDIS
IN TURKEYS OF UTAH**

by

Ross S. Hadfield

A thesis submitted in partial fulfillment of requirements

for the degree of

Master of Science

in

Zoology

**Utah State Agricultural College
1952**

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I wish to express appreciation to my wife who helped gather data and conduct the experiments described in this paper. I acknowledge, also, the valuable suggestions and guidance of Dr. D. M. Hammond and Dr. M. L. Miner in conducting the experiments and in preparation of the manuscript.

Ross S. Hadfield

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INTRODUCTION

Turkey raising has become big business. During the period 1942 to 1946, the average annual return in Utah amounted to about nine and one-half million dollars (4). This amount would have been increased considerably if the death loss among poults had been lower. As an example, using the percentage of mortality given by Miner (9, p. 5), it is estimated that the death-loss of turkeys in 1944 resulted in a loss of gross income by the farmers of Utah of more than four million dollars provided that the price the farmer received had remained the same.

Among the prevalent causes of this enormous loss are those caused by the protozoa. One of the most common, yet least understood, of the protozoa infecting turkeys is Pentatrachomonas gallinarum (Martin and Robertson). It is found in many turkeys with no apparent harm to the host, while in others it has been reported to cause an "acute type of infection, characterized by diarrhea" (15, p. 1021). The lack of consistency in producing apparent effects in turkeys has caused some investigators to believe that the protozoan may not be pathogenic.

Morgan and Hawkins (11, p. 94) summed up the problem by stating:

"Hadley and Amison (1911) and Jowett (1911) held that infectious enterohepatitis was caused by trichomonads, but this work was discredited for a number of years by the work of Tyzzer (1919, etc.). Recently this theory has been revived, although not conclusively demonstrated, by Allen (1936, 1940). She considers there are two types of the disease, one produced by Histomonas meleagridis and a second produced by Pentatrachomonas gallinarum."

As yet, little, if any, information is known concerning the incidence and effects of Pentatrachomonas gallinarum (Martin and Robertson) in Utah flocks or its relationship to Histomonas meleagridis in causing infectious enterohepatitis.

Taxonomy and Morphology of Pentatrichomonas gallinarum

(Martin and Robertson).

The classification of the protozoan is as follows:

Phylum--Protozoa
 Sub-phylum--Plasmodroma
 Class--Mastigophora
 Order--Trichomonadida
 Genus--Pentatrichomonas*
 Species--gallinarum

The body of Pentatrichomonas gallinarum is somewhat similar in shape to that of an egg or a pear, although the form may vary. (Compare figures 1 and 2). The average measurements are about 7 microns in width and about 11 microns in length. Allen (2, p. 67) found the average of those she studied to be 5 microns wide and 6.6 microns long; whereas, DeVolt and Davis (6, p. 560) report that the organism is from 4 to 8 microns wide and 10 to 15 microns long. From the writers observation the main distinguishing characteristics are an undulating membrane, five free anterior flagella, and one trailing flagellum. The trailing flagellum arises from the anterior blepharoplast complex and proceeds caudad as the marginal border of the undulating membrane. An axostyle projects from the posterior margin of the body. There are two blepharoplasts and a nucleus has the chromatin distributed on the nuclear membrane. The cytoplasm appears vacuolated and may have dark granules located in the central region of the cell body along the axostyle. As yet, no cysts have been found. (for detailed morphology, see figure 1. p. 3.).

The movements of the organism are characterized by an irregular,

* This organism has been generally called Trichomonas. Morgan and Hawkins call it Pentatrichomonas and the organism also fits Kudo's description of the genus Pentatrichomonas. Hence the generic name used here is Pentatrichomonas.

Twisting motion created by the undulating membrane and flagella. Allen (2, p. 67) states that the organism shows little forward progression, which readily distinguishes it from the quick darting movements shown by Trichomonas gallinae, sy. T. Columbae. In apparently degenerating individuals, there appears an amoeboid-type or peristaltic wave motion. This motion appears to be created by an extension of the cell wall and the undulating membrane. It begins at the anterior end of the body and moves to the posterior portion of the body in a wave-like motion. (see fig. 3 p. 4)

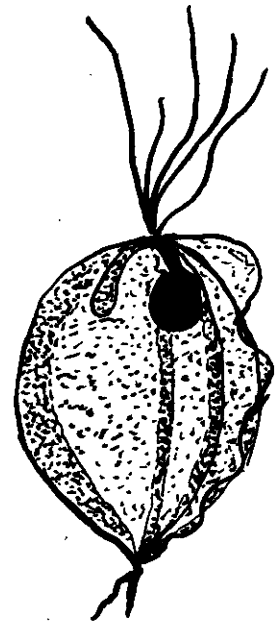
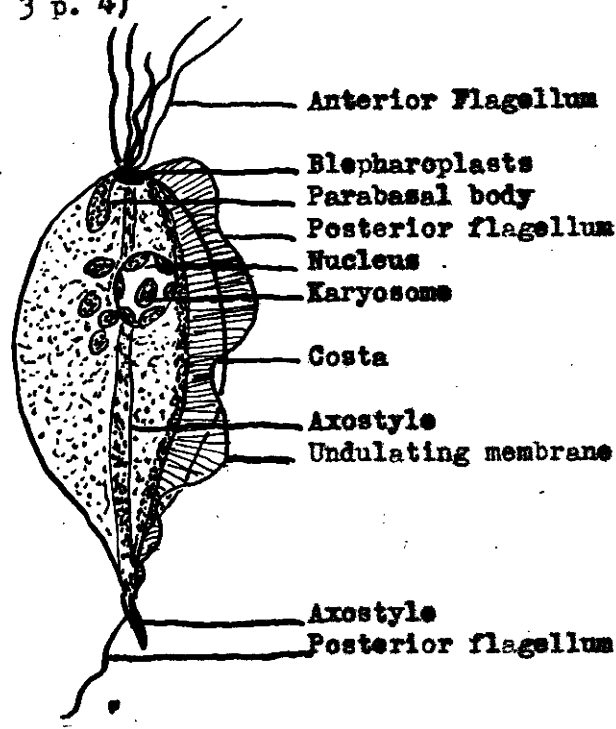


Fig. 1. Diagram of Pentatrichomonas gallinarum (Martin and Robertson) From prepared slides X550

Fig. 2. Pentatrichomonas gallinarum. Redrawn from Morgan and Hawkins to show different shape organism may assume.

Distribution of Infection. Pentatrichomonas gallinarum has a wide geographical distribution. Allen (2, p. 64) reports that it has been found in Maryland, the district of Columbia, Virginia, Tennessee, Ohio,

Colorado, and Pennsylvania. Other workers have found it infecting turkeys in California, (7, p. 104), as well as in Utah and Idaho.

Survey work done in Utah indicates that it is present in turkeys of Cache, Box Elder, Weber, Davis, Utah, Sanpete, Sevier, Wayne, Piute, and Washington Counties. (see fig. 4, p. 5) Infections were found to be present in all counties where examinations were made. Because of the unrestricted movement of turkeys throughout the United States, it is likely that Pentatrichomonas is wide spread and probably exists in all of the major turkey raising areas.

