

Chapter 19

Research Progress on the Relationship between Avian trichomonosis and the Microbiota of the Oral Cavity and Intestine

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ABSTRACT

Avian trichomonosis is a parasitic protozoan infection that can affect various avian species, including poultry, and frequently results in high mortality rates among juvenile birds, thereby posing a significant threat to the pigeon farming industry. The oral and intestinal microbiota represent crucial components of animal health; they are complex microbial communities that establish long-term residence within the host and exhibit mutual dependence with it. There exists an intricate interplay between parasites and microbiota: the latter can influence the processes of invasion, colonization, and pathogenicity of parasites, while parasites have the capacity to modify the composition of microbiota. In-depth study of the interaction between *Trichomonas gallinae* and the host's oral and intestinal microbiota is not only of great significance for the prevention and control of avian trichomonosis but also has potential value for understanding broader host-parasite interaction mechanisms, developing new prevention and control strategies, and promoting human health. Through these studies, we can better protect the health of pigeons and other birds, while also providing valuable insights for the field of human medicine.

KEYWORDS

Avian trichomonosis, Microbiota, Oral Cavity, Intestines

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INTRODUCTION

Avian trichomonosis is a contagious disease caused by the protozoan *Trichomonas gallinae* (*T. gallinae*) which primarily affects pigeons and other birds. This disease is distributed worldwide and poses a serious threat to the pigeon breeding industry. The rock pigeon (*Columba livia*), as the main host of *T. gallinae*, is widely distributed and migratory in nature, which is considered a key factor in the global spread of the disease (Harmon et al., 1987). In pigeon populations, squabs have not fully developed immune systems, hence the infection rate in squabs is usually higher than in adolescent and breeding pigeons (Stabler, 1954). Pigeons infected with *T. gallinae* often develop yellowish-white caseous lesions in the oropharyngeal area, which not only affect the birds' feeding and breathing but may also impact the balance of the oral microbiota (Rogers et al., 2018). An imbalance in the oral microbiota can lead to other oral diseases, further affecting the health of pigeons. The intestinal microbiota is one of the most complex and important ecosystem in an animal, playing a key role in the health and disease of the host. Under normal conditions, the intestinal microbiota is generally in a state of dynamic equilibrium, which is crucial for the host's health, however, this balance can be disrupted by various factors, such as age, diet, pathogens, etc. Studies have shown that parasites colonizing the host can directly or indirectly affect the dynamic balance of the microbiota (Mehlhorn et al., 2009). For example, parasites may affect the composition and function of the intestinal microbiota by consuming nutrients within the host, secreting toxins or altering the host's physiological environment.

Currently, there is no effective vaccine available for avian trichomonosis. Therefore, a deep understanding of the interaction between *Trichomonas gallinae* and the host's oral and intestinal microbiota is of great significance for developing new prevention and control strategies. Studying these interactions can better understand how *Trichomonas gallinae* survives and reproduces within the host and how they affect the host's health. This knowledge can provide a reference for the research and development of new therapeutic drugs, vaccines, or management strategies to reduce the harm of avian trichomonosis to pigeons and other birds.

Overview of *T. gallinae*

The *T. gallinae* belongs to the Phylum Protozoa, Class Mastigophora, Subclass Zoomastigophorea, Order Trichomonadida, Family Trichomonadidae, and Genus Trichomonas, has a pear-shaped body with numerous wrinkles and indentations on its surface. It measures 5-9 μ m in length and 2-9 μ m in width. Under the microscope, the live organism moves rapidly with a spiral swimming pattern. It is characterized by having four free flagella of varying sizes, which are typical features and serve as its primary locomotive organs. The four anterior flagella are closely packed together, emerging from the flagellar pocket. The wall of the pocket having crescent-shaped membranous structures is visible to the cell's anterior end, and the flagella extends from a single axostyle on one side (Mehlhorn et al., 2009). The organism possesses an undulating membrane that starts at the front end and ends just in front of the rear end, resembling a fish fin, with several distinct undulations and sometimes fewer and shallower ones. The outer edge of the undulating membrane is composed of paraxial rods and attached recurrent flagella, which are not free and are located within the grooves of the undulating membrane, extending beyond the end of the membrane. Under the optical microscope, a single axostyle can be seen, often extending beyond the rear edge of the cell, running from the anterior end to the caudate posterior end of the trichomonad, positioned near the longitudinal axis of the cell. The terminal part of the axostyle protrudes about one-third of the cell length beyond the posterior surface of the organism. *T. gallinae* has a simple morphology, being a single-celled structure without mitochondria, but it does have hydrogenosomes. The organism is semi-transparent and relatively bright. With no complex life cycle, the organism exists only in the hape of the rophozoite stage and reproduces by binary fission. It can reproduce a generation approximately every 4 hours.

