Title
Function-guided selection of midgut antigens from *Ornithodoros erraticus* ticks and an evaluation of their protective efficacy in rabbits.

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Abstract

The identification of candidate protective antigens for the development of tick vaccines may be approached by selecting antigen candidates that play key biological functions. Tick midgut proteins that play essential functions in tick survival and disease transmission are upregulated in response to blood feeding and digestion. In this study, *Ornithodoros erraticus* midgut transcriptomic and proteomic data upon feeding were inspected to select functionally relevant antigens to be assessed as vaccine candidate antigens. For this, we primarily focused on proteins with relevant biological functions in key physiological processes for ticks and tick-host-pathogen interactions. Later, we used additional criteria based on overexpression after feeding, predicted antigenicity and cellular localisation, resulting in the selection of four theoretical candidates, two aquaporins (OeAQP, OeAQP1), one ABC transporter (OeABC) and one selenoprotein T (OeSEL). Rabbit vaccination with synthetic immunogenic peptides designed from the extracellular antigenic regions of the selected candidates induced humoral responses that reduced tick feeding and reproduction performance. Both AQPs and OeSEL demonstrated significant protection efficacy against the homologous species *O. erraticus*, but lower non-significant cross-species protection against *Ornithodoros moubata*. Conversely, OeABC showed no protection against the homologous species *O. erraticus*, but significant cross-species protection against *O. moubata*. These results are the first demonstration of the protective potential of argasid aquaporins, suggesting that they might be included in vaccines for the control of multiple tick species. Additionally, these results also unveiled two novel protective antigens from argasid ticks, OeABC and OeSEL, belonging to functional protein families that have never been explored as a source of vaccine candidates and are deserving of further studies. Finally, our data add value to the midgut as a protective candidate antigen source in argasids for the control of tick infestations.

Keywords

Ornithodoros erraticus; mialome; vaccines; aquaporins; ABC transporters; selenoproteins

1. Introduction

Tick infestations and tick-borne diseases pose a serious threat to human and animal health (de la Fuente et al., 2016; Schorderet-Weber et al., 2017). Among argasid ticks, several species in the genus *Ornithodoros* are able to transmit the African swine fever (ASF) virus and a number of *Borrelia* spirochetes that cause tick-borne human relapsing fever (TBRF).

In particular, *Ornithodoros erraticus*, reported from the Iberian Peninsula, northern and western Africa and western Asia, is the main vector of these pathogens in the Mediterranean Basin (Boinas et al., 2014; Arias et al., 2017; Oleaga et al., 2018; Talagrand-Reboul et al., 2018). In addition, *O. erraticus* is the type species of the *O. erraticus* complex, and several species in this complex are distributed throughout an extensive area, including the Middle East, Caucasus, the Russian Federation and China, where they act as vectors of TBRF-causing borreliae (Chen et al. 2010; Rebaudet and Parola, 2006) and where the ASF virus has recently penetrated and spread (Gallardo et al., 2015). In 2007, ASF first entered into The Caucasus and it has since uncontrolledly spread both westwards and eastwards, having already reached the European Union and several provinces of China due to the absence of protective vaccines (EFSA, 2014, 2015; FAO, 2018; Jurado et al., 2018). Although not yet experimentally proven, if these tick species in the *O. erraticus* complex are competent vectors of the ASF virus, their presence in anthropic environments would greatly impact the dissemination and long-time persistence of ASF in this extensive area. Thus, the prevention and control of TBRF and ASF would require eliminating *O. erraticus* tick populations from at least anthropic environments (Díaz-Martín et al., 2015).

As chemical acaricides are inefficient against these argasids, alternative methods for tick control are needed (Oleaga et al., 2018). Tick vaccines have emerged as an effective and environmentally adequate method for the prevention and control of tick infestations and tick-borne diseases (de la Fuente and Contreras, 2015; Šmit and Postma, 2016; de la Fuente, 2018).

Success in research for vaccine development toward the control of argasid ticks largely depends on the identification of new and highly protective antigens. This task may be approached by the selection of candidate protective antigens that have important biological functions and share conserved structural and sequence motifs,

which would facilitate the simultaneous control of several tick species (Guerrero et al., 2014; de la Fuente and Contreras, 2015; de la Fuente et al., 2016).

Previous studies with *Ornithodoros* ticks have shown that plasma membrane-associated proteins expressed by the midgut endothelial cells (enterocytes) are able to induce protective immune responses in vaccinated animals (Manzano-Román et al., 2006, 2007; Obolo-Mvoulouga et al., 2018). Indeed, the tick midgut proteins play essential functions in blood digestion-related processes, as well as in the infection and transmission of blood-borne pathogens (Kocan et al., 2004), which turn midgut proteins into potential protective antigens for tick vaccines (de la Fuente et al., 2016).

Recently, the midgut transcriptomes and proteomes (mialomes) of *O. erraticus* female ticks, taken before feeding and 48 hours post-feeding, have been obtained and functionally annotated (BioProject PRJNA377416; Oleaga et al., 2015, 2018). These mialomes can thus be searched for candidate protective antigens for *Ornithodoros* vaccine development, following a function-guided selection strategy. In this way, among the functionally relevant proteins that are upregulated in the *O. erraticus* midgut in response to blood feeding and digestion, we focussed on aquaporins (AQPs), the ATP-binding cassette (ABC) family of transporters and selenoproteins.

Aquaporins, or water channels, are evolutionarily conserved proteins that form pores in cell membranes through which water and small neutral solutes can be transported (Campbell et al., 2008; Gomes et al., 2009; Ni et al., 2017). In ticks, AQPs have been found in the salivary glands, digestive tract and Malpighian tubules, where they are involved in multiple physiological processes that are essential for ticks, including saliva production, feeding and managing the osmoregulatory stress following blood ingestion, which means that about 75% of the ingested water and ions have to be returned to the host via tick saliva (Bowman and Sauer, 2004; Benoit et al., 2014). AQPs have been considered rational targets for ixodid tick vaccine development (Campbell et al., 2010; Ball et al., 2009; de la Fuente et al., 2016). In fact, cattle vaccination with recombinant *R. microplus* AQP1 provided 68–75% vaccine efficacy in reducing tick infestations (Guerrero et al., 2014). More recently, rabbit vaccination with two recombinant forms of *I. ricinus* AQP1 showed 32 and 80% efficacy for the control of infestations by *I. ricinus* larvae (Contreras and de la Fuente, 2017). These findings support that AQPs from ixodids might be candidate protective antigens for the

control of several tick species. Regarding argasid ticks, no similar studies on their AQP have been performed hitherto, so the protective efficacy of AQPs from argasids remains to be demonstrated.

The ABC transporters are membrane proteins that are able to bind and translocate a large variety of substrates, including xenobiotics, metabolites, signalling molecules and drugs, across the cellular membrane (Fletcher et al., 2016; Szöllősi et al., 2018). ABC transporters are very well known in vertebrates for their role in multidrug resistance in cancer patients (Fletcher et al., 2016), but less is known about their role in arthropods (Dermauw and Van Leeuwen, 2014). Recent studies in ticks have shown that ABC transporters mediate the transport of the heme group from the digestive vesicles to the hemosomes, which are organelles in the cytosol of the digestive cells where the heme is aggregated and detoxified (Lara et al., 2015). This ABC-mediated transport and detoxification pathway is also used by ticks in the detoxification of acaricides, representing a new mechanism of resistance to pesticides (Mangia et al., 2016, 2018; Le Gall et al., 2018). Thus, control strategies that can disrupt the accumulation of heme in the hemosome could also increase the sensitivity of ticks to commonly used acaricides. Developing vaccines targeting ABC transporters might be useful, but as of yet, no studies on the protective efficacy of tick ABC transporters have been undertaken.

Selenoproteins are selenocysteine-containing proteins whose primary function is defence against oxidative stress damage, although they are also responsible for many other functions, including selenium transport, protein folding and the endoplasmic reticulum-associated degradation of misfolded proteins (Budachetri et al., 2018). Tick selenoproteins have been little investigated, but current evidence links them to the ticks' tolerance to the extreme variations in redox homeostasis that happen between the off-host long periods of starvation and the acquisition and digestion of host blood (Adamson et al., 2013, 2014; Budachetri and Karim, 2015). Tick blood feeding generates toxic levels of reactive oxygen species (ROS), which are one of the first lines of defence against invading microbes, but which can also damage lipids, proteins and DNA, being detrimental for tick fitness and reproduction, as well as for the tick microbiome (Budachetri et al., 2018). Tick selenoproteins and antioxidant enzymes may play critical roles in detoxifying ROS and maintaining the tick condition and

two different tick species, *O. erraticus* and *Ornithodoros moubata*. Here, we showed that rabbit vaccination with synthetic immunogenic peptides designed from the extracellular antigenic regions of the selected candidates induced robust humoral responses that significantly reduced tick feeding and reproduction performance. These results (i) support that argasid AQP_s are promising protective antigens, which might be included in vaccines for the control of multiple tick species, and (ii) disclose two novel potentially protective antigens from argasid ticks, namely, one ABC transporter family member and one selenoprotein T, which deserve further investigation.

2. Materials and Methods

2.1. Ethics statement

All animal experiments performed in this study followed the regulations established by the Ethical and Animal Welfare Committee of the institution where the experiments were conducted (IRNASA, CSIC, Spain) and the corresponding EU law (Directive 2010/63/EU).

2.2. Ticks and tick material

The *O. erraticus* and *O. moubata* ticks were obtained from laboratory colonies maintained at IRNASA, CSIC, Spain. The *O. erraticus* colony was originally established from specimens captured in Salamanca, western Spain, and the *O. moubata* colony was established from specimens obtained from the Institute for Animal Health in Pirbright (Surrey, UK). The ticks were regularly fed on rabbits (New Zealand white) and kept in a culture chamber at 28 °C, 85% relative humidity and a 12 hour photoperiod.

Midguts from both *O. erraticus* and *O. moubata* unfed females and fed females 48 hours post feeding (h.p.f) were obtained to prepare extracts of soluble and membrane-associated proteins, as described by Oleaga et al. (2015). Briefly, batches of 25 midguts from each species and physiological condition were homogenised and sonicated in ice-cold PBS containing proteinase inhibitors (Roche Diagnostics, Indianapolis, USA). Tissue homogenates were centrifuged at 10^4 g to eliminate particulate material, and the 10^4 g supernatants were then centrifuged at 10^5 g into two new fractions, supernatant and pellet, which were respectively enriched in either soluble or membrane proteins. The protein concentration in these samples was measured using a BCA Protein Assay Reagent kit (Thermo-Fisher, Rockford, USA) and the samples were stored at -20°C .

