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Survey for *Trichomonas gallinae* in Northern Bobwhites (*Colinus virginianus*) from the Rolling Plains Ecoregion, Oklahoma and Texas, USA

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ABSTRACT: Northern Bobwhites (*Colinus virginianus*) have been in decline throughout the southeastern US. Prevalence of *Trichomonas gallinae* in wild bobwhites is unknown, although *T. gallinae* caused morbidity and mortality in experimentally infected bobwhites. Many species of Columbidae (pigeons and doves) in Texas are hosts to *T. gallinae*. Bobwhites potentially may become exposed to this protozoan through supplemental feed or water sources contaminated by columbids infected with *T. gallinae*. All of 506 bobwhites collected in Oklahoma and Texas, 2011–13, were PCR negative for *T. gallinae*. These data suggest *T. gallinae* is not contributing to the population decline of bobwhites in this region.

Key words: *Colinus virginianus*, Northern Bobwhite, Oklahoma, PCR, Rolling Plains Ecoregion, Texas, trichomonosis, *Trichomonas gallinae*.

Studies examining factors causing or contributing to Northern Bobwhite (*Colinus virginianus*) population decline seldom included diseases caused by parasitic infections. One disease that could potentially be threatening to bobwhites is avian trichomonosis caused by the flagellated protozoan *Trichomonas gallinae*. Although *T. gallinae* is not documented to occur in wild bobwhites, experimental transmission from chickens (*Gallus gallus domesticus*) to eight bobwhites was successful and resulted in 75% mortality within 5–11 d (Levine et al. 1941). Trichomonosis commonly occurs in Columbidae (pigeons and doves) and is associated with major epizootics in some species (Haugen and Keeler 1952; Greiner and Baxter 1974). *Trichomonas gallinae* occurs in the upper digestive tract of infected birds causing pathologic changes, such as inflammation of the mucosa and caseous lesions, which can block the esophagus and subsequently kill the host through starvation (Narcisi et al. 1991; Grabensteiner et al. 2010). Birds become infected via mouth-to-mouth contact or through feed and water contaminated with *T. gallinae* (Stabler 1954; Kocan 1969). Transmission between hosts and naïve birds is facilitated and enhanced by concentrating infected and uninfected hosts at supplemental feed and water sites (Greiner and Baxter 1974).

Supplemental feed and water are provided for bobwhites as a means to improve habitat and survivability on Texas, US ranches (DeMaso et al. 2002; Hernández and Guthery 2012) and attract many species of doves (Rollins et al. 2006; Henson et al. 2010; Morris et al. 2010). In Texas, bobwhites co-occur with known hosts of *T. gallinae,* such as Mourning Doves (*Zenaida macroura*) and White-winged Doves (*Zenaida asiatica*; Glass et al. 2001; Gerhold et al. 2008). Therefore, *T. gallinae* could be present at supplemental feed and water sites, where bobwhites and infected columbid hosts may interact.

We surveyed bobwhites of the Rolling Plains Ecoregion of Texas and western Oklahoma for *T. gallinae*. Samples were collected from 30 counties (31°11′–56°48′N, 90°24′–102°32′W) within the Rolling Plains Ecoregion of Texas and western Oklahoma (Fig. 1). Samples were collected every August and October from 2011 to 2013, following the protocol of the study Operation Idiopathic Decline (Bruno 2014).

