Host-parasite interactions of *Trichomonas gallinae* (Rivolta, 1878)

Glenn Ernest Kietzmann Jr.

*Iowa State University*

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Host-parasite interactions of *Trichomonas gallinae* (Rivolta, 1878)

Kietzmann, Glenn Ernest, Jr., Ph.D.

Iowa State University, 1988
Host-parasite interactions of *Trichomonas gallinae* (Rivolta, 1878)

by

Glenn Ernest Kietzmann, Jr.

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Zoology
Major: Zoology (Parasitology)

Approved:

Signature was redacted for privacy. Members of the Committee:

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For The Graduate College

Iowa State University
Ames, Iowa
1988

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GENERAL INTRODUCTION

Avian trichomoniasis is caused by the parasitic protozoan *Trichomonas gallinae*. Birds from several orders are susceptible to this disease, however pigeons and doves (Columbiformes) are more frequently infected in natural situations. The disease apparently is distributed worldwide and appears to have followed closely the introduction of rock doves (*Columba livia*). In North America, rock doves were introduced by settlers in the early 1600s (Schorger, 1952). Since that time, epizootics of avian trichomoniasis in wild columbiforms have been observed, and it appears that rock doves may have served as reservoir hosts.

Columbiform birds which have been experimentally infected with virulent strains of *T. gallinae* and which have had no prior exposure to the parasite usually die within 35 days post infection after large caseous nodules (cankers) form in the upper alimentary tract, liver, air sacs, lungs or other vital organs. Cankers appear to be a host response to the presence of trichomonads, and are composed of blood cells (primarily leucocytes), tissue debris, degenerate parasites and other components. Signs of avian trichomoniasis other than cankers and intense inflammation include emaciation, hunched posture, ruffled feathers, closed eyes and gasping for air. Birds near death lose perching and/or standing abilities.

*Trichomonas gallinae* is the most pathogenic member of the genus *Trichomonas*, yet literature concerning its relationship with its hosts during canker formation is scarce. The most significant work on the lesions of this disease was performed by Mesa et al. (1961). Many
questions concerning canker formation were not addressed or were left largely unanswered. This dissertation describes attempts to answer some of the questions concerning trichomonad activities prior to, during and after canker formation in the ring dove (Streptopelia risoria) pregastric alimentary tract. Some questions concerning *T. gallinae* in culture systems were also addressed.

Explanation of Dissertation Format

The alternate format was used in writing this dissertation. Included with a general introduction, general literature review and general summary are six papers concerning *Trichomonas gallinae*. A complete literature cited section follows the general summary. This dissertation is written in the style required by the Journal of Parasitology.
LITERATURE REVIEW

Taxonomy and Evolutionary Position

Trichomonad classification has been addressed several times (Cheng, 1986; Honigberg, 1963; Krier and Baker, 1987; Levine et al., 1980), and in this classification *T. gallinae* (Rivolta, 1878) exists as follows: Kingdom Protista, Phylum Sarcomastigophora Honigberg and Balamuth, 1963, Class Zoomastigophorea Calkins, 1909, Order Trichomonadida Kirby, 1947, Family Trichomonadidae Chalmers and Pekkola, Subfamily Trichomonadinae Chalmers and Pekkola, Genus *Trichomonas* (Donné, 1836).

Donné erected the genus *Trichomonas* in 1836, however, *Trichomonas gallinae* was not discovered and described by Rivolta until 1878 (cited in Stabler, 1954). Organisms observed from pigeons were initially designated *Cercomonas gallinae* when in the upper alimentary tract and intestine and *C. hepaticum* when in the liver. According to Stabler (1954), diagrams and descriptions of similar parasites followed in 1880 by Rivolta and Delprato, however, reference to intestinal forms of *C. gallinae* apparently was not made.

After descriptions of *Cercomonas gallinae* and *C. hepaticum* had been made, a short period of time elapsed before nomenclatural errors and incorrect name designations made unclear the true parasite identity. This was alleviated for a time with the establishment of the name *Trichomonas columbae* Rivolta, which too was incorrect. The binomial, *Trichomonas columbae*, survived until Stabler (1938a) resurrected *T. gallinae* Rivolta as the correct name. For a thorough account of the nomenclature of *T. gallinae*, the reader is directed to Stabler's (1954) review.
Synonymy of *T. gallinae* will not be examined in detail. One other name worth mentioning is *T. diversa* (Volkmar, 1930), which was associated with disease in turkeys. Descriptions and diagrams of this parasite and preliminary transmission experiments (Stabler, 1938b) indicated that it was synonymous to *T. gallinae*.

Evolutionary relationships of trichomonads have been constructed using similarities to other living groups (Honigberg, 1963). *Trichomonas gallinae* and its relatives do not possess cyst stages in their life cycles and it appears that these parasites probably evolved along with their preferred host species. General schemes as to possible relationships among the protozoa (Krier and Baker, 1987) and the Trichomonadidae (Honigberg, 1963) are available. It appears that ancestors of *Trichomonas* sp. were members of the Monocercomonadidae, which inhabit the cloaca and large intestines of squamate reptiles, i.e., lizards and snakes (Honigberg, 1963). Honigberg (1963) also stated that the most primitive members of the Trichomonadidae belong to the genus *Monocercomonas*. As birds and mammals are believed to have evolved from reptiles, and *Monocercomonas* sp. parasitizes squamates, it is plausible that trichomonads associated with modern birds and mammals coevolved with their host's ancestors.

*Trichomonas gallinae* from columbiform birds, *T. tenax* from the human oral cavity and *T. vaginalis* from the human reproductive tract are the only members of the genus *Trichomonas*. Honigberg (1963) suggested that *T. gallinae* and *T. tenax* were closer morphologically to each other than to *T. vaginalis*. Figure 1 shows the most accepted ideas on the evolutionary
Fig. 1. Possible evolution of members of the Trichomonadidae. This figure was constructed in part from data available from Honigberg (1963), Krier and Baker (1987) and Levine et al. (1980). Solid lines indicate generally accepted relationships and dotted lines indicate theoretical relationships.
relationships of the Trichomonadidae. For a more detailed discussion of trichomonad evolution, see Honigberg's (1963) review.

Morphology and Life Cycle

Light microscopy (LM) and transmission electron microscopy (TEM) have been utilized to study Trichomonas gallinae structure. In this review, attempts will be made to address information available from both methods. Diagrams of T. gallinae (Fig. 2) will be helpful throughout this discussion.

Light microscopy Trichomonas gallinae has been described and diagramed several times since the work of Rivolta and Delprato. The parasite can assume several shapes (Abraham and Honigberg, 1964; Oguma, 1931; Stabler, 1938b; 1941b; Volkmar, 1930). The most frequently encountered forms are pyriform or pear-shaped, spherical and amoeboid (Stabler, 1941b; 1954). The latter forms were shown to possess fine filamentous stalks when attached to free squamous cells, whereas pyriform-shaped trichomonads, which comprise the majority of published diagrams, are apparently encountered when parasites are not dividing (Stabler, 1941b). It has been suggested that spherical forms appear when conditions for parasite survival become unfavorable (Stabler, 1954).

Measurements of T. gallinae vary considerably. Length is from 5.0 μm (Oguma, 1931) to 20.0 μm (Florent, 1938), whereas width is from 3.0 μm (Oguma, 1931) to 13.0 μm (Volkmar, 1930). Discrepancies in size may be due to parasite preparation prior to observation (Abraham and Honigberg, 1964). Abraham and Honigberg (1964) stated that fixed and stained organisms can shrink approximately 33% from their living dimensions.
Fig. 2. Diagramatic representations of *Trichomonas gallinae* showing morphological features. Diagrams were drawn from actual parasites but are not drawn to scale. Culture forms are represented by A, rounded forms by B and amoeboid forms by C. Letter designations are as follows: Ax, axostyle; Ca, capitulum; Co, costa; F, anterior flagella; H, hydrogenosomes (Paracostal and paraxostylar granules); P, pelta; Pf, parabasal filament; Rf, recurrent flagellum; Um, undulating membrane; V, membrane bound vacuoles.
Measurements of length from living trichomonads were reported as 9.0 to 15.3 µm for cultured organisms and 12.6 to 19.8 µm for living organisms placed under a coverglass (Abraham and Honigberg, 1964).

**Trichomonas gallinae** possesses four anterior flagella and one posteriorly coursing recurrent flagellum. All flagella originate in the kinetosomal or basal granule complex (Honigberg, 1978), which is sometimes termed the blepharoplast (Oguma, 1931; Stabler, 1941b). Anterior flagella leave the kinetosomal complex as a bundle before they separate (Honigberg, 1978; Volkmar, 1930). Flagella vary in length from approximately 8.0 to 13.0 µm and appear equal in diameter (Abraham and Honigberg, 1964; Oguma, 1931).

The recurrent flagellum and undulating membrane, which are situated dorsally, begin in the kinetosomal complex (Abraham and Honigberg, 1964; Oguma, 1931). Posterior termination of the undulating membrane marks also the termination of the recurrent flagellum as no free posterior flagellum exists (Abraham and Honigberg, 1964; Honigberg, 1978; Stabler, 1941b; Oguma, 1931). The free edge of the undulating membrane is delimited by the recurrent flagellum (Abraham and Honigberg, 1964; Honigberg 1978).

Appearing as a fine, dorsally situated rod, the costa also originates in the kinetosomal complex (Abraham and Honigberg, 1964; Oguma, 1931), and is believed to function in cell support (Abraham and Honigberg, 1964). Surrounding the costa in two rows are paracostal granules which are visible in living organisms and in those stained with iron hematoxylin (Abraham and Honigberg, 1964; Honigberg, 1978).
The parabasal complex, composed of parabasal bodies and filaments, also arises from the kinetosomal complex (Honigberg, 1978; Oguma, 1931). Filaments have no definite arrangement within the cell (Oguma, 1931) and are best visualized after special staining (Honigberg, 1978). The parabasal body is hook-shaped and lies near the nucleus (Honigberg, 1978). Cheng (1986), indicated that the parabasal complex is now referred to as the Golgi apparatus.

The axostyle is a hyaline rod that runs ventral to the nucleus and appears to function in cell support (Cheng, 1986). Anteriorly, the axostyle flattens into the capitulum, whereas posteriorly it tapers to a point as it exits the cell (Abraham and Honigberg, 1964; Hawn, 1937; Honigberg, 1978; Oguma, 1931; Stabler, 1941b; Volkmar, 1930). The capitulum is wider than the axostyle and connects directly to the crescent-shaped pelta which wraps around the anterior aspect of the nucleus (Abraham and Honigberg, 1964; Honigberg, 1978). Surrounding the axostyle are paraxostylar granules. These resemble paracostal granules in shape and arrangement and appear to be concentrated near the capitulum (Abraham and Honigberg, 1964; Honigberg, 1978).

Remaining organelles resolvable with LM include food vacuoles, inclusion bodies and other granular structures (Honigberg, 1978). These structures appear to vary in size, shape, number and location in individual trichomonads (Abraham and Honigberg, 1964; Oguma, 1931).

Reports of *T. gallinae* possessing a cytostome or mouth were made by Stabler (1941b) and Volkmar (1930). No such organelle has been reported recently however. Although Stiles (1939) and Volkmar (1930) reported the presence of a cyst or resting stage, *T. gallinae* is believed not to possess a cyst stage in its life cycle (Cheng, 1986; Honigberg, 1978). Examination of Volkmar's (1930) resting stage diagram indicated that he may have been looking at *Chilomastix* sp.

**Transmission electron microscopy** The cytology of *T. gallinae* has not been studied extensively. In this review, all information has come from Mattern et al. (1967) and Ruiz (1977) unless otherwise noted.

These authors did not discuss trichomonad cell membranes and membrane components. Examination of their micrographs revealed a typical bilayered arrangement.

Anterior and recurrent flagella are composed of microtubules arranged in nine doublets plus two singles, and are enclosed in a unit membrane. Kinetosomal microtubules are arranged in nine triplets. Kinetosomes of the four anterior flagella surround the kinetosome of the recurrent flagellum. The recurrent flagellum resides in a small groove along the free edge of the undulating membrane. No physical attachment between these organelles has been shown. The undulating membrane appears to be a cytoplasmic fold with a lamellar structure (Mattern et al., 1967).
The costa and parabasal filaments are striated throughout their lengths, although the periodicity between striations differ in the two structures. According to Mattern et al. (1967), the costa is composed of transverse bands which are approximately 100 Å thick and have an average period of 420 Å. Parabasal filaments in comparison, consist of thin, densely staining transverse lines. Each set of four lines apparently has the same 420 Å spacing as the thick costal bands. The costa and parabasal filaments originate in different locations. The costa arises from the kinetosome of the recurrent flagellum whereas the two parabasal filaments originate from kinetosomes of two of the anterior flagella. It is not known where these organelles terminate.

Golgi bodies (parabasal bodies) lie close to the nucleus and endoplasmic reticulum, and appear to be stacked membrane-bound clusters.

The pelta, capitulum and axostyle are all composed of microtubules and are closely associated with each other. The capitulum appears to be an extremely dense staining sheet of microtubules situated between the pelta and axostyle. Longitudinal microtubules make up the pelta which supports the anterior end of the organism and may shape the periflagellar canal wall (Honigberg, 1978). Peltar microtubules are ensheathed within the axostyle, and their precise length is not known (Mattern et al., 1967). Covering the capitulum microtubules are bands of transverse axostylar microtubules.

Paraxostylar and paracostal granules are cytoplasmic membrane-bound, microbody-like organelles of various sizes. Their function was not determined (Mattern et al., 1967), although similar structures in T.
vaginalis and *Trichomonas foetus* were shown to function in pyruvate metabolism (Lindmark and Muller, 1973; Lindmark and Muller, 1975).

The nucleus of *T. gallinae* is enclosed in an envelope possessing few pores (Honigberg, 1978). Rough endoplasmic reticulum surrounds the nucleus.

Hosts and Transmission of *Trichomonas gallinae*

In natural settings, *Trichomonas gallinae* infects columbiform birds (pigeons and doves) primarily, although there are reports of other bird species being infected. Experimentation has expanded the host list to include additional bird species and some mammals. Table 1 lists the known hosts of *T. gallinae*.