Tick saliva samples from *O. erraticus* and *O. moubata* female ticks were collected after stimulation with 1% pilocarpine, following the protocol described by Díaz-Martín et al. (2013). A Bradford assay (Bio-Rad) was used to assess the protein concentration in the saliva samples. Then, samples were stored at -20°C .

2.3. Analysis of *O. erraticus* aquaporins, ABC transporters and selenoproteins sequences, and the selection of candidates

Transcriptomic data for the AQPs, ABC transporters and selenoproteins protein families of *O. erraticus*, including transcript sequences and transcription levels (fragments per kilobase of transcript per million mapped reads, FPKM), were all obtained from the *O. erraticus* midgut transcriptome, which was produced by our team in a recent study (Oleaga et al., 2018). In that work, the midgut transcriptomes of *O. erraticus* female ticks before feeding (unfed) and 48 hours after the blood meal (fed) were obtained, characterised and compared, providing the genes differentially expressed upon feeding.

For the encoded proteins, antigenicity prediction was performed with the VaxiJen 2.0 tool (<http://www.ddg-pharmfac.net/vaxijen/vaxijen/vaxijen.html>) using the 0.5 antigenicity threshold established by default for endoparasitic organisms (Doytchinova and Flower, 2007a, 2007b). VaxiJen is an alignment-independent tool suitable for the prediction of potential antigens in organisms whose genomes are poorly or not sequenced. VaxiJen has shown 70–97% prediction accuracy for bacterial,

viral, tumour, fungal and endoparasitic antigens (Flower et al., 2010); more recently, it has also been used to predict protein antigens from ticks (Maritz-Olivier et al., 2012; Obolo-Mvoulouga et al., 2018).

One or two members in each of these protein families were selected as potentially protective candidate antigens. In this selection, priority was given to proteins with the highest expression level, the highest fold-change after feeding and the highest predicted antigenicity (Vaxijen Score). Additionally, the presence of the proteins in the *O. erraticus* midgut proteome (Oleaga et al., 2015) was also scored, although no candidate of interest was discarded for not being detected in the proteome.

For every selected protein, BLASTp searching for orthologues in argasid and ixodid ticks was performed using the Uniprot and NCBI nr databases. Multiple amino acid sequence alignment of the orthologous sequences was performed with Clustal Omega, and conserved protein regions were identified after sequence alignment. For phylogenetic analysis, neighbour-joining trees were built with the Mega6 package (Tamura et al., 2013). Gaps were treated as pairwise deletions, amino acid distances were calculated using a Poisson model, and branch supports were estimated using bootstrap analysis (10,000 bootstraps).

Topographical analyses of the amino acid sequence of all selected candidates were performed using the TMHMM and SACS TMHMM tools in order to define their transmembrane and extracellular exposed regions (<http://www.sacs.ucsf.edu/cgi-bin/tmhmm.py>) (Krohg et al., 2001; Moller et al., 2001).

2.4. Design and synthesis of immunogenic peptides

The selected candidate proteins were subjected to linear B-cell epitope prediction and the amino acid sequences of the B-cell epitopes located on the extracellular exposed regions of the candidates were used for the design of synthetic immunogenic peptides.

The linear B-cell epitope predictions were performed using the following tools: ABCpred (<http://www.imtech.res.in/raghava/abcpred/index.html>) (Saha and Raghava, 2006), BCEpred (<http://www.imtech.res.in/raghava/bcepred/>) (Saha and Raghava, 2004) and BepiPred-2.0 (<http://www.cbs.dtu.dk/services/BepiPred/>) (Larsen et al.,

2006). B-cell epitopes predicted by at least two of these tools were included in the design of the immunogenic peptides. A cysteine residue was added to each end of the peptides to allow for oligomerisation through spontaneous oxidation of the cysteine sulfhydryl groups (Caro-Aguilar et al., 2005). Oligomerisation increased the size and immunogenicity of the peptides, avoiding the need to conjugate them to carrier proteins.

2.5. Vaccine trial

The aim of this trial was to evaluate the protective potential of the synthetic peptides designed from the *O. erraticus* selected candidate antigens (i.e. one ABC transporter, two aquaporins and one selenoprotein T) against infestations by *O. erraticus* and *O. moubata* ticks.

The peptides were formulated in Montanide ISA 50 V2 and administered to three groups of New Zealand white rabbits as follows (Table 1). Five peptides designed from the ABC transporter were formulated together and administered to one group of three rabbits. Four peptides designed from the two aquaporins were formulated together and administered to a second group of three rabbits. The only peptide designed from the selenoprotein T was individually administered to a third group of rabbits. An additional control group of rabbits was treated with the adjuvant alone. Each animal was vaccinated with three doses at 15-day intervals, each including 100 µg of each individual peptide, administered subcutaneously. These doses and the administration route were chosen according to experience in previous work (Manzano-Román et al., 2015; Obolo-Mvoulouga et al., 2018).

Rabbits were bled immediately before administration of the first antigen dose (pre-immune sera), 14 days after the third antigen dose – immediately before tick infestation – and 14 days after the infestation (14 and 28 days post-immunisation, d.p.i., respectively). Blood samples were allowed to clot and sera were removed and stored at -80 °C.

In the immune sera, the antibody titres to the homologous peptides and their reactivity to the other peptides were tested using an ELISA, according to standard procedures (García-Varas et al., 2010). Briefly, polystyrene plates (Sigma) were coated with 100 ng of peptide per well in 100 µl of carbonate buffer, pH 9.6, at 4 °C overnight

and post-coated with 1% bovine serum albumin in PBS. The sera were diluted in TPBS (PBS containing 0.05% Tween 20) in a two-fold dilution series starting at 1/100, and each dilution was analysed in duplicate wells. Peroxidase-conjugated anti-rabbit IgG (Sigma) was used, diluted 1/10,000 in TPBS. Ortho-phenylene-diamine (OPD) was used as a chromogen substrate for peroxidase and the reactions were stopped with 3N sulphuric acid. Incubations were performed at 37 °C for 1 h, and washes with TPBS were carried out at room temperature for 10 min per wash. The serum titre was defined as the highest dilution giving more than twice the reactivity of the corresponding pre-immune serum at the same dilution.

After titration, the reactivity of the immune sera to the saliva and the four midgut protein extracts (soluble and membrane proteins from fed and unfed females) from each species (*O. erraticus* and *O. moubata*) were tested using an ELISA and western blot, following standard procedures with minor modifications (Obolo-Mvoulouga et al., 2018). The ELISA plates were coated with 1 µg of saliva or midgut extract per well in 100 µl of carbonate buffer, pH 9.6, at 4 °C overnight and post-coated with 1% bovine serum albumin in PBS. The sera were diluted 1/300 in TPBS and the PO-anti-rabbit IgG was diluted 1/10,000.

Fourteen days after the third antigen dose, all rabbits received one tick infestation with 15 females, 25 males and 50 nymphs-3 of *O. erraticus*, and 15 females, 25 males and 50 nymphs-3 of *O. moubata* per rabbit. The parasites were allowed to feed on the rabbits for a maximum of 2 hours. After that time, any tick still remaining on the animal was removed. The degree of protection was determined by measuring: (i) the amount of blood ingested; (ii) the female oviposition and fertility rates (that is, the number of eggs laid per female and the number of newly hatched larvae/nymphs-1 per female); (iii) the moulting rate of nymphs-3; and (iv) the mortality rates of all tested developmental stages.

The obtained values for the parasites that fed on the animals from each group were summarised as means $\pm$ standard deviations. Statistical differences between the vaccinated and control group were assessed using one-way ANOVA followed by Dunnett's t-test. Values of $p < 0.05$ were considered significant.

For each peptide formulation, its vaccine efficacy (E) was calculated according to the formula established by Canales et al. (1997), and later updated by Aguirre et al.

(2015) and Contreras and de la Fuente (2016); this is based on the reduction in the studied developmental processes in ticks fed on vaccinated animals, compared to ticks fed on controls. Here, vaccine efficacy was calculated as $E = 100 \times [1 - (S \times F \times N \times M)]$, where S and F represent the reduction in the survival and fertility of female ticks, respectively, and N and M represent the reduction in survival and moulting of nymphs-3, respectively.

3. Results

3.1. Aquaporins: selected candidates and designed peptides

Five polypeptides around 300 amino acids long, annotated as putative full length AQPs, were recovered from the *O. erraticus* midgut transcriptome (Table 2). These five AQPs all showed a fold-change in their transcription level between 1 and 2, indicating non-significant increases in expression after feeding. Four of these polypeptides were predicted to be antigenic (Vaxijen score > 0.5), and the two predicted antigens showing the highest expression levels after feeding were selected as candidates, i.e. transcripts ci|000079254 (Uniprot A0A293N0K9) and ci|000090257 (Uniprot A0A293N7C6). In addition, the proteins encoded by these two transcripts had already been detected in the *O. erraticus* midgut proteome (Oleaga et al., 2015).

Searches of the Uniprot/NCBI nr databases for orthologues of either A0A293N0K9 or A0A293N7C6 retrieved the same top 12 matches, comprising four argasid and eight ixodid aquaporin sequences, all of them showing E values < 10^{-50} . The alignment of these 12 AQP amino acid sequences with A0A293N0K9 and A0A293N7C6 showed highly conserved regions throughout the whole protein sequence (Supplementary Fig. 1A), including the two NPA (asparagine-proline-alanine) motifs and the four residues within the so-called aromatic arginine (ar/R) constriction; all together, these shape the aqueous pore and determine its permeability (Campbell et al., 2008).

Phylogenetic analysis of these 14 tick AQPs grouped them, with high confidence, into three main clades, which we referred to as AQP1s, AQP2s and other AQPs (Fig. 1). The *O. erraticus* A0A293N0K9 protein was classified into the AQP1s clade, which included the four argasid AQPs and most of the ixodid AQP1s, so we termed it OeAQP1. OeAQP1 was most similar to the argasid AQPs (83–88% sequence identity)

followed by the ixodid AQP1s (63–66%) and AQP2s (52–53%) (Supplementary Fig. 1A). By contrast, the *O. erraticus* A0A293N7C6 protein (termed OeAQP) fell into the third clade (other AQPs), showing 60–55% sequence identity with the other members in this clade, and decreasing sequence identity with members in the AQP2 and AQP1 clades (50–48% and 51–44%, respectively). OeAQP1 and OeAQP had 45% sequence identity with each other (Supplementary Fig. 1A).

The topology prediction for both the OeAQP1 and OeAQP proteins showed a conserved overall structure with six transmembrane domains and five connecting loops (A to E). Loops A, C and E were on the extracellular side and had lengths of around 10, 30 and 30 amino acids, respectively (Supplementary Fig. 1B).