Bobwhites were collected using baited funnel traps and a subsample selected by

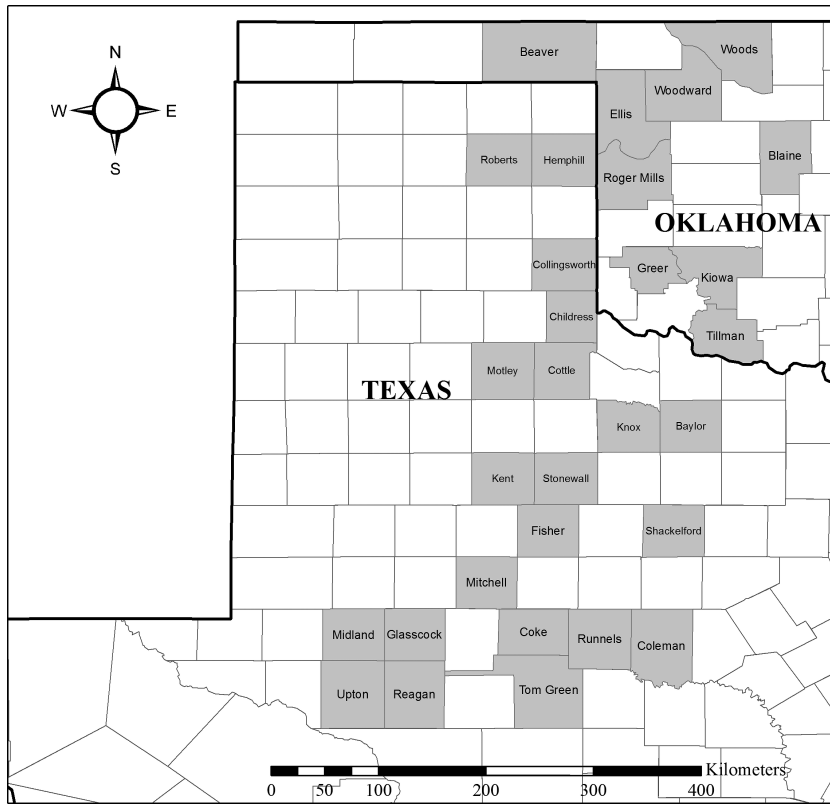

FIGURE 1. Samples for *Trichomonas gallinae* were collected from Northern Bobwhites (*Colinus virginianus*) throughout the Operation Idiopathic Decline study area in the Rolling Plains Ecoregion of Texas and western Oklahoma, USA during August and October 2011–13. The map highlights study sites where bobwhites were sampled by county.

computer randomization. Oral cavities were swabbed, and bobwhites released. Testing protocols followed Erwin et al. (2000) and Glass et al. (2001) in which the oral swabs were cultured in individually marked In-Pouch™ TF diagnostic pouches (BioMed Diagnostics, San Jose, California, USA). The InPouch TF kit contains media comparable to Diamonds media in a pouch that is functionally practical for field conditions (Cover et al. 1994). Each inoculated pouch was maintained at 21–32 C, while transported from the field to an incubator unit at The Institute of Environmental and Human Health at Texas Tech University. Pouches were placed upright in the incubator and incubated at 37 C for 48 h before freezing to preserve *T. gallinae* genetic material. This method of culturing *T. gallinae* was successful in 97 of 100 White-winged Doves (Erwin et al. 2000) and 42 isolates from several species of birds (Gerhold et al. 2008).

Bobwhites were handled in accordance with protocols approved by the Texas A&M University–Kingsville (TAMUK) Institutional Animal Care and Use Committee (2009-09-21A), Texas A&M University (AUP 2011-193), TAMUK Institutional Biosafety Committee (IBC-ID 009-2011), and Texas Parks and Wildlife Department Scientific Research permit (SPR-0690-152).

Samples were analyzed at the University of Tennessee–Knoxville. DNA pellets were obtained by thawing pouches, transferring media into 1.5-mL microcentrifuge tubes, and centrifuging (7,000 × G for 2 min). DNA amplification of the internal

