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A New Method for the Diagnosis of *Trichomonas gallinae* Infection by Culture

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ABSTRACT: Diagnosis of *Trichomonas gallinae* infection can reliably be made by the demonstration of organisms in material taken from a bird's mouth and crop. This can be examined directly or incubated in a growth medium until organisms are numerous enough to be easily found in aliquots examined with the microscope. We compared two methods of culture diagnosis of *Trichomonas gallinae* infection in pigeons. A commercially available kit, InPouch®TF, proved as sensitive for this purpose as in vitro Diamond's medium, but had practical advantages over the usual in vitro system.

Key words: *Trichomonas gallinae*, diagnostic methods, pigeons, raptors.

Trichomonas gallinae is a pathogenic protozoan often affecting the mouth and crop of domestic pigeons, wild columbids and raptors. Diagnosis of infection can be accomplished by direct microscopic examination of material scraped from the oral cavity of infected birds (Stabler, 1951) or by the inoculation of such material into a suitable growth medium (Honigberg, 1978) followed by examination of aliquots of the medium after incubation.

Diagnosis either by direct examination or by culture provides satisfactory results, but both methods have some drawbacks. Our objective was to evaluate a new culture system that has overcome some of the problems related to diagnosis by in vitro cultivation.

The specified use for both culture methods tested is the detection of *Tritrichomonas foetus* infection in cattle. We rarely have failed to establish a new culture of *Trichomonas gallinae* in either medium from a bird known to be infected, however. Thus, both methods are potentially useful in diagnosis of the avian infection.

The new culture system evaluated is InPouch®TF (BioMed Diagnostics, Santa Clara, California, USA). The vessel is a plastic pouch, 4.5 cm wide by 21 cm long, with two tapered chambers separated by a narrow channel, Fig. 1). The channel is effectively sealed by squeezing the medium from it. The lower chamber is filled with 4 ml of a proprietary medium containing antibiotics and a fungal growth inhibitor. Its sensitivity in detecting infection was compared to that of Diamond's medium (Kimsey, 1986) with horse serum, prepared in our laboratory. Diamond's medium was dispensed in 10 ml aliquots in glass screw-capped culture tubes.

Fifty pigeons were examined for infection. Twenty-two were feral birds trapped on the California State University, Fresno farm in Fresno, California (USA) (36°44′N, 119°46′W); 28 were from a loft in Woodlake, California (36°24′N, 119°5′W). Direct microscopic examination for organisms was performed by instilling a drop or two of 0.85% saline into the back of the mouth of a bird held on its back. This was immediately withdrawn into the pipette, placed on a microscope slide, and examined. Direct examination, as done in the present study, was not intended as a primary diagnostic technique. It only provided insight into the minimum number of cultures we might expect to be positive.

Culture specimens were collected by swabbing the mouth and upper crop of the bird with a dry cotton-tipped swab. The swab was then introduced into one medium or the other. The procedure was then repeated with a new swab for the other culture system. The medium for first inoculation was alternated between the two.

When the swab was introduced into a glass tube, its tip was pressed and rolled against the wall in the medium to express material from the cotton. It then was removed from the tube. Culture tubes were

