Chapter 60

Recent Advancements on Alternative Control Measures of *Histomonas meleagridis*

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ABSTRACT

Histomonas (*H.*) *meleagridis*, a unicellular protozoan parasite, is the causative agent of histomonosis, commonly known as blackhead disease, predominantly affecting turkeys and also chickens. Transmission primarily occurs through the intermediate host, *Heterakis gallinarum* (roundworm), with both either direct or indirect routes. The parasite's unique characteristics, including pleomorphism and reliance on hydrogenosomes for energy metabolism, pose challenges for control and treatment. Despite the impact of this disease on the poultry industry, there are limited approved prophylactics, therapeutics, or vaccines available for disease management. Traditional control measures, such as antihistomonal compounds like nitarsone and dimetridazole, due to regulatory restrictions and toxicity concerns necessitate the exploration of alternative strategies. Recent advances in disease control encompass use of vaccines, prebiotics and probiotics, plant extracts, nutraceuticals, and improved management practices. Prebiotics and probiotics offer promising avenues by modulating the gut microbiota to create an inhospitable environment for *H. meleagridis* colonization. Plant extracts have shown efficacy *in vitro* in treating *H. meleagridis* infection, providing a potential natural alternative to chemical treatment. Nutraceuticals, food-based substances with health benefits, are being explored for controlling histomoniasis on farms. Vaccination and management strategies like bedding/litter hygiene and biosecurity protocols are promising for disease prevention, but standardization and field administration remains a challenge. Overall, a multifaceted approach integrating novel control methods tailored to specific farm conditions is essential for effective histomoniasis management to reduce the impact of *H. meleagridis* on poultry production. By utilizing a combination of different control measures, the poultry industry can strive towards reduced disease incidence, under a healthy production environment.

KEYWORDS
Histomonas meleagridis, Poultry, Nutraceuticals, Histomoniasis, *Heterakis gallinarum,* Efficacy

Received: 07-Jun-2024
Revised: 17-Jul-2024
Accepted: 13-Aug-2024

A Publication of Unique Scientific Publishers

Cite this Article as: Khalil I, Abbas S, Zaman MA, Vemprala S, Hayat S, Inayat I, Sarfaraz MZ, Zulqarnain M, Bibi S, and Jafar Z, 2024. Recent advancements on alternative control measures of *Histomonas meleagridis*. In: Abbas RZ, Khan AMA, Qamar W, Arshad J and Mehnaz S (eds), Complementary and Alternative Medicine: Botanicals/Homeopathy/Herbal Medicine. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 510-516. https://doi.org/10.47278/book.CAM/2024.473

INTRODUCTION

Histomonas (*H.*) *meleagridis* is a parasite that mostly affects turkeys and chickens and causes histomonosis, which kills 80 to 100% of flocks. There are no approved medicines, vaccines, or preventative measures to fight against this disease. It is often known as blackhead disease because it causes sulfur-colored diarrhea and, on rare occasions, a blue-colored head. Histomonosis is typically characterized by cecal and liver damage. It has become challenging to treat *H. meleagridis* since the removal of nitarsone in 2015 (Bleyen et al., 2010). *H. meleagridis* belongs to the phylum Parabasalia, class Tritrichomonadea, order Tritrichomonadi,da and family Monocercomonadidae (Beer et al., 2022). *H. meleagridis* is a pleomorphic parasite with single-celled body, axosytle, pelta, parabasal bodies, and hydrogenosomes, exhibiting characteristics of both amoeba and flagellates (Hess and McDougald, 2013). Cushman described histomonosis in turkeys over 100 years ago (Cushman S. 1893). After that, Chester and Robin reported that *H. meleagridis* parasite can also infect chickens. Based on the evaluation of 110 fresh stool samples, it was discovered that 31% of poultry in Lorestan Province, Western Iran, had *H. meleagridis* (Badparva and Kheirandish, 2017). Turkey's production fell from 6.5 million in 1900 to 3.6 million in 1920, indicating that histomoniasis posed a serious risk to poultry farming (Animal and Plant Health Inspection

