Parasitology. Author manuscript; available in PMC 2010 October 19.

Published in final edited form as:
Parasitology. 2009 April ; 136(4): 379–391. doi:10.1017/S0031182008005477.

Identification and molecular characterization of numerous *Histomonas meleagridis* proteins using a cDNA library

I. BILIC, **M. LEBERL**, and **M. HESS***
Clinic for Avian, Reptile and Fish Medicine, University of Veterinary Medicine, Vienna, Veterinaerplatz 1, 1210 Vienna, Austria

SUMMARY

Histomonas meleagridis is a protozoan parasite of various galliform birds causing a type of enterohepatitis termed histomonosis or 'blackhead disease'. Due to the ban of chemotherapeutic substances and an increase in free-range poultry production, histomonosis is currently a re-emerging disease. So far limited molecular knowledge is available. In the present work, mRNAs coding for antigenic proteins of *H. meleagridis* were identified. For this purpose, a cDNA expression library was constructed from a mono-eukaryotic culture of *H. meleagridis*. The library was screened with polyclonal rabbit serum raised against purified *H. meleagridis* trophozoites. Polyclonal rabbit serum specifically recognized the same major *H. meleagridis* antigens as chicken and turkey sera originating from animal trials, but displayed a significantly lower bacteria-dependent background signal. After 2 rounds of screening, a total of 95 positive clones were sequenced. Bioinformatics analyses were performed on nucleotide and deduced amino acid sequences, identifying 37 unique clones. Based on the homology to other protozoan parasites, mostly *Trichomonas vaginalis*, the clones were grouped according to functional aspects: structural proteins, possible surface proteins, oxygen reducing proteins, ribosomal proteins, protein kinases and various other intracellular proteins.

Keywords

Histomonas meleagridis; histomonosis; chicken; turkey; cDNA expression library; immuno-screening; amino acid sequence; *Trichomonas vaginalis*

INTRODUCTION

The flagellated protozoon, *Histomonas meleagridis*, is the aetioligical agent of an enterohepatitis termed histomonosis or 'blackhead disease' (Tyzzer, 1920). This parasitic disease is of economic importance in the poultry industry. Untreated, histomonosis causes a high rate mortality in turkeys, whereas clinical signs in chickens may vary considerably. For decades, the disease has been well controlled by the use of chemotherapeutics as preventative and curative drugs (McDougald, 2005). However, due to potential consumer health risks, effective drugs have been withdrawn from different markets, leading to the re-emergence of histomonosis in poultry.

Two different morphological forms of *H. meleagridis* have been described within its host, (i) a flagellated form with one anterior flagellum, residing in the caecal lumen, and (ii) an amoeboid form invading the intestinal mucosa and the liver (Bishop, 1938; Lund *et al*.

*Corresponding author. Tel: +43 1250775150. Fax: +43 1250775192. Michael.Hess@vu-wien.ac.at.

Europe PMC Funders Author Manuscripts

1967). A cyst stage, common to other related parasites as a survival form, has not been identified for *H. meleagridis* so far. Light and electron microscopy studies described the structure and division of flagellated forms of *H. meleagridis* in detail (Wenrich, 1943; Schuster, 1968; Honigberg and Bennett, 1971). Due to the amoeboid morphology under the light microscope, *H. meleagridis* was commonly placed among the *Rhizopoda*, which includes *Entamoeba* and its relatives. However, electron microscopy studies of *H. meleagridis* revealed morphological similarities with trichomonads, such as the presence of hydrogenosomes and numerous mastigont structures with 4 kinetosomes (Schuster, 1968; Rybicka *et al*. 1972). More recently, comparative analysis of small subunit rRNAs demonstrated a close phlyogenetic relationship with *Dientamoeba fragilis* (Gerbod *et al*. 2001), an atypical intestinal trichomonad that lacks flagella throughout its life cycle. Furthermore, the study linked *Histomonas* and *Dientamoeba* to the genus *Trichomonas*, suggesting that they might be representatives of a reductive evolution marked by the loss of several trichomonad cytoskeletal structures.

Until now, most of the work published on *H. meleagridis* characterizes its phylogenetic position, while detailed molecular studies, such as studies on proteins that cause an immune response in the host, are still missing. Most antigenic proteins of related protozoa like *Tritrichomonas foetus*, *Trichomonas vaginalis* and *Entamoeba histolytica*, are located on the cell surface or are secreted. These proteins are frequently involved in the colonization of mucosal surfaces as well as in the mechanisms of tissue damage and are therefore called virulence factors. *Entamoeba histolytica* produces an abundance of antigenic virulence factors that are involved in both host invasion and tissue destruction. These factors include a surface galactose-binding lectin (Gal/GalNAc lectin), amoebapores and a family of secreted cysteine proteinases (Chaudhry and Petri, 2005). In addition to surface antigens, a few antigenic proteins have been shown to be cytoplasmic proteins: elongation factor 1-alpha, enolase, 70 kDa heat-shock like protein, ribosomal protein L-23-a, cyclophillin and NADP+ dependant alcohol dehydrogenase (De Meester *et al*. 1991; Beanan and Bailey, 1995; Carrero *et al*. 2000; Sanuki *et al*. 2001). Major antigenic proteins, as well as the molecular mechanisms with which *E. histolytica* colonizes mucosal surfaces and causes tissue damage, are well defined. Characterizations of these proteins and mechanisms are not yet as developed for *Trichomonas vaginalis* and *Tritrichomonas foetus*. Nevertheless, several immunogenic virulence factors have been identified for *T. vaginalis* and include a variety of cysteine proteinases, alpha-actinin, P270 immunogen, alpha-enolase and tv44 (Alderete *et al*. 1991 *a*, b; Addis *et al*. 1999; Musatovova and Alderete, 1999; Mundodi *et al*. 2006, 2008).

In the present study, the aim was to identify and characterize antigenic proteins of *H. meleagridis*. For this purpose, a phage display method employing a cDNA expression library was used in combination with a polyclonal rabbit serum raised against purified *H. meleagridis* parasites. Screening of the cDNA expression library resulted in the identification of various surface and intracellular proteins.

MATERIALS AND METHODS

Culture of *Histomonas meleagridis*

A xenic mono-eukaryotic culture of *Histomonas meleagridis* was used for all experiments. The culture was established out of the caecal content and faecal material of a bronze turkey which died of histomonosis at the age of 20 weeks. *In vitro* isolation, establishment and propagation of the cloned parasite were performed as described recently (Hess *et al*. 2006*b*). Briefly, 1 g of caecal content, and some material scraped from the caecal wall were placed in 9 ml of Medium 199 containing Earl's salts, L-glutamine, 25 mM HEPES and L-amino acids (all Gibco™, Invitrogen GmbH, Lofer, Austria). Additionally, 15% foetal bovine

serum (FBS) (Gibco™, Invitrogen GmbH, Lofer, Austria), antibiotics (200 IE penicillin and 200 μg streptomycin/ml of medium) (Sigma-Aldrich, Vienna, Austria), an anti-mycotic drug (2·5 μg amphotericin B/ml of medium) and 11 mg of rice starch (Sigma-Aldrich, Vienna, Austria) were added. This was the standard medium for *in vitro* cultivation of *H. meleagridis*. Cells were passaged every 2–3 days by transferring 1 ml of the old culture into a new sterile 50 ml tube (Sarstedt, Vienna, Austria) containing 9 ml fresh standard medium.

