Oxidative Stress Control by Apicomplexan Parasites

Soraya S. Bosch,1 Thales Kronenberger,1 Kamila A. Meissner,1 Flávia M. Zimbres,1 Dirk Stegehake,2 Natália M. Izui,1 Isolmar Schettert,1 Eva Liebau,2 and Carsten Wrenger1

1Unit for Drug Discovery, Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, Avenida Professor Lineu Prestes 1374, 05508-000 São Paulo, SP, Brazil
2Department of Molecular Physiology, Westfälische Wilhelms-University Münster, Schlossplatz 8, 48143 Münster, Germany

Correspondence should be addressed to Eva Liebau; liebaue@uni-muenster.de and Carsten Wrenger; cwrenger@icb.usp.br

Received 3 August 2014; Accepted 27 October 2014

Academic Editor: Kevin Tyler

Copyright © 2015 Soraya S. Bosch et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Apicomplexan parasites cause infectious diseases that are either a severe public health problem or an economic burden. In this paper we will shed light on how oxidative stress can influence the host-pathogen relationship by focusing on three major diseases: babesiosis, coccidiosis, and toxoplasmosis.

1. Apicomplexan Parasites Are Subject to Oxidative Stress from Their Host Cells

Apicomplexan parasites are the causative agents of several different diseases: malaria (Plasmodium spp.), toxoplasmosis (Toxoplasma spp.), cryptosporidiosis (Cryptosporidium spp.), and babesiosis (Babesia spp.). Apicomplexa form a large group of complex unicellular eukaryotes and belong to the higher group of the Alveolata along with Chromerida, dinoflagellates, and ciliates. Within the Apicomplexa phylum, all parasites have an infective stage, the sporozoite. The sporozoites enter the host via typical invasion machinery consisting of the apical complex, which is composed of distinct organelles such as rhoptries, micronemes, and dense granules [1]. This process is actin-myosin dependent and subsequently a new host-derived membrane, the parasitophorous vacuole, surrounds the parasite. The life cycles of these parasites are complex, containing asexual and sexual reproduction. However, all these parasites invade cells and have to adapt to the intracellular environment of their hosts. In particular, the apicomplexan parasites have to deal with the oxidative level inside their host cells. Reactive oxygen species (ROS) and oxidative stress are the result of an aerobic metabolism that generates highly reactive metabolites of molecular oxygen (O2) in the cytosol or in organelles such as the mitochondria or the peroxisomes [2]. These oxygen metabolites comprise superoxide anions (O2−) and hydrogen peroxides (H2O2) or the highly reactive hydroxyl radical (OH·) that is formed in the presence of metal ions via the Fenton and/or Haber-Weiss reactions [3]. Severe discrepancies in the ROS level can induce oxidative modifications in the indispensable cellular macromolecules such as DNA, proteins, and lipids [4], ultimately leading to cell death.

In order to tackle this challenge, parasites have developed a variety of different antioxidant systems such as the thioredoxin- and glutaredoxin-systems. These systems act as thiol/disulfide pairs and are thereby involved in controlling the redox state of the cell. Glutathione/glutathione disulfide (GSH/GSSG) is one of the major redox pairs that control the antioxidative capacity of the cell, while thioredoxins (Trxred/Trxox) form an additional redox system that interacts with a different subset of proteins [5]. Trx plays an important role in different biological processes including the reduction of ribonucleotides, transcription control, and hydrogen peroxide detoxification [6, 7]. In addition to these thiol/disulfide pairs, enzymatic antioxidants are also present, which can be classified into primary or secondary antioxidants. Whereas the latter are involved in the regeneration of low molecular weight antioxidant species [8] the primary antioxidants react directly with prooxidants. The enzymes catalase (CAT) and superoxide dismutase (SOD) belong to this class. The CAT catalyses the reaction of 2H2O2 → 2H2O + O2 and thereby...
diminishes the cellular level of hydrogen peroxide. SODs are metallo- (M-) proteins that catalyse the dismutation of superoxide anions to form molecular oxygen and hydrogen peroxide as indicated below ($M_{\text{ox}}$, the oxidized and $M_{\text{red}}$ the reduced state of the metalloproteins):

$$M_{\text{ox}} + O_2^{•−} \rightarrow M_{\text{red}} + O_2$$

$$M_{\text{red}} + O_2^{•−} + 2H^+ \rightarrow M_{\text{ox}} + H_2O_2$$

Several common forms of SODs exist that are classified according to their metal cofactors such as Cu/Zn, Fe, or Mn. Different SODs can be present in a single cell; for example, the mammalian cells contain cytosolic Cu/ZnSODs and MnSODs in their mitochondria [9].

Antioxidants also include peroxiredoxins (Prx) which are involved in the conversion of peroxides and alkyl hydroperoxides to water or the respective alcohol and have two characteristic catalytic cysteine residues [10]. Furthermore, glutaredoxins utilize the reducing power of glutathione to catalyze disulfide reductions in the presence of NADPH and glutathione reductase (the glutaredoxin system).

Due to the fact that the redox systems play such a fundamental role for parasites [3] this paper highlights the importance of oxidative stress for host-pathogen interactions in apicomplexa; however, due to the recent article by Nepveu and Turrini [11] we would like to focus on different apicomplexan parasites other than *Plasmodium*.