Service, 1984). The prevalence of *H. meleagridis* in Pakistan is significant. Studies on the parasite's survival away from the host have shown that it can live on a variety of surfaces, which may contribute to its widespread distribution across Pakistani poultry farms (Umar et al., 2016). Blackhead treatment and prevention in veterinary medicine have been significant in the 20th century, with the use of commercial drugs. *H. meleagridis* strains in Pakistan exhibit varying sensitivities to different treatments. Research indicates that Pakistani strains of *H. meleagridis* are more susceptible to nitarsone than to metronidazole, suggesting the use of nitarsone instead of metronidazole to enhance the prevention and management of poultry histomoniasis. However, the European Union's ban on prophylactics and chemotherapeutics in food animals in the 1990s and early 21st century has increased outbreaks of *H. meleagridis*, making blackhead a significant turkey disease in the poultry industry (Bleyen et al., 2010). From June 2014 to September 2016, 13 outbreaks of histomoniasis affected eight meat turkey farms in Austria, resulting in 75,300 turkeys affected. 28,000 died or had to be euthanized, confirming the high fatality of the disease (Sulejmanovic et al., 2017) So, it becomes necessary to control *H. meleagridis* by reducing mortality, preventing disease and its spread, improving overall bird health and food safety, and maintaining a healthy poultry industry. High mortality rates in histomoniasis outbreaks are exacerbated by concurrent infection, as confirmed in a newly constructed barn with *H. meleagridis* and hemorrhagic enteritis virus (Durairaj et al., 2024). Stress in poultry production affects bird behavior, gut health, and disease susceptibility. Studies have shown that feed withdrawal and reduced crude protein diets can increase infection rates and cecal lesions in turkeys. Nutrition and gut health are crucial in histomoniasis disease progression. The study also found that fecal-oral infection could be a potential transmission pathway during commercial production (Fudge, 2022).

Life Cycle

Bilic and Hess, (2020) explained that *H. meleagridis* can be introduced into the caeca of its host by direct (oral) uptake of the protozoa from the other birds' caecal discharges, cloacal drinking, and indirect methods (earthworms) and start dividing in the lumen of turkey and chickens (Hu et al., 2004). *H. meleagridis* transmission primarily occurs through a cecal roundworm, *Heterakis gallinarum*, which acts as a vector for the protozoa, leading to infection in carrier birds like turkeys and chickens. Direct transmission can occur rapidly, after that *H. gallinarum* replicates and degrades the cecal lining of the host (Liebhart et al., 2017). Eggs of *H. gallinarum* will be eliminated from the host body along with the fecal material/feces and then become deposited in the soil as described in Fig. 1. At specific environmental factors, these eggs become embryonated. Other birds become infected by ingesting these embryonated eggs of the *H. gallinarum* (caecal worm), which contains *H. meleagridis*. *H. meleagridis* lacks mitochondria, hence it reproduces using a method known as binary fission, which relies on their hydrogenosomes as modified organelles for energy metabolism. Within the host or intermediate host, in vitro cultured *H. meleagridis* can live only for a few hours at most (Lotfi et al., 2012). *H. meleagridis* can infect chickens directly through the droppings of infected birds or indirectly through earthworms (Nguyen et al., 2015). *H. meleagridis* excretion in chicken lines after co-infection with *Heterikus. gallinarum* and *Ascaridia galli* have been studied.

