STUDIES ON CULTURAL, SEROLOGICAL, AND OTHER ASPECTS OF TRICHOMONADS ASSOCIATED WITH ATROPHIC RHINITIS AND SIMILAR STUDIES ON OTHER TRICHOMONADS

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STUDIES ON CULTURAL, SEROLOGICAL, AND OTHER ASPECTS OF TRICHOMONADS ASSOCIATED WITH ATROPHIC RHINITIS AND SIMILAR STUDIES ON OTHER TRICHOMONADS

By

Warren Roberts Sanborn

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I. INTRODUCTION AND REVIEW OF LITERATURE

Trichomonads are protozoans found in the class Mastigophora. The genus Trichomonas, including such forms as Pentatrichomonas and Tritrichomonas is widely distributed in nature. Various species are found to infect mammals, fish, birds, and amphibia. Some of these are parasitic, while others are considered to have more of a commensal relationship with their hosts. Among the parasitic forms some perform minor harm, while others cause extensive pathological changes in the host animal. Some of this work will be concerned with the possible relationship of certain trichomonads producing pathological changes.

This work is concerned primarily with the two forms of Trichomonas found in the domestic hog, Trichomonas suis from the intestinal tract and Trichomonas sp. from the nasal cavities of hogs affected with atrophic rhinitis. Since the presence of trichomonads shows correlation with this disease, it was felt important to give a short summary of the disease history and work on its possible etiology.

Gruby and Delafond (1843) described vaguely a protozoan in the intestine of hogs. In 1877 Davaine named this organism Trichomonas suis. The first report of a trichomonad in the facial area of swine was recorded by Hegner and Alicata (1938).

These organisms were possibly the same form as the ones discovered by Switzer (1951) in the nasal cavities of hogs affected with atrophic rhinitis.

Atrophic rhinitis as a disease was first recognized in Europe in 1842 according to Doyle (1944). It rendered swine raising unprofitable in certain areas of Denmark, Germany, Poland, Sweden, and Norway. Duthie first described the disease in this continent in Canada in 1934 and again in 1936. Since that time it has become well distributed.

Quin (1951) reported it to be in Ontario and Ohio on the eastern border, through the swine belt to Nebraska on the western edge and from Ontario to the Gulf of Mexico, and he further stated that although there is no positive proof, the disease was supposed to have been introduced into this hemisphere via Canada through the importation of breeding stock from Sweden and since that time it has put 40 percent of the hog breeders out of business in some of the best swine areas of Ontario.

The disease itself is an insidious one. It often does not become apparent in a herd until several years after its introduction. By that time, there are many pigs infected and the whole herd usually must be eradicated. It is of main concern to the man raising high grade breeding stock because they can no longer be used for such and must be slaughtered at a great loss to the owner. However, the man who raises for meat is also affected because often the affected hog does not make

proper weight gains. The disease seems to be easily transmissable to young pigs as shown by Gwatkin et al (1949 a).

The main lesion of the disease is the complete or partial destruction of the nasal turbinates and even the ethmoturbinates. The nasal passages become occluded with pus and other waste material. Deaths are usually due to secondary pneumonia. Some minor outward symptoms include sneezing, epistaxis, rubbing the snout, and watery discharge from the nose and eyes. Shuman et al. (1953) mentioned the possibility of rhinoscopic examination for diagnosis but concluded that it was not accurate enough to be practical. To date the only positive method of diagnosis is the observance of the nasal passages at autopsy. Tentative diagnosis may be made by observation of a twisted snout due to changes in growth rates in the affected and unaffected portions of the face. This condition or the lack of it, however, is not entirely indicative of a positive or negative rhinitic condition.

Duthie (1947) pointed out that due to selective breeding the Yorkshire has developed a short upturned snout which may be confused with and given a tentative diagnosis of chronic atrophic rhinitis. He also pointed out that chronic atrophic rhinitis in man is predisposed by short, open nasal passages. He showed that this might be an aid to the acquirement of the infection in hogs. However, this view has not been borne out by later work on the etiology of the disease. Gilman (1949)

pointed out that there is no evidence to support theories of breed resistance in Yorkshire or Tamworth hogs, and there is no evidence pointing toward the resistance of long-nosed swine in general to atrophic rhinitis.

Ever since the beginning of work on the etiology of this disease, bacteria have been suggested. At first the evidence seemed to refute any ideas along these lines, but more recently there has been good evidence in support of an etiological agent of a bacterial nature. One of the earliest reports in this line is that of Moynihan (1947). He indicated no success in transmission of the disease with aerobic broth cultures of the following organisms isolated from an active case of the disease: Corynebacterium pyogenes, Staphlococcus albus, S. aureus, Alcaligenes bronchisepticus, and Pseudomonas aeruginosa.

Phillips (1946) produced a typical rhinitis in baby pigs by instilling bacteria-free filtrates into their nasal passages. This seems to be the first positive report on infection trials. This work tied in with the filterable agent aspect of the disease and work by Switzer (1953). After this work, Gwatkin et al. (1949 a) reported positive results by using nasal washings from infected pigs. These washings in this case caused partial or complete disappearance of the turbinates in baby pigs inoculated when one day old. Later experiments (Gwatkin, 1949 b) showed negative results on nasal structures in other laboratory animals.

Gwatkin's next paper (1951) brought more light to the picture. He showed definitely that the disease could be transmitted by contact of young, clean animals with infected animals. Here he also showed that streptomycin and penicillin destroyed the activity of the infective agent. This tended to point to a bacterial agent as an important etiological factor, but did not throw out the possibility of other types of organisms entering into the picture. In this same paper, there were also results that tended to obscure the picture as it appeared later. Bacterial cultures failed to reproduce the disease, and material from subcutaneous lesions in rabbits, using the MacKay rabbit lesion method, did not produce the disease. MacKay (1948), as reported by Gilman (1949), got positive results by instilling material from the lesions of rabbits injected subcutaneously with rhinitic nasal suspension into the nasal passages of healthy, young pigs. Later Gwatkin (1953) reproduced MacKay's results.

Gwatkin and Dzenis (1952) showed a possibility of treatment in early stages of the disease with penicillin and streptomycin. However the limited size of the experiment and the difficulty of early diagnosis made the use of these agents unproved and impractical at the time.

In 1953, Gwatkin *et al.* reported having isolated *Pasteurella multocida* from a field case of atrophic rhinitis and were able to successfully produce the disease in baby pigs with this

culture. At this time they were also able to produce a rhinitis in rabbits with this organism. They reisolated the organism from two of the five pigs inoculated.

The next report by Gwatkin and Dzenis (1953) added evidence to their previous paper. Using a culture of P. multocida seven months old, they produced the disease in baby pigs with the same strain which had been passaged in one pig and four rabbits for the same length of time. They then isolated P. multocida from the next six field cases and reproduced the disease in baby pigs with pure cultures of these strains. P. multocida from the lung of a pig with acute Pasteurella infection also produced the disease. P. multocida was reisolated in the course of these experiments from animals that became infected. This was not true in all cases but was so in many of them. These authors have on a number of different occasions fulfilled Koch's postulates. Antiserum injected conjointly with nasal instillation of P. multocida in one of these experiments seemed to have no effect on the course of the infection. However, the results were inconclusive. Antiserum did protect mice from 1000 M.L.D. of the culture of P. multocida used.

While this work by Gwatkin was progressing, MacKay and Carter were also progressing along similar lines. In 1953, they noted L type colonies of Spherophorus necrophorus appearing upon bacterial examination of cases of atrophic rhinitis. In this paper they noted that the disease could not be

produced regularly with these organisms, however, they found that a combination of L type S. necrophorus and P. multocida regularly produced the characteristic lesions. In a paper submitted earlier but printed later (September, 1953) these workers showed the use of the MacKay rabbit lesion method mentioned above in isolation of the two aforementioned organisms. They reported these to be isolated consistently from the rabbit subcutaneous lesions.