Linear B-cell epitope predictions for extracellular loops C and E are shown in Fig. 2. Each of the three immune-informatics tools predicted somewhat different, but overlapping linear B-cell epitopes for each loop. Based on these predictions, four immunogenic peptides (one *per* loop) were designed and synthesised, as indicated in section 2.4. All these peptides included a cysteine residue at each end, either from the original sequence or added later, to allow for peptide oligomerisation (Fig. 2 and Table 3).

3.2. ABC transporters: selected candidate and designed peptides

A total of 20 transcripts annotated as ABC transporters were obtained from the *O. erraticus* midgut transcriptome (not shown), five of which were significantly upregulated after feeding (fold-change > 2; Table 2). Four of these transcripts encoded proteins predicted to be antigenic (Vaxijen score > 0.5). Among these last transcripts, transcript ci|000077665 showed the highest expression levels in the tick midgut, both before and after feeding. This transcript encodes a full-length ABC transporter that was 1,518 amino acids long, which was in fact detected in the *O. erraticus* midgut proteome (Oleaga et al., 2015). Accordingly, this transcript/protein was selected as a candidate antigen and termed OeABC.

BLASTp searching of the Uniprot and NCBI nr databases for orthologues of OeABC retrieved a range of highly conserved sequences, from which we selected the top six matches; they all had E values = 0 and sequence identities > 70%, and included 1 argasid and 5 ixodid sequences. Multiple alignments of OeABC and these six proteins

384 included the two ABC membrane domains and nucleotide binding domains that are
385 characteristic of the full ABC transporters (Dermauw and Van Leeuwen, 2014).

386 Phylogenetic analysis of these proteins showed a close relationship between
387 OeABC and its *O. moubata* orthologue, A0A1Z5KXC6_ORNMO, which shared 88.9%
388 sequence identity (Fig. 3), suggesting that these proteins might belong to the C
389 subfamily of arthropod ABC transporters (Dermauw and Van Leeuwen, 2014).

390 The topology prediction for OeABC provided a multispanning transmembrane
391 protein model with 16 transmembrane segments, 7 extracellular loops shorter than 30
392 amino acids, and 2 larger extracellular regions, 72 and 284 amino acids long,
393 respectively, in the carboxi terminus (Supplementary Fig. 2B).

394 Linear B-cell epitope predictions were made for the 418 amino acid-long
395 carboxiterminal fragment of the protein that contained these two extracellular
396 regions; the results are shown in Fig. 4. Each algorithm predicted a different set of 5 to
397 10 linear B-cell epitopes, although for five of these epitopes, the three predictions
398 clearly overlapped. Based on these overlapping sequences, five immunogenic peptides
399 were designed and synthesised, as already indicated, including the addition of a
400 cysteine residue at both ends to allow for peptide oligomerisation (Fig. 4 and Table 3).

401 402 3.3. Selenoproteins: selected candidate and designed peptide

403 Five transcripts annotated as selenoproteins were found in the *O. erraticus*
404 midgut transcriptome, and three of them were upregulated upon feeding (Table 2).
405 Among them, the transcript with the highest fold-change (6.285) was also the most
406 antigenic (Vaxijen score, 0.7096). This transcript, A0A293LXQ0_ORNER, encoded a
407 selenoprotein T that was 111 amino acids long, which was selected as a candidate and
408 termed OeSEL.

409 The top 10 tick orthologues for OeSEL, with E values $< 10^{-60}$ and sequence
410 identities $> 80\%$, were obtained from the Uniprot and NCBI nr databases. Multiple
411 alignments of all these sequences and OeSEL showed very high conservation
412 throughout the whole OeSEL sequence, including the fragment where the designed
413 immunogenic peptide was located (Supplementary Fig. 3A).

The corresponding neighbour-joining tree indicated a very close relationship between all these tick T selenoproteins, and suggested that they belong to two main clades, one clade for argasids (2 sequences) and a second clade for metastriate ixodid ticks (8 sequences). The only sequence from prostriate ixodid ticks that was included in this analysis, A0A147BVB9_IXORI, fell in between the two former clades (Fig. 5A). The OeSEL topology predicted by TMHMM showed a transmembrane protein with two transmembrane segments and one extracellular loop that was 18 amino acids long (Supplementary Fig. 3B). The three linear B-cell epitope predictions indicated that the most antigenic part of the OeSEL protein was in fact its extracellular loop. Thus, its sequence was the basis for the design of an immunogenic peptide termed OE7237/1 (Fig. 5B and Table 3).

3.4. Humoral immune response to the immunogenic peptides

Ten immunogenic peptides were synthesised and administered to three groups of rabbits, according to the scheme in Table 1. The rabbit IgG antibody responses to these peptides are shown in Fig. 6.

The sera from the rabbits in group 1, immunised with the five peptides derived from the OeABC transporter (anti-ABC), reacted intensely with two of these peptides, OE7665/2 and OE7665/4, as they showed optical densities (OD) between 1.6 and 2.6 and IgG antibody titres over 1/12,800 (Fig. 6B, 6D). On the other hand, these sera did not react or barely reacted to the other three OeABC peptides, OE7665/1, OE7665/3 and OE7665/5 (Fig. 6A, 6C, 6E). The sera from rabbits in group 2, immunised with the four peptides derived from the OeAQP and OeAQP1 aquaporins (anti-AQPs), specifically and strongly recognised all four peptides, showing ODs between 1.1 and 2.4 and, for most of them, antibody titres around 1/12,800 (Fig. 6F-6I). Lastly, the sera from rabbits immunised with peptide OE7237/1 (group 3, anti-OeSEL) specifically recognised this peptide, showing medium reactivity, with average ODs of 0.6 and antibody titres reaching 1/3,200 (Fig. 6J).

Fig. 6 also shows that no sera taken 14 d.p.i., immediately before the infestation, reacted to the saliva of *O. erraticus* or *O. moubata* (Fig. 6K, 6L). Conversely, the sera taken 28 d.p.i. (14 days post-infestation) did not show any significant variation in reactivity to the immunogenic peptides (Fig. 6A-6J).

The reactivity of the rabbit IgG antibody response to native forms of the OeABC, OeAQP, OeAQP1 and OeSEL proteins in the midgut extracts from *O. erraticus* and *O. moubata* was analysed using an ELISA (Supplementary Fig. 4) and western blot (Fig. 7).

All the immune sera showed low or very low reactivity to soluble midgut proteins from both *Ornithodoros* species according to the ELISAs (Supplementary Fig. 4); most of this reactivity was due to the nonspecific recognition of host IgG, which was also recognised by the preimmune sera (Fig. 7). By contrast, the immune sera reacted somewhat more intensely to the membrane midgut proteins from both fed and unfed *O. erraticus* and *O. moubata* females (Supplementary Fig. 4, Fig. 7), supporting the predicted location of the candidate proteins to cell membranes.

The anti-ABC serum pool recognised a 160 kDa band, compatible with the native OeABC protein, in extracts of membrane proteins from fed and unfed *O. erraticus* females (Fig. 7B) and a similar 160 kDa band among the membrane proteins from *O. moubata* females (Fig. 7D). This suggests that the OeABC protein and its native orthologue in *O. moubata* (Fig. 3, Supplementary Fig. 2) share cross-reactive epitopes and similar localisation in the enterocyte.

The anti-AQPs serum pool recognised three bands of around 30, 45 and 66 kDa in the extracts of membrane proteins from both fed and unfed *O. erraticus* (Fig. 7B), which are compatible with the oligomeric forms of aquaporins (Yoo et al., 2016). These sera also recognised similar bands in the extracts of membrane proteins from both fed and unfed *O. moubata*, strongly suggesting that OeAQP and/or OeAQP1 share cross-reactive epitopes with their orthologous proteins in *O. moubata* (Fig. 7D).

On the other hand, the anti-OeSEL serum pool did not recognise any band with the characteristics of the native OeSEL protein from any midgut extract from *O. erraticus* or *O. moubata* (Fig. 7B, 7D).

3.5. Protective effects of the immune response on the ticks

The protective effect of the immune response induced by the synthetic peptides is summarised in Table 4.

On the *O. erraticus* ticks, the anti-ABC response caused small reductions (2.4–3.3%) in the survival of all developmental stages. The anti-AQP response caused significant reductions (13.4%) in the fertility of females without affecting other

parameters, and the anti-SEL response significantly reduced the feeding (26.4%), fertility (40.5%) and survival (6.7%) of the females. Accordingly, the vaccine efficacy of the OeABC, OeAQPs and OeSEL peptide formulations against *O. erraticus* infestations was 3.1, 15.5 and 47.5%, respectively.

On the *O. moubata* ticks, the immune responses induced by the peptide formulations did not affect the survival of any developmental stage, but reduced the amount of blood ingested in all the developmental stages and groups, as well as the fertility of females in all groups. This reduction was only significant in the females fed on rabbits vaccinated with the OeABC peptides (26.7%). Thus, the vaccine efficacy of the OeABC, OeAQPs and OeSEL peptide formulations against *O. moubata* infestations was 22.5, 4.6 and 9.6%, respectively.

4. Discussion

The identification of candidate protective antigens for the development of tick vaccines may be approached by selecting antigen candidates that play key biological functions and share conserved sequence motifs for the simultaneous control of different tick species (de la Fuente et al., 2016; Contreras and de la Fuente, 2017).

The tick midgut expresses a wealth of proteins that play essential functions in tick survival and disease transmission (Kocan et al., 2004; Sojka et al., 2013, 2016; de la Fuente et al., 2016); many of these proteins are upregulated in response to the stimulus provided by host attachment and blood ingestion (Sojka et al., 2013, 2016; Oleaga et al., 2017a, 2017b). Previous evidence strongly suggested that these upregulated proteins could be targeted by vaccines (Akov, 1982; Matsuo et al., 2003; Manzano-Román et al., 2006, 2007; Obolo-Mvoulouga et al., 2018). Accordingly, in order to explore new strategies for the control of *O. erraticus* ticks, in previous works, we aimed to improve our knowledge of the *O. erraticus* midgut protein composition and function. With that purpose, the midgut proteome and transcriptome (mialome) of *O. erraticus* female ticks before and after feeding were characterised (Oleaga et al., 2015, 2018).

In the current work, our aim was to select several candidate antigens from the *O. erraticus* mialome and to test their protective potential in animal vaccination trials. We focused on AQPs, ABC transporters and selenoproteins because these protein families

are involved in important physiological processes that occur in the tick midgut upon blood feeding and digestion (Benoit et al., 2014; Lara et al., 2015; Budachetri et al., 2018). In addition, previous evidence has already established AQPs as promising candidate vaccine antigens for the control of multiple haematophagous vectors (Guerrero et al., 2014; Hussein et al., 2015; Contreras and de la Fuente, 2017).

