Functional genomics of the horn fly, *Haematobia irritans* (Linnaeus, 1758)

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**Abstract**

**Background:** The horn fly, *Haematobia irritans* (Linnaeus, 1758) (Diptera: Muscidae) is one of the most important ectoparasites of pastured cattle. Horn flies infestations reduce cattle weight gain and milk production. Additionally, horn flies are mechanical vectors of different pathogens that cause disease in cattle. The aim of this study was to conduct a functional genomics study in female horn flies using Expressed Sequence Tags (EST) analysis and RNA interference (RNAi).

**Results:** A cDNA library was made from whole abdominal tissues collected from partially fed adult female horn flies. High quality horn fly ESTs (2,160) were sequenced and assembled into 992 unigenes (178 contigs and 814 singlets) representing molecular functions such as serine proteases, cell metabolism, mitochondrial function, transcription and translation, transport, chromatin structure, vitellogenesis, cytoskeleton, DNA replication, cell response to stress and infection, cell proliferation and cell-cell interactions, intracellular trafficking and secretion, and development. Functional analyses were conducted using RNAi for the first time in horn flies. Gene knockdown by RNAi resulted in higher horn fly mortality (protease inhibitor functional group), reduced oviposition (vitellogenin, ferritin and vATPase groups) or both (immune response and 5′-NUC groups) when compared to controls. Silencing of ubiquitination ESTs did not affect horn fly mortality and oviposition while gene knockdown in the ferritin and vATPase functional groups reduced mortality when compared to controls.

**Conclusions:** These results advanced the molecular characterization of this important ectoparasite and suggested candidate protective antigens for the development of vaccines for the control of horn fly infestations.

**Background**

The horn fly, *Haematobia irritans* (Linnaeus, 1758) (Diptera: Muscidae) is one of the most important ectoparasites of pastured cattle [1]. This fly was originally introduced from Europe and currently represents a tremendous health problem for cattle in the Americas from Southern Canada to Argentina [2]. Although horn flies parasitize mainly cattle, occasionally they feed on horses, sheep and dogs [3]. The developmental cycle of *H. irritans* is very short, taking from 10 to 14 days to complete. Larvae and pupae develop on dung and once the flies emerge from pupae, immediately start and remain feeding on cattle during their whole life. Flies leave the host only to move to others or to lay eggs on fresh manure [1]. Both males and females feed 24 to 38 times per day ingesting an average of 14.3 mg blood per fly [4].

Horn flies infestations interfere with animal feeding, thus producing significant reductions in weight gain and milk production [5,6]. The economic impact of *H. irritans* on livestock in the United States was estimated in approximately US$1 billion annually [7,8]. In dairy cattle, infestations higher than 200 flies per animal produce a loss of 520 ml milk and 28 kg weight daily [6]. In beef cattle, *H. irritans* infestations can cause a reduction of 8.1 kg weight daily [5]. Moreover, the skin lesions caused by the intermittent feeding of horn flies produce significant hide damages, affecting considerably the leather industry [9]. Additionally, horn flies are mechanical vectors of different pathogens that cause disease in cattle [10-14].
The control of horn flies has been primarily based on the use of chemical insecticides [15,16]. This control strategy has been partially successful but has resulted in the selection of flies resistant to most commercially available insecticides [15-17]. In addition to resistance, chemical insecticides affect other living organisms, contribute to environmental pollution and contaminate cattle products for human consumption.

Recently, research has been conducted to develop new horn fly control strategies that are cost-effective and environmentally friendly. The efficacy of the entomopathogenic fungi, *Metarhizium anisopalinae*, against horn fly larvae was very high in vitro [18]. However, field application of entomopathogenic fungi for biological control of horn flies is difficult. The use of female-specific conditional lethality systems has been also considered but not yet developed [19].

The immunological control of ectoparasite infestations was demonstrated through cattle vaccination against tick infestations [20,21]. The effect of anti-tick vaccines on the reduction of cattle tick infestations and the transmission of some tick-borne pathogens [21-23] and preliminary results obtained in insect vector species [24-32] have provided evidence that protective antigens may be used for development of vaccines with the dual target control of both arthropod infestations and reduction of vector capacity to transmit pathogens that impact human and animal health. Recently, Cupp et al. [33] demonstrated that horn flies fed on cattle immunized with the anti-clotting factor thrombostasin, took smaller blood meals and the egg development was delayed. Although other molecules have been proposed as vaccine candidates against horn flies [16,34,35], further research is needed to identify new vaccine candidates for effective control of horn fly infestations.

Recently, RNA interference (RNAi) was proposed as a method to identify candidate tick protective antigens [36] and was used for the screening of tick genes with potential applications in vaccine development [37-39].

The aim of this study was to conduct a functional genomics study in female horn flies using Expressed Sequence Tags (EST) analysis and RNAi. The results of this study will advance the molecular characterization of this important ectoparasite and suggested candidate protective antigens for the development of vaccines for the control of horn fly infestations.

**Results**

**Assembly and annotation of female horn fly Expressed Sequence Tags (ESTs)**

A cDNA library was made from whole abdominal tissues collected from partially fed adult female horn flies. From 2,462 sequenced ESTs, 302 and 2,160 were low and high quality ESTs, respectively (Table 1). Empty or vector ESTs were not obtained.

Since the female horn fly cDNA library was not normalized, the EST distribution per contig was quantified to determine the redundancy level of our EST dataset. High quality ESTs were assembled into 992 unigenes (178 contigs and 814 singlets) (Table 1; Additional file 1: Table S1), representing 46% novelty (unigenes/assembled ESTs) in our dataset. ESTs (814) present as singleton sequences represented 82% of all unigenes, while 72 unigenes (7%) contained only two ESTs. On average, the number of ESTs per unigene was 2.2, which suggested a low diversity in our dataset.

