VARIOUS GRAINS AND LIQUID AS POTENTIAL VEHICLES OF TRANSMISSION FOR Trichomonas gallinae

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VARIOUS GRAINS AND LIQUID AS POTENTIAL VEHICLES OF TRANSMISSION FOR *Trichomonas gallinae*

Although the main route of transmission of *Trichomonas gallinae* is from infected adults to their young, it has been suggested (Stabler, 1954, Exptl. Parasitol. III: 368-402) that domestic poultry and uninfected adult columbids may become infected via water and feed contaminated by infected feral pigeons. The occurrence of trichomonads in the upper digestive tract makes it possible for them to be expelled onto grain or into water during feeding or drinking.

The following experiments were designed to determine whether various liquid and solid materials ingested by pigeons could support *T. gallinae* long enough to make these materials suitable vehicles of transmission.

All tests were conducted in triplicate using two strains of *T. gallinae*. One was isolated from a feral pigeon and proved non-virulent for both doves and pigeons. The other was the highly virulent Jones' Barn strain obtained through the courtesy of Dr. Robert Stabler. All organisms were washed with 0.9% NaCl three times before being placed in or on the various test materials. The last organisms capable of being cultured from the test materials were placed in the mouths of *Trichomonas*-free pigeons to determine whether viability or virulence had been altered.

Experiment 1: Various salt concentrations. Distilled water (pH 6.5) and 0.01%, 0.05%, 0.1%, 0.6%, and 0.9% NaCl (pH 6.5) were tested as vehicles at 25 C and 35 C. The final trichomonad concentration was 6 to 9 x 10^4 organisms per ml (total 5 ml). Microscopic observations were made at 5-minute intervals to determine survival time. When motility was lost and only flagellar movement could be seen, the organisms were transferred to Diamond's medium. Although some organisms survived for at least 120 minutes in all concentrations, motility and survival were greatest and of longest duration in concentrations of 0.05% NaCl and above. All organisms were culturable and infectious for pigeons for up to 120 minutes and, in those maintained at 0.05% to 0.9% NaCl survival time was 180 minutes to 24 hours. There was no difference in survival or viability when cultures were maintained at 25 C and 35 C.

Experiment 2: Grain (moist). Two grams each of wheat, buckwheat, sorghum, cracked corn, and peas were soaked in 0.9% NaCl for 1 hour after which 1 ml of washed *T. gallinae* was added and thoroughly mixed with the grain (7.5 to 8.0 x 10^5 organisms). The seeds were then placed on pre-washed moist paper towels or filter paper in petri dishes and incubated at 35 C, 30 C, 25 C, and 10 C. Glass beads treated similarly served as controls. Ten to 15 seeds or beads were removed from the petri dishes at each temperature daily and placed in tubes of Diamond's medium at 35 C. Each tube was incubated and observed microscopically for at least 3 days, or until it became positive. The glass beads and whole peas maintained the trichomonads for only 24 hours while the cracked corn and wheat cultures were positive after 96 hours and the sorghum and buckwheat were positive after 120 hours. Lower temperatures (10 C and 25 C) resulted in 48 hours less survival time than did the higher temperatures (30 C and 35 C). The organisms on glass beads showed no better survival than would occur in 0.9% NaCl alone. Organisms were viable and infectious for pigeons and doves at each of the maximum survival times.

Experiment 3: Water extracts of grain. Ten percent (w/v) water extracts of each of the above grains were made and used as stock culture media. This was done by grinding each grain to a powder with a mortar and pestle and then soaking the powder in water for 24 hours. Concentrations of 5% in saline of the supernatants were used as supportive media at 35 C and 30 C to determine if the extended survival on soaked grain as compared to liquid was due to the grain constituents. Each tube (5 ml) received 3.75 to 4.0 x 10^5 trichomonads. All of

the water extracts of grains used were positive for motile *T. gallinae* after 168 hours with the exception of cracked corn which supported the organisms for less than 24 hours. There was no apparent difference in survival rate at 30 C and 35 C, and no apparent change in virulence occurred.

Discussion

It is apparent that *T. gallinae* is capable of surviving for sufficiently long periods on the vehicles tested here to be transmitted from an infected to an uninfected individual if both frequent the same feed lots or watering places; thus, losses of domestic poultry due to trichomoniasis (Gierke, 1933, Calif. Dept. Agr. Bull. 22: 205-208; Hawn, 1937, J. Infec. Dis. 61: 184-197; Levine and Brandly, 1939, J. Am. Vet. Med. Ass. 95: 77-78) may have been the result of contaminated feed or water.

The experimental conditions reported here probably would not be exactly duplicated under natural conditions, but it is certain that standing water in pastures or grain fields would have some dissolved materials which would raise the ionic concentration. Even if this were not the case, birds drinking side by side with infected columbids could become infected as indicated by the limited survival of the organism in distilled water.

The extended survival of trichomonads on the moist grain apparently resulted from their being capable of using the eluted grain constituents as an energy or food source. This is supported by the survival of trichomonads in the various grain extracts. Another possible explanation is that microbial contaminants which grow readily on the seeds secrete organic compounds which are used by the trichomonads, or they may break down constituents of the grain, producing substances which are then available to the trichomonads.

In addition to being good supportive media, the grains and their extracts apparently stimulated some multiplication of the organisms. This was assumed to be the case when numerous organisms could be seen in the seed cultures on the 3rd and 4th days of incubation, while prior to and following this time culture in Diamond's medium was necessary to observe the trichomonads.

Two of the seed types showed opposite effects when used whole and when solubilized. It is probable that the surface to volume ratio and shape of the whole peas resulted in little elution of the seed constituents. Crushed peas with a greater surface to volume ratio would be a more suitable substrate than whole peas. Presently I have no explanation for the effect with cracked corn but feel that this is also related in some way to the microbial flora which must exert a great deal of influence on such systems, much as they do in nature.

Although it is clear that *T. gallinae* can survive outside of the host on or in materials which are potential vehicles for transmission, it remains to be proven whether or not actual transmission can occur as a result of food or water contamination by infected pigeons.

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