Hence, all the sequences in the *O. erraticus* mialome annotated as AQPs, ABC transporters and selenoproteins were retrieved and subjected to a second round of selection as a function of their fold-change after feeding, expression level in fed ticks and predicted antigenicity (Table 2). The four final selected candidates were all multispinning transmembrane proteins (Supplementary Figs. 1B, 2B, 3B). Midgut transmembrane proteins expressed on the luminal side of the enterocyte plasma membrane are considered first-election candidate antigens for vaccine design because their antigenic extracellular regions are easily accessible to host antibodies ingested in blood (Rappuoli and Bagnoli, 2011; Richards et al., 2015). Accordingly, topology predictions were used to identify the extracellular regions/domains on the four candidates (Supplementary Figs. 1B, 2B, 3B) and lineal B-cell epitope predictions verified the presence of this kind of epitopes on these extracellular regions, which allowed us to design and synthesise potential immunogenic peptides (Figs. 2, 4, 5B).

Multiple alignments of each candidate with their orthologues in other tick species showed that they shared conserved structural and sequence motifs, including most of their antigenic extracellular regions (Supplementary Figs. 1A, 2A, 3A); this would facilitate the simultaneous control of different tick species if the immunogenic peptides designed from these regions induced protective immune responses.

Seven of the 10 synthetic peptides administered to rabbits demonstrated high immunogenicity (Fig. 6A-6J), in good agreement with the linear B-cell epitope predictions, and in turn supporting the antigenicity prediction of VaxiJen for the four candidate antigen proteins: OeABC, OeAQP, OeAQP1 and OeSEL. These responses specifically recognised the inducing peptides and did not cross-react to the saliva of *O. erraticus* or *O. moubata* (Fig. 6K, 6L). Accordingly, the tick bites did not boost the vaccine-induced humoral responses either (Fig. 6A-6J), indicating that natural tick-host contacts would not serve as boosting antigen doses in immunised hosts.

identities. As expected due to the high sequence identity between the OeABC and OeAQPs and their homologous proteins in *O. moubata* (Supplementary Figs. 1 and 2), these sera also cross-reacted with protein bands compatible with native forms of the ABC transporters and AQPs of *O. moubata* (Fig. 7D), anticipating the possibility of some cross-protection between these species.

Actually, the anti-OeABC immune response provided significant cross-protection against *O. moubata*, which consisted of a reduction in the females' oviposition and fertility (Table 4). It has recently been demonstrated that ticks require dietary haemoglobin as an exogenous source of heme group for successful embryonic development and larval hatching, while they lack the heme oxygenase gene necessary for heme degradation relying on ABC transporters for maintaining heme homeostasis through the transport and detoxification of excess heme (Lara et al., 2015; Perner et al., 2016a, 2016b). So, an antibody-mediated loss of function of the involved ABC transporter could lead to imbalanced heme homeostasis, resulting in embryonic development impairment and a subsequent reduction in the reproduction performance of female *O. moubata* ticks.

Unexpectedly, the anti-OeABC immune response provided less protection against the homologous species, *O. erraticus*, which is challenging to explain. According to the phylogenetic tree in Fig. 3, OeABC would belong to the C subfamily of arthropod ABC transporters, and this subfamily is known to frequently show specific expansions within species and lineages (Dermauw and Van Leeuwen, 2014). Thus, if a specific expansion in the ABC-C subfamily in *O. erraticus* had occurred, other functionally redundant members of this subfamily would have compensated the vaccine-induced loss of function of the OeABC candidate. Although this hypothesis

needs to be demonstrated, the high number of ABC transporter family members that have been found in the *O. erraticus* midgut would lend it some initial support (Oleaga et al., 2015, 2018).

Regarding the anti-OeAQPs immune response, it essentially affected the homologous species, *O. erraticus*, causing a significant reduction in oviposition and fertility, in agreement with the results of similar studies with ixodid AQPs (Guerrero et al., 2014). This effect could be the result of an antibody-mediated loss of function of AQPs to concentrate blood components during tick feeding, resulting in reduced water elimination. This would increase tick weight, but reduce the ingestion of blood components, which in turn would reduce the supply of nutrients for tick reproduction (Contreras and de la Fuente, 2017). A similar, although less intense, protective effect against *O. moubata* was observed, confirming some degree of cross-species protection by ornithodoros AQPs-based vaccines.

On the other hand, the immune sera to OeSEL did not recognise its native protein target in any midgut protein extract from *O. erraticus* or *O. moubata* (Fig. 7B, 7D). This lack of recognition and the absence of OeSEL in the *O. erraticus* midgut proteome (Oleaga et al., 2015) indicate that OeSEL is expressed at a low level in the tick midgut during the two sampled time points of the tick trophogonic cycle, even though the OeSEL mRNA level increased by six-fold in fed females 48 h.p.f. (Table 2). Nonetheless, since gene transcription and translation have different regulation mechanisms (Maier et al., 2009; Kumar et al., 2016b), the expression of the OeSEL protein might have taken place at a different point of the trophogonic cycle, one that was not sampled in the current study. In fact, the significant protection provided by the anti-OeSEL immune response against *O. erraticus* (Table 4) strongly suggests that native OeSEL protein must be expressed at some time point along the trophogonic cycle, and that its function is somehow neutralised by the vaccine-induced antibodies. One of the proposed functions for tick selenoproteins is detoxifying the reactive oxygen species (ROS) that are generated after blood feeding and digestion (Adamson et al., 2014; Budachetri et al., 2018). Thus, an antibody-mediated loss of OeSEL function would result in increased (toxic) levels of ROS, being detrimental for tick fitness and reproduction, which was indeed the main protective effect observed. The same protective effect, although less significant, was also observed against *O.*

moubata (Table 4), pointing to cross-species protection, at least among ornithodoros ticks, as suggested by the high sequence identity between OeSEL and its orthologue in *Ornithodoros turicata* (Fig. 5A, Supplementary Fig. 3A). These results suggest tick selenoproteins may be suitable targets for vaccines aimed to control tick infestations, and provide a new candidate protective antigen, OeSEL that is deserving of further studies in order to validate its use for argasid tick vaccine development.

5. Conclusions

The recent availability of transcriptomic and proteomic data from the *O. erraticus* midgut allowed us to implement a function-based strategy to select potentially protective protein antigens from the tick midgut: two aquaporins, one ABC transporter and one selenoprotein T.

Vaccination of rabbits with these candidates confirmed their predicted immunogenicity, as they all induced robust humoral immune responses. The four candidates showed low to medium level protection against ornithodoros ticks, which was assumed to be the result of an antibody-mediated loss of function of the antigen protein targets.

The results of this study suggest the potential of argasid aquaporins as protective candidate antigens to be included in vaccines for the control of multiple tick species. They also unveil two novel protective antigens from argasid ticks, OeABC and OeSEL, belonging to functional protein families that have never been explored as a source of vaccine candidates, which deserve further studies.

Moreover, new candidate protective antigens from ornithodoros are still needed and will likely be identified by maintaining a focus on proteins with relevant biological functions in key physiological processes for tick survival and tick-host-pathogen interactions. For this purpose, proteogenomics approaches aimed at host-tick-pathogen interactions seem to be the best option to acquire abundant omic data whose integration and analysis will facilitate the identification of key tick proteins and functions, and their assessment as vaccine candidate antigens.

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Figure captions

Figure 1. Neighbour-joining analysis of the phylogenetic relationship of tick AQPs. Uniprot accession numbers followed by the species acronym are shown for each sequence. Q06EX9_DERVA from *Dermacentor variabilis*; C5J8E5_IXORI from *Ixodes ricinus*; A0A168DD71_IXOSC, B7PDX8_IXOSC and B7QMK7_IXOSC from *Ixodes scapularis*; A0A1D2AI66_ORNBR from *Ornithodoros brasiliensis*; A0A293N7C6_ORNER and A0A293N0K9_ORNER from *Ornithodoros erraticus*; A0A1Z5L547_ORNMO and A0A1Z5KVQ3_ORNMO from *Ornithodoros moubata*; A0A2R5L6A3_ORNTU from *Ornithodoros turicata*; A0A097ITI9_RHIMP and A0A141GE76_RHIMP from *Rhipicephalus microplus*; B8ZJG8_RHISA from *Rhipicephalus sanguineus*. Evolutionary distances were computed using the Poisson correction method. Branch support values (10,000 bootstraps) for the nodes are indicated. The *O. erraticus* OeAQP (A0A293N7C6) and OeAQP1 (A0A293N0K9) are marked by a blue square and red dot, respectively.

Figure 2. Linear B-cell epitope predictions for the extracellular domains of OeAQP1 and OeAQP. The sequence of each protein is represented in triplicate, showing the ABCpred (yellow), BCEpred (blue) and BepiPred-2.0 (green) predictions. Amino acids with antigenicity predictions made by at least two of these three algorithms were included in the design of the immunogenic peptides (red and blue boxes topped with the peptide name). The predicted topology is indicated below each protein sequence: **o** (outside), extracellular; **M**, transmembrane; **i**, intracellular.

Figure 3. Neighbour-joining analysis of the phylogenetic relationship of tick ABC transporters. The *O. erraticus* ABC transporter (OeABC) is marked with a red dot. Uniprot accession numbers followed by the species acronym are shown for the other sequences. A0A1E1X7G1_AMBAU from *Amblyomma aureolatum*; A0A023GP66_AMBTR from *Amblyomma triste*; V5HHU7_IXORI and V5HBA0_IXORI from *Ixodes ricinus*; B7PUI6_IXOSC from *Ixodes scapularis*; A0A1Z5KXC6_ORNMO from *Ornithodoros moubata*. Evolutionary distances were computed using the Poisson correction method. Branch support values (10,000 bootstraps) for the nodes are

indicated. The *Ornithodoros turicata* ABC transporter, A0A2R5L4J2_ORNTU, was included as an outside reference.

Figure 4. Partial amino acid sequence of the OeABC protein, showing its carboxy terminus, which contains the two largest extracellular regions, comprising residues 1111–1183 and 1234–1518, respectively. The sequence of the protein is represented in triplicate, showing the linear B-cell epitope predictions by ABCpred (yellow), BCEpred (blue) and BepiPred-2.0 (green) algorithms. Amino acids with antigenicity predictions made by two or three of these three algorithms were included in the design of the immunogenic peptides (highlighted in blue boxes topped with each peptide name). The predicted topology is indicated below the protein sequence: **o** (outside), extracellular; **M**, transmembrane; **i**, intracellular.

