

Forum

Interplay between
Histomonas meleagridis
and Bacteria: Mutualistic
or Predator–Prey?Ivana Bilic^{1,*} and Michael Hess^{1,2}

***Histomonas meleagridis* is an extra-cellular protozoan parasite and the aetiological agent of histomonosis, an important poultry disease whose impact is greatly accentuated by inaccessibility of any treatment. A special feature of the parasite is its intricate interplay with bacteria *in vitro* and *in vivo*, the focus of this article.**

Histomonosis: A Bacteria-Driven Parasitic Disease

The protozoan *Histomonas meleagridis* is the causative agent of histomonosis (synonyms blackhead disease and histomoniasis) in gallinaceous birds [1]. The disease was controlled for decades using chemotherapeutics as preventive and curative drugs, but changes in drug legislation in the EU and the USA have led to its reappearance [2]. The devastating outcome of histomonosis is obvious in turkeys, in which it can lead to mortality of up to 100%, whereas in chickens the disease is less severe [1]. The essential presence of live bacteria either to propagate *H. meleagridis in vitro* or to successfully establish an infection in its host questioned the aetiology of histomonosis for a long time [3]. The infection biology of *H. meleagridis* is complicated by the fact that the nematode *Heterakis gallinarum*, an intermediate host and most important vector, also needs a certain bacterial flora to complete its life cycle in birds. Due to the low tenacity of the protozoan parasite, *Heterakis* eggs are considered a crucial vector for initial

introduction of *H. meleagridis* into a poultry flock (Figure 1).

Initial studies set up to investigate the parasite–bacteria interaction aimed to resolve the aetiology of histomonosis. For this purpose, sterilised *Heterakis* eggs harbouring *H. meleagridis* were combined with selected bacteria *in vitro* and in gnotobiotic chickens and turkeys. As a general outcome, it was noted that the pathogenicity of the parasite is not influenced by the microflora; however, its replication relies on the presence of bacteria, which differ in their level of support. Establishing a system based on a monoxenic clonal culture of *H. meleagridis* enabled the targeted exchange of the cocultivated bacterial strain [4]. It was confirmed by *in vitro* experiments that *Escherichia coli* was superior to other bacterial species. Growth of the parasite in a monoxenic background deferred the onset of histomonosis as compared with infection with a xenic culture, indicating a delayed adaptation of *H. meleagridis* to caecal conditions and a subsequent infection [4]. Dysbiosis, modulation of mucosal immunity and disruption of the epithelial barrier in the context of bacteria–parasite interaction, has been reported for other extracellular parasites, such as *Entamoeba histolytica* and *Trichomonas vaginalis* [5,6]. Such studies highlight the importance of the microflora in the context of infection and clinical consequences. Special attention has to be given to the immune and physiological reactions of the host and its nutrition in order to fully elucidate the complete interaction triangle.

E. coli, and bacteria in general, are found attached to the *H. meleagridis* surface but also enclosed in the protozoan cell. However, it is unclear whether all these bacteria die upon ingestion. Observations during monoxenisation and maintenance of monoxenic cultures suggest that some ingested bacteria survive the enclosure into the *H. meleagridis* cell [4], which is in

agreement with studies on the homologous protozoan parasite *T. vaginalis* [7]. Consequently, (i) why does *H. meleagridis* need bacteria for its survival and growth, and (ii) is this interaction mutualistic or rather of a predator–prey nature as outlined below and summarised in Figure 2?

Bacteria as a Food Source for the Parasite

Experiments feeding an *H. meleagridis* culture with dead instead of live bacteria resulted in the death of the parasite, suggesting that killed bacteria are not an essential nutrient source. However, bacteria are taken up into food vacuoles and metabolised by the parasite. In agreement with this, peptidoglycan-degrading enzymes were identified in the transcriptome, proteome and exoproteome of *H. meleagridis*, suggesting their involvement in the destruction of cocultivating or accompanying bacteria [8–10]. The acquisition of these bacterial genes involved in degradation of the bacterial cell envelope into the parasite's genome allegedly by lateral gene transfer is not new and was reported for the closely related parasite *T. vaginalis* [11]. This could be beneficial for the protozoan in respect to nutrient acquisition and to control the presence or abundance of certain bacteria. Selective predation of bacteria by protozoans was implicated in the regulation of bacterial strain composition and was shown to be abundant, involving both parasitic and free-living species [12]. Considering that *H. meleagridis* prefers certain bacterial background [4], it is possible that the parasite exploits a similar process. Altogether, it appears that *H. meleagridis* phagocytises bacteria for nutrient acquisition but, at the same time, relies on proteins and metabolites released during bacterial replication.