*Trichomonas gallinae* reproduces by binary fission and has no cyst stage in its life cycle (Cheng, 1986; Honigberg, 1978; Kocan and Herman, 1971; Stabler, 1954). Therefore, trophozoites must be transmitted to other susceptible hosts. Because drying kills the trophozoite (Kocan and Herman, 1971; Stabler, 1954), direct transmission or transmission through a liquid medium probably is necessary.

*Trichomonas gallinae* transmission is divided into two categories, natural and experimental, in the following discussion. Figure 3 diagrams natural transmission and Figure 4 presents a flow chart of both categories.

**Natural transmission**

Rock doves (*Columba livia*) appear to be the primary source of *T. gallinae* infections (Stabler, 1938b; 1947a). In North America, *T. gallinae* was probably introduced with rock doves by settlers at Port Royal, Nova Scotia, in 1606 (Schorger, 1952).
Table 1. Natural and experimental hosts of *Trichomonas gallinae*

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<td>Barnes, 1951; Carpenter et al., 1972; Cauthen, 1934; 1936; Conti et al., 1985; Greiner and Baxter, 1974; Harwood, 1946; Haugen, 1952; Haugen and Keeler, 1952; Kocan, 1969c; Kocan and Amend, 1972; Morgan, 1944; Stabler, 1951b; Stabler and Herman, 1951</td>
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<td></td>
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<tr>
<td>bobwhite</td>
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<tr>
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<td>House mouse</td>
<td>Frost and Honigberg, 1962; Frost et al., 1961; Honigberg, 1961; Schnitzer et al., 1950;</td>
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Table 1. (Continued)

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<td>(Continued)</td>
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<td></td>
<td>Norway Rat</td>
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<tr>
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<td>Cat</td>
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Fig. 3. Diagramatic representation of the natural transmission of *Trichomonas gallinae*. Rock doves serving as reservoir hosts possibly transmit *T. gallinae* to other wild species in food and/or water sources (A). Doves infected by this method then become immune carriers of *T. gallinae* (B), become sick and die of trichomoniasis or become easy prey for raptors which become infected also (C). Doves which become carriers, infect their young squabs by feeding crop milk after hatching. Squabs then die within several days with avian trichomoniasis (D) or become carriers themselves (E). These "new" carriers when mature (F) can then continue the cycle. Drawings were made for the author by Ms. Luci Branyan.
Fig. 4. Flow chart of natural and experimental transmission of *Trichomonas gallinae*. Solid lines indicate documented means of transmission while dotted lines indicate theoretical means of transmission.
Pigeon carrier

EXPERIMENTAL TRANSMISSION

Injection

Oral intubation

Mice

Columbiforms

Galliforms

NATURAL TRANSMISSION

Columbiforms

Galliforms

Feeding of crop milk

Transovarially or through egg shell

Water or food

Feeding on infected columbiforms

Doves

Pigeons

Passenger pigeon

Doves

Pigeons

Passenger pigeon

Raptors

Scavengers

Pigeons

Passenger pigeon

Doves

Galliforms

Raptors

Other birds
Adult columbiforms produce pigeon or crop milk, which is given to squabs through regurgitacional feeding (Miller, 1969). Through this mechanism, adults harboring T. gallinae can pass parasites to their young immediately after they hatch (Kocan and Herman, 1971; Stabler, 1947a; 1954). This method of transmission appears to be so effective that it has been suspected of initiating and maintaining epizootics of avian trichomoniasis in wild mourning doves (Haugen and Keeler, 1952; Kocan and Herman, 1971; Stabler and Herman, 1951; Tongson et al., 1969). The cycle continues as all infected squabs do not die of trichomoniasis. Some gain immunity to the parasites and eventually become asymptomatic carriers (Stabler, 1954).

Adult-to-adult and juvenile-to-juvenile transmission of T. gallinae also could occur through billing activities during courtship and through food and water (Haugen and Keeler, 1952; Kocan, 1969a; Kocan and Herman, 1971; Stabler, 1947a; 1954; Stabler and Herman, 1951). However, experimental evidence confirming this is not available. Evidence was offered by Kocan (1969a) that T. gallinae can survive in distilled water, saline solutions and ground moist grains, and that these sources could serve as vehicles for transmission. Kocan (1969a) did not perform transmission experiments through these media.

Epizootics of avian trichomoniasis in mourning doves have been observed (Haugen, 1952; Haugen and Keeler, 1952; Stabler and Herman, 1951; Greiner and Baxter, 1974) and it was suggested that water and/or food could have played a role.
In natural situations infected columbiforms either die or become carriers of *T. gallinae* (Stabler, 1954). These infected individuals can become a source of infection for birds of other orders. Eagles, hawks, falcons and owls become infected when they prey on infected columbiforms (Cooper and Petty, 1988; Jessup, 1980; Rettig, 1978; Stabler, 1941a; 1953c; Stabler and Shelanski, 1936; Stone and Janes, 1969; Stone and Nye, 1981). It is not known if birds of prey can be asymptomatic carriers.

Turkeys too have been found to be infected with *T. gallinae* (Gierke and Hinshaw, 1936; Hawn, 1937; Stabler, 1938b; Volkmar, 1930). Reports of parasites being present in both the pre and postgastric digestive tract have been published (Gierke and Hinshaw, 1936; Hawn, 1937). However, Stabler (1954) believes that *T. gallinae* is restricted to the upper digestive tract, since *T. gallinarum* is usually found in the intestines. Again, transmission of the organism is thought to occur through contaminated food or water. Pigeons may serve as reservoir hosts when trichomoniasis breaks out in free-ranging turkeys (Stabler, 1954).

**Experimental transmission** Transmission of *T. gallinae* by experimental means has been performed in avian and mammalian hosts. In columbiform birds, experimental transmission by oral intubation of cultured or fresh parasite suspensions has been used (Cauthen, 1936; Conti et al., 1985; Hare, 1937; Kocan, 1969b; Powell and Hollander, 1982; Sileo and Fitzhugh, 1969; Stabler, 1951b; 1953b; 1977; Stabler and Braun, 1975; 1979; Stabler and Kihara, 1954). Many of the aforementioned papers report simple cross-transmission experiments to establish whether or not *T. gallinae* would produce disease. Conti et al. (1985) transmitted parasites
from white-winged doves to mourning doves, Stabler (1953b) followed infections passed serially in 119 consecutive pigeons and Powell and Hollander (1982) transmitted *T. gallinae* from pigeons to ring doves. Other accounts of transmission are those of Sileo and Fitzhugh (1969) and Stabler and Braun (1975; 1979) who transmitted parasites from band-tailed pigeons to band-tailed pigeons. Stabler (1951b) also transmitted *T. gallinae* from mourning doves to domestic pigeons.

Chickens and turkeys have been orally intubated with *T. gallinae* from pigeons also (Gierke and Hinshaw, 1936; Hawn, 1937; Levine et al., 1941; Levine and Brandly, 1939; 1940; Stabler, 1938b). Levine (1985) reported that infections in chickens are rare.

Intrahepatic, intramuscular, intraperitoneal and subcutaneous injections of *T. gallinae* have been used to address questions of trichomonad pathogenicity. Cauthen (1936) utilized columbiform birds while Gabaldon and Andrews (1935) used chickens. The most frequently used chordate for studying trichomonad pathogenicity was *Mus musculus* (Frost et al., 1961; Frost and Honigberg, 1962; Honigberg, 1961; Schnitzer et al., 1950; Warren et al., 1961). Mice when infected, develop abscesses or pustules rather than pronounced caseous nodules as in birds (Frost and Honigberg, 1962; Honigberg, 1961). Neutrophils, multinucleated giant cells and monocytic phagocytes are host cells associated with abscesses (Frost et al., 1961; Frost and Honigberg, 1962; Honigberg, 1961). Abscess size and duration appear to vary with the strain of *T. gallinae* injected (Frost and Honigberg, 1962; Honigberg, 1961; Schnitzer et al., 1950).
Culture of *Trichomonas gallinae*

Studies concerning in vitro cultivation of *Trichomonas gallinae* and other trichomonads center around maintenance medium development and determinations of unique growth requirements (Stabler, 1954). Media used for cultivation includes liquid and semi liquid types (Honigberg, 1978). Cultivation medium for *T. gallinae* has been used effectively with *T. vaginalis*. Discussion in this dissertation will be directed toward cultivation of *T. gallinae*.

According to Stabler (1954), Bos was the first to grow *T. gallinae* in pure culture, using a medium designed for *Entamoeba histolytica*. Cauthen and Harris (1935) cultivated *T. gallinae* free from bacteria in Locke-egg-serum and liver bouillon media. Since these early reports, liquid media such as cysteine-peptone-liver infusion-maltose (CPLM) medium (Johnson and Trussell, 1943), simplified trypdicase-serum (STS) medium (Kupferberg et al., 1948), trypdicase-yeast extract-maltose (TYM) medium (Diamond, 1957) and 28 saline-serum-carbohydrate based media (Diamond, 1954) have been developed. Honigberg (1978) stated that tryptose is an adequate replacement for peptone in CPLM medium. Semi-solid CPLM-agar medium also supports the growth of *T. gallinae* when in the presence of 95% nitrogen and 5% carbon dioxide atmosphere (Honigberg, 1978).

Much of what is known of carbohydrate utilization by *T. gallinae* was contributed by Cailleau (1935). She determined that trichomonads were able to use glucose, maltose, galactose, sucrose, dextrin and soluble glycogen, whereas some use of inulin, lactose and fructose was noted also.
Xylose, arabinose, rhamnose, glycerol, sorbitol, dulcitol, mannitol and erythritol were not used.

In studies of maltose metabolism (Daly, 1970), greater parasite growth was attained in maltose medium rather than glucose medium. Read (1957) indicated maltose, starch and glycogen to be the most efficient substrates for *T. gallinae*, whereas glucose, galactose, fructose, turanose, sucrose, cellobiose and trehalose were used infrequently. Arabinose was used little, while lactose, melibiose, mannose, xylose, inositol, d-sorbitol and dulcitol were not utilized. Matthews (1986) determined that parasites grown only on starch or maltose used these substrates, yet parasites grown on other sugars combined with starch or maltose did not use the added components. All his parasite cultures utilized mannose, galactose, glucose and fructose, although trichomonads grown on starch or maltose did not use them efficiently. Some ribose utilization was evident whereas xylose and arabanose were not used. Turanose also was not used during nongrowth periods.

Cailleau (1936a; 1936b; 1937a; 1937b; ) contributed much on the cholesterol requirement of *T. gallinae*. She indicated that cholesterol or compounds such as cholestanol, ergostanol, sitostinol and cis-cholestane 3, 4-diol were required for growth of *T. gallinae* and that serum and/or liver were ingredients to be included in cultures.

Different sera types in culture situations were also examined by Cailleau (1937a). Rabbit, horse, cat, human or sheep serum gave poor or no growth, while pigeon serum gave the best growth of *T. gallinae*. 
Work concerning added vitamins in culture media is limited. Cailleau (1939) believed ascorbic acid to be necessary for cell division. Recent work indicates that *T. vaginalis* has a requirement for cyanocobalamin (Hollander and Legett, 1985).

In addition to culture development and culture additives used to improve *T. gallinae* growth, observations of laboratory procedures on trichomonad virulence have been made (Honigberg et al., 1970; Stabler et al., 1964). These investigators indicated that the Jones' barn strain of *T. gallinae* had a tendency to lose its virulence when in continuous culture. Virulence was retained when freshly isolated trichomonads were maintained at -19 C or -72 C. Virulence is apparently retained and/or increased by bird-to-bird transfer of trichomonads also (Honigberg et al., 1970; Stabler et al., 1964). Virulence of *T. gallinae* may be increased in culture systems as well. According to Honigberg and Livingston (1968) and Honigberg and Read (1960), exposing avirulent strains of *T. gallinae* to cell-free homogenates of the virulent Jones' barn strain the pathogenicity of the avirulent strain increased. It is believed the DNA and/or RNA in the homogenates was responsible for the transformation (Honigberg and Livingston, 1968).

Antibiotics often are included in cultures for bacterial control. Some of those used are potassium penicillin G, streptomycin sulfate (Diamond, 1957), and chloramphenicol.

Cultivation of *T. gallinae* is relatively easy in the above mentioned media (Stabler, 1954) although temperature and pH are factors to be considered. The most widely used temperature was 37 C. Some workers
prefered 35 C (Stabler and Engley, 1946). A range of temperatures from 32 C to 40 C apparently supports trichomonad growth (Llwoff, 1951; Stabler, 1954). The risk of cold shock exists at temperatures well below this range (Daly, 1980).

Under certain conditions, trichomonads can withstand cryopreservation (Diamond, 1964; Honigberg et al., 1970; Stabler et al., 1964). Success of this technique depends largely on successful treatment of the parasites with dimethylsulfoxide (DMSO) or glycerol and rapid freezing in liquid nitrogen to prevent ice crystal formation (Diamond, 1964). Rapid thawing of parasites in a warm water bath is also required for parasite survival (Diamond, 1964). Thawing has been little studied.

In addition to temperature, pH can be variable in trichomonad cultures. Based on hydrogen production by T. gallinae, optimum pH appears to be approximately 7.2, although 6.5 to 7.5 gives good growth (Read, 1957). Read (1957) indicated also that extremes for hydrogen production were approximately 5.5 and 8.0. Cailleau (1935) indicated that cultures with added calcium carbonate and a pH of 7.0 survived for long periods while a pH of 4.5 quickly killed trichomonads. Some investigators routinely transfer parasites to fresh medium at 48 to 72 hour intervals (Read, 1957; Stabler and Engley, 1946).