2. *Babesia*: Infection and Host Response

Parasites of the genus *Babesia* are classified as apicomplexan and belong to the suborder *Piroplasmdia* within the family *Babesiidae*. This classification is based on their capacity for invading erythrocytes, multiplication via budding rather than schizogony, and the lack of hemozoin [12]. *Babesia gibsoni* is a pathogen occurring in Indian dogs. The first species was described by Patton in 1910, and since then the disease has been widely reported all over the world [13–15].

*Babesia* spp. are naturally transmitted by the bite of infected ticks, from the species *I. ricinus*. Other occasionally occurring mechanisms of transmission are via transplacental and perinatal routes and from contaminated blood products [15]. Among the known species, *B. microti*, *B. divergens*, and *B. venatorum* cause human babesiosis in Europe [12, 16–18].

Similar to other members of this group, *Babesia* undergoes a complex life cycle involving arthropods and other mammalian hosts [19]. The cycle starts with ticks taking a blood meal thereby infecting the mammalian host with sporozoites [20], which then invade erythrocytes and reproduce through asynchronous binary fission. This results in two or sometimes four merozoites. Once present in a reservoir host, *Babesia* will develop into male and female gametes [18, 21].

The zoonotic *Babesia* reservoirs are quite diverse species including the white-footed mouse, cattle, wild ruminants, canids, shrews, and possibly cottontail rabbits. Several reservoirs are unknown for some human *Babesia* pathogens [18]. The cycle is completed when an ixodid tick feeds on a competent reservoir and the gametes fuse to form the zygote. Finally the pathogen undergoes a sporogonic cycle, forming the infectious sporozoites [20, 22].

The members of *Babesia sensu stricto* spp. group have a characteristic feature in common; they can infect ovary cells and thus be transmitted transovarially by eggs [12]. Despite this information, there are several species of *Babesia*, such as *B. duncanii*, which are almost not characterised [16].

Like many aerobic parasites, *Babesia* lives in an oxygen-rich environment within its mammalian host (mainly during the erythrocytic stage). As a result, the pathogen is exposed to the toxic effects of ROS, which can cause damage to membrane lipids, nucleic acids, and proteins [23, 24]. *Babesia* is an example of the importance of the parasite’s antioxidant system for proliferation within erythrocytes [25–27].

According to Regn et al., the biochemical properties of proteins of the Trx system from the bovine parasite *B. bovis*, the *BboTrx(R)* system, share several features with their counterparts in *P. falciparum*, such as kinetics and physical properties and the capacity for reducing S-nitrosogluthionine (GSNO) and GSSG [26] (Table 1).

This latter competency is highly relevant, since GSH is the most abundant intracellular nonprotein thiol that represents a key-molecule within redox homeostasis. To date, three genomes of the genus *Babesia* were sequenced: *B. bovis*, *B. microti*, and *B. equi*. After sequence analysis of the *B. bovis* genome database, no homology to a specific glutathione reductase was identified, suggesting that *B. bovis* might lack this enzyme. Further, the 2-Cys peroxiredoxin present in the cytoplasm of the merozoite was characterized to be an important component of the Trx network in *B. bovis* and *B. gibsoni*, which is able to reduce ROS [23, 28].

In general, *Babesia* spp. parasites, as well as species of *Theileria* and *Plasmodium*, invade erythrocytes and cause anaemia of the host [18, 29, 30]. Anaemia is considered as a severe complication of babesiosis, being the major cause of mortality in infected animals. However, its pathogenesis still remains uncertain [18]. Despite the correlation between parasitisandanaemia,theseverityofanaemiaisnotalways proportional to the parasitaemia [31, 32]. This phenomenon suggests that nonparasitized erythrocytes may also be damaged by an unknown mechanism of action [32].

The parasite does not always need to control elevated ROS levels present in the host. Studies in dogs naturally infected with *B. gibsoni* demonstrated the presence of a host response via an increased expression level of the SOD and CAT enzymes implying the generation of ROS by the pathogen. Additionally, an elevated level of lipid peroxides within the erythrocyte was detected. Moreover, in this study it was suggested that the low level of iron, zinc, and copper in the blood seems to have an additional role in the genesis of anaemia and oxidative stress [32].

In parasites that are proliferating in erythrocytes, the liver plays an essential role in clearing infected red blood cells [30]. It has been shown by flow cytometry analysis that *B. divergens* induces hepatic tissue damage via oxidative stress, leading to an alteration in the cell metabolism. Further, it has been demonstrated that ROS damage hepatocytes, thereby affecting the function of the liver [33]. This study also observed a significant decrease in the total antioxidant capacity during
The first report of cryptosporidiosis was in the early 20th century while the first case in humans was reported in 1976 [43–45]. Today Cryptosporidium spp. are known as the major waterborne parasite worldwide with an important economic impact and a source of diarrhea in calves and lambs [46]. Although Cryptosporidium spp. are also pathogenic for humans, clinical symptoms such as diarrhea are restricted to immune-deficient people. However, immune-compromised patients (e.g., HIV/AIDS patients) are at higher risk and cryptosporidiosis can lead to dehydration, wasting, and even death [47–54].

While various Cryptosporidium spp. were detected in humans, over 90% belong to the most common species C. hominis and C. parvum [55, 56]. Little is known about their pathogenic factors due to difficulties in in vitro culturing and only oocysts have been biochemically analyzed so far [57–59]. To date, the genome sequencing project of C. hominis and C. parvum has discovered about 25 putative virulence factors [58].