Bacteria as Regulators of the Parasite's Environment

The need for live bacteria in the *H. meleagridis* environment advocates for

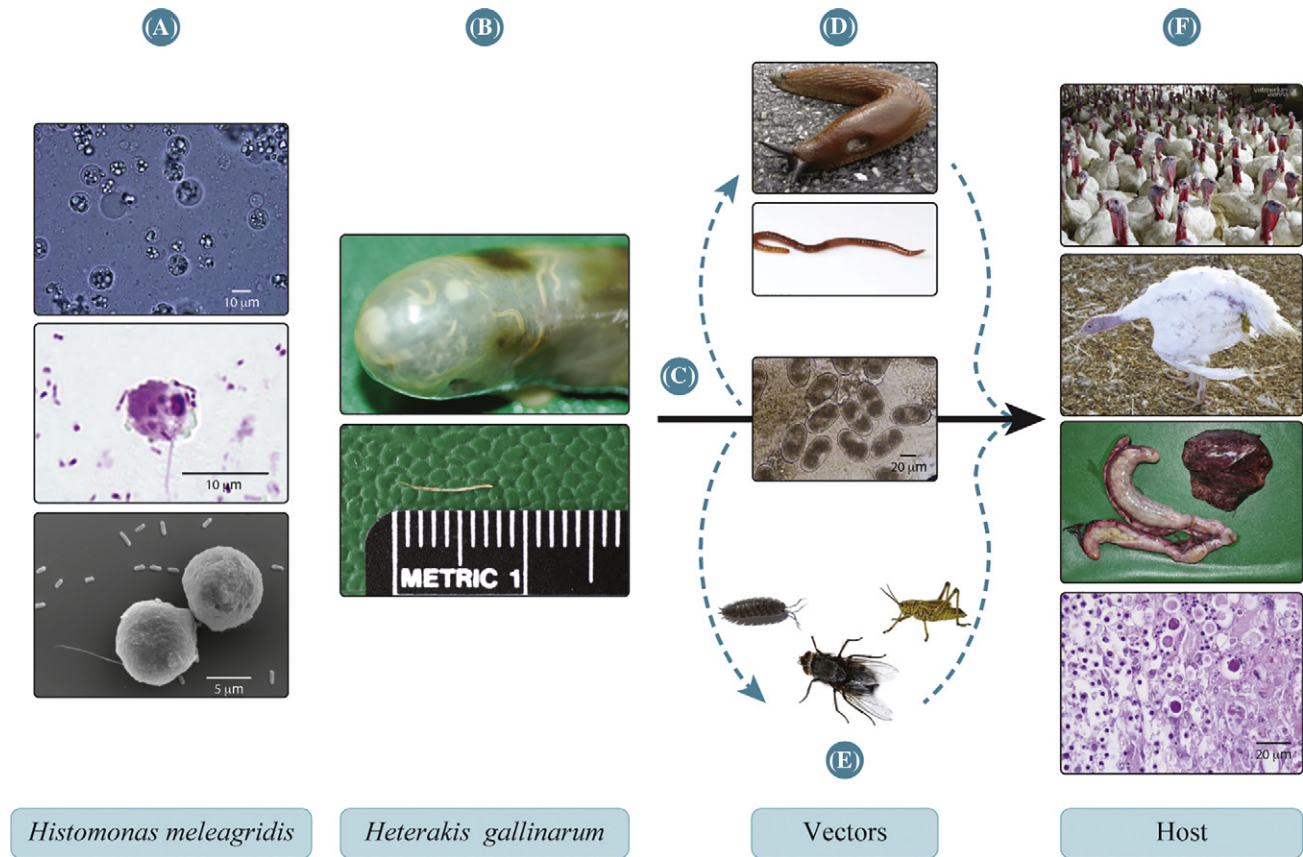


Figure 1. Life Cycle of *Histomonas meleagridis*. (A) *H. meleagridis* appears in different forms *in vivo* and *in vitro*. (Cultures are shown with bacteria and rice starch.) (B) The nematode *Heterakis gallinarum* cohabitates the caecum of Galliformes and incorporates the protozoan into its eggs, supplying long-term protection to the highly vulnerable flagellate. (C–E) *Heterakis* eggs (C) are taken up by the host as the most efficient way of infection or (D) reside in earthworms as a paratenic host and might be taken up by snails or (E) are mechanically carried by insects. (F) Following introduction into a flock, *H. meleagridis* can spread directly between birds, and turkeys display severe clinical signs before death. After evasion from the gut, the flagellum is lost, and *H. meleagridis* appears in spherical form in various organs of diseased birds, predominantly the caecum and liver.