Biochemistry of Trichomonas gallinae

Trichomonas gallinae biochemistry has not been studied as extensively as that of some other trichomonad species. The following is a brief survey of the present state of knowledge concerning the biochemistry and cytochemistry of T. gallinae.
Listings of carbohydrates utilized by *T. gallinae* were presented under the culture section of this review. Recapping the list, however, the best growth of *T. gallinae* occurs in glucose, fructose, maltose, sucrose, glycogen, starch, dextrin and inulin (Shorb, 1964). Carbohydrate breakdown is primarily by anaerobic fermentation although aerobic respiration has been suggested (Read, 1957; Shorb, 1964).

Anaerobic fermentation of glucose by *T. gallinae* is apparently vigorous (Read, 1957), however, other sugars support growth and produce hydrogen and carbon dioxide also. Read (1957) did not actually determine that hydrogen was being evolved. He termed the gas hydrogen because it was not absorbed by acid or alkali, and because *T. vaginalis* and *Tritrichomonas foetus* produce hydrogen. Hydrogen production may be related to electron transport in *T. gallinae* (Baernstein, 1963), although studies supporting this theory are not yet available. In *Tr. foetus*, electrons in the electron transport chain apparently combine with protons to form molecular hydrogen (Lindmark and Müller, 1973). Electron transport in trichomonads involves dehydrogenase coupling and excretion of reduced compounds (Baernstein, 1963). It is not known if *T. gallinae* possesses cytochromes.

Whether *T. gallinae* possesses an operational tricarboxylic acid cycle (TCA cycle) is unknown. Read (1957) indicated that *T. gallinae* could oxidize many TCA cycle intermediates, whereas later investigators found only malic dehydrogenase (Betterton and Dowda, 1976; Dowda and Betterton, 1974) and lactic dehydrogenase (Betterton, 1976). The reason for the discrepancy is unknown, however, Read (1957) utilized whole cells in his
study while the other investigators (Betterton, 1976; Betterton and Dowda, 1976; Dowda and Betterton, 1974) used cell fractions. Pyruvate synthase and hydrogenase were also found in *Tr. foetus* (Lindmark and Müller, 1973).

In considering pyruvate metabolism in trichomonads, a discussion of localization is necessary. In aerobic eukaryotic cells, mitochondria would be expected to be the site of activity. However, these organelles are not found in *Trichomonas gallinae* (Mattern et al., 1967; Ruiz, 1977). The microbody-like paracostal and paraxostylar granules (hydrogenosomes) in *Tr. foetus* (Lindmark and Müller, 1973) and *T. vaginalis* (Lindmark et al., 1975) have been examined and it appears that alpha-glycerophosphate, malate dehydrogenase, pyruvate synthase and pyruvate hydrogenase activities are centered in these membrane-bound inclusions (Lindmark and Müller, 1973).

The structure of trichomonad glycogen was examined by Manners and Ryley (1955). Glycogen in *Tritrichomonas foetus* and *T. gallinae*, have branched alpha-1:4 glucosans, which are similar, but not identical, to those in metazoans. Chain length varies from 15 glucose units in *Tr. foetus* to 9 in *T. gallinae* and structure rotation is apparently 197 to 199 degrees. Purified polysaccharides have molecular weights of approximately $3.0 \times 10^6$, are degraded by salivary alpha-amylase and stain yellow brown in iodine (Manners and Ryley, 1955). Cytoplasmic glycogen in *T. gallinae* has been localized with transmission electron microscopy also (Mattern et al., 1967; Ruiz, 1977).

Little is known of lipid and nitrogen metabolism in *T. gallinae*. Warren and Allen (1959), using the Jones' barn strain, studied amino acids
and glutamic dehydrogenase activity. Gas production apparently increased two or three times when studied under anaerobic conditions.

Concerning steroid conversion, it was found that conversion in trichomonads was similar to that in mammals (Sebek et al., 1957). Apparently *T. gallinae* has a C-17 series dehydrogenating system and acts on C-3 of 3-ketosteroids of allopregnane and pregnane series (Sebek et al., 1957). The requirement of cholesterol or related compounds was indicated previously.

Amino acid composition of *T. gallinae* was summarized by Shorb (1964). She noted that alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tyrosine, valine and some unidentified amino acids were present. Warren and Allen (1959) found gamma-amino-n-butyric acid and alpha-methyl-alpha-amino-n-butyric acid also.

Cailleau (1939) suggested that *T. gallinae* probably requires ascorbic acid. Since her report, Jones and Smith (1959) suggested requirements for nicotinamide, choline, pyridoxamine, pyridoxine, calcium pantothenate, folic acid and biotin as well. Shorb (1964) examined vitamin requirements also.

Literature concerning nucleic acids of *T. gallinae* is limited to the report of Mandel and Honigberg (1964). They isolated and characterized DNA from the Jones' barn and YG strains.

**Disease Associated with Trichomonas gallinae**

It was noted in the introduction that *Trichomonas gallinae* is the causative agent of avian trichomoniasis, and the disease primarily affects
columbiform birds, although raptors, galliforms and other birds occasionally become infected. Aspects of avian trichomoniasis such as host appearance, gross pathology and histopathology are addressed here.

Columbiform birds known to be infected with virulent *T. gallinae* and near death exhibit several clinical signs. Such birds are generally emaciated, have ruffled feathers, perch in a huddled position and lose their ability to stand or walk (Levine, 1985; Ruhl et al., 1982). Greenish fluid teeming with trichomonads occasionally is found in the mouth and crop (Levine, 1985; Stabler, 1947a). Stabler (1947a) stated that so much fluid accumulates in some cases that infected birds nearly drown. Caseous nodules (cankers) and intense inflammation of the upper digestive tract are other features of avian trichomoniasis.

*Trichomonas gallinae* was described by Rivolta in 1878 (cited in Stabler, 1954), however, according to Schorger (1952; 1955), lesions of avian trichomoniasis were known well before that time. Cankers have been found in the oropharynx, nares, palate, esophagus, crop, proventriculus, liver, pancreas, air sacs, lungs, heart, spleen, pericardium, peritoneum, pleura, blood and navel of infected birds (Bushnell, 1942; Butler, 1979; Callender and Simmons, 1937; Cauthen, 1934; 1936; Conti et al., 1985; Cooper and Petty, 1988; Florent, 1938; Hare, 1937; Hawn, 1937; Hollander, 1945; Jaquette, 1950; Jessup, 1980; Kocan, 1969c; Kocan and Herman, 1971; Levine et al., 1941; Levine and Brandley, 1939; 1940; Niemeyer, 1939; Mesa et al., 1961; Rettig, 1978; Stabler, 1941a; 1947a; 1954; Stabler and Engley, 1946; Volkmar, 1930; Waller, 1934). Lesions from the intestine have also been reported (Gierke and Hinshaw, 1936; Hawn, 1937). Stabler
(1954) believed that *T. gallinae* did not invade this organ, rather the intestine harbored nonpathogenic *T. gallinarum*. Intestinal lesions should be differentiated from those caused by *Histomonas meleagridis*, a known pathogen of this organ (Allen, 1941).

Cankers form at variable times following host infection. They first appear as small, hard, yellow to white lumps on the esophageal mucosa and are accompanied by intense inflammation (Callender and Simmons, 1937; Stabler, 1947a; 1954). With time, the small lumps grow larger, or several small cankers may coalesce to form larger ones. Large cankers may completely block the esophagus and prevent affected birds from closing their mouths (Callender and Simmons, 1937; Stabler, 1947a). If the liver becomes involved, large foci of caseation develop, or entire lobes may be affected by caseous necrosis. Stabler (1947a) indicated that contact lesions may occur inside the body cavity.

Cankers have been noted in different organs, but documentation of associated pathogenesis is lacking. Studies of canker formation and progression over time in columbiform birds appear to be limited (Callender and Simmons, 1937; Mesa, et al., 1961). Reports of individual lesions in various other species can also be found (Conti et al., 1985; Cooper and Petty, 1988; Hawn, 1937; Jaquette, 1950; Jessup, 1980; Levine et al., 1941; Levine and Brandley, 1939; 1940).

According to Mesa et al. (1961), punctate lesions near the pharyngeal papillae and minor changes in the hepatic subcapsular vasculature were noted two days post infection (PI). Microscopically, ulcers were not found but trichomonads were arranged perpendicularly on the esophageal
mucosa. No inflammatory response was noted except at mucous gland openings, where there were indications of monocytic infiltration. They also indicated that parasites were found in gland lumina and adjacent to small capillaries near the glands. Photomicrographs supporting this finding were not presented. Mesa et al. (1961) based their conclusion that parasites were present solely on cell shape since they apparently could not demonstrate trichomonad morphological characters with their staining methods. The pigeon livers examined microscopically were apparently normal two days PI.

The third day PI revealed the oropharynx to be covered with mucous and some minor ulcers. Mesa et al. (1961) described intercellular trichomonads which appeared to cause squamous cell separation. Parasites were not positively demonstrated in this region. Heavy leucocytic infiltration in the submucosa surrounding mucous glands was described, and it was stated that parasites were seen around dilated blood vessels. Again, this was not demonstrated in photomicrographs. The respiratory system was supposedly involved with *T. gallinae* and cankers as well. The trachea had palisading parasites and small cankers whereas the lungs possessed abscesses composed of necrotic centers, palisading monocytes, giant cells and lymphocytes. Minute cankers and inflammation were noted in the liver. Mononuclear cells and heterophils were predominant cell types found there.

Lesions on the fourth and fifth days PI were similar. Grossly, ulcerations were absent although the pharyngeal mucosa was covered with what Mesa et al. (1961) called a "yellow pseudomembrane". Small mucosal
cankers were seen microscopically, and the associated inflammation was intense. Mucous glands in the submucosa had apparently disappeared. Liver abscesses had increased in size and some had coalesced with others. Inflammatory cell types in liver lesions were the same as those noticed on three days PI.

Liver lesions from seven to 10 days PI were enlarged forms of those noted on previous days. Mesa et al. (1961) indicated that pharyngeal lesions had enlarged as well and that parasites were seen near exposed blood vessels. Palisading parasites were visible on intact epithelium.

Mesa et al. (1961) used the Jones' barn strain of *T. gallinae* which has a preference for pigeon livers. This was supported by observations of liver cankers and the lack of extensive oral lesions.

Other descriptions of oral lesions have been made (Callender and Simmons, 1937; Conti et al., 1985; Hawn, 1937; Levine et al., 1941), however, studies similar in design to that of Mesa et al. (1961) are not available.

Photomicrographs included in Callender and Simmons (1937) paper were too low in magnification to identify *T. gallinae*, and their microscopic description indicated that simple mucous glands in the throat became dilated early and unidentifiable as glands later. Purulent exudate was noted at times in the throat as well. Pharyngeal mucosal epithelium was "more or less" necrotic and desquamated, whereas glands contained mucous, trichomonads, epithelium and detritus.

Hawn (1937) studied trichomoniasis in turkeys, and included a description of crop and esophagus histopathology. Apparently, areas of
leucocytic infiltration were composed of polyblasts (macrophages) and some eosinophils. Hawn indicated that polyblasts were found in highest numbers near edges of necrosis and near affected mucous glands. Epithelium was eroded in some areas and not eroded in others. Small nodules were found within mucous glands and consisted of cellular debris. Lesion extension into the muscularis appeared to be common also. Levine, Boley and Hester (1941) found similar conditions in chickens and other birds. The leucocytic infiltrate in these birds was composed primarily of polymorphonuclear leucocytes, not polyblasts as stated by Hawn (1937). In addition to leucocytic infiltration, Levine et al. (1941) described congestion of blood vessels and areas of submucosal hemorrhage.

A recent study of mourning doves (Conti et al., 1985) indicated oropharyngeal abscesses to be associated with giant cells, macrophages and colonies of coccoid bacteria. Stratified squamous epithelium in this region showed signs of spongy degeneration and ulceration. It is not known if bacteria play a role in canker formation.

Cooper and Petty (1988) mentioned the presence of cellular debris, blood clots and bacteria in oral lesions of goshawks. Trichomonads were present in low numbers based on evaluation of wet smears.

As indicated, Trichomonas gallinae is an organism not readily stained by standard methods. Histomonas meleagridis and various fungal agents do not stain readily either (Kemp and Reid, 1966). In studies concerning these or related organisms, demonstration of the etiologic agent is paramount in importance as several different afflictions may present similar clinical signs. Other diseases which may resemble canker are
hypovitaminosis A, avian fowl pox, capillariasis and candidiasis (Hubbard et al., 1985; Ruhl et al., 1982).
PROBLEM DESCRIPTION

Much of what is known about the lesions of avian trichomoniasis was presented by Mesa et al. (1961). Their work was based on observations in rock doves infected with the Jones' barn strain of *Trichomonas gallinae*, a hepatotropic strain. The present study was performed with a strain of *T. gallinae* which had an affinity for the palate, palatal papillae (pharyngeal fringe) and the palato-esophageal junction (p-e junction). Figures 1 and 2 demonstrate these locations where cankers caused by this strain formed almost exclusively. Parasites used in this dissertation were isolated from infected pigeons supplied by an area pigeon fancier. Experimental infections were produced in specific pathogen-free ring doves (*Streptopelia risoria*).

Three studies were conducted to document trichomonad activities prior to, during and after canker formation in ring doves. Firstly, a parallel study to that of Mesa et al. (1961) was conducted in which paraffin and plastic resin embedded tissues were examined rather than paraffin exclusively. The purpose of this study was to re-examine the lesions associated with avian trichomoniasis. Secondly, the palatal mucosal surface was examined by scanning electron microscopy to document parasite activities during canker formation. Thirdly, the host-parasite interface during the same period was examined by transmission electron microscopy to determine more precisely the host-parasite interactions. Examination of the literature did not disclose reports of SEM and TEM studies with *T. gallinae* in a natural host system.
Fig. 1. Sagittal section of a pigeon head with characteristic lesions of avian trichomoniasis. B, brain; C, caseous nodule or canker; Cr, crop; T, trachea. The black arrow indicates palatal flaps highly involved with the canker whereas the asterisk indicates the additis laryngis, which is frequently called the epiglottis. The photograph is approximately normal in size.