BLAST searches to TrEMBL and Swiss-Prot databases assigned 367 proteins to molecular function Gene Ontology (GO) terms (Figure 1). One hundred unigenes (10%) containing 535 ESTs (25%) corresponded to serine proteases. Other molecular functions represented in the unigenes included those involved in cell metabolism, mitochondrial function, transcription and translation, transport, chromatin structure, vitellogenesis, cytoskeleton, DNA replication, cell response to stress and infection, cell proliferation, and cell-cell interactions, intracellular trafficking and secretion, and development (Figure 1). Of the 367 unigenes with molecular function GO assignments, 184 could be assigned to Clusters of Orthologous Groups of proteins (COG) (Figure 2). The COG comprising posttranslational modification, protein turnover and chaperones contained 40% of proteins with COG assignments, followed by translation, ribosomal structure and biogenesis (17%) and energy production and conversion (12%) (Figure 2).

A relatively large set of 449 unigenes (45%) lacked any significant sequence similarity (Blast E values > 10^-5) to any sequence available in the public databases. Of all the 543 unigenes with significant sequence similarity to

| Table 1 Statistics of horn fly EST assembly |
|--------------------------------------------|
| Number of sequences | 2,462 |
| Mean length ± S.D. before vector stripping | 877 ± 44 bp |
| Mean length ± S.D. after vector stripping | 653 ± 32 bp |
| High quality EST reads | 2,160 |
| Assembled ESTs | 2,160 |
| No. unigenes | 992 |
| Mean length ± S.D. | 758 ± 46 bp |
| No. unigenes with more than 20 ESTs | 13 |
| No. unigenes with 5-20 ESTs | 44 |
| No. unigenes with less than 5 ESTs | 935 |
| No. contigs | 178 |
| No. singlets | 814 |
| Novelty (unigenes/assembled ESTs) | 46% |
| Redundancy (1-Novelty) | 54% |

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previously published sequences, 88.8% were most similar to Diptera (Drosophila spp., H. irritans, Musca domestica, Lucilia cuprina, Glossina spp., Stomoxys calcitrans, Aedes aegypti, Sarcophaga spp., Phlebotomus papatasii, Chrysomyia bezziana, Anopheles gambiae, Ceratitis stricta, Trichopalpus fraterna, Automola atomaria, Nanna tibiella, Bactrocera dorsalis, Lutzomyia longipalpis, Eristalinus punctulatus, and Ophiomyia sp.). 1.5% to other insect species (Spodoptera frugiperda, Tribolium castaneum, Gryllus bimaculatus, Lonomia obliqua, Nasonia vitripennis, and Lymantria dispar), 5.5% to other eukaryotic organisms, and 4.2% to microorganisms (Table 2).

Thirteen unigenes assembled from 505 sequence reads, contained more than 20 ESTs, most probably representing transcripts with highest abundance in abdominal tissues of partially fed female horn flies (Table 3). As expected from the results of the annotation of the entire EST dataset, 10 (77%) of these unigenes corresponded to serine proteases (Table 3). The second largest group of ESTs was derived from mitochondrial transcripts (Table 3). The analysis of serine protease unigene sequences showed that although some of them may be paralogs (for example unigenes 1-2 and 3-5; Table 3), other probably reflect sequence polymorphisms within the horn fly population because they
had 97%-98% nucleotide sequence identity (for example between unigenes 3-5; Table 3).

**Functional characterization of horn fly ESTs by RNAi**

For functional genomics studies, selected unigene functional groups were used in RNAi experiments in female horn flies (Table 4). These groups included serine protease, protease inhibitor, vitellogenin (VTG), ubiquitination, ferritin (FER), vacuolar (H\(^+\))-ATPase (vATPase), proteasome component, immune response and 5\(^'-\)nucleotidase (5\(^'-\)NUC) ESTs and were selected based on their putative function in insect biology and previous results of RNAi experiments in other arthropods (see Discussion). As controls, ESTs with sequence identity to Nora virus and *Wolbachia* endosymbionts were selected (Table 4). The injection of these control dsRNAs did not affect horn fly mortality ($\beta = -0.01$, Wald Chi\(^2\) = 0.01, P = 0.91) and oviposition ($P > 0.05$) when compared to buffer-injected flies in 14 independent RNAi experiments (Table 5), thus supporting their use as controls. Significant gene knockdown was obtained for at least one targeted unigene sequence on each group except for the serine protease group 1 in which significant gene expression silencing was not obtained for any of the unigenes included in the analysis (Table 5). For some sequences, gene knockdown was observed as early as 6 h post-injection (hpi) and lasted at least until 36 hpi (Table 5). For other sequences in groups 8 and 9, gene knockdown was not detected until after 12 hpi (Table 5). In most cases, gene expression silencing was higher than 70% when compared to the control group (Table 5).

To analyze RNAi off-target effects, the expression of genes not targeted by the injected dsRNA was analyzed at 12 hpi in functional groups 7-9 (Figure 3). The results showed that the expression of genes not targeted by the injected dsRNA was silenced in all three groups analyzed (Figure 3), thus suggesting RNAi off-target effects in horn flies. Pairwise sequence alignments identified regions with homology $\geq$ 11 bp in some sequences (Figure 4). However, only one region had 21 bp homology between unigene sequences 13_D07 and 7_A04 (Figure 4).