One of these factors is an acid phosphatase in C. parvum [60]. Aguirre-Garcia and Okhuysen have shown the activity of the membrane-bound enzyme in the oocyst [60]. They suggest that the acid phosphatase similar to those in Leishmania spp. Coxiella burnetii, Legionella micdadei, and Francisella tularensis has an important role for the survival of the intracellular pathogens [61–64]. Acid phosphatases are known to inhibit the respiratory burst of human neutrophils and macrophages [65–67].

Only limited information is present of the antioxidant enzymes in Cryptosporidium spp. While the enzymes glutathione transferase, glutathione reductase, and glutathione peroxidase seem to be absent in C. parvum (Table 1), some SOD activity has been detected [68]. Further, it has been shown that the parasite contains and synthesizes GSH and possesses a thioredoxin peroxidase that could be important for detoxification and thereby protection against ROS [69–72] (Table 1). Previous studies suggested that the antioxidant enzymes might have a protective effect against ROS that occurs via inflammation or phagocytosis by macrophages [68].

### Table 1: Predicted antioxidant enzymes according to the genome databases of Babesia and Cryptosporidium.

| Proteins                     | Abbr.                  | B. bovis          | B. equi          | C. hominis | C. parvum | References |
|------------------------------|------------------------|-------------------|------------------|------------|-----------|------------|
| Directly dealing with prooxidants |                        |                   |                  |            |           |            |
| Glucose-6-phosphate dehydrogenase | BBOV_IV001600         | BEWA_012440A     |                  |            |           |            |
| Superoxide dismutase (SOD1) Fe | SODBI U70131          | BEWA_043090      | XM_660499       | AY399065  |           |            |
| Peroxiredoxin                | Prx1                   |                   | BEWA_033010     |            |           |            |
| Glutathione system           |                        |                   |                  |            |           |            |
| Glutathione peroxidase 1     |                        |                   | XM_663205.1     | XM_62663L1|           |            |
| Glutathione reductase        |                        |                   |                  |            |           |            |
| Glutathione synthase         |                        |                   | XM_004829930    |            |           |            |
| Glutaredoxin                 | Grx BBOV_IV00432023    | BEWA_031250      | XM_660840       | XM_627733 |           |            |
| Thioredoxin system           |                        |                   |                  |            |           |            |
| Thioredoxin                  | Trx BBOV_I003650       | BEWA_047060      | —                | XP_626444 |           |            |
| Thioredoxin peroxidase 1     | AK440717               | BEWA_001220      | XM_660495       | GQ388272  |           |            |
| Thioredoxin reductase        | TrxR BBOV_I002190      | XM_004831637     | GQ388271        | AY145120  |           |            |

B. divergens infections. This was shown by decreased GSH and CAT levels as well as a significant increase in the concentration of malondialdehyde (MDA). The increased level of MDA strongly suggests lipid peroxidation and alteration of the nitrite/nitrate levels [34]. Increased levels of MDA have also been reported in B. gibsoni infections [32] and in double infections of Ehrlichia canis and B. gibsoni [34, 35].

Currently there are several drugs used for the treatment of human babesiosis. Atovaquone, azithromycin, clindamycin, and quinine are drugs that show activity in Babesia animal models [12]. Due to spreading drug resistance, there are currently only two major antimicrobial treatments present, which consist of a combination therapy of antimalarial drugs and antibiotics such as quinine and clindamycin or atovaquone and azithromycin [12, 36, 37]. The use of antibiotics is also present in the apicomplexan parasites Toxoplasma and Plasmodium [38].

### 3. Cryptosporidium: A Resistant Pathogen

The protozoan pathogen Cryptosporidium was described first by Tyzzer, which is a worldwide occurring coccidian parasite causing gastroenteritis in mammals. The main pathway of infection is the uptake of oocysts via food consumption [39, 40].

Cryptosporidium spp., in contrast to other apicomplexan parasites, have a monoxenous life cycle that takes place in the gastrointestinal tract of the host. During the excystation, four infective sporozoites, which are released from the oocyst, glide over the intestinal cells until they invade the cell using the apical complex. After infection the parasite develops at the apical surface in the host cell; thus the parasite using the apical complex. After infection the parasite develops at the apical surface in the host cell; thus the parasite is in an intracellular but extracytoplasmic state. Inside the parasitophorous vacuole, Cryptosporidium spp. are protected from the gut environment and use nutrients via an Apicomplexa-unique feeder organelle. Uniquely amongst other apicomplexan parasites, Cryptosporidium spp. lack an apicoplast and also have lost the mitochondrial genome and most of its functions [41, 42].
To enhance the negative effect of oxidative stress on *C. parvum*, the use of selenium (Se) seems promising [73, 74]. Selenium compounds are able to react with thiols such as GSH which lead to elevated levels of superoxide and hydrogen peroxide [75]. Interestingly, Se-supplemented *C. parvum*-infected mice show a decreased number of oocysts in feces and a longer survival time than the respective control [73]. On the other hand Se-deficiency results in a heavy oocyst shedding, a higher susceptibility of the host to *C. parvum*, and a decreased immune response [73, 74].

A major problem is the high resistance of the parasite to common disinfectants such as chlorine [48, 76, 77]. The disinfecting effect of chlorine is based on free radicals, which seem to react with the plasma membrane [78]. As a response *C. parvum* initiates the expression of the chaperone Hsp70 [79]. Bajszár and Dekonenko suggest that this chaperone protects membrane proteins against protein denaturation caused by oxidative stress, as already known for the bacterial Hsp33 [79, 80].