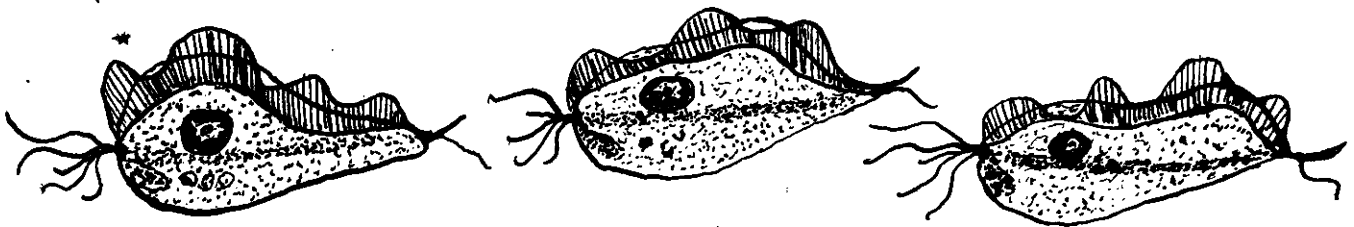


Fig. 3. Diagram showing the type of movement exhibited by an apparently degenerating individual.

REVIEW OF LITERATURE

Infectious enterohepatitis in turkeys was first described by Cushman in 1894, but the causative organism of this disease was not noted at that time. In 1895, Theobald Smith (1, p. 215), in giving the etiology of infectious enterohepatitis, named a protozoan, Amoeba meleagridis as the causative organism.

In 1911, Martin and Robertson described Pentatrichomonas gallinarum in a very brief way and named it Trichomonas gallinarum. They did not attempt to assay its relationship to infectious enterohepatitis. Since that time, further study indicates that the organism has five anterior

STATE OF UTAH

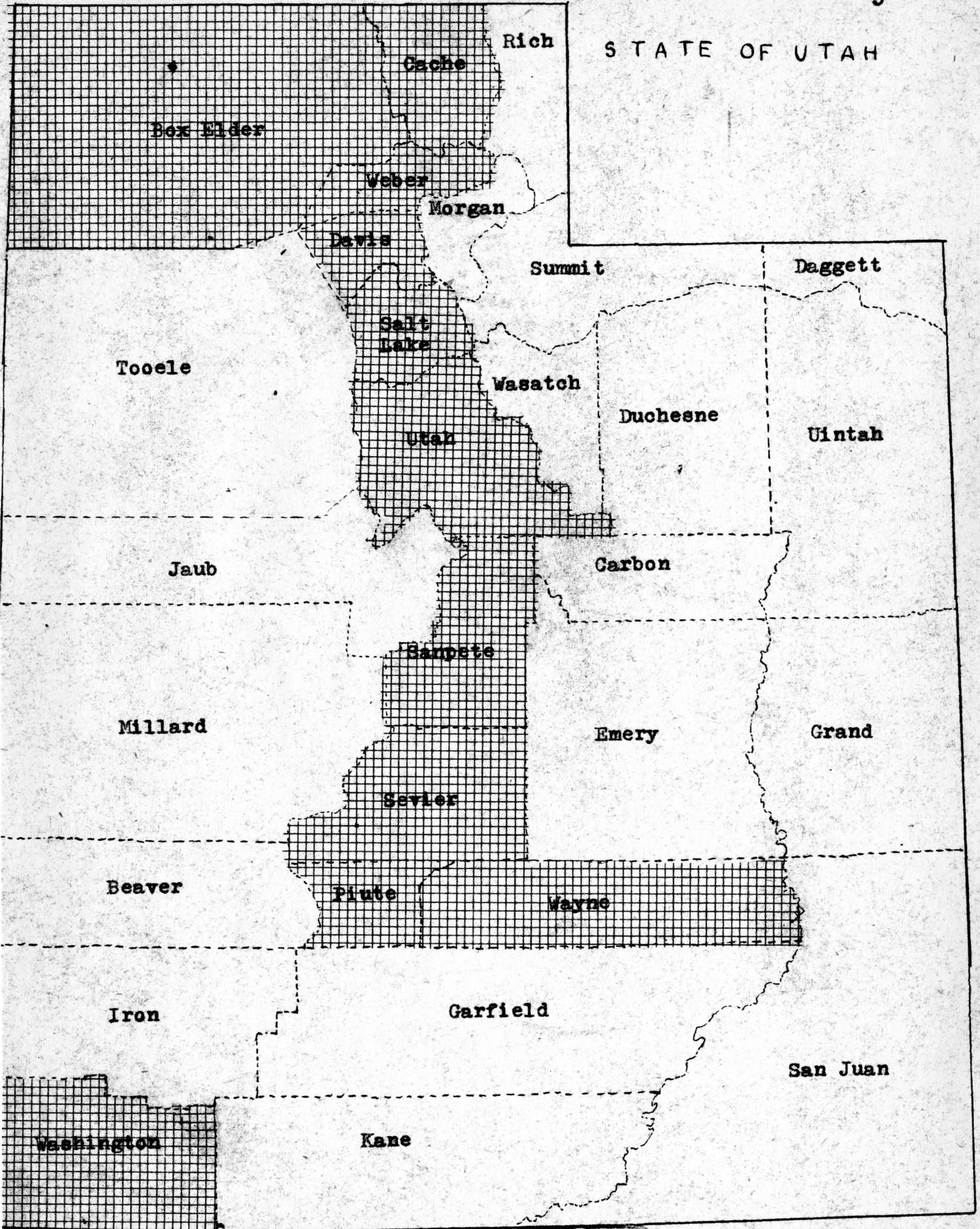


Fig. 4. Counties of Utah where *P. gallinarum* has been observed.

flagella instead of the four described by Martin and Robertson. According to Allen (2, p. 65) both she and Wenyon are of this opinion and believe that Martin and Robertson merely overlooked the fifth flagellum, which is shorter than the other four.

According to Morgan and Hawkins (11, p. 94), Pentatrichomonas gallinarum was first incriminated as a causative organism of infectious enterohepatitis by Hadley and Amison, and Jowett in 1911. Again in 1916, Hadley identified Pentatrichomonas gallinarum as the causative organism in enterohepatitis. This was the generally accepted viewpoint until Tyzzer's work came out in 1920. Tyzzer renamed the organism that Smith, in 1895, had called Amoeba meleagridis. Tyzzer called the organism Histomonas meleagridis. Tyzzer also gave experimental evidence that Histomonas meleagridis is the cause of infectious enterohepatitis instead of Pentatrichomonas as had been thought previously (14).