Fig. 2. Sagittal section through the palate and an associated palatal papilla. C is an early canker, L is the lumen of the alimentary tract and Pe indicates the palato-esophageal junction. Black arrows indicate trichomonads on the epithelial surface. This photomicrograph is turned 180 degrees from that in figure 1, however, it is in correct anatomical position. Scale bar = 100 \( \mu \text{m} \).
Prior to and during the course of these major studies, other smaller studies concerning transmission of *T. gallinae*, culture of *T. gallinae* and preparatory techniques of *T. gallinae* for scanning electron microscopy were conducted.
SECTION I. CULTURE OF TRICHOMONAS GALLINAE (RIVOLTA) UTILIZING BOVINE SERA AND A COMMERCIAL GROWTH SUPPLEMENT

Abstract

Trichomonas gallinae was cultured in broth medium supplemented with calf, newborn or fetal bovine serum, either with or without an added commercial growth supplement, to examine their effects on trichomonad growth. The growth supplement was compatible with T. gallinae, and gave increased growth over cultures grown in medium containing serum alone. Cultures containing fetal serum were superior to those containing calf or newborn serum. Differences between calf and newborn serum were minor.

Introduction

Isolation and maintenance culture media containing a variety of components and types of blood sera have been utilized in Trichomonas gallinae cultivation (Cailleau, 1937; Diamond, 1954; 1957; Matthews, 1986; Matthews and Daly, 1974; Read, 1957). Specifically, pigeon serum was found to give better growth of T. gallinae than sera from other sources (Cailleau, 1937). Routine use of pigeon serum in maintenance medium, however, necessitates the keeping of large numbers of donor birds which may be expensive and/or impractical. An alternate choice of serum which gives good growth, is readily available and is inexpensive is therefore desirable. Newborn calf serum has these characteristics and has been used primarily in this laboratory. Calf and fetal bovine serum are used occasionally.
Growth supplements and serum extenders for general cell culture are available. However, their compatibility and performance in trichomonad culturing systems have not been reported. The purpose of this study was to determine if one such growth supplement was compatible with *T. gallinae* in culture and to see which of three different bovine sera gave the best growth of *T. gallinae* with and without added growth supplement.

Materials and Methods

Kupferberg *Trichomonas* broth (Difco) served as the test medium, while serum requirements were met with heat inactivated calf serum, newborn calf serum or fetal bovine serum (Gibco). The growth supplement used was SerXtend™ (DuPont). Trichomonads were obtained from a freshly killed ring dove (*Streptopelia risoria*) by washing the crop and throat with mammalian saline, pH 7.2.

The medium was prepared in six 100 ml erlenmeyer flasks, and calf, newborn or fetal serum was then added to three respective flasks to give final concentrations of 10% serum. The remaining flasks were treated similarly for final serum concentrations of 5%. Two 30 ml, screw-capped, straight culture tubes per treatment were aseptically inoculated with 9.5 ml of the medium. The remaining medium in the six flasks was then inoculated with 0.62 ml each of growth supplement. Following mixing, the medium was similarly transferred to 30 ml culture tubes. All media prior to the introduction of parasites was approximately pH 6.3.

Medium blanks were inoculated with 0.5 ml of the throat washings to give final tube volumes of 10 ml. Each tube received $3.0 \times 10^5$ parasites as determined by hemocytometer counts. Prior to incubation at 36°C, screw
caps were removed and the tubes were fitted with rubber serum caps. Each tube was then inverted several times, and a small volume of medium was removed with loc tuberculin syringes and the parasites were counted with a hemocytometer to establish a baseline. Another ring dove was orally intubated with 2.0 ml of throat washings so the experiment could be repeated.

At various intervals, tubes were inverted for mixing prior to medium withdrawal and counting with a hemocytometer. As there were two tubes per treatment, the hemocytometer was filled once from each tube and the values obtained averaged.

Results and Discussion

Growth characteristics of *T. gallinae* in vitro were monitored on two separate occasions, one week apart. Highly vacuolated and spherical trichomonads having sluggish movements constituted the inoculum for trial I (Fig. 1A). After passage through another dove, trichomonads assumed the pyriform shape, were non-vacuolated and had characteristic rapid movements. No round forms were observed. Throat washings from this bird served as the inoculum for trial II (Fig. 1B).

The growth supplement, a combination of hormones, insulin, transferrin and albumin, supports growth of various cell lines and primary cultures when used in conjunction with fetal bovine serum (Anonymous, 1985). In this study it was shown also to support growth of *Trichomonas gallinae* when cultivated in a commercially available STS medium (Kupferberg et al., 1948) containing calf, newborn or fetal bovine serum (Fig. 1).
Growth rates of 3T3 fibroblasts were examined (Anonymous, 1985), and in all instances growth was better in cultures containing fetal serum and growth supplement than serum alone. Similarly, higher trichomonad populations were observed in cultures containing serum and growth supplement rather than serum alone, with one exception.

The best growth in both trials occurred in media containing fetal serum. In trial I, 5% and 10% serum with added growth supplement produced higher peak populations than with serum alone, whereas in trial II, serum alone was better than media containing growth supplement. The reason for the discrepancy is unknown. The peak trichomonad populations in trial II fetal treatments were higher than those of trial I.

Low parasite populations were evident in trial I when calf and newborn serum was used, whereas better growth occurred in the same media of trial II. Since the STS medium was identical in all treatments, the low populations of trial I may possibly be attributed to the inability of vacuolated trichomonads to utilize serum or medium components effectively, the age of the serum or a combination thereof. Media containing calf or newborn serum and growth supplement produced peak parasite populations as high (Fig. 1B) or higher (Fig. 1A) than serum alone.

The study presented here suggests that a commercial growth supplement when used in conjunction with STS medium containing calf, newborn or fetal bovine serum gives as good or better growth of T. gallinae in most cases. Of three sera tested in isolation medium, fetal bovine serum was shown to be superior to calf and newborn sera. There appeared to be no appreciable difference between calf and newborn serum.
Literature Cited


Fig. 1. Growth profiles of *Trichomonas gallinae* grown in STS-medium containing calf, newborn and fetal bovine serum, with and without growth supplement. A.) Cultures inoculated with highly vacuolated, spherical trichomonads having sluggish movements. B.) Cultures inoculated with non-vacuolated, highly motile trichomonads. *10% newborn serum served as the control culture since it is used routinely in this laboratory.*
SECTION II. TRANSMISSION OF TRICHOMONAS GALLINAE TO RING DOVES  
(STREPTOPELIA RISORIA)

Abstract

Experimental transmission of Trichomonas gallinae to ring doves (Streptopelia risoria) was successful. Further, a naturally transmitted outbreak of trichomoniasis in a colony of ring doves is described. Fourteen squabs died with palatal canker as a result of being fed crop milk from infected parents, while seven others apparently gained resistance. Transmission through drinking water was observed on two occasions, whereas transmission through food was not seen.

Introduction

Since the early 1950s, epizootics of avian trichomoniasis in mourning doves (Zenaidura macroura) have been reported and speculation as to their causes offered (Greiner and Baxter, 1974; Haugen and Keeler, 1952). In epizootics of avian trichomoniasis, the transmission of T. gallinae from bird-to-bird may be a factor. Transmission of T. gallinae occurs from adult columbiforms to newly hatched squabs via feeding of crop milk (Stabler, 1954), and there also exists the possibility of transmission through water or food sources (Kocan, 1969; Stabler, 1954). Much speculation concerning transmission of T. gallinae through water exists (Kocan, 1969; Stabler, 1954; Stabler and Herman, 1951), but experimental accounts of transmission through this source are lacking. The purpose of this study was to further examine means of T. gallinae transmission to determine if T. gallinae can be transmitted through water.
Materials and Methods

Virulent *Trichomonas gallinae* used in all observations was isolated from an infected pigeon (*Columba livia*) which was supplied by an area pigeon fancier. Juvenile ring doves (*Streptopelia risoria*) from my personal breeding colony served as the experimental birds. In preparation for inoculation into doves, parasites were cultured in simplified-trypticase-serum medium (Difco) enriched with 10% fetal bovine serum (Gibco) and containing chloramphenicol for bacterial control. Experimental infections of the doves was done by orally intubating $3.0 \times 10^6$ parasites.

During experiments, birds were fed commercial pigeon pellets, red milo and tap water. No antibiotics other than those present in the pellets were administered to doves, so that the bird's normal oral flora remained unaltered. Vessels containing food and water were replenished daily.

Elevated cages used to house doves were constructed of one inch wire mesh and in a manner that allowed for removal of waste food and droppings. Waste food was not reused.

Results and Discussion

Two juvenile ring doves were infected with *T. gallinae* but did not succumb to the infection. These birds were retained in the colony and housed together in one cage. They were allowed to mature and eventually mate. The doves were determined to be trichomonad carriers by finding parasites in throat swabs and by growing parasites in STS-culture medium.
Transmission of *T. gallinae* from these carrier adult ring doves to newly hatched squabs through crop milk was observed. During a nine month period, 21 squabs were hatched by the adults. Seven of these survived to maturity while 14 died of avian trichomoniasis within seven days of hatching. Necropsies of these birds revealed massive palatal cankers in each, and trichomonads in throat washings.

Transmission of *T. gallinae* through drinking water was observed also. A single juvenile ring dove was orally intubated with approximately $3.0 \times 10^6$ trichomonads several hours before being caged with four other uninfected juveniles. The dove initially infected died with palatal canker 13 days post infection, whereas two of the remaining doves died on day 21 of the experiment, and a third died on day 35. All three birds had massive palatal cankers and high numbers of trichomonads in throat washings. The fourth juvenile never exhibited signs of infection. It was later learned that this bird may have been resistant to infection.

After completion of this experiment, the watering trough of an infected juvenile was switched with one from a cage holding four uninfected juveniles. Within eight days, all four juveniles were dead. Necropsies revealed palatal cankers and high numbers of trichomonads. In this instance, water was the only possible means of infection.

Transmission of *T. gallinae* usually occurs through feeding of crop milk to squabs (Stabler, 1954). Transmission by this mechanism probably requires young squabs to completely hatch before crop milk could be obtained from the parents. This phenomenon was witnessed here as 14 squabs died with palatal canker. Seven additional squabs from the same
parents apparently became resistant by this mechanism after going through subclinical infections.

Kocan (1969) determined that *T. gallinae* survives in distilled water, saline solutions and ground moist grains. Transmission from infected to uninfected birds, however, was not discussed. According to observations presented here, drinking water appears to be a reliable method of transmission between juvenile ring doves. Since columbiform birds drink in a continuous draught (Miller, 1969), unlike many birds, and only one virulent trichomonad is required for infection (Stabler and Kihara, 1954), it is conceivable that carriers of even low numbers of parasites are capable of producing and maintaining epizootics of avian trichomoniasis.

Through regurgitational feeding of squabs by infected parents and transmission of *T. gallinae* through water, epizootics of avian trichomoniasis like those in the past, will probably continue to occur.

Literature Cited


SECTION III. EFFECTS OF AIR DRYING AND CRITICAL POINT DRYING ON TRICHOMONAS GALLINAE MORPHOLOGY

Abstract

Upper alimentary tract tissues of ring doves (Streptopelia risoria) experimentally infected with virulent Trichomonas gallinae were processed for scanning electron microscopy by three procedures. Improved fixation of tissues with tannic acid and guanidine hydrochloride followed by air drying from freon revealed improved preservation of surface details and decreased drying artifacts when compared to tissues prepared by standard techniques and critical point drying.

Introduction

Most shrinkage and swelling artifacts in tissues prepared for scanning electron microscopy (SEM) are due to poor fixation rather than the specific drying technique employed (Gamliel, 1985). It was also indicated that air drying from volatile solvents was possible after improved fixation (Gamliel, 1985). Megakaryocytes, human myeloblastic leukemia cells and other soft tissues air dried from freon following optimum fixation in tannic acid and guanidine hydrochloride had less swelling and shrinkage of cells and improved preservation of surface detail when compared to critical point dried tissues (Gamliel, 1985).

Such techniques may be beneficial in studies of trichomonad parasites (Protozoa: Sarcomastigophora), where improved preparatory methods are needed. In this study the effect of improved fixation and air drying on the structure of Trichomonas gallinae, a parasite of columbiform birds,
and on avian stratified squamous epithelium morphology was investigated. A modification of Gamliel's (1985) technique was used, and the results obtained were compared to techniques used for other trichomonad species.

Materials and Methods

Uninfected ring doves (*Streptopelia risoria*) were each orally intubated with approximately \(3.0 \times 10^6\) virulent culture grown *T. gallinae*. After three days, the birds were killed by thoracic compression to reduce investigator-induced esophageal damage. At necropsy, tissues from the upper alimentary tract of each bird were excised and processed separately for SEM by one of the following protocols.

**Procedure 1** Tissue samples were fixed by immersion at 4°C for 24 hours in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. Tissues were washed in buffer and then placed into a freshly filtered solution of 2% tannic acid, 2% guanidine hydrochloride and buffer for two hours. Samples were again washed, osmicated in 1% osmium tetroxide, washed, dehydrated in ethanol, infiltrated with freon 113 (trichlorodifluoromethane) and air dried.

**Procedure 2** Tissue samples were treated similarly to those in procedure 1 except the tannic acid-guanidine hydrochloride step was eliminated and critical point drying replaced air drying from freon.

**Procedure 3** Samples were processed like those in procedure 2 except that 0.1 M cacodylate buffer, pH 7.2, replaced phosphate buffer and fixation was for six hours. This method is that frequently reported as for other trichomonads.
After all samples were dried, they were mounted on brass specimen stubs with colloidal silver paint and coated with approximately equal amounts of gold palladium. A JEOL JSM-35 scanning electron microscope set at 15 Kv was used for sample examinations. Photomicrographs were taken from approximately the same location on each palate.

Results and Discussion

Excellent surface details of *Trichomonas gallinae* and avian squamous epithelium were obtained in this study by utilizing a technique modified from that of Gamliel (1985), which incorporated secondary fixation in tannic acid-guanidine hydrochloride and air drying (Fig. 1). Comparative samples critical point dried without tannic acid-guanidine hydrochloride fixation, regardless of whether phosphate (Fig. 2) or cacodylate (Fig. 3) buffer was used, were in much poorer condition.

