Conclusions
10. Conclusions

The amitochondriate protist *Giardia lamblia* is the etiological agent of giardiasis, one of the most common intestinal infectious diseases worldwide, responsible for 280 million symptomatic infections per year (Adam R.D, 2001). The outcome of giardiasis can vary a lot: the infection may remain asymptomatic or lead to severe and sometime persistent symptoms. The major clinical traits of giardiasis are watery diarrhea, nausea, abdominal discomfort and vomiting accompanied by dehydration, malabsorption and weight loss (Buret A.G, 2008). The disease can lead to failure to thrive and cognitive impairment in children or chronic fatigue in adults. Giardiasis has been also implicated, through yet unknown mechanisms, in the pathogenesis of a number of chronic disorders of the gastrointestinal tract, such as irritable bowel syndrome, inflammatory bowel disease and food allergies (Buret AG, 2008). Due to the high incidence of this infectious disease, particularly in developing countries, *Giardia lamblia* is one of the major contributors to diarrheal diseases that collectively are the second-leading cause of death in children under five years of age worldwide (Kosek M et al., 2003). For these reasons, *Giardia lamblia* represents a threat to human health.

Following oro-fecal transmission, in the stomach lumen the cyst develops into the trophozoite which causes the disease by proliferating attached to the mucosa of duodenum and jejunum, below the Vater ampulla. Several hypotheses have been raised to rationalize why *Giardia*, unlike most of the other commensal or pathogenic microorganisms inhabiting the human gut, preferentially colonizes the proximal small intestine. Colonization of this tract of the gut by *Giardia* may be favored by the reduced competition with the intestinal microbial flora that is much less abundant in the small than in the large intestine. Another advantage may arise from the fact that compared to the large intestine; the proximal small intestine is much richer in bile and nutrients necessary for parasite survival. In this regard, it has long been known that the bile has a stimulatory effect on *Giardia* trophozoites proliferation, presumably providing the parasite with those lipids that it is unable to synthesize de novo. As an additional hypothesis, it was proposed that the peculiar localization of *Giardia lamblia* is related to the higher (compared to the large intestine) redox buffering capacity of the small intestine (Mastronicola D et al., 2011), due to biliary antioxidants (glutathione, cysteine, bilirubin, etc.).

Despite these possible advantages, given their O₂-susceptibility, *Giardia* trophozoites are expected to be challenged by the O₂ present in the proximal small intestine. Here O₂ tension is higher (up to 60 μM) than in distal tracts of the gut and undergoes ample
oscillations with time, peaking at every meal in order to meet metabolic need. In addition, at the level of the intestinal mucosa $O_2$ tension is presumed to be much higher than in the lumen. $O_2$ is indeed released by the extensive microcirculatory vascular network pervading the sub-mucosa and a steep $O_2$ gradient is therefore established moving from the sub-mucosa towards the luminal midpoint (Espey MG, et al., 2013). Living attached to the intestinal mucosa, it is thus likely that *Giardia* trophozoites are physiologically exposed to fairly high and fluctuating $O_2$ levels. In addition to facing $O_2$ stress, in vivo the parasite is also likely challenged by the nitric oxide (NO) produced by the NO-synthases (NOSs) in intestinal epithelial cells and/or derived from reduction of dietary nitrate/nitrite (Espey MG, et al., 2013). Nitrite reduction to NO is strongly favored under the acidic conditions of the stomach lumen, from where part of the gas could diffuse to the proximal small intestine. Summing up, due to its peculiar localization at the mucosa of the proximal small intestine, *Giardia* is likely challenged in vivo by relatively high concentrations of $O_2$, NO and possible related reactive species. Elucidating the *Giardia* antioxidant defense systems that enable parasite survival to oxidative and nitrosative stress conditions is thus important, particularly in the perspective of unveiling novel potential pharmacological targets. In this regard, it is important to recall that this microaerophilic pathogen lacks not only respiratory terminal oxidases (being amitochondriate), but also most of the conventional antioxidant enzymes typically implicated in management of oxidative stress, including catalase, superoxide dismutase (SOD) and glutathione peroxidase (Brown DM et al., 1995). This makes the story of interest also from an evolutionary point of view. Finally, it is worth noting that several contact points have been recently highlighted between the antioxidant defense and drug resistance in *Giardia* pointing to an involvement of antioxidant enzymes in modulation of parasite susceptibility to drugs. Hence, a comprehensive elucidation of the antioxidant defense systems will likely allow a better understanding of the molecular mechanisms that underlie drug resistance in *Giardia*.