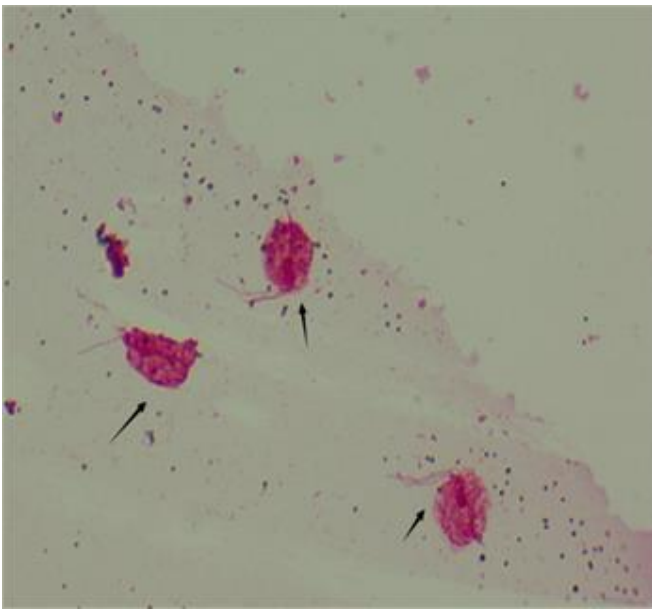


Fig. 1: Microscopic image of *Trichomonas gallinae* stained with Gram stain (1000X).

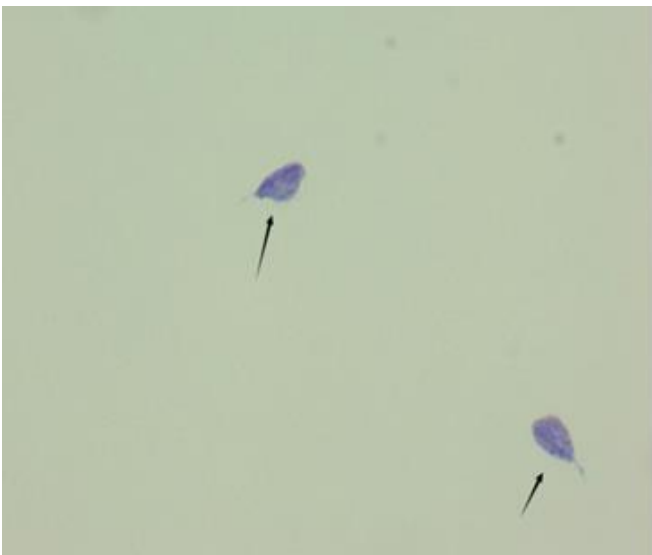


Fig. 2: Microscopic image of *Trichomonas gallinae* stained with Giemsa stain (1000X).