Purification of *H. meleagridis* trophozoites from mono-eukaryotic culture and RNA isolation

Since *H. meleagridis* grows in culture with ill-defined bacterial flora and rice starch, cells harvested directly from the *in vitro* culture are not suitable for the isolation of nucleic acids, proteins or antigens. Additional purification of *H. meleagridis* is required for the production of *H. meleagridis*-specific antibodies and the isolation of nucleic acids and proteins. For this purpose, a purification method including a Histopaque® 1077 (Sigma-Aldrich, Vienna, Austria) and different centrifugation steps was developed. Briefly, *H. meleagridis* cells were collected from 2-day-old cultures by centrifugation at 200 ***g*** for 5 min at room temperature. The supernatant containing bacteria was carefully discarded and the pellet containing *Histomonas* cells and rice starch was re-suspended in 1 ml of Medium 199 (Gibco™, Invitrogen, Lofer, Austria). This suspension was loaded onto a 3 ml Histopaque® 1077 (Sigma-Aldrich, Vienna, Austria) and centrifuged for 30 min at 650 ***g***, room temperature. Then the supernatant containing *Histomonas* cells and the remaining bacteria was transferred to a fresh 15-ml tube (Sarstedt, Vienna, Austria) and centrifuged for 5 min at 200 ***g*** at room temperature. Following centrifugation, the supernatant containing bacteria was removed and the cell pellet was subsequently washed 3 times with 5 ml of Medium 199 (Gibco™, Invitrogen, Lofer, Austria) and centrifuged at 200 ***g*** for 5 min at room temperature. Finally, cells were re-suspended in 1 ml of phosphate-buffered saline (PBS) and 10 μl of this suspension was examined microscopically for the presence of bacteria and to quantify *H. meleagridis* cells. Purified cells were stored as a pellet at −80 °C.

Total RNA from purified *H. meleagridis* was prepared using TRIzol® Reagent (Invitrogen, Lofer, Austria) according to manufacturer's instructions.

Production of rabbit antisera against *H. meleagridis* trophozoites

Two rabbits were each immunized with 2×10^7 purified *H. meleagridis* cells suspended in 50% GERBU LQ (GERBU Biochemicals GmbH, Gaiberg, Germany). Rabbits were immunized subcutaneously and boosted 3 times at 6-week intervals. Serum to be used in the investigation was obtained 6 weeks after the final injection.

Production of chicken and turkey sera against *H. meleagridis* trophozoites

Chicken and turkey sera, used for immuno-detection of the Western blots, were collected during animal trials described recently. Briefly, chicken sera were gained from specified pathogen-free chickens cloacally infected at 14 days of age with 380 000 histomonads (Hess *et al.* 2006*a*). One serum sample taken from a non-infected control bird and 3 samples taken from infected birds (nos. 6, 10, 11) were used in the present study. As the birds did not die from histomonosis, they were killed 6 weeks post-infection. Serum samples were taken on the same day.

The 2 turkey sera were obtained from a vaccination experiment testing the efficacy of an inactivated vaccine (Hess *et al.* 2008). One serum sample was taken from a non-infected control bird (no. 849). The second sample was obtained from a turkey vaccinated at 7 days of age and boosted 2 weeks later (no. 828). This turkey was infected into the cloaca with 10 000 histomonads another 2 weeks later. The serum used was collected when the bird had to

be euthanized due to its poor condition 19 days post-infection (p.i.), when histomonosis was diagnosed.

Purification of serum samples by pre-absorption

In order to decrease non-specific background reactions, pre-absorption of the sera was performed. For this purpose, serum samples were incubated with acetone powders made from the bacteria that cohabitate the mono-eukaryotic *H. meleagridis* culture. The purification was performed according to the protocol of Zhao and Siu (1995) including minor changes. Briefly, the bacterial supernatant harvested from the mono-eukaryotic culture of *H. meleagridis*, collected after the first step of purification, was centrifuged at 4000 ***g*** for 30 min at 4 °C. The bacterial pellet was washed in 1 ml of PBS and cells were collected by centrifugation at 16 000 ***g*** for 15 min at 4 °C. Cells were re-suspended in 300 μl of 0·9% NaCl and divided into 2 Eppendorf tubes. In one aliquot, cells were disrupted with 4 subsequent freeze-thaw cycles while the other aliquot was kept on ice. After freezing and thawing, both cell aliquots were pooled together to achieve a mixture of proteins and intact bacteria in a single sample. This sample was then mixed vigorously with 1·2 ml of cold acetone (−20 °C) by vortexing and incubated on ice for 30 min. The cell and protein pellet was collected by centrifugation at 10 000 ***g*** for 10 min at 4 °C. The pellet was re-suspended in acetone (−20 °C) and incubated on ice for 10 min. The cell and protein pellet was again collected by centrifugation, air-dried and ground with a sterile spatula to make a fine powder. The powder was stored in Eppendorf tubes at −20 °C until use. For pre-absorption, the final concentration of acetone powder was 1% (w/v). Sera were incubated by rocking for 1 h at room temperature or, alternatively, at 4 °C overnight. After incubation, sera were centrifuged at 10 000 ***g*** for 10 min at 4 °C and the supernatant was collected as a source for the primary antibody. Pre-absorption efficiency was examined by dot-blots, comparing pre-absorbed and non pre-absorbed sera.

SDS-PAGE, Western blotting and dot-blotting

For native protein preparations from purified *H. meleagridis* cells, pellets were re-suspended in PBS supplemented with 1% Nonidet P-40 and Complete protease inhibitors cocktail (Roche Diagnostics GmbH, Vienna, Austria). Cells were opened by 4 freeze-thaw cycles. Native protein samples of bacteria were prepared from the supernatant of the mono-eukaryotic *H. meleagridis* culture, collected after the first centrifugation step of *H. meleagridis* purification. The bacterial pellet was re-suspended in PBS supplemented with 1% Nonidet P-40 and Complete protease inhibitors cocktail (Roche Diagnostics GmbH, Vienna, Austria) and lysed by sonication (3 × 30 sec cycles with continuous power). Soluble proteins were separated from insoluble ones by centrifuging at 20 000 ***g*** for 10 min at 4 °C. Both fractions of *Histomonas* and bacterial proteins were mixed with SDS-PAGE loading buffer and analysed on 10% SDS-PAGE. After electrophoresis, proteins were transferred onto a polyvinyl difluoride (PVDF) membrane (Pall Corporation, VWR International GmbH, Vienna, Austria). For immuno-detection, membranes were saturated with 3% skimmed milk and incubated for 2 h with an adequate dilution of either pre-absorbed or non pre-absorbed immune sera: rabbit (1 : 50 000), chicken or turkey (each 1 : 500). After washing, membranes were incubated with horseradish conjugated donkey anti-rabbit IgG (1 : 100 000 dilution; Jackson ImmunoResearch Laboratories, Inc., Dianova, Hamburg, Germany), donkey anti-chicken IgY (IgG) (1 : 20 000 dilution; Jackson ImmunoResearch Laboratories, Inc., Dianova, Hamburg, Germany) or goat anti-turkey IgG (1 : 20 000 dilution; Southern Biotech, Biomedica, Vienna, Austria), respectively, and detected with SuperSignal® West Pico Chemiluminescent Substrate (Pierce, Thermo Fisher Scientific, Vienna, Austria).