### 4. Eimeria: Effect of Diet Supplementation upon Oxidative Balance

*Eimeria* spp. are etiologic agents of coccidiosis [81]. Although some organisms such as reptiles, mammals, and fish can be infected with coccidia, the majority of the studies were focussed on species infecting poultry, such as *E. tenella*, *E. brunetti*, *E. praecox*, *E. mitis*, *E. acervulina*, *E. necatrix*, and *E. maxima*. Due to the huge economic significance, there is an urgent need for strategies to control the parasite [82, 83]. *Eimeria* spp. usually differ in pathogenesis and tissue tropism. The transmission of coccidiosis is facilitated by a faecal-oral mechanism of contaminated water or food which initiates the comprehensive life cycle [83].

The current strategy for coccidiosis control relies on (i) the parasite's cofactor metabolism (synthetic drugs), such as ethopabate, sulphonamides, pyrimethamine, and amprolium [84], (ii) the mitochondrial metabolism, employing drugs such as quinolone which blocks electron transport [85] and the triazinetrione compound toltrazuril, which reduces the activity of certain enzymes in the respiratory chain [86]; and (iii) the balance of ions, using drugs like polyether antibiotics or ionophores which induce osmotic damage [87]. Furthermore, some drug combinations of currently unknown mode of action have shown promising effects [88]. However, as already known from other drug based therapies, the indiscriminate use of anticoccidian compounds leads to the development of resistant strains [89].

*Eimeria* infections can harm the host, among other classically described mechanisms [22], due to an imbalance of the antioxidant defence system [90, 91]. In order to control the oxidative environment of the host, *E. tenella* increases the level of antioxidative enzymes such as CAT during infection [90].

As outlined above, interfering with the redox homeostasis makes the cell vulnerable to ROS and can cause cell damage. Classical biological markers such as MDA and lipid peroxidation (LPO) have elevated concentrations in *E. tenella*- and *E. acervulina*-infected birds [92–94].

Sepp and colleagues showed that greenfinches fed on an excess of carotenoids as an antioxidant were able to manage chronic *Eimeria* infection [95]. Based on this it has been suggested to use food supplements such as 2Gly-ZnCl$_2$·2H$_2$O [92], vitamin A, vitamin E, vitamin C, and low-molecular endogenic antioxidants for controlling *E. tenella* infections [82, 96, 97].

### 5. Toxoplasma: Oxidative Stress, a Source of Drug Targets

Infection with the parasite *Toxoplasma gondii* has a worldwide distribution [22]. The parasite is transmitted by warm-blooded animals, from the mother to fetus (congenital) and also by food-borne transmission [22]. In many cases the immune system can prevent the symptoms; however, *T. gondii* infections can be lethal for immune deficient people such as HIV patients [98]. Moreover, during pregnancy the fetus is at particular risk since the disease can affect the nervous system, eyes, and other organs [99].

The life cycle of *T. gondii* begins with the ingestion of tissue cysts by cats, the definitive host. Once inside the (human) intestine, the oocyst excysts and subsequently the tachyzoites invade cells using a characteristic active invasion mechanism. Within the tissues *T. gondii* is infective for various cell types. Like the malaria parasite *T. gondii* forms a parasitophorous vacuole during the invasion process [100].

In the parasitophorous vacuole the parasite already induces the production of IL-10 (interleukin 10) and TGF-β (transforming growth factor β). In this manner, *T. gondii* is able to modulate the innate and adaptive host immune system in order to reduce the immune response rather than its complete inhibition [101]. The stress deriving from the host’s immune system can induce differentiation from the highly replicative and invasive form (tachyzoite) towards the persisting bradyzoite stage [102].

In the early phases of invasion, macrophages and natural-killer cells are primarily responsible for defeating the parasite [103]. Classically, these cells use ROS against pathogens; therefore the antioxidant defence system of *T. gondii* became attractive for drug discovery.

Regardless of the CAT’s importance in detoxifying H$_2$O$_2$, this enzyme is lacking in most pathogenic protozoans [8]. Unusually, the *T. gondii* genome encodes for a catalase [104]. Based on similarity analysis with respective homologues, the enzyme requires NADPH and a haem group as cofactors [105]. Much had been discussed about the localisation and role of the catalase in the parasite. Initially a classical peroxisomal localisation was suggested, whereupon the parasite would use the catalase for detoxifying by-products within peroxisomes [10]. However, the cytosolic localisation has been recently confirmed by microscopy and corroborated by the absence of peroxisome biogenesis factor (PEX) proteins in *T. gondii* (Figure 1) [106]. A cytosolic catalase can act on the detoxification of the majority of host born peroxides due to its high substrate turnover [104, 105].

Additionally, catalase seems to have an important role in invasion and replication inside the parasitophorous vacuoles according to knock-out studies [107]. Here, the knock-out
Table 2: Antioxidant enzymes in T. gondii.

| Proteins | Abbr.       | T. gondii | Comments                  | Stage            | Localisation        | References |
|----------|-------------|-----------|---------------------------|------------------|---------------------|------------|
|CAT       | CAT         | AF161267  |                           | Constitutive     | Cytoplasmatic       | [104, 105, 107] |
|Glucose-6-phosphate dehydrogenase | XP_002370586 | Putative |                           | Tachyzoite/bradyzoite | Cytoplasmatic       | [115] |
|Superoxide dismutase (SOD1) Fe | SODB1 | AF029915 | Fe in the active site      | Constitutive     | Mitochondrial       | [107] |
|Superoxide dismutase (SOD2) | SOD2 | AY176062 |                           | Constitutive     | Mitochondrial       | [107] |
|Superoxide dismutase (SOD3) | SOD3 | AY254045 |                           | Sporulated oocyst | Mitochondrial       | [107] |
|Peroxiredoxin | Prx1 | AF305718 | 2-Cys mechanism            | Constitutive     | Cytoplasmatic       | [107, 127] |
|Peroxiredoxin | Prx2 | AF397213 | 1-Cys mechanism            | Tachyzoite/bradyzoite | Cytoplasmatic       | [107] |
|Peroxiredoxin | Prx3 | AY251021 | 2-Cys mechanism            | Constitutive     | Mitochondrial       | [107] |