Injection of dsRNAs in the serine protease and ubiquitination functional groups did not affect fly mortality ($\beta = 0.09$, Wald Chi\(^2\) = 2.68, $P = 0.10$ and $\beta = 0.07$, Wald Chi\(^2\) = 3.60, $P = 0.08$, respectively) or oviposition ($P > 0.05$) when compared to controls (Table 5). The knockdown of a protease inhibitor gene (elastase) resulted in higher fly mortality ($\beta = 0.60$, Wald Chi\(^2\) = 13.35, $P = 0.0002$) but did not affect oviposition ($P > 0.05$) when compared to controls (Table 5). VTG-2 and proteasome component genes knockdown did not affect fly mortality ($\beta = 0.03$, Wald Chi\(^2\) = 1.42, $P = 0.22$ and $\beta = 0.13$, Wald Chi\(^2\) = 2.59, $P = 0.107$, respectively) but significantly ($P < 0.005$) reduced oviposition (Table 5). When the expression of immune response and 5\(^'-\)NUC genes was silenced,
higher fly mortality ($\beta = -0.46$, Wald $\chi^2 = 7.39$, $P = 0.006$ and $\beta = 0.35$, $Wald \chi^2 = 4.65$, $P = 0.03$, respectively) and reduced oviposition ($P < 0.005$) were obtained when compared to controls (Table 5). Interestingly, knockdown of FER light chain and vATPase genes resulted in lower fly mortality ($\beta = -0.21$, Wald $\chi^2 = 5.12$, $P = 0.02$ and $\beta = -0.16$, Wald $\chi^2 = 14.70$, $P = 0.0001$, respectively), and 6- and 16-fold decrease in oviposition ($P < 0.005$) when compared to control dsRNA-injected flies (Table 5).

**Discussion**
The effective control of horn fly infestations requires the design of new control strategies. Genomics and functional genomics studies are important to understand basic biological questions and to identify new targets for improved control strategies. Recently, gene expression analysis was reported in horn fly embryos, larvae and adult females [19,35]. However, this is the first report of functional genomics studies in this species.

ESTs sequenced and assembled in this study provided new sequence information for horn fly. The assembled unigenes without sequence similarity to sequences in public databases probably represented unique transcripts for horn fly or corresponded to proteins that have not yet been identified in related organisms due to incomplete genomic information. However, it cannot be excluded that the identified ESTs represent parts of known proteins whose similarities are located in parts of the sequence that are not covered by the analyzed ESTs.

The number of ESTs assembled into a certain unigene roughly reflected the relative abundance of corresponding mRNAs since the cDNA library from female horn flies used in this study was not normalized. We found that 100 unigenes, containing 25% of the ESTs, corresponded to serine proteases, indicating that this group represented the most abundantly expressed genes in abdominal tissues of partially fed female horn flies. In fact, the unigenes with the largest number of ESTs represented members of the serine protease family, thus suggesting that posttranslational modification and protein turnover were highly active in partially fed female flies.

The high proportion of ESTs present as singleton sequences when compared to contigs reflected a low diversity in our dataset, probably due to the presence of paralogs and sequence polymorphisms for some unigenes. In fact, sequence analysis of serine protease unigenes makes at this point difficult to discriminate between paralogs and ESTs representing sequence polymorphisms within the horn fly population.

RNAi was used to functionally characterize selected horn fly genes in adult female flies. To our knowledge, this is the first report of RNAi in horn flies. RNAi has been used to study gene function in insects and other arthropods [37,40-49] and to screen for candidate protective antigens in ticks [36-39]. Although with some fly mortality probably due to dsRNA injection with a Hamilton syringe, the RNAi method used here produced reproducible results in female horn flies. The failure to demonstrate gene knockdown for some sequences in serine protease and other functional groups studied could be due to unknown factors affecting RNAi in horn flies, because gene expression silencing did not occur until after 24-36 hpi for these genes or due to the existence of paralogs in these groups that affected the efficacy of gene knockdown. Gene silencing mediated by RNAi depends on short interfering RNAs (siRNAs) and micro RNAs (miRNAs). These RNAs have unique features, namely a defined size of 19-21 pb, and characteristic two-nucleotide single-stranded 3’ overhangs and 5’ monophosphate

**Table 2 Distribution of annotated unigenes to different species**

| Species                  | Total No. of unigenes | Ratio (%) |
|-------------------------|-----------------------|-----------|
| All organisms           | 543                   | 100       |
| Insects (Diptera)       | 482                   | 88.8      |
| Drosophila spp.         | 289                   | 53.2      |
| H. irritans             | 89                    | 16.4      |
| Musca domestica         | 22                    | 4.0       |
| Lucilia cuprina         | 16                    | 2.9       |
| Glossina spp.           | 15                    | 2.8       |
| Stomoxys calcitrans     | 14                    | 2.6       |
| Aedes aegypti           | 11                    | 2.0       |
| Sarcophaga spp.         | 9                     | 1.7       |
| Phlebotomus papatasi    | 3                     | 0.6       |
| Chrysomyia bezziana     | 3                     | 0.6       |
| Anopheles gambiae       | 3                     | 0.6       |
| Ceratitis stricta       | 1                     | 0.2       |
| Trichopalmus fraterna   | 1                     | 0.2       |
| Automola atomaria       | 1                     | 0.2       |
| Nanna tibiella          | 1                     | 0.2       |
| Bactrocera dorsalis     | 1                     | 0.2       |
| Lutzomyia longipalpis  | 1                     | 0.2       |
| Enistalinus punctulatus | 1                     | 0.2       |
| Ophiomyia sp.           | 1                     | 0.2       |
| Insects (Other)         | 8                     | 1.5       |
| Tribolium castaneum     | 2                     | 0.4       |
| Gryllus bimaculatus     | 2                     | 0.4       |
| Spodoptera frugiperda   | 1                     | 0.2       |
| Lonomia obliqua         | 1                     | 0.2       |
| Nasonia vitripennis     | 1                     | 0.2       |
| Lymnaea dispar           | 1                     | 0.2       |
| Other eukaryotes        | 30                    | 5.5       |
| Microorganisms          | 23                    | 4.2       |

Over 90% of best hits matched to insects. Only less than 10% of all best hits matched to other eukaryotes and microorganisms.
groups [50]. Although RNAi off-target effects were shown in horn flies, most sequence alignments resulted in homology regions of 11 bp only and in some cases no homology ≥ 11 bp was found. These results suggested differences in RNAi specificity and sensitivity, a fact that needs to be fully characterized to understand and efficiently use RNAi in horn flies and other organisms [37,48,51].