**Procedure 1**  Trichomonads air dried from freon following triple fixation (Fig. 1) showed recurrent flagella and undulating membranes to be intact and cell body distortions to be minimal. Apical microridges of squamous epithelium which are composed of microvilli-like processes (Ackerman et al., 1976) exhibited excellent detail, as did cell borders.

**Procedure 2**  Parasites which were subjected to phosphate buffering and critical point drying (Fig. 2) revealed separations of recurrent flagella from undulating membranes allowing for collapse of the membranes. Alterations to parasite cell bodies, was evident, although pseudopodia appeared unaffected. Epithelial processes were similar in condition to those in Figure 1.
Procedure 3  Cacodylate buffered parasites fixed for six hours and critical point dried were in similar condition to those reported in the literature (Heath, 1981; John and Squires, 1978; Juliano et al., 1986; Ovčinnikov et al., 1975; Warton and Honigberg, 1979). Undulating membranes and recurrent flagella were intact although drying artifacts were apparent (Fig. 3). Distortions of parasite cell bodies were noted also. Squamous epithelial processes were somewhat obscured by the presence of overlying mucus. Visible microvilli-like projections and cell borders appeared to be intact but more swollen than those processed by preceding methods.

The overall condition of the squamous epithelium and its associated processes was similar among the three outlined procedures. Differences in detail due to preservation were seen primarily in the appearance of *T. gallinae* undulating membranes and the associated recurrent flagella due to their delicate nature.

The undulating membrane of *T. gallinae* is a folded fin-like organelle on the dorsal surface which possesses a slight depression where the recurrent flagellum lies (Mattern et al., 1967). Direct connections between the two organelles have not been sufficiently demonstrated. It is possible that due to the nature of this relationship, shrinkage and swelling artifacts would be more pronounced in this region. It is also conceivable that chemical fixation of trichomonads for study by SEM, will cause some unavoidable artifacts. It should be apparent that artifacts can be minimized by using improved fixation and air drying from freon, rather than critical point drying. Preparation of other eukaryotic
protozoa for scanning electron microscopy by this technique may also prove beneficial.

Literature Cited


Fig. 1. *Trichomonas gallinae* and palatal mucosa processed by procedure 1, showing minor shrinkage of the undulating membrane and recurrent flagellum of one parasite (arrow). Good preservation of parasite cell bodies, squamous cell apical processes and cell boundaries (arrowheads) is also evident. Scale bar = 10 μm.

Fig. 2. Sample processed by procedure 2 showing separation of recurrent flagella from undulating membranes (black arrows) due to probable severe shrinkage. Pseudopodia (black and white arrows) and parasite cell bodies show some evidence of shrinkage and swelling. Epithelial processes indicate good preservation. Scale bar = 10 μm.

Fig. 3. Treatment of samples by procedure 3 reveals swelling of undulating membranes and recurrent flagella (arrows). Some parasite cell bodies exhibited swelling while others showed shrinkage artifacts (arrowhead). Squamous cell microvilli-like processes also appear somewhat swollen. Scale bar = 10 μm.
SECTION IV. HISTOPATHOLOGY OF THE RING DOVE PALATE FOLLOWING INFECTION WITH TRICHOMONAS GALLINAE

Abstract

Two sets of 10 and 14 ring doves (Streptopelia risoria) were orally intubated with virulent Trichomonas gallinae and killed at 24 hour intervals. Oral tissues were prepared for light microscopy by standard techniques and embedded in paraffin and plastic resin. At 24 hours post infection trichomonads were attached to the palatal mucosa and appeared to aid in pathological desquamation of epithelial cells. Cankers formed above the pharyngeal fringe and at the palato-esophageal junction by 48 hours post infection. The major cellular components of early cankers and the concomitant leucocytic infiltration appeared to be heterophils while later more histiocytes were encountered. Areas beneath cankers seemed to be sites where trichomonads were destroyed. Trichomonads were not demonstrated within simple mucous glands, although the glands appeared to undergo destruction during abscess formation. Later mucosal changes included widening of epithelial intercellular spaces and the migration of leucocytes through them. Pathological desquamation became more pronounced with time.

Introduction

Columbiform birds infected with virulent Trichomonas gallinae usually die when large caseous nodules (cankers) form in the pregastric alimentary tract, liver, air sacs, lungs or other vital organs (Cauthen, 1934; 1936; Stabler, 1954). Histopathologically, aspects of canker formation and
progression are not well documented. This general lack of histopathological data prompted this study.

The most comprehensive study dealing with histopathological changes in columbiforms following infection with *T. gallinae* appears to be that of Mesa, Stabler and Berthrong (1961). They experimentally infected pigeons (*Columba livia*) with the hepatotropic Jones' barn strain. Using light microscopy, they examined the liver and pharynx on several days post infection (PI). Apparently, large pharyngeal cankers failed to form. Small pharyngeal ulcerations and large hepatic abscesses were described.

Early reports concerning the lesions of avian trichomoniasis (Callender and Simmons, 1937; Mesa et al., 1961) were based on observations made from paraffin embedded tissue. Demonstration of trichomonads prepared in this manner is difficult since parasites often become distorted, and morphological characters such as flagella are not stained or stain poorly (Mesa et al., 1961). Likewise, trichomonads are not readily distinguished from host leucocytes and tissue debris in areas beneath cankers or at canker-epithelial interfaces.

With the advent of plastic embedding resins and the ability to cut much thinner sections than possible with paraffin, re-examination of the histopathology associated with canker formation in the upper alimentary tract of columbiform birds is possible. Therefore, paraffin and resin embedded tissues were compared in this study to early works to address some areas of uncertainty. Juvenile ring doves (*Streptopelia risoria*) were experimentally infected with virulent *T. gallinae* and examined at 24
hour intervals. The parasites utilized had a distinct preference for palatal tissues.

Materials and Methods

Prior to this study, virulent *Trichomonas gallinae* was isolated from infected pigeons and cultured in commercial STS medium (Difco) containing 10% fetal bovine serum (Gibco) and chloramphenicol.

Two groups of 10 and 14 juvenile ring doves were orally intubated with approximately $1.0 \times 10^7$ trichomonads (determined with hemocytometer) and were killed at 24 hour intervals after infection. Care was taken to avoid mechanical abrasion of the oral tissues during intubation. Two additional uninfected doves per group served as controls. At necropsy, the palate and upper esophagus were excised and prepared for light microscopy by the following methods.

**Paraffin embedded tissues** Prior to embedding in paraffin, excised tissues were fixed in formalin, alcohol and acetic acid (FAA) and dehydrated in a graded series of tertiary butyl alcohol (TBA). Following embedding of tissues in paraffin, 6 μm to 8 μm thick sections were cut with an A. O. Spencer rotary microtome, affixed to glass slides with egg albumin, deparaffinized in xylene, stained in Harris hematoxylin and eosin and dehydrated in ethanol. Stained slides were then placed in xylene prior to the addition of Canada balsam and glass cover slips.

**Resin embedded tissues** Tissues subjected to resin embedment were fixed by immersion at 4 C for 24 hours in 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.2. Tissues were then washed in buffer, post fixed in 1% osmium tetroxide, washed, dehydrated in
ethanol, infiltrated with propylene oxide and subsequently embedded in Epon 812 plastic resin. Following polymerization, 1.5 µm thick sections were cut with glass knives on a Reichert Ultracut E ultramicrotome. Sections were affixed to glass slides and stained with toluidine blue 0. After drying, slides were immersed in xylene and covered with Canada balsam and glass cover slips.

All micrographs were taken with a Leitz Orthomat automatic camera mounted on a Leitz Wetzlar Orthoplan compound microscope.

Results

Controls Control sections (Figs. 1 and 2) from the palate indicated minor desquamation of mucosal epithelium and the submucosal vasculature to be undilated.

24 hours PI Small and red spots resembling petechial hemorrhage appeared to be the only gross change to the palate, palatal papillae and palato-esophageal junction (p-e junction). Cankers were not seen macroscopically. Microscopically, a generalized hyperemia near the epithelial basement membrane and possibly slight dilation of blood vessels was noted (Fig. 4). Trichomonads at the mucosal surface were easily identified in both paraffin (Fig. 3) and resin sections (Fig. 4), however, those in resin exhibited morphological features such as cytoplasmic vacuolation and flagella more clearly. Pathological desquamation became apparent and trichomonads appeared to be attached to the upper layer of epithelium. It was noted also that trichomonads were associated with crevices on the mucosal surface and that they appeared to be forming a monolayer of individuals. Differences in staining intensity between
superficial and deep epithelial layers was evident. The apical layers and basement membrane stained deep blue whereas the middle layers retained almost no stain and appeared pale by comparison.

48 hours PI At necropsy the palate was deep red and swollen. Cankers still were not visible macroscopically. Paraffin sections revealed no great change from 24 hours PI with the exception of larger numbers of parasites in the monolayer and greater leucocytic infiltration near the epithelial basement membrane. Resin sections revealed a small canker superior to the palatal papillae (Figs. 5, 7 and 8) and a monolayer of perpendicularly arranged parasites on adjacent epithelium which appeared to be actively engaged in pathological desquamation. Desquamation seemed to be accelerated from 24 hours PI. Parasites were seen beneath loosened squames and on rafts of squamous cells (Figs. 5, 6, 7 and 8). There was massive leucocytic infiltration (Fig. 5), echymotic hemorrhage and dilated vessels at the epithelial basal lamina (Figs. 7 and 8). Polymorphonuclear leucocytes (heterophils) and some macrophages (histiocytes) were predominant cell types in the infiltrate. The canker proper appeared to possess many heterophils and some histiocytes (Fig. 7). Leucocytes were seen within epithelial intercellular spaces. Epithelial vacuolation appeared to be a result of epithelial destruction and/or edema.

72 to 96 hours PI By 96 hours PI small hard, yellowish, raised projections were seen macroscopically superior to the palatal papillae (Figs. 9 and 11). Microscopically, much of the submucosa was now infiltrated by leucocytes. In paraffin sections, many leucocytes could
not be positively identified, however, heterophils, histiocytes and erythrocytes were seen. Blood cells were seen within dermal papillae and the enlarged squamous cell intercellular spaces (Figs. 9 and 10). Trichomonads were localized on intact epithelium near the canker. It could not be determined if trichomonads were present at the canker-epithelial interface. Features observed in resin sections (Fig. 11) were more advanced than those seen 48 hours PI. Heterophils appeared to be more numerous than histiocytes and intercellular spaces had enlarged. Trichomonads were identified at the epithelial surface, but not identified elsewhere. By 96 hours PI infected doves had ceased feeding.

120 to 144 hours PI Grossly, cankers increased in size over 96 hours PI and the redness and swelling remained pronounced. Inflammation by this time had involved the pharynx. Other features remained about the same as parasites were still associated with intact epithelium and were not identified under cankers. Examination beneath cankers revealed dilated blood vessels near the free surface, tissue debris, heterophilia and increased numbers of histiocytes (Figs. 12 and 13).

168 to 240 hours PI Ring doves by this time were emaciated, had ruffled feathers and kept their eyes closed. Large cankers at the posterior border of the palate had nearly occluded the throat. The pharyngeal fringe was no longer identifiable and the redness of inflammation involved most of the oral cavity and esophagus. Microscopically, the submucosa was heavily infiltrated by leucocytes. Mucous glands appeared as if they were being replaced with leucocytes (Fig. 14), and the glandular epithelium appeared to be denuded.
Trichomonads were not demonstrated within glands. It was extremely
difficult to distinguish trichomonads from host cells and debris in
paraffin sections (Fig. 15) although parasites were still somewhat
distinguishable from host cells in resin sections (Figs. 16 and 17).
Histiocytes appeared to be the predominant leucocyte by 240 hours PI
although some heterophils were still present (Figs. 16 and 17). Blood
vessels were greatly dilated (Fig. 17), and structures resembling
trichomonads were seen outside of them.

Discussion
Pathological desquamation was the first major palatal change noticed
after infection. This appeared to be accompanied by parasite
multiplication and the formation of trichomonad monolayers. Pathological
desquamation also appeared to become more accelerated with time. Lifting
of squamous cells may have been due to mechanical activities of the
parasites, but initial loosening of squames may involve parasite
hydrolytic secretions as well. Mesa, Stabler and Berthrong (1961) were
intrigued with trichomonad palisading on the mucosal surface. This
phenomenon was recognized in this study also, although the significance of
this activity is unknown.

According to Mesa et al. (1961) mononuclear cells were involved with
the early inflammatory response in rock doves, whereas Hawn (1937),
described the inflammatory zone in T. gallinae infected turkeys as
consisting of histiocytes and eosinophils. Levine et al. (1941) working
with quail, turkeys, canaries and sparrows indicated the primary cell type
to be polymorphonuclear leucocytes. The present study tends to support
these latter investigators as polymorphonuclear leucocytes and possibly other granulocytes were more prevalent early in the infection. Increased numbers of histiocytes were not noticed until cankers had formed. Macrophages, giant cells and coccoid bacteria were predominant cell types found in old caseous lesions by Conti et al. (1985). The contribution of bacteria to canker formation is not known.

Demonstration of parasites beneath cankers and at canker-epithelial interfaces is difficult because trichomonads become distorted and do not stain well (Mesa et al., 1961). Examinations made during the present study indicated parasite numbers to be low in these areas, possibly due to their destruction by host leucocytes. Many large histiocytes were visible in these areas. In later stages of disease, dilated blood vessels could be seen near the ulcerated luminal surface, and histiocytes and heterophils appeared to migrate from them. Trichomonads appeared to be present on the outside surface of vessels. Whether trichomonads are found within the circulation during canker formation is not known. Mesa et al. (1961) stated that trichomonads were identified in mucous gland lumina and near adjacent capillaries. Glandular epithelium in the present study appeared to be denuded although parasites were not found. Further study is needed to determine if T. gallinae can survive in this location.