Figure 5. (A) Neighbour-joining analysis of the phylogenetic relationship of tick T selectins. Uniprot accession numbers followed by the species acronym are shown for each sequence. A0A1E1WZU0_AMBAU from *Amblyomma aureolatum*; A0A023HQI3_AMBMA from *Amblyomma maculatum*; A0A023FUX9_AMBPA from *Amblyomma parvum*; A0A023GF10_AMBTR from *Amblyomma triste*; A0A131X9Z0_HYAEX from *Hyalomma excavatum*; A0A147BVB9_IXORI from *Ixodes ricinus*; A0A293LXQ0_ORNER from *Ornithodoros erraticus*; A0A2R5LKM1_ORNTU from *Ornithodoros turicata*; A0A131Z2T8_RHIAP from *Rhipicephalus appendiculatus*; L7MED7_RHIPU from *Rhipicephalus pulchellus*; A0A224Z6C8_RHIZA from *Rhipicephalus zambiensis*. Evolutionary distances were computed using the Poisson correction method. Branch support values (10,000 bootstraps) for the nodes are indicated. OeSEL is marked with a red dot. **(B)** Amino acid sequence of OeSEL, showing the linear B-cell epitope predictions by ABCpred (yellow), BCEpred (blue) and BepiPred-2.0 (green) algorithms. The predicted topology is indicated below the protein sequence: **o** (outside), extracellular; **M**, transmembrane; **i**, intracellular. All the amino acids in the extracellular loop had at least one prediction of antigenicity and were included in the design of the immunogenic peptide (blue box), except the leucine at position 48, which was omitted to allow the peptide to end in cysteine.

Figure 6. ELISA. IgG antibody response in rabbits vaccinated with the synthetic peptides derived from antigens OeABC (anti-ABC), OeAQP+OeAQP1 (anti-AQPs) and OeSEL (anti-OeSEL). **(A-J)** Reactivity of rabbit sera to each individual synthetic peptide. **(K-L)** Reactivity of rabbit sera to the saliva of *Ornithodoros erraticus* and *Ornithodoros moubata*. Values are the average OD $\pm$ SD at 492 nm for each rabbit group. Sera were taken before immunisation (preimmune), 14 days post-immunisation, immediately before the infestation with ticks (14 d.p.i.) and 14 days post-infestation (28 d.p.i.), and were used at 1/300 dilution.

Figure 7. (A, C) Coomassie Blue-stained 5–20% SDS-PAGE gels showing the soluble (S) and membrane (M) proteins of the midgut from *Ornithodoros erraticus* **(A)** and *Ornithodoros moubata* **(C)** female ticks taken before feeding (Unfed) and 48 hours after engorgement (Fed). **(B, D)** Western blots: antigens revealed by the sera from the rabbits immunised with the synthetic peptides derived from the ABC transporter (anti-ABC), aquaporins (anti-AQPs) and selenoprotein T (anti-SEL) antigens in the protein extracts from *O. erraticus* **(B)** and *O. moubata* **(D)**. Sera were taken before immunisation (preimmune) and 14 days post-immunisation (d.p.i.), immediately before the infestation with ticks. Arrow: OeABC transporter native protein. Arrow heads: monomeric and likely oligomeric forms of AQPs. Asterisks: IgG heavy chain from the rabbit host, ingested with blood.

Supplementary Fig. 1. (A) Alignment of the amino acid sequences of tick AQP proteins. Uniprot accession numbers are shown for each sequence (DERVA, *Dermacentor variabilis*; IXORI, *Ixodes ricinus*; IXOSC, *Ixodes scapularis*; ORNBR, *Ornithodoros brasiliensis*; ORNER, *Ornithodoros erraticus*; ORNMO, *Ornithodoros moubata*; ORNTU, *Ornithodoros turicata*; RHIMP, *Rhipicephalus microplus*; RHISA, *Rhipicephalus sanguineus*). The amino acids conserved in all the sequences are labelled with asterisks (*); the conservative and semi-conservative substitutions are labelled with colons (:) and periods (.), respectively. The NPA motifs are highlighted in yellow and the four residues within the ar/R constriction are highlighted in green. The immunogenic peptides designed from *O. erraticus* A0A293N7C6 (OeAQP) and A0A293N0K9 (OeAQP1) are bolded and underlined. The percentage of sequence identity showed by

OeAQP or OeAQP1 to the other tick AQPs is indicated in the two columns on the low right corner. **(B)** Topology predictions for the OeAQP1 and OeAQP monomers using the TMHMM server. The values indicate the amino acids forming part of each region, and the red and blue boxes indicate the position on the extracellular third and fifth loops, respectively, of the two immunogenic peptides designed for each sequence.

Supplementary Fig. 2. (A) Alignment of the amino acid sequences of tick ABC transporters. Uniprot accession numbers followed by acronyms are shown for the other sequences (AMBAU, *Amblyomma aureolatum*; AMBTR; *Amblyomma triste*; IXORI, *Ixodes ricinus*; IXOSC, *Ixodes scapularis*; ORNMO, *Ornithodoros moubata*; ORNTU, *Ornithodoros turicata*). OeABC, *Ornithodoros erraticus* ABC transporter. The amino acids conserved in all the sequences are labelled with asterisks (*); the conservative and semi-conservative substitutions are labelled with colons (:) and (.) periods, respectively. The immunogenic peptides designed from OeABC are bolded and underlined. The percentage of sequence identity showed by OeABC to the other tick ABC transporter family members is indicated in the column on the lower right corner. **(B)** Topology prediction for OeABC using the TMHMM server. Blue boxes indicate the approximate position of the designed immunogenic peptides. The values on the right panel indicate the amino acids forming part of each region. Transmembrane helices are numbered and the external loops are highlighted in grey.

Supplementary Fig. 3. (A) Alignment of the amino acid sequences of tick selenoproteins T. Uniprot accession numbers are shown for each sequence (AMBAU, *Amblyomma aureolatum*; AMBMA, *Amblyomma maculatum*; AMBPA, *Amblyomma parvum*; AMBTR; *Amblyomma triste*; HYAEX, *Hyalomma excavatum*; IXORI, *Ixodes ricinus*; ORNER, *Ornithodoros erraticus*; ORNTU, *Ornithodoros turicata*; RHIAP, *Rhipicephalus appendiculatus*; RHIPU, *Rhipicephalus pulchellus*; RHIZA, *Rhipicephalus zambiensis*). The amino acids conserved in all the sequences are labelled with asterisks (*); the conservative and semi-conservative substitutions are labelled with colons (:) and periods (.), respectively. The immunogenic peptide designed from OeSEL (A0A293LXQO_ORNER) is bolded and underlined. The percentage of sequence identity showed by OeSEL to the other tick selenoproteins is indicated in the column on the

lower right corner. **(B)** Topology prediction for OeSEL using the TMHMM server. The values indicate the amino acids forming of each region and the blue box indicates the location of the designed immunogenic peptide.

Supplementary Fig. 4. ELISA. IgG antibody response in rabbits vaccinated with synthetic peptides derived from antigens OeABC, OeAQPs (AQP+AQP1), and OeSEL against midgut protein extracts from *Ornithodoros erraticus* **(A)** and *O. moubata* **(B)** female ticks taken either before blood feeding (Unfed) or 48 hours post-feeding (Fed). Protein extracts were fractionated into soluble proteins (Sol) and membrane-associated proteins (Mem). Values are the average OD $\pm$ SD at 492 nm (OD_{492 nm} immune sera – OD_{492 nm} preimmune sera) from each rabbit group. Sera were taken before immunisation (preimmune), 14 days post-immunisation (14 d.p.i.), immediately before the infestation and 14 days post-infestation (28 d.p.i.) and were used at 1/300 dilution.

Table 1. Vaccination trial. Groups of rabbits, selected candidate antigens and immunogenic peptides that were administered to each group of rabbits.

Group	Candidate antigens	Immunogenic peptides	Number of rabbits	Adjuvant
Group 0 (control)	-	-	3	Montanide ISA 50 V2
Group 1	ABC transporter (OeABC)	OE7665/1 OE7665/2 OE7665/3 OE7665/4 OE7665/5	3	
Group 2	Aquaporin: OeAQP	OE0257N OE0257C	3	
	Aquaporin: OeAQP1	OE9254N OE9254C		
Group 3	Selenoprotein T (OeSEL)	OE7237/1	3	

Table 2. Aquaporins, ABC transporters and selenoprotein transcripts recovered from the transcriptome of the *Ornithodoros erraticus* midgut (Oleaga et al., 2018). Unfed: female ticks before feeding; fed: female ticks 48 hours post-feeding. FPKM: fragments per kilobase of transcript per million mapped reads.

Transcript	Entry name (Uniprot)	Protein names	Length (aa)	MW (kDa)	Transcript expression level (average FPKM): unfed	Transcript expression level (average FPKM): fed	Fold-change	FDR	Vaxijen Score
Aquaporins									
ci 000012977	A0A293LVU2_ORNER	AQP9-like protein	295	31.46	23.06	30.24	1.36	4.39x10 ⁻⁰¹	0.6103
ci 000016152	A0A293LYK5_ORNER	VM23	321	34.83	25.18	49.01	1.81	3.12x10 ⁻⁰¹	0.6568
ci 000078002	A0A293MYA1_ORNER	Aquaporin AQP Ae.a	271	28.50	416.34	729.27	1.74	4.33x10 ⁻⁰⁴	0.4611
ci 000079254*	A0A293NOK9_ORNER	Water-specific aquaporin	285	30.84	399.68	507.86	1.28	9.51x10 ⁻⁰¹	0.5891
ci 000090257*	A0A293N7C6_ORNER	Aquaporin	303	32.84	849.94	1,343.66	1.58	4.59x10 ⁻⁰⁵	0.6365
ABC transporters									
ci 000079929	A0A293MZ42_ORNER	ABC transporter, putative	520	57.78	717.67	6,652.10	9.23	5.83x10 ⁻²²²	0.4074
ci 000038204	A0A293MIC1_ORNER	ABC transporter, putative	334	36.50	5.77	24.49	3.51	3.50x10 ⁻⁰²	0.5159
ci 000048641	A0A293MM56_ORNER	ABC transporter, putative (fragment)	175	20.33	11.01	33.94	2.76	5.60x10 ⁻⁰²	0.5371
ci 000082016	A0A293MJG3_ORNER	ABC transporter, putative (fragment)	464	52.04	2,163.16	5,905.55	2.72	2.67x10 ⁻³⁸	0.5435
ci 000077665*	No name	ABC transporter, putative	1,518	168.63	21,594.58	44,470.94	2.06	1.27x10 ⁻²⁵	0.5941
Selenoproteins									
ci 000017237*	A0A293LXQ0_ORNER	Selenoprotein T, putative	111	12.30	69.19	452.54	6.29	4.74x10 ⁻¹⁵	0.7096
ci 000016549	No name	Selenoprotein M	39	4.19	860.80	3,531.50	4.09	1.07x10 ⁻⁸⁴	0.3573
ci 000079224	No name	Selenoprotein P precursor, putative	338	38.12	48,126.37	76,721.99	1.59	3.59x10 ⁻⁰⁶	0.5623

* Transcripts selected as vaccine antigenic candidates.