The aim of this study was to conduct a functional genomics study in female horn flies combining EST analysis with RNAi. Therefore, we will focus the discussion on unigene functional groups characterized by RNAi.

Table 3 Transcripts with highest abundance in abdominal tissues of partially fed female horn flies

| Unigene No. [GenBank accession number] | Number of clustered ESTs | Sequence identity | Clusters of Orthologous Groups of proteins (COG) | Molecular function Gene Ontology (GO) term |
|----------------------------------------|--------------------------|------------------|-----------------------------------------------|------------------------------------------|
| 1 [HO004732]                          | 21                       | L. cuprina clone sbsp9 serine proteinase | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 2 [HO004733]                          | 21                       | L. cuprina clone sbsp9 serine proteinase | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 3 [HO004734]                          | 22                       | H. irritans serine protease            | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 4 [HO004735]                          | 25                       | H. irritans serine protease            | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 5 [HO004736]                          | 25                       | H. irritans serine protease            | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 6 [HO004737]                          | 26                       | D. pseudoobscura GA21163-PA (DpseGA21163) | Amino acid transport and metabolism | Zinc carboxypeptidase |
| 7 [HO004738]                          | 28                       | H. irritans serine protease mRNA       | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 8 [HO004739]                          | 30                       | L. cuprina clone sbsp9 serine proteinase | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 9 [HO004740]                          | 33                       | H. irritans serine protease            | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 10 [HO004741]                         | 37                       | H. irritans mitochondrion, complete genome | Posttranslational modification, protein turnover, chaperones | Mitochondria |
| 11 [HO004742]                         | 40                       | H. irritans serine protease            | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 12 [HO004743]                         | 90                       | H. irritans serine protease            | Posttranslational modification, protein turnover, chaperones | Secreted trypsin-like serine protease |
| 13 [HO004744]                         | 107                      | H. irritans mitochondrion, complete genome | Posttranslational modification, protein turnover, chaperones | Mitochondria |

Table 4 Horn fly unigene functional groups selected for RNAi

| Group N° | Functional group | N° of unigenes a | Unigenes selected for RNAib |
|----------|-----------------|------------------|-----------------------------|
| 1        | Serine protease | 100              | 5, 10, 14, 19, 42, 90, 224, 230 |
| 2        | Protease inhibitor | 2              | 2_B12, 24_H02               |
| 3        | Vitellogenin    | 13               | 7, 20, 37, 76, 89, 145, 176, 7_D07 |
| 4        | Ubiquitination  | 5                | 84, 146, 4_E04, 5_G03, 7_B08 |
| 5        | Ferritin        | 5                | 26, 39, 154, 156, 10_A09    |
| 6        | vATPase         | 3                | 7_F08, 9_A08, 17_H03       |
| 7        | Proteasome component | 3          | 6_G04, 7_A04, 12_H09       |
| 8        | Immune response | 2                | 6_F11, 10_G05              |
| 9        | S’-nucleotidase | 1                | 13_D07                    |
| 10 (negative control) | Infectious agents: Nora virus Wolbachia endosymbionts | 3, 3 | 191, 2_E12 |

aNumber of unigenes grouped into this category.
bWhen the number of unigenes in the functional group was greater than 5, unigenes were selected for RNAi to include all different genes present in this category. Unigenes GenBank accession numbers are shown in Table 6.
Serine proteases

Serine proteases are a group of endopeptidases involved in several processes such as digestion, immune response, blood clotting and inflammation. In female horn flies, 10% of the assembled unigenes, containing more than 500 ESTs, were identified as serine proteases. In agreement with these results, Guerrero et al. [19] recently showed that serine proteases are differentially expressed in fly adult stages when compared to larvae. Significant gene knockdown was not obtained for any of the genes targeted by dsRNA injection in this group. Consequently, RNAi did not affect fly mortality or oviposition. In other arthropods, silencing of serine proteases expression by RNAi showed that these proteins are involved in blood digestion, oocyte maturation, development and immune response [40,42-44,46,52-55].

Protease inhibitors

The protease inhibitor genes identified in female horn flies corresponded to serpins, inhibitors of serine proteases and thus involved in the same biological processes discussed before for serine proteases. A horn fly serine protease inhibitor gene was previously cloned and characterized, suggesting that these genes may be involved in the control of fly endogenous and pathogen proteases [56,57]. In mosquitoes, serpin RNAi affected insect immune response [58]. The elastase inhibitor gene knockdown significantly increased horn fly mortality at 12, 24 and 36 hpi. Thus, the effect of elastase inhibitor RNAi described here in horn flies may be the result of impaired fly protease control and/or the effect of increased susceptibility to persistent pathogen infections resulting from diminished immune response.

