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TRICHOMONAS GALLINAE IN AVIAN POPULATIONS IN URBAN TUCSON, ARIZONA

by

Charise Ann Hedlund

A Thesis Submitted to the Faculty of the

SCHOOL OF RENEWABLE NATURAL RESOURCES

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE
WITH A MAJOR IN WILDLIFE AND FISHERIES SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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STATEMENT BY AUTHOR

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ACKNOWLEDGMENTS

I wish to thank my committee: W. Shaw (advisor), R. W. Mannan, and T. H. Noon. Their advice and support throughout this study were essential.

I also wish to thank Jim Dawson for his advice and insights into trichomoniasis, both were invaluable to me during this study.

The following people helped me tremendously with this study: the entire staff at the Veterinary Diagnostic Laboratory in Tucson, AZ; David Bentley; Clint Boal; and all of the Tucson-area residents who donated their time and bird baths.

Funding for this study was provided by a grant from the Heritage Fund, administered by The Arizona Game and Fish Department.

I owe the greatest debt of all to Ardis Adams and Suzi Shoemaker, both of whom provided me with unending support and encouragement during this study. I could not have done it without them beside me.

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ABSTRACT

I studied Trichomonas gallinae, a flagellated protozoan that is the causative agent of the avian disease trichomoniasis. The purpose of my study was to assess (1) the incidence of trichomonads in wild birds, (2) the prevalence of trichomonads in water sources utilized by wild birds, and (3) possible methods to control the transmission of trichomonads in water sources utilized by wild birds. I trapped 403 birds during 1994 and 1995. Approximately 1/3 of these birds tested positive for T. gallinae, however, none exhibited any signs of lesions. I collected water samples from 10 bird baths, isolating flagellated protozoa from 2 of them. I could not identify the species of flagellated protozoa. I determined that high temperatures (50°C), near ultra-violet radiation, and natural sunlight are effective against trichomonads. In addition, the highest effective dilutions of Chlorox, Nolvasan, and distilled white vinegar active against trichomonads were determined.

INTRODUCTION

History

Trichomonas gallinae is a flagellated protozoan that belongs to the subphylum Sarcomastigophora, super class Mastigophorasica, class Zoomastigophorasida, order Trichomonadorida, and family Trichomonadidae (Petrak 1969, Tudor 1991). T. gallinae was isolated by Rivolta in 1878 (Stabler 1954) from cankers present in the upper digestive tract of a common pigeon (Columba livia). Rivolta initially identified the organism as a cercomonad: Cercomonas gallinae (Stabler 1938). Later, it was correctly identified as a trichomonad and renamed Trichomonas gallinae (Stabler 1938). T. gallinae is synonymous with T. columbae (Stabler 1954).

Hosts

Historically, trichomoniasis has been referred to as 'canker' or 'roup' in Columbiformes and 'frounce' in Falconiformes. Columbids, especially Mourning doves (Zenaida macroura) and common pigeons are the primary carriers of T. gallinae (Waller 1934; Cauthen 1936; Stabler 1938, 1941a; Harwood 1946; Stabler 1951). Trichomonads have also been isolated from Inca doves (Columbina Inca) (Locke and James 1962), White-Winged doves (Z. asiatica) (Locke and Kiel 1960, Stabler 1961) and Band-Tailed pigeons (Columba fasciata) (Sileo and Fitzhugh 1969).

Trichomoniasis has been reported in wild raptors in the United States including: nestling Peregrine Falcons (Falco

peregrinus) (Stabler 1941a), American Kestrels (F. sparverius) (Stone and Janes 1969), Grey hawks (Buteo nitidus) (Stensrude 1965), Bald eagles (Haliaeetus leucocephalus) (Stone and Nye 1981), and Golden eagles (Aquila chrysaetos) (Beecham and Kochert 1975). In Tucson, Arizona, investigators have reported trichomoniasis in nestling Harris' hawks (Parabuteo unicinctus) and Coopers' hawks (Accipiter cooperi) (Unpublished data from James Dawson and Clint Boal, School of Renewable Natural Resources, University of Arizona, Tucson).

Birds that have died after being experimentally infected with T. gallinae include: Red-Tailed hawks (B. jamaicensis) (Stabler 1941a), House sparrows (Passer domesticus), Bobwhite quail (Colinus spp.), and Song sparrows (Melospiza melodia) (Levine et al. 1941).

Lesions and Clinical Signs

T. gallinae is the causative agent in the avian disease trichomoniasis. Trichomoniasis is characterized by yellowish caseous lesions in the upper digestive region, particularly in the mouth, esophagus, and crop of infected birds. The sinuses may also be affected. Pharyngeal lesions are caused by erosion and ulceration of the epithelium (Kocan and Herman 1971, Honigberg 1978) and normally appear within 7 to 10 days (Stabler 1954). Lesions produced by trichomoniasis may resemble gross or macroscopic mucosal lesions produced by avian pox, vitamin A deficiency, candidiasis, or herpesvirus

infection (Locke, et al. 1960, Petrak 1982, Harrison and Harrison 1986, Hansen 1987, and Tudor 1991). Microscopic examination of wet mounts of lesions or cultures of material obtained from affected areas is necessary to differentiate between these disorders and trichomoniasis. Histopathologic examination may also be of value.

Clinical signs of trichomoniasis include labored breathing, weakness, and emaciation that result from lesions occluding the upper digestive region and make feeding or respiration difficult (Stabler 1954, Stabler et al. 1964, Davidson and Nettles 1988). More virulent strains may cause lesions to form in the liver, lungs, heart, pancreas, spleen, kidney, and bone marrow (Stabler 1947, Honigberg 1978). Trichomonads have never been detected in the intestinal tract (Stabler and Herman 1951, Honigberg 1978). In birds that have recovered from trichomoniasis, the palatal flap may show some necrosis (Stabler and Herman 1951, Kocan and Herman 1971).

Virulence

Although the expression of a disease is related to "physiological, nutritional, and immune factors of the host, researchers have demonstrated an inherent difference in the virulence potential between strains of T. gallinae" (Honigberg 1978:165). Strains of T. gallinae can range from avirulent, with no clinical signs, to extremely virulent,

causing fatal disease (Stabler 1948a, 1948b, 1954; Stabler et al. 1964; Honigberg et al. 1970; Honigberg 1978).

More than 1 strain may exist in a bird at any one time (Stabler and Kihara 1954, Honigberg 1978). Stabler (1948a) initially identified different strains based on the symptoms exhibited in pigeons that were experimentally inoculated with trichomonads. These trichomonads were obtained from birds with varying degrees of the disease. The most virulent strain (termed Jones' Barn strain), which killed 4 out of 5 pigeons, was collected from a cankerous wild squab (Stabler 1948a). Stabler and Kihara (1954) have demonstrated that only one trichomonad of Jones' Barn strain can produce an infection in pigeons.

Using quantitative fluorescent antibody methods and gel diffusion techniques, researchers have demonstrated that avirulent strains are richer in antigens than virulent strains; suggesting the possibility of a greater stimulation of antibodies (Goldman and Honigberg 1968, Honigberg and Goldman 1968, Stepkowski and Honigberg 1972). In studies involving mice, researchers have found that the more virulent strains tend to exhibit a faster growth rate and stimulate a more intense infiltration of macrophages (Honigberg 1961, Honigberg et al. 1964).

Immunity to a lethal strain may occur in birds that have recovered from a less virulent strain or harbor trichomonads from an avirulent strain (Stabler 1948b, 1951; Kocan 1970;

Kocan and Knisley 1970; Stabler 1975). According to Stabler (1954) and Honigberg (1978), the elimination of infection by trichomonads results in a gradual decrease in protection reflected by a parallel decrease in agglutinin titers. Trichomonads cultured *in vitro* will eventually become attenuated but virulence can be retained when trichomonads are stored in liquid nitrogen or are subsequently passed through successive pigeons (Stabler 1954, Goldman and Honigberg 1968, Honigberg and Goldman 1968, Stepkowski and Honigberg 1972, Honigberg 1978).

Epidemiology

Transmission of trichomoniasis occurs through pigeon crop milk (Stabler 1954, Levine 1961, Harrison and Harrison 1986), billing during courtship (Stabler 1954), and predation by raptors on infected birds (Levine 1961, Harrison and Harrison 1986). Stabler (1954) and Honigberg (1978) have shown that transmission through egg production and fecal debris is not possible. Trichomonads are able to survive in water and moist grain, and it has been suggested that contaminated water and seed sources may be significant in the transmission of trichomonads (Stabler 1954, Kocan 1969, Honigberg 1978). Contamination of water and seed sources may occur when an infected bird drops water and seed.

Morphology

T. gallinae is a eukaryotic organism with 6 chromosomes and longitudinal binary fission is the propagation method (Stabler 1941b). It is ellipsoid in shape with average dimensions of stained organisms being 8.3 x 4.5 μm (Abraham and Honigberg 1964) to 10.5 x 5.2 μm (Honigberg 1978). The basic structure (Figure 1) of T. gallinae includes a kinetosome complex, 4 anterior flagella, an axostyle, a pelta, a nucleus, a well developed undulating membrane, a costa, and both paraxostylar and paracostal granules that correspond to hydrogenosomes (Stabler 1954, Mattern et al. 1967).