Shortly after this Carter and Bigland (1953) showed the dissociation and virulence of various strains of P. multocida. Of four strains from rhinitis cases, three were smooth and one was mucoid. Of twenty-eight cultures isolated, twelve were type B, three were type A, and the rest were unidentifiable.

A filterable agent was one of the first things suspected in the etiology of atrophic rhinitis. Quin (1951) pointed out that all attempts to produce the disease with filtrates of known infective material had failed. He stated that this ruled out a virus. However, he did not mention the types of filters used.

In 1953 Switzer, who previously had described trichomonads in connection with the disease, described a filterable agent from the nasal passages of pigs with atrophic rhinitis. The agent passed a Selas No. .02 filter, but the titer, as measured by pathological studies on chicken embryos, was reduced by passage through a Selas No. .03 filter. The agent

was held back by a Mandler filter of 7 pounds bubbling pressure and a Seitz sterilizing pad. Penicillin and bacitracin had no effect on this agent, streptomycin affected it mildly by reducing the titer, aureomycin affected it strongly, and terramycin had a complete inhibitory effect. This filtrate introduced into the nasal passages of 8-day old pigs produced a definite atrophy of the nasal turbinates. However, the atrophy was less severe than that produced by instillation of the whole nasal exudate. The filtrate produced definite pathological changes in chicken embryos, especially in the heart and liver showing pericarditis, epicarditis, and necrosis in the liver. Death and other pathological changes were recorded, but the above mentioned changes were the most characteristic. When the agent was inoculated into young pigs intraperitoneally it produced severe pericarditis.

Earlier in 1951, Switzer reported the discovery of trichomonads in the nasal passages of 80 percent of Iowa swine affected with atrophic rhinitis. At the same time he reported that only 2.8 percent not affected with atrophic rhinitis were positive for trichomonads. This possibly was not the first description of these trichomonads. There was an earlier report by Hegner and Alicata (1938) of the discovery of trichomonads in facial lesions on pigs in Hawaii. Swellings were found on the jaws and face of the animal. They reported sneezing and nasal discharge. The bacteria present were

Pseudomonas pyocyaneus and Actinomyces sp. These nasal trichomonads appeared in some respects to be like T. suis, the form normally found in the hog intestine. However, they also appeared different in some respects.

Switzer went on to report that after the two forms from the pig were cultured over a long period of time, they became indistinguishable as to size and morphology. On original examination the nasal forms were found to average 15.3 by 5.3 microns. The organism had a well-developed axostyle with a bulbous tip. An undulating membrane, diffuse nucleus, and an 9 to 10 micron trailing flagellum were described. The vast majority had three anterior flagella. Morgan and Hawkins' (1952) description of T. suis showed it as being 8 to 10 microns long with a slender axostyle.

Switzer at this time was not able to establish the trichomonads in the noses of swine from the culture tube. It did become established in the vagina of cattle, in chicken embryos, and in C.P.L.M. medium.

Spindler et al. (1953) reported several experiments attempting to elucidate the etiology of atrophic rhinitis with trichomonads being foremost in their minds. In the experiments the washings from the animals that were used for inocula were not pure in the sense that they contained only one possible type of infective agent. All of the inocula could have contained bacteria, mold, filterable agents, and protozoans. This was one admitted weak point of the experiment.

The pigs used ranged in age from five days to 42 days at inoculation. The following results were obtained in the experiments. Washings from the nasal cavities of rhinitic swine with or without trichomonads and washings from non-rhinitic swine with trichomonads all produced rhinitis in the experimental animals and trichomonads were established.

Washings from rhinitic swine with trichomonads and rhinitic swine without trichomonads were stored at 4° C. for several months to insure elimination of the protozoa present. These washings were then used as inocula. Stored washings from non-rhinitic swine with trichomonads were also used. These washings stored at 4° C did not produce rhinitis or establish trichomonads in experimental animals. Trichomonads from the cecum of a non-rhinitic hog produced rhinitis in experimental animals.

This work by Spindler was thought by him to be evidence that the trichomonads were a major cause of atrophic rhinitis. It also was thought to point out that the two forms in the pig were the same.

Simms (1952) reported that 44 percent of rhinitis-affected swine in the Beltsville herd harbored trichomonads. He pointed out, however, that the direct smear method used was not the best method for demonstrating the organisms. In 1953 Simms commented on the work of Spindler and reported on some further work that was not in Spindler's paper. Surveys of affected

and unaffected herds at the Agricultural Research Center showed that members of the former harbored nasal trichomonads while members of the latter did not. Twelve pigs were inoculated with trichomonads, four from a rhinitis-negative trichomonad-positive source, and four from cecal contents of a rhinitis negative source. All pigs showed marked turbinate degeneration and trichomonads upon autopsy. A contact control showed mild degeneration of turbinates and trichomonads upon autopsy. Pigs 9 weeks old were inoculated with trichomonads from the nose or cecum of rhinitis-affected pigs. All eleven showed trichomonads upon autopsy but no changes in turbinates. This discrepancy might have been accounted for by consideration of the increased age of the experimental pigs.

Hammond and Fitzgerald (1953) published an abstract on observations of trichomonads from pigs. The organisms were isolated from the nose, stomach, and cecum. They reported successful culture in a modified Plastridge medium. Morphological studies showed the cecal form to have a spheroid to ovoid body and the nasal form to have an elongate shape. The undulating membranes in the cecal form were high and from one-half to two-thirds body length, while that of the nasal form was low and nearly as long as the body. The stomach forms were reported to more closely resemble the cecal forms than the nasal forms. It was also reported that there were differences in growth characteristics in culture. These were not detailed.

The etiology of atrophic rhinitis is at present rather obscure. There are good clues pointing in at least three directions. Gwatkin (1953) and MacKay (1953) have shown evidence in the direction of bacteria. Switzer (1953) has pointed out a possible filterable agent aspect, and Switzer (1951) and Spindler (1953) have pointed out a correlation between the presence of trichomonads and the disease.

The present study was initiated largely due to the obscureness of the etiology. It consisted of further attempts to infect swine with bacteria-free strains of the nasal trichomonads, general survey work, experiments with other possible susceptible hosts, and comparative studies of the cultural and serological relationships of the trichomonads from swine and other trichomonad forms.

II. MATERIALS AND METHODS

A. Packing House Survey

Since there was little information available on the prevalence of atrophic rhinitis or nasal trichomonads in Michigan and no immediate sources of diseased hogs at hand, it was thought advisable to make a survey. Arrangements were made with Rosevale slaughter house to examine hogs coming off their line. This proved to be an abortive idea. Details of this will be discussed later. The hogs were examined as they came off the line by splitting the heads in sagittal section. This was done with a bladed device with a long handle and a clamp for the head and was already set up by the slaughter house for that purpose. The nasal passages were examined grossly for damage to the turbinates and one or both turbinates were removed and placed in sterile Ringer's solution. This was brought back to the laboratory and examined by centrifuging and microscopically examining the sediment for trichomonads.

B. Hog Handling

The first rhinitic material to be used was obtained from a pig in the animal pathology autopsy room of Michigan State College. The pig was from a herd in Mason, Michigan. Later three more pigs were obtained from this same herd for examination.

These hogs were housed in an isolated animal room which was quite easily hosed out for cleaning purposes. The hogs were fed from a standard hog feeder placed across the doorway to minimize the necessity of entry into the room. They were fed a standard hog ration. Weight was measured by placing the hogs in a large burlap sack which was tied securely. It was found that the animals could best be restrained in this manner and would lie still long enough for their weight to be read on a standard feed scales. Bacteria were obtained via loop at autopsy and plated out on serum agar and blood agar under aerobic and anaerobic conditions. They were tested in the usual diagnostic media. Molds were tested for on specific media by Dr. Beneke of the Michigan State College Botany Department. Examination for parasites was made by fecal examination and external gross inspection plus scrapings. Material for filterable agent examination was frozen at -40° C. for several months. It was then thawed and put through eggs on the chorio-allantoic membrane and in the allantoic cavity. Sterility tubes were run concurrently. Examination for protozoa of the intestinal tract was made by direct smear as was that for protozoa of the nasal passages.