Glutathione system

- Glutathione peroxidase 1 | AY043228 | Putative | Tachyzoite/bradyzoite |
- Glutathione reductase | AF041450 | Putative |
- Glutathione synthase | XP_002366333 | Putative |
- Glutathione-S-transferase | XP_002369230 | Putative |
- γ-Glu-Cys synthase | XP_002368128 | Putative |
- Glutaredoxin | Grx1 | BM131493 | Putative |
- Glutaredoxin | Grx2 | BM040167 | Putative |

Thioredoxin system

- Thioredoxin | Trx | BG657266 | Putative | Constitutive | [117] |
- Thioredoxin peroxidase 1 | BM039715 | Putative | Oocyst | [117] |
- Thioredoxin reductase | TrXR | AAS19618 | Putative |

The cell line was more susceptible to peroxide exposure and was also less virulent to mice [107]. This is consistent with studies demonstrating that reduced catalase expression could diminish the infection efficacy in mice [108].

Peroxiredoxins can support the catalase in its detoxification of reactive oxygen species. Currently, three peroxiredoxins have been found in T. gondii. They have different catalytic mechanisms, subcellular localisations, and roles in the parasite metabolism.

The peroxiredoxin 2 (Prx2, Figure 1) belongs to the 1-cys group which, unlike the other peroxiredoxin groups, possesses just one catalytic cysteine residue. Despite this dissimilarity, Prx2 is still able to detoxify H₂O₂ in the presence of dithiothreitol [107, 109]. It is worth mentioning that up to date no endogenous reducing partner has been identified, despite the fact that glutathione, lipoic acid, thioredoxin, and glutaredoxin have all been tested as electron donors for the regeneration of the protein [109]. Prx2-overexpressing parasites showed an increased resistance against H₂O₂ stress, which allowed the pathogen to survive after applying oxidative stress [107]. Additionally, further experiments suggest another role for Prx2. The 1-cys peroxiredoxins are bifunctional enzymes with phospholipase activity and its overexpression is related to membrane protection against oxidative stress [110, 111].

Furthermore, T. gondii possesses two other peroxiredoxins, Prx1 and Prx3, which belong to the typical 2-cys peroxiredoxins harbouring two cysteine residues for forming intermolecular disulphide bonds [10, 112]. Normally peroxiredoxins are considered as proteins with a slow conversion rate [10]. The TgPrx1 is a cytosolic enzyme with constitutive expression which, differentially from Prx2 and Prx3, has an unusually high efficiency in dealing with peroxides (Figure 1, Table 2) [109].

Prx3 is localised in the mitochondrial and there likely to be involved in the detoxification of its own metabolism-derived ROS and their respective by-products [107, 113, 114]. The function of Prx3 might be assisted by another set of enzymes; two SODs are also present in the parasite's mitochondrion (Table 2) [107].

SOD2 and SOD3 have been localised in the parasite's mitochondrion. They have conserved residues to bind iron and although very similar in the primary sequence to SODs from P. falciparum, their configuration is more characteristic to prokaryotes [107]. There is a third cytosolic superoxide dismutase, SODB1, previously characterized as Fe-binding enzymes, which is different to the Mn-binding enzyme in humans. Studies have demonstrated that this gene is essential, as SODB1 knock-outs are lethal [115]. SODs are present in almost all developmental stages of T. gondii and in all organelles, pointing out the importance of a rapid detoxification of superoxide anions in order to protect the parasite.

The classical antioxidant systems such as Trx and GSH are also suggested to occur in Toxoplasma, which is corroborated by the presence of trx genes in the transcriptome as reviewed previously [116]. The glutathione biosynthetic enzymes are present in the genome of T. gondii (Table 2). However, no experimental data are available about these enzymes.

Currently, the treatment of toxoplasmosis relies on inhibitors such as pyrimethamine and atovaquone, in a similar way to the treatment of malaria [117]. In malaria artemisinin has been proposed to interfere with the parasite's oxidative homeostasis, today being one of the most effective drugs for
malaria treatment. Artemisinin has also been experimentally used against *T. gondii*, however, with only moderate effect in comparison to malaria [118, 119]. This, besides other factors, might undermine the importance of the antioxidant systems for the parasite [120].

Regardless of the variety of antioxidant systems presented in this review, *T. gondii* is still highly susceptible to oxidative stress as demonstrated by the use of stress inducers, such as juglone, phenazine methylsulfate and *t*-BuOOH, affecting the tachyzoites stage even at the nanomolar range, without harming the host [109]. The antioxidant systems in *T. gondii* are essential and therefore good candidates for the discovery of novel drugs.