**Table 5 Results of RNAi experiments in female horn flies**

| Group No. | Cumulative percent mortality | Oviposition (eggs per survived fly) | Expression silencing (average% ± SD) with respect to group 10 control | dsRNA injecteda |
|-----------|-------------------------------|------------------------------------|--------------------------------------------------------------------|----------------|
| 1 (Serine protease) | 57 ± 6, 69 ± 11, 78 ± 2 | 0.88 ± 0.23, ND | ND | 57 ± 30, 0 ± 0 | 90 |
| 2* (Protease inhibitor) | 73 ± 11, 84 ± 7, 94 ± 1 | 2.56 ± 1.80, ND | ND | 83 ± 3*, ND | 2_B12 |
| 3 (Vitellogenin) | 55 ± 11, 67 ± 8, 74 ± 8 | 0.31 ± 0.9**, ND | ND | 0 ± 0, 100 ± 2* | 7 |
| 4 (Ubiquitination) | 42 ± 12, 67 ± 11, 83 ± 16 | 0.64 ± 0.63, ND | ND | 92 ± 6*, 70 ± 24 | 84 |
| 5* (Ferritin) | 24 ± 23, 39 ± 37, 47 ± 41 | 0.22 ± 0.17**, ND | ND | 68 ± 13*, 86 ± 86 | 26 |
| 6* (vATPase) | 17 ± 13, 24 ± 11, 34 ± 3 | 0.08 ± 0.05**, ND | ND | 78 ± 6*, 100 ± 1* | 154 |
| 7 (Proteasome component) | 64 ± 3, 76 ± 8, 84 ± 11 | 0.38 ± 0.03**, 98 ± 2* | ND | 100 ± 2*, ND | 7_F08 |
| 8* (Immune response) | 66 ± 7, ND, 99 ± 8 | 0.23 ± 0.09**, 100 ± 8* | ND | 92 ± 5, 96 ± 9* | 7_A08 |
| 9* (5’nucleotidase) | 50 ± 11, ND, 91 ± 22 | 0.12 ± 0.09**, 0 ± 0 | ND | 98 ± 9*, 70 ± 7* | 13_D07 |
| 10 (negative control) | 45 ± 24, 57 ± 30, 64 ± 27 | 1.26 ± 0.90, — | ND | —, 1.50 ± 1.01 | 191 |
| Injection buffer | 46 ± 28, 57 ± 30, 65 ± 29 | 0 ± 0, 0 ± 0 | ND | 0 ± 0, 0 ± 0 | None |

*aThe average ± S.D. of two independent RNAi experiments for each of the test groups 1-9 and 14 experiments for the negative control and injection buffer groups is shown. One hundred flies were used on each RNAi experiment. Cumulative percent mortality was evaluated in female horn flies at 12, 24 and 36 hpi. Survival curves (temporal rates of mortality) were compared between different treatments and the control group 10 using Cox Proportional Hazards Survival Regression analysis (P < 0.05). Abbreviation: ND, not determined.

*bOviposition data from test groups 1-9 and the injection buffer control were compared with the unrelated dsRNA-injected control group 10 by Student’s t-test (P < 0.005).

For analysis of expression silencing, the mRNA levels of each knockdown gene were determined by real-time RT-PCR in 4 individual flies each at 6, 12 and 24 or 36 hpi. Percent expression silencing shown at different hpi corresponds to each individual gene targeted by dsRNA injection. The mRNA levels were normalized against horn fly 16S rRNA and the mean of the duplicate normalized Ct values from test dsRNA-injected groups 1-9 and in the injection buffer control were compared with the unrelated dsRNA-injected control group 10 by Student’s t-test (P < 0.05, **P < 0.005).

Unigenes GenBank accession numbers are shown in Table 6.
Vitellogenin

VTGs constitute a multigene superfamily encoding for egg yolk precursor proteins expressed in the females of arthropods and other oviparous organisms [59]. In cockroaches, ants and ticks, knockdown of VTG receptor (VTG-R), essential for VTG uptake into developing oocytes, disrupts egg formation [41,60-62]. In honeybees, silencing of VTG expression by RNAi affects honeybee workers developmental behavior [63,64]. Similar to results of VTG-R knockdown in other arthropods, silencing of VTG-2 expression in horn flies reduced oviposition in 4-fold when compared to controls.

Ubiquitination

Ubiquitination is a post-translational modification carried out by a set of enzymes that affect protein proteasomal degradation, stability, function, and intracellular localization [65]. In this functional group, horn fly genes involved in the ubiquitination pathway such as ubiquitin-1 (UBQ-1), UBQ-protein ligase, and UBQ hydrolase were included. In this group, only the UBQ-protein ligase expression was significantly silenced after RNAi. Although UBQ-protein ligase has been shown to regulate apoptosis in Drosophila [66], knockdown of this gene did not affect horn fly mortality or oviposition. Thus, it may be possible that the phenotype resulting from silencing the UBQ-protein ligase expression was not evident in horn flies under our experimental conditions. Additionally, knockdown of other ubiquitination genes may be required in order to have a significant effect on horn fly mortality and oviposition. For example, UBQ knockdown in ticks causes mortality and reduces oviposition and Anaplasma marginale infection/multiplication in the guts [39,67].

Ferritin

FER is the main protein for intracellular iron storage and consists of 2 types of subunits, a heavy (ferroxidase sites) and a light chain (nucleation sites) [68]. FER light
(3 unigenes containing 5 ESTs) and heavy (2 unigenes containing 4 ESTs) chains were not among the most abundant ESTs identified in female horn flies. However, Guerrero et al. [35] found FER light chain as one of the most abundant transcripts in horn fly larvae. These results suggested differences in the FER expression between horn fly larvae and adult females. FER light chain knockdown in horn flies significantly reduced oviposition (6-fold with respect to controls), but surprisingly, fly mortality was reduced when compared to controls. In ticks, FER RNAi reduces not only oviposition but also feeding and A. marginale infection levels in IDE8 cells [67,68].

vATPase

vATPase is a multisubunit enzyme that mediates acidification of eukaryotic intracellular organelles and has been shown to be required for the normal function of the Golgi complex, endoplasmic reticulum, vacuoles and endocytotic and exocytotic vesicles [69]. vATPase was also implicated in immunity [70]. Guerrero et al. [35] identified vATPase as one of the most abundant transcripts in horn fly larvae. However, in adult females, only 3 ATPase unigenes were assembled with one EST each, those suggesting like previously for FER, differences in the vATPase expression between horn fly larvae and adult females. Genetic knockout of vATPase subunits resulted in lethal phenotypes in fruit flies (Drosophila melanogaster), flour beetles (T. castaneum), pea aphids (Acyrthosiphon pisum), and tobacco hornworms (Manduca sexta) [69,71] and reduced influenza virus replication in Drosophila cells [72]. RNAi of vATPase expression in ticks resulted in testis and salivary gland degeneration, suggesting a role for this molecule in the function of these organs [73] and reduced A. marginale infection in Dermacentor variabilis tick guts but not pathogen multiplication in IDE8 tick cells [67]. vATPase knockdown in horn flies resulted in 16-fold reduction in oviposition, but, as with FER light chain, fly mortality was reduced when compared to controls. These results suggested that despite the important function of vATPase in all arthropods, developmental stage-specific and species-specific differences might exist that could explain the results obtained after gene knockdown in horn flies.