Table 3. Names and sequences of the synthetic peptides designed from the extracellular loops and domains of proteins OeAQP1, OeAQP, OeABC transporter and OeSEL. The design includes one cysteine in each peptide end to allow spontaneous lineal oligomerization of the peptides. Added cysteines, not present in the original sequence, are underlined.

Protein name	Peptide name	No. of aa	Sequence	MW/Da
OeAQP	OE0257N	22	<u>C</u> RSVVGEKGTAAIFSTYPREE <u>C</u>	2,403
	OE0257C	26	CMAALNPARDIGPRIFTAISGWGSQ <u>C</u>	2,736
OeAQP1	OE9254N	25	CTYKSAFDHYDGGLRATTGVNATA <u>C</u>	2,622
	OE9254C	26	CGAPLNPARDLGPRVFTAMAGWGTE <u>C</u>	2,691
OeABC	OE7665/1	23	<u>C</u> GAGDRFVARSNELTDTNHSSY <u>C</u>	2,503
	OE7665/2	21	<u>C</u> AAWEKPHNKPPIQWPAAGR <u>C</u>	2,360
	OE7665/3	22	CDLQPGEKVGVVGRTGAGKSS <u>C</u>	2,148
	OE7665/4	21	<u>C</u> GNIRSNLDPFQQFSDEEVW <u>C</u>	2,487
	OE7665/5	20	<u>C</u> HDVTEGGDNISVGQRQLVC	2,130
OeSEL	OE7237/1	19	<u>C</u> RPTPSFVAWMLNNKLYSC	2,230

Table 4. Effect of the vaccination with synthetic peptides derived from candidate antigens OeABC, OeAQPs (AQP+AQP1), and OeSEL on the *Ornithodoros erraticus* and *O. moubata* specimens fed on control and vaccinated rabbits. Results are shown as mean $\pm$ standard deviation for each rabbit group. Means were compared between ticks fed on vaccinated and control rabbits by one-way ANOVA followed by the Dunnett's t-test. In parentheses, % of change in the corresponding parameter respect to the control: percentage of reduction for ingested blood, moulting, oviposition and fertility; increase for mortality.

Parameter	Life stage	Control	OeABC (% change)	OeAQPs (% change)	OeSEL (% change)
<i>Ornithodoros erraticus</i>					
Ingested blood (mg)	Males	3.2 $\pm$ 0.4	3.8 $\pm$ 0.9	3.7 $\pm$ 0.3	3.2 $\pm$ 0.3
	Females	9.1 $\pm$ 1.1	9.8 $\pm$ 0.4	9.1 $\pm$ 0.4	6.7 $\pm$ 1.3 (26.4)**
	Nymphs-3	1.9 $\pm$ 0.1	1.9 $\pm$ 0.3	1.9 $\pm$ 0.3	2.2 $\pm$ 0.5
Mortality (%)	Males	1.2 $\pm$ 1.7	3.6 $\pm$ 3.0 (2.4)**	1.1 $\pm$ 1.6	2.3 $\pm$ 1.7 (1.1)
	Females	0 $\pm$ 0	2.6 $\pm$ 3.7 (2.6)**	0 $\pm$ 0	6.7 $\pm$ 5.5 (6.7)**
	Nymphs-3	13.2 $\pm$ 9.1	16.5 $\pm$ 0.5 (3.3)	11.9 $\pm$ 5.4	12.2 $\pm$ 6.5
Moulting (%)	Nymphs-3	37.7 $\pm$ 5.2	38.4 $\pm$ 9.6	36.5 $\pm$ 17.4 (3.2)	34.9 $\pm$ 10.7 (7.4)
Oviposition (no. eggs/female)	Females	36.9 $\pm$ 1.9	39.5 $\pm$ 3.9	33.4 $\pm$ 1.4 (9.5)**	22.2 $\pm$ 6.5 (39.8)**
Fertility (no. larvae/female)	Females	33.6 $\pm$ 1.3	36.1 $\pm$ 4.6	29.1 $\pm$ 3.0 (13.4)**	20 $\pm$ 7.9 (40.5)**
Efficacy (%)			3.1	15.5	47.5
<i>Ornithodoros moubata</i>					
Ingested blood (mg)	Males	27.9 $\pm$ 1.1	23.7 $\pm$ 2.6 (15.4)**	25.3 $\pm$ 4.1 (9.3)*	22.8 $\pm$ 1.6 (18.6)**
	Females	169.6 $\pm$ 34	144.8 $\pm$ 17.4 (14.5)**	165.3 $\pm$ 9.9 (2.4)	164.3 $\pm$ 5.4 (3.0)
	Nymphs-3	16.7 $\pm$ 01.5	16.5 $\pm$ 1.9 (1.0)	16.3 $\pm$ 0.7 (2.4)	16 $\pm$ 02 (4.0)
Mortality (%)	Males	3.5 $\pm$ 3	4.4 $\pm$ 6.4 (0.9)	2.1 $\pm$ 3.1	2.3 $\pm$ 1.6
	Females	6.8 $\pm$ 0.2	2.2 $\pm$ 3.2	4.4 $\pm$ 3.2	4.4 $\pm$ 3.2
	Nymphs-3	2 $\pm$ 1.7	0 $\pm$ 0	0.7 $\pm$ 1	0.7 $\pm$ 1
Moulting (%)	Nymphs-3	96 $\pm$ 4	98 $\pm$ 2	96 $\pm$ 4	94 $\pm$ 42 (2.1)
Oviposition (no. eggs/female)	Females	172.3 $\pm$ 23.5	127.6 $\pm$ 10.9 (25.9)**	161.6 $\pm$ 22.7 (6.2)	155.2 $\pm$ 17.3 (9.9)
Fertility (no. nymphs/female)	Females	159.6 $\pm$ 21.4	116.7 $\pm$ 11.8 (26.7)**	150.9 $\pm$ 21.3 (5.4)	144.2 $\pm$ 8.1 (9.6)
Efficacy (%)			22.5	4.6	9.6

* p < 0.05; ** p < 0.01

Figure 1

Figure 2

A0A293N0K9 (OeAQP1, 285 aa)

[illegible]

A0A293N7C6 (OeAQP, 303 aa)

[illegible]

Figure 3

Figure 4

70

Figure 6

Figure 7

Supplementary Fig. 1. (A) Alignment of the amino acid sequences of tick AQP proteins. Uniprot accession numbers are shown for each sequence (DERVA, *Dermacentor variabilis*; IXORI, *Ixodes ricinus*; IXOSC, *Ixodes scapularis*; ORNBR, *Ornithodoros brasiliensis*; ORNER, *Ornithodoros erraticus*; ORNMO, *Ornithodoros moubata*; ORNTU, *Ornithodoros turicata*; RHIMP, *Rhipicephalus microplus*; RHISA, *Rhipicephalus sanguineus*). The amino acids conserved in all the sequences are labelled with asterisks (*); the conservative and semi-conservative substitutions are labelled with colons (:) and periods (.), respectively. The NPA motifs are highlighted in yellow and the four residues within the ar/R constriction are highlighted in green. The immunogenic peptides designed from *O. erraticus* A0A293N7C6 (OeAQP) and A0A293N0K9 (OeAQP1) are bolded and underlined. The percentage of sequence identity showed by OeAQP or OeAQP1 to the other tick AQPs is indicated in the two columns on the low right corner. **(B).** Topology predictions for the OeAQP1 and OeAQP monomers using the TMHMM server. The values indicate the amino acids forming part of each region, and the red and blue boxes indicate the position on the extracellular third and fifth loops, respectively, of the two immunogenic peptides designed for each sequence.