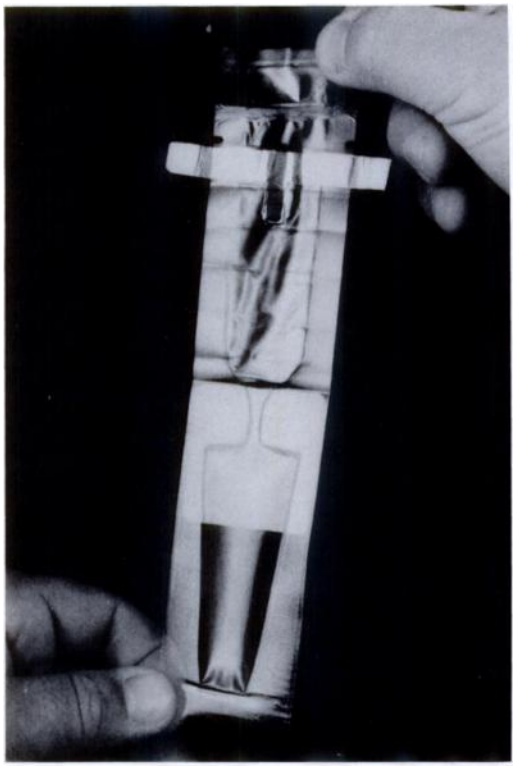

FIGURE 1. The InPouch®TF diagnostic pouch. Note the two tapered chambers separated by a narrow channel. The lower chamber holds 4 ml of medium.

closed, labeled, and incubated vertically at 37 C.

Following the manufacturer's directions, a small volume of medium was forced into the upper chamber from the lower one of an InPouch (Fig. 1) and the pouch opened. The swab was introduced into the medium in the upper chamber and its tip rolled between the fingers through the wall of the pouch. After the swab was removed, the medium was squeezed back down into the lower chamber. The top of the pouch was rolled down and the pouch sealed with the attached tabs. After labeling, pouches also were incubated vertically at 37 C.

Cultures were examined for trichomonads at 12, 36, 60, and 84 hr after incubation. In vitro cultures were examined by mounting a drop of medium, collected aseptically from near the bottom of a tube, on a slide and searching microscopically for motile organisms with both low (100×) and high dry powers (400×). Pouches were examined at both magnifications directly through the wall after attaching a plastic viewing clamp, supplied by the manufacturer, to the lower end of the pouch and fitting the clamp into the mechanical stage of the microscope.

Of the 50 birds examined in this survey, 30 were found infected with *T. gallinae* by one or more of the techniques. We detected one infection by direct examination that was not found by either culture method. We detected two infections with the InPouch system not found by culture in Diamond's medium (Table 1). Using a Chi Square test (Sokal and Rohlf, 1981), there was no significant difference ($P > 0.05$) between the two culture methods.

The two culture media also were very similar in the time required to detect infection (Table 2). The median incubation time required was only 12 hr in both culture systems. New positive samples were not found in InPouch after 60 hr. Fungal contamination apparently was the basis for the differences in results between media.

TABLE 1. Success of three methods in the detection of 30 *Trichomonas gallinae* infections in 50 pigeons.

	Direct examination	Diamond's medium	InPouch system
This method[a]	22/30	27/30	29/30
This method only[b]	1/30	0/30	2/30

[a] Total number of samples positive by the method identified in the column heading/total number of birds found positive in the survey.

[b] Total number of samples found to be positive only by the method identified in the column heading/total number of birds found positive in the survey.

TABLE 2. Numbers of cultures found positive for the first time after the specified incubation time.

Method	Incubation time				Totals
	12 hours	36 hours	60 hours	84 hours	
Diamond's	22	0	2	3	27
InPouch	26	2	1	0	29

Eight Diamond's cultures had heavy fungal overgrowth. In only one of these, where *T. gallinae* also could be detected, were organisms detected in 12 hr. In five, trichomonads were found at 60 or 84 hr of incubation. In two contaminated cultures, no trichomonads were found, although infection in these birds was demonstrated by InPouch. Fungal hyphae were found in only one InPouch. Nevertheless, trichomonads were detected in that culture 12 hr after inoculation.

Although the two culture systems are equally sensitive in the diagnosis of *T. gallinae* infection, the InPouch system has a number of advantages over Diamond's medium in glass tubes. The plastic pouch is lighter and easier to transport and handle in the field than is the usual glass culture tube. Pouches do not break nor easily spill. If pouches are kept vertical after inoculation, cells are concentrated by gravity in the bottom of the tapered chamber enhancing detection of parasites. Unlike tubes of Diamond's medium, once inoculated, an InPouch need not be opened again for repeated examinations. Aseptic technique in examining established cultures is not needed. The one year shelf life at room temperature of InPouch allows the convenient keeping of a supply of pouches.

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