The starting and care of young pigs was at first somewhat of a problem. They were raised in an animal room approximately fifteen feet square. A small platform three feet square and two inches off the floor was placed in one corner and covered

with sawdust. This served as a bedding space for the pigs and they used it for the most part. The pigs were obtained when approximately three weeks old. They were started on corn meal four parts to soy bean meal one part. This was mashed up in cow's milk since they had just been removed from the sow. The feed was placed in heavy iron feeders on the floor. This seemed to be quite satisfactory and little trouble was had in getting the pigs to eat it. The milk was gradually diminished and water put in its place until at a week and a half to two weeks, water was being used alone. Shortly after this the pigs would eat dry meal.

Isolation of the pigs was accomplished by placing the two groups in separate rooms with the step-ups at the doors. The rooms were first washed down with detergent. They then were rinsed with hot water and cold. They were then scrubbed with Roccal solution at 1:5000. This was allowed to set for about one-half hour, and they were again rinsed. Separate pairs of boots were used for entry into each room, and these were washed with Roccal solution at 1:2000.

C. Culture of Trichomonads

1. Trichomonads used and sources. A number of trichomonad species were obtained for trial at culture. Trichomonas suis was obtained from the cecum of a rhinitic hog from Mason, Michigan. Trichomonas sp. from the nasal passages of hogs was collected and cultured upon two different occasions.

T. sp. #1 was obtained from the nasal passages of the first rhinitic hog mentioned above. T. sp. #2 was obtained from the nasal passages of one of the rhinitic hogs in the second group mentioned as having been obtained from Mason. T. foetus was obtained from a sample received by the Parasitology Section for diagnosis. T. vaginalis came from a urine sample received by a medical diagnostic laboratory in Lansing. The cecum of a turkey received for autopsy by the poultry diagnostic laboratory at Michigan State College yielded Trichomonas gallinarum. T. ruminantium was obtained from the large intestine of a cow at autopsy in the animal pathology autopsy room and T. muris came from the colon of a laboratory rat.

2. Methods of counting protozoa. Protozoa were counted after the suggestion by Trussell (1946). The method was not elaborated on by him and thus possibly bears some elaboration at this point. The culture or suspension to be counted was first thoroughly mixed by use of a pipette. A drop of the suspension was then placed in each chamber of a haemocytometer. In the case of the lighter cultures, the total number of organisms in the central large square in each chamber was counted. This was divided by two to obtain the average number within one of the large squares. This result was then multiplied by 10,000 to get the number of organisms per cc. However, this method was found to be impractical if not impossible in

case of heavy populations of highly motile trichomonads. With large numbers of protozoans present, it was nearly impossible to count all organisms in each central large square. Advantage was then taken of the next smaller squares into which the large central square was divided. There are 25 of the smaller squares. Ten were counted in one chamber and five in the other. These were counted in lines of five horizontally. If the numbers of organisms in the three counts were not reasonably close to each other, the count was discarded and a new one made. The amount of variance of course, depended upon the size of the population. The number obtained in this count was divided by 3 and multiplied by 5. This product was the average number of organisms in one large square. Multiplication by 10,000 then gave the number of organisms per cc.

3. Media used and preparation. Several different media were used during the course of the study. Some were commercial, while others were of a more intricate nature. Thioglycollate broth was made according to Difco. Brain-heart infusion was also according to Difco with the addition of 0.5 percent agar. Plastridge medium was made according to Morgan (1946). The medium of Laidlaw (1928) was sometimes utilized. C.P.L.M. (cysteine HCl, peptone, liver infusion, maltose) medium, which was most frequently used in the study, was modified in its preparation for general usage slightly from Trussell (1946). The medium contained horse serum which was easily available.

Whereas the formula given by Trussell (loc. cit.) calls also for the addition of 11-13 cc. of 1N NaOH before adjustment, it was found more advantageous to omit this step and merely adjust with 1N NaOH. Also it was found that adjustment to a pH several tenths higher than the 5.8 - 6.0 suggested by Trussell was satisfactory. Indeed upon one occasion the pH of the medium was adjusted to 7.0 - 7.1 by accident in the bacteriology media room and used without knowledge of this until discovered while checking a pH meter. On this occasion all the strains grew with the same characteristics that they had at 5.8 - 6.1.

4. Isolation Procedures. To grow the trichomonads axenically was necessary for most of the work, and several procedures were adopted which were found to free them from other organisms. To get rid of bacteria, a culture tube of C.P.L.M. was inoculated with the contaminated Trichomonas inoculum. Fifty thousand units of penicillin and 50 mg. of streptomycin were then added tc each tube containing 9 cc. of medium. The penicillin and streptomycin were diluted so that the prescribed amounts were contained in one-fourth of a cc. for each tube. After two days another transfer was made into new C.P.L.M. medium containing these antibiotics. Every time transfers were made, they were microscopically checked for motile protozoa. Sometimes in the case of fecal samples, it was necessary to make the third transfer into C.P.L.M. containing antibiotics. The tubes were shaken and then incubated

for two days. Sterility was checked using thioglycollate broth, brain-heart infusion semi-solid, and tryptose serum agar slants.

Molds and yeasts were also common contaminants of the cultures. These were excluded by the use of a simple cotton plugged "U" tube containing 18 cc. of C.P.L.M. The inoculum was dropped in one side and material for transfer was removed from the other side after 24 - 48 hours incubation at 37° C. Usually only one such passage was necessary to free the culture from all traces of yeasts and molds. Microscopic inspection or gross inspection was all that was necessary to note the presence or absence of these contaminants.

The problem of other contaminating protozoa only occurred twice, and it resolved itself by the dying out or loss on transfer of the extraneous forms.

D. Serological Methods

1. Preparation of sera. Horse serum for use in C.P.L.M. and other media was collected in several different ways, only one of which proved to be really satisfactory. The unsatisfactory methods involved sterile flasks, large flasks containing a partial vacuum, flasks with tubes for evacuation while drawing blood, and several other such devices. These all proved to be cumbersome, conducive to hemolysis, or at times completely unworkable. The most successful method involved the use of a sharp bleeding needle of 10 - 16 gauge, 12 gauge being the best size, with an attached four-inch rubber

hose having an inside diameter about the same as that of the needle. The area of the jugular vein to be punctured was then given a small amount of local anesthetic to obviate the necessity of using a twitch on the horse. The skin was swabbed with roccal and the vein punctured. The blood was run directly into 100 cc. centrifuge tubes, filling as many as desired. The blood was run down the side of the tube and not allowed to drop the tube's length. As much as 1400 cc. was taken from a large horse at a time. This blood was allowed to clot at room temperature for approximately one hour. The clot was then broken from the sides of the tube by gently going around the edge with an applicator stick. The blood was placed in the refrigerator at about 4° C. overnight. In the morning it was centrifuged in an International No. 2 centrifuge with a regular eight-place head for about 1 1/2 hours to 2 hours at 2000 R.P.M. At the end of this period the supernatant serum was pooled in a common flask. The serum was filtered through a 25 cm. Seitz DK.-8 filter pad by gravity at 4° C. This usually took 18 to 24 hours. The filter flasks were opened in a sterile transfer room and sterile volumetric pipettes of 25 or 50 cc. capacity were used to transfer to sterile serum bottles of a maximum capacity of 60 cc. Rubber stoppers were put in place, and the serum was incubated at 37° C. for from 48 to 72 hours as a sterility

check. At the end of this period it was inactivated by heating at 56° C. for 25 minutes. The serum was then stored at 4° C.