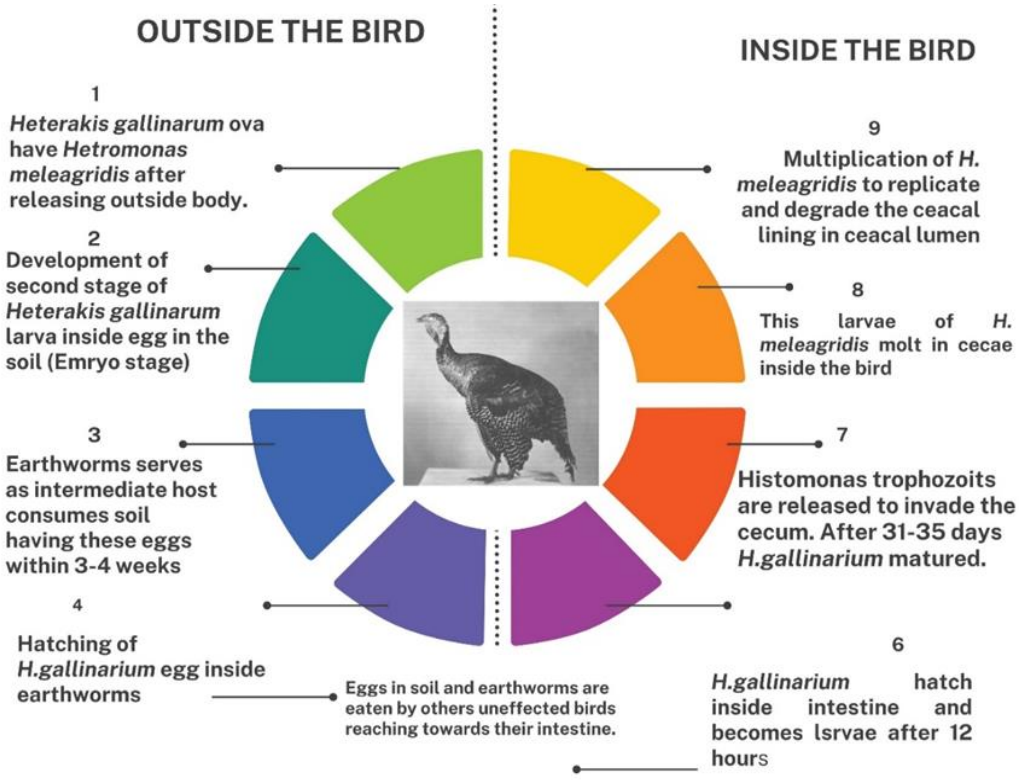

Fig. 1: Transmission of *H. meleagridis* via embryonated eggs of *Heterakis gallinarum*.

Chickens are less susceptible than turkeys to the disease but can serve as reservoirs. Turkeys and chickens show resistance to re-infection from *H. meleagridis*, with live-attenuated isolates as potential vaccines. Natural resistance against both parasites varies among chicken breeds, leading to distinct genetic lines for egg and meat production. (Zuidhof et al., 2014; Das et al., 2021; Hernandez, 2014; Beer et al., 2022).

Right; Inside the Bird; **Left: Outside the Bird**

Chickens experience less clear clinical signs of infection, leading to high mortality. Bloody stool, body weight imbalance, and egg production drop occur. Clinical signs typically develop 7-14 days after infection, with co-infection of coccidia potentially, broadening symptoms (Beer et al., 2022) while clinical signs in turkeys include drooping head and wings, prolonged standing, closed eyes, dark skin pigmentation, ruffled feathers, emaciation, and sulfur-colored feces (McDougald et al., 2020). With the help of microscopic examination of cecal content, cecal and liver scrapings, and histological examination of cecal or liver tissue from turkeys and chickens, *H. meleagridis* can be detected. Typical lesions are seen in the caeca caused by *H. meleagridis* after an experimental infection in chickens. PCR could be a useful tool for rapid and routine screening of *H. meleagridis* (Huber and Zenner, 2005).

Empirical Control Measures

In the past, efforts to prevent the disease focused on the effect of chemical substances. The empirical control measures of histomoniasis involves use of antihistomonal compounds like arsenicals, nitroimidazol es,nitrofu,rans and carbamates which were effective in the 1960s and 1970s. Additionally, chemotherapies like Histostat-50 and paromomycin were explored, but concerns about antibiotic resistance were raised in birds and human health (Collins et al., 2021). Many antihistomonal Compounds were used against histomoniasis as described in Fig. 2 from which following were used in the past:

Arsenicals (Nitarsone)

It disrupts DNA repair processes and cellular energy metabolism, causing cell death. Nitarsone, also inhibits ATP synthesis in *H. meleagridis*, leading the parasite death. The poultry industry has employed arsenicals particularly nitarsone to control blackhead disease, by reducing ceca lesion severity and mortality rates (Peek and Landman, 2013). Nitarsone is an organoarsenic compound. It is a poultry feed additive thastillt improves weight gain and feeding efficiency while preventing blackhead disease. In 2015, the U.S. market withdraw nitarsone, but its use in other countries including Pakistan stil persists (Abraham et al., 2014).