6. Conclusion

This review highlights the importance of the antioxidant systems of apicomplexan parasites, which are essential for tackling oxidative stress and consequently indispensable for the survival of the pathogens in their hosts. Parasites such as *T. gondii* and *Babesia* have developed a complex antioxidant defence system for surviving inside the host cell. Oxidative stress not only is a problem for intracellular survival but also occurs during infection when the parasite is exposed to the host's immune system, which uses ROS to fight the infection. In summary all apicomplexa are highly susceptible to oxidative stress and therefore selective interference with the redox homeostasis of these pathogens presents excellent drug target qualities.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Soraya S. Bosch, Thales Kronenberger, Kamila A. Meissner, and Flávia M. Zimbres contributed equally to this work.

Acknowledgments

The authors would like to thank Dr. Matthew R. Groves, University of Groningen, for critical reading of the paper and helpful comments. This work was financially supported by Grant nos. 2009/54325-2, 2010/20647-0, 2011/13706-3, 2011/19703-6, 2012/12807-3, 2013/17577-9, and 2013/10288-1 from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grant no. 445391/2014-6). The authors would also like to acknowledge the CAPES/DAAD support within the UNIBRAL programme entitled “INFECT-BIO-USP-WWU” (081/14) between the Universities of Münster (WWU) and São Paulo (USP).

References

[1] H. Mehlhorn, *Encyclopedia of Parasitology*, Springer, 3rd edition, 2008.
[2] A. Y. Andreyev, Y. E. Kushnareva, and A. A. Starkov, “Mitochondrial metabolism of reactive oxygen species,” *Biochemistry*, vol. 70, no. 2, pp. 200–214, 2005.
[3] K. M. Massimine, M. T. McIntosh, L. T. Doan et al., “Eosin B as a novel antimalarial agent for drug-resistant *Plasmodium falciparum*,” *Antimicrobial Agents and Chemotherapy*, vol. 50, no. 9, pp. 3132–3141, 2006.
[4] D. Trachootham, W. Lu, M. A. Ogasawara, N. R.-D. Valle, and P. Huang, “Redox regulation of cell survival,” *Antioxidants and Redox Signaling*, vol. 10, no. 8, pp. 1343–1374, 2008.
[5] D. P. Jones and Y.-M. Go, “Redox compartmentalization and cellular stress,” *Diabetes, Obesity and Metabolism*, vol. 12, supplement 2, pp. 116–125, 2010.
[6] E. S. I. Arnér and A. Holmgren, “Physiological functions of thioredoxin and thioredoxin reductase,” *European Journal of Biochemistry*, vol. 267, no. 20, pp. 6102–6109, 2000.
[7] J. Nordberg and E. S. J. Arnér, “Reactive oxygen species, antioxidants, and the mammalian thioredoxin system,” *Free Radical Biology and Medicine*, vol. 31, no. 11, pp. 1287–1312, 2001.
[8] B. Halliwell, “Antioxidant defence mechanisms: from the beginning to the end (of the beginning),” *Free Radical Research*, vol. 31, no. 4, pp. 261–272, 1999.
[9] I. Fridovich, “Superoxide radical and superoxide dismutases,” *Annual Review of Biochemistry*, vol. 64, pp. 97–112, 1995.
[10] B. Hofmann, H. J. Hecht, and L. Flohe, “Peroxiredoxins,” *The Journal of Biological Chemistry*, vol. 383, pp. 347–364, 2002.
[11] F. Nepveu and F. Turrini, “Targeting the redox metabolism of *Plasmodium falciparum*,” *Future Medicinal Chemistry*, vol. 5, no. 16, pp. 1993–2006, 2013.
and Environmental Microbiology, vol. 71, no. 9, pp. 5022–5028, 2005.

[79] G. Bajszár and A. Dekonenko, “Stress-induced Hsp70 gene expression and inactivation of Cryptosporidium parvum oocysts by chlorine-based oxidants,” Applied and Environmental Microbiology, vol. 76, no. 6, pp. 1732–1739, 2010.

[80] J. Winter, M. Ilbert, P. C. F. Graf, D. Özdeliş, and U. Jakob, “Bleach activates a redox-regulated chaperone by oxidative protein unfolding,” Cell, vol. 135, no. 4, pp. 691–701, 2008.

[81] P. M. S. Katja Becker, Apicomplexan Parasites, Wiley-Blackwell, 2011.

[82] P. C. Allen, H. D. Danforth, and P. C. Augustine, “Dietary modulation of avian coccidiosis,” International Journal for Parasitology, vol. 28, no. 7, pp. 1131–1140, 1998.

[83] R. A. Walker, D. J. P. Ferguson, C. M. D. Miller, and N. C. Smith, “Sex and Eimeria: a molecular perspective,” Parasitology, vol. 140, no. 14, pp. 1701–1717, 2013.

[84] G. Greif, A. Harder, and A. Haberkorn, “Chemotherapeutic approaches to protozoa: coccidiae—current level of knowledge and outlook,” Parasitology Research, vol. 87, no. 11, pp. 973–975, 2001.

[85] C. C. Wang, “Studies of the mitochondria from Eimeria tenella and inhibition of the electron transport by quinolone coccidiostats,” Biochimica et Biophysica Acta, vol. 396, no. 2, pp. 210–219, 1975.

[86] A. Harder and A. Haberkorn, “Possible mode of action of toltrazuril: Studies on two Eimeria species and mammalian and Ascaris suum enzymes,” Parasitology Research, vol. 76, no. 1, pp. 8–12, 1989.

[87] R. F. Shumard and M. E. Callender, “Anticoccidial drugs: screening methods,” Experimental Parasitology, vol. 28, no. 1, pp. 13–24, 1970.

