JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2005, p. 1005–1006 Vol. 43, No. 2
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.2.1005–1006.2005

Letters to the Editor

Comparison of the InPouch TF Culture System and Wet-Mount Microscopy for Diagnosis of *Trichomonas gallinae* Infections in the Pink Pigeon *Columba mayeri*

Trichomoniasis is commonly known as a sexually transmitted disease of humans (caused by *Trichomonas vaginalis*) or cattle (*Tritrichomonas foetus*), but it is also a ubiquitous disease of pigeons and raptors worldwide and is caused by the flagellate protozoan *Trichomonas gallinae*. The disease in birds is characterized by necrotic ulceration of the mouth, esophagus, crop, proventriculus, and occasionally other organs. The oral lesions prevent feeding and impede normal breathing by obstructing the upper digestive and respiratory tracts and frequently cause death through starvation. At this time, the parasite is being studied in the endangered Mauritian pink pigeon *Columba mayeri*, in which it appears to be causing high mortality in squabs (pigeon chicks), thus influencing adult recruitment (10). In the past, the wet preparation method has been used to screen pink pigeons (and other bird species) for *T. gallinae* using direct microscopic examination of swabbed mucosal samples from the crop and esophagus. More recently, a commercial method, the InPouch TF method (BioMed Diagnostics, San Jose, Calif.) has become available. The InPouch TF method was developed for the purpose of screening cattle for *Tritrichomonas foetus*, but it has also been shown to be a reliable test for *T. gallinae* (4). The InPouch TF culture pack essentially consists of a double-chambered plastic pouch, in which the two chambers are connected by a narrow channel. It functions as both a chamber for immediate viewing of the sample (equivalent to the wet preparation method) and a means of culturing the parasites. A similar test, the InPouch TV test, was developed to improve the sensitivity of screening humans for *T. vaginalis* and has compared favorably to alternative methods of screening (1, 2, 8). This study compares the sensitivity of the InPouch TF culture system with the wet-mount method in diagnosing *T. gallinae* in an upland population of pink pigeons.

Pairs of specimens were taken from 45 wild pink pigeons in October and November 2003. This number represented all the individuals from the only site in Mauritius that had adequate facilities to conduct rapid microscopy. Each individual was swabbed twice in the mouth, esophagus, and crop to obtain a sample of saliva and mucosal cells. The first swab was examined immediately by wet-mount preparation, and the second swab was used to inoculate the InPouch TF culture pack. The latter specimens were incubated at 38°C for up to 96 h and examined every 24 h. Trichomonads were identified microscopically in both methods by their characteristic morphology and motility.

Of 45 birds screened, a total of 27 (60%) tested positive for *T. gallinae* by the InPouch TF culture packs. Of these 27 birds, 12 were positive by wet-mount microscopy (26.7% of all samples and 44.5% of samples positive by culture). No infections were detected by the wet-mount method alone. All 12 of the infections detected by the wet-mount method were positive within the first 24 h of culture in the InPouch TF pack. Of the 15 infections that were not detected by the wet-mount method, 8 were detected after 24 h of incubation, 4 were detected after 48 h, and 3 were detected after 72 h. No further infections were found after 96 h of incubation.

Although wet-mount microscopy has been the standard diagnostic technique for determining *T. gallinae* infections in birds, the InPouch TF method detected more than twice as many positive infections as wet-mount microscopy ($P = 0.001$, two-sided Fisher's exact test). Previous investigations of *T. gallinae* in pigeons using wet-mount preparations may have underestimated the number of birds infected. For example, a previous study detected a prevalence of *T. gallinae* in pink pigeons of 18% using the wet-mount method (11). Our results suggest that the true prevalence of infection may have been closer to 40%. Wet-mount preparations have also been used to detect *T. gallinae* in other rare species of Columbidae, e.g., the Galapagos dove *Zenaida galapagoensis* (5). Results using this method will inevitably depend on the exact technique and equipment used, the screener's ability to detect the parasites in the preparation, and the intensity of infection in the birds; in this respect, the wet-mount method is arguably more subjective than culture techniques.