For the next fifteen years following Tyzzer's report, P. gallinarum was considered to be of a non-pathogenic nature. In 1936, Devolt and Davis (6, p. 562) and Allen (1, p. 315-322) reported on experiments in connection with the pathogenicity of P. gallinarum. Devolt and Davis reported as follows:

"In connection with this phase of the problem, the writer wishes to present the data that have accumulated to date. These show that trichomonads are quite frequently associated with both liver and cecal lesions with histomonads and some cases have been recorded wherein the trichomonads were present in large numbers without the histomonads. ...The circumstantial evidence against trichomonads as possible pathogens seems to be increasing rather than diminishing at the present time."

Allen indicated in her experiments covering a period of six years that P. gallinarum is pathogenic in turkeys. In Missouri, McDougale and Durant (8), in August 1946 reported that P. gallinarum affects turkeys

and may cause a mortality of 50 to 70 percent in birds 10 to 12 weeks old. 7

Since the advent of these reports on Pentatrachomonas and its association with enterohepatitis, the role of P. gallinarum in causing mortality in turkeys has been questionable.

OBJECTS OF STUDY

Since there seems to be little, if any, agreement among the authorities concerning the relationship of P. gallinarum to turkey mortality, it became evident that investigations should be conducted on this problem. At the present time, some turkey growers and feed company field representatives in Utah distinguish between Pentatrachomoniasis and Histomoniasis mortality, while others lump the mortality all under the heading of "blackhead". Those individuals reporting Pentatrachomoniasis as the cause of loss have used Allen's (2, p. 214-217) description to differentiate between Histomonas lesions and Pentatrachomonas lesions (see page 21).

So far as is known, no investigations have been conducted in the state of Utah concerning the incidence or pathogenicity of Pentatrachomonas gallinarum, and no definite information is at hand in reference to the relationship of P. gallinarum to Histomonas meleagridis. Preliminary work showed that the incidence of infection of P. gallinarum in Utah turkeys was probably quite high, but no exact figures were available before this study was undertaken.

This study was undertaken with four objectives: (1) To obtain an accurate index of infection within a given locality; (2) to determine the degree of infection in selected flocks at various times; (3) to check the virulence of various strains of Pentatrachomonas gallinarum

from different localities within the state of Utah; and (4) if possible, to determine the relationship of Pentatrichomonas type lesions to Histomonas type lesions.

METHODS OF PROCEDURE: Studies of Incidence

Diagnostic methods. Infections with Pentatrichomonas gallinarum are diagnosed by demonstration of the protozoan under the microscope. The organisms normally localize in the ceca and bursa of Fabricius and can be readily observed if they are present. Examinations for protozoa in the ceca were made either by autopsy, in which case samples were taken directly from the cecal contents, or by taking freshly passed cecal droppings. Material from the bursa of Fabricius was obtained for examination from live birds by using a small curett to scrape the bursal wall.

A small drop of material to be examined was placed on a clean glass slide, and one drop of normal saline was added. The material and saline solution were thoroughly mixed and a coverslip placed over the sample. The sample was immediately examined microscopically under low and medium powers.

Ordinarily, the protozoan will be moving at such a rapid pace that little can be discerned about it except the general outline, shape, and characteristic movement. To count the flagella and to identify the internal structures, the following procedure, worked out by Allen (2, p. 65), was used at various times:

"Two parts of a saturated solution of eosin in physiological saline and 1 part of a 5 per cent solution of potassium iodide in physiological saline saturated with iodine, were mixed just before using. Small amounts of cecal material or culture fluid containing living trichomonads were mixed with 1 drop of the stain and to this mixture was added 1 drop of a saturated solution of thymol in distilled water. This treatment extended and emphasized the flagella."

To supplement the above diagnosis, dark field illumination was used

in the laboratory at the beginning of the study. For field identification, however, the location of the organism in the cecal contents and cecal droppings, characteristic movements, and presence of undulating membrane were the criteria used for field identification.

The materials used to conduct a field examination were as follows: Slides, coverslips, normal saline solution, curette, small pieces of wood (toothpicks or applicators), lens paper, microscope, and a towel or cloth to wipe the dust off the microscope.

Information from the field examination was recorded on a form that was originated with the idea that, over a period of years, an accumulation of information may be obtained that may shed light on this or other problems of the protozoan diseases of turkeys.

The type of form used is as follows:

SURVEY OF TURKEY PROTOZOA

Date _____

Name of owner _____ Place _____

Type of coop _____

Type of location _____

Kind of heating _____ Kind of litter _____

Cleaning (times, kind, disinfectant used, etc.) _____

Size of flock _____ Condition of flock _____

Previous sickness and mortality (cause and number) _____

Breeding: How long _____ Conditions _____

Range: How long on range at time of examination _____

Type _____

Location _____

Other animals found in the same locality _____

Practices and sanitation _____

Microscopical findings:

Type of examination: Fecal _____ Internal _____ Autopsy _____

Number of birds examined: _____

Number of birds positive for E. gallinarum:
 numerous _____ few _____ very few _____

Number of birds positive for H. meleagridis:
 numerous _____ few _____ very few _____

Number of birds positive for Hexamita:
 numerous _____ few _____ very few _____

Other protozoa: Kind _____
 number of birds positive
 numerous _____ few _____ very few _____

Number of birds negative for protozoa _____

Cultural methods. Attempts were made to concentrate the organisms being studied by inoculating them into a cultural medium suitable for their growth and reproduction. Egg slants were prepared in the following manner as suggested by Morgan (10, p. 19) for Tritrichomonas foetus:

"Four eggs were washed, cleaned with alcohol, and broken into a sterile flask containing glass beads. Fifty cc. of Locke's solution (see below) were added and the flask shaken to make a good mixture. Test tubes filled with about 2 cc. (enough to produce slants from 1 to 1½ inch long after coagulation by heat) were then slanted and heated at 15 pounds pressure for 30 minutes.
 ... The Locke solution in the above medium is made as follows:

NaCl-----	9.0 grams
CaCl ₂ -----	0.2 grams
KCl-----	0.4 grams
NaHCO ₃ -----	0.3 grams
Glucose-----	2.5 grams
Distilled water-----	1000 cc.

The original reaction of this medium varies from a pH of 7.2 to 7.8 and requires no adjustment."

In an attempt to acquire a suitable culture medium, egg slants prepared as above, were layered with various kinds of media. The solutions used consisted of a one to one mixture of Locke's solution with each of the following: Defibrinated horse's blood, defibrinated rabbit's blood, defibrinated turkey's blood, and horse's serum. Ringer's solution was also tried in place of the Locke's solution in the above experiments. In all cases, the cultural medium showed myriads of bacteria after twenty-four hours but no protozoa. Attempts to prevent the growth of the bacteria were made by adding 5000 and 10,000 units of penicillin to each slant; however, the culture media still showed overgrowth of bacteria.

Allen (1, p. 315) gives directions for culturing the organism, which she claims is very successful. The knowledge of this method was acquired too late in the experiment to be successfully attempted. This method is included here for future reference because the original reference was not readily available. The method with which she acquired the best results consisted of making egg slants according to Boeck and Drbohlav and using Locke's solution containing quinanil in the proportion of 1:10,000 as the liquid medium. She used this solution for eight to ten transplants to free the medium from bacteria. The reduction of bacteria made it possible for her to make transplants once a week instead of every three or four days.