[88] H. D. Chapman, “Biochemical, genetic and applied aspects of drug resistance in Eimeria parasites of the fowl,” Avian Pathology, vol. 26, no. 2, pp. 221–244, 1997.

[89] A. C. Cuckler and C. M. Malanga, “Studies on drug resistance in Coccidia,” The Journal of parasitology, vol. 41, no. 3, pp. 302–311, 1955.

[90] G. Eraslan, Y. Cam, M. Eren, and B. C. Liman, “Stress-induced Hsp70 gene expression and inactivation of Cryptosporidium parvum oocysts by chlorine-based oxidants,” Applied and Environmental Microbiology, vol. 76, no. 6, pp. 1732-1739, 2010.

[91] J. Winter, M. Ilbert, P. C. F. Graf, D. Özdeliş, and U. Jakob, “Bleach activates a redox-regulated chaperone by oxidative protein unfolding,” Cell, vol. 135, no. 4, pp. 691–701, 2008.

[92] P. M. S. Katja Becker, Apicomplexan Parasites, Wiley-Blackwell, 2011.

[93] P. C. Allen, H. D. Danforth, and P. C. Augustine, “Dietary modulation of avian coccidiosis,” International Journal for Parasitology, vol. 28, no. 7, pp. 1131–1140, 1998.

[94] R. A. Walker, D. J. P. Ferguson, C. M. D. Miller, and N. C. Smith, “Sex and Eimeria: a molecular perspective,” Parasitology, vol. 140, no. 14, pp. 1701–1717, 2013.

[95] G. Greif, A. Harder, and A. Haberkorn, “Chemotherapeutic approaches to protozoa: coccidiae—current level of knowledge and outlook,” Parasitology Research, vol. 87, no. 11, pp. 973–975, 2001.

[96] H. D. Chapman, “Biochemical, genetic and applied aspects of drug resistance in Eimeria parasites of the fowl,” Avian Pathology, vol. 26, no. 2, pp. 221–244, 1997.

[97] A. C. Cuckler and C. M. Malanga, “Studies on drug resistance in Coccidia,” The Journal of parasitology, vol. 41, no. 3, pp. 302–311, 1955.

[98] G. Eraslan, Y. Cam, M. Eren, and B. C. Liman, “Stress-induced Hsp70 gene expression and inactivation of Cryptosporidium parvum oocysts by chlorine-based oxidants,” Applied and Environmental Microbiology, vol. 76, no. 6, pp. 1732-1739, 2010.

[99] J. Winter, M. Ilbert, P. C. F. Graf, D. Özdeliş, and U. Jakob, “Bleach activates a redox-regulated chaperone by oxidative protein unfolding,” Cell, vol. 135, no. 4, pp. 691–701, 2008.

[100] V. Carruthers and J. C. Boothroyd, “Pulling together: an integrated model of Toxoplasma cell invasion,” Current Opinion in Microbiology, vol. 10, no. 1, pp. 83–89, 2007.

[101] C. Lang, U. Grosz, and C. G. K. Lüder, “Subversion of innate and adaptive immune responses by Toxoplasma Gondii,” Parasitology Research, vol. 100, no. 2, pp. 191–203, 2007.

[102] W. Bohne, M. Holpert, and U. Gross, “Stage differentiation of the protozoan parasite Toxoplasma gondii,” Immunobiology, vol. 201, no. 2, pp. 248–254, 1999.

[103] E. Y. Dekerse, L. Kim, and B. A. Butcher, “In the belly of the beast: Subversion of macrophage proinflammatory signalling cascades during Toxoplasma gondii infection,” Cellular Microbiology, vol. 5, no. 2, pp. 75–83, 2003.

[104] M. Ding, C. Clayton, and D. Soldati, “Toxoplasma gondii catalase: are there peroxisomes in Toxoplasma?” Journal of Cell Science, vol. 113, part 13, pp. 2409–2419, 2000.

[105] A. J. Kaasch and K. A. Joiner, “Targeting and subcellular localization of Toxoplasma gondii catalase. Identification of peroxisomes in an apicomplexan parasite,” Journal of Biological Chemistry, vol. 275, no. 2, pp. 1112–1118, 2000.

[106] S. Subramani, A. Koller, and W. B. Snyder, “Import of peroxisomal matrix and membrane proteins,” Annual Review of Biochemistry, vol. 69, pp. 399–418, 2000.

[107] L. Y. Kwok, D. Schlüter, C. Clayton, and D. Soldati, “The antioxidant systems in Toxoplasma gondii and the role of cytosolic catalase in defence against oxidative injury,” Molecular Microbiology, vol. 51, no. 1, pp. 47–61, 2004.

[108] N. Nischik, B. Schade, K. Dytnerska, H. Długoska, G. Reichmann, and H.-G. Fischer, “Attenuation of mouse-virulent Toxoplasma gondii parasites is associated with a decrease in interleukin-12-inducing tachyzoite activity and reduced expression of actin, catalase and excretory proteins,” Microbes and Infection, vol. 3, no. 9, pp. 689–699, 2001.

[109] S. E. Akerman and S. Müller, “Peroxiredoxin-linked detoxification of hydroperoxides in Toxoplasma gondii,” Journal of Biological Chemistry, vol. 280, no. 1, pp. 564–570, 2005.

[110] J. W. Chen, C. Dodia, S. I. Feinstein, M. K. Jain, and A. B. Fisher, “1-Cys peroxiredoxin, a bifunctional enzyme with glutathione peroxidase and phospholipase A2 activities,” Journal of Biological Chemistry, vol. 275, no. 37, pp. 28421–28427, 2000.