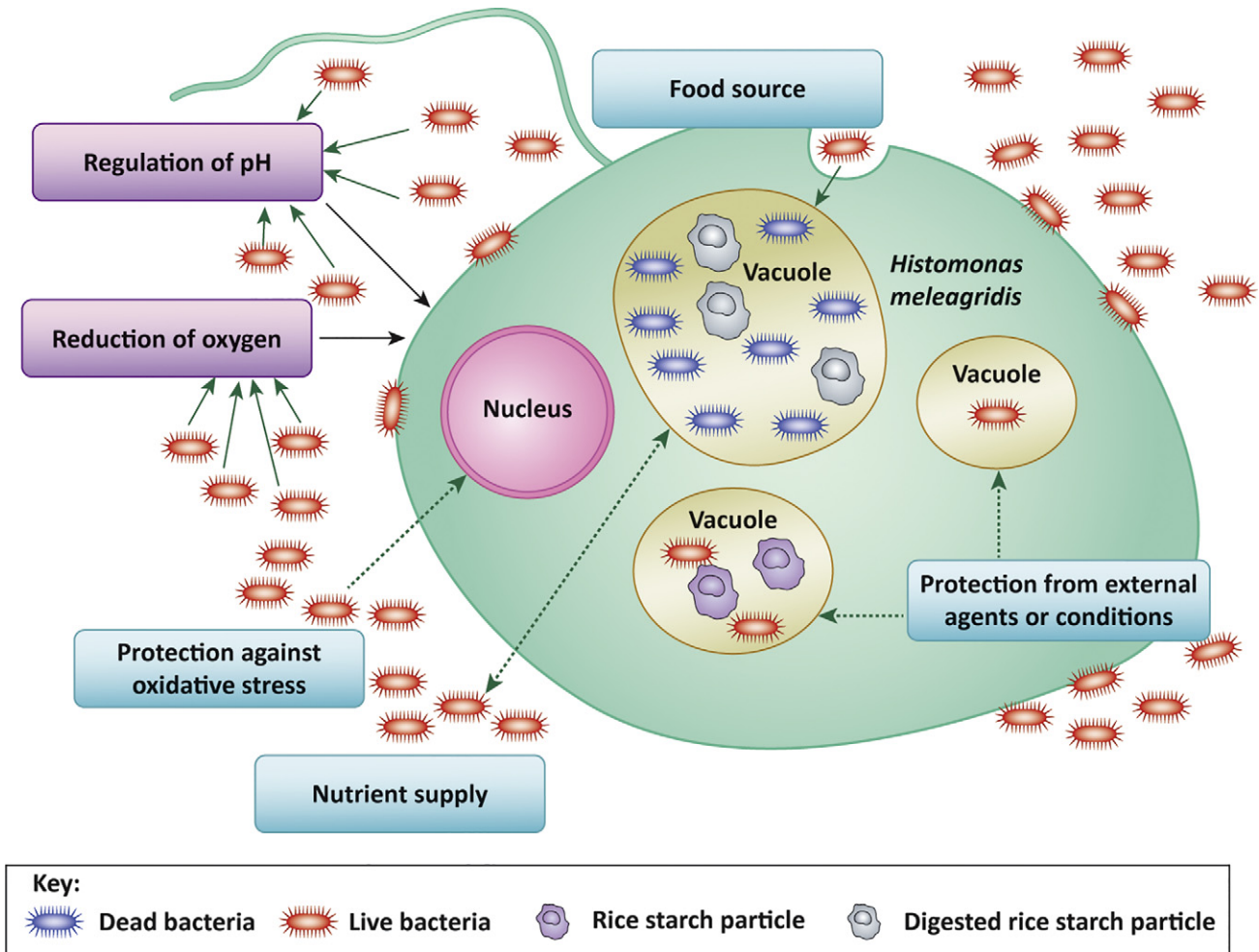
roles of bacterial support other than as a nutrition source. Experiments with various monoxenic cultures suggested that the interaction might also involve other indirect effects, such as adjusting environmental conditions by regulating the pH and decreasing the availability of free oxygen [4]. As *Histomonas* favours a neutral pH, buffered media are used for *in vitro* growth to optimise cell yields. Similarly, a neutral pH in the caecum as the target organ and entrance gate of the parasite should favour replication and infection. Such conditions are noted in gnotobiotic turkeys; yet, an infection could not be established. Aside from this, *H. meleagridis*, a microaerophilic