Initial canker formation began in the area superior to the pharyngeal fringe and progressed from there. Why cankers become large necrotic masses is unknown, but perhaps rapidly reproducing parasites simply "outrun" the host response once it has been initiated. Rapidly multiplying trichomonads would be able to attach to adjacent epithelium
and inflict further tissue damage while keeping ahead of advancing lesions. Honigberg et al. (1964), utilizing virulent and avirulent strains of *T. gallinae* in tissue cultures, indicated that rate of reproduction may be a factor in pathogenicity. Whether this is true in the natural disease is not known. The walling off process performed by the host appears to be inadequate as it seems to lag behind advancing parasites. Eventually, the entire palate can become involved with trichomonads and cankers, as can other oral tissues. It seems plausible that similar activities may be present during abscess formation in the liver as well.

To further understand canker formation in the *T. gallinae*-columbiform system, scanning and transmission electron microscopic studies have been initiated so that trichomonad activities at the epithelial interface and the identities of the host leucocytes involved with canker formation can be determined.

**Literature Cited**


**Fig. 1.** Control. Section of palatal papilla showing minor non-pathologic desquamation. Small undilated blood vessels (V) are visible. Paraffin section. Scale bar = 50 μm.

**Fig. 2.** Control. Section of palate in the region of the pharyngeal fringe. Non-pathological desquamation is visible as are small undilated blood vessels (V). Part of a simple mucous gland (M) is also identified. Resin section. Scale bar = 30 μm.

**Fig. 3.** 24 hours PI. Section from near the palato-esophageal junction. Black arrows indicate a trichomonad monolayer on the mucosal surface. Paraffin section. Scale bar = 30 μm.

**Fig. 4.** 24 hours PI. Resin section from near the palato-esophageal junction. Some trichomonads (black arrow) appear to be undergoing binary fission. Blood vessels (V) appeared to be slightly dilated and some blood cells appear to be undergoing margination at the vessel wall. Resin section. Scale bar = 30 μm.

**Fig. 5.** 48 hours PI. Sagittal section through a palatal papilla. Trichomonads are visible on the surface of intact epithelium (black arrows) whereas an early canker (C) is also visible. A zone of blood cells (black and white arrows) possibly indicates an attempt at walling off the lesion. Similar zones are present beneath much of the epithelium at the basal lamina. The alimentary tract lumen is represented by the letter L. Resin section. Scale bar = 100 μm.

**Fig. 6.** 48 hours PI. Higher magnification view of figure 5 showing trichomonads at the palatal mucosal surface. Parasites appear to be actively engaged in pathological desquamation of epithelial cells. Resin section. Scale bar = 30 μm.
Fig. 7. 48 hours PI. Higher magnification view of figure 5 showing the early canker and trichomonads on intact epithelium in the region of the palato-esophageal junction (black arrow). Hemorrhage is evident in and around the partially liquefied canker as are increased numbers of granulocytes. The black and white arrow indicates leucocytic infiltration near parasites attached to the epithelial surface. Resin section. Scale bar = 50 μm.

Fig. 8. 48 hours PI. Higher magnification view of figure 5 showing spreading intercellular spaces within the epithelium and at the canker edge. Leucocytes, possibly heterophils and histiocytes (black and white arrows), are present within intercellular spaces. Many trichomonads are attached to a raft of squamous cells (black arrow) as well as the mucosal surface. Resin section. Scale bar = 30 μm.

Fig. 9. 96 hours PI. Pronounced leucocytic infiltration, intercellular space expansion and cankers (C) were prominent. Trichomonads were identified with certainty in areas of intact epithelium only (black arrow). Leucocytic infiltration in areas resembling dermal papillae (black and white arrow) and directly beneath active trichomonads was a prominent feature. Paraffin section. Scale bar = 50 μm.

Fig. 10. 96 hours PI. Paraffin section showing possible trichomonad stacking in craterform excavations at the epithelial surface (black arrow). Black arrowheads indicate what may be histiocytes, whereas black and white arrows indicate leucocytes within a dermal papilla. Note the closeness of dermal papilla to the mucosal surface. Scale bar = 30 μm.

Fig. 11. 96 hours PI. More advanced canker than seen at 48 hours PI showing dilated vessels, free heterophils and possibly some histiocytes. Trichomonads stained well at the epithelial surface (black arrow), whereas deeper cellular elements (black arrowhead) stained poorly. Identities of these cells could not be determined. Resin section. Scale bar = 30 μm.
Fig. 12. 144 hours PI. Resin section of the area beneath a canker. Possible trichomonads in this region were few in number (black arrowheads). Tissue debris and heterophils were prevalent. Scale bar = 30 μm.

Fig. 13. 144 hours PI. Area beneath a canker. There appears to be increased numbers of histiocytes. The black arrow indicates a histiocyte in the process of engulfing a squamous cell. Resin section. Scale bar = 30 μm.

Fig. 14. 240 hours PI. The entire palate had been infiltrated by leucocytes and large cankers (C) which nearly occluded the esophagus. Intact epithelium was highly vacuolated and simple mucous glands (M) appeared as if they were being destroyed during abscess formation. Glandular epithelium was somewhat denuded. Paraffin section. Scale bar = 500 μm.

Fig. 15. 240 hours PI. Higher magnification view of the canker-epithelial interface. Trichomonads are associated with intact epithelium (black arrow). The black arrowhead may indicate a trichomonad. Possible heterophils can be seen near the scale bar. Paraffin section. Scale bar = 30 μm.

Fig. 16. 240 hours PI. Section of the canker-epithelial interface. Note trichomonads (black arrowheads) at the epithelial interface and what appears to be giant cells above them. Resin section. Scale bar = 30 μm.

Fig. 17. 240 hours PI. Large dilated blood vessels which appear to be leaking leucocytes can be seen. Possible trichomonads are indicated by black arrowheads while more prevalent histiocytes, some in stages of phagocytosis, are indicated by black arrows. Scale bar = 30 μm.
SECTION V. MICROTOPOGRAPHY OF THE RING DOVE PALATE FOLLOWING INFECTION WITH TRICHOMONAS GALLINAE

Abstract

Ring doves (Streptopelia risoria) were experimentally infected with virulent Trichomonas gallinae so trichomonad activities prior to, during and after canker formation could be examined with scanning electron microscopy. Between 6 and 15 hours post infection low numbers of amoeboid trichomonads attached to microvilli-like apical processes and cell borders of palatal squamous epithelium. Initial parasite activities at tightly attached cell borders suggests that some parasite factor(s) may initiate cell removal. Once squamous cells began abnormal desquamation, trichomonads were free to invade beneath loosened cells and ultimately remove them, possibly by mechanical means. Pathological desquamation continued throughout the study. Cankers began forming four days post infection, however, trichomonads were not always seen at canker-epithelial interfaces. These areas were probably sites of trichomonad destruction. Areas of the upper alimentary tract not necrotic by 10 days post infection, were covered by an apparent trichomonad monolayer.

Introduction

Columbiform birds infected with virulent strains of Trichomonas gallinae (Rivolta) usually die several days post infection (PI) when large caseous nodules (cankers) form in the pregastric alimentary tract, liver, air sacs, lungs or other vital organs (Cauthen, 1934; 1936; Stabler, 1954). Other than the histopathological study of experimentally infected
pigeons (*Columba livia*) performed by Mesa et al. (1961), and descriptions of lesions associated with avian trichomoniasis such as those of Callender and Simmons (1937), activities undertaken by *T. gallinae* prior to canker formation have not been studied extensively. Studies involving scanning electron microscopy (SEM) of avian trichomoniasis are not available. Purposes of this study, therefore, were to examine events prior to, during and after canker formation in ring doves (*Streptopelia risoria*) experimentally infected with *T. gallinae*. Canker formation in the oropharynx and palate were addressed specifically.

**Materials and Methods**

An infected pigeon from the loft of an area pigeon grower was the source of the virulent trichomonads. Throat washings collected from the pigeon were cultured in 10 ml glass screw-cap culture tubes containing STS-medium (Difco), 10% fetal calf serum (Gibco) and chloramphenicol. Stationary phase cultures were slowly centrifuged for five minutes to concentrate trichomonads, at which time known uninfected ring doves were orally intubated.

A total of 14 ring doves were infected with approximately $1.1 \times 10^7$ parasites each (determined with hemocytometer) and killed by thoracic compression at 6, 15, 19 and 24 hours PI, and every 24 hours thereafter. Two uninfected doves served as controls. Doves were not administered antibiotics to avoid alterations of the normal oral flora.

Palatal flaps, their associated pharyngeal (palatal) papillae and esophagus were excised and processed for SEM by a technique modified from that of Gamliel (1985). Half of the excised tissues from each dove were
fixed for 24 hours at 4°C in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. Following washing in buffer, tissues were immersed in a freshly filtered solution of 2% tannic acid, 2% guanidine hydrochloride and buffer for two hours. Tissues were again washed, osmicated in 1% osmium tetroxide, washed, dehydrated in ethanol, infiltrated with freon 113 (trichlorodifluoromethane) and air dried. The remaining tissues were processed for transmission electron microscopy by conventional methods and will be discussed in a separate report.

Dried tissue samples were mounted on brass specimen stubs with colloidal silver paint, coated with gold palladium and examined with a JEOL JSM-35 scanning electron microscope.

Results

Tissues examined included palatal flaps, their associated papillae, the palato-esophageal junction (p-e junction) and the proximal end of the esophagus as these sites are where cankers form most commonly with the strain of _T. gallinae_ used (unpublished observations). Cankers formed, although infrequently, in other regions of the upper alimentary tract, however, descriptions of these lesions will not be included.

**Control tissues** Micrographs of control tissues (Fig. 1) revealed that squamous epithelial cells were tightly attached to adjacent cells by their borders and that non-pathological desquamation was minimal. Microvilli-like apical processes arranged in microridges were prevalent on squamous cells.

**6 to 15 hours PI** Although high numbers of trichomonads were inoculated into each dove, photomicrographs revealed extremely low
parasite numbers to be present between 6 and 15 hours PI. Parasites were attached to squamous cells and pseudopodia were forming. Pseudopodia that had formed appeared to be in intimate contact with squamous cell apical processes, and in some cases with cell borders (Figs. 2 and 3). Mucosal surface irregularities of the upper esophagus harbored trichomonads also (Fig. 4).

19 to 24 hours PI A prominent change occurring to the palate between 19 and 24 hours PI was the apparent dislodgment of surface epithelial cells by trichomonads (Figs. 6 and 8). Preceding actual cell dislodgment, increased parasite attachment at cell borders and microvillous apical processes was evident (Fig. 5). Although involvement with squamous cells appeared to be the predominant activity undertaken by trichomonads, they also appeared to retain their ability to engulf bacteria (Fig. 7). Some areas of the palate harbored few parasites and some areas possessed small localized parasite populations superior to the palatal papillae (Figs. 9 and 10). Pathological desquamation in this area was increased over other areas.

48 to 72 hours PI By 48 hours PI, trichomonad populations superior to the palatal papillae had increased as did pathological desquamation of the mucosa. Small squames were still being removed and larger, multicellular squames were being removed with higher frequency (Fig. 11). Pathological desquamation of adjacent epithelial sheets from apparently the same mucosal layer also became pronounced (Fig. 11).

Palatal mucosal changes and trichomonad structure at 72 hours PI differed from those previously noted. Rather than localizing beneath
squamous cells exclusively, trichomonads appeared to be attached to the luminal surface of loosened cells (Fig. 12). Additionally, numerous parasites assumed bell-shapes and did not form characteristic pseudopodia. These bell-shaped trichomonads formed broad, foot-like pseudopods with many smaller pseudopodia branching from them (Fig. 13). Sites where large pseudopodia contacted the epithelium appeared to be eroded as craterform depressions were visible (Fig. 13).

96 hours PI First evidence of canker formation was observed at 96 hours PI. The small canker, visible to the unaided eye, formed in the mucosa where the palate meets the pharynx (Fig. 15). Examination of the canker-epithelial interface revealed few parasites, while areas only a short distance away harbored large numbers of trichomonads (Fig. 14). The p-e junction and papillar bases were well populated by parasites.

120 hours PI Caseous nodules were larger by 120 hours PI. Small white buttons had erupted superior to the palatal papillae (Fig. 16) and unlike the canker seen at 96 hours PI, trichomonads were present in large numbers at the canker-epithelial interface (Fig. 17). Pronounced invasion of the proximal esophagus had occurred and many partially dislodged squames with parasites beneath them were visible (Fig. 18). Doves were not eating large quantities of food, and examination of the ventral surface of the palate revealed localized trichomonad populations (Fig. 19). Parasites had not been observed here previously.

144 to 192 hours PI Canker enlargement and increased pathological desquamation were the primary changes occurring during this period. Invasion of trichomonads from above palatal papillae to the ventral palate
via interpapillary spaces was pronounced (Fig. 20). Mucous gland duct openings on the ventral surface of the palate were greatly affected by invading trichomonads (Fig. 24), as squamous epithelium was being removed in these areas by the parasites. Extremely large sheets of squamous cells were being removed elsewhere on the ventral palate (Fig. 23). Desquamation of the proximal esophagus (Fig. 22) had become severe. Cankers were now large necrotic masses, composed of blood cells and covered with what appeared to be mucosal epithelium remnants (Fig. 25). Much of the posterior palate and pharyngeal fringe had been destroyed through enlargement of these cankers (Figs. 21 and 25). Presence of trichomonads at the canker-epithelial interface was variable as they were observed in some instances and not in others.

216 to 240 hours PI By 216 hours PI much of the palate had been transformed into a large caseous mass. Portions of the palate away from the canker proper still possessed normal appearing squamous epithelium (Fig. 27). Papillae not destroyed during canker formation possessed large trichomonad infested excavations. Although excavations appeared not to traverse the basal lamina, several epithelial layers had been removed (Fig. 26).

Final tissue samples were collected 240 hours PI. The entire palate was affected by caseous necrosis and cankers had formed in adjacent areas also. Some smaller cankers appeared to have coalesced to form larger ones. Although the proximal esophagus was also involved with cankers, most of the remaining esophageal mucosa was covered by a trichomonad monolayer (Fig. 28).
Extremely high numbers of trichomonads were orally inoculated into each ring dove, however, examinations of tissues 6 to 15 hours PI indicated that few parasites had adhered to palatal squamous cells. It is probable that few parasites are needed to establish infections. Stabler and Kihara (1954) indicated that oral implantation of one trichomonad was sufficient for infection and death of pigeons.