Proteasome component

Proteasomes are large protein complexes involved in protein proteolysis that are functionally related to ubiquitination and thus essential for eukaryotic cells [74]. Experiments in D. melanogaster showed that knockdown of proteasome subunits leads to increased levels of ubiquitin conjugates, cell cycle defects, DNA overreplication, and apoptosis [74,75]. In tick cells, 26S proteasome knockdown resulted in lower A. marginale infection levels when compared to controls but did not affect tick survival, feeding and reproduction [67]. However, based on the essential proteasome function in eukaryotic cells, it was not surprising to observe a decrease in oviposition in horn flies injected with proteasome components dsRNAs targeting proteasome subunit beta (two unigenes) and proteasome maturation protein (one unigene). As previously shown in D. melanogaster [74,75], proteasome subunits knockdown in horn flies may affect cell cycle and DNA replication thus resulting in reduced oviposition.

Immune response

Innate immune response is essential for insect survival. Only two unigenes were assembled into this category and knockdown in female horn flies. Assembled unigenes encoded for putative T-cell immunomodulatory protein and RNAse L inhibitor. Silencing of these genes resulted in higher horn fly mortality and lower oviposition when compared to controls. These RNAi results may be due to an effect of gene knockdown on increased susceptibility to persistent pathogen infections resulting from impaired immune response in horn flies. Knockdown of immune response genes may affect the mechanisms involved in the control of persistent infections such as those caused by Nora virus and Wolbachia spp. [76-78], which could affect horn fly mortality and ovisposition. RNAi knockdown of immune response genes in other arthropods results in increased mortality and higher pathogen infection levels [79-81].

5’-nucleotidase

5’-NUT and other ectonucleotidases control the levels of extracellular nucleotides and nucleosides that act as signaling molecules involved in a wide spectrum of biological effects [82]. 5’-NUT is commonly expressed in the salivary glands of blood-sucking ectoparasites [83-88]. Herein, as previously shown in ticks [36], 5’-NUT knockdown resulted in higher fly mortality and lower oviposition when compared to controls. As in other organisms, these results suggested an essential function for 5’-NUT in horn fly females.

Conclusions

In summary, a cDNA library was constructed from whole abdominal tissues collected from partially fed adult female horn flies and 2,160 high quality ESTs were sequenced and assembled into 992 unigenes (178 contigs and 814 singlets) representing molecular functions such as serine proteases, cell metabolism, mitochondrial function, transcription and translation, transport, chromatin structure, vitellogenesis, cytoskeleton, DNA replication, cell response to stress and infection, cell proliferation and cell-cell interactions, intracellular
trafficking and secretion, and development. A method was developed for RNAi that produced reproducible results in horn flies. Functional analyses by RNAi showed the effect of some genes on horn fly mortality and oviposition. These results advanced the molecular characterization of this important ectoparasite and suggested candidate protective antigens for the development of vaccines for the control of horn fly infestations. Based on RNAi results, some of the candidate antigens to be considered for cattle vaccination experiments against horn flies include those within VTG, immune response and 5’-NUC functional groups.

Methods

Rearing of horn flies

H. irritans were reared under laboratory conditions as reported by Schmidt et al. [89]. A horn fly colony was established with flies originally collected in a cattle farm close to Ciudad Victoria, Tamaulipas, Mexico. About 2,000 flies were collected from 2 infected animals and transported in a 20 × 30 cm mosquito netting aluminum cage. Flies were allowed to lay eggs over a water container during 12 h. Eggs were collected and incubated into fresh bovine feces during 5 days. Pupae were collected and placed in Petri dishes located inside mosquito netting aluminum cages for molting into adult flies. After molting, flies were fed twice a day using pieces of cotton impregnated with fresh defibrinated bovine blood obtained from a naive cow. All the horn fly developmental phases were kept under a photoperiod of 12 h light: 12 h darkness at 28-32°C and 70-80% relative humidity [89].

Analysis of expressed sequence tags (ESTs) in adult female horn flies

Total RNA was isolated from whole abdominal tissues collected from 1,500 partially fed adult female horn flies using Trizol (Sigma, St. Louis, MO, EUA). The cDNA library was synthesized using the SMART cDNA Library Construction Kit (Clontech, Mountain View, CA, USA) at Creative Biolabs (Port Jefferson Station, NY, USA; http://www.creativebiolabs.com). cDNAs were cloned into the pBluescript II SK vector (Agilent Technologies, Inc., Santa Clara, CA, USA). The library had more than 1×106 primary clones, with >90% recombinants with inserts >500 bp (average cDNA length >1,000 bp). A total of 2,462 ESTs were 5’ sequenced (Creative Biolabs). The cDNA Annotation System software (CAS; Bioinformatics and Scientific IT Program (BSIP), Office of Technology Information Systems (OTIS), National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD, USA) http://exon.niaid.nih.gov was used for automated sequence clean up, assembly, blasting against multiple sequence databases (ncbi non-redundant nucleotide and protein sequence databases, H. irritans EST sequences [35] and databases of mosquito- and tick-specific sequences http://www.ncbi.nlm.nih.gov/http://www.vectorbase.org/index.php) and Gene Ontology (GO) assignments. Comparison with the ncbi Clusters of Orthologous Groups of proteins (COG; http://www.ncbi.nlm.nih.gov/COG) was also performed. Nucleotide sequences were aligned using the program AlignX (Vector NTI Suite V 5.5, InforMax, North Bethesda, MD, USA). Gene sequences were deposited in the GenBank with accession numbers HO000420-HO001165 and HO004499-HO004744.

