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Experimental Infection of Turkeys and Chickens with a Clonal Strain of *Tetratrichomonas gallinarum* Induces a Latent Infection in the Absence of Clinical Signs and Lesions

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Summary

The pathogenicity of a mono-eukaryotic culture of *Tetratrichomonas gallinarum* in specific pathogen free (SPF) chickens and turkeys was studied. Two experiments of identical design were performed: the first with SPF chickens and the second with commercial turkeys. Each experiment included three groups. Groups 1 and 2 each contained 12 infected and three in-contact birds. The birds in these groups were infected on the first day of life, either cloacally (group 1) or orally (group 2). Group 3 consisted of four control birds. Re-isolation of the parasite from cloacal swabs was performed to verify the excretion of *T. gallinarum*. The infected birds excreted trichomonads from the second day post-infection. Spread of the flagellate from infected to in-contact birds was detected after 5 days post-infection (dpi), based on the re-isolation of the protozoa. No clinical signs or deaths were recorded in chickens or turkeys. Three birds were killed at 4, 8, 14 and 21 dpi and various tissues were collected for pathological examination. No gross lesions were noted. Protozoal DNA was demonstrated in the oesophagus, duodenum, jejunum, caecum, liver, lung, bursa of Fabricius and brain by polymerase chain reaction and in-situ hybridization. No antibodies were detected in the serum of infected birds by enzyme linked immunosorbent assay. Microscopical changes were only present in the caecum, where there was sloughing of the epithelium associated with the presence of numerous flagellates on the epithelial surface, within the crypts of Lieberkühn and in the lamina propria. These changes were found in caecal samples from infected and in-contact birds. These studies have demonstrated the rapid transmission of *T. gallinarum* between both turkeys and chickens and the establishment of a latent infection in both species.

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Introduction

Tetratrichomonas gallinarum, a member of the Trichomonadidae, was first described by Martin and Robertson (1911). The pathogenic potential of this protozoon has been discussed controversial (Allen, 1941; Kemp and Reid, 1965; Norton, 1997), but *T. gallinarum* is a common inhabitant of the intestinal tract of different poultry species (Friedhoff *et al.*, 1991). This controversy may relate to the frequent occurrence of mixed infections with other protists such as *Histomonas meleagridis* and *Blastocystis* spp. (Tyzzer, 1920). Co-infection with *T. gallinarum* and *H. meleagridis* has been demonstrated in the caecum and liver of naturally infected chickens and turkeys with histomonosis (Grabensteiner and Hess, 2006).

Although it is not clear whether *T. gallinarum* should be regarded as a primary pathogen, Weinzirl (1917) proposed *T. gallinarum* as a cause of fatal catarrhal enteritis in 1–4-week-old chicks and turkey poults. Trichomonads are known to induce lesions in the caecum and liver of domestic fowls and turkeys and should be considered as a causative agent of enterohepatitis (Allen, 1941). A further study reported

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the pathogenic potential of *T. gallinarum* in chickens infected cloacally with the caecal content of broiler breeder chickens naturally infected with *T. gallinarum* (Lee, 1972). Lesions induced within the caecum of these birds included loss of microvilli and reduction of the glycocalyx, and complete loss of the polysaccharide matrix was detected electron microscopically. In contrast, the pathogenic potential of *T. gallinarum* was not confirmed by Delappe (1957) who reported that a strain of *T. gallinarum*, which had been isolated originally from a liver lesion of a turkey, together with *H. meleagridis*, produced no symptoms or lesions after oral administration to young chickens and turkey poults. No clinical signs or histopathological changes were detected following rectal inoculation of turkeys with a *T. gallinarum* culture (Goedbloed and Bool, 1962). Kemp and Reid (1965) considered *T. gallinarum* as a non-pathogenic organism based on the absence of gross lesions and lack of mortality in infected birds. These authors did not report a decrease in body weight or drop in egg production in chickens and turkeys infected experimentally with a strain of *T. gallinarum* obtained from naturally infected birds.

The aim of the present study was to determine whether *T. gallinarum* isolated from turkeys could induce infection in chickens or turkeys and, if such infection was induced, whether the flagellate could be transmitted to non-infected in-contact birds. Additionally, the study aimed to investigate the clinical and pathological changes and the serological response following cloacal or oral infection of the host birds.

Materials and Methods

Animals

The study involved two experiments of identical design: the first with specific pathogen free (SPF) chickens and the second with turkeys obtained from a commercial hatchery. All birds were housed on deep litter (wood shavings) in rooms under negative pressure. On the first day of life each bird was individually identified by a numbered tag (Swiftack, Heartland Animal Health Inc., Fair Play, Missouri). Water and unmedicated feed for chickens (chicken starter feed) and turkeys (turkey starter feed) were provided *ad libidum*. Both experiments were approved by the institutional ethics committee and registered by Austrian law (license numbers 68.205/0017-BrGT/2005 and 68.205/0026-II/10b/2008).

Experimental Design

In both experiments, two groups of 15 birds (groups 1 and 2) were housed in one room in separate pens. Twelve birds in each of these groups were infected on the first day of life, while the other three were kept as in-contact birds. Birds of group 1 were infected cloacally, whereas those of group 2 were inoculated via the oral route. Four control birds (group 3) were kept in another room separate from the infected birds. Clinical examination was performed daily. All birds were weighed once each week throughout the experiments.

Culture of T. gallinarum

The clonal culture *T. gallinarum*/turkey/Germany/4114-C5/05 was established as described by Hess *et al.* (2006). The flagellate was isolated from a flock of turkeys in Germany that suffered from histomonosis. Following axenization this clone was grown in modified *Trichomonas vaginalis* medium (TV; Amin *et al.*, 2010). Each bird was infected with 10^5 trichomonads determined by a Neubauer cell counting chamber (Reichert, Buffalo, New York). The required number of live cells was adjusted in 300 µl modified TV medium for each bird. After infection, the birds were deprived of feed for 5 h. All birds were infected with the same passage number (18×) after thawing the clone from liquid nitrogen and propagating it for two passages.

Re-isolation of the Parasite

For re-isolation of the parasite, cloacal swabs were taken and incubated in 2 ml Eppendorf tubes containing 1.5 ml of fresh Medium 199 supplemented with Earle's salts + L-Glutamine + 25 mM HEPES + L-Amino acids (Gibco; Invitrogen, Lofer, Austria). 15% fetal calf serum (FCS; Gibco) and 2 mg of rice starch (Sigma Aldrich, Steinheim, Germany). The tubes were subsequently incubated at 40°C until the microscopical investigation. This sampling procedure was performed at intervals of 2–3 days throughout the experiments. The re-isolated *T. gallinarum* cells were examined microscopically after 48 and 96 h of incubation.

Serology

Blood samples were collected from all birds in both experiments weekly for the duration of the experiments. Samples were kept at 4°C for 24 h and then centrifuged (3,300g) for 12 min (Hettich Rotanta 460; Tuttlingen, Germany). The sera were stored at −20°C until tested. Serum antibody specific for *T. gallinarum* was detected by indirect enzyme linked immunosorbent assay (ELISA; Windisch and Hess, 2009) with a polyclonal rabbit serum raised against *T. gallinarum* as a positive control. The production of the rabbit antiserum specific for *T. gallinarum*

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