The rabbit sera for the agglutination reactions were prepared in much the same way as was the horse serum. The main differences were only in scale. The average amount of blood obtained from the rabbits when completely bled out was 100 cc. On one occasion 130 cc. was obtained. The rabbits were immobilized by stretching them on their backs on a board designed for the purpose and tying their legs out away from the body as tightly as possible. The left chest was then clipped with an electric animal clipper. The area was swabbed with 70 percent alcohol and a sharp, two-inch 18 gauge needle was used for heart puncture going through the rib cage on the left side. An attached 50 cc. syringe was used to withdraw blood. When this was full, the syringe was removed leaving the needle in place, and while an assistant expressed the blood into a 100 cc. centrifuge tube, a second syringe was used to obtain further amounts of blood. When no more blood could be obtained, the rabbit was sacrificed with chloroform. The blood in the tubes was then handled as was the horse blood used in media.

2. Preparation of antigen. The preparation of antigens varied slightly depending on whether their use was for rabbit inoculation or for agglutination reactions. The antigen for inoculation was living, and that for agglutination was dead. The antigen used for rabbit inoculation was trichomonad

cultures grown in regular C.P.L.M. medium. This was washed three times with Ringer's Solution by centrifugation. A drop of this final suspension was counted in a haemocytometer chamber. It was then concentrated or diluted as the case demanded so that the desired number of living protozoans could be contained within one cc. or less of inoculum.

The antigen used for agglutination reactions was trichomonads grown in a modified C.P.L.M. medium after Trussell (1946). These were grown in a number of tubes and then five or six tubes were poured into a 50 cc. graduated centrifuge tube for concentration. They were washed three times with M/15 NaH_2PO_4 and suspended in this solution. They were then counted in a haemocytometer chamber and the whole made up to a concentration of 2,000,000 organisms per cc. The antigen was formalinized to a concentration of 0.3 per cent formalin and stored in serum bottles at 4° C.

3. Rabbit inoculation. All rabbits used were adult, non-pregnant females. Rabbits were inoculated once a week for a period of eleven weeks. The dosage was as follows with noted exception: inoculation # 1, 2.5 million; inoculation # 2, 6.5 million; inoculations # 3 through #6, 6 million; inoculations #7 and #8, 10 million; inoculation #9, 15 million; inoculations #10 and #11, 20 million. (The eighth inoculation of rabbit 3 with T. foetus was only 1 1/2 million due to an unexplained difficulty in growth of the cultures at that time.)

Serum was taken from rabbits 1, 2, and 3, four days after the last inoculation, rabbits 5 and 6, three days after the last inoculation, and rabbits 7 and 8, six days after the last inoculation.

The rabbits were inoculated with the suspensions of living trichomonads by first immobilizing them in a laboratory coat and then using a 1 cc. tuberculin syringe with a sterile 22 gauge, one-inch needle to go into the external ear vein. This method allowed the advantages of only one person having to be present and the minimum of discomfort to the rabbit.

4. Agglutination procedure. The agglutination procedure was much like that described by Trussell (1946) for T. vaginalis. The main difference between the two procedures was that this method allowed for direct dilution of the serum with the antigen suspension. This was done by placing 0.8 ml. of antigen suspension in the first serial tube and 0.5 ml. in each of the following tubes. 0.2 ml. of serum was added to the first tube to make a 1:5 dilution. Serial transfers of 0.5 cc. amounts were then made down the line. Antigen controls and normal serum controls were run concurrently with the test mixtures. The mixtures were pipetted onto slides marked by heating the slide and then making six enclosed squares with a wax pencil. The heating was necessary to keep the moisture from raising the wax during the four hour incubation

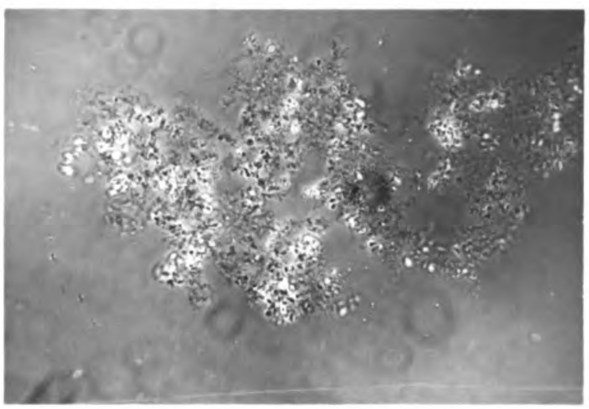
at 37° C. thus preventing the various mixture drops from running together. The amount of mixture used for incubation was 0.015 ml. Hanging drops were attempted but found to be completely unsatisfactory.

Incubation was accomplished by placing the slide in a moist chamber in order to keep it from drying out. The moisture chamber was constructed of a petri dish with two pieces of round filter paper in the bottom. These were thoroughly moistened with distilled water, but none was allowed to lie inpuddles. The slide was supported on the two halves of an applicator stick. The top of the petri dish was put in place, and the whole was incubated.

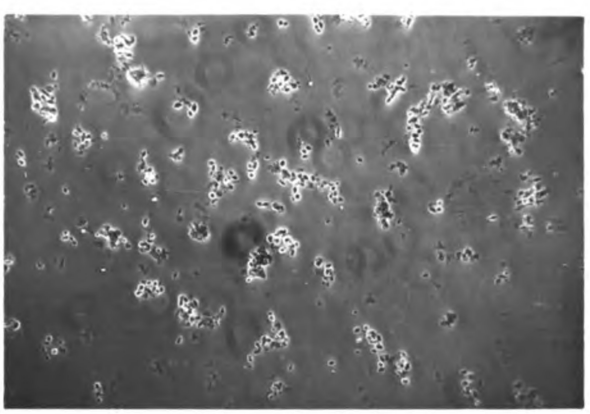
At the end of the four hour incubation period, the slide was removed, placed on paper toweling to remove moisture on its under side, and read at 100x magnification. This reading was best accomplished with wide field oculars and phase contrast. Dark field was often also helpful in determining doubtful end points. Floating debris, the nature of which was never ascertained, sometimes caused the reading of false positives when regular illumination was used. End points were at first sometimes difficult to determine, however experience made it possible to tell the difference between true agglutination and loose aggregations of the organisms in suspension. The titer was read according to Trussell (1946). It was read as the highest dilution of serum in which a few

small clumps occurred. Please see Plates 1 and 2 for photographs of agglutination reactions.

PLATE 1

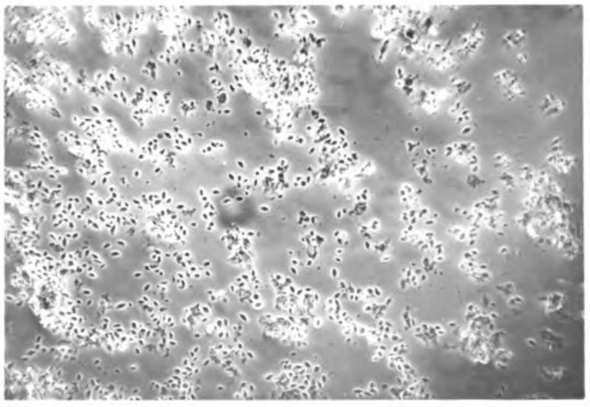


Strongly positive agglutination.
x 300

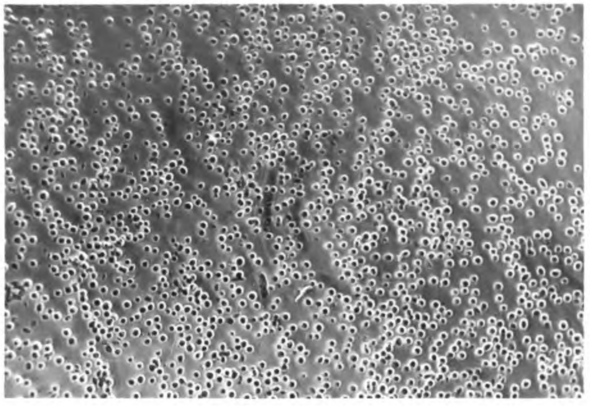


Clear end point agglutination.
x 300

PLATE 2



weak end point agglutination with tendency toward clumps but few true clumps. x 300