Attempts to isolate a pure culture were conducted using the "V"

tube, the "U" tube, and the petri dish marked with concentric rings for radial migration as given by Morgan (10, p. 12-16). However in all attempts, the inoculation media contained motile bacteria that prevented getting a pure culture. These findings corroborated the statements of Morgan (10, p. 13) in which he states that if motile bacteria are present or the flagellates appear meager or sluggish, attempts at isolation are usually fruitless.

Incidence of Infection. A definite area was selected in an effort to determine how many birds within this area were infected with P. gallinarum. The area selected was in the Bear River Valley, and centered in Tremonton, Utah. This survey was conducted by visiting the flocks in the area selected and checking individual birds. Ten birds were selected at random from each flock and examined for protozoa. The examinations were made by examining cecal droppings and the exudate scraped from the bursa of Fabricius. A sample of only ten birds from each flock was probably inadequate to give anything but an approximate incidence, but this number was convenient and enabled a wider coverage than would have been possible if larger samples were taken from each flock.

The number of organisms in each sample was not estimated because of the extreme difficulty of making an accurate count in the field; however, there was an attempt to classify the number of protozoa present in each sample as to whether the protozoa were numerous, few, or very few. This classification was based on the following: When there were more than four or five individuals found in a low power (100X) field or two or more individuals found in a medium power (440X) field, the sample was classified as numerous; when there were only one or

two individuals in several low power fields or medium power fields, the sample was noted as few; and when there were less than ten individuals found in the entire sample, it was classified as very few.

Of all the flocks listed as being in the area, about two-thirds of the flock were included in the survey. The lack of time and knowledge of the location of the remaining flocks prevented a complete coverage.

By the time the flocks were about sixteen weeks old, and had been on the range for an average of about eight or nine weeks, all flocks examined showed a high level of incidence of infection with P. gallinarum. The survey resulted in a negative finding in only four per cent of all birds examined. The data from this survey are summarized in Table 1.

It should be pointed out that this survey was mainly concerned with P. gallinarum and was not made to determine all types of protozoan infections. From Table 1, it is evident that there is little difference in the percentage of infection in flocks on wheat stubble or irrigated alfalfa, the two most common types of range ground utilized. Birds on wheat stubble showed 98.6 per cent infection, while those on irrigated alfalfa showed 92.5 per cent infection which is not a significant difference according to Snedecor (13, p. 5). The main result is the high incidence of P. gallinarum infections regardless of the type of range used.

Flock Studies. In an attempt to determine the degree of infection at various ages, three flocks were selected for careful study. These flocks were located at Plymouth, Fielding, and Garland, Utah. The three flocks that were selected for the study were examined at 3, 4, 5, 7, 10, 13, 16, and 19 weeks of age. The various examinations consisted

of fecal and bursal examinations, and included autopsy examinations if sick birds were encountered. The information from each examination was recorded on the form "Survey of turkey protozoa" as given on page 9.

Table 1. Incidence of infection with P. gallinarum in turkeys of Bear Valley, Utah.

Flock	Size of Flock	Age of Flock (wks)	Weeks on Range	Type of Range	No. of Birds Examined	No. of birds infected with <u>P. Gallinarum</u>			Percent of Infection	
						Num. ¹	Few	V.F. ²	Tot	
1	5000	18	10	Wheat stb. ³	10	9	1	0	10	100%
2	6000	18	10	Wheat stb.	10	10	0	0	10	100%
3	2000	13	5	Wheat stb.	10	7	2	0	9	100%
4	3500	13	6	Irr. alf. ⁴	10	9	1	0	10	100%
5	2300	17	9	Wheat stb.	10	9	1	0	10	100%
6	2500	17	9	Wheat stb.	10	8	2	0	10	100%
7	3500	19	11	Wheat stb.	10	9	1	0	10	100%
8	2200	17	9	Irr. alf.	10	0	7	2	9	90%
9	2000	17	9	Irr. alf.	10	4	4	0	8	80%
10	2300	17	9	Irr. alf.	10	7	2	1	10	100%
Aver.	3130	16.6	8.7		10	7.2	2.1	0.3	9.6	96%

1 Numerous

2 Very few

3 Wheat stubble

4 Irrigated alfalfa

The brief case history of each selected flock is as follows:

Flock A. This flock was hatched on April 1st and arrived at the brooder on April 3rd. The birds appeared in good condition and apparently had no rough treatment. The brooder house was constructed of wood and had a concrete floor. The house was located near Fielding, Utah, on clay-loam ground in a dry-farming section with no other farmstead within a

mile radius. Brooder heat was furnished by bottled gas and coal cinders were used as litter. Water was supplied from a deep well located on the premises.

Previous to the arrival of the poults, attempts to clean the brooder house were carried out. This consisted of sweeping the house and scraping off previous deposits of dirt. No disinfectant was used.

The general practice of sanitation consisted of disinfecting the waterers once a week and using wire screen under the feeders and waterers. No disinfecting of clothes, shoes, or equipment other than the waterers was attempted.

The brooder house was not rat or mouse-proof and sparrows had access to the building. Other animals found in the immediate vicinity were ground squirrels, dogs, meadow larks, pheasants, cows, and horses.

The first microscopical examination was conducted when the poults were three weeks old. Previous losses amounted to approximately fifty birds from unspecified causes. Succeeding examinations were made at two or three week intervals until the birds were 19 weeks old. All examinations made before the seventh week showed no protozoa. The examination at the seventh week showed one bird positive for Chilomastix. The examination at the tenth week showed eight birds positive for P. gallinarum, two birds positive for Histomonas, and two birds negative for protozoa. This examination was made when the birds had been on the range for two weeks.

The examinations made during the tenth and thirteenth weeks were from different units of the flock. By the sixteenth week, both units were combined and the examination was made from the whole flock.

The degree of infection with P. gallinarum after the tenth week showed an increase until by the sixteenth week all ten birds examined each time were infected.

The data from this flock in comparison with flocks B and C are summarized in Figure 5, page 19.

Flock B. This flock was hatched on April 3rd and arrived at the brooder on April 5th. There were some apparently weak poults included in the shipment and approximately 200 birds died the first week. The poults were brooded in four separate buildings. Two were constructed of wood with concrete floors, one was made of brick with a concrete floor, and the fourth was a double-car garage with concrete floor. Heat for the first three brooders was furnished by bottled gas. Fuel oil was used for heat in the remodeled garage. Coal cinders were used for the litter in all brooders. The brooding houses were located on well drained sandy soil within the limits of Plymouth, Utah. Water was from a municipal water supply.