Europe PMC Funders Author Manuscripts

Dot-blots were prepared on PVDF membranes and contained 2 spots of histomonad proteins and 2 spots of bacterial proteins (30 μg and 3 μg protein, respectively). The best serum concentration for the immunodetection of Western blots and the screening of the cDNA expression library, as well as the efficiency of the serum pre-absorption were determined using dot-blots.

Construction and screening of the cDNA expression library of *H. meleagridis*

Histomonas meleagridis cDNA library was constructed using the ZAP Express® cDNA Synthesis Kit and ZAP Express® cDNA Gigapack® II Gold Cloning Kit (both from Stratagene), according to manufacturer's instructions. Five micrograms poly (A)+ RNA were purified from total RNA using the Absolutely mRNA™ Purification Kit (Stratagene) and applied to cDNA synthesis. The library was immunologically screened with purified rabbit anti-*H. meleagridis* serum diluted 1 : 100 000 as described (Sambrook *et al.* 1989). After 2 rounds of screening and plaque purification, phagemids were excised with ExAssist interference-resistant Helper Phage according to manufacturer's instructions. Positive clones were sequenced using fluorescence-based sequencing with T3 and T7 primers or specific internal primers. Both DNA strands of each clone were sequenced.

Analysis of sequence data

Assembly and analyses of cDNA sequences as well as alignments of both nucleotide and amino acid sequences were performed with Accelrys Gene, version 2.5 (Accelrys, San Diego, CA) and Lasergene (DNASTAR Inc.) software packages. The cDNA sequences of unique positive clones were deposited in EMBL database and their accession numbers are listed in Table 2. GenBank™ database searches of obtained sequences were carried out with BlastN, BlastP and specialized BLASTs for conserved domains and conserved domain architecture with default settings.

RESULTS

Analysis of polyclonal rabbit, chicken and turkey sera raised against *H. meleagridis*

In order to determine which serum would be best for immuno-screening of the *H. meleagridis* cDNA expression library, both dot and Western blot analyses were performed with anti-*H. meleagridis* sera from rabbits, chickens and turkeys. Rabbit sera were included in the dot blot analysis to compare signals to poultry sera. As expected, the unspecific staining due to antibodies recognizing bacterial antigens was less prominent in rabbit sera (Fig. 1A). Since *H. meleagridis* is a poultry pathogen, sera from either infected chickens or turkeys would have been the obvious choice to identify *H. meleagridis* immuno-reactive antigens. But dot blot analyses of these sera demonstrated an extremely high bacteria-specific background, proving them inadequate for immuno-screening purposes (Fig. 1B and C). This result was not unexpected, since infections in these animal trials were performed with non-purified *H. meleagridis* cells. The sera from non-infected control chickens (data not shown) and a control turkey (Fig. 1C) were also included in dot-blot analyses and showed high bacterial background as well. This could be explained by the natural presence of these bacteria in the caeca of chickens and turkeys and in mono-eukaryotic cultures of *H. meleagridis*. In contrast to the infections induced in chickens and turkeys, rabbits were injected with purified and inactivated *H. meleagridis* trophozoites.

All positive sera were further analysed by Western blotting. For this purpose, *H. meleagridis* and bacterial proteins originating from the same preparation were separated on SDS-PAGE. Following electrophoresis, proteins were transferred to a PVDF membrane and incubated with an adequate dilution of rabbit, chicken or turkey immune sera (Fig. 2). The pattern of protein bands in Western blot analyses demonstrated major *H. meleagridis* antigens at 180

kDa, 150 kDa, 125 kDa, 105–110 kDa and 90 kDa (Table 1). All rabbit, chicken and turkey sera recognized these proteins with varying intensity. Since rabbit immune sera recognized the same major *H. meleagridis* antigens as chicken and turkey sera but displayed significantly lower bacteria-specific background, a rabbit serum was used for the immuno-screening of the *H. meleagridis* cDNA expression library.

Screening of the cDNA expression library of *H. meleagridis*

In order to identify and characterize antigenic proteins of *H. meleagridis*, a cDNA expression library constructed from 6×10^7 purified *H. meleagridis* cells was immuno-screened with polyclonal rabbit serum raised against purified *H. meleagridis*. From the initial 612 000 cDNA library phages plated, 226 positive clones were obtained in the original screening. Upon a second round of screening, 95 clones were positive for coding for antigenic proteins. Before sequence analysis, all 95 clones were analysed for the presence and size of their cDNA insert by digesting the phagemid DNA with *Xho*I and *Pst*I restriction endonucleases.

Analysis of positive clones

All 95 positive clones obtained after 2 rounds of immuno-screening were completely sequenced. In order to gain more information about the sequence of each clone, bioinformatics analyses such as BLAST search algorithm and conserved domain analysis were performed using nucleotide and deduced amino acid sequence. These analyses resulted in 37 *H. meleagridis*-specific sequences that were unique. Based on the homology to other protozoan parasites, mostly to *Trichomonas vaginalis*, positive clones were placed into several groups according to their functional aspects. Detailed information about all 37 unique clones is listed in Table 2. During the screening procedure, clones were additionally categorized according to the intensity of their reaction with polyclonal anti-*H. meleagridis* rabbit serum.

Detailed analysis of clones 28-1 and 93a-2

The BLAST search of the deduced amino acid sequence of clone 93a-2 demonstrated an homology to the *T. vaginalis* adhesin AP65-2/hydrogenosomal malic enzyme (Table 2). Since the existence of *H. meleagridis* hydrogenosomal malic enzyme was recently reported (Mazet *et al*. 2008), the identity of these 2 proteins was examined. Amino acid alignment of clone 93a-2, *H. meleagridis* malic enzyme (Hm_ME), *T. vaginalis* malic enzyme (Tv_ME) and the *T. vaginalis* adhesins AP65-1 (Tv_AP65-1), AP65-2 (Tv_AP65-2) and AP65-3 (Tv_AP65-3), was performed. The alignment of clone 93a-2 and Hm_ME in Fig. 3 shows that the 2 *H. meleagridis* homologues of adhesin AP65/hydrogenosomal malic enzyme are not identical, suggesting the existence of different AP65 adhesins in *H. meleagridis*.

BLAST search of the deduced amino acid sequence of clone 28-1 demonstrated homology to *T. vaginalis* thioredoxin reductase (Table 2). In order to examine whether the key active site containing cysteine is conserved in the *H. meleagridis* homologue, the amino acid alignment of clone 28-1 and the thioredoxin reductases of *T. vaginalis*, *E. histolytica* and *G. lamblia*, was performed. The amino acid alignment in Fig. 4 shows that this active site in the protein is the only region well conserved among all 4 parasites.

DISCUSSION

In recent years, histomonosis has re-emerged as the consequence of both the ban on prophylactic and therapeutic substances and the increased popularity of free-range housing. In contrast to this parasite's importance, its investigation on a molecular level is still rather poor. So far, merely the sequences of the 18S and 5.8S rRNA genes and those of the internal

transcribed spacer regions 1 and 2 are available in databases. In a recent article (Mazet *et al*. 2008), information is given about protein-coding genes for malic enzyme, alpha-succinyl coenzyme A synthetase and iron-hydrogenase, which are all required for the hydrogenosomal carbon metabolism.