Following infection, activities undertaken by *Trichomonas gallinae* prior to caseous nodule formation include attachment to squamous cell microvilli-like apical processes and to cell borders, formation of pseudopodia and invasion beneath loosened squames. With increased trichomonad multiplication and spread at the p-e junction and superior to palatal papillae, pathological desquamation of large squames and expansive mucosal sheets occurred commonly.

In early stages of squamous cell removal, amoeboid *Trichomonas gallinae* were associated with cell borders and microvilli-like apical processes of what appeared to be tightly attached cells, while lifting of cell borders followed later. Adherence and amoeboid movement have been considered principal modes of mucosal epithelium damage in studies concerning *T. vaginalis* (Heath, 1981; Kotcher and Hoogasian, 1957; Nielsen and Nielsen, 1975), whereas a combination of mechanical damage and parasite secreted digestive enzymes have been suggested also (Christian et al., 1963; Honigberg et al., 1964). Mechanical damage from flagellar and undulating membrane motion may play a role in pathological desquamation once squamous cells have been loosened, however, cells remaining in tight juxtaposition
with adjacent cells may not be so affected. In this study, mechanical removal of squamous cells became more pronounced with time. These observations suggest that some factor(s) may occur at cell borders to initiate squamous cell removal. Once squamous cells begin to separate from one another, an opening is provided through which additional trichomonads can migrate. Through actions of amoeboid movement, flagellar and undulating membrane motion and possible hydrolase activity, cells can then be completely removed. More complete observations of this mechanism need to be made.

Trichomonads were not always associated with active squamous cell removal. Occasionally, bell-shaped parasites with large foot-like pseudopodia were seen in areas away from pathological desquamation. These parasites were associated with craterform erosions on squamous cell surfaces. With further study, these areas may prove to be sites of active epithelial digestion by trichomonads.

Canker formation and trichomonad activity were most pronounced initially superior to the pharyngeal fringe and near the p-e junction, and only later did parasites invade ventral aspects of the palate. Doves did not curtail feeding until cankers began to form. It appears that birds must stop or greatly reduce feeding before ventral palatal surfaces can become involved with trichomonads. Tongue actions, the passage of food into the crop or other phenomena prior to this time may be sufficient for parasite removal. Once cankers formed, areas directly beneath them were very difficult to examine, and precise descriptions of trichomonad activities in these regions were not made. The canker-epithelial
interface had been examined previously (Callender and Simmons, 1937; Mesa et al., 1961), and definite parasites were not confirmed in these areas. Due to poor staining characteristics of T. gallinae in paraffin sections, one must be cautious in interpreting events occurring in this region. It was noted in this study that the presence of trichomonads at canker edges was variable. On some occasions parasites were present and on others they were not. When absent, large numbers of parasites could be found elsewhere superior and inferior to the palate. It is conceivable that interfaces possessing trichomonads were sites of active trichomonadocidal activity by host leucocytes. Sites devoid of parasites yet possessing cankers were probably areas where trichomonads had been destroyed. It has been shown in cell culture (Honigberg et al., 1964), that an avirulent strain of T. gallinae with low reproductive potential is easily kept in check by host macrophages, whereas the virulent Jones' barn strain which has a high reproductive potential is not. In natural systems, rapid reproduction of trichomonads may be a factor in canker production and enlargement since rapidly dividing parasites may avoid destruction by advancing ahead of canker development. Parasite monolayer formation also may be a result of rapid reproduction. Further observations of this phenomenon with sectioned material would be beneficial.

Data have been presented which gives a clearer picture of the activities undertaken by T. gallinae prior to, during and after canker formation in upper alimentary tract tissues of a representative species of columbiform bird.
Literature Cited


Fig. 1. Control. Palatal mucosa showing tight juxtaposition of squamous epithelial cells. Cell borders and microridges composed of microvilli-like apical processes are pronounced. Scale bar = 5 μm.

Fig. 2. 15 hours PI. Fine branching pseudopodia (black and white arrow) and lobopodia (black arrow) are in contact with squamous cell apical processes. Scale bar = 3 μm.

Fig. 3. 6 hours PI. Trichomonad attached to palatal mucosa shows broad lobopodium (black and white arrow) which is in direct contact with squamous cell microvilli-like apical processes and cell borders. Scale bar = 3 μm.

Fig. 4. 15 hours PI. Trichomonads had invaded surface irregularities of the palato-esophageal junction. Note presence of bacteria in low numbers. Scale bar = 5 μm.
Fig. 5. 19 hours PI. Trichomonads localized at cell borders. Note how one parasite (black and white arrow) appears to be pulling on the edge of a squamous cell in early stages of removal, while another appears to be attached to a cell border (B). Scale bar = 5 μm.

Fig. 6. 19 hours PI. Trichomonad beneath loosened squamous cell. Black and white arrows indicate cell border remnants where this cell probably was once attached to others. Scale bar = 5 μm.

Fig. 7. 19 hours PI. Trichomonads attached to squamous cells also engulfing bacteria. Some bacteria can be seen in early phagocytic cups (black arrows). Scale bar = 3 μm.

Fig. 8. 19 hours PI. Large squames apparently being removed by parasites. Here at the p-e junction, many parasites have invaded deeply into intercellular spaces. Black arrows indicate extent of parasite advancement. Black and white arrow shows additional lifting of cells. Scale bar = 5 μm.

Fig. 9. 19 hours PI. Trichomonads were above two adjacent palatal papillae (P). Photomicrograph has been inverted 180 degrees from anatomical position. Scale bar = 50 μm.

Fig. 10. 19 hours PI. Higher magnification of figure 9. Great numbers of parasites are in association with abnormally removed squames. Scale bar = 10 μm.
Fig. 11. 48 hours PI. Trichomonads had invaded larger areas above palatal papillae (P). Pathological desquamation of squamous cell sheets became pronounced. Conditions suggestive of trichomonad palisading were evident (black and white arrows). Micrograph has been inverted 180 degrees from anatomical position. Scale bar = 50 μm.

Fig. 12. 72 hours PI. Superior surface of palatal papilla and p-e junction. Trichomonads were attached primarily to squamous cell apical surfaces rather than harboring beneath them. Scale bar = 50 μm.

Fig. 13. 72 hours PI. Bell-shaped trichomonads possessing broad foot-like pseudopodia with many smaller filopodial branches. A craterform depression (black and white arrow) indicates an area of possible digestion by parasite secretions. Numerous exocytotic vesicles (black arrowheads) are visible on parasite surfaces. Scale bar = 2 μm.
Fig. 14. 96 hours PI. Enface view of two papillae (P), p-e junction and upper reaches of esophagus (E). Arrow indicates elevated numbers of parasites at the p-e junction and bases of papillae. Trichomonads seen here were some distance away from the canker seen in figure 15. Scale bar = 100 µm.

Fig. 15. 96 hours PI. Canker visible to the unaided eye. The canker-epithelial interface (black and white arrow) revealed an absence of trichomonads, suggesting their control by the host. Black arrows indicate blood cells at the canker edge. Scale bar = 500 µm.

Fig. 16. 120 hours PI. Low magnification enface view of one palatal flap. Cankers (C) were visible macroscopically as small white buttons. Trichomonads were visible at canker-epithelial interfaces and an area midway between cankers (black and white arrow). Scale bar = 500 µm.

Fig. 17. 120 hours PI. Higher magnification view of figure 16. Canker-epithelial interface possesses high numbers of parasites. Canker (C) and papilla (P) are visible. Scale bar = 50 µm.

Fig. 18. 120 hours PI. Invasion of upper esophagus by parasites. Squames consisted of many epithelial cells. Scale bar = 50 µm.

Fig. 19. 120 hours PI. Trichomonads on inferior surface of palate. Parasites in this region were found in an almost continuous monolayer. Palisading trichomonads were not observed. Scale bar = 50 µm.
Fig. 20. 144 hours PI. Interpapillary involvement by trichomonads had become pronounced. Papillae (P) also, were generally surrounded by parasites at their bases. Scale bar = 50 μm.

Fig. 21. 144 hours PI. Papillae (P) had been largely destroyed during canker progression. Larger necrotic masses involved most of the posterior palate. Arrows indicate trichomonads at the canker-epithelial interface. Scale bar = 500 μm.

Fig. 22. 168 hours PI. Pathological desquamation of the upper esophagus. Several layers of squamous cells are visible. Scale bar = 50 μm.

Fig. 23. 168 hours PI. Large sheet of squamous epithelium being removed from the ventral palate. Scale bar = 50 μm.

Fig. 24. 168 hours PI. Many mucous duct openings (D) on the ventral palate were involved with trichomonads. Scale bar = 50 μm.

Fig. 25. 192 hours PI. Canker showing cellular debris, blood cells and apparent squamous epithelial remnants. Note papilla (P) being consumed by the advancing lesion. Black arrow indicates trichomonads. Scale bar = 100 μm.
Fig. 26. 216 hours PI. Papilla (P) not yet destroyed shows large parasite infested erosion with several layers of epithelium having been removed. The basal lamina probably has not been exposed as evidenced by the relative lack of blood cells. Scale bar = 50 μm.

Fig. 27. 216 hours PI. Much of the palate has been reduced to cankers (C). Areas of normal appearing epithelium (black and white arrows) were still visible. Scale bar = 1 mm.

Fig. 28. 240 hours PI. Areas not involved with cankers were covered by an apparent monolayer of trichomonads. Parasites here are covering the esophagus. Scale bar = 50 μm.
SECTION VI. ULTRASTRUCTURE OF THE RING DOVE PALATE FOLLOWING INFECTION WITH TRICHOMONAS GALLINAE

Abstract

Fourteen ring doves (Streptopelia risoria) were experimentally infected with virulent Trichomonas gallinace and killed at various intervals during a 10 day period for purposes of examining palatal canker formation with transmission electron microscopy. By 24 hours post infection, trichomonads appeared to phagocytose microvilli-like apical processes of squamous epithelial cells. Examination of the host-parasite interface revealed the presence of an area of electron dense granulation near the trichomonad cell membrane as well as structures which resembled Golgi bodies. Trichomonads appeared to aid in pathological desquamation. Small cankers formed superior to palatal papillae by 48 hours post infection and examination of the canker-epithelial interface revealed widening of intercellular spaces and infiltration by granulocytes and macrophages. Trichomonads beneath cankers were being destroyed by host leucocytes. Dilated blood vessels near the ulcer surface contained primarily granulocytes, and trichomonads were seen associated with the outer surface of these capillaries.

Introduction

Studies concerning caseous nodule (canker) formation in columbiform birds after infection with Trichomonas gallinace have been performed with light microscopy in the past (Callender and Simmons, 1937; Mesa et al., 1961). These studies were limited by the fact that individual parasites
Electron microscopy allows the observation of the *T. gallinae*-epithelial interface. The purpose of this study then was to examine interactions between host oral tissues and *T. gallinae* with transmission electron microscopy (TEM) and to document these relationships.

**Materials and Methods**

A total of 14 ring doves (*Streptopelia risoria*) were orally intubated with virulent *T. gallinae* which had been axenically cultured in commercial STS medium (Difco) containing 10% fetal bovine serum (Gibco) and chloramphenicol. The *T. gallinae* had been obtained from rock doves in a local colony. Doves were given approximately $1.1 \times 10^7$ trichomonads each (determined with hemocytometer) and killed at 6 hours, 15 hours, 19 hours and 24 hours post infection (PI), and every 24 hours thereafter. Birds were killed by thoracic compression to reduce tissue damage in the upper alimentary tract.

At necropsy, the palatal flaps, pharyngeal fringe (palatal papillae) and esophagus were excised and processed for TEM by standard methods. Tissues were fixed for 24 hours at 4 C in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer, at a pH of 7.2. Samples were subsequently washed in buffer, post fixed in 1% osmium tetroxide, washed again, dehydrated in ethanol, infiltrated with propylene oxide, embedded in Epon 812 plastic resin and polymerized at 60 C. Half of all excised
tissues from each bird in this study were processed for scanning electron microscopy, which will be discussed in another report.

Thick sections were cut with glass knives and ultrathin sections with a DuPont diamond knife on a Reichert Ultracut E ultramicrotome. Thin sections were collected on uncoated 200 mesh copper grids, stained with 15% methanolic uranyl acetate and 2% aqueous lead citrate and observed in Hitachi HU-11C and HS-8 transmission electron microscopes.

Results

Tissue samples were examined at 6, 15, 19 and 24 hours PI and every 24 hours thereafter during this study. It should be noted that many trichomonad activities observed prior to canker formation were also seen during and after canker formation, and that bacterial contaminants did not appear to be prevalent.

**Controls** Stratified squamous epithelium of the palate possessed the typical layered appearance and exhibited signs of non-pathological desquamation (Fig. 1). Prominent cellular features were microvilli-like apical processes which appeared to be cytoplasmic extensions.

**24 hours PI** At 24 hours PI, desquamation of mucosal epithelial cells, apparently due to trichomonad activities, could be seen. The underlying submucosal vasculature was dilated (Fig. 2). Parasites were associated with the upper layers of the mucosa. Ultrathin sections indicated trichomonads to be associated primarily with the microvilli-like apical processes (Figs. 3 and 4). At times they conformed closely to cell processes (Fig. 6). Trichomonads appeared to engulf apical processes by phagocytosis, as the processes were seen within membrane bound vacuoles.
(Fig. 4). These vacuoles are visible in living organisms observed with light microscopy.

Higher magnification of trichomonad-epithelial interfaces (Fig. 5) revealed electron dense and granular regions near trichomonad cell membranes. Structures resembling the Golgi apparatus could be seen in close association with parasite cell membranes also. Cell membranes of epithelial apical processes seemed to be in contact with parasite cell membranes.