transcribed spacer (ITS)1, 5.8S rRNA, and ITS2 regions was performed using trichomonad-specific primers ITSF (TTCAGTTCAGCGGGTCTTCC) and ITSR (GTAGG TGGACCTGCCGTTGG; Cepicka et al. 2005). PCR components included 3 μL of DNA in a 25-μL reaction containing 1 μL of primers ITSF and ITSR, and 20 μL water added to 1 Illustra PuReTaq Ready-To-Go PCR Bead (10 mM Tris-HCl [pH 9.0], 50 mM KCl, and 1.5 mM $MgCl_2$; GE Healthcare, Pittsburg, Pennsylvania, USA). Cycling parameters used for amplification were 95 C for 3 min, followed by 40 cycles of 95 C for 3 min, 50 C for 30 s, 72 C for 1 min, and 72 C for 5 min. A negative control (distilled water) and a positive control (laboratory propagated *T. gallinae*) were included in PCR extractions to detect contamination and presence of *T. gallinae*. For gel electrophoresis, PCR amplicons were separated using a 1% agarose gel and stained with ethidium bromide. The amplified samples were mixed with 3 μL of loading dye, loaded in the gel, run at 120 V for 30 min, and read under ultraviolet light.

Of 874 bobwhites sampled, 506 samples were processed using PCR and gel electrophoresis. All samples taken in 2011 ($n=194$) and 187 and 125 samples from 2012 and 2013, respectively, were processed for *T. gallinae*. All bobwhite samples were PCR negative.

The absence of *T. gallinae* DNA in these bobwhites suggests that certain barriers preventing transmission exist in the wild. For example, prevalence of *T. gallinae* is typically lower in Mourning Dove populations (Conti and Forrester 1981; Schulz et al. 2005) than in White-winged Dove populations (Conti and Forrester 1981; Glass et al. 2001). Mourning Doves more commonly use supplemental feed and water than White-winged Doves (Rollins et al. 2006; Henson et al. 2010; Morris et al. 2010); therefore, bobwhites may not come into contact with White-winged Doves chronically infected with *T. gallinae*.

Trichomonas gallinae does not have a true cyst stage and is suspected to only persist for a short time outside its host (Stabler 1954; Tasca and De Carli 2003). Even though Kocan (1969) demonstrated the survivability of trichomonads on moist grain (sorghum and buckwheat) for up to 120 h, his experiments were conducted in a controlled environment, and transmission from this feed to a host was never demonstrated. Although *T. gallinae* can survive in carcasses up to 48 h (Erwin et al. 2000), *T. gallinae* outside of its host likely cannot survive for long in the hot, dry environment in the Rolling Plains Ecoregion. Furthermore, Stabler (1954) proposed that water contaminated with *T. gallinae* was the sole avenue of infection for Galliformes. Kocan (1969) reported that motility and survivability of trichomonads in water was greatest at concentrations of 0.05%, 0.1%, 0.6%, and 0.9% salinity during periods of 3–24 h. Gerhold et al. (2013) concluded that *T. gallinae* survived best in artificial waters (distilled and chlorinated) when organic matter (detritus, leaves, and soil) was present. These experiments represent trichomonad survivability in water sources that best resemble those on Texas rangelands (i.e., standing water in nature contain some dissolved solids and organic matter). However, frequency of use of supplemental water by bobwhites may not occur simultaneously with infected hosts or may be too inconsistent (e.g., only in drought conditions; Hernández and Guthery 2012) for *T. gallinae* transmission to occur.

Although *T. gallinae* DNA was not detected in wild bobwhite populations in the Rolling Plains, it may be present in other areas where bobwhites potentially interact with infected hosts. Future studies may survey bobwhites in rural areas of South Texas, where White-winged Doves are more common than in the Rolling Plains.

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LITERATURE CITED

Bruno A. 2014. *Survey for Trichomonas gallinae and assessment of helminth parasites in northern bobwhites from the Rolling Plains ecoregion*. MS Thesis, Range and Wildlife Science, Texas A&M University–Kingsville, Kingsville, Texas, 133 pp.

Cepicka I, Kutisova K, Tachezy J, Kulda J, Flegr J. 2005. Cryptic species within the *Tetratrichomonas gallinarum* species complex revealed by molecular polymorphism. *Vet Parasitol* 128:11–21.

Conti JA, Forrester DJ. 1981. Interrelationships of parasites of white-winged doves and mourning doves in Florida. *J Wildl Dis* 17:529–535.