The present study attempts to broaden the molecular knowledge on *H. meleagridis* by identifying mRNAs that code for antigenic proteins. For this purpose, a cDNA library of *H. meleagridis* was constructed and immuno-screened with polyclonal rabbit serum raised against purified parasite cells. Extensive bioinformatics sequence analysis of positive clones revealed 37 unique clones of *H. meleagridis*-specific sequences. Most of these show a high level of homology to protein-coding genes of *Trichomonas vaginalis*. This finding supports the results of former investigations, indicating that *H. meleagridis* belongs to the class of *Trichomonadea* (Gerbod *et al*. 2001). Positive clones were grouped into several categories based on homologies to known proteins of other protozoan parasites: proteins for translation, ribosomal structure and biogenesis; structural proteins; proteins for energy construction and conversion; proteins for the biogenesis of the outer membrane and cell envelope; protein kinases; oxidative stress proteins and various other intracellular proteins.

Due to the broad spectrum of expression sequence tags (ESTs) identified in this study, only mRNAs coding for proteins involved in host cell adhesion and host tissue invasion are discussed in more detail. These proteins represent a first line of intrusion into the host by the parasite. Several clones described in this study show homology to such proteins. All 5 protein coding sequences from the category ‘structural proteins’ have homology to *T. vaginalis* proteins involved in the reorganization of actin fibres within the cell. It has been shown that redistribution of actin within *T. vaginalis* results in the transformation of the cell from a flagellated to an amoeboid form. This amoeboid form has been proven to be required for the parasite's cytopathogenicity (Fiori *et al*. 1999). In the obligatory amoebic parasite *E. histolytica*, the rearrangement of actin plays a central role in both movement and cellular interaction with the environment. This includes interaction with host cells (Guillen, 1996). Rearrangement of actin within *T. vaginalis* cells is accomplished by coordinated action of different actin-bundling proteins such as alpha-actinins (Bricheux *et al*. 1998; Addis *et al*. 1999). Since 2 morphological forms, flagellated and amoeboid, have been described for *H. meleagridis* (Bishop, 1938; Lund *et al*. 1967), a similar actin rearranging feature is likely. The flagellated form with one anterior flagellum, residing in the caecal lumen, can be considered a locomotive form, while the amoeboid parasite is found when invading the intestinal mucosa and the liver. In this study, homologous protein-coding sequences involved in cyto-skeletal rearrangements have been identified. This fact and the presence of 2 morphological forms of *H. meleagridis* suggest mechanisms of host tissue invasion similar to those of *T. vaginalis*. A sequence homologous to an additional actin-bundling protein, fimbrin, was identified as well. The presence and role of this protein for *T. vaginalis* has yet to be described, although its putative sequence has been detected in the completed genome of *T. vaginalis* (Carlton *et al*. 2007).

Another group of proteins that play a role in the invasion of host tissue are adhesins. In this study, 2 clones, termed 93a-2 and 117a-1, were detected to be homologous to *T. vaginalis* adhesins AP65 and AP120, respectively. Both adhesins have been described as having additional functions as metabolic enzymes within the *T. vaginalis* hydrogenosomes. The hydrogenosome is a double membrane-bound organelle involved in the fermentative oxidation of pyruvate derived from glycolysis (Muller, 1997). The AP65 adhesin of *T. vaginalis* is the decarboxylating malic enzyme (ME), while the AP120 adhesin of *T. vaginalis* functions as pyruvate : ferredoxin oxidoreductase A (PFOA) (Engbring *et al*. 1996; Alderete *et al*. 2001; Moreno-Brito *et al*. 2005). Due to possessing 2 unrelated functions, that of a metabolic enzyme and an adhesion function, these proteins represent moonlighting

proteins of *T. vaginalis* (Jeffery, 1999; Hirt *et al*. 2007). Since they are both members of multi-gene families in *T. vaginalis*, it is still an open question whether only some or all genes code for both enzymatic and adhesive functions. The presence of a *H. meleagridis* homologue of malic enzyme (Hm_ME) together with another *T. vaginalis* moonlighting protein, an alpha-subunit of a succinyl coenzyme A synthetase (Hm_aSCS), was recently reported (Mazet *et al*. 2008). Yet the present study is the first to report the existence of another *H. meleagridis* homologue of a *T. vaginalis* moonlighting protein, namely AP120/ pyruvate : ferredoxin oxido-reductase A (PFOA).

Sequence divergences shown in the protein sequence alignment of clone 93a-2, *H. meleagridis* malic enzyme (Hm_ME), *T. vaginalis* malic enzyme (Tv_ME) and *T. vaginalis* adhesins AP65-1, (Tv_ AP65-1), AP65-2 (Tv_AP65-2), AP65-3 (Tv_AP65-3) suggest that the hydrogenosomal malic enzyme in *H. meleagridis*, as in *T. vaginalis*, is most likely a member of a multi-gene family. Another clone isolated during this study, termed clone 177-1, showed homology to cytosolic malic enzyme. This enzyme is a member of another family of malic enzymes usually found in prokaryotes. The differences between the two families of malic enzymes are obvious in several aspects: (i) localization: the cytosolic enzyme is localized within the cytoplasm, while the hydrogenosomal one is related to the enzyme found in mitochondria and plastids, (ii) size: the cytosolic enzyme is smaller (42 kDa subunit) and forms a dimer, while the hydogenosomal enzyme is bigger (60 kDa subunit) and forms a tetramer and (iii) coenzyme specificity: the cytosolic enzyme has a strict specificity to nicotinamide adenine dinucleotide phosphate (NADP+), while the hydrogenosomal one preferentially uses nicotinamide adenine dinucleotide (NAD+). The existence of 2 different families of malic enzymes in different compartments of a single eukaryotic cell appears to be unique in nature and was described in detail for *T. vaginalis* (Dolezal *et al*. 2004).

The fact that homologous protein-coding sequences of adhesins/hydrogenosomal enzymes and of a cytosolic malic enzyme were identified in *H. meleagridis* is very interesting on several points. Firstly, it suggests that the mechanisms of both host-cell adhesion and energy production and conservation are very similar between *H. meleagridis* and *T. vaginalis*. Secondly, it emphasizes the close phylogenetic relationship between these two parasites. However, it still remains to be investigated whether the same phenomenon of moonlighting is true for *H. meleagridis* proteins.

Another interesting clone detected during this study, clone 28-1, shows strong homology (65%) to thioredoxin reductase (TrxR) of *T. vaginalis*, an enzyme involved in the response to oxidative stress. As a microaerophilic parasitic protozoon, *T. vaginalis* depends on an anaerobic metabolism. When exposed to oxygen, *T. vaginalis* must be able to cope with the resultant oxidative stress. Thioredoxin reductase functions together with thioredoxin and thioredoxin peroxidase to detoxify potentially damaging oxidants (Coombs *et al*. 2004). This system does not use a glutathione as the reductant and is thus of particular importance to amitochondriate eukaryotes, such as *Trichomonas*, *Entamoeba* and *Giardia*, which lack glutathione (Ellis *et al*. 1994; Flohe *et al*. 1999; Muller *et al*. 2003). The protein alignment of the *H. meleagridis* clone 28-1 with thioredoxin reductases of other protozoan parasites demonstrates its strong homology to the *T. vaginalis* protein, as compared to the *E. histolytica* and *G. lamblia* homologues. In this protein, the only well-conserved part among all 4 parasites is the key active site containing cysteine residues (boxed in Fig. 4). *H. meleagridis* is regarded as an anaerobic parasite, but strict anaerobic incubation is not obligatory for the parasite to multiply *in vitro* (Hess *et al*. 2006*b*). The presence of the EST sequence in *H. meleagridis* with a high homology to thioredoxin reductase indicates that *Histomonas* is capable of combating oxidative stress. Furthermore, the mechanism to detoxify damaging oxidants seems to be very similar to that of other homologous parasites.