Sanitation procedures carried out before the arrival of the poults consisted of scraping and sweeping out the houses. No attempt was made to disinfect brooders or other equipment. During the brooding period, practices of sanitation were generally poor. No screens were used under the feeders or waterers, nor were they disinfected. The brooders were not cleaned during the brooding period. No disinfecting of shoes, clothes, or equipment was attempted. Of the four houses only one was constructed to prevent the entrance of rats, mice, or small birds. Other animals found in the immediate vicinity were dogs, cats, horses, cows, pigs, sheep, and chickens.

The first microscopical examination was conducted when the poults

were about two and one-half weeks old. The results were negative for protozoa. Cumulative losses at this time amounted to approximately three hundred birds, apparently caused by poor hatching. The second examination showed negative results for protozoa, as did the third examination at five weeks.

In the two week interval between the fifth and seventh week, one of the oil brooder stoves developed a leak and a large amount of the litter around the stove became saturated with fuel oil. Several birds died and the owner reported that an autopsy, performed by himself, disclosed that the birds crops were heavily loaded with oil soaked cinders. The garage was immediately cleaned and pea gravel installed as litter; however, the birds continued to die. The cause of death of those birds that died after the brooder was cleaned was diagnosed by the feed company representative as "blackhead". This diagnosis was not confirmed by the writer.

The examination at the seventh week was conducted on the birds brooded in the garage because the other birds had been moved to the range. The examination included twelve fecal samples and one autopsy examination. Results showed two birds positive for Chilomastix gallinarum, four birds positive for Eimeria, and seven birds negative for protozoa. No P. gallinarum was found at this time.

The tenth week examination was made in the unit where the owner had been having trouble with birds dying of infectious enterohepatitis. The birds had been on the range for four weeks. All ten fecal examinations were positive for P. gallinarum. The examinations on the thirteenth and sixteenth weeks were made from the other two units with which the owner had experienced little trouble. All units of the flock

were combined by the seventeenth week and the final examination was taken from the single combined unit. The infection with P. gallinarum from the tenth until the nineteenth week ranged between fifty and one hundred per cent.

The data from this flock are compared with flock A and C in Figure 5, page 19.

Flock C. This flock arrived from the brooder on April 7th in very good condition. Only five birds were lost in the first two weeks. The brooder house, located at Garland, Utah, was a wood frame building with concrete floors. The building was on clay-loam ground with poor drainage. Heat was furnished by steam pipes. Cedar shavings were used as litter.

Sanitation practices before the arrival of the poults consisted of cleaning and scraping the brooder house and disinfecting the house and equipment with lye water. The entire building was sprayed with DDT. After the poults arrived, the sanitation procedures consisted of using a mild disinfectant in the drinking water and cleaning the waterers in hot water and disinfecting them every other day. There was no disinfecting of shoes, clothing, supplies, or other equipment. The construction of the building enabled small birds, such as sparrows, to gain entrance, but there was good protection against rats and mice. Other animals found in the vicinity included dogs, cats, and pigeons.

The first examination at three weeks was negative for protozoa. The second examination at four weeks showed one bird positive for Chilomastix. At five weeks, two of ten birds were positive for Chilomastix and at seven weeks, three of ten birds examined were positive for this organism. The examination made at ten weeks disclosed two

birds positive for P. gallinarum and one positive for Eimeria. After the tenth week the percentage of P. gallinarum infection went up until at the sixteenth and nineteenth weeks all ten birds examined were infected.

The data from this flock are compared with flock A and B in Fig. 5.

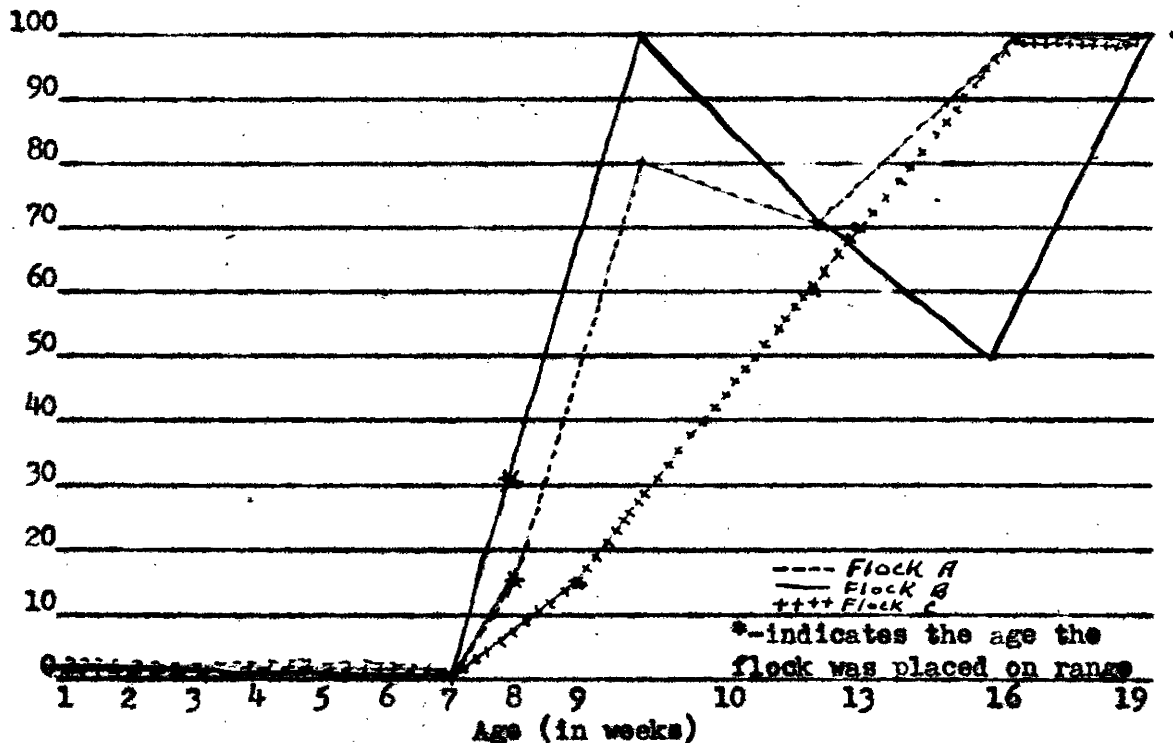


Figure 5. Infection of P. gallinarum in three selected flocks at various age levels.

Other Types of Protozoa Encountered. As has been previously recorded in the flock histories and incidence of infection, protozoa other than P. gallinarum were encountered. These included Chilomastix (possibly two types), Histomonas meleagridis, and Eimeria (sp.). The number of times each of the above types was encountered is tabulated in Table 2. This includes the findings in the flock studies and in the survey of the incidence of infection.

In one flock, there was encountered a type of protozoan which had not been seen before by the writer. This organism appeared to be a

Chilomastix but with some variation; however, this organism was included in the incidence of Chilomastix infection in Table 2. This protozoan was estimated to be about twenty microns long and eight or nine microns wide. When the organism was oriented with the open section of the spiral groove up, the groove ran anteriorly and to the left about to the median line. The number of flagella was not ascertained. The organism moved at a fairly steady rate of forward movement with only occasional rolling or turning.