A

A0A293N7C6_ORNER	-----MQIRNVLAARELINEFGLTMTILIGDSIVAVI-----IAGQGQHN	40
A0A097ITI9_RHIMP-AQP1	-----MKIENLLIRQLINEFLGTMLITIGDSIMAI-----IAGDNESL	40
B7QMKT_IXOSC	-----MLARDELIIYTLKGLIGDLSILAII-----IAGDNEPL	31
Q06EX9_DERVA_AQP9	MKPNAVTRAWRRVCGCCIERPLARQALAEVMGTVLTVLVGDCVLASL----AVFQLGST	55
A0A141GE76_RHIMP_AQP2	MKPNTVTTRAQRQTGCCIENTLARQALAEVMGTVLTVLVGDCVLASL----AVFQLGSV	55
A0A293N0K9_ORNER	-----MITDRIKIRSPAVREMLAELLGGTFVLVLFGTGVLASL----KFDPKVGTI	45
A0A2R5L6A3_ORNTU	-----ILDVKVKSTAVREMLAELLGGTFVLVLFGTGVLAAAL----KFDPKTGNI	44
A0A1D2AI66_ORNB	-----DKMKIKSLAVREMLAELGGTFVLVLFGTGVLAAAL----KFDNAGNV	42
A0A1Z5L547_ORNMO	-----MILDKVKISLALRETFAELLGGTFVLVLFGTGVLAAAL----KFDRAGNI	45
A0A1Z5KVQ3_ORNMO	-----MILDKVKIKSLALRETFAELLGGTFVLVLFGTGVLAAAL----KFDRAGNI	45
B8ZJG8_RHISA_AQP1	-----MLDSVKIKNALVREVLAELLGGTFVLVLFGNTVLAVV----AFDRAPNL	44
A0A168DD71_IXOSC_AQP1	-----MLDGFKIKNAVIRESLAELMGTFILVLFGNTVLAIL--TFEKASTD	44
B7PDX8_IXOSC	-----MQLFGNTVLAIL--TFEKASTD	20
C5J8E5_IXORI_AQP1	-----MQLFGNTVLAILNHVCMTFEKASTD	25
	. * : : *	
A0A293N7C6_ORNER	AHVIGPIGTAIFCAVFVAGGVSAHINPAVTTLALASAKEFPKKVPFWLAQYLGAFF	99
A0A097ITI9_RHIMP-AQP1	AACVGPLNGVAIYVAVQISGGVS-SHLNPATTLAQASVRKFPIAKVPLYFAAQYLGGFV	99
B7QMKT_IXOSC	APIVGPGVGTAFIVAVTIAGGVSAHINPAVTTLALASTKKFPINKVPLYFAVQYLGAFV	90
Q06EX9_DERVA_AQP9	GIAAGPLNGLAVFLGVLVAGGVSGAHLNPATVTAMATVGKLAWCKLAYLVAAQYVGAFV	115
A0A141GE76_RHIMP_AQP2	GLAAPLGNGLAVFLGVLVAGGVSGAHLNPATVTALATIGKLGCNCNVLVTAQYLGAFV	115
A0A293N0K9_ORNER	GYAAGFWNGGALVLGVLVAGGVSGAHLNPSITIALATIGKCCEWKVLHYMVQAQYLGAFV	105
A0A2R5L6A3_ORNTU	GYAVGFWNGGLALALAVLVAGGVSGAHLNPSITIAMATIGKCQWKKVVHYMVQAQYLGAFV	104
A0A1D2AI66_ORNB	GYAAGFWNGGLALAMGVLVSGGVSGAHLNPSITVALATIGKCEWRKHVLYVAQYLGAFV	102
A0A1Z5L547_ORNMO	GYAAGFWNGGLALGMGVLVSGGVSGAHLNPSITVALATIGKCQWRKHVPHYLVAQYLGAFV	105
A0A1Z5KVQ3_ORNMO	GYAAGFWNGGLALGMGVLVSGGVSGAHLNPSITVALATIGKCQWRKHVPHYLVAQYLGAFV	105
B8ZJG8_RHISA_AQP1	GYYAAFNGGLALTGLVLVAGGVSGAHLNPATVTAVTTVGKFPWRKIPVYMVAQYIGAFT	104
A0A168DD71_IXOSC_AQP1	GLAACFWNGGLALTGLVLVAGGVSGAHLNPATVTAVTTIGKFPWRKIPVYLAQYIGAFT	104
B7PDX8_IXOSC	GLAACFWNGGLALTGLVLVAGGVSGAHLNPATVTAVTTIGKFPWRKIPVYLAQYIGAFT	80
C5J8E5_IXORI_AQP1	GLAACFWNGGLALTGLVLVAGGVSGAHLNPATAVAVTTIGKFPWRKIPVYVIAQYIGAFT	85
	. ** . *: . * :*:*. * :*:**:*: * : : : : : **:*. *	
A0A293N7C6_ORNER	GAVLVYMYRDVINHFDDGGVRSVVGEGKGTAEIFSTYPREEVTTLTCFFDQVISTAVLMILT	159
A0A097ITI9_RHIMP	GAALVFATYKDAIEHFDQGIHQVTEGKATAGIFATYPRPHVSTLTCFIDQVIATGIMMVC	159
B7QMKT_IXOSC	GAALVYLLYHDQIAHFDGGVRQITGKHGTAPIFATYPREQISTLTCFFDQVLVGTGILMILT	150
Q06EX9_DERVA_AQP9	ASCLEVYVYTDLALSQIDANLTTVGINATAPVFSFCPPAGVSTLTCCLVDQIVSTAVLLVG	175
A0A141GE76_RHIMP_AQP2	ASGLVYLVADALSQVDVNLAIVYGTNATAPVFSFCPPAGVSTLTCCLDQTVSTAVLLVG	175
A0A293N0K9_ORNER	AAAVMFCITYKSAPHDYDGLLRATTGVNATAFI FASYPRDYVTANCFLDVGGTGLFVMC	165
A0A2R5L6A3_ORNTU	GAAVLFACTYKNADFDFDGGRLRATTGVNATADVFASTREFVSTGNCFLDQVVGCTGLFVMC	164
A0A1D2AI66_ORNB	GAAVLFACTYKGAFDFDFDGGNRRTTGATATADI FASYPREFVTGNCFLDQVVGCTGLFVMC	162
A0A1Z5L547_ORNMO	GAAVLFLTXYKGAFDNYDGGFRATTGVNGTAGADVFASTPRDFVGTGNCFLDQVVGCTGLFVMC	165
A0A1Z5KVQ3_ORNMO	GAAVLFLTXYKGAFDNYDGGFRATTGVNGTAGADVFASTPRDFVGTGNCFLDQVVGCTGLFVMC	165
B8ZJG8_RHISA_AQP1	ASVILFITRYRGALNDYDGGVRAVTGSNATAGIFASYPQEFTVSTGNGLDVQIVGTALLVLA	164
A0A168DD71_IXOSC_AQP1	ASVLFITTYRGALDNFDGGNRIVTGVTGNTAGIFASYPKEFLSTGNGLDVQIVGTALLMLC	164
B7PDX8_IXOSC	ASVLFITTYRGALDNFDGGNRIVTGVTGNTAGIFASYPKEFLSTGNGLDVQIVGTALLMLC	140
C5J8E5_IXORI_AQP1	ASVLFITTYRGALDNFDGGNRIVTGVTGNTAGIFASYPKEFLSTGNGLDVQIVGTALLMLC	145
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A0A293N7C6_ORNER	VAAIVDERNFAPA-VPKALLPLMLGMLIVAEAFASHNCAAALNPARDIGPRIFTAISGWGS	218
A0A097ITI9_RHIMP-AQP1	VEAITGDTRNFGGIPPPIHPICLGLMIMAIFSAYNCMPLNPARDISPRLFETLAMAGWP	219
B7QMKT_IXOSC	AEAITDPRNFGGMPKHLHLPALGFMIMALIFGFSYNMAPLNPARDIGPRIFTAAGWG	210
Q06EX9_DERVA_AQP9	ICAITDTRNMD- VSRGQQPLLVLGTVSACYAFSYNCPNLNPARLAPRIFTAMSGWS	234
A0A141GE76_RHIMP_AQP2	ICAITDGRNMA-VSRGQQPLLVLGTVSACYAFSYNCPNLNPARLAPRIFTAMCGWS	234
A0A293N0K9_ORNER	IWAITDSHNMAMPVLOGLPILGIALATTLCFGYNCAPLNPARLGPVRTAMAGWG	222

A0A2R5L6A3_ORNTU	IVAITDSSNMA-VPQGLQPLLIGISLATTA	LCFGYNCGAPLNPARDLGPRIFTAMAGWGT	223
A0A1D2AI66_ORNBR	IIAITDNSNMS-VAQGLQPLLIGISLATTA	LCFGYNCGAPLNPARDLGPRIFTSMAGWGT	221
A0A1Z5L547_ORNMO	IVAITDNHNMA-VPQGLQPLLIGISLATTA	LCFGYNCGAPLNPARDLGPRIFTAMAGWGT	224
A0A1Z5KVQ3_ORNMO	IVAITDNHNMA-VPQGLQPLLIGISLATTA	LCFGYNCGAPLNPARDLGPRIFTAMAGWGT	224
B8ZJG8_RHISA_AQP1	ILAITDSRNMA-VPQGVQPLFIGLALGAL	LCFGYNCGAPLNPARDLGPRVFTAMAGWGV	223
A0A168DD71_IXOSC_AQP1	ILAITDARNMA-VPQGVQPLFIGFALAAI	LSFGYNCGAPLNPARDLAPRVFTAMAGWGG	223
B7PDX8_IXOSC	ILAITDARNMA-VPQGVQPLFIGFALAAI	LSFGYNCGAPLNPARDLAPRVFTAMAGWGG	199
C5J8E5_IXORI_AQP1	ILAITDARNMA-VPQGVQPLFIGFALAAI	LSFGYNCGAPLNPARDLAPRVFTAMAGWGG	204
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A0A293N7C6_ORNER	QVFTFRNYQWVWVPIFGPHIGAIIGLWIYKLCIRDHWPVQEMTSLKQVVSAA--NGDNP	276
A0A097ITI9_RHIMP-AQP1	ETFTLRGWNYYVWPVPLGPHIGAILGVWLYKVAIGDHWPEKPKPAISMDHPVAVVAKKAP	279
B7QMK7_IXOSC	EVFTYRNWNYIWVPIFGPHIGAIIGAWIYKVGIGDNFPDDEPKLRAAESGVQLVYQPGA	270
Q06EX9_DERVA_AQP9	AVFSFRAYNWFVWPVVGPHLGAVIGVWIYKLAVDNHWRADDEIDAERKPLLGS-----	288
A0A141GE76_RHIMP_AQP2	AVFSFRSYNWFVWPVVGPHLGAVIGVWIYKLAVDNHWNKDEDEDEDEKRPLLSN-----	288
A0A293N0K9_ORNER	EVFSFRDYNWFVWPVIVAPHIGAVVGTWLYRLLVGLHWPQDSYDMEQGRRSKDGG-----	278
A0A2R5L6A3_ORNTU	EVFSFRDYNWFVWPVIVAPHIGAVVGTWLYRLLVGLHWPQDSYDMDQGRRKDKGG-----	277
A0A1D2AI66_ORNBR	EVFSFRDYNWFVWPVIVAPHLGAIIGTWLYKLMVGLHWPQDSYDMEQGGGRKDKGG-----	275
A0A1Z5L547_ORNMO	EVFSFRDYNWFVWPVIVAPHIGAVGTWLYRLLVGLHWPQDSYDMEQQRNRAKDGG-----	278
A0A1Z5KVQ3_ORNMO	EVFSFRDYNWFVWPVIVAPHIGAVGTWLYRLLVGLHWPQDSYDMEQQRNRAKDEY-----	278
B8ZJG8_RHISA_AQP1	EVFSYRDYNWFVWPVIVGPHIGAVIGAWLYTLAVELHWPQSSYDMDSGNTVSAKD-----	277
A0A168DD71_IXOSC_AQP1	EVFSFRDYNWFVWPVILGPHMGAILGAWIYTLAVELHWPQATYEMGGGNPVSVKD-----	277
B7PDX8_IXOSC	EVFSFRDYNWFVWPVILGPHMGAILGAWIYTLAVELHWPQATYEMDGGNPVSIKD-----	253
C5J8E5_IXORI_AQP1	EVFSYRDYNWFVWPVILGPHIGAILGAWIYTLAVELHWPQATYEMDGGNPVSTKD-----	258
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			OeAQP	OeAQP1
A0A293N7C6_ORNER	GKTVESIVLRPAGENRT-----EGDQNTNGKI-----	303	-	45%
A0A097ITI9_RHIMP-AQP1	SRTPPALSPSAAPTSTSTSPQPAAPQATDVNAGSPAKA-----	316	55%	44%
B7QMK7_IXOSC	EQPVP-EAPAPVP-----	282	60%	45%
Q06EX9_DERVA_AQP9	---PKLVA-----	293	48%	52%
A0A141GE76_RHIMP_AQP2	---AKICA-----	293	50%	53%
A0A293N0K9_ORNER	---YTLERN-----	285	45%	-
A0A2R5L6A3_ORNTU	---YTLERRN-----	284	47%	88%
A0A1D2AI66_ORNBR	---YSLEGRN-----	282	44%	83%
A0A1Z5L547_ORNMO	---YSLE-----	282	45%	85%
A0A1Z5KVQ3_ORNMO	---MPIGGYDAAP-----KEQQSASS-PNEKQSKV-	304	45%	83%
B8ZJG8_RHISA_AQP1	---AP-----RFQ-----LANQNEAIF	291	47%	66%
A0A168DD71_IXOSC_AQP1	---VE-----NSVQMRGIKLGDSKN---	294	47%	63%
B7PDX8_IXOSC	---GG-----YTGAIPVNHVC-----	266	51%	65%
C5J8E5_IXORI_AQP1	---GG-----YTGAIP-----	266	50%	64%