The kinetosome complex lies in the anterior portion of the protozoan and includes 5 kinetosomes. The anterior flagella originate from kinetosomes 1 through 4 and the costa originates from the 5th kinetosome (Mattern et al. 1967, Honigberg 1978).

The 4 anterior flagella have an average stained length of 9.9μm, and appear to be in pairs of 2 (Stabler 1941b, 1954). The axostyle is a hyaline rod composed of microtubules (Abraham and Honigberg 1964, Mattern et al. 1967, Honigberg 1978). It originates as a flattened capitulum in the kinetosome complex and extends out posteriorly from the protozoan to form a tail-like tip. The axostyle is accompanied by 2 rows of paraxostylar granules (Abraham and Honigberg 1964, Honigberg 1978). The pelta,

also composed of microtubules, is a crescent-shaped organelle that originates near the anterior end of the capitulum and encircles the kinetosome complex (Abraham and Honigberg 1964, Mattern et al. 1967, Honigberg 1978).

The nucleus, approximately 4.6μm in length, (Stabler 1941b), is bounded by a double envelope and surrounded by rough endoplasmic reticulum. It has no cysts and contains a single nucleolus surrounded by a clear halo (Abraham and Honigberg 1964, Honigberg 1978).

The undulating membrane defines the dorsal side of the protozoan. The costa runs along the base of the undulating membrane and both originate in the kinetosome complex (Stabler 1954, Mattern et al. 1967). The costa is accompanied by 2 rows of paracostal granules that may vary in size (Abraham and Honigberg 1964, Honigberg 1978).

T. gallinae obtains energy by converting exogenous and endogenous carbohydrates into organic acids (Lindmark and Muller 1973). Hydrogenosomes, in the form of paracostal and paraxostylar granules, take the place of mitochondria in T. gallinae and in other trichomonads (Honigberg 1978). These respiratory organelles produce molecular hydrogen as a metabolic end product and can function under anaerobic and aerobic conditions (Lindmark and Muller 1973, Honigberg 1978, 1986).

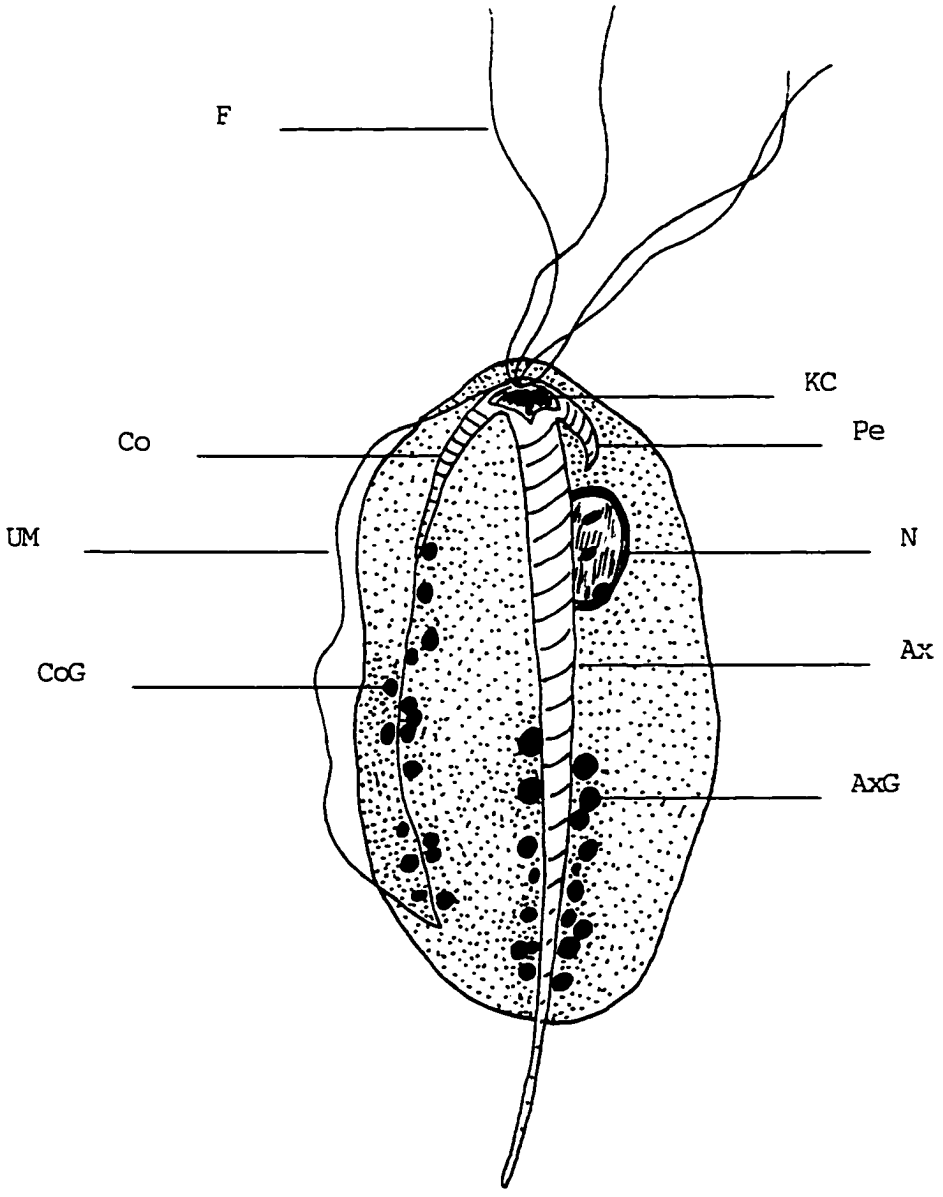

Figure 1. Schematic diagram of T. gallinae showing the anterior flagella (F), kinetosome complex (KC), pelta (Pe), nucleus (N), axostyle (Ax), costa (Co), and undulating membrane (UM). The axostyle and costa are accompanied by paraxostylar (AxG) and paracostal (CoG) granules.

Incidence

In Arizona, 32 of 42 White-Winged doves tested positive for T. gallinae (Toepfer et al. 1966) and Straus (1966) reported that at an urban Tucson location the incidence of T. gallinae in Mourning doves was 26%. In Colorado, Stabler (1951a) has recorded incidences of 69% and 23% of T. gallinae in pigeons and Mourning doves, respectively.

Infection by virulent strains of trichomonads can result in considerable mortality among certain bird species. During the 1950's a substantial number of Mourning dove deaths were reported over a large portion of the Southern United States (Stabler 1954). Haugen and Keeler (1952) estimated the number of dead Mourning doves in Alabama from 1950-1951 to be between 25 and 50 thousand individuals. In Hollywood, California in 1941, Stabler and Herman (1951) reported that trichomoniasis was the causative agent in the mortality of 200 Mourning doves.

Problem Statement

Each summer a few birds are found dead from trichomoniasis and occasionally there are epizootic events resulting in the mortality of hundreds to thousands of birds. Trichomoniasis is therefore a concern to both wildlife agencies, which are responsible for formulating management plans, and to wildlife enthusiasts who use bird baths and bird feeders and who do not want to contribute to the spread of this disease. Although alternative backyard feeding

methods have been suggested (Engel-Wilson 1991), scientific data on the prevalence of the disease and methods to reduce its spread were needed.

Objectives

There were 3 objectives to this project.

(1) To determine the incidence of T. gallinae in wild populations of columbids in the Tucson urban area.

(2) To assess the occurrence of T. gallinae in backyard bird baths in urban Tucson.

(3) To study the effectiveness of various types of treatments in controlling T. gallinae in water sources.

MATERIALS AND METHODS

Culture Techniques

To achieve my objectives, I needed a culture technique that would allow me to detect the presence or absence of motile trichomonads in samples collected during my study. I used InPouch TF culture medium packets (BIOMED Diagnostics) to culture all samples (Cover et al. 1994). The packets were kept at 4.5°C until needed, then allowed to warm up to room temperature (25°C). Details concerning how samples were obtained are provided in later sections. Cultures were placed in an incubator maintained at 37°C and specimens were checked for motile trichomonads at 24 to 48-hour intervals using a light microscope. Cultures were incubated for a period of 10 days and if trichomonads were not detected I

recorded the cultures as being negative. Cultures positive for motile T. gallinae were subcultured after 3 days by centrifuging the sample and using a disposable pipette to transfer the pellet into fresh medium. All centrifugation was done with a Beckman centrifuge, model TJ-6 (head diameter of 13in.), using the following settings: speed at 4.4, brake in off position, and time set at 7.5 minutes, resulting in a spin down time of 2.5 minutes and a maximum RPM of 1400.

Storage

In addition to a culture technique, I needed a technique that would allow me to store trichomonads safely until needed at a later date. Cultures of trichomonads were stored in liquid nitrogen (-196°C) as outlined by Diamond et al. (1963). Cultures were centrifuged and the resultant pellets were transferred to 1.8ml screw-cap vials (McEntegart 1954, Diamond et al. 1963). Each inoculum was then re-suspended in fresh medium until the volume of both combined (trichomonad inoculum and fresh medium) was approximately 1.7ml. Approximately 0.1ml of dimethyl sulfoxide (DMSO) (Appendix A, Table 1) was added to each vial as a cryoprotectant, creating an approximate concentration of 5% DMSO in each vial (Lovelock and Bishop 1959; Diamond et al. 1961, 1963; Honigberg 1978; Warton and Honigberg 1979). The vials were then placed upright in a container that was partially filled with 90-95% ethyl alcohol, enough to immerse the lower 1/4 of the vials, and placed in a freezer for 24-48 hours. The

specimens were then placed in liquid nitrogen. The specimens were thawed by slowly agitating the vials manually in a 40-45°C water bath until contents were completely liquefied (5-10 minutes) (McEntegart 1954; Diamond et al. 1961, 1963). The vials were then centrifuged and the resultant pellet was placed into a fresh InPouch TF culture packet and incubated according to the standard procedure described previously in Culture Techniques.

Objective #1: Incidence

I studied the incidence of T. gallinae in wild birds in the Tucson metropolitan area in Pima County, Arizona. I trapped birds from May through August in 1994 and 1995, reported to be the peak time of trichomoniasis outbreaks in other areas (Stabler and Herman 1951, Haugen 1952, Haugen and Keeler 1952, Straus 1966). In addition to trapping, several necropsies were performed on dead birds found in my study area.

I trapped birds at 6 different locations (Appendix B): 3 were representative of urban or suburban areas with a housing density of $\geq$ 1 house per acre, 2 were representative of rural areas with a housing density of $\leq$ 1 house per 3 acres, and one was on a golf course.

To assess the prevalence of trichomoniasis I trapped 403 wild birds using funnel traps (Straus 1966, Hawthorne 1980) and sparrow traps. Wild columbids composed the majority of birds trapped, but other species were included. Traps were

set in the early morning and taken in by 9 am, to avoid exposing the birds to excessive heat. Traps were continuously monitored and birds were removed from the traps within 20 minutes of being caught.

To prevent movement during examination, each bird was placed in a sock with only the head exposed. I examined each bird for lesions and collected a mucus sample from the crop and throat area using a cotton swab. Cotton swabs were pre-moistened in a 0.9% sterile saline solution (Appendix A, Table 1). I also examined the keel (breastbone) on each bird to determine its physical condition. A prominent keel on a bird is an indication of inadequate nutritional intake. Prior to release, each bird was marked on the lower left leg with a permanent felt-tip marker to ensure birds were not re-swabbed during each trapping period. The mucus sample was transferred to, and cultured in, InPouch TF culture packets. The presence of visible or palpable caseous lesions in the mouth, throat, or crop area was considered an indication of active disease. InPouch TF cultures were determined to be positive if motile trichomonads were visible by microscopic examination.

Necropsies. In addition to information obtained from trapping, several necropsies on birds found dead were performed over the summers of 1994 and 1995 at the University of Arizona's Veterinary Diagnostic Laboratory for determination of the cause of death. Specimens were

submitted by Tucson-area residents and Arizona Game and Fish Department personnel.

Objective #2: Water Sources

Isolation Procedures. To accomplish objective #2, I needed to first determine if T. gallinae could be detected in a water source. I evaluated 2 methods of isolating trichomonads from water. I used water samples collected from the watering dish of 4 captive pigeons, which were determined by throat cultures to be carriers of T. gallinae. Each sample consisted of 900 to 1000 ml of water. I inoculated these samples with various amounts (1.75ml, 1.5ml, and a drop from a 4ml disposable pipette) of InPouch TF culture medium containing motile trichomonads that had been incubated at 37°C for 24 to 48 hours. The methods for isolating trichomonads from water are given below.

Step 1. The water sample containing motile trichomonads was poured slowly into a Buchner funnel fitted with a 7cm diameter, 25µm pore-size filter. This size filter was used to remove the larger debris from the water while allowing trichomonads to pass through. The sample was filtered under low vacuum (15-20 in.Hg) and collected in a 1-liter Erlenmeyer flask. When the flask was full or the water sample was depleted I used one of two methods to obtain an inoculum for culture.

Step 2: Method 1. The collected filtrate from Step 1 was drawn through a plastic tube into a filter holder fitted with a 47mm diameter, 3μm pore-size filter. This size filter paper was used to trap trichomonads as the water passed through the filter. The sample was filtered under low vacuum (15-20 in.Hg) and collected in a 1-liter Erlenmeyer flask. When the flask was full, the water sample depleted, or the filter became clogged the filter paper was removed and placed in InPouch TF culture medium. If more of the water sample remained, a new filter paper was put in place and the process continued until the sample was depleted.

Step 2: Method 2. The collected filtrate from Step 1 was divided into 4, 50ml vials and centrifuged. The resultant pellets were removed and cultured in InPouch TF culture medium. If more of the water sample remained the process was repeated until the sample was depleted.

I repeated the procedures for both methods 3 times.

Bird Baths. To determine if T. gallinae is naturally present in water sources utilized by birds, I studied 10 bird baths in the Tucson metropolitan area in Pima County, Arizona from July, 1995 through August, 1995. I collected water samples from bird baths at households that provide food and water for birds. At each location, water samples were collected each day for a period of 7 consecutive days. During sampling periods either myself or homeowners, at each

sampling location, refilled bird baths after each water sample was collected. Homeowners were asked to refrain from adding water or cleaning bird baths between collection of water samples.

The sampling locations (Appendix B, Table 2) were in areas with housing densities that ranged from $\leq$ 1 house per 3.3 acres to 1 house per acre. These sites were selected, at random, from 400 households that responded to a notice requesting volunteer cooperation with this study. This notice was printed in newsletters of local bird supply stores, Tucson Audubon Society's newsletter "Flycatcher", and a local newspaper, "The Arizona Daily Star" (April 12, 1994).

Several types of bird baths were included in the study (Appendix B, Table 2). For reference purposes, I included a water source used by 4 captive pigeons (Location #1), known to be carriers of T. gallinae as established by prior throat cultures. All samples were collected between 8am and 11am, while water temperatures were still within a range tolerable to trichomonads (Honigberg 1978).

The entire contents of water from each bird bath was siphoned into a disposable plastic bag and transported to the laboratory in a Coleman 2-gallon Igloo container. For water sources containing in excess of 2 gallons of water, samples were siphoned from the lowest part of each source and only a total of 2 gallons were collected. Water samples were

siphoned from the lowest part of each water source because trichomonads tend to settle downward in a liquid medium (Cover et al. 1994).

In the laboratory I collected filtrate material for culturing using the techniques previously discussed in Objective #2: Isolation Procedures Steps 1 and 2, with the exception that water samples were not inoculated with trichomonads. Step #2, the second method (obtaining a final culture inoculum using centrifugation), was used when water samples contained large amounts of suspended particles that greatly hindered the filtering process. This was determined by how quickly the filter became clogged during the filtering process in Step #1. InPouch cultures created from Steps 1 through 2 were examined microscopically at 24-hour intervals for presence or absence of motile, flagellated protozoa.

Objective #3: Treatments to Control Trichomonads in Water

To fulfill this objective, I evaluated the effects that different treatments had on the survival of T. gallinae. The treatments used were: (1) exposure of T. gallinae to various temperatures, (2) exposure of T. gallinae to near-ultraviolet radiation and natural sunlight, and (3) exposure of T. gallinae to various commercially available chemicals. I based these treatments on environmental conditions that were most likely to affect the survival of trichomonads in bird baths and my experience with homeowners who added

disinfectants to water sources that they provided for wild birds.

I tested these treatments using (1) a 0.9% sterile saline solution, (2) Tucson municipal water derived from Central Arizona Project (CAP) water, and (3) Tucson municipal water derived from ground water, all of which were inoculated with 0.25ml (selected arbitrarily) of InPouch TF culture medium containing motile trichomonads. The water types differed in hardness, salinity, and pH (Appendix B, Table 3). Saline solution was used as a reference medium, while CAP and ground water were included to represent the sources of domestic drinking water available to Tucson residents at the start of my study.

Trichomonad inocula to be used in the different treatments (described above) were obtained by the culture of a throat swab collected from a captive pigeon, known to be a carrier of T. gallinae. The culture was incubated for 48 hours in InPouch TF medium. The contents of the InPouch TF culture packet were then transferred from the culture pouch to a 15ml vial. The vial was slowly inverted 10 times to provide a uniform distribution of trichomonads. Each treatment was inoculated with aliquot of 0.25ml of InPouch TF culture medium. Each 0.25ml aliquot contained an average of 1.34×10^7 trichomonads (standard deviation = 1.10×10^6). This was determined by averaging a total of 20 different counts made using a hemacytometer and done over a period of a

month. Aliquots used in each count were obtained from 1 of 3 cultures inoculated from throat swabs (described on previous page) collected on random, separate, non-successive days and incubated for 48 hours prior to use.

Each treatment was evaluated for its' effectiveness against T. gallinae as determined by the presence or absence of motile trichomonads in InPouch TF cultures. A trichomonad was considered motile when I could detect progressive movement in a forward direction when examining the culture using a light microscope. I included a positive control with each experiment to ensure trichomonads were surviving under non-treatment conditions. This was a sample maintained at 24°C and away from light sources.

Air and water temperatures were monitored during each treatment using mercury thermometers.

Treatment 1a: Exposure to 24°C for 10 Hours. To assess the effects of different temperatures, I first wanted to determine if trichomonads could survive for a prolonged period (10 hours) at room temperature (24°C) in media other than culture medium. Five, 15ml vials of CAP water and 5, 15ml vials of ground water were each inoculated with 0.25ml of InPouch TF culture medium containing motile trichomonads. The vials were placed in a Styrofoam container and a pair of vials, one containing CAP water with trichomonad inoculum and one containing ground water with trichomonad inoculum, was removed and examined every 2 hours until the end of the

experiment. Test containers were centrifuged and the resultant pellet was used to determine the survival of trichomonads by 1) observation of motile organisms on a wet mount slide and 2) observation of motile organisms after a 48-hour incubation period in InPouch TF culture medium.

I did not use saline solution because I had already observed, using a wet mount slide preparation, motile trichomonads that had been kept in a vial of 0.9% sterile saline solution for 24 hours at ambient laboratory temperatures. This observation was made after I neglected to dispose of a vial of trichomonads in saline solution and was not part of any formal experimental design. Temperatures in the laboratory from 5pm to 8am were mainatined within a few degrees of 24°C (K. Pruitt, Univ. of Ariz., pers. commun.).

Treatment 1b: Exposure to Different Temperatures. To assess the effect different temperatures had on T. gallinae, I exposed trichomonads to temperatures of 4.5°C, 10°C, 15°C, 30°C, 35°C, 40°C, 45°C, and 50°C. This experiment was modeled after Honigberg's (1978) study of survival of trichomonads in tap water. I did not exceed 50°C because the thermal death point of T. gallinae has been shown to be 48-49°C (Matthews and Daly 1974, Andrews 1926). I omitted 20 and 25°C because I felt this would be a repeat of the 10-hour test described in Treatment 1a: Exposure to 24°C for 10 Hours.

For each temperature I inoculated 1, 15ml vial of 0.9% sterile saline solution; 1, 15ml vial of CAP water; and 1, 15ml vial of ground water with 0.25ml of InPouch TF culture medium containing motile trichomonads. Vials were then placed in a pre-heated water bath (for temperatures $\geq$ 10°C) or a refrigerator (for temperature = 4.5°C) for 2.5 hours (Honigberg 1978). Next, vials were centrifuged and the resultant pellet was used to determine the survival of trichomonads by 1) observation of motile organisms on a wet mount slide and 2) observation of motile organisms after a 48-hour culture in InPouch TF medium. I repeated the experiment 3 times per temperature.

Treatment 2a: Exposure to Artificial Near Ultraviolet Radiation. I was interested in the effects of near-ultraviolet (UV) radiation, delineated by wavelengths ranging from 3000 - 3900A. A majority of the UV radiation reaching the earth falls within this range. Potentially more detrimental to living organisms than near-UV radiation is far-UV radiation (2000 - 3000A), a certain amount of which reaches the surface of the earth (Geise 1967). Although near-UV radiation is not absorbed by the nucleus or cytoplasm to the extent that far-UV radiation is, it may be detrimental to organisms after prolonged exposure (Geise 1967).

The objective of this treatment was to assess the effect near-UV radiation has on T. gallinae maintained in a 0.9% sterile saline solution when varying (1) saline depth, (2)

exposure time, and (3) cover. I used a General Electric BLB lamp that emits 97% of its radiation in the 3000-4000A range, produces a negligible amount of heat, and is comparable to the near-UV radiation emitted by the sun (Jagger 1967). Depths of saline used were 1cm, 3cm, 5cm, 8cm, 11cm, and 13cm. These depths were selected to represent the most common bird bath depths I had observed in use at my sampling locations during the previous summer. Length of exposure times were 30, 60, 240, and 480 minutes. The exposure times were chosen to assess the short- and long-term effects of near-UV radiation and natural sunlight on trichomonads. The diameter of the test containers was 6.25cm $\pm$ 1.25cm.

For each depth, 4 plastic containers of 0.9% sterile saline solution were inoculated with 0.25ml of InPouch TF culture medium containing motile trichomonads incubated for 48 hours. I then placed the 4 containers, uncovered, 2.5cm from the artificial near-UV light source. One container was removed from the light source and the temperature of the saline solution recorded at the end of each of the 4 exposure periods. The contents were transferred to vials and centrifuged. The resultant pellet was transferred to and incubated in InPouch TF culture medium. Cultures were checked at 24-hour intervals and survival of trichomonads was determined by observation of motile organisms visible by microscopic examination. I repeated the experiment 3 times per depth.

Shading by debris and algae in a water source may provide some amount of protection from UV radiation and was mimicked in the laboratory experiments by using opaque covers. In my initial experiments, described on the previous page, the containers were uncovered. In order to simulate this shading effect I repeated those initial experiments that resulted in negative cultures for motile trichomonads, using fresh inocula and with 1/2 of each container covered with a plastic opaque cover.

Treatment 2b: Exposure to Natural Sunlight. The objective of this treatment was to assess the effect natural sunlight has on T. gallinae maintained in a 0.9% sterile saline solution when varying (1) saline depth and (2) exposure time. To fulfill this objective I repeated the laboratory experiments, described in Treatment 2a: Exposure to Artificial Near Ultraviolet Radiation, outside using sunlight instead of a UV lamp.

For each depth, 3 plastic containers of 0.9% sterile saline solution were inoculated with 0.25ml of InPouch TF culture medium containing motile trichomonads. I then placed the containers in direct sunlight, uncovered, from 7:30am to 3:30pm. One container was removed and the temperature recorded at the end of each of the following exposure times: 60, 240, and 480 minutes. I omitted the 30-minute period because trichomonads had survived at least until the 60-minute time period when exposed to UV light from the BLB

lamp. The contents were transferred to vials and centrifuged. The resultant pellet was transferred to and incubated in InPouch TF culture medium. Cultures were checked at 24-hour intervals and survival of trichomonads was determined by observation of motile organisms visible by microscopic examination. I repeated the experiment 3 times for each depth.

Treatment 3: Exposure to Different Chemicals - Introduction. I examined the effectiveness of different commercially available chemicals against T. gallinae (information concerning active ingredients and manufacturers is given in Appendix A, Table 1): Chlorox, Nolvasan, distilled white vinegar, and Potable Aqua iodine tablets. The Potable Aqua iodine tablets are germicidal tablets intended for emergency disinfection of water for human consumption.

My objectives were (1) to determine the highest effective dilution (HED) of Chlorox, Nolvasan, and distilled white vinegar active against T. gallinae after a 30-minute exposure and (2) to assess the effectiveness of Potable Aqua iodine tablets against T. gallinae after a 30-minute exposure. I am defining highest effective dilution (HED) as the highest dilution lethal to trichomonads, i.e. resulting in cultures negative for motile trichomonads.

Treatment 3: Exposure to Different Chemicals - Objective 1. To determine the HED for each chemical

(Chlorox, Nolvasan, and distilled white vinegar) 1, 15ml vial of 0.9% sterile saline solution; 1, 15ml vial of CAP water; and 1, 15ml vial of ground water were inoculated with 0.25ml of InPouch TF culture medium containing motile trichomonads incubated for 48 hours. Next, I added the chemical (undiluted) to each vial using a microliter pipette. The amount of chemical added to each vial was calculated by dividing 15ml (the total amount of solution in each vial) by the desired dilution. For example, for a final dilution of 1:100 of Chlorox in a 15ml vial, I would add 0.15ml of undiluted Chlorox to 14.85ml of solution (0.9% saline solution, CAP water, or ground water) containing trichomonad inoculum. Initial dilutions for each chemical (Appendix C) were selected arbitrarily and subsequent dilutions were tested until the highest effective dilution was obtained.

The vials containing one of the 3 different types of solution (0.9% sterile saline solution, CAP water, or ground water), trichomonad inoculum, and the calculated amount of chemical were then placed in a 25°C water bath for 30 minutes (Matthews and Daly 1974). Next, vials were centrifuged and the resultant pellet was transferred to and incubated in InPouch TF culture medium. Cultures were checked at 24-hour intervals and survival of trichomonads was determined by observation of motile organisms by microscopic examination. For each chemical, I repeated the experiment 3 times per HED.

Treatment 3: Exposure to Different Chemicals - Objective 2. Product information for Potable Aqua iodine tablets recommends 1-2 tablets per liter of solution. Using 2 tablets per liter of solution, the product is reported to be effective in preventing infection in humans by Giardia lamblia, a protozoan parasite of mammals (Davidson and Nettles 1988). To determine its effectiveness against T. gallinae I mixed 2 tablets with 1 liter of (1) 0.9% sterile saline solution, (2) CAP water, and (3) ground water. One 15ml vial of each mixture was inoculated with 0.25ml of InPouch TF culture medium containing motile trichomonads incubated for 48 hours. Vials were then placed in a 25°C water bath for 30 minutes (Matthews and Daly 1974). Next, vials were centrifuged and the resultant pellet was transferred to and incubated in InPouch TF culture medium. Cultures were checked at 24-hour intervals and survival of trichomonads was determined by observation of motile organisms visible by microscopic examination. I repeated the experiment 3 times per water type.

RESULTS

Objective #1: Incidence

I trapped a total of 403 birds during the summers of 1994 and 1995 (Table 1). Some individuals were difficult to classify as an adult or immature and were classified as unknowns. Although approximately 1/3 of all birds tested

positive for trichomonads, none exhibited any lesions. To ascertain physical condition, I palpated the keel of each bird to determine if it was prominent or not. Analysis of data obtained from this examination indicated that there was no correlation between presence of a prominent keel and presence of trichomonads (Table 2) ($N = 403$, $\chi^2 = 0.29$, $\alpha = .05$).

Columbids constituted the majority of species trapped. Mourning doves were trapped with the highest frequency, but had the lowest incidence of trichomonads (Table 3, Table 4). Two Mourning doves were classified as being of unknown age. There was no significant difference in the incidence of trichomonads between adults and immatures ($N = 141$, $\chi^2 = 1.37$, $\alpha = .05$). Inca doves numbered the second highest, with approximately half testing positive for trichomonads (Table 3, Table 4). Two Inca doves were classified as being of unknown age. White-Winged doves numbered the lowest but nearly all were positive for trichomonads (Table 3, Table 4). There was no significant difference in the incidence of trichomonads between adults and immatures ($N = 52$, $\chi^2 = 3.8$, $\alpha = .05$).

I also isolated T. gallinae from adult House Finches, but the incidence was low (Table 3, Table 4). The remainder of birds trapped were composed of a variety of different species, none of which tested positive for T. gallinae (Table 5).

Necropsies. A total of 12 necropsies were performed on birds that were found dead in the study area. Nine of the birds examined, 8 House Finches and 1 House Sparrow, had mucosal lesions consistent with trichomoniasis and cultures of swabs taken from these lesions were positive for T. gallinae. The mucosal lesions were found exclusively in the upper digestive region, i.e. mouth, esophagus, and crop, of each bird.

Gross and histopathologic examination of the remaining birds found dead in my study area (2 House Finches and 1 pigeon) showed no evidence of trichomonad-type lesions and cultures of swabs taken from mucosal regions of the upper digestive region of each bird were negative for T. gallinae. The cause of death for each bird was undetermined.

Table 1. Percent of cultures positive for motile T. gallinae. Cultures were obtained from throat swabs taken from wild birds trapped in 1994 and 1995. "Total" includes individuals classified as being of unknown age.

	1994		1995		Total	
	N	Percent Positive	N	Percent Positive	N	Percent Positive
Total	258	31.4	145	32.4	403	31.8
Adult	157	36.9	106	36.8	263	36.9
Immature	90	21.1	27	29.6	117	23.1

Table 2. Results obtained from (1) cultures of throat swabs taken from wild birds trapped and (2) examination of each bird's keel. "Positive" = culture positive for motile trichomonads. "Negative" = culture negative for motile trichomonads.

	Prominent Keel	
Culture Results	**Yes**	**No**
Positive	32	96
Negative	62	213

Table 3. Percent of cultures positive for motile T. gallinae. Cultures were obtained from throat swabs taken from columbids and House Finches trapped during 1994 and 1995. Numbers in bold are the total number trapped, including individuals classified as being of unknown age.

	1994		1995	
Species	**N**	**Percent Positive**	**N**	**Percent Positive**
Mourning Dove	**128**	**13.3**	**15**	**20.0**
Adult	51	9.8	11	9.1
Immature	75	14.7	4	50.0
Inca Dove	**83**	**47.0**	**25**	**60.0**
Adult	75	46.7	23	60.9
Immature	6	50.0	2	50.0
White-Winged Dove	**22**	**100.0**	**30**	**96.7**
Adult	17	100.0	24	100.0
Immature	5	100.0	6	83.3
House Finch	**11**	**27.3**	**35**	**0.0**

Table 4. Percent of cultures positive for motile T. gallinae. Results were calculated using combined trapping data from 1994 and 1995. Cultures were obtained from throat swabs taken from columbids and House Finches. Numbers in bold in the second column are the total number trapped, including individuals classified as being of unknown age.

Species	N	Percent Positive
Mourning Dove	**143**	**14.0**
Adult	62	9.7
Immature	79	16.5
Inca Dove	**108**	**50.0**
Adult	98	50.0
Immature	8	50.0
White-Winged Dove	**52**	**98.1**
Adult	41	100.0
Immature	11	90.9
House Finch	**46**	**6.5**

Table 5. Number of other species trapped in 1994 and 1995. All cultures obtained from throat swabs from these birds were negative for motile T. gallinae.

Species	N
House Sparrow (Passer domesticus)	22
Gambel's Quail (Callipepla gambelii)	20
Bronzed Cowbird (Molothrus aeneus)	1
Cactus Wren (Campylorhynchus brunneicapillus)	3
Curved-billed Thrasher (Toxostoma curvirostre)	3
Northern Cardinal (Cardinalis cardinalis)	2
Pyrrhuloxia (Cardinalis sinuatus)	1
Pigeon (Columba livia)	2

Objective #2: Water Sources

Isolation Procedures. The 7cm diameter, 25μm pore-size filter used in Step #1 was effective in removing macroscopic suspended particles while allowing trichomonads to pass through. Both methods (Method #1 and Method #2) used in Step #2 to isolate trichomonads from water were equally successful. All 3 replicates of each method resulted in cultures positive for motile T. gallinae.

Bird Baths. I isolated flagellated protozoa in cultures from 2 of the 10 bird baths examined. In both of these, samples were collected and cultured on each of 7 consecutive days and the culture from 1 day of the 7 was positive for flagellated protozoa. One of the water sources that cultured positive for flagellated protozoa was utilized by the 4 captive pigeons known to be carriers of T. gallinae (Appendix B, Table 2 - Location #1). The other, Location #3, (Appendix B, Table 2) was a static water source. The water in each bird bath examined varied with respect to amount, temperature, and depth of water (Table 6). In addition, algae were visible in 5 of the bird baths. Algae observations were cursory and not part of any formal experiment.

I was unable to positively identify the species of protozoa isolated from the 2 bird baths. I could, however, determine that they were flagellated and had characteristics consistent with trichomonads: i.e. flagella numbered from 4

to 5, and an axostyle and a nucleus were present. This method, therefore, should be considered as a possible but not a positive method for detecting trichomonads. The ability of the isolated protozoa to infect birds was not determined.

Objective #3: Treatments to Control Trichomonads in Water

Treatment 1a: Exposure to 24°C for 10 Hours. Trichomonads survived for 10 hours while being maintained at 24°C in CAP and ground water. In addition, cultures made at 2-hour intervals were positive for motile trichomonads. Activity of the trichomonads, when viewed microscopically using wet mount preparations, began to change after 4 hours; trichomonads appeared more sluggish and moved in a circular pattern. This became increasingly pronounced and by the end of the experiment, many of the trichomonads had only flagella in motion without any forward or circular movement. When these were cultured for 48 hours in InPouch TF culture medium, however, I observed motile trichomonads, i.e. trichomonads moved with progressive forward motion. I observed no difference in the survival of trichomonads maintained in CAP water versus those maintained in ground water.

Treatment 1b: Exposure to Different Temperatures. Trichomonads maintained at different temperatures for 2.5 hours did not appear to be affected by temperatures $\leq$ 40°C (Table 7). This data is consistent with results reported by

Honigberg (1978). Trichomonads, when viewed microscopically using wet mount preparations, showed some decrease in motility after exposure to each temperature; i.e. some organisms moved only in a circular pattern or displayed movement of flagella alone without any progressive forward movement. When these were cultured for 48 hours in InPouch TF culture medium, however, I observed motile trichomonads, i.e. trichomonads moved with progressive forward motion. The thermal death point in my study was 50°C; at this temperature all cultures were negative for motile trichomonads. Water type (CAP versus ground water) seemed to have no effect on trichomonad survival regardless of the temperature.

Table 6. Average amount of water collected from each bird bath, average depth of water recorded prior to sample collection, and average water temperature. Results were averaged over the 7 day sampling period.

Location	Average Water Sample (ml)	Average Depth (cm)	Average Temperature (°C)
1	807.1 ± 77.3	1.9 ± 0.34	24.7 ± 1.5
2	2328.6 ± 1103.0	2.7 ± 0.76	29.4 ± 3.9
3	4200.0 ± 951.75	3.9 ± 0.89	30.7 ± 3.6
4	2292.9 ± 183.55	4.5 ± 0.65	21.7 ± 1.25
5	871.4 ± 288.5	2.1 ± 0.67	30.1 ± 1.2
6	1978.6 ± 205.9	4.1 ± 0.34	28.3 ± 1.8
7	2557.1 ± 316.8	2.6 ± 0.94	25.4 ± 1.0
8	2157.1 ± 386.5	2.4 ± 0.69	33.6 ± 1.5
9	1242.9 ± 566.0	1.5 ± 0.64	35.4 ± 1.0
10	3500.0 ± 675.8	3.5 ± 0.5	26.6 ± 1.3

Table 7. Number of trials[2] positive for motile T. gallinae. Trichomonads were exposed to each temperature for 2.5 hours. WM = wet mount, C = culture.

	Saline		CAP		Ground	
Temperature (°C)	**WM**	**C**	**WM**	**C**	**WM**	**C**
4.5°C	3	3	2	3	2	3
10°C	3	3	2	3	1	3
15°C	3	3	3	3	3	3
30°C	3	3	3	3	3	3
35°C	2	3	3	3	3	3
40°C	1	3	2	3	2	3
45°C	1	3	0	3	0	2
50°C	0	0	0	0	0	0

[2] Of a total of 3 per temperature and water type.

Treatment 2a: Exposure to Artificial Near Ultra-Violet Radiation. Exposure to near-UV radiation was effective against trichomonads in a 0.9% sterile saline solution when exposure was prolonged (≥ 240 minutes) and containers were not covered (Table 8). Trichomonads in depths greater than 8cm survived longer than those in shallower depths, but failed to survive until the end of the experiment (480 minutes). The temperatures of the saline solution, recorded at the end of each trial, were between 24°C-25°C.

Addition of a 1/2 cover to the containers resulted in trichomonads at depths ≤ 8cm surviving for a longer period of time (240 minutes) (Table 8). Results from trichomonads exposed to near-UV radiation for 480 minutes did not appear to follow any particular pattern with respect to depth of saline and exposure time. The temperatures of the saline solution, recorded at the end of each trial, were between 24°C-25°C.

Treatment 2b: Exposure to Natural Sunlight. Natural sunlight was effective against trichomonads over a prolonged exposure period (≥ 240 minutes), regardless of saline depth (Table 9). The exception to this was the survival of trichomonads from one trial (Depth = 11cm, 240 minutes). The average temperatures recorded at the end of each trial, were within a range tolerable (< 45°C) to trichomonads (Table 10).

Table 8. Number of trials[3] resulting in cultures positive for motile T. gallinae after exposure to near-UV radiation. Containers of trichomonads maintained in 0.9% sterile saline solution were provided: (1) no cover or (2) 1/2 cover.

	Exposure period (minutes)					
	no cover				1/2 cover	
Depth (cm)	30	60	240	480	240	480
1	3	3	0	0	3	3
3	3	3	0	0	3	1
5	3	3	0	0	3	2
8	3	3	0	0	3	3
11	3	3	3	0	-	0
13	3	3	3	0	-	1

[3] Of a total of 3 per depth and exposure period.

Table 9. Number of trials[4] resulting in cultures positive for motile T. gallinae after exposure to full sunlight. Solution = 0.9% sterile saline.

Depth (cm)	Exposure period (min)		
	60	240	480
1	3	0	0
3	3	0	0
5	3	0	0
8	3	0	0
11	3	1	0
13	3	0	0

[4] Of a total of 3 per depth and exposure period

Table 10. Temperatures of 0.9% sterile saline solution exposed to natural sunlight measured at the end of each time period.

Trial	60 min	240 min	480 min
1	28°C	37°C	39°C
2	26°C	36°C	37°C
3	27°C	37°C	39°C
Average	27°C	36.7°C	38.3°C

Treatment 3: Exposure to Different Chemicals - Objective 1. As described previously, I am defining the highest effective dilution (HED) as the highest dilution lethal to trichomonads, i.e. resulting in cultures negative for motile T. gallinae. Chlorox, Nolvasan, and distilled white vinegar were all effective against trichomonads (Table 11). Chlorox was the most effective and distilled white vinegar was the least effective (See Appendix A, Table 1 for active ingredients).

The HED's of Chlorox, Nolvasan, and distilled white vinegar effective against trichomonads differed when CAP and ground water were substituted for the 0.9% sterile saline solution. Using saline plus chemical as a reference, the HED for Chlorox decreased in both CAP and ground water indicating that more Chlorox was required. The HED for Nolvasan decreased for CAP water and increased with ground water. The HED increased for distilled white vinegar in CAP and ground water.

Treatment 3: Exposure to Different Chemicals - Objective 2. Two tablets of Potable Aqua iodine tablets per liter of solution (See Appendix A, Table 1 for active ingredient) were ineffective against trichomonads, regardless of whether trichomonads were maintained in 0.9% sterile saline solution, CAP water, or ground water. All of the cultures were positive for motile trichomonads.

Table 11. The highest effective dilutions (**HED**) lethal to T. gallinae (in 0.9% sterile saline, CAP water, and ground water) when exposed to different commercially available chemicals. Exposure time = 30 minutes.

	HED's		
	Saline	**CAP**	**Ground**
Chlorox	1:3000	1:2500[5]	1:2500
Nolvasan	1:700	1:300	1:1000
Distilled White Vinegar	1:5	1:15	1:15

[5] Dilution is result of 2 of 3 trials

DISCUSSION

Objective #1: Incidence

In fulfilling this objective I examined primarily Columbiformes, therefore my discussion will focus on the incidence of T. gallinae in these species.

Columbids. There is an important distinction between the incidence of trichomoniasis due to T. gallinae and the incidence or presence of T. gallinae. In birds with trichomoniasis there will be lesions present. Carriers of T. gallinae, however, will test positive for this organism but there will be no evidence of active disease. There is a considerable amount of literature on T. gallinae that indicates that columbids are the main reservoir of the organism. Approximately 1/3 of the columbids trapped and examined during my study tested positive for T. gallinae but none of the birds exhibited any signs of trichomoniasis. Results from my study are consistent with those reported by other researchers regarding (1) the incidence of T. gallinae in Mourning doves and White-winged doves and (2) the absence of lesions associated with trichomoniasis in Mourning doves and White-winged doves (Stabler 1951a, 1961; Straus 1966; Sileo 1970; Ostrand et al. 1995).

Columbids may be the main reservoir of T. gallinae because both parents feed their young regurgitated crop milk, a secretion formed in the crop glands. Adult breeding birds that harbor the protozoan in their upper digestive tract may

readily contaminate the crop milk with trichomonads. In addition, columbids are among those species that often concentrate at water and seed sources, increasing the likelihood that trichomonads are transmitted from one bird to the next as food and water is shared among infected and non-infected birds.

Although results from my study did not indicate any significant difference between the incidence of T. gallinae in adult and immature columbids, I did not collect any samples from nestlings during my study. Therefore, the incidence of T. gallinae in immatures is biased toward fledged birds and does not include nestling mortality that may have resulted from trichomoniasis.

Trichomoniasis, however, does not appear to have a significant impact on columbid populations in Arizona. Despite the near 100% incidence of T. gallinae in White-Winged doves detected during this study and Toepfer's (1966) study, there are no published accounts of trichomoniasis outbreaks in this species (Brown 1989). There is 1 published account of a trichomoniasis outbreak in Mourning doves (Brown 1989). Based on call-count surveys and hunter success, populations of Mourning doves and White-Winged doves in rural areas of Arizona have declined during the last 30 years, but most biologists believe this is primarily due to (1) the destruction of riparian ecosystems that are prime nesting

habitats and (2) a shift in harvest practices away from grain producing crops (Brown 1989). These surveys, however, are done exclusively in rural areas and do not take into account the number of columbids inhabiting urban areas.

Non-Columbid Species. Aside from raptors, non-columbid species may not be exposed to T. gallinae as often as columbid species. First, of the 54 non-columbid birds I trapped, none exhibited any sign of lesions associated with trichomoniasis and only 3 of the 46 House Finches trapped were positive for T. gallinae. Second, there are no reports of epizootic events, i.e. large die-offs, involving wild birds other than columbids. Finally, there are only a few reports of T. gallinae isolated in individual, non-columbid, wild birds.

Non-columbid species, however, are not immune to trichomoniasis. Several non-columbid species experimentally inoculated with T. gallinae were reported to have developed lesions consistent with trichomoniasis (Levine et al. 1941). In addition, my study included necropsy results from several House Finches and a House Sparrow that indicated mortality resulted from gross lesions compatible with trichomoniasis; histopathologic examination supported the diagnosis and cultures of lesions were positive for the organism. Finally, although raptors were not included in my study, researchers in Tucson, Arizona have suggested that trichomoniasis may significantly affect urban populations of Harris' and Coopers

Hawks (Unpublished data from James Dawson and Clint Boal, School of Renewable Natural Resources, University of Arizona, Tucson).

Objective #2: Water Sources

Isolation Procedures. Results from the laboratory studies were unique because I developed methods for recovering T. gallinae from water samples inoculated with motile trichomonads (See Methods: Isolation Procedures). However, in a water source utilized by wild birds trichomonads may coexist with other species of flagellated protozoa. Since there is currently no technique available that can positively differentiate T. gallinae from these other species, only the presence or absence of flagellated protozoa in a bird bath can be determined.

Bird Baths. Researchers have suggested that trichomonad contamination of water sources utilized by wild birds may be significant in the transmission of this protozoan (Stabler 1954, Honigberg 1978). Although the species of flagellated protozoa isolated from bird baths during this study were not identified, the low incidence of flagellated protozoa detected suggests that (1) contamination of bird baths by T. gallinae may not be as common and (2) bird baths may not be as significant in the transmission of this protozoan as previously believed.

This does not imply that trichomonads are not transmitted through a water source. First, water sources may

be a significant source of transmission during an epizootic event when more diseased birds are present. My study did not coincide with such an event. However, several House Finches and a House Sparrow found dead during this study were diagnosed with trichomoniasis. Although the source of infection for these birds is unknown, water sources contaminated by infected birds may have played a role. Second, it is possible that carriers of T. gallinae do not contaminate water sources as readily or as heavily as birds with active disease. Carriers may harbor a lower number of trichomonads and might contaminate water sources to a lesser extent. Third, only a few water sources contaminated with a virulent strain of T. gallinae may initiate an epizootic event. Finally, I sampled water sources at one point in time during the day. Although I collected samples at mid-morning following the time birds are most active, it is possible that water sources were contaminated at any point during the day. Based on results from this study that indicate trichomonads exposed to natural sunlight are able to survive for at least 60 minutes, further studies in this area should increase the sampling intervals at bird baths to once every hour.

Objective #3: Treatments to Control Trichomonads in Water

Treatment 1a: Exposure to 24°C for 10 Hours. Although the temperature of most bird baths does not remain constant at 24°C, results from this experiment and Honigberg's (1978)

suggest that at favorable or mild temperatures trichomonads are capable of surviving for extended periods of time in a water source. The exact length of time trichomonads are able to survive in water may vary depending on several factors including the strain of trichomonad, the angle of incident sunlight, shading, and water characteristics (salinity, pH, etc.).

Treatment 1b: Exposure to Different Temperatures. Results from my study and Honigberg's (1978) have demonstrated the ability of trichomonads to survive exposure to low temperatures. This raises the question of why more epizootic events are not detected in the desert southwest during the winter months since air temperatures are mild and water in bird baths rarely freezes completely over.

Although summer months are reported to be the peak time of trichomoniasis outbreaks (Stabler and Herman 1951, Haugen 1852, Haugen and Keeler 1952, Straus 1966), more than an increase in ambient temperatures may be required to initiate such events. The shift in the number and composition of birds during the summer months may also play a role. First, an increase in bird numbers due to the influx of migrant birds and recently fledged birds may result in a higher concentration of birds at water and seed sources, increasing the likelihood of transmitting trichomonads from one bird to the next. Second, migrant birds may introduce a new or more

virulent strain of trichomonads into a non-migrant bird population that may be unable to effectively resist an infection.

Treatment 2a: Exposure to Artificial Near Ultraviolet Radiation. Near-UV radiation appears to be effective against trichomonads. However, it is only effective after a prolonged exposure period.

Protection provided by partially covering the containers, combined with increasingly deeper depths of solution should have resulted in greater survival of trichomonads when exposed to near-UV radiation. However, the results from this experiment were not consistent. When exposure was prolonged (480 minutes), the results varied considerably from one depth to the next and did not provide any information as to the effectiveness of cover. Additional trials might resolve the variation in results.

Treatment 2b: Exposure to Natural Sunlight. Natural sunlight was more effective against trichomonads than near-UV radiation. Although the temperatures of saline solution exposed to natural sunlight were greater (Table 11) than the temperatures of saline solution (24°C-25°C) exposed to only near-UV radiation, they were still within a range tolerable to trichomonads. Geise (1967) has suggested that a combination of heat and UV radiation may result in lower lethal temperatures effective against protozoa. Sunlight is composed of near-UV and far-UV, the latter being more

detrimental to living organisms. Therefore, this may explain why natural sunlight was more effective against trichomonads and why I did not isolate more flagellated protozoa from bird baths.

The effectiveness of natural sunlight against trichomonads in a water source may vary depending on the strain and angle of incident light, which varies depending on the time of day or year. However, the results from exposing trichomonads to natural sunlight suggest that high summer daytime temperatures in the desert southwest combined with exposure to radiation from the sun may be an effective strategy for controlling trichomonads in water sources used by wild birds.

Treatment 3: Exposure to Different Chemicals - Objective 1. Results from exposing trichomonads to different commercially available chemicals suggests that certain dilutions of Chlorox, Nolvasan, and distilled white vinegar are effective against trichomonads. However, I did not determine the duration of time each chemical was effective against trichomonads.

There are additional issues raised by the use of these chemicals. First, it is unknown what long- or short-term harmful effects these chemicals may have on different bird species. I obtained material safety data sheets from the manufacturers of Chlorox and Nolvasan, but little data has

been gathered regarding the effects these chemicals may have on avian species. Ritchie and Harrison (1994) have reported that chlorhexidine (Nolvasan) ingested by birds may result in death due to dehydration, especially in finches. There was no attempt made during my study to determine the long- or short-term toxic effects of these chemicals. Second, it is unknown whether residual amounts of these chemicals persist within a bird's system. This may be important in game species that are eaten by humans. Third, we do not know the effects these chemicals may have on other species, such as cats, dogs, or invertebrates, that drink from a bird bath treated with one of these chemicals. Finally, use of these chemicals in a bird bath may produce an unpalatable taste resulting in a shift in use from a treated bird bath to an untreated bird bath, concentrating birds at fewer water sources.

Given the many unanswered questions regarding the effects of these chemicals (Chlorox, Nolvasan, and distilled white vinegar), I cannot recommend their use.

Treatment 3: Exposure to Different Chemicals - Objective 2. Potable Aqua iodine tablets were not effective against trichomonads at the manufacturer's recommended dosage of 2 tablets per liter of solution. No attempt was made during this study to determine the effectiveness of a higher dosage and their use cannot be recommended unless questions (discussed on the previous page) of possible harmful effects,

residues, effects on non-avian species, and palatability are answered.

RECOMMENDATIONS

Primary Research

Over the past 6 decades researchers have isolated T. gallinae from numerous species of wild birds, passed the organism from one bird to the next, and described the protozoan in some detail. However, strain identification and the attributes of various isolates of T. gallinae are not well understood. Currently, isolation of trichomonads from wild birds does not give us any means to identify the strain or the organisms' ability to cause disease. Therefore, the following studies would be important to our understanding of strains and how they relate to the disease process.

1. Develop a reliable technique for differentiating individual strains, including distinguishing an avirulent strain from a virulent one.
2. Assess whether an avirulent strain can revert to a virulent strain.
3. Begin a comprehensive study on the prevalence of various strains within avian populations, including (a) if strain prevalence is dependent on species, age, or region examined and (b) what strains or combination of strains are able to produce disease within various bird species.

4. Continue research, based on methods developed during my study, to determine how various strains are affected by exposure to various temperatures, near-UV radiation, natural sunlight, and various chemicals.

Secondary Research

Once strains of T. gallinae can be reliably identified and virulence attributes of each are understood there are topics of research that would expand on information currently available concerning the relationship between T. gallinae and various bird species. These topics include: incidence, immunity, transmission, and the use of chemicals.

Incidence. The majority of the literature on trichomoniasis describes the incidence of T. gallinae in adult and immature columbids. The following studies would continue this research and expand our knowledge of the incidence of T. gallinae in other species.

1. Determine the incidence of T. gallinae in non-columbid species, including nestlings, and the extent T. gallinae affects non-columbid populations.
2. Determine the impact trichomoniasis has on urban raptor populations, including the effect trichomoniasis-related mortality in nestling raptors has on the overall population of raptors.
3. Determine if there is a difference between the incidence of T. gallinae in bird populations

inhabiting urban versus rural environments.

4. Determine the incidence and impact of T. gallinae in nestling columbids.

Immunity. There is evidence to suggest that birds can acquire resistance to trichomoniasis (See section on Virulence). However, how immunity is acquired is still unclear. Therefore, the following study is recommended:

1. Determine the mechanisms of immunity to trichomoniasis, including (a) if resistance varies with species or age, (b) the length of time birds remain immune, and (c) what strain or strains are involved, i.e. to determine whether "avirulent" strains are immunogenic.

Transmission. During my study I developed methods for isolating flagellated protozoa from water sources utilized by wild birds. The following studies would expand on these methods:

1. Develop a reliable technique for distinguishing T. gallinae from other species of flagellated protozoa isolated from water sources.
2. Develop techniques that would allow continuous monitoring of water sources in a laboratory environment. Determine if there is a difference in the ability of carriers of T. gallinae versus birds with active lesions to contaminate a water source.

3. Assess whether crop milk can be contaminated with T. gallinae.

Use of Chemicals. Results from my study suggest that commercially available chemicals (Chlorox, Nolvasan) are effective against T. gallinae. Although their use is not recommended, I encountered homeowners during my study that were already utilizing these chemicals in their bird baths. Therefore, the following studies would assess the effects of Chlorox and Nolvasan on avian species when they are added to water sources utilized by wild birds. It should be noted, however, that any study assessing the possible effects of these chemicals may produce legal and liability obstacles.

1. Determine whether consumption of and contact with these chemicals have toxic effects on various bird species during short- and long-term use.
2. Determine if chemical residues occur within various bird species and, if so, then (a) the length of time chemical residues remain within a bird, (b) if this varies with species or age, and (c) any toxic effects residues might have on birds and the possible hazard to humans who consume these birds.
3. Determine if the presence of a chemical in a bird bath affects the palatability of the water and use of the water source by birds.

4. Determine the length of time a one-time addition of chemicals are active against trichomonads in a water source.
5. Determine if the effectiveness of a chemical in a water source is altered by the presence of suspended organic material.

Control Methods

It is important to note that, at least in columbids, trichomoniasis does not appear to significantly affect long-term population density. Lesions consistent with trichomoniasis have been described since the 1600's (Stabler 1954). In addition, results from my study and others suggest that T. gallinae may be a natural parasite of columbids. Therefore, measures to reduce the incidence of this protozoan may be ineffective.

However, providing artificial food and water sources for birds may promote the spread of disease by concentrating birds. Therefore, the following recommendations are ideally intended to minimize the transmission of trichomonads via these sources.

1. Place bird baths in direct sun and keep water in bird baths shallow. Based on results from my study, it appears that sunlight is effective against T. gallinae in water depths of $\leq$ 13cm.
2. Keep bird baths free of plant material and periodically flush bird baths out. There is

evidence, based on this study and other research (Geise 1967), to suggest that T. gallinae may be able to survive longer when shade or cover is available.

3. **Do not** add any chemicals to water sources provided for birds. No information is currently available regarding what effects, toxic or otherwise, chemicals may have on birds.
4. Place seed feeders in direct sun; since sunlight appears to be effective against trichomonads in water, it should be effective against trichomonads that may contaminate seed dropped by infected birds.
5. Minimize attracting large concentrations of birds in one area by placing seed feeders away from water sources as far as is practical.

Epizootic Recommendations

In the event of an outbreak of trichomoniasis, there are management practices that could be put into effect at or near the outbreak location. The recommendation to suspend providing food and water is not given here for several reasons. It is not known how significant artificial water and seed sources are in the transmission of T. gallinae. Especially given that outbreaks of trichomoniasis have been reported in rural as well as urban areas. In addition, it is possible that with fewer water and seed sources available, birds may concentrate at these sources and the transmission

of trichomonads may be enhanced. With these considerations, the following recommendations are provided.

1. Residents should report dead birds found within their yard to their local wildlife agency.
2. Wildlife department personnel should attempt to establish the number of dead birds at or near an outbreak area and the cause of mortality by submitting specimens to a diagnostic laboratory.

APPENDIX A

Table 1. List of reagents used during this study, including active ingredients of each and the manufacturers information.

NAME	ACTIVE	INGREDIANT	MANUFACTURER
Saline	0.9%	sodium chloride	Baxter Healthcare Corp. Deerfield, IL 60015
DMSO	99.5%	dimethyl sulfoxide	Sigma Chemical Company St. Louis, MO 63178
Chlorox	5.25%	sodium hypochlorite	The Chlorox Company Oakland, CA 94612
Nolvasan	2.0%	chlorhexidine diactetate	Fort Dodge Laboratories 800 5th St. NW Fort Dodge, IA 50501
Distilled White Vinegar	5.0%	acetic acid	H.J. Heinz Co. Pittsburgh, PA 15212
Potable Aqua	16.7%	tetraglycine hydroperiodide (per tablet)	Wisconsin Pharmacal Co. P.O. Box 198 Jackson, WI 53037

APPENDIX B

Table 1. Township-Range coordinates and housing densities of trapping locations.

Location	Housing Density (house per acre)	Coordinates
1	≥ 1	T14S, R14E, S.06, NE 1/4
2	≥ 1	T14S, R14E, S.15, NE 1/4
3	≥ 1	T13S, R13E, S.03, N 1/2
4	≤ 1 house per 3.3 acres	T13S, R14E, S.29, NW 1/4
5	≤ 1 house per 3.3 acres	T13S, R13E, S.34, N 1/2
6	Golf Course	T14S, R14E, S.16, NE 1/4

Table 2. Township-Range coordinates of water sample locations and type of bird baths at each. Container type indicates whether bird baths had static water sources (static) or were fountains.

Location	Township-Range	Container Type
1	T14S, R14E, S.06, NE 1/4	Static
2	T14S, R14E, S.15, NE 1/4	Static
3	T14S, R15E, S.07, W 1/2	Static
4	T14S, R14E, S.11, NW 1/4	Fountain
5	T13S, R14E, S.01, NW 1/4	Static
6	T13S, R14E, S.15, SE 1/4	Fountain
7	T13S, R14E, S.14, N 1/2	Static
8	T13S, R13E, S.24 SW 1/4	Static
9	T15S, R13E, S.11, SW 1/4	Static
10	T14S, R13E, S.16, NW 1/4	Static

Table 3. Characteristics of Central Arizona Project (CAP) water and ground water.

Characteristic	CAP	Ground
Hardness (ppm)	240	120
Total solids (ppm)	580	270
Chloride (ppm)	87	<28
Chlorine (ppm)	<10	<10
pH	7.084	7.691

APPENDIX C

Table 1. The dilutions of Chlorox in 0.9% saline solution, CAP water, and ground water tested to determine the highest effective dilution of Chlorox active against T. gallinae. Dilutions tested are designated with an "X".

DILUTIONS	SALINE	CAP	GROUND
1:100	X		
1:200	X		
1:300	X		
1:400	X		
1:600	X		
1:700	X		
1:800	X		
1:900	X		
1:1000	X		
1:1500	X		
1:2500		X	X
1:3000	X	X	X
1:3500	X	X	X
1:4000	X		
1:6000	X		

Table 2. The dilutions of Nolvasan in 0.9% saline solution, CAP water, and ground water tested to determine the highest effective dilution of Nolvasan active against T. gallinae. Dilutions tested are designated with an "X".

DILUTIONS	SALINE	CAP	GROUND
1:300		X	
1:600	X	X	X
1:700	X	X	X
1:800	X	X	X
1:1000	X	X	X
1:1500	X		X

Table 3. The dilutions of distilled white vinegar in 0.9% saline solution, CAP water, and ground water tested to determine the highest effective dilution of vinegar active against T. gallinae. Dilutions tested are designated with an "X".

DILUTIONS	SALINE	CAP	GROUND
1:1	X		
1:3	X		
1:5	X	X	X
1:6	X		
1:10	X		
1:12.5	X		
1:15	X	X	X
1:30		X	X
1:45		X	X
1:60		X	X
1:100	X	X	X

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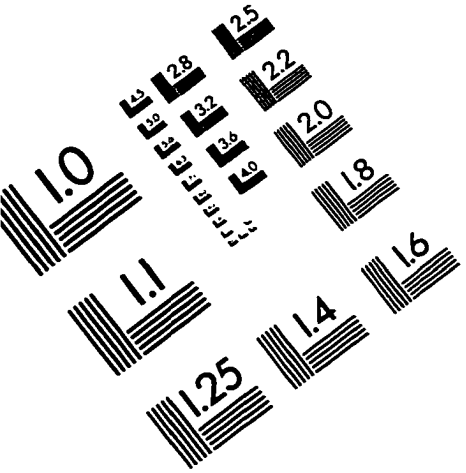

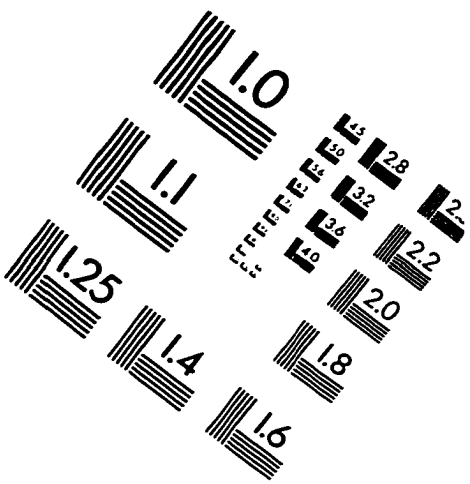

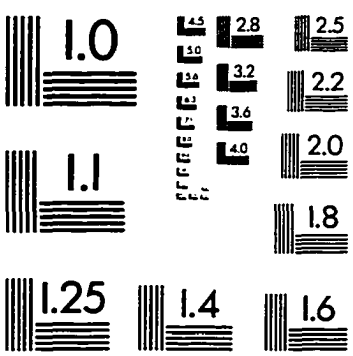

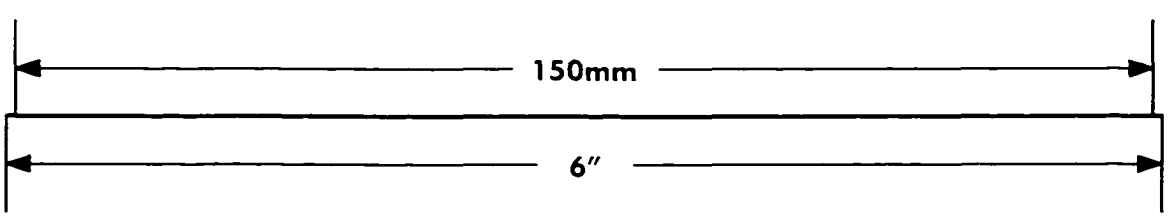

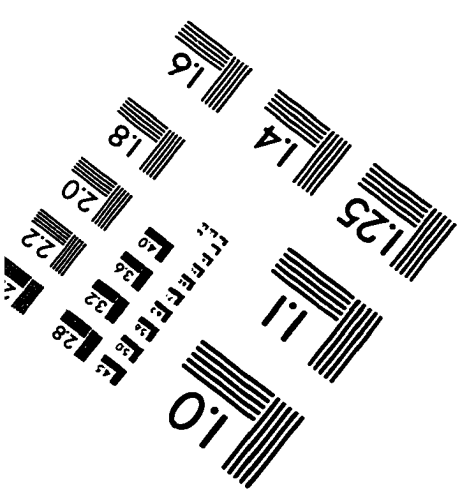

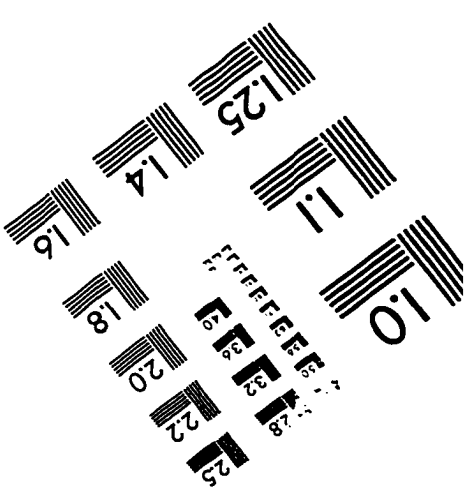

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