48 hours PI  By 48 hours PI cankers were visible microscopically. Parasites were associated with pathological desquamation and appeared to be arranged in monolayers (Fig. 7). During pathological desquamation, trichomonads appeared to extend pseudopodia into areas beneath superficial squamous cells (Fig. 9). Frequently trichomonads extended fine pseudopodia between adjacent squamous cells which had been loosened (Fig. 10). Epithelium near cankers featured a widening of intercellular spaces and apparent concomitant invasion by host macrophages (histiocytes) and granulocytes (Figs. 8, 10, 11 and 13). The granulocytes (Figs. 11 and 13) did not appear to be fully differentiated. The granulocyte nucleus was more round and spindle-shaped granules in the cytoplasm were seen. Trichomonads were observed in areas beneath superficial squamous cells in protected areas, whereas epithelium destruction appeared to be in progress in deeper layers (Fig. 12).

72 to 96 hours PI  Trichomonad activities did not differ greatly from 48 hours PI. At the epithelial basal lamina, intercellular spaces
Mere widening and deposition of collagen by fibroblasts was evident (Figs. 14 and 15).

**120 to 240 hours PI**  
Cankers became visible macroscopically by 120 hours PI and were located superior to the pharyngeal fringe of the palate in the region of the palato-esophageal junction (p-e junction). Thin sections from lesion cores (Fig. 17) had much tissue debris and what is believed to be collagen. Areas between cankers and the underlying tissue possessed many dead parasites and some apparently healthy ones, as well as tissue debris (Figs. 16, 18 and 19). Living trichomonads appeared to be in direct contact with epithelial cells (Fig. 16), whereas parasites being killed appeared to undergo lysis as a result of interactions with histiocytes (Fig. 18). Microbody-like hydrogenosomes, which are membrane bound inclusions within the trichomonad cytoplasm, seemed to survive parasite lysis, however, ruffling of the membranes appeared to be an early event in their eventual destruction (Fig. 19).

At 240 hours PI, trichomonads were seen on the outside of dilated blood vessels (Fig. 20). It could not be determined with certainty if parasites were actually attacking them however. It also was noted that erythrocytes were exiting blood vessels and were not numerous. Granulocytes were the predominant cell type within the dilated vessels. Much tissue debris was in the area surrounding the vessels.

**Discussion**

The predominant features of *Trichomonas gallinae* infections during the course of this study were the apparent phagocytosis of squamous epithelial cell microvilli-like apical processes and the pathological
desquamation of epithelial cells by the trichomonads. Apical processes appeared to be removed during phagocytosis and many superficial squamous cells were removed. Ovchinnikov et al. (1975) studied T. vaginalis and noted phagocytosis of vaginal squamous cells. In the present study, phagocytic cups contained partially engulfed squamous cell apical processes. It is not known if parasite secreted hydrolases play a role in apical process removal.

Squamous cell removal by trichomonads has been addressed by other investigators. Some believe cell removal to be a result of mechanical dislodgment (Nielsen and Nielsen, 1975), whereas others believe in a combination of mechanical damage and enzymatic activity (Christian et al., 1963; Honigberg et al., 1964). The results of the present study tend to support the latter investigators, since trichomonads appeared to separate squamous cells by extending fine pseudopodia into deeper epithelial layers at cell borders. Since squamous cells normally are tightly attached to others, it may be necessary for parasites to secrete some factor(s) to initiate cell removal. With cells loosened, parasites can move beneath them and possibly remove them by further mechanical and/or enzymatic means. Without further investigation, this aspect will remain conjectural.

Another major change to the epithelium occurred after cankers began forming. Squamous cell intercellular spaces appeared to spread apart, possibly due to edema. Host leucocytes including histiocytes and granulocytes, possibly non-differentiated, were seen beneath areas harboring many trichomonads and in intercellular spaces. Speculation as
to the identity of these granular leucocytes has been made in the past. Levine, Boley and Hester (1941), while studying quail, turkey, sparrow and canary trichomoniasis, stated that the predominant cells at the edge of necrotic areas were polymorphonuclear leucocytes whereas Hawn (1937) indicated the inflammatory zone in turkey trichomoniasis to be composed of macrophages and eosinophils. In this study granulocytic cells were encountered frequently. Based on criteria such as spindle-shaped cytoplasmic granules and amoeboid shape, the granulocytes probably are polymorphonuclear leucocytes. Supporting this assumption are the observations made by Shaw (1933). In his description of polymorphonuclear leucocytes, he stated that "blunt ectoplasmic pseudopodia are thrust out into which the granules slowly stream either in their long axes or transversely". He stated also that "the nucleus occupies a lateral or posterior site during motility". Nuclei appeared more rounded than lobulated in the present study, suggesting that these cells may be immature polymorphonuclear leucocytes. Many granulocytic cells were also present in dilated capillaries beneath cankers late in this study.

Identification of cells and parasites beneath cankers has been difficult due to parasite alterations and poor staining characteristics of T. gallinae (Mesa et al., 1961). Examination of this region with TEM indicated that parasites were present in these deeper sites and that they were being destroyed by histiocytes. Areas beneath larger and presumably older cankers revealed the presence of what appeared to be trichomonad hydrogenosomes.
An attempt has been made to outline important events of caseous nodule formation in ring doves following their experimental infection with *Trichomonas gallinae*. By no means have all trichomonad activities during this period been completely examined, however, with the availability of immunocytochemical and biochemical techniques further data can be gathered.

**Literature Cited**


Fig. 1. Control. Micrograph shows the upper layers of the palatal mucosa. Microvilli-like apical processes (black arrowheads) are prevalent. Upper squamous cells appear to be undergoing non-pathologic desquamation. The lumen of the alimentary tract is indicated by the letter L. Scale bar = 1 μm.

Fig. 2. 24 hours PI. Section 1.5 μm thick from near the palato-esophageal junction. Trichomonads are visible in a monolayer at the epithelial surface and appear to be engaged in pathological desquamation. Slightly dilated blood vessels (BV) shows margination of blood cells. Scale bar = 30 μm.

Fig. 3. 24 hours PI. Trichomonad in association with squamous cell apical processes. Note the formation of pseudopodia and what appears to be an early phagocytic vesicle. What is believed to be another phagocytic vesicle (V) is present some distance from the epithelial surface. Scale bar = 1 μm.

Fig. 4. 24 hours PI. Trichomonads showing early phagocytic vesicles containing what appears to be squamous cell apical processes (black arrows) and later phagocytic vesicles (V). All phagocytic vesicles are membrane bound. Scale bar = 2 μm.

Fig. 5. 24 hours PI. Higher magnification of the trichomonad-epithelial interface. Squamous cell apical process is represented by the letter S. Area between black arrowheads indicates electron dense granulation. Black arrows indicate what appears to be portions of Golgi bodies. Scale bar = 1 μm.

Fig. 6. 24 hours PI. Parasites prepared for TEM after they were observed with SEM. This micrograph shows the high degree of vacuolation that can be present and how close parasites conform to the outline of host cells. Scale bar = 2 μm.
Fig. 7. 48 hours PI. Section 1.5 μm thick of the canker-epithelial interface. Note trichomonads on raft of squamous cells and spreading of intercellular spaces. The black arrowhead indicates a host leucocyte beneath the trichomonad monolayer. Scale bar = 30 μm.

Fig. 8. 48 hours PI. Many host leucocytes at the canker-epithelial interface appeared to be histiocytes. Scale bar = 2 μm.

Fig. 9. 48 hours PI. Trichomonads with longer pseudopodia can be seen on the surface of an affected squamous cell (S) whereas another parasite with a much broader pseudopod (Ps) has apparently worked between epithelial cells and is engulfing portions of deeper squamous cells. Scale bar = 1 μm.

Fig. 10. 48 hours PI. A degenerate squamous cell appears as if being removed from underlying squamous cells by what appears to be a trichomonad with long pseudopodia (black arrows). A host granulocyte is visible just beneath this parasite. Scale bar = 2 μm.

Fig. 11. 48 hours PI. Trichomonad monolayer at the epithelial surface with an underlying granulocyte (G). Tissue breakdown appears to be underway. The black arrowhead indicates a trichomonad costa whereas H indicates a hydrogenosome. Scale bar = 1 μm.
Fig. 12. 48 hours PI. Alterations to squamous epithelium with several trichomonads between overlying and underlying squamous cells (S). Scale bar = 2 μm.

Fig. 13. 48 hours PI. Additional trichomonads at the epithelial surface and underlying granulocytes. Scale bar = 1 μm.

Fig. 14. 96 hours PI. Epithelial basal lamina indicating what appears to be much collagen (Co) deposition. Scale bar = 2 μm.

Fig. 15. 96 hours PI. Epithelial basal lamina with adjacent capillary (black arrowheads) which contains an erythrocyte (E). What appears to be collagen (Co) is also evident. Scale bar = 2 μm.

Fig. 16. 168 hours PI. Canker-epithelial interface. One trichomonad (black arrow) is residing beneath a squamous cell whereas another one appears to have been destroyed (black arrowheads). Scale bar = 2 μm.

Fig. 17. 240 hours PI. Micrograph of the center of a caseous nodule. Cellular components were not identifiable, although some collagen was present (black arrowheads). Scale bar = 1 μm.
Fig. 18. 168 hours PI. Area directly beneath a canker showing two trichomonads, one which has lysed and appears as if it is being engulfed by a macrophage (M). Hydrogenosomes (H) appear to have survived cell lysis. Black arrows indicate the Golgi apparatus of trichomonads. The lower example appears to be forming vesicles near the parasite cell membrane. Scale bar = 1 μm.

Fig. 19. 240 hours PI. Area directly beneath an older canker. Hydrogenosomes (H) are the only recognizable trichomonad feature. One has a ruffled membrane while the other appears to have no membrane. Black arrows indicate what is believed to be collagen. Scale bar = 1 μm.
Fig. 20. 240 hours PI. Dilated capillary near an overlying canker. Endothelial cells (arrowheads) are visible. A break in the endothelium can be seen between the two black arrows as an erythrocyte (E) is escaping. A majority of the remaining cells within the capillary are granulocytes (G). A trichomonad (Tr) can be seen outside the capillary. Scale bar = 1 μm.
Published literature concerning **Trichomonas gallinae** is concerned with taxonomy, morphology and life cycles, host accounts, transmission studies, parasite culture and metabolic studies, biochemistry and pathology. It is apparent that little information is available in any of these areas with the exception of host accounts. Experimental work is limited and work concerning pathology of avian trichomoniasis is scant. Three of the papers in this dissertation deal with experimental avian trichomoniasis while ancillary studies of trichomonad culture, transmission and preparation for SEM are also presented.

Section I addressed questions concerning growth of **T. gallinae** in culture. It was shown that trichomonads grown in STS medium containing 10% fetal bovine serum and an added commercial growth supplement grew better than in medium containing either 10% or 5% bovine calf serum or newborn calf serum. Differences between calf and newborn serum were minor, as similar growth profiles were obtained with each.

Section II dealt with host-to-host transmission of **T. gallinae**. Transmission from parent ring doves to their offspring via crop milk was extremely efficient as was the passage from infected juveniles to other uninfected juveniles by a common water source. This appeared to be the first report of transmission through water, as in the past it was merely speculation.

Section III described an improved preparatory technique for trichomonads undergoing scanning electron microscopy. The technique was required as methods appearing in much of the literature were inadequate.
for my needs. A comparison of three preparatory techniques was made. Trichomonads fixed for 24 hours in glutaraldehyde and paraformaldehyde in phosphate buffer and subjected to secondary fixation (mordanting) in tannic acid and guanidine hydrochloride prior to osmication, allowed parasites to be air dried from freon 113 with only minor swelling or shrinkage artifacts. Tissues fixed with paraformaldehyde and glutaraldehyde in phosphate or cacodylate buffers and critical point dried revealed many more artifacts. It was suggested that artifacts are probably unavoidable in chemically fixed material, but that the use of this technique reduces artifacts to a more acceptable level.

Section IV was the first of three sections dealing with pathology of canker formation in experimentally infected ring doves. Lesions of the palate were examined with paraffin and plastic resin embedded tissues under light microscopy. Trichomonads were associated with pathological desquamation throughout all stages of the infection under study. Early cankers formed by 48 hours post infection and were associated with leucocytic infiltration, primarily heterophils and histiocytes, whereas older cankers had increased numbers of histiocytes. Areas beneath cankers were apparently sites of trichomonad destruction. Dilated blood vessels near the ulcerated surface showed migrating blood cells, some of which appeared to migrate through dilated intercellular spaces of the mucosa. Speculation as to why cankers become large necrotic masses was offered.

Section V addressed canker formation and progression with scanning electron microscopy. It was shown that trichomonads attacked microvilli-like apical processes and tightly attached cell borders of
palatal epithelium initially, and then "crawled" beneath squamous cells once loosened. This cycle of pathological desquamation continued throughout the study and became intensified. Trichomonads at times were associated with craterform depressions in the mucosa which were possibly caused by parasite secretions. Speculation concerning canker enlargement was offered as was speculation as to trichomonad activities beneath cankers. Trichomonads were not always present at canker-epithelial interfaces.

Section VI addressed canker formation and progression with transmission electron microscopy. Trichomonads again were initially involved with microvilli-like apical processes, as they appeared to phagocytose them, and pathological desquamation. Host cell damage appeared to be associated with granulation near parasite cell membranes. Structures resembling golgi bodies were seen near phagocytic cups. Areas beneath cankers were sites of trichomonad destruction as parasites were undergoing lysis and were in direct contact with histiocytes. Areas near the canker-epithelial interface revealed infiltration of granulocytes and histiocytes into areas possessing dilated epithelial intercellular spaces. Many granulocytes appeared to be undifferentiated polymorphonuclear leucocytes. Dilated blood vessels near ulcerated surfaces contained granulocytes primarily.

Sections IV, V and VI attempted to answer some basic questions concerning canker formation in experimentally infected ring doves. It should be apparent that much more work on this interesting host-parasite system needs to be done. It should also be apparent that the problems...
addressed in this dissertation and the knowledge gained from them that more is known about avian trichomoniasis and canker formation now than in the past. Perhaps with the use of biochemical and immunocytochemical techniques we shall know better this organism known as *Trichomonas gallinae*. 


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