B

A0A293N0K9 (OeAQP1, 285 aa)

A0A293N7C6 (OeAQP, 303 aa)

Supplementary Fig. 2. (A) Alignment of the amino acid sequences of tick ABC transporters. Uniprot accession numbers followed by acronyms are shown for the other sequences (AMBAU, *Amblyomma aureolatum*; AMBTR, *Amblyomma triste*; IXORI, *Ixodes ricinus*; IXOSC, *Ixodes scapularis*; ORNMO, *Ornithodoros moubata*; ORNTU, *Ornithodoros turicata*). OeABC, *Ornithodoros erraticus* ABC transporter. The amino acids conserved in all the sequences are labelled with asterisks (*); the conservative and semi-conservative substitutions are labelled with colons (:) and (.) periods, respectively. The immunogenic peptides designed from OeABC are bolded and underlined. The percentage of sequence identity showed by OeABC to the other tick ABC transporter family members is indicated in the column on the lower right corner. **(B)** Topology prediction for OeABC using the TMHMM server. Blue boxes indicate the approximate position of the designed immunogenic peptides. The values on the right panel indicate the amino acids forming part of each region. Transmembrane helices are numbered and the outside loops are highlighted in grey.

A

OeABC	-----MESFCGSTFWDPQLVWDVASSPDTSCFQDTVLLWI PCGFLWLLPLELYYYG	53
A0A1Z5KXC6_ORNMO	-----	0
B7PUI6_IXOSC	SPNTPPAMDAFCGSTFWDTLSWNS-ESPDLTPCFQETAMLWAPCVLLWVAMPFELSSYK	59
V5HHU7_IXORI	SPNTPPAMDAFCGSTFWDTLSWNS-ESPDLTPCFQETAMLWAPCVLLWVAMPFELSSYK	59
V5HBA0_IXORI	-----MLWAPCVLLWVAMPFELSSYK	21
A0A1E1X7G1_AMBAU	---TTPSMDSFCGSPFWDDSVTWNT-ETPDFTPCFQETALLWAPCLFLWLCSVFDIVAFK	56
A0A023GP66_AMBTR	-----	0
OeABC	THKDRFVPWTFNLALKVVLCLILSILSIVEIVHAMLLYGDGLDTFFVTTYAPFIRLATFL	113
A0A1Z5KXC6_ORNMO	-----	0
B7PUI6_IXOSC	MSRFKMLPWTFLNLLKA-LLCILVLISAVEVTHAMLLYGEGTTFVFPVDYYSPLRLVTFI	118
V5HHU7_IXORI	MSRFKTLPTWTFNLKVVLLCILVLISAVEVTHAMLLYGEGAMVFPVDYYSPLRLATFI	119
V5HBA0_IXORI	MSRFKTLPTWTFNLKVVLLCILVLISAVEVTHAMLLYGEGAMVFPVDYYSPLRLATFI	81
A0A1E1X7G1_AMBAU	KSRNSALPWTFLNIAKVLLALLIILVSILEIAQALTRYAEGQIVFSVEYYAPAIRMVTFI	116
A0A023GP66_AMBTR	-----	0
OeABC	AVLALVVSGRRGVHTSAVLFI FWLLMTIASGVQYRSMLIKVFGQ-AETISDDHFAFYFT	172
A0A1Z5KXC6_ORNMO	-----	0
B7PUI6_IXOSC	VALVLVITGRRQGIHTSGSLFLFWLLMSLTGGVRYRSLIMQVFDEEQDTISDPHIGFRFI	178
V5HHU7_IXORI	LALVLVIIGRRQGIHTSGSLFLFWLLMSLTGVVRYRSLIMQVFDEEQDTISDPHIGFRFT	179
V5HBA0_IXORI	LALVLVIIGRRQGIHTSGSLFLFWLLMSLTGVVRYRSLIMQVFDEEQDTISDPHIGFRFT	141
A0A1E1X7G1_AMBAU	LALVLIMTARRRGVCTSGPLFLFWLLMSLVSVRYRSLVLLQIFSEDVESISDDQIEFRFI	176
A0A023GP66_AMBTR	-----	0
OeABC	AAMIYFPLIVGEFLLSCSADTSYIIAGAPKHPRLVCPEATASFLSQLLFWWFNGMAILGF	232
A0A1Z5KXC6_ORNMO	-----MAILGF	6
B7PUI6_IXOSC	TVVLYFPFI FLGQFFLSCFADKLPE--DAKAAMKVCPEAKASFLSRILFWWFNGMAILGF	236
V5HHU7_IXORI	TVVLYFPFI FLGQFFLSCFADKLPE--DAKAAMKICPEAKASFLSRILFWWFNGMAILGF	237
V5HBA0_IXORI	TVVLYFPFI FLGQFFLSCFADKLPE--DAKAAMKICPEAKASFLSRILFWWFNGMAILGF	199
A0A1E1X7G1_AMBAU	TIVYICPVLVGQFFLSCFADTRPAV--GGKRIMRQCPEGRASFLSKLLFWWFNGMAILGF	234
A0A023GP66_AMBTR	-----	0
OeABC	LRPLRMEFMWKLQKHQNTEDVVDKFNVDQFEKEKQKRGAVRSKKADT-----KGN--	281
A0A1Z5KXC6_ORNMO	LRPLRMEFMWKLGRQNQTENIVDKFSVQFEKEKQKRGAVRSKKPDA-----KGN--	55
B7PUI6_IXOSC	IRPLQMYHMMNLDKPNKTEYI VENFDYHFNKEKQKRGFWLKNLSLHKFSLNQSHLQSGSEP	296
V5HHU7_IXORI	IRPLQMYHMMNLDKPNKTEYI VENFDYHFNKEKQKRGAVPKKKPD-----ADGRRP	288
V5HBA0_IXORI	IRPLQMYHMMNLDKPNKTEYI VENFDYHFNKEKQKRGAVPKKKPD-----ADGRRP	250
A0A1E1X7G1_AMBAU	MRPLQMYHMMFLDQANQTAKI SEDFAYHFEKEKQKRGAVVSKEPD-----ADGRRP	285
A0A023GP66_AMBTR	-----	0
OeABC	----FPVVSQVGIMRPLLRTFSLELVII GLLKLVASVLTFFVNPLVLDLLIGYVNSNDPLW	337
A0A1Z5KXC6_ORNMO	----YPVLSQVGIMRPLLRTFSLELVIIALLKLTA SVLTFVNPLILDMLIGYVNSNDPLW	111
B7PUI6_IXOSC	SECPRPEETFHCKSNKKKHVYASRSC---PKCSQMTPSHLSHFHFSLLIAFVNSNDPLW	353
V5HHU7_IXORI	IV-----SMVGIMTPLLRTFWAELTLVAFVKFFASVLTFFVNPMILDLLIAFVNSNDPLW	342
V5HBA0_IXORI	IV-----SMVGIMTPLLRTFWAELTLVAFVKFFASVLTFFVNPMILDLLIAFVNSNDPLW	304
A0A1E1X7G1_AMBAU	----IVSMIGIMTPLFRFTWSELTLVALVKLVSSVLTFFVSPLILDMLIAFVSSNDPLW	339
A0A023GP66_AMBTR	-----	0
OeABC	KGLLFAFTMFFSSMIESLLNGQYDYRI FCVAMRMRSAMINAVYQKALRLSSSTARGKFTTG	397
A0A1Z5KXC6_ORNMO	KGLLFAFTMFFSSMVESLLNGQYDYRI FCVAMRMRSAMINAVYQKALKLSSSTARGQFTTG	171
B7PUI6_IXOSC	HGLLFAFTMFFSSMVESFLNSQYERYIFVVSMMRMSAMISAIYRKALALSSVARGKFTTG	413
V5HHU7_IXORI	HGLLFAFTMFFSSMVESFLNSQYERYIFVVSMMRMSAMISAIYRKALALSSVARGKFTTG	402
V5HBA0_IXORI	HGLLFAFTMFFSSMVESFLNSQYERYIFVVSMMRMSAMISAIYRKALALSSVARGKFTTG	364
A0A1E1X7G1_AMBAU	RGLLFAFTMFFSAMESILNGQYDYRIYIVISMRMSAMINAIYQKALALSSVARGQYTTG	399
A0A023GP66_AMBTR	-----RSSRGQYTTG	10
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OeABC	EIVNLSVDTQRVMDYMQVFNLLWVTP LQIGIAIFLLWQGLGVATMGGVAVMLLLLPING	457

A0A1Z5KXC6_ORNMO	EIVNLSVSDTQRVMYMQVFNLWVTPLQIGIAIYLLWGQLGVATMGGVGMVLLLPING	231
B7PU16_IXO5C	EIVNLSVSDTQRIMDYMQVFNLWVTPLQIGIAIFLLWGQLGVATMGGVAVMILLLPING	473
V5HHU7_IXORI	EIVNLSVSDTQRIMDYMQVFNLWVTPLQIGIAIFLLWGQLGVATMGGVAVMILLLPING	462
V5HBA0_IXORI	EIVNLSVSDTQRIMDYMQVFNLWVTPLQIGIAIFLLWGQLGVATMGGVAVMILLLPING	424
A0A1E1X7G1_AMBAU	EIVNLSVSDTQRVMYDIQVFNLWITPLQIGIAIWLWGQLGIATLGGVGMIILLLPING	459
A0A023GP66_AMBTR	EIVNLSVSDTQRVMYDIQIFNLWITPLQIGIAIWLWGQLGIATLGGVGMIILLLPING	70
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OeABC	VITAYIRKYQVALMKQDKRRTKLMNEILGGIKVKLKLYAWEKSFQGRVEDIREKEVSSLKV	517
A0A1Z5KXC6_ORNMO	VITAYIRKYQVALMKQDKRRTKLMNEILSGIKVKLKLYAWEKSFQGRVEKIRDNEVSSLKV	291
B7PU16_IXO5C	VVTAYIRKYQVRLMKQDKRRITKLMNEILGGIKVKLKLYAWEKSFQARVQEIRDQEMSSLKV	533