In *T. vaginalis*, thioredoxin reductase is one of the proteins also found to be secreted in response to the parasite's contact with host cells (Kucknoor *et al*. 2007). It is most likely involved in modifying disulphide bonds of host proteins, thereby altering the microenvironment to one more suitable for the parasite's survival. Although the invasion of caecal epithelium by *H. meleagridis* seems to resemble the invasion of vaginal mucosa surfaces by *T. vaginalis* in many ways, it still remains elusive whether thioredoxin reductase is secreted by *Histomonas*, thereby actively contributing to the virulence of this parasite.

During the present study, a broad spectrum of protein-coding sequences showing homology to both intracellular and surface proteins was identified. Since the original intention of the study was the identification of *H. meleagridis* antigens, one would expect these to be located on the cell surface. However, in addition to surface antigens, numerous intracellular proteins were detected. It is evident from the literature that some intracellular proteins of *E. histolytica* were also found to be antigenic (De Meester *et al*. 1991; Beanan and Bailey, 1995; Carrero *et al*. 2000; Sanuki *et al*. 2001). It is possible, though, that some of the intracellular proteins identified in this study, especially weak signal translation machinery proteins, might not represent true *H. meleagridis* immunogenic proteins. Reporting sequences of such proteins, nevertheless, has its scientific importance, since until now only sequences of 3 *H. meleagridis* proteins are available in the database. The detection of intracellular proteins is obviously a consequence of the rabbit serum used for screening the cDNA library, because the inoculum administered to rabbits contained both destroyed and intact parasite cells. The detection of intracellular proteins during screening would have also been expected using chicken or turkey sera, which were gained from the natural host of the parasite after lethal infection. Prior to screening the *H. meleagridis* cDNA expression library, we used dot blot analysis to compare the polyclonal sera of the 3 different species. Polyclonal rabbit serum had the advantage of displaying a much weaker bacteria-specific background as compared to chicken and turkey sera. This background would obscure the screening procedure. Therefore it was decided to use polyclonal rabbit serum in subsequent experiments. Furthermore, the comparative analyses of the sera by Western blot analysis from all 3 animal species showed that the same major *H. meleagridis* antigens were detected by all polyclonal sera. It is nevertheless possible that polyclonal chicken and turkey sera would detect additional proteins. This, however, is the scope of further investigations.

Finally, this study represents a major breakthrough for the molecular investigation of *H. meleagridis*, since it is the first to report 37 *H. meleagridis*-specific protein-coding sequences. Various ESTs that might have an impact on host-cell invasion were detected and have been commented on in more detail. Discussing all 37 *H. meleagridis*-specific EST sequences identified during the present study would definitely reach beyond the capacity of this investigation. Nevertheless, the remaining clones listed in Table 2 should not be ignored. Since little molecular data are available on *H. meleagridis*, these cDNA sequences represent a foundation for further molecular studies on this parasite.

Acknowledgments

This investigation was supported by the Austrian Science Fund (FWF), grant no. P19315.

REFERENCES

Addis MF, Rappelli P, Pinto De Andrade AM, Rita FM, Colombo MM, Cappuccinelli P, Fiori PL. Identification of *Trichomonas vaginalis* alpha-actinin as the most common immunogen recognized by sera of women exposed to the parasite. The Journal of Infectious Diseases. 1999; 180:1727–1730. [PubMed: 10515842]

Alderete JF, Millsap KW, Lehker MW, Benchimol M. Enzymes on microbial pathogens and *Trichomonas vaginalis*: molecular mimicry and functional diversity. Cellular Microbiology. 2001; 3:359–370. [PubMed: 11422079]

Alderete JF, Newton E, Dennis C, Neale KA. Antibody in sera of patients infected with *Trichomonas vaginalis* is to trichomonad proteinases. Genitourinary Medicine. 1991a; 67:331–334. [PubMed: 1916796]

Alderete JF, Newton E, Dennis C, Neale KA. The vagina of women infected with *Trichomonas vaginalis* has numerous proteinases and antibody to trichomonad proteinases. Genitourinary Medicine. 1991b; 67:469–474. [PubMed: 1774051]

Beanan MJ, Bailey GB. The primary structure of an *Entamoeba histolytica* enolase. Molecular and Biochemical Parasitology. 1995; 69:119–121. [PubMed: 7723780]

Bishop A. *Histomonas meleagridis* in domestic fowls (*Gallus gallus*). Cultivation and experimental infection. Parasitology. 1938; 30:181–194.

Bricheux G, Coffe G, Pradel N, Brugerolle G. Evidence for an uncommon alpha-actinin protein in *Trichomonas vaginalis*. Molecular and Biochemical Parasitology. 1998; 95:241–249. [PubMed: 9803416]

Carlton JM, Hirt RP, Silva JC, Delcher AL, Schatz M, Zhao Q, Wortman JR, Bidwell SL, Alsmark UC, Besteiro S, Sicheritz-Ponten T, Noel CJ, Dacks JB, Foster PG, Simillion C, Van de PY, Miranda-Saavedra D, Barton GJ, Westrop GD, Muller S, Dessi D, Fiori PL, Ren Q, Paulsen I, Zhang H, Bastida-Corcuera FD, Simoes-Barbosa A, Brown MT, Hayes RD, Mukherjee M, Okumura CY, Schneider R, Smith AJ, Vanacova S, Villalvazo M, Haas BJ, Pertea M, Feldblyum TV, Utterback TR, Shu CL, Osoegawa K, de Jong PJ, Hrdy I, Horvathova L, Zubacova Z, Dolezal P, Malik SB, Logsdon JM Jr. Henze K, Gupta A, Wang CC, Dunne RL, Upcroft JA, Upcroft P, White O, Salzberg SL, Tang P, Chiu CH, Lee YS, Embley TM, Coombs GH, Mottram JC, Tachezy J, Fraser-Liggett CM, Johnson PJ. Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. Science. 2007; 315:207–212. [PubMed: 17218520]

Carrero JC, Petrossian P, Acosta E, Sanchez-Zerpa M, Ortiz-Ortiz L, Laclette JP. Cloning and characterization of *Entamoeba histolytica* antigens recognized by human secretory IgA antibodies. Parasitology Research. 2000; 86:330–334. [PubMed: 10780744]

Chaudhry OA, Petri WA Jr. Vaccine prospects for amebiasis. Expert Review of Vaccines. 2005; 4:657–668. [PubMed: 16221067]

Coombs GH, Westrop GD, Suchan P, Puzova G, Hirt RP, Embley TM, Mottram JC, Muller S. The amitochondriate eukaryote *Trichomonas vaginalis* contains a divergent thioredoxin-linked peroxiredoxin antioxidant system. Journal of Biological Chemistry. 2004; 279:5249–5256. [PubMed: 14630923]