organism by nature, has its energy production based in hydrogenosomes, whose major enzymes are inhibited by the presence of oxygen, and its survival is strictly dependant on its effective removal [8]. Bacterial species, identified as most supportive of *H. meleagridis* growth, are themselves able to use both aerobic and anaerobic respiration to obtain the energy [4]. The high division rate of cocultivating bacteria effectively consumes oxygen from the environment and improves the conditions for anaerobic metabolism of the parasite [4]. Another possible, and more direct, bacterial effect would be influencing the expression of parasite's genes involved

in oxidative stress management and by doing so stimulating its intrinsic ability to cope with an intoxicating environment. Support for this comes from *E. histolytica*, where a direct bacterial effect on the regulation of parasite's genes was demonstrated [13]. Assuming that the intrinsic capacity of *H. meleagridis* to cope with oxidative stress is provided [8], such direct effects of bacteria might be feasible.

Impact of *H. meleagridis* on Bacteria

So far, only very few relevant studies are available, but molecular data point towards an intricate relationship between



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Figure 2. Potential Aspects of the ‘Eu-prokaryotic’ Interaction. The cocultivating bacteria or intestinal prokaryotes invoke an indirect influence on *Histomonas meleagridis* by maintaining optimal environmental conditions (e.g., regulation of pH or reduction of oxygen), shown in the violet rectangles. A direct effect of bacteria on *H. meleagridis* and vice versa is shown in the blue rectangles: bacteria acting as a food source; bacteria invoking protection of *H. meleagridis* against oxidative stress by stimulating the expression of the parasite’s antioxidant proteins; bacteria and *H. meleagridis* influencing components of substrates enabling mutual nutrient supply; and *H. meleagridis* providing protection of bacteria by allocating them into its vacuoles. Live and dead bacteria are shown in red and blue, respectively. Green and black arrows indicate direct and indirect effects, respectively. Solid arrows show processes reported in the literature, whereas dashed arrows mark processes speculated here.

H. meleagridis and bacteria. On the basis of proteomic analyses of *H. meleagridis*, it seems that the presence of the parasite itself substantially influences the expression pattern of genes of cocultivated bacteria [9,10,14]. Due to the monoxenic nature of the *H. meleagridis* *in vitro* culture, the detection of a few *E. coli* proteins was anticipated. However, a substantial variation of their abundance, which could be associated with the parasite’s phenotype,

is remarkable and thought-provoking. Especially the exoproteome study demonstrated substantial changes in the expression of *E. coli* proteins. Considering that about one-third of bacterial proteins undertake their function outside the cytoplasm, it seems unsurprising that major differences in the exoproteome were of *E. coli* origin, whereas variations in protozoal exoproteins were almost nonexistent [9,15]. Analysis of detected

divergence in *E. coli* exoproteins suggests that, during cultivation, *E. coli* relies on the consumption of bioproducts from the parasite’s metabolism, indicating a mutual role as nutrient supply [9]. However, the data also reveal a potential resilience of prokaryotes to predation by the parasite. Whether bacteria themselves acquire some advantage from this tight interaction remains hypothetical and needs to be investigated in more detail. The obvious

hypotheses are (i) the protection from external agents or conditions, (ii) the nutrient supply and/or (iii) the use of *H. meleagridis* as a Trojan horse for prokaryotes. Some evidence for the last hypothesis comes from animal experiments and case reports of histomonosis from the field, which often find a secondary *E. coli* infection [3].

Future Perspectives

Aside from a nutrition source and/or the creation of favourable environmental conditions, the need for bacteria in the turkey/chicken caecum to induce histomonosis might be seen as a cooperative aid in the disruption of the gut epithelial barrier. This 'eu-prokaryotic' interaction can have fatal consequences for the host, altogether a unique alliance in medicine. However, the underlying functional mechanisms are still to be resolved, considering that the host itself may trigger and contribute certain features inducing substantial consequences on the outcome. On the basis of available data, we hypothesise that the parasite–bacteria interplay is mutualistic and not

of a predator–prey nature. Future research should focus on resolving the unknowns of this interaction in order to elaborate whether a targeted manipulation of the gut microbiome can be achieved in order to minimise clinical consequences. Similarly, such knowledge could also be used to optimise the infection of attenuated *H. meleagridis* strains used for vaccination.

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<https://doi.org/10.1016/j.pt.2019.12.015>

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