Negative agglutination. x 300

III. RESULTS

A. Packing House Survey

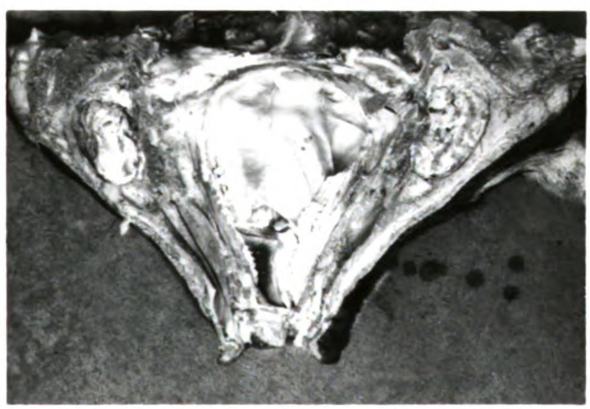
The purpose of this survey was first to obtain trichomonad samples for experimentation and secondly to ascertain the possible prevalence of atrophic rhinitis in Michigan. In the packing house survey there were 29 hogs examined. Fourteen of these were from Michigan, and fifteen were from Illinois. No trichomonads were found in the nasal cavities of any of the hogs. None of the Michigan hogs showed any evidence of atrophy of the turbinates. Of the fifteen Illinois hogs, two showed definite atrophy of the turbinates. Since there were no trichomonads found in these animals, affected hogs coming into the M.S.C. pathology autopsy room were examined, and material was sought in the field.

B. Rhinitic Hogs from Mason, Michigan

Four rhinitic hogs were received from a herd in Mason, Michigan. The first of these was obtained for autopsy on March 2, 1953. The other three were given by the owner on March 22, 1953, for experimentation and observation. The general conditions under which these hogs were being raised were very dirty and inviting to any sort of parasitism. The feed was mostly scraps, garbage, and left-over fodder. The infected pigs of the latter group obtained were weighed prior

PLATE 3

Dorsal view of pig's head showing lateral deviation of snout.



Saggital section of pig's head showing unilateral destruction of turbinates on left side.

to autopsy. They were respectively 67 1/2 pounds, 80 pounds, and 84 1/2 pounds. Their litter mates that were seemingly uninfected weighed between 250 and 300 pounds. The pigs were all approximately one year old.

Autopsy of the first animal showed nearly complete destruction of the turbinates and some pneumonia. The animal was unthrifty and underweight. The other three hogs were kept under observation for a while and autopsied at intervals. Autopsies on these hogs showed them all to have a well advanced pneumonia. In one case three-fourths of the lungs were consolidated. All of the pigs showed some external deviation of the snout. Two of these hogs had the dorsal and ventral turbinates missing from both sides of the nose. The other one upon sagittal section showed the left dorsal and ventral turbinates to be present and normal and the right turbinates to be absent. Photographs of these findings are found on Plate 3.

Direct smears of the nasal passages of these animals showed the presence of motile trichomonads in all four cases. The feces also contained motile trichomonads. Fecal examination also demonstrated Balantidium coli.

Examination and cultural procedures also showed the presence of molds in the nasal passages. These were examined by Dr. Beneke of Michigan State College who felt that the species present, Aspergillus sp., were not of pathological significance.

Worm parasites present were Ascaris lumbricoides and Oesophagostomum dentatum. Also Eimeria scabra was identified from fecal samples.

Tests were made for the filterable agent described by Switzer (1953). Material was collected and stored in 1/15M NaH_2PO_4 buffer at -40° C. for seven months. It was then thawed, prepared by centrifugation and after addition of streptomycin and penicillin, inoculated into embryonating chicken eggs by the allantoic cavity route. No Selas filters were available, so the material was put into the eggs without filtering it. Thioglycollate sterility controls showed no growth of bacteria or fungi. This may have been due to the long period of very cold storage. Only three eggs were available for inoculation the first time. They were inoculated as 11th day embryos and were examined on their 20th day. All embryos were alive. Pathological changes observed were a thickened chorio-allantoic membrane and a slight myocarditis. Three more eggs were inoculated later from the same original inoculum. These were inoculated by the allantoic route on the 11th day and the embryos were autopsied on the 19th day. Examination of these eggs showed a more striking pathological picture than that of the first autopsy of chicken embryos. Focal necrosis of the liver was slight in two of the embryos and very decided in the third embryo. All three embryos showed mild epicarditis and early petechial epicarditis. On a second

passage from the allantoic fluid of these eggs, the only discernible pathological change was a possible diffuse hyperemia.

During the next month the arrival of a good case of atrophic rhinitis for autopsy afforded the opportunity of using some fresh material for egg passage. This material was collected, ground,suspended, centrifuged, and treated with antibiotics. Again there were no Selas filters available, so a Swinney filter (Seitz type pad) was used. Two eggs were used for the unfiltered material, and two eggs were used for the filtered material. They were again inoculated by the allantoic route. Examination revealed no pathological changes in the filtered group, while the unfiltered group exhibited a thick chorio-allantoic membrane, myocarditis, focal necrosis in the liver, and dwarfing of the embryo. The thioglycollate sterility control for these eggs showed the presence of some mold. A second passage from the allantoic fluid of the above unfiltered group was made into 8 eggs. Of these eggs, 4 were inoculated with filtered allantoic fluid, and 4 with unfiltered allantoic fluid. Examination of the embryos showed no pathological changes. On first passage there seemed to be an indication of an infective agent that was not affected by penicillin or streptomycin. This agent was apparently removed by a Seitz filter pad, and it was either lost on passage or became dormant upon egg passage.

Since some important indications had been found in the direction of bacterial agents by other workers, the nasal passages were also examined for bacteria. Bacteria were isolated on plates of tryptose blood agar. Aerobic and anaerobic methods were employed. There was not sufficient time to identify the anaerobic forms, and so they were introduced into brain-heart infusion semi-solid medium and stored at 4° C. The aerobic forms isolated were examined using the various standard stains and diagnostic media. The following bacteria were found: Escherichia coli, E. intermedium, Pseudomonas incognita, Alcaligenes faecalis, Staphlococcus epidermidis, Corynebacterium renale, Neisseria catarrhalis, two unidentified bacilli, two unidentified diptheroids, alpha viridans Streptococcus, three unidentified small gram negative rods, and several other unidentifiable forms.

C. Experimental Pig Inoculation

Since the previous experiment did not disclose any of the possible bacterial etiological agents described by other workers, it was thought desirable to try some experimental infections using the trichomonads that were found to be present in conjunction with the disease. Two pig inoculation experiments were run. In the first experiment four pigs were used, two for experimental and two for controls. Fecal examinations revealed no work eggs or protozoa. Methods used were sugar flotation and direct smears. The pigs were of the

Chester White breed. At 28 days of age they weighted approximately 25 pounds each. Throughout the experiment the weights of the experimental pigs and the controls did not vary significantly from each other. The experimental pigs were kept segregated in a room, and the controls were run in the field on a spot where there had never been rhinitis. The temperatures of the experimental pigs remained within the normal range throughout the course of the experiment.

The first inoculation of trichomonads was on the 28th day. Each of the experimental pigs was given living trichomonads in pure culture intranasally on three different occasions at intervals of nine days and five days respectively. One pig received the bottom 1 cc. of a pure culture that had been centrifuged at approximately 1500 rpm. for 5 - 10 minutes. This was dripped into his nose on the right side with a hypodermic syringe by holding the pig upright on his hind legs and closing the left nostril. The drops were dropped on each inhalation. The other pig received trichomonads prepared in the same way but placed in about 5 grams of hog fecal material which had previously been autoclaved at 123° C. for 20 minutes and cooled. This mixture was forced into his right nostril with a tongue depressor. It was felt that this might closely resemble the way that the infection was obtained in nature. The object was to give the trichomonads something to hold them

in place so that they could get a better start. Autopsy was done at 65 days. There were no pathological changes in any of the animals. There was no parasitism of any sort, and there were no living trichomonads either in the intestine or in the nasal passages.

