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Survival of *Trichomonas gallinae* in White-winged Dove Carcasses

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ABSTRACT: Survival of *Trichomonas gallinae* was examined in white-winged dove (*Zenaida asiatica*) carcasses to assess whether birds that have been dead up to 8 hr can be sampled reliably for this protozoan. Carcasses of 100 *T. gallinae*-positive white-winged doves were separated into four groups of 25 birds, representing 2, 4, 6, and 8 hr post mortem sampling intervals and placed into an environmental chamber maintained at 27 C and 75% relative humidity. Live *T. gallinae* were isolated in 96, 100, 100, and 92% of the carcasses at each of the respective post mortem intervals. The experiment was repeated with another 100 carcasses of *T. gallinae*-positive white-winged doves placed in the environmental chamber, this time maintained at 27 C and 40% relative humidity. Live *T. gallinae* occurred in 96, 100, 96, and 100% of the carcasses at each of the respective post mortem intervals. Across both trials, the overall ability to detect positive birds from sampling carcasses up to 8 hrs post mortem was 97%. An *a posteriori* experiment was conducted in which 23 and 18 carcasses from the second trial were maintained in the environmental chamber at 27 C and 40% relative humidity and resampled at 24 and 48 hr post mortem, respectively. Live trichomonads were isolated from 91 and 44% of the carcasses at 24 and 48 hr, respectively. Results suggest live *T. gallinae* can be obtained from dove carcasses reliably up to 8 hr and possibly up to 24 hr after host death. The ability for *T. gallinae* to survive within this time interval can aid wildlife personnel in monitoring this protozoan at hunter check stations or obtaining samples from recently killed birds.

Key words: Post mortem isolation, *Trichomonas gallinae*, trichomoniasis, white-winged dove, *Zenaida asiatica*.

Trichomonas gallinae is a pathogenic sarcomastigophoran parasite commonly found in the upper digestive tract of columbids and in certain avian predators that feed on columbids (Conti, 1993). Rock doves (*Columbia liva*) appear resistant to disease with *T. gallinae* (Tudor, 1991) and sometimes may serve as reservoir hosts for transmission to other avian species (Kietzmann, 1988). This parasite is common (97–100%) in white-winged doves (*Zenaida asiatica*) surveyed in Texas (Stabler, 1961; Glass, 1999) and Florida (Conti and Forrester, 1981). However, clinical signs of disease in wild populations of white-winged doves have not been sufficiently documented, leading to speculation about host-specific immunity or resistance to *T. gallinae*-induced disease. Alternatively, numerous epizootics have been reported in mourning doves (*Zenaida macroura*) across the United States (Stabler and Herman, 1951; Haugen, 1952; Greiner and Baxter, 1974) with reported prevalences up to 58% (Straus, 1966; Sileo, 1970; Conti and Forrester, 1981).

State and federal agencies have expressed interest in surveying populations of columbids for *T. gallinae*, given the prevalence and potential impact of this pathogenic protozoan on the continental mourning dove population (Conti, 1993), the ongoing range expansion of white-winged doves into areas where mourning doves traditionally occur (Conti and Forrester, 1981; George et al., 1994), and its negative impact on certain birds of prey (Conti, 1993; Boal et al., 1998). How best to monitor these large host populations for *T. gallinae* has been of concern, given agency personnel constraints, budgets, and reliability of data collected. One potential method of monitoring is to use hunter-shot birds processed at check stations. However, problems arise when data are collected from animals with highly variable and indefinite intervals from time of death to sample collection. This is particularly important in parasitological studies where parasites may emigrate or die in response to host death. Organisms that remain in hosts may be misidentified due to changes

in their morphological characteristics and varying rates of decay.

Current field methods of testing for *T. gallinae* require collection and observation of a live specimen. It is uncertain whether sampling carcasses across a wide host post mortem time interval (as would occur at hunter check stations) would yield accurate results as to the prevalence of *T. gallinae* at the time of host death. To our knowledge, information concerning the survival of *T. gallinae* in host carcasses has not been published. Therefore, our objective was to determine the survivability of *T. gallinae* in white-winged dove carcasses across a time period (up to 8 hr post mortem) that would be expected at a typical hunter check station.

White-winged doves were captured alive in walk-in funnel traps (Schemnitz, 1994) from February to April 1999 in and around Kingsville (27°31′N, 97°52′W) and San Antonio (29°25′N, 98°30′W) (Texas, USA), individually marked with a coded leg band, and temporarily housed at the Texas A&M University–Kingsville aviary. Doves were captured and handled in accordance with Texas Parks and Wildlife (Austin, Texas, USA) Scientific Permit No. SPR-0993-636 and U.S. Fish and Wildlife Service (Albuquerque, New Mexico, USA) Permit No. PRT-827294. This study was approved by Texas A&M University–Kingsville Animal Care and Use Committee (#3-98-07R).