Cover AJ, Harmon WA, Thomas MW. 1994. A new method for the diagnosis of *Trichomonas gallinae* infection by culture. *J Wildl Dis* 30:457–459.

Demaso SJ, Townsend DII, Cox SA, Parry ES, Lochmiller RL, Peoples AD. 2002. The effect of quail feeders on Northern Bobwhite density in western Oklahoma. In: *Proceedings of the National Quail Symposium*, Texas Parks and Wildlife Department, Austin, Texas, 23–27 January 2002, pp. 241–244.

Erwin KG, Kloss C, Lyles J, Felderhoff J, Fedynich AM, Henke SE, Roberson JA. 2000. Survival of *Trichomonas gallinae* in white-winged dove carcasses. *J Wildl Dis* 36:551–554.

Gerhold RW, Maestas LP, Harnage P. 2013. Persistence of two *Trichomonas gallinae* isolates in chlorinated and distilled water with or without organic material. *Avian Dis* 57:681–683.

Gerhold RW, Yabsley MJ, Smith AJ, Ostergaard E, Mannan W, Cann JD, Fischer JR. 2008. Molecular characterization of the *Trichomonas gallinae* morphologic complex in the United States. *J Parasitol* 94:1335–1341.

Glass JW, Fedynich AM, Small MF, Benn SJ. 2001. *Trichomonas gallinae* in an expanding population of white-winged doves from Texas. *Southwest Nat* 46:235–237.

Grabensteiner E, Bilic I, Kolbe T, Hess M. 2010. Molecular analysis of clonal trichomonad isolates indicates the existence of heterogenic species present in different birds and within the same host. *Vet Parasitol* 172:53–64.

Greiner EC, Baxter WL. 1974. A localized epizootic of trichomoniasis in mourning doves. *J Wildl Dis* 10:104–106.

Haugen AO, Keeler JE. 1952. Mortality of Mourning Doves from trichomoniasis in Alabama during 1951. *Trans N Am Wildl Conf* 17:141–151.

Henson KD, Rollins D, Lyons EK, Ransom D. 2010. Species visitation at free-choice quail feeders in west Texas. *Wildl Soc Bull* 36:735–740.

Hernández F, Guthery FS. 2012. *Beef, brush, and bobwhites: Quail management in cattle country*. Texas A&M University Press, College Station, Texas, 244 pp.

Kocan RM. 1969. Various grains and liquid as potential vehicles of transmission for *Trichomonas gallinae*. *J Wildl Dis* 5:148–149.

Levine ND, Boley LE, Hester HR. 1941. Experimental transmission of *Trichomonas gallinae* from the chicken to other birds. *Am J Hyg* 33:23–32.

Morris G, Conner LM, Oli MK. 2010. Use of supplemental Northern Bobwhite (*Colinus virginianus*) food by non-target species. *Fla Field Nat* 38:99–105.

Narcisi EM, Sevolan M, Honigberg BM. 1991. Pathologic changes in pigeons infected with a virulent *Trichomonas gallinae* strain (Eigberg). *Avian Dis* 35:55–61.

Rollins D, Taylor BD, Sparks TD, Wadell TE, Richards G. 2006. Species visitation at quail feeders and guzzlers in southern New Mexico. In: *Proceedings of Gamebird 2006: Quail VI and Perdix XII*. Warnell School of Forestry and Natural Resources, Athens, Georgia, 31 May–4 June, pp. 210–219.

Schulz JH, Bermudez AJ, Millspaugh JJ. 2005. Monitoring presence and annual variation of trichomoniasis in mourning doves. *Avian Dis* 49:387–389.

Stabler RM. 1954. *Trichomonas gallinae*: A review. *Exp Parasitol* 3:367–402.

Tasca T, De Carli GA. 2003. Scanning electron microscopy study of *Trichomonas gallinae*. *Vet Parasitol* 11:37–42.

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