The second pig inoculation experiment involved eight pigs. Four were put in the pasture mentioned above. Weights and other observations were the same as in the above experiment. Two of the remaining pigs were placed in a thoroughly cleaned room. The remaining two pigs were inoculated intranasally with a Ringer's solution emulsion of nasal exudate from a Mason rhinitic hog. This exudate contained many motile trichomonads. The pigs were 31 days old when inoculated. One pig received 2 cc. of this suspension in the right nostril by dripping from a hypodermic syringe as described above. The other experimental pig had the inside of his right nostril scarified with a small test tube brush before instillation of 2 cc. of the suspension. This was done with the thought in mind that an inflammation or mild rhinitis might be a necessary precursor to establishment of the trichomonads. None of the pigs showed any pathological changes upon autopsy when they were 86 days old. Neither were there any trichomonads established in the pigs. No other parasitism was found. The necessity of keeping cultures available for further experimentation after material had been used to start the above experiment and

to obtain pure cultures for parts of the above experiment prompted a study of the cultural requirements, behavior of organisms, and qualities of various media to be used.

D. Culture of Trichomonads

1. Keeping qualities of media. One problem in the culture of trichomonads is that of keeping media that will support growth. Most of these media are anaerobic and are unstable. Five media were checked for keeping qualities.

a. Thioglycolate - Twenty tubes of this kept anaerobically at room temperature for 3 weeks.

b. Brain-heart infusion semi-solid (0.5 percent agar) - Long keeping quality at 4° C.

c. C.P.L.M. - Of twenty tubes of this medium, 11 remained sufficiently anaerobic for use for three weeks. The others were no good at approximately two weeks' time. Warming restores anaerobiasis for only a short period (12 - 24 hours). On one occasion a whole batch of several hundred tubes of this medium became aerobic in one week's time.

d. C.P.L.M. semi-solid (0.4 percent agar) - Ten tubes were tested at room temperature and ten tubes at 4° C. Of the room temperature tubes, two became contaminated and were thrown out. At the end of 7 weeks, six tubes were aerobic and two were slightly anaerobic. Of the medium stored at 4° C., five tubes were aerobic in 29

days, and the rest were aerobic within 42 days. This medium was then warmed at 56° C. until anaerobic again. It was then stored at room temperature. Ten days after this rejuvenation the tubes were one-half anaerobic. Mold contamination, which will aid anaerobiasis, then grew through the plugs and further observations were impossible.

e. C.P.L.M. medium was frozen and stored at -40° C. Thirty-four days later two tubes were thawed and found to be in good condition and still anaerobic for one-half the depth. After 106 days of freezing, the remaining six tubes were thawed and found capable of supporting growth of Trichomonas sp. #1.

2. Growth trials - longevity of protozoa. Of the various media mentioned above, thioglycollate will not support the growth of trichomonads from the pig or Trichomonas foetus. It will keep them for 2-3 days, but they will not multiply. The same is true for brain-heart infusion semi-solid. However, this medium is useful in that it will keep the above organisms for 15 days, and on one occasion it kept Trichomonas sp. #2 for 19 days. It did not support continual transfer. All the C.P.L.M. media supported the growth of the above organisms superbly and also that of T. vaginalis and T. gallinarum. These latter two organisms grew perhaps a little better in the semi-solid C.P.L.M. than in the regular C.P.L.M.

Cultures in C.P.L.M. at 37° C. usually lasted about 5-7 days. This varied somewhat with amount of inoculum and the organism used. These variations will be brought out below.

It was stated by Trussell (1947) that acid production caused the death of protozoa in C.P.L.M. To check this, C.P.L.M. was adjusted to pH 5.2, a pH lower than that of the medium after the organisms had all died, and inoculated with T. sp. #1, T. sp. #2, T. suis, T. foetus, T. vaginalis, and T. gallinarum. Three tubes of each were used. All grew as well and as long as did the controls in regular C.P.L.M.

E. Amount of Inoculum Variation

Since it was observed that the growth of cultures varied with varying amounts of inoculum, it was thought advisable to run an experiment on this variable to see what amounts would be best for transfer and later experiments. In this experiment the constants were the temperature of incubation (37° C.) and the time of incubation (48 hours). The test organisms were T. sp. #1, T. sp. #2, T. suis, T. foetus, T. vaginalis, and T. gallinarum. Two tubes were run on each organism for each amount of inoculum in 9 cc. of medium. The results appear in Table I.

F. Room Temperature Populations

Switzer (1951) noted that the trichomonads from the nose of the pig laster longer at room temperature incubation

TABLE I

TWENTY-FOUR HOUR POPULATIONS OF TRICHOMONADS GROWN IN C.P.L.M. AT 37 DEGREES CENTIGRADE FROM VARYING INITIAL INOCULA*

Initial Inocula	100,000	200,000	300,000	500,000	700,000	1,000,000
Trichomonas sp. #1 (nose of pig)	12.35 4.83	1.25 1.08	3.08 2.38	1.32 1.4	3.43 2.8	.93 .97
T. sp. #2 (nose of pig)	10.98 7.77	1.48 1.63	2.13 1.73	2.22 1.98	2.67 1.3	1.38 1.35
T. suis	4.30 8.02	9.28 8.62	2.63 2.77	2.85 2.33	2.08 2.2	1.8 1.88
T. foetus	9.27 10.13	8.98 8.78	2.48 3.	7.02 6.85	1.62 1.8	1.52 1.63
T. vaginalis	.03 .03	.06 .12	.71 1.14	.77 .77	.11 .09	.87 .87
T. gallinarum	.31 .73		.32 .23		.42 .22	

* Results in millions of organisms per cc.

than did the ones incubated at 37° C. No other data were given, and so it was thought to be of some importance to get more data on longevity and population peaks at this incubation temperature. It was also hoped that some insight could be gained into comparisons of growth rates of the two forms in the pig. In this experiment the amount of inoculum was a constant, and the temperature was somewhat constant, that is to say, a record from the constant recording thermometer registered a gradual drop in temperature over the duration of the experiment of 0.4° C. The drop was from 20.4° C. to 20° C. Populations were counted at varying time intervals. The organisms used were the same as in the above experiment, and pH was recorded in addition. Starting pH was 6.6. Inoculum was 500,000 organisms in 9 cc. of medium. The medium used was the same as used in all the population experiments, C.P.L.M. Results are found in Table II.

G. 37.5° C. Populations

The purpose in this experiment is much the same as the previous one with the exception that the emphasis is placed on normal incubator temperature. The experiment was set up as the previous one. Starting pH was 7.35, and the inoculum was 200,000 in one case. In the other case, the inoculum was 500,000, and the starting pH was 6.55. Results were checked every 12 hours and are recorded in Tables III and IV respectively.

TABLE II

POPULATIONS OF TRICHOMONADS AND pH OF THE MEDIUM FROM GROWTH IN C.P.L.M. AT ROOM TEMPERATURE AT VARIOUS AGES OF CULTURES. STARTING pH 6.6. INOCULUM 500,000.

Results in millions of organisms per cc.