Other types encountered followed the description given in standard works on turkey diseases.

Table 2. Incidence of various types of protozoa found while investigating infections of P. gallinarum.

Protozoa	Number of times encountered	Percentage of examined birds infected
<u>Histomonas meleagridis</u>	8	2.6%
<u>Chilomastix gallinarum</u>	18	5.8%
<u>Hexamita meleagridis</u>	0	0.0%
<u>Eimeria (sp.)</u>	5	1.6%
<u>Trichomonas gallinae</u>	0	0.0%

STUDIES OF PATHOGENICITY

To study the pathogenicity of various strains of P. gallinarum and its relationship to Histomonas meleagridis in causing infectious enterohepatitis, inoculation tests were carried out over a period of eight months. These tests were made using strains of both P. gallinarum and Histomonas meleagridis, as well as the mixed type of infection including both of the above mentioned protozoa.

Differentiation of lesions. The differentiation of the various types of lesion supposedly caused by P. gallinarum and H. meleagridis was based on Allen's (3, p. 214-217) description. She described the macroscopic liver lesions caused by P. gallinarum as granular, cream-colored, necrotic areas of irregular outline. These necrotic areas are level with, or elevated above, the surface of the liver. The size of the lesions vary from the size of a pin-point to a diameter of three-fourths of an inch. They appear to be composed of closely packed rice-shaped granules. She described the lesions of the liver that are caused by infections of H. meleagridis as slightly depressed, necrotic areas from seven-sixteenths to one inch in diameter. They are generally circular, cream-colored lesions with quite narrow borders enclosing web-like formations of necrotic tissue. For a comparison of Pentatrachomonas and Histomonas lesions see figures 6 and 7.

According to Allen, the mixed type of infection produces lesions that appear as large, circular areas with slightly elevated, granular borders. The center of the necrotic area is markedly depressed and in some instances cream colored granules are present.

Strain 1. In January, 1948, an examination was made on a flock of turkeys at Marysvale, Piute County, Utah that had been suffering losses from unknown causes. One bird, showing numerous pentatrachomonads in the cecal droppings and bursa of Fabricius, was selected for further transmission and pathogenicity studies, and was brought to the laboratory. This bird appeared emaciated and had a large contusion over the right ribs. Force-feeding by hand was necessitated in order to keep the bird alive until transmission of the infection could be accomplished. The bird died on February 9, 1948. On examination all internal organs were apparently

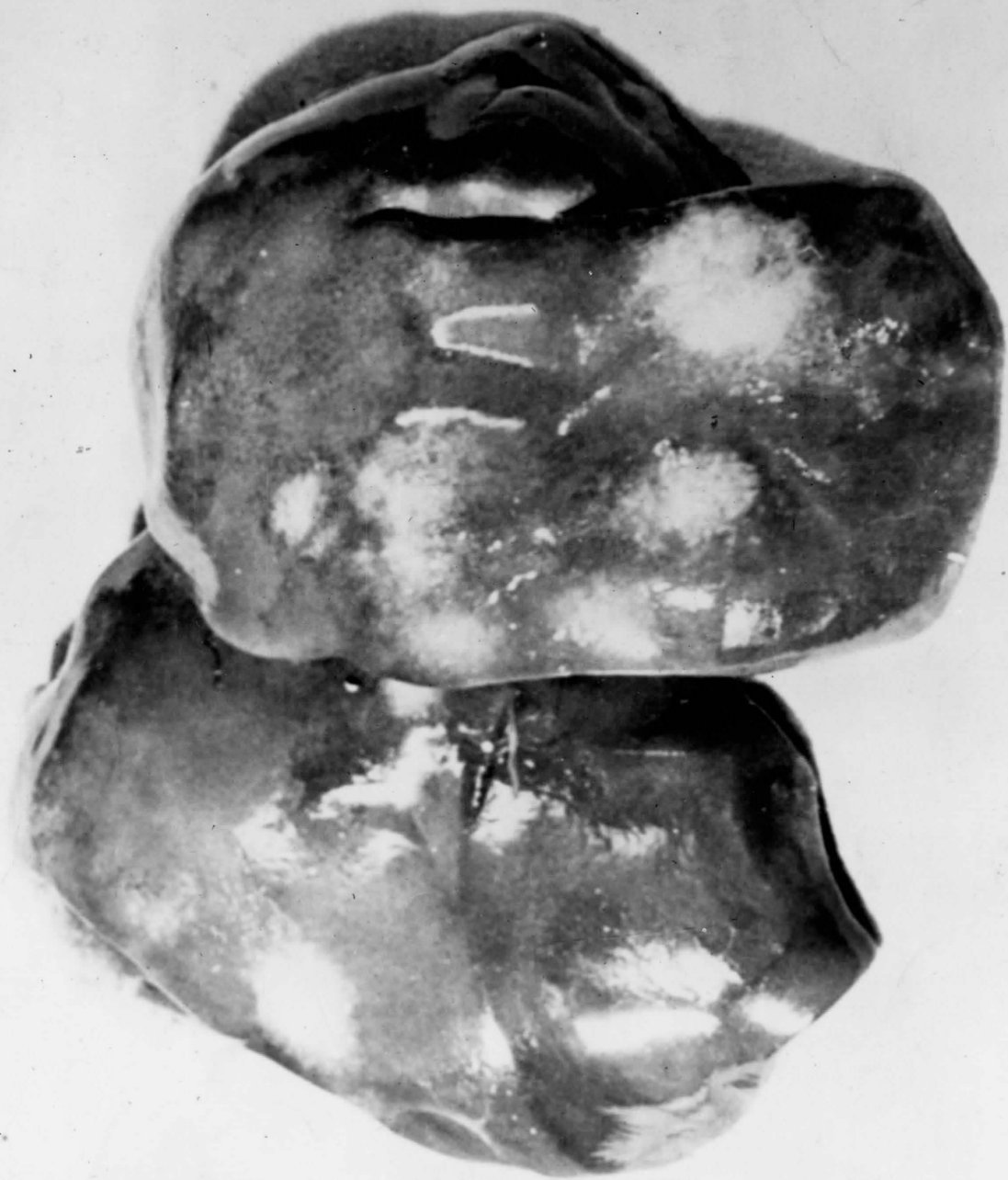


Figure 6. Turkey liver showing typical Pentatrichomonas lesions



Figure 7. Turkey liver showing typical Histomonas lesions

normal except for one lung which was necrotic and nonfunctional, and for the presence of some enteritis in the lower portion of the small intestine. The microscopical examination was positive for Trichomonas gallinae in the crop and upper esophagus. Pentatrachomonas gallinarum was located in the upper portion of the large intestine, in the caeca, and in the bursa of Fabricius.