Overview of Avian Trichomonosis

Avian trichomonosis predominantly affects birds' upper respiratory and digestive tracts, with a common occurrence in the pharynx, esophagus, and liver. It can cause severe ulceration and yellow-white caseous necrotic lesions in the digestive tract of pigeons. In severe cases, it may lead to systemic infection. In 2022, it was observed that *T. gallinae* can invade the

heads of avian species, parasitizing the brain and eyes. The most important route of transmission between birds is through the ingestion of trophozoites via shared water and food sources facilitated by saliva (Villanúa et al., 2006; Gerhold et al., 2007). It has been suggested that the transmission of parasites via crop milk from infected parent birds to squabs seems most efficient for establishing an infection (Harmon et al., 1987). Infected adult pigeons often do not show significant symptoms, becoming asymptomatic carriers and a new source of infection. Additionally, birds of prey can become infected by consuming infected prey, as *T. gallinae* can survive in carcasses for at least 48 hours. The parasite can persist in various water sources for up to an hour (Purple et al., 2015), such as drainage ditches and water drinkers. However, higher temperatures (30–35°C) can extend its survival time (Kocan, 1969). Although it can form pseudocysts under adverse conditions, a moist environment is crucial for maintaining its infectivity (Stabler, 1954). Clinical manifestations of infection vary from mild to severe, with severe, potentially fatal inflammation causing birds to die from starvation or asphyxiation by obstructing the esophagus and respiratory tract. *T. gallinae* are generally considered normal residents (symbionts) on the mucosal surface of the upper digestive tract. However, by causing inflammation in the underlying tissues or when entering the more distal parts of the avian digestive tract, this protozoan parasite can cause mild to severe lesions, depending on the strain's virulence and the host's susceptibility. Infection with highly pathogenic strains can lead to death. Laboratory diagnosis typically includes direct smear microscopic observation and microscopic observation after in vitro culture to observe the living *T. gallinae* as the key to confirmation. In addition, molecular biological techniques such as PCR, RAPD, RFLP, and high-throughput sequencing can also be used for more sensitive diagnosis (Turner et al., 2023). In terms of prevention and treatment, there is currently no effective vaccine, and nitroimidazole drugs are usually used for prevention and treatment. The method of drug use needs attention because some drugs, such as metronidazole, have certain toxic side effects and should not be used arbitrarily in increased dosage or extended course. Generally, a course of treatment is preferable for five days (Gómez-Muñoz et al., 2022). In addition to using drugs, it is also necessary to strengthen breeding management, reduce breeding density, supplement nutrients, maintain a dry and sanitary environment, regularly check health conditions, and timely detect and treat infections. At the same time, for dead pigeons, they should also be treated harmlessly in a timely manner, and effective disinfection work should be carried out to avoid causing widespread transmission.



Fig. 2: Pictorial dissection of the head in pigeons with avian trichomonosis.

The Interaction between Avian Trichomonosis Infection and the Oral Microbiota

It is generally understood that the intricate interactions between microbiomes and host-parasite microorganisms can be bidirectional and significantly impact animal health. Dietary changes, host morphology and phylogeny, captivity, antibiotic treatment, age, gender, and the presence of certain diseases can all affect oral microbiota composition. There is little evidence available about how trichomonosis infection affects the composition, structure, and dynamics of avian oral

microbial communities. Previous research indicates that Cooper's hawks' susceptibility to trichomonosis is substantially connected to the age-specific pH of the oral cavity (Urban et al., 2014).

The mean pH of fluid in the oral cavity of nestling Cooper's hawks is 6.8, whereas that of fledglings and adults is 6.0–6.1, which is at least seven times more acidic. *Trichomonas gallinae* thrives when pH is between 6.5 and 7.5 (optimum 7.2). Fluid in the oral cavity of Cooper's hawks becomes more acidic after birds have fledged and are nearing independence (≥ 50 days of age), but the reason for the change is unknown (Urban et al., 2014). Many animals undergo similar changes in body chemistry (i.e., a change in acidity) during maturation, which are often associated with changes in their bacterial communities. Therefore, Taylor et al. (2019) hypothesized that oral microbiota composition may be related to age-specific differences in susceptibility to *T. gallinae* by comparing the oral microbiota of nestling and adult Cooper's hawks. The study found significant differences in the oral microbiome composition between nestling and juvenile/adult Cooper's hawks, which is surprising given the feeding behavior of the species. Breeding adults consume the same prey as that fed to the nestlings, so dietary changes at different ages are unlikely to play a role in the observed changes in the oral microbiome.