De Meester F, Bracha R, Huber M, Keren Z, Rozenblatt S, Mirelman D. Cloning and characterization of an unusual elongation factor-1 alpha cDNA from *Entamoeba histolytica*. Molecular and Biochemical Parasitology. 1991; 44:23–32. [PubMed: 2011152]

Dolezal P, Vanacova S, Tachezy J, Hrdy I. Malic enzymes of *Trichomonas vaginalis*: two enzyme families, two distinct origins. Gene. 2004; 329:81–92. [PubMed: 15033531]

Ellis JE, Yarlett N, Cole D, Humphreys MJ, Lloyd D. Antioxidant defences in the microaerophilic protozoan *Trichomonas vaginalis*: comparison of metronidazole-resistant and sensitive strains. Microbiology. 1994; 140:2489–2494. [PubMed: 7952198]

Engbring JA, O'Brien JL, Alderete JF. *Trichomonas vaginalis* adhesin proteins display molecular mimicry to metabolic enzymes. Advances in Experimental Medicine and Biology. 1996; 408:207–223. [PubMed: 8895795]

Fiori PL, Rappelli P, Addis MF. The flagellated parasite *Trichomonas vaginalis*: new insights into cytopathogenicity mechanisms. Microbes and Infection. 1999; 1:149–156. [PubMed: 10594979]

Flohe L, Hecht HJ, Steinert P. Glutathione and trypanothione in parasitic hydroperoxide metabolism. Free Radical Biology and Medicine. 1999; 27:966–984. [PubMed: 10569629]

Gerbod D, Edgcomb VP, Noel C, Zenner L, Wintjens R, Delgado-Viscogliosi P, Holder ME, Sogin ML, Viscogliosi E. Phylogenetic position of the trichomonad parasite of turkeys, *Histomonas meleagridis* (Smith) Tyzzer, inferred from small subunit rRNA sequence. Journal of Eukaryotic Microbiology. 2001; 48:498–504. [PubMed: 11456328]

Guillen N. Role of signalling and cytoskeletal rearrangements in the pathogenesis of *Entamoeba histolytica*. Trends in Microbiology. 1996; 4:191–197. [PubMed: 8727599]

Hess M, Grabensteiner E, Liebhart D. Rapid transmission of the protozoan parasite *Histomonas meleagridis* in turkeys and specific pathogen free chickens following cloacal infection with a mono-eukaryotic culture. Avian Pathology. 2006a; 35:280–285. [PubMed: 16854640]

Hess M, Kolbe T, Grabensteiner E, Prosl H. Clonal cultures of Histomonas meleagridis, Tetratrichomonas gallinarum and a *Blastocystis* sp. established through micromanipulation. Parasitology. 2006b; 133:547–554. [PubMed: 16854251]

Hess M, Liebhart D, Grabensteiner E, Singh A. Cloned *Histomonas meleagridis* passaged *in vitro* resulted in reduced pathogenicity and is capable of protecting turkeys from histomonosis. Vaccine. 2008; 26:4187–4199. [PubMed: 18586362]

Hirt RP, Noel CJ, Sicheritz-Ponten T, Tachezy J, Fiori PL. *Trichomonas vaginalis* surface proteins: a view from the genome. Trends in Parasitology. 2007; 23:540–547. [PubMed: 17962075]

Honigberg BM, Bennett CJ. Lightmicroscopic observations on structure and division of *Histomonas meleagridis* (Smith). Journal of Protozoology. 1971; 18:687–700. [PubMed: 4943763]

Jeffery CJ. Moonlighting proteins. Trends in Biochemical Sciences. 1999; 24:8–11. [PubMed: 10087914]

Kucknoor AS, Mundodi V, Alderete JF. The proteins secreted by *Trichomonas vaginalis* and vaginal epithelial cell response to secreted and episomally expressed AP65. Cellular Microbiology. 2007; 9:2586–2597. [PubMed: 17590165]

Lund EE, Augustine PC, Chute AM. *Histomonas meleagridis* after one thousand *in vitro* passages. Journal of Protozoology. 1967; 14:349–351. [PubMed: 6038042]

Mazet M, Diogon M, Alderete JF, Vivares CP, Delbac F. First molecular characterisation of hydrogenosomes in the protozoan parasite *Histomonas meleagridis*. International Journal for Parasitology. 2008; 38:177–190. [PubMed: 17697679]

McDougald LR. Blackhead disease (histomoniasis) in poultry: a critical review. Avian Diseases. 2005; 49:462–476. [PubMed: 16404985]

Moreno-Brito V, Yanez-Gomez C, Meza-Cervantez P, Avila-Gonzalez L, Rodriguez MA, Ortega-Lopez J, Gonzalez-Robles A, Arroyo R. A *Trichomonas vaginalis* 120 kDa protein with identity to hydrogenosome pyruvate:ferredoxin oxidoreductase is a surface adhesin induced by iron. Cellular Microbiology. 2005; 7:245–258. [PubMed: 15659068]

Muller M. Evolutionary origins of trichomonad hydrogenosomes. Parasitology Today. 1997; 13:166–167. [PubMed: 15275085]

Muller S, Liebau E, Walter RD, Krauth-Siegel RL. Thiol-based redox metabolism of protozoan parasites. Trends in Parasitology. 2003; 19:320–328. [PubMed: 12855383]

Mundodi V, Kucknoor AS, Alderete JF. Immunogenic and plasminogen-binding surface-associated alpha-enolase of *Trichomonas vaginalis*. Infection and Immunity. 2008; 76:523–531. [PubMed: 18070902]

Mundodi V, Kucknoor AS, Chang TH, Alderete JF. A novel surface protein of *Trichomonas vaginalis* is regulated independently by low iron and contact with vaginal epithelial cells. BMC Microbiology. 2006; 6:6. [PubMed: 16448556]

Musatovova O, Alderete JF. The *Trichomonas vaginalis* phenotypically varying P270 immunogen is highly conserved except for numbers of repeated elements. Microbial Pathogenesis. 1999; 27:93–104. [PubMed: 10458920]

Rybicka K, Honigberg BM, Holt SC. Fine structure of the mastigont system in culture forms of *Histomonas meleagridis* (Smith). Protistologica. 1972; 8:107–120.

Sambrook, J.; Fritsch, EF.; Maniatis, T. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY, USA: 1989.

Sanuki J, Nakano K, Tokoro M, Nozaki T, Okuzawa E, Kobayashi S, Asai T. Purification and identification of major soluble 40-kDa antigenic protein from *Entamoeba histolytica*: its application for serodiagnosis of asymptomatic amebiasis. Parasitology International. 2001; 50:73–80. [PubMed: 11438429]

Schuster FL. Ultrastructure of *Histomonas meleagridis* (Smith) Tyzzer, a parasitic amebo-flagelate. The Journal of Parasitology. 1968; 54:725–737.

Tyzzer EE. The flagellate character and reclassification of the parasite producing ‘Blackhead’ in turkeys- *Histomonas* (gen. nov.) *meleagridis* (Smith). The Journal of Parasitology. 1920; 6:124–131.

Wenrich DH. Observations on the morphology of *Histomonas* (Protozoa, Mastigophora) from phesants and chickens. Journal of Morphology. 1943; 72:279–303.