On January 30, 1948, inoculations were made from this bird into two nine months old turkeys. The inoculate was obtained by scraping the bursal wall and mixing the scrapings with normal saline. Two cc. of the mixture were injected into each bird's bursa with a medicine dropper. No P. gallinarum was located in either bird before the inoculation, but four days later both birds had numerous pentatrachomonads in the bursa. Neither bird showed any symptoms of abnormalities up to the time they were autopsied at fourteen months of age. Periodic examinations were positive for P. gallinarum from the time of infection until the birds were killed. Postmortem findings were negative for any macroscopical pathological abnormalities.

On July 1, 1948, four eleven-week old poults were each inoculated rectally with five cc. of a mixture made by diluting one part of cecal droppings with four parts of normal saline solution. These droppings were from the two birds that were inoculated in January. The inoculations were made by introducing the suspension into the rectum with a medicine dropper. No P. gallinarum was found in any of the four birds before the inoculations were made. After five days all four of the birds were positive for P. gallinarum. Until these birds were nineteen-weeks old, no symptoms of abnormalities were observed. One bird developed perosis and was killed. This perosis was attributed to keeping the birds for about

two weeks in a cage with insufficient vertical space. The autopsy on this bird failed to show any internal lesions or abnormalities.

Strain 2. On June 13, 1948, an examination of a flock of turkeys in the vicinity of Tremonton, Utah, disclosed that many of the birds had died from infectious enterohepatitis. At autopsy all of five birds had livers with typical mixed Pentatríchomonas-Histomonas type of lesions according to Allen's description. In an effort to study this condition further, a sick bird from this flock was brought to the laboratory. This bird died during the first day at the laboratory. At autopsy the liver had the mixed type of lesion. The cecal examination showed many P. gallinarum, but no histomonads were found.

To continue this study eight nine-weeks old poults were inoculated with infective material from this bird. Four of the birds were each inoculated rectally with five cc. of a saline suspension of cecal contents. The other four birds were each inoculated rectally with five cc. of a saline suspension of ground tissue from liver lesions. This suspension was made by grinding the liver lesions in a mortar and adding normal saline solution. All rectal inoculations were made with a medicine dropper. These inoculations were made on June 14, 1948, about thirty minutes after the bird from which the infective material was taken had died.

Of the eight birds, the four inoculated with the suspension from the liver lesions showed no P. gallinarum or H. meleagridis in cecal droppings from the time of inoculation until they were nineteen weeks old when examinations were discontinued. These negative results are probably the result of placing the infective material from liver lesions into only the rectum. Santter, et al., (13) were able to transmit enterohepatitis when the infected liver suspension was inoculated into the vicinity of the cecal

openings. Experiments conducted at Utah State Agricultural College by Hammond and Miner in 1950 (unpublished data) corroborate this.

The four birds inoculated with the cecal suspension developed infectious enterohepatitis and died. One bird died on June 27, two on June 30, and the fourth on July 7, 1948. The bird that died on June 27 had typical Histomonas lesions in the liver. There were many P. gallinarum and a few H. meleagridis in the ceca. Of the two birds that died on June 30, one had typical Pentatrichomonas lesions in the liver; microscopical examination disclosed many P. gallinarum in the ceca but no H. meleagridis were found. The other bird had typical Histomonas lesions in the liver; many P. gallinarum were found in the ceca but no H. meleagridis were located. In both cases microscopic examinations of the livers were negative for any kind of protozoa. It is very difficult to demonstrate Histomonas in the cecum and even more so in the liver and such negative results are not conclusive evidence of the absence of Histomonas. The bird that died on July 7 had lesions of the mixed infection type. Many P. gallinarum were found in the ceca, but no histomonads were found.

Inoculation of four birds with cecal suspension taken from a bird that had mixed-type of lesions in the liver resulted in two birds having Histomonas-type lesions, one bird having Pentatrichomonas-type lesions, and one bird with the mixed-type of lesions.

The two birds which died on June 30 were selected for further study because of the marked difference in the lesions. Twelve eleven-weeks old birds were selected to be inoculated. All inoculations were made within 30 minutes after the bird from which the infective material was taken had died. Each of the twelve birds received a five cc. inoculation orally and a five cc. inoculation rectally of a saline suspension of the infective material.

From the dead bird that had typical Pentatrichomonas lesions, three birds were inoculated with a saline suspension of cecal contents and three other birds were inoculated with a saline suspension of liver lesions prepared as in the previous inoculations. The same procedure as in the preceding paragraph was used in inoculating six birds with infective material taken from the bird that had typical Histomonas lesions.

None of the six birds inoculated with material from the bird that had Histomonas lesions developed any clinical symptoms, although the three infected with the cecal suspension were positive for P. gallinarum after four days, whereas those inoculated with liver suspension were negative for all protozoa. In the case of the six birds inoculated with material from the bird having typical Pentatrichomonas lesions, two of the three birds inoculated with the cecal suspension died. One bird died on July 19, 1948, and the post-mortem examination showed typical Pentatrichomonas lesions, but no protozoa were found in the ceca. The second bird was in a morbid condition and was killed on July 21. This bird had typical Pentatrichomonas lesions and many P. gallinarum were found in the ceca immediately after death. In a subsequent examination three hours after the bird was killed no protozoa could be located. This may explain why no protozoa were found during the examination of the previous bird that died on July 19, which was examined approximately four hours after it died. The third bird of this group developed no clinical symptoms but was found to be infected with Pentatrichomonas five days after being inoculated. The three birds that were inoculated using liver lesion suspension from the bird having Pentatrichomonas lesions were negative for all protozoa and no symptoms of disease were noted.

The depth of the rectal inoculation was probably a factor in the negative results in these experiments as in those conducted previously.

Strain 3. In an effort to determine more fully the relationship of Paratrichomonas lesions and Histomonas lesions, another turkey from a flock in the vicinity of Tremonton, Utah, was acquired for inoculation studies. This bird had typical Histomonas lesions at the time of autopsy on June 17, 1948. Inoculations were made into eight nine-weeks old poults. Four were inoculated each with five cc. of saline cecal suspension and four each with five cc. of saline liver suspension. Of the four birds inoculated with the saline cecal suspension, one died on July 10, 1948. This bird had Histomonas lesions in the liver and many P. gallinarum were found in the ceca. The other three birds had many P. gallinarum in their cecal dropping but showed no symptoms of disease. Examinations of the cecal droppings from the four birds inoculated with the saline liver suspension were negative for all types of protozoa and showed no symptoms of disease.

Strain 4. On July 8, 1948, an investigation was made of a flock at Garland, Utah, which was experiencing losses due to infectious enterohepatitis. Of the four birds examined all had Histomonas lesions in the liver. One sick bird in which no protozoa were located in the droppings or in the cecal contents at autopsy was used for further study. Four twelve-weeks old poults were each inoculated rectally with five cc. and orally with two cc. of a suspension made from the cecal contents of the bird having Histomonas liver lesions. These inoculations were made on July 8, 1948, immediately after the bird from which the inoculating material was taken had been killed. On July 23, two of the birds died. Both birds had typical Paratrichomonas lesions in the liver. One bird which had died two or three

hours before it was examined had a few, apparently dead, P. gallinarum in the cecal contents. The other bird which was examined immediately after death had numerous P. gallinarum in the ceca and intestine. No H. mal-saridis was found in either bird. The other two birds developed no clinical symptoms but were positive for P. gallinarum in their cecal droppings four days after being inoculated.