Nitroimidazoles (Dimetridazole)

Historically, nitroimidazoles like ronidazole, ipronidazole, ornidazole and dimetridazole were effective for treatment were very effective against *H. meleagridis* (Ellis et al., 1964; Liu et al., 2023). Nitroimidazoles, particularly 4-(3,4-dichlorophenyl)-1-methyl-5-nitroimidazole, have good activity against *H. meleagridis*, indicating the potential for controlling this protozoal infection. Nitroimidazole compounds serve as both antibiotics and antiprotozoal medications. The 5-nitro functional group of the imidazole ring is the active part of the nitroimidazoles that damage the parasite's DNA. Dimetridazole, an effective antihistomonal compound, to treat *H. meleagridis* infections in galliform birds, particularly turkeys and chickens (Van der Heijden, 2009; Umar et al., 2016; Liebhart et al., 2017).

Nitrofurans

The furan ring's 5-nitro group determines the mechanism of action for nitrofurans. The chemical is reduced by the enzyme nitrofuran reductase inside the cells to many reactive intermediates, which damage the DNA of microorganisms. It reduces cecal and liver lesion scores in infected birds similar to nitarsone (Umar et al., 2016). Nitrofurans like Nifursol were used against *H. meleagridis*, but in 2003, their ban left a gap in preventive measures, causing a resurgence of histomoniasis in poultry (Zenner, 2005).

Carbamates

Diethyldithio-carbamates, organic compounds derived from carbamic acid, inhibit enzymes like superoxide dismutases and metalloproteinases by replacing oxy-gen atoms with sulfur atoms. (Hogarth, 2012). In Vivo tests showed that these substances had the potentialto treat histomonosis. Additionally, dithiocarbamates, including diethyldithiocarbamates, have been recognized for their antioxidant properties and their ability to counteract oxidative stress induced by compounds like diclofenac. The use of above mentioned antihistomonal compounds was later banned due to toxicity concerns. Blackhead disease, a serious health concern in poultry, has currently no approved drug for use in the affected turkeys and chickens as the US has banned the use of antihistomonal compounds. Research on blackhead control is limited, focusing on preventive measures to reduce the parasite's impact on poultry populations, rather then availability of effective drugs (Clark and Kimminau, 2017).

Alternative Measures

Using antihistomonal compounds such as arsenicals (nitarsone), nitroimidazoles (dimetridazole), nitrofurans (nifursol), and carbamates(dithiocarbamates) for controlling *H. meleagridis* infections in poultry can pose several hazards, including

toxicity concerns, the development of antibiotic resistance, environmental contamination, regulatory restrictions, and many other human health risks. All these compounds have historically been used to control *H. meleagridis* infections in poultry, but their excess use causes many risks. So, it is essential to explore alternative control methods to ensure safe poultry products and protect poultry, human, and environmental health. Following is an account of alternative control measures that can be taken for the treatment and control of histomoniasis.

Vaccines

Despite potential preventive measures like attenuated histomonads, production challenges hindered the widespread use of vaccines for treatment in turkey and chicken (Nguyen et al., 2020). Birds that recover from infection by antihistomonal treatment can develop resistance against histomonosis, supporting the idea of the use of vaccines. Attenuated histomonads stimulate immunity in turkey and chicken but do not offer protection. The parasite can be grown and attenuated in vitro, but only with bacteria. This is effective but intracloacal applications make use of live vaccines difficult (Hauck and Macklin, 2024). Liu et al. (2023) cloned the 1839-bp α-actinin 1-encoding gene of *H. meleagridis* an d expressed it in the Prokaryotic Expression Vector PET28a(+). Results revealed that Hmα-actinin 1 may be an important virulence factor and stimulate humoral and cellular immune responses against *H. meleagridis* infection in chickens (Liu et al., 2023; Hauck and Macklin, 2024; Liebhart et al., 2017). Mitra et al. (2021) examined the interaction between virulent and attenuated *H. meleagridis* and innate immune systems in turkeys and chickens. They found significant changes in TLR expression as the results of attenuated *H.meleagridis* exposure. The study suggests that Toll Like Receptors (TLR) expression is crucial for immune protection. Experimental trials show effective histomoniasis vaccination using attenuated *H. meleagridis* strain, but standardization and field administration are still required. No vaccines are currently approved for trethe atment of histomoniasis and comcommerciallyailable in poultry birds (Van der Heijden, 2009; Liebhart et al., 2017).