Zhao X, Siu CH. Colocalization of the homophilic binding site and the neuritogenic activity of the cell adhesion molecule L1 to its second Ig-like domain. Journal of Biological Chemistry. 1995; 270:29413–29421. [PubMed: 7493978]

Europe PMC Funders Author Manuscripts

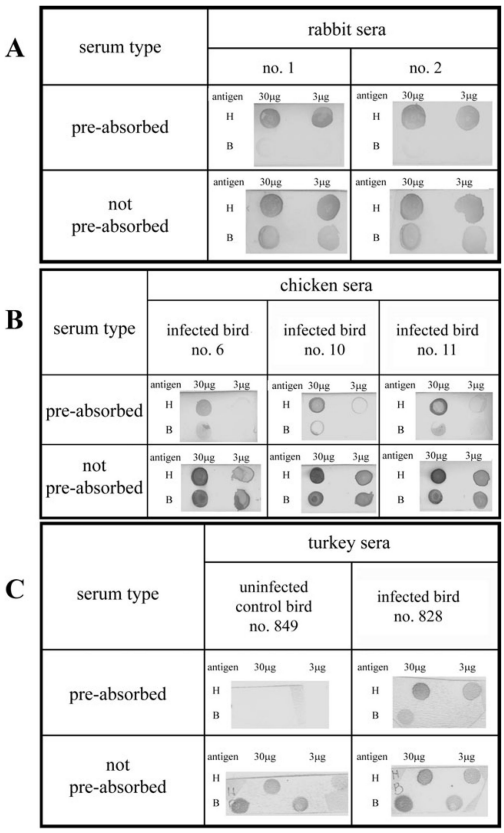

Fig. 1.

Immunodetection of dot-blots with pre-absorbed and not pre-absorbed sera. (A) Polyclonal rabbit anti-*Histomonas meleagridis* immune sera (1 : 50 000), (B) polyclonal chicken anti-*H. meleagridis* immune sera (1 : 500) and (C) polyclonal turkey anti-*H. meleagridis* immune serum (1 : 500). Dots H and B correspond to *Histomonas meleagridis* and bacteria, respectively.

Europe PMC Funders Author Manuscripts

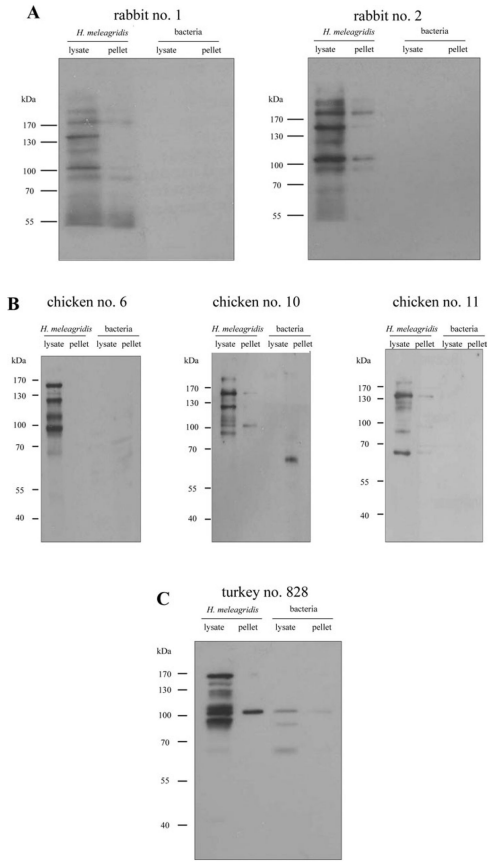

Fig. 2.

Immuno-detection of Western blots with pre-absorbed sera. (A) Polyclonal rabbit anti-*Histomonas meleagridis* immune sera (1 : 50 000), (B) polyclonal chicken anti-*H. meleagridis* immune sera (1 : 500) and (C) polyclonal turkey anti-*H. meleagridis* immune serum (1 : 500). Lysate is the 1% Nonidet P40 soluble fraction of *H. meleagridis* and bacterial proteins. Pellet is the insoluble fraction of *H. meleagridis* and bacterial proteins. Molecular standards are in kDa.

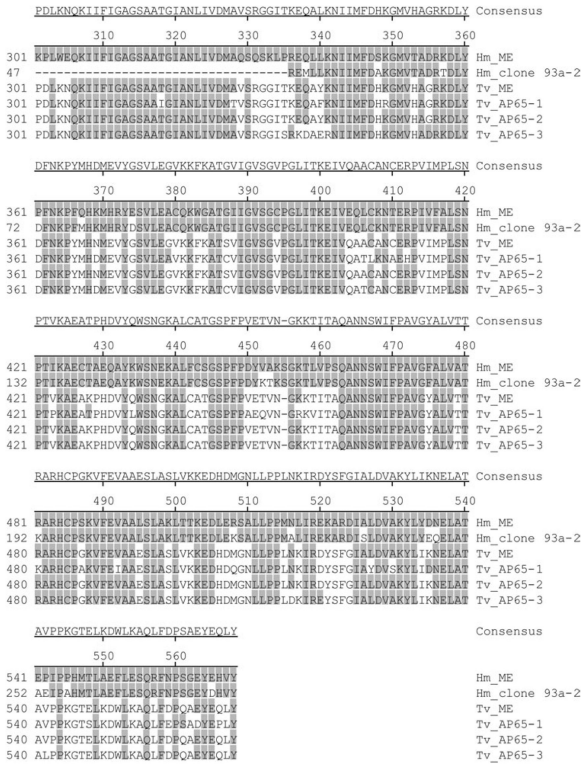

Fig. 3.

Amino acid sequence alignment of the *Histomonas meleagridis* homologue of adhesin AP65-2 (Hm_clone 93a-2) with *H. meleagridis* malic enzyme (Hm_ME), *Trichomonas vaginalis* malic enzyme (Tv_ME) and the *T. vaginalis* adhesins AP65-1 (Tv_AP65-1), AP65-2 (Tv_AP65-2), AP65-3 (Tv_AP65-3). Amino acids are numbered on the left. The sequence of Hm_ME is shown at the top and only identities to this sequence are shaded grey. The deletions are indicated with dashes. The Hm_clone 93a-2 sequence originates from this study. GenBank™ Accession numbers for Hm_ME, Tv_ME, Tv_AP65-1, Tv_AP65-2, Tv_AP65-3 are FJ185157, AAA927141, AAA87406, AAA87407, and AAA91133, respectively.

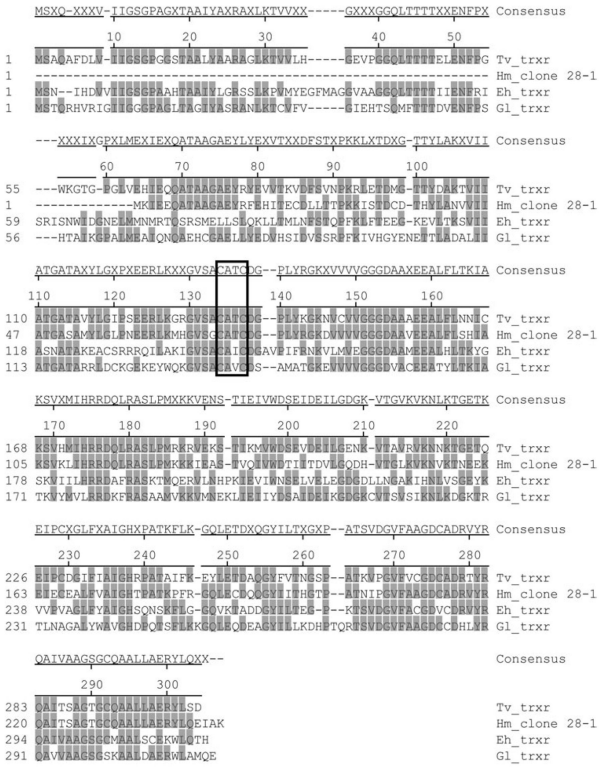

Fig. 4.