There is mounting evidence that diagnosing trichomonads by culture is more sensitive than wet-mount methods in detecting low-intensity infections (1, 2, 8), although the disparity in sensitivity between the methods varies. Other culture systems available for the diagnosis of *Trichomonas* species have been found to have sensitivities similar to that of the InPouch method (2, 4, 7), and PCR techniques have also been developed as alternative techniques (6, 9). However, despite the advantages of PCR methods (potentially faster than culture, and diagnosis is independent of parasite survival), it is rarely possible to use such techniques under field conditions, which is where most of the work on wild birds is carried out. It can be difficult to obtain the ingredients for other culture media, which may also be problematic to transport, require refrigeration, and still have a shorter shelf-life than the InPouch TF culture kits. This explains why the wet-mount method has been the only realistic diagnostic test in field conditions until recently. The advantages of the InPouch TF system for the diagnosis of *T. gallinae* in wild birds include convenience of sampling, ease of transport, relatively low cost, storage at room temperature, and a long shelf-life. Its greater expense over the wet-preparation method can be justified by these benefits together with its increased sensitivity and potentially lower subjectivity in diagnosing infections.

Avian trichomoniasis is becoming increasingly recognized in terms of the problems it causes for both conservation management of threatened species (in the threat it poses to immunologically naïve pigeons and doves), e.g., the pink pigeon and the Galapagos dove, and for game bird management, e.g., the mourning dove *Zenaida macroura* (there was a severe outbreak of the disease in the early 1950s [3]). Therefore, it is essential to use a screening method that is both accurate and suitable for use in the field. In conclusion, the higher sensitivity of the InPouch TF method compared to that of the wet preparation

makes it an ideal tool for the diagnosis of *T. gallinae* infections in wild birds.

REFERENCES

1. **Barenfanger, J., C. Drake, and C. Hanson.** 2002. Timing of inoculation of the pouch makes no difference in increased detection of *Trichomonas vaginalis* by the InPouch TV method. J. Clin. Microbiol. **40:**1387–1389.
2. **Beale, C., R. Goldsmith, M. Kotby, M. Sherif, A. el-Tagi, A. Farid, S. Zakaria, and J. Eapen.** 1992. The plastic envelope method, a simplified technique for culture diagnosis of trichomoniasis. J. Clin. Microbiol. **30:** 2265–2268.
3. **Conti, J. A.** 1993. Disease, parasites and contaminants, p. 205–224. *In* T. S. Baskett, M. W. Sayre, R. E. Tomlinson, and R. E. Mirarchi (ed.), Ecology and management of the mourning dove. Stackpole Books, Mechanicsburg, Pa.
4. **Cover, A. J., W. M. Harmon, and M. W. Thomas.** 1994. A new method for the diagnosis of *Trichomonas gallinae* by culture. J. Wildl. Dis. **30:**457–459.
5. **Harmon, W. M., W. A. Clark, A. C. Hawbecker, and M. Stafford.** 1987. *Trichomonas gallinae* in Columbiform birds from the Galapagos Islands. J. Wildl. Dis. **23:**492–494.
6. **Ho, M. S., P. A. Conrad, P. J. Conrad, R. B. LeFebvre, E. Perez, and R. H. BonDurant.** 1994. Detection of bovine trichomoniasis with a specific DNA probe and PCR amplification system. J. Clin. Microbiol. **32:**98–104.
7. **Levi, M. H., J. Torres, C. Pina, and R. S. Klein.** 1997. Comparison of the InPouch TV culture system and Diamond's modified medium for detection of *Trichomonas vaginalis*. J. Clin. Microbiol. **35:**3308–3310.
8. **Ohlemeyer, C. L., L. L. Hornberger, D. A. Lynch, and E. M. Swierkosz.** 1998. Diagnosis of *T. vaginalis* in adolescent females: InPouch TV culture versus wet-mount microscopy. J. Adol. Health **22:**205–208.
9. **Riley, D. E., B. Wagner, L. Polley, and J. N. Krieger.** 1995. PCR-based study of conserved and variable DNA sequences of *Tritrichomonas foetus* isolates from Saskatchewan, Canada. J. Clin. Microbiol. **33:**1308–1313.
10. **Swinnerton, K. J.** 2001. The ecology and conservation of the pink pigeon *Columba mayeri* in Mauritius. Ph.D. thesis. University of Kent, Canterbury, United Kingdom.
11. **Swinnerton, K. J., A. G. Greenwood, R. Chapman, and C. G. Jones.** The incidence of parasitic disease trichomoniasis and its treatment in reintroduced and wild pink pigeons *Columba mayeri*. Ibis, in press.

Nancy Bunbury*
Diana Bell
School of Biological Sciences
University of East Anglia
Norwich, United Kingdom

Carl Jones
Mauritian Wildlife Foundation
Vacoas, Mauritius

Andrew Greenwood
International Zoo Veterinary Group
Keighley, United Kingdom

Paul Hunter
School of Medicine, Health Policy and Practice
University of East Anglia
Norwich, United Kingdom