RNAi in adult female horn flies

Oligonucleotide primers (pBLUET75: 5’-TAATACGACT CACCTATAGGGTACTCGAGGGGTATCGAT-3’ and pBLUET73: 5’-TAATACGACTCACTATAGGGTACTCAATTAACCCTACCTAAAGGGA-3’) were synthesized specific for vector DNA sequences flanking the horn fly cDNA insert and containing T7 promoter sequences (in italics) for in vitro transcription and synthesis of dsRNA. PCR reactions were performed from individual or pooled cDNA clones (when more than one unigene was included in the functional group analyzed; Table 2) using the Access RT-PCR system (Promega, Madison, WI, USA) in a 50 μl reaction mixture. The resultant amplicons were purified using the Wizard 96-well PCR purification system (Promega). In vitro transcription and purification of dsRNA was done using the Megascript RNAi kit (Ambion, Austin, TX, USA). The dsRNA was quantified by spectrometry. Adult partially fed female flies were injected with approximately 0.1 μl of dsRNA (1 × 105-1 × 1011 molecules per μl) in the abdominal segment. The injections were done with a Hamilton syringe with a 1 inch, 33 gauge needle. Control flies were injected with unrelated dsRNA or injection buffer (10 mM Tris-HCl, pH 7, 1 mM EDTA) (Table 5). One hundred flies were used in each group. After injection with dsRNA, female flies were kept in petri dishes for one hour and then transferred to wired 20 × 30 cm boxes. Flies were fed using impregnated cotton with fresh defibrinated blood obtained from a naive cow and reared as described before. Fly mortality was evaluated at 12, 24 and 36 hpi. Survival curves (temporal rates of mortality) were compared between different treatments and controls using Cox Proportional Hazards Survival Regression analysis (SPSS Inc., Chicago, IL, USA). Oviposition (number of eggs per survived fly) was also evaluated and the results in test dsRNA-injected groups and in the injection buffer control were compared with the unrelated dsRNA-injected control group by Student’s t-test (P = 0.05).
Gene expression silencing was evaluated in 4 individual flies each at 6, 12 and 36 or 24 hpi. The mRNA levels of each knockdown gene were determined using sequence-specific oligonucleotide primers (Table 6) and the iScript One-Step RT-PCR Kit with SYBR Green and the iQ5 thermal cycler (Bio-Rad, Hercules, CA, USA) following manufacturer’s recommendations. A dissociation curve was run at the end of the reaction to ensure that only one amplicon was formed and that the amplicon denatured consistently in the same temperature range for every sample [90]. The mRNA levels were normalized against horn fly 16S rRNA (Table 4) using the genNorm method (ddCT method as implemented by Bio-Rad iQ5 Standard Edition, Version 2.0) [91]. In all cases, the mean of the duplicate values was used and normalized Ct values from test dsRNA-injected groups and in the injection buffer control were compared with the unrelated dsRNA-injected control group by Student’s t-test (P = 0.05).

**Additional material**

Additional file 1: Table S1. Assembling and analysis of *H. irritans* high quality ESTs.

**Table 6** Primer sets and real-time RT-PCR conditions used for analysis of selected *H. irritans* ESTs

| Unigene ID (Genbank accession number) | Gene description | Upstream/downstream primer sequences (5’-3’) | PCR annealing conditions |
|--------------------------------------|------------------|---------------------------------------------|-------------------------|
| 13_D07 (HO000820)                    | S’-nucleotidase  | AGTGGAACAAATGTCCGGAGG AAGCATTGGGTTTGAATGAG | 55°C, 30 s              |
| 6_F11 (HO000069)                     | T-cell immunomodulatory protein | CCGGATTTGTATGGAGATGAA GTAGATGGCTCCTCCCTTTTGGT | 55°C, 30 s              |
| 10_G05 (HO000738)                   | RNase L inhibitor | GGCGCACGTGTTATGTGCCT CCACGGACCTAAGTATGAG | 55°C, 30 s              |
| 7_A04 (HO000619)                     | Proteasome subunit beta | CAGGCGAGGGTGTTTGTGA AGATGGGTGCTGAGGATAG | 55°C, 30 s              |
| 12_H09 (HO000808)                   | Proteasome maturation protein | GAGGAACTCTGAGGGGTTTGA ACATGGGGTGTCCGGATTA | 55°C, 30 s              |
| 7_F08 (HO000644)                     | vATPase subunit d | GTTTCGGCTACGGACATTGCA GGCACAAACCTCCGAGAAA | 60°C, 30 s              |
| 9_A08 (HO000679)                     | vATPase subunit f | GTTGGATTCGGCTGTTGTTG GCAGCTGGGTATGGTGTC | 60°C, 30 s              |
| 17_H03 (HO001053)                   | vATPase proteolipid subunit | GCACCAGAGCTGATGGTAAGA AATGACGGGGACACAAAG | 60°C, 30 s              |
| 26 (HO004524)                        | Ferritin light chain | TGATCAGTGGAAACCCGGAGA CGGCTGGTCAATTCTTGAT | 60°C, 30 s              |
| 154 (HO004652)                       | Ferritin heavy chain | GTTTGCTACCCCTGCTCTATT TGAAAGGGCTGCTCCATAG | 60°C, 30 s              |
| 84 (HO004582)                        | Ubiquitin-protein ligase | TGCACTCTTGTGGATGTTG GGGAAAACCTTTTGAAGTCCA | 60°C, 30 s              |
| 146 (HO004644)                       | Ubiquitin family (UBQ-1) | CCCGACCAACAAAAGTATTA CGAGAAAGGGGGAATTTT | 60°C, 30 s              |
| 4_E04 (HO000538)                     | Ubiquitin carboxyl-terminal hydrolase | AGCCAGACGTGGTAATGGAG AGCCAAGAATGTTGCCCATA | 60°C, 30 s              |
| 7 (HO004505)                         | Vitellogenin 3   | GAGCTTTTTCGTTGATGCC ACAAAGTGGGACACAACCC | 60°C, 30 s              |
| 20 (HO004518)                        | Vitellogenin 1   | GAGCTTTTTCGTTGATGCC ACAAAGTGGGACACAACCC | 60°C, 30 s              |
| 76 (HO004574)                        | Vitellogenin 2   | ACGGCCGGTTTGAGGATAT AGCATTTTTCGTCGTCG | 60°C, 30 s              |
| 2_B12 (HO004579)                     | Elastase inhibitor | CAGGGGTTAGGGAAGAAGA TAAAGGGCCTCAGGTTCTTG | 60°C, 30 s              |
| 90 (HO004588)                        | Serine protease, midgut specific trypsin, secreted | TGCTGTATATAGGGGGCAAGAG GTTCCATCAATGGTAAGGCG | 60°C, 30 s              |
| 230 (HO004728)                       | Serine protease  | TGCCATCAATGGTAAGGCG GTTCCATCAATGGTAAGGCG | 60°C, 30 s              |
| (FJ025436)                           | *H. irritans* 16S rRNA | TTTTATTGGCCCGAGATATCC AATTTATAGGGGCTGCTCGT | 60°C, 30 s              |
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Authors' contributions
LT reared horn flies, prepared RNA samples, did the RNAi experiments and drafted the manuscript. JF participated in design and coordination of the study, analyzed EST and RNAi data and drafted the manuscript. RRC and HQR participated in coordination of the study. All authors read and approved the manuscript. JF.