Amino acid sequence alignment of the *Histomonas meleagridis* homologue of thioredoxin reductase (Hm_clone 28-1) with the thioredoxin reductases (TrxR) of *Trichomonas vaginalis* (Tv_trxr), *Entamoeba histolytica* (Eh_trxr) and *Giardia lamblia* (Gl_trxr). Amino acids are numbered on the left. The conserved active site, a redox centre is boxed. The sequence of Tv_trxr is shown at the top and only identities to this sequence are shaded grey. The deletions are indicated with dashes. The Hm_clone 28-1 sequence originates from this study. GenBank™ Accession numbers for Tv_trxr, Eh_trxr and Gl_trxr are XP_001316923, CAA56112 and AJ507833, respectively.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Summary of major antigens recognized by rabbit, chicken and turkey immune sera

Histomonas meleagridis antigens	Rabbit sera		Chicken sera			Turkey serum
	1	**2**	**6**	**10**	**11**	**828**
>170 kDa	++[b]	++	−	+	+/−	−
ca. 150 kDa	+++[a]	+++	+++	+++	+++	++
ca. 125 kDa	+[c]	+	+++	+++	+	++
ca. 115 kDa	−[e]	−	−	+	+	−
ca. 110 kDa	−	−	+++	+	−	+++
ca. 105 kDa	+++	+++	−	+	−	−
ca. 90 kDa	++	++	+++	++	+	+++
ca. 65 kDa	+/−[d]	+/−	+/−	−	++	−

[a](+++) very strong band

[b](++) strong band

[c](+) weak band

[d](+/−) very weak band

[e](−) no band.

Europe PMC Funders Author Manuscripts

Table 2

List and detailed information of all unique clones isolated during immuno-screening of the cDNA expression library

Category	Clone name	Antigen homology	Signal intensity	% identity to homologous parasites	Accession number
Translation, ribosomal structure and biogenesis	3/1	*T. vaginalis* putative ribosomal protein S3	Strong	83% ns[a]	FM200059
				91% aa[b]	
	7/1	*T. vaginalis* and *E. histolytica* glutaminyl tRNA synthetase family protein	Strong	54% aa (*T. vaginalis*)	FM200060
				42% aa (*E. histolytica*)	
	29b/1	*T. vaginalis* putative 60S acidic ribosomal protein P1	Weak	66% aa	FM200061
	32a/1	*T. vaginalis* putative ribosomal protein L10	Weak	61% aa	FM200062
	79a/1	*T. vaginalis* elongation factor 1-alpha (*tef1*)	Weak	82–84% ns	FM200063
				83% aa	
	110a/1	*T. vaginalis* putative ribosomal protein conserved domain L4/L1 family	Weak	58% aa	FM200064
	177/2	*T. vaginalis* putative ribosomal protein S27a	Weak	57% aa	FM200065
	183c/2	*T. vaginalis* putative ribosomal protein L18	Weak	64% aa	FM200066
	195a/2	*T. vaginalis* ribosomal protein L8	Strong	68% aa	FM200067
Structural proteins	9a/3	*T. vaginalis* alpha-actinin	Strong	46% aa	FM200068
	48/3	*T. vaginalis* putative fimbrin	Strong	63% aa	FM200069
	73/2	*T. vaginalis* actin	Strong	78% ns	FM200070
				81% aa	
	163/1	*T. vaginalis* alpha-actinin	Strong	41% aa	FM200071
	192/1	*T. vaginalis* alpha-actinin	Strong	46% aa	FM200072
Energy construction and conversion	11/2	*T. vaginalis* putative hydrogenosomal oxygen reductase	Strong	58% aa[b]	FM200073
	93a/2	*T. vaginalis* hydrogenosomal malic enzyme subunit A (AP65-2)	Weak	58% aa	FM200074
	117a/1	*T. vaginalis* pyruvate : ferredoxin oxidoreductase E (PFOE)	Weak	76% aa	FM200075
	177/1	*T. foetus* cytosolic malate dehydrogenase 1 and 2 (*mdh1*, *mdh2*)	Weak	70% aa	FM200076
Protein kinases	25a/2	*T. vaginalis* CAMK family protein kinase	Weak	36–81% aa	FM200077
	37b/1	*T. vaginalis* C2 domian containing protein	Weak	30% aa	FM200078
	141/2	*T. vaginalis* CAMK family protein kinase	Weak	40% aa	FM200079
Oxidative stress	28/1	*T. vaginalis* thioredoxin reductase (*trxr*)	Weak	65% aa	FM200080
	76b/1	*T. vaginalis* DJ-1 family protein	Weak	55% aa	FM200081

Europe PMC Funders Author Manuscripts

Category	Clone name	Antigen homology	Signal intensity	% identity to homologous parasites	Accession number
Outer membrane, cell envelope biogenesis	93a/2	*T. vaginalis* AP65-2 adhesin	Weak	58% aa	FM200074
	117a/1	*T. vaginalis* pyruvate : ferredoxin oxidoreductase A (PFOA)	Weak	69% aa	FM200075
	145/2	*T. vaginalis* sugar isomerase domain containing protein	Strong	60% aa	FM200082
Carbohydrate transport and metabolism	58c/1	*T. vaginalis* phosphoglucomutase/phosphomannomutase family protein	Weak	74% aa	FM200083
	110c/1	*T. vaginalis* triosephosphat isomerase	Weak	76% aa	FM200084
Signal transduction	23b/2	*T. vaginalis* putative guanine nucleotide-binding protein β subunit	Strong	61% aa[b]	FM200085
Modification	10a/3	*T. vaginalis* ubiquitin family protein (UBA/TS-N domain containing protein)	Strong	23% aa	FM200086
Chromatin structure	191b/1	*T. vaginalis* putative histone 2A-IV	Weak	86% ns[a]	FM200087
				93% aa	
Lipid metabolism	15c/1	*T. vaginalis* AMP-binding enzyme protein	Weak	48% aa	FM200088
Metall-beta-lactamase superfamily III	218/1	*T. vaginalis* metallo-beta-lactamase superfamily III protein	Weak	36% aa	FM200089
Hypothetical proteins	5a/3	Hypothetical protein TVAG_151060	Strong	25% aa	FM200090
	15a/2	*T. vaginalis* conserved hypothetical protein EAY23136	Weak	40% aa (to both EAY23136 and EAY21702)	FM200091
		T. vaginalis XYPPX repeat family protein EAY 21702			
	97/1	*T. vaginalis* hypothetical protein TVAG_533930	Weak	33% aa	FM200092
	152b/1	*T. vaginalis* hypothetical protein TVAG_424960	Weak	68% aa	FM200093
	175/2	*T. vaginalis* hypothetical protein TVAG_264950	Weak	35% aa	FM200094
	186a/1	*T. vaginalis* hypothetical protein TVAG_161040	Weak	40% aa	FM200095

[a] ns – nucleotide sequence.

[b] aa – amino acid sequence.