In the foregoing inoculation studies concerning strains 1, 2, 3, and 4, live protozoa were found in the ceca for approximately thirty minutes after death occurred; longer periods resulted in negative microscopic findings. In the examination of liver lesions for protozoa, one of the contributing factors to the negative findings may have been the method used in examining the lesions. The examination consisted of making a normal saline suspension of the liver lesion in a mortar and examining this material under a microscope. If a warm stage had been used it may have resulted in positive findings.

The number of days that elapsed from the time a bird was inoculated until it died varied in these experiments from thirteen days to twenty-three days with a mean of eighteen days. There was no apparent difference in the time elapsing from inoculation until death when the infecting material was taken from a bird having Pentatrichomonas, Histomonas, or mixed-type of liver lesions.

Table 3. Comparison of infections with P. gallinarum resulting from inoculation of material taken from liver lesions and from cecal contents.

Type of inoculat- ing material	Number of birds inoculated	Number of result- ing infections	Percent of in- fection
Liver	14	0	0.00%
Cecal	24	22	91.67%

Table 4. Kinds of lesions resulting from inoculations with material from turkeys with different type of liver lesions.

Original type of lesions	Number of birds furnishing infective material	Number of birds inoculated	Type of lesions resulting		
			Hist.	Penta.	Mixed
Histomonas	3	18	1	2	0
Pentatrichomonas	1	12	0	2	1
Mixed	1	8	2	1	1

Other Autopsy Findings. During the period of investigation numerous turkeys were sent to the Veterinary Science Department, Utah State Agricultural College, for analysis of infectious enterohepatitis. In addition to the regular analysis they were also examined to determine the type of lesions present in the liver, if any, and the kinds of protozoa found in the digestive tract. The results of these examinations are tabulated in Table 5.

Table 5. Type of liver lesions in relation to protozoa found in the cecum of turkeys dying of infectious enterohepatitis.

Number of birds examined	Kind of protozoa found in the cecum	Type of lesions found in the liver
8	Histomonas	Histomonas
12	Pentatrichomonas	Pentatrichomonas
4	Hist. and Pentatrach.	Mixed
2	Hist. and Pentatrach.	Histomonas
4	Pentatrichomonas	Histomonas

DISCUSSION

Turkey raising is big business, but the annual death toll results in a great economic loss. This loss amounts to about four million dollars each year in the state of Utah. Protozoan diseases are one of the important agents of this loss; hence, any work on the protozoan diseases that

may help in preventing these losses is important from an economic standpoint.

Pentatrichomonas gallinarum was reported from widely separated areas of the United States (3, p. 64), and from every county in Utah where investigations were conducted (fig. 4.). Investigators are not in agreement as to the pathogenicity of P. gallinarum. Some workers believe that the protozoan is entirely non-pathogenic; others believe it causes infectious enterohepatitis independently of, or in combination with, Histomonas meleagridis.

The diagnosis of the infection was made by finding the protozoan in material taken from the turkey's digestive tract. Unsuccessful attempts involving several methods were made to isolate the protozoan and grow it on egg slants.

The incidence of infection with P. gallinarum in ten flocks from thirteen to nineteen weeks of age was ninety-six percent (96%). These flocks were all located in the Bear River Valley of Northern Utah. There was very little difference in the percentage of infection in birds ranging on alfalfa as compared to that for birds on wheat stubble. In three selected flocks examined at regular intervals, the incidence of infection was very low for the first six weeks; however, after the sixth week the percentage of infection went up rapidly, especially after the flocks had been placed on the range. One discernible factor, as might be expected, was the type of procedure used in brooding. The flock with the lower standards of sanitation became infected with protozoa before the other two flocks.

Several other protozoa were encountered. They were: H. meleagridis, C. gallinarum, and Eimeria (sp).

In the inoculation studies that were conducted several points seemed significant. When cecal materials were used for inoculating, ninety-six percent (96%) of the birds inoculated became infected with P. gallinarum. Of the birds infected, thirty-nine percent (39%) developed clinical symptoms and died or were killed in a morbid condition. However no evidence was found that death was the result of being infected with P. gallinarum instead of possible Histomonas infections. Further studies are necessary before any definite conclusions can be drawn as to the nature of the difference in Pentatrichomonas lesions and Histomonas lesions. There was no discernible difference as to the virulence of the disease resulting with inocula from birds with Pentatrichomonas, Histomonas or mixed-type of liver lesion. Neither was there any consistent production of the same type of liver lesions from a bird having a definite type of liver lesions when material from this bird was inoculated into other birds. One problem that developed was the inability to get positive infections with either oral or rectal inoculations when the infective material was taken from liver lesions. However, as was pointed out other workers were successful in acquiring positive infections when the liver material was inoculated rectally into the vicinity of the cecal openings instead of just into the rectum as was the case in these experiments. The interval from the time of inoculation until death resulted from infectious enterohepatitis showed no consistent difference with reference to the type of liver lesion used as the source of the inoculate. There was no evidence that P. gallinarum, per se, causes enterohepatitis.

These experiments, however, were very limited in scope and a wider and more comprehensive study would have to be made before any definite conclusions could or should be made.

SUMMARY

1. Because of the wide spread incidence of Pentatrichomonas gallinarum in turkeys and the lack of agreement among investigators concerning its effects on turkeys, studies were conducted in 1948 at Logan, Utah, on this protozoan.
2. In every county of Utah where examinations were conducted turkeys were found infected with P. gallinarum.
3. The incidence of infection with P. gallinarum among ten flocks of turkeys from thirteen to nineteen weeks in age was ninety-six percent (96%).
4. The percentage of infection with P. gallinarum went up very rapidly after the turkeys were placed on the range.
5. There was no significant difference in the percentage of infection among flocks ranging on alfalfa as compared to those flocks ranging on wheat stubble.
6. One flock with a lower standard of sanitation was affected by protozoa at an earlier age than two flocks in which there were better sanitation practices.
7. When cecal material from birds infected with infectious enterohepatitis was used as the inoculating material, ninety-six percent (96%) of twenty-four birds inoculated became infected with P. gallinarum and thirty-nine percent (39%) of the infected birds developed infectious enterohepatitis.
8. No infection with enterohepatitis was observed when a saline suspension of liver lesions from birds infected with this disease was inoculated into the rectum or given orally. Nor were any infections with P. gallinarum observed when this material was used as the inoculating

medium.

9. There was no apparent evidence that a bird having Pentatricho-
monas lesions in the liver had a more or less virulent infection than
birds having the Histomonas or mixed-type of lesions.

10. There was no apparent evidence that liver lesions of a given
type (Pentatrichomonas, Histomonas, or mixed) resulted consistently from
inoculations with material from turkeys having liver lesions of the same
type.

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