Prebiotics and Probiotics

Prebiotics redreducet infections, and boost immunological response in chickens, resulting in faster infection clearance (Pourabedin and Zhao, 2015; Ajuwon, 2016). Prebiotic Natustat showed efficacy against *H. meleagridis* in male turkeys, improving feed conversion ratios, reducing cecal and liver lesions, and increasing body weight compared to infected non-supplemented turkeys (Duffy and Power, 2005). Probiotics balance cytokines, improve barrier integrity and enhance both innate and humoral immunity (Prado-Rebolledo et al., 2017). Probiotics such as Lactobacillus and Bifidobacterium strains have shown promise in reducing the incidence and severity of histomoniasis. Researchers are investigating the use of prebiotics and probiotics to manipulate the gut microbiota of poultry animals by creating an environment that is less conducive to *H. meleagridis* colonization.

Plants Extracts

Recently, due to a lack of authorized and efficient medications to control *H. meleagridis* plant products have received a great attention. This was particularly preferred since active plant components may combat certain parasite infections through direct and indirect ways (Anthony et al., 2005). Different herbal compounds are effective against different protozoans (Grabensteiner et al., 2008; Harold et al., 2008; Aline et al., 2023). Essential oils commercial plants known as phytosynthese such as cinnamon (*Cinnamomum verum*), lemon (*Citrus limon*), rosemary (*Salvia rosmarinus*), garlic (*Allium sativum*), and thyme (*Thymus*) are surveyed for their antihistomonal effects and ability to suppress the growth of parasites when added to *in vitro* culture of histomonads (Bolouri et al., 2022; Hafez et al., 2006; van der Heijden, 2011). Additionally, one study investigated various plant extracts for their efficacy against *H. meleagridis*, with ethanolic extracts of thyme (thymus), saw palmetto (*Serenoa repens),* grape seed (*Vitis vinifera*), and pumpkin fruit (*Cucurbita pepo*) showing effectiveness *in vitro* (Zaragatzki et al., 2010).

Nutraceuticals

Nutraceuticals are dietary supplements used to improve health, prevent diseases, and support bodily function. With a global market of approximately USD 117 billion, they are categorized as herbal bioactive compounds. Nutraceuticals have shown promising results in treating various diseases. (Sachdeva and Bharadvaja, 2020). Researchers suggest the use of nutraceuticals for the control of hisomoniasis on farms. Schildknecht and Squibb (1979), found that when vitamin E was added to an antihistomonal compound such as ipronidazole, it improved the efficacy of the drug by reducing the morbidity and mortality of the birds to significant extent.

Managemental Practices

For turkey farming, regular change of bedding or litter is recommended after each flock is removed. It helps to prevent the spreading of histomoniasis (Clark and Kimminau, 2017). The *H. meleagridis* parasite has low tenacity and short survival time outside the hosts. However, it can survive in contaminated water or feces for up to 9 hours, potentially causing indirect transmission between farms therefore proper hygiene of farms should be practicised (Liebhart et al., 2017; Lotfi et al., 2012). Avoidance of overcrowding can reduce the risk of *H. meleagridis* transmission between birds. Recent research has shown significant correlations between flock management and histomoniasis (Callait-Cardinal et al., 2010). The farm's biosecurity strategies should adopted to control earthworms, beetles, flies, and rodents by minimizing flooding.