[111] Y. Manevich, T. Sweitzer, J. H. Pak, S. I. Feinstein, V. Muzykantov, and A. B. Fisher, “1-Cys peroxiredoxin overexpression protects cells against phospholipid peroxidation-mediated membrane damage,” Proceedings of the National Academy of Sciences of the United States of America, vol. 99, no. 18, pp. 11599–11604, 2002.

[112] W. Jung, T. Sweitzer, J. H. Pak, S. I. Feinstein, V. Muzykantov, A. B. Fisher, and S. A. M. Müller, “Peroxiredoxin-linked detoxification of hydroperoxides in Toxoplasma gondii,” Journal of Biological Chemistry, vol. 280, no. 1, pp. 564–570, 2005.

[113] B. J. Luft and J. S. Remington, “Toxoplasmic encephalitis in AIDS,” Clinical Infectious Diseases, vol. 15, no. 2, pp. 211–222, 1992.

[114] G. Desmonts and J. Couvreur, “Congenital toxoplasmosis. A prospective study of 378 pregnancies,” The New England Journal of Medicine, vol. 290, no. 20, pp. 1110–1116, 1974.
[113] A. E. Vercesi, C. O. Rodrigues, S. A. Uyemura, L. Zhong, and S. N. J. Moreno, “Respiration and oxidative phosphorylation in the apicomplexan parasite Toxoplasma gondii,” The Journal of Biological Chemistry, vol. 273, no. 47, pp. 31040–31047, 1998.

[114] E. J. L. Melo, M. Attias, and W. de Souza, “The single mitochondrion of tachyzoites of Toxoplasma gondii,” Journal of Structural Biology, vol. 130, no. 1, pp. 27–33, 2000.

[115] C. Ödberg-Ferragut, J. Philippe Renault, E. Viscogliosi et al., “Molecular cloning, expression analysis and iron metal cofactor characterisation of a superoxide dismutase from Toxoplasma gondii,” Molecular and Biochemical Parasitology, vol. 106, no. 1, pp. 121–129, 2000.

[116] S. Rahlfs, R. H. Schirmer, and K. Becker, “The thioredoxin system of Plasmodium falciparum and other parasites,” Cellular and Molecular Life Sciences, vol. 59, no. 6, pp. 1024–1041, 2002.

[117] C. P. Hencken, L. Jones-Brando, C. Bordón et al., “Thiazole, oxadiazole, and carboxamide derivatives of artemisinin are highly selective and potent inhibitors of Toxoplasma gondii,” Journal of Medicinal Chemistry, vol. 53, no. 9, pp. 3594–3601, 2010.

[118] E. Holfels, J. McAuley, D. Mack, W. K. Milhous, and R. McLeod, “In vitro effects of artemisin ether, cycloguanil hydrochloride (alone and in combination with sulfadiazine), quinine sulfate, mefloquine, primaquine phosphate, trifluoperazine hydrochloride, and verapamil on Toxoplasma gondii,” Antimicrobial Agents and Chemotherapy, vol. 38, no. 6, pp. 1392–1396, 1994.

[119] M. E. Sarciron, C. Saccharin, A. F. Petavy, and F. Peyron, “Effects of artesunate, dihydroartemisinin, and an artesunate-dihydroartemisinin combination against Toxoplasma gondii,” The American Journal of Tropical Medicine and Hygiene, vol. 62, no. 1, pp. 73–76, 2000.

[120] W. E. Ho, H. Y. Peh, T. K. Chan, and W. S. F. Wong, “Artemisinins: pharmacological actions beyond anti-malarial,” Pharmacology & Therapeutics, vol. 142, no. 1, pp. 126–139, 2014.

[121] L. D. Sibley, R. Lawson, and E. Weidner, “Superoxide dismutase and catalase in Toxoplasma gondii,” Molecular and Biochemical Parasitology, vol. 19, no. 1, pp. 83–87, 1986.

[122] K. A. Brayton, A. O. T. Lau, D. R. Herndon et al., “Genome sequence of Babesia bovis and comparative analysis of apicomplexan hemoproteozoa,” PLoS Pathogens, vol. 3, no. 10, pp. 1401–1413, 2007.

[123] L. S. Kappmeyer, M. Thiagarajan, D. R. Herndon et al., “Comparative genomic analysis and phylogenetic position of Theileria equi,” BMC Genomics, vol. 13, no. 1, article 603, 2012.

[124] J.-M. Kang, H.-I. Cheun, J. Kim et al., “Identification and characterization of a mitochondrial iron-superoxide dismutase of Cryptosporidium parvum,” Parasitology Research, vol. 103, no. 4, pp. 787–795, 2008.

[125] P. Becuwe, C. Slomianny, A. Valentín, J. Schrevel, D. Camus, and D. Dive, “Endogenous superoxide dismutase activity in two Babesia species,” Parasitology, vol. 105, no. 2, pp. 177–182, 1992.

[126] P. Xu, G. Widmer, Y. Wang et al., “The genome of Cryptosporidium hominis,” Nature, vol. 431, no. 7012, pp. 1107–1112, 2004.

[127] E. S. Son, K. J. Song, J. C. Shin, and H. W. Nam, “Molecular cloning and characterization of peroxiredoxin from Toxoplasma gondii,” Korean Journal of Parasitology, vol. 39, no. 2, pp. 133–141, 2001.