*Giardia* trophozoites are $O_2$-sensitive cells that in vitro grow preferentially under anoxic reducing conditions (Raj et al., 2014). $O_2$-sensitivity was ascribed on the one hand to the expression of metabolic enzymes inactivated by $O_2$, such as pyruvate:ferredoxin oxidoreductase (Raj et al., 2014), and on the other hand to the ability of NAD(P)H:menadione oxidoreductase (DT-diaphorase) and other possible enzymes to produce ROS by reacting with $O_2$. The occurrence of efficient $O_2$-detoxifying systems thus seems to be a requirement for survival of *Giardia* trophozoites at the fairly aerobic mucosa of the proximal small intestine. The genes identified from the transcriptomic study playing role
in oxidative stress regulation can be used for new drug targets. Arginine deiminase, released during oxidative stress interferes directly in the mucosal immune system. Besides its numerous physiological functions, in higher organisms NO is produced by the immune system to counteract microbial pathogens (MacMicking J et al., 1997). Living attached to the mucosa of the proximal small intestine, *Giardia lamblia* is likely challenged by the NO, enzymatically produced by host cell NOSs or generated through nitrite reduction. It is therefore reasonable to assume that the parasite is endowed with multiple defence mechanisms against NO and related reactive species. *Giardia* trophozoites are known to inhibit NO production by human intestinal epithelial cells through the consumption of arginine, a substrate of NOS. Arginine is both taken up by the parasite for metabolic purposes and metabolized extracellularly by arginine-deiminase, a protein secreted (together with other metabolic enzymes) by the parasite upon interaction with the intestinal epithelial cells (Ringqvist E et al., 2008). Arginine depletion, however, as such represents a potentially risky defense strategy, because under arginine limitation NOSs can become a source of not only NO, but also ROS, thereof leading to formation of peroxynitrite (ONOO-) (Ringqvist E et al., 2008), a highly toxic reactive nitrogen species produced by the reaction of NO with $O_2\cdot^{-}$. Probably for this reason *Giardia* suppresses the expression of the inducible isoform of NOS (NOS-2) in intestinal epithelial cells at later stages of the infection (Ringqvist E et al., 2008). Taken together, the results presented above suggest that *Giardia* is endowed with a variety of defense mechanisms against NO, that are likely relevant for parasite survival in the human intestine. As arginine deiminase is absent in human could be good target for drug designing. Oxidative stress management was thought to be controlled by NADH oxidase, flavodiiron protein etc (Mastronocola et al., 2011).

However, the transcriptomic results have shown that the oxidative regulation is not only controlled by some metabolic genes, but also different types of other proteins take a significant role in ROS detoxification (Raj et al., 2014). In the two experimental conditions (H$_2$O$_2$ and cysteine-ascorbate deprivation) it has been observed that different types of stress generating conditions regulate the pyruvate metabolism pathway differently. Some metabolic genes like pyruvate dikinase, acetyl-coA synthase etc. are always up-regulated during both stresses. Pyruvate, can alter the effective lifetime of reactive oxygen species by scavenging them. Pyruvate attenuates ROS production in the *Giardia* trophozoites. Exogenously added pyruvate was also able to inhibit lipid peroxidation of stressed *Giardia lamblia* (Raj et al., 2015). *Giardia* had a greater need for pyruvate during oxidative stress. Pyruvate decreased the number of DNA breaks. Pyruvate plays a role in DNA protection and repair in *Giardia*
trophozoites during oxidative stress (Raj et al., 2015). As Giardia does not contain the components of respiratory chain (e.g. cytochromes), by controlling pyruvate level it keeps the harmful consequences of ROS at bay. Modulation of the fate of pyruvate in one direction or the other can be important for homeostatic response of Giardia to oxidative stress. This could alter functioning of the antioxidant system and have protective effects against DNA damage induced by oxidative stresses. Alterations of pyruvate metabolism are observed in Giardia due to high oxygen environment. This could be advantageous for Giardia trophozoites in such stressful conditions.