Age in Hours		60	84	108	180	204	252	324	372	468
Trichomonas sp. #1 (nose of pig)	Count	.16	.14	.20	.18	.23	.19	.13	.05	.01
	pH	6.7		6.7	6.9				6.6	5.6
T. sp. #2 (nose of pig)	Count	.79	1.13	1.44	1.73	1.08	1.31	.54	.60	.05
	pH	6.5		6.2	5.9				5.7	5.8
T. suis	Count	.24	.28	.27	.35	.34	.26	.22	.11	.05
	pH	6.7		6.7	6.7				6.6	6.5
T. foetus	Count	.20	.25	.49	.33	.33	.31	.19	.13	.02
	pH	6.6		6.6	6.6				6.5	6.4
T. vaginalis	Count	.05	.05	.02	neg					
	pH	6.7		6.7	6.6					
T. gallinarum	Count	neg								
	pH	6.6								

TABLE III

POPULATIONS OF TRICHOMONADS AND pH OF THE MEDIUM FROM GROWTH IN C.P.L.M. AT 37.5 DEGREES C. AT VARIOUS AGES OF CULTURES STARTING pH 7.4. INOCULUM 200,000.

Results in millions of organisms per cc.

Age in Hours		12	24	36	48	60	72	84	108	156	180
Trichomonas sp. #1 (nose of pig)	Count	.16	.53	3.71	2.55	2.57	1.45	1.35	1.02	.06	.05
	pH	7.3	7.3	6.6	5.6	5.6	5.9	5.6	5.5	5.5	5.4
T. sp. #2 (nose of pig)	Count	.15	.84	2.25	3.47	1.97	2.27	.88	.27	.03	.03
	pH	7.4	7.2	6.9	5.8	5.5	5.5	5.5	5.4	5.4	5.4
T. suis	Count	.13	.46	3.72	2.65	1.63	1.10	.90	.55	.45	neg
	pH	7.3	7.3	6.6	6.5	5.9	5.8	5.5	5.4	5.4	5.3
T. foetus	Count	.14	.57	3.20	2.50	3.40	1.70	1.10	.14	pos	neg
	pH	7.4	7.2	6.6	5.9	5.6	5.6	5.5	5.4	5.3	5.3
T. vaginalis	Count	.03	.03	.04	.04	.04	.01	.05	.28	1.30	.70
	pH	7.4	7.4	7.3	7.3	7.3	7.4	7.3	7.2	5.8	6.4
T. gallinarum	Count	.01	.01	.08	.31	.29	.35	.26	.09	.02	.04
	pH	7.4	7.4	7.3	7.2	6.9	6.9	6.9	6.3	6.2	6.0

TABLE IV

POPULATIONS OF TRICHOMONADS AND pH OF THE MEDIUM FROM GROWTH IN C.P.L.M. AT 37.5 DEGREES C. AT VARIOUS AGES OF CULTURES STARTING pH 6.6. INOCULUM 500,000.

Results in millions of organisms per cc.

Age in Hours		12	24	36	48	60	72	84
Trichomonas sp. #1 (nose of pig)	Count	.18	3.45	14.60	1.00	.40	.16	.05
	pH		6.0		5.0		4.9	
T. sp. #2 (nose of pig)	Count	.41	4.05	12.75	.80	.14	.15	.08
			5.8		4.9		5.0	
T. suis	Count	.30	2.95	9.65	.45	.18	.08	.09
	pH		5.9		5.0		5.0	
T. foetus	Count	.14	1.10	4.14	7.40	2.20	.28	.10
	pH		6.3		4.9		5.1	
T. vaginalis	Count	.10	.41	1.65	2.05	.55	.08	.04
	pH		6.3		5.3		5.1	
T. gallinarum	Count	.12	.55	2.95	2.45	1.15	.27	.18
	pH		6.2		5.5		5.3	

H. Experimental Animal Inoculation

Experimental animal inoculation was suggested by previous workers and was thought advisable at this point. A rabbit subcutaneous inoculation was done to observe the MacKay rabbit lesion method reaction. The haunches of the rabbit were shaved, and the rabbit was given 0.125 cc. of nasal exudate suspension from an atrophic rhinitis-affected hog containing trichomonads in the right side and 0.25 cc. in the left side subcutaneously. Slight swelling was observed in both areas at 24 hours. At 48 hours there was a large inflamed area around the inoculation site on the left side but not much change on the right side. The animal was dead at approximately 60 hours. The areas of inoculation were dissected and found to be very necrotic with large amounts of pus. Microscopic examination revealed no trichomonads.

A guinea pig was inoculated in the same manner. The results and observations were much the same as those encountered in the rabbit. There was no opportunity at the time to make bacterial examinations. Cause of death was thought to be septicemia.

In another experiment three guinea pigs were utilized. These were given material by nasal instillation. The control was given 0.5 cc. of sterile Ringer's solution. The other two animals were given nasal exudate from a rhinitis-affected hog, containing motile trichomonads. No changes were observed

in the control. Of the other two animals, one had its nose scarified with a cotton-tipped applicator stick before instillation. Both of these guinea pigs aborted on the fourth day. The one with the scarified nose died on the 5th day, and the other died on the sixth day. There were no trichomonads present in the nasal passages or placentas of these animals.

In the next experiment seven guinea pigs were used. Each was given a subcutaneous injection of 0.5 ml. in the shaved back. The inocula were as follows: C.P.L.M. culture of bacteria-free trichomonads, brain-heart infusion broth cultures of each of four different unidentified bacteria isolated aerobically from the nose of a rhinitis-affected hog (one was known to be Pseudomonas sp.), and sterile C.P.L.M. There was no reaction in any of the animals in this experiment.

I. Agglutination Tests

The experiments of Trussell (1946) suggested inquiry into the little explored field of trichomonad immunological studies. There were three strains of trichomonads used as antigens in these experiments: T. sp. #2, T. suis, and T. foetus. The agglutination tests were conducted at all times with both antigen and normal serum controls. The results are tabulated in Tables V and VI.

TABLE V

AGGLUTINATION TITERS OF TRICHOMONADS FORMALINIZED AND SUSPENDED IN 1/15 MOLAR PHOSPHATE BUFFER SOLUTION WITH VARIOUS ANTISERA AND A NORMAL SERUM CONTROL

Serum number code:
Anti-sera 1 and 2 - Trichomonas sp. #2 (nose of pig)
Anti-serum 3 - T. foetus
Anti-sera 5 and 6 - T. suis
Anti-sera 7 and 8 - C.P.L.M. washed with Ringer's solution as were the cultures for the above antigens. This is a control on the medium used.

Antigen		Titer	Control Titer
Trichomonas sp. #2 (nose of pig)	vs. Serum 1	640	40
	2	640	40
	3	20	40
	5	40	20
	6	40	40
	7	160	40
	8	80	20
T. suis	vs. Serum 1	160	40
	2	40	40
	3	160	40
	5	640	40
	6	640	40
	7	160	40
	8	80	40
T. foetus	vs. Serum 1	160	40
	2	80	40
	3	1280	160
	5	160	40
	6	80	40
	7	40	40
	8	80	40

TABLE VI

AGGLUTINATION TITERS OF TRICHOMONADS FORMALINIZED AND SUSPENDED IN RINGER'S SOLUTION WITH VARIOUS ANTISERA AND A NORMAL SERUM CONTROL

Serum number code:

Anti-sera 1 and 2 - Trichomonas sp. # 2 (nose of pig)
Anti-serum 3 - T. foetus
Anti-sera 5 and 6 - T. suis
Anti-sera 7 and 8 - C.P.L.M. washed with Ringer's solution as were the cultures for the above antigens. This is a control on the medium used.

Antigen		Titer	Control Titer
Trichomonas sp. #2 (nose of pig)	vs. Serum 1	640	10
	2	320	10
	3	20	10
	5	40	10
	6	20	10
	7	10	10
	8	20	10
T. suis	vs. Serum 1	160	80
	2	80	80
	3	80	80
	5	320	80
	6	320	80
	7	40	80
	8	40	80
T. foetus	vs. Serum 1	40	10
	2	20	10
	3	640	10
	5	160	10
	6	80	10
	7	40	10
	8	40	10

IV. DISCUSSION AND INTERPRETATION OF EXPERIMENTS

The packing house survey was not extensive enough to give any conclusive results as to rhinitis prevalence. The figures could also be further clouded in that affected hogs might not be accepted for slaughter due to being underweight. The other purpose of this survey, to obtain trichomonads for culture, was possibly foiled by the passage of the animals through hot water and hot rosin before it was possible to examine them.