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References
1. Harwood RF, James MT: Insecticides and acaricides: resistance and environmental impact. Rev Sci Tech 1994, 13:1249-1286.
2. Barros AT, Guglielmone AA, Martins JR: The effects of hair density of cattle on Haematobia irritans (Diptera:Muscidae). Med Vet Entomol 1998, 12:1249-1286.
3. Johnson NN, Mayer DG: Rating of ticks for resistance to a defined anti-Leishmania vaccine targeting vector antigens: characterization of a protective salivary protein. J Exp Med 2001, 194:331-342.
4. Sierr Azad AF: Antimicrobial resistance in organophosphates. J Vet Parasitol 2009, 23:77-89.
5. Lal AA, Stevens JS, Ashford PW, Wernsdorfer WH: A novel approach to the control of tsetse (Glossina morsitans). J Appl Entomol 1997, 121:303-306.
6. Oyarzún MP, Quiroz A, Birkett MA: A ten-year review of commercial vaccine performance for control of tick infestations on cattle. Anim Health Res Rev 2007, 8:1-20.
7. Suneja A, Gulia M, Gakhar SK: Antigenic variability of Rickettsia rickettsii in organs of the cattle tick Boophilus microplus. J Med Microbiol 2007, 56:322-327.
8. Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recombinant 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gland protein Panangala V: Evaluation of a recombinant salivary gland protein Panangala V: Evaluation of a recon
79. Eleftherianos I, Millichap PJ, ffrench-Constant RH, Reynolds SE. RNAi suppression of recognition protein mediated immune responses in the tobacco hornworm Manduca sexta causes increased susceptibility to the insect pathogen Photorhabdus. Dev Comp Immunol 2006, 30:1099-107.

80. Ayres JS, Freitag N, Schneider DS. Identification of Drosophila mutants altering defense of and endurance to Listeria monocytogenes infection. Genetics 2008, 178:1807-15.

81. Han-Ching Wang K, Tseng CW, Lin HY, Chen IT, Chen YH, Chen YM, Chen TY, Yang HL. RNAi knock-down of the Litopenaeus vannamei Toll gene (LvToll) significantly increases mortality and reduces bacterial clearance after challenge with Vibrio harveyi. Dev Comp Immunol 2010, 34:49-58.

82. Schetinger MR, Morsch VM, Bonan CD, Wyse AT. NTPDase and 5'-nucleotidase activities in physiological and disease conditions: new perspectives for human health. BioFactors 2007, 31:77-98.

83. Liyou N, Hamilton S, Mckenna R, Elvin C, Willadsen P. Localisation and functional studies on the 5'-nucleotidase of the cattle tick Boophilus microplus. Exp Appl Acarol 2000, 24:235-46.

84. Faudry E, Lozzi SP, Santana JM, DSouza-Ault M, Kieffer S, Felix CA, Sousa MV, Vernet T, Teixeira AR. Triatoma infestans apyrases belong to the 5'-nucleotidase family. J Biol Chem 2004, 279:19607-13.

85. Valenzuela JG, Garfield M, Rowton ED, Pham VM. Identification of the most abundant secreted proteins from the salivary glands of the sand fly Lutzomyia longipalpis, vector of Leishmania chagasi. J Exp Biol 2004, 207:3717-29.

86. Van Den Abbeele J, Caljon G, Dienick JF, Moens L, De Ridder K. Coosemans M. The Glossina morsitans tsetse fly saliva: general characteristics and identification of novel salivary proteins. Insect Biochem Mol Biol 2007, 37:1075-85.

87. Francischetti MI,_Valenzuela JG, Pham VM, Garfield MK, Ribeiro JM. Toward a catalog for the transcripts and proteins (sialome) from the salivary gland of the malaria vector Anopheles gambiae. J Exp Biol 2002, 205:2429-51.

88. Schmidt CD, Harris RL, Hoffman RA. Mass rearing of the horn fly, Haematobia irritans (Diptera: Muscidae), in the laboratory. Ann Entomol Soc Am 1967, 60:508-510.

89. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta CT) Method. Methods 2001, 25:402-408.

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