There is little study on the relationship of *T. gallinae* with the oral microbiome. Claudio Alba et al. (2023) investigated how captive breeding and trichomonad infection affected the oral microbiome of Bonelli's eagle nestlings. Bonelli's eagle's core oral microbiota comprises Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria, with *Megamonas* and *Bacteroides* being the most numerous taxa. The study discovered that trichomonad infection had a minor impact on the microbiota composition, with a considerable increase in the relative abundance of the *Gemella* genus in eaglets with trichomonosis lesions. This genus lives on the oral mucosal surface and is an opportunistic pathogen known to cause human abscess problems, inflammation, and abscesses in various places. Abscesses, comparable to those seen in human endocarditis, meningitis, and orbital or maxillary abscesses (Maraki et al., 2019; McQuinn et al., 2019) were found in highly infected birds. *T. gallinae*-infected eaglets also contained a larger proportion of planktonic bacteria. This phylum is found in a variety of environments, including marine, freshwater, and wastewater treatment plants, and its presence in water sources aids in the spread of *T. gallinae*, which may explain its link with the parasite.

The Interaction between Avian Trichomonosis Infection and the Intestinal Microbiota

The intestinal microbiota, a complex community of microorganisms living in the animal's gut, plays a crucial role in the function of the intestinal barrier, including promoting nutrient absorption and digestion, maintaining intestinal physiological functions, and regulating the body's immune system. Under normal conditions, the population density and composition of the animal gut microbiota are in a state of balance, which can be altered by various factors such as age, diet, and pathogens. The interaction between parasites and gut microbiota is significant for maintaining intestinal homeostasis. Parasitic infections can lead to changes in the gut microbiota, dysbiosis, and inflammatory diseases (Faivre et al., 2019). Gaining a deep understanding of how parasites affect the composition and function of the host's intestinal flora may, in turn, affect the infection and pathogenicity of the parasites through these changes. Research into the interplay between parasites and the host's intestinal microbiota can also provide references for other areas of study. For example, understanding how parasites affect the host's immune system can help develop new immune modulation strategies to enhance the host's resistance to other pathogens. At the same time, these studies can also provide insights for the field of human medicine, as many human diseases are also related to the imbalance of the gut flora, such as inflammatory bowel disease (Sultan et al., 2021), obesity, and diabetes, etc (Ortega et al., 2020; Madhogaria et al., 2022). However, recent research had found that helminths had potential therapeutic effects on inflammatory bowel diseases, challenging the conventional notion that parasites are generally harmful to humans (Wang et al., 2020).

Infection with *T. gallinae* can affect the diversity and composition of the pigeon's gut microbiota. When comparing the richness of different microbial communities in the small intestine and rectum of healthy pigeons, it was found that the *Lactobacillus* genus was dominant. However, after infection with *T. gallinae*, the richness of Firmicutes and *Lactobacillus* decreased, while the richness of Proteobacteria, *Enterococcus*, *Atopobium*, *Roseomonas*, *Bifidobacterium*, and *Peptostreptococcus* increased (Ji et al., 2020). When 14-day-old pigeons were infected with *T. gallinae*, the richness of the crop microbiota significantly decreased, with the proportion of *Lactobacillus* reducing by at least 90%. *Lactobacillus* is a beneficial microorganism that competes with pathogens through adhesion and replication, produces pathogen-resistant complex substances, and regulates immune functions, playing an important role in maintaining intestinal health (Sengupta et al., 2013). The reduction in the abundance of *Lactobacillus* may lead to drastic changes in the intestinal environment, for example, pH, followed by an apparent increase in the abundance of harmful bacteria, such as *Enterococcus* and *Atopobium*. *Enterococcus* is an important pathogen for chickens, ducks, and pigeons, and it exhibits intrinsic or acquired resistance to many antibiotics (Osman et al., 2019). *Atopobium vaginae* may be a marker for bacterial vaginosis in women (Marconi et al., 2012). Besides, *T. vaginalis* was reported to be associated with vaginal microbiota consisting of low proportion of *lactobacilli* and high proportions of *Mycoplasma*, *Parvimonas*, *Sneathia*, and *Atopobium* (Brotman et al., 2012).

The growth and development status of pigeons is closely linked to their ability to resist invasion by foreign pathogens. Squabs have weaker immunity, and the normal bacterial composition of the intestinal barrier is not fully established. Several experimental studies have confirmed that probiotics can promote the growth and development of the body (Chen et al., 2017). Before infection with *T. gallinae* in the early age of pigeons, administering *Lactobacillus* can help beneficial bacteria form a dominant population in the pigeon's gut, thereby preventing infection with *T. gallinae* (Ji et al., 2020). Administering *Lactobacillus* after infection can improve the body's immunity to a certain extent, stimulate the body's

immune response, inhibit the growth and reproduction of *T. gallinae*, reduce the infection rate of *T. gallinae*, and promote the body's clearance of *T. gallinae*.

Summary and Outlook

With the development of high-throughput sequencing technology, especially the second-generation sequencing technology (Next-Generation Sequencing, NGS), the impact of parasitic infections on the species diversity and community structure of the host microbiota is increasingly receiving attention, and the understanding of the complex interactions between parasites and host microbiota is also becoming more in-depth. In the host's oral and intestinal tracts, microbial communities are closely related to the host's health status. These microbial communities participate in a variety of physiological processes, including nutrient metabolism, the development and regulation of the immune system, and defense against pathogens. Parasitic infections may disrupt the balance of these microbial communities, leading to a decrease or increase in species diversity and changes in community structure (Berrilli et al., 2012). This imbalance may affect the host's health and increase the risk of disease. Avian trichomonosis, as a global parasitic disease, poses a serious threat to the pigeon breeding industry and wild bird populations. Currently, the interaction between *Trichomonas gallinae* and the host's oral and intestinal microbiota is still in the initial stage of exploration. Studies have shown that *Trichomonas gallinae* may affect the microbial composition in the host's oral and intestinal tracts, thereby affecting the host's health. For example, infection may increase the number of certain pathogenic bacteria or reduce the number of beneficial microbes. These changes may affect the host's immune response and disease progression, making the host more susceptible to other diseases.

Probiotics are a class of beneficial microorganisms that can affect the ability of intestinal microbes to participate in the regulation of various biological processes in the host through multiple mechanisms. This includes interacting with host sex hormones, regulating stress responses and cognitive conditions, and affecting central nervous system-related functions through the microbiota-gut-brain axis (Le Morvan de Sequeira et al., 2022; Ashique et al., 2024). Probiotic interventions have shown great potential in combating parasitic infections and have been proven to reduce the pathogenicity of parasitic infections, providing new strategies for the treatment and prevention of avian trichomonosis. They can enhance the host's immune system, inhibit the growth of pathogens, or improve the structure and function of the microbial community through interactions with the host's microbiota. In addition, the antiviral effects of probiotics have also attracted attention. Probiotics may function by enhancing the intestinal mucosal barrier, stimulating the body's immune system, inducing anti-inflammatory cytokines, downregulating pro-inflammatory cytokines, and inhibiting signaling pathways (Petrariu et al., 2024).

Based on the understanding of the interaction between *Trichomonas gallinae* and the host microbiota, it also lays the foundation for the development of new targeted antiparasitic products, which may have higher selectivity and lower side effects. In the research and development of targeted antiparasitic products, innovative antiparasitic drugs and treatment methods are continuously being introduced. For example, the canine antiparasitic drug BRAVECTO QUANTUM launched by Merck, which is administered by injection, can provide protection against fleas and ticks for up to one year, showing new progress in antiparasitic product innovation (Fisara et al., 2023).

By delving into the interplay between *Trichomonas gallinae* and the host's oral and intestinal microbiota, we can provide more effective strategies for the treatment and prevention of avian trichomonosis, laying a scientific foundation for the development of new targeted antiparasitic products, and also provide references for the study of other parasitic diseases. These studies not only help to improve the benefits of poultry farming but may also have a positive impact on human health, as many principles of parasitic disease prevention and treatment are common between birds and humans. With the application of NGS technology and further research on probiotic interventions, we hope to achieve more precise and personalized prevention and treatment of parasitic diseases in the future.

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