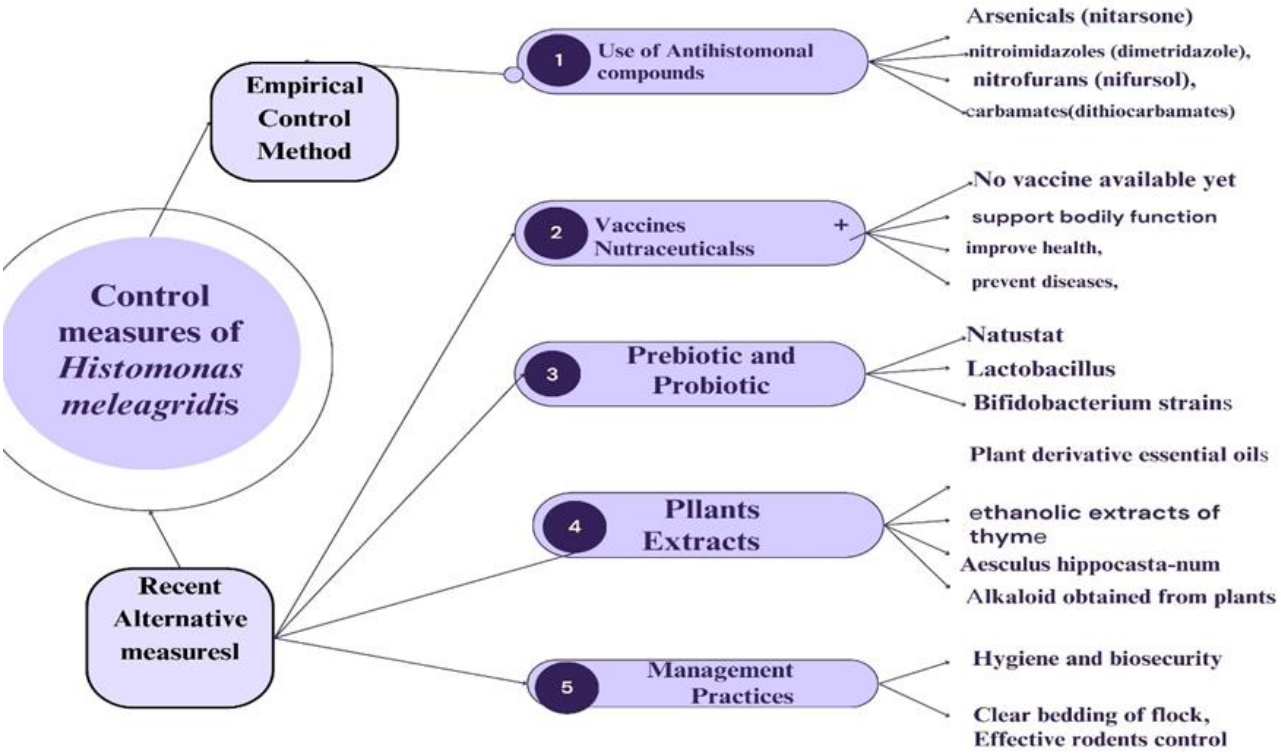

Fig. 2: Control Measures for *H.meleagridis*

Conclusion

The control of *H. meleagridis* requires a multifaceted approach due to the limitations and hazards associated with empirical antihistomonal compounds. Although compounds like arsenicals, nitroimidazoles, nitrofurans and carbamates have historically been effective, concerns regarding toxicity, antibiotic resistance development, and environmental contamination necessitate the exploration of alternative methods for the control of histomoniasis. Recent advances, including vaccines, use of prebiotics and probiotics, plant extracts, nutraceuticals, and management practices offer promising avenues for histomoniasis control. Utilizing a combination of these approaches tailored to specific farm conditions can enhance resistance in birds, reduce disease incidence and spread, and safeguard poultry and its related products. Continous research related to novel and alternative control strategies, along with stringent adherence to biosecurity protocols is crucial to reduce the impact of *H. meleagridis* on poultry production (turkey and chickens) to ensure the welfare of birds as well as consumers.

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