The autopsy reports and other findings on the rhinitic hogs from Mason are largely self-explanatory. The photographs and data give a generally good picture of the disease itself. This was mainly a personally informative investigation and a means to collect specimens for culture.

In the bacterial findings, none of the forms that were found and identified had previously been described in connection with atrophic rhinitis. Also the reactions and characteristics of the unidentified bacteria that were isolated from this rhinitis case did not conform with those of any of the previously described forms.

Results of the experimental pig inoculation were all negative. This could possibly be due to the age of the experimental animals. Much of Gwatkin's (1951) success with nasal instillations was with much younger pigs. However,

Spindler (1953) did obtain positive results in the production of rhinitis and establishment of trichomonads in the nasal passages with pigs approximately the same age as those used in this experiment. This latter idea might show that the experiment tends to point to something working in conjunction with the trichomonads.

Of the several media tried in the culture of trichomonads, C.P.L.M. and its modifications was by far the best. Thioglycollate was of little value, but brain-heart infusion semi-solid did serve a useful specialized purpose. The C.P.L.M. that was made into a semi-solid form served well the purpose of continual keeping of the protozoa, since it grew the organisms well and also kept quite well itself. Frozen C.P.L.M. was satisfactory for this purpose also, but requirement of special equipment and time for thawing could be a definite hindrance to its use. It was therefore adjudged less practical than the semi-solid C.P.L.M. The medium C.P.L.M. had a definite disadvantage of requiring frequent transfers, but coupling with the brain-heart infusion medium obviated much of this objection.

The group of experiments on inoculum variation and longevity counts showed no significant differences in the growth rates of the several swine trichomonads. However, the counting and measuring methods used were felt to be rather crude, and this may have accounted for the poor results. It

did serve, however, as a guide to the proper amount of inocula to be used in other experiments and general transfers. It showed that better results could be obtained using smaller amounts of inocula, around 10,000 to 20,000 organisms per cc. The experiment also pointed out that with T. vaginalis and T. gallinarum larger amounts of inocula were needed to obtain satisfactory populations. The studies on pH showed that pH does not play as important a role in the longevity of the protozoa as previously supposed.

Experimental animal inoculation did produce the type of lesion described by MacKay (1948). However, at the time this experiment was attempted there was no suggestion in the literature of the possible etiological agent of a bacterial nature being quite pure in this lesion. Time limitations did not permit a routine bacterial examination at the time.

The agglutination experiments were quite satisfactory in general. The titers were not quite as high as those obtained by Trussell (1946) with T. vaginalis. The titers and cross titers of the two hog forms showed some antigenic difference, indeed as much as with T. foetus which acted as a sort of a control in that it was a known different form. The differences of titer between these experiments and Trussell's suggested that perhaps another electrolytic suspending medium would give higher titers. Ringer's solution was used because it contained several salts known to be good electrolytes.

The variation and instability of titers here, however, seemed to suggest that perhaps the profusion of different ions had a detrimental effect on the experiment. In the case of T. suis, Ringer's solution suspension, as compared to phosphate buffer suspension, had a definite effect on the antigen so as to raise the titer consistently in the control serum. It was not determined which ion or combination of ions did this.

V. CONCLUSIONS

Seven different bacteria were identified in this work from a severe case of atrophic rhinitis. These had not previously been reported. Also at least eight unidentified bacteria were isolated, and none of these conformed in characteristics with any bacteria previously described in association with this disease.

Experimental infections of pigs with pure cultures of trichomonads failed in all cases. This would seem to point to a combination of factors being necessary for the establishment of nasal trichomonads in pigs. The several different methods used in attempting to infect the pigs made this possible combination of factors seem to be biological rather than mechanical. Since Spindler (1953) managed to establish trichomonads in the noses of pigs of the same age as those with whole infective nasal exudate, his work seems to strengthen this point.

The agglutination experiments showed conclusively a definite antigenic difference between the two trichomonad forms used from the pig. These were taken from the nose and cecum respectively of the same hog affected with atrophic rhinitis. The experiments utilized also a known different form, T. foetus. There was little or no evidence of a group

antigen among these three forms. The minor differences in growth rates of cultures of the two hog forms of trichomonads reported by Hammond and Fitzgerald (1953) coupled with the minor morphological differences described by the above authors, Switzer (1951), and Morgan and Hawkins (1952) along with this demonstrated immunological difference might prove to be enough evidence to separate the two species of trichomonads and describe and name the new one.

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STUDIES ON CULTURAL, SEROLOGICAL, AND OTHER ASPECTS OF TRICHOMONADS ASSOCIATED WITH ATROPHIC RHINITIS AND SIMILAR STUDIES ON OTHER TRICHOMONADS

By

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AN ABSTRACT

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

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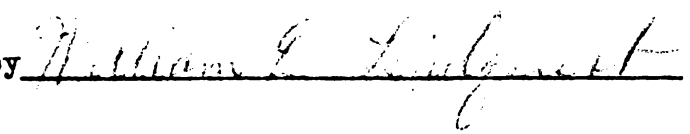
Approved by 

The purpose of this work was to study the disease of hogs known as atrophic rhinitis, paying especial attention to the trichomonads often found in conjunction with it. This was accomplished through general observation, cultural studies, attempts at experimental infection, and serological studies.

Hogs affected with atrophic rhinitis were obtained through the cooperation of the Animal Pathology Department of Michigan State College and the cooperation of several hog raisers in the surrounding area. The two forms of Trichomonas found in the hog were isolated from the animals obtained from the above mentioned sources. T. foetus, T. vaginalis, and T. gallinarum, the other trichomonads used, were isolated from their respective normal hosts. Media used in cultural studies were thioglycollate broth, brain-heart infusion semi-solid, Plastride medium, Laidlaw's medium, and C.P.L.M. (cysteine HCl, peptone, liver infusion, maltose). Rabbits were used for antibody production in the serological studies.

At least fifteen different bacteria were isolated from the nasal passages of a hog affected with atrophic rhinitis. None of these bacteria had previously been described in connection with atrophic rhinitis. Seven of these bacteria were identified as follows: Escherichia coli, E. intermedium, Alcaligenes faecalis, Staphlococcus epidermidis, Corynebacterium renale, and Neisseria catarrhalis. Amongst the forms that did not yield to identification were two bacilli, two diptheroids,

an alpha viridans Streptococcus, three small, gram negative rods, and several other undetermined forms.

Cultural studies showed no significant differences between the two trichomonads in the hog, but the studies did serve to clarify the amounts of inoculum that would give the best growth results upon transfer of the various organisms.

Experimental infection of hogs with axenic cultures of Trichomonas sp. from the nose of a hog affected with atrophic rhinitis failed in all cases. Since other workers have been successful at this, using nasal exudate from an affected hog, it would seem that a combination of factors was necessary for the establishment of trichomonads in the nasal passages of hogs, and also that another factor or combination of factors is necessary for the production of atrophic rhinitis in hogs. However, the number of experimental animals used (eight) was not thought to be significant.

The agglutination experiments using rabbit sera showed conclusively an antigenic difference between the two Trichomonas forms found in the hog. These were taken from the nose and cecum respectively of the same hog affected with atrophic rhinitis. The experiments utilized a known different form, T. foetus, which acted in the capacity of a control. Titers and cross-titers showed the two forms from the hog to be as different from each other antigenically as each was antigenically different from T. foetus. There was little evidence

of a group antigen discovered for the three forms. The minor differences in growth rates and morphology described by other authors, coupled with this immunological dissimilarity, might be enough evidence to separate these two trichomonads of the hog and name the one from the nasal passages.