In the aviary, each bird was tested for *T. gallinae* using an InPouch™ TF diagnostic pouch (BioMed Diagnostics, San Jose, California, USA) as described by Cover et al. (1994). Test pouches were viewed under the microscope at 100–400× magnification for 15 min or until trichomonads were observed. Samples where trichomonads were not observed were incubated at 37 C and reexamined at 24, 48, and 72 hr. If no *T. gallinae* were found by the 72 hr examination period, the bird from which that sample was taken was considered negative and was excluded from the study.

In the first trial, 100 *T. gallinae*-positive white-winged doves were separated randomly into four groups of 25 birds each, representing 2, 4, 6, and 8 hr testing intervals. We chose this experimental design rather than resampling individual dove carcasses over time to avoid introducing the effect of multiple swabbing into the study design. Each dove was euthanized by thoracic compression. The internal surface of the mouth and throat region was sampled immediately (time 0) using an algonite-tipped swab and inserted into an InPouch™ TF pouch; this was done to reverify that doves, which were found to be infected when sampled in the aviary, were still infected at the beginning of the experiment. Each carcass was placed into an individual compartment within an environmental chamber (Scientific Instrument Service, Pearland, Texas, USA) that was maintained at 27 C and 75% relative humidity. At the appropriate post mortem interval for each group, a sample was taken as previously described from the internal surface of the mouth and throat region of each dead bird and inserted into an InPouch™ TF pouch; this procedure was repeated to obtain a replicate sample from each bird. Both samples (pouch "A" and "B") from each bird were examined as outlined previously. Birds that tested negative at time 0 were excluded from the study and replaced with a bird that tested positive at time 0. A positive sample of either the "A" or "B" diagnostic pouch confirmed that the carcass still contained viable *T. gallinae*. Methods for the second trial were the same as above except relative humidity was lowered to 40% within the environmental chamber. Climatological parameters for this study were selected based upon the average temperature and relative humidities within humid and semi-arid regions of the southern USA during dove hunting season (Critchfield, 1974; Rudloff, 1981).

In an *a posteriori* experiment to test further the survivability of *T. gallinae*, we resampled 23 and 18 carcasses maintained in the environmental chamber (27 C and

40% humidity) at 24 and 48 hr post mortem, respectively. Additionally, we determined if a replicate sample was necessary for accurately identifying carcasses positive for *T. gallinae* by determining the proportion of cases in which both pouches from the same bird were positive. Statistical analyses were not performed on the data due to the obvious results (Cherry, 1998).

In the first trial, live *T. gallinae* were isolated in 96, 100, 100, and 92% of the carcasses sampled at 2, 4, 6, and 8 hr, respectively. In the second trial, live *T. gallinae* were found in 96, 100, 96, and 100% of the carcasses at each of the respective time intervals. Across both trials, the overall ability to detect truly positive birds from sampling 200 carcasses 2 to 8 hr post mortem was 97%.

Of the 23 carcasses resampled at 24 hr post mortem, 91% contained live *T. gallinae*. However, only 44% of the 18 carcasses resampled at 48 hr post mortem contained live *T. gallinae*.

There was a 3% discrepancy between the A and B pouches from 100 positive birds. In two cases, the A pouch was negative and the B pouch positive, and one case where the A pouch was positive and the B pouch was negative. Therefore, if only one sample was taken (i.e., A pouch only), we would have misdiagnosed two birds (2% false negative rate).

The environmental parameters selected in this study reflected those typically encountered in many southern and western states during the dove hunting season (Critchfield, 1974; Rudloff, 1981). Because *T. gallinae* can persist in carcasses of white-winged doves for at least 8 hr postmortem at an ambient temperature of 27 C and relative humidities of 75 and 40%, hunter-collected birds can be used to assess the prevalence of *T. gallinae* in white-winged doves when environmental conditions are more extreme than during our experiment.

Based on our resampled carcasses (under the controlled conditions of our experiment), *T. gallinae* may be collected reliably up to 24 hr after host death. In cases where *T. gallinae* is suspected as a cause of mortality; sampling a carcass reliably up to 24 hr after host death may be feasible. Environmental variables, changing over a 24 hr period in some regions, might alter the ability of *T. gallinae* to survive in carcasses. Such changes were not specifically addressed in our study. For those wishing to sample carcasses that have extended post-mortem intervals, we suggest caution in interpretation of their findings.

Because we had a 3% overall error rate between the A and B pouches in identifying a truly positive bird, by taking only one sample, we would have misdiagnosed two birds in 100 birds sampled. Therefore, it is doubtful that taking two samples from each bird would substantially reduce the incidence of false negatives compared to the added cost in taking a replicate sample in situations where large numbers (i.e., >50) of carcasses are being tested.

Live *T. gallinae* can be reliably obtained from white-winged dove carcasses at least up to 8 hr after host death, which can aid wildlife personnel in monitoring this protozoan at hunter check stations or obtaining samples from recently dead birds to aid in determining potential cause of death. It is highly likely that our findings are applicable to mourning doves and other columbids, based on the persistence of *T. gallinae* within this particular host Family. Further investigation of the effects of other host species, temperature, humidity, and sampling intervals on *T. gallinae* survival may be necessary to meet unique conditions found in other areas where managers are interested in monitoring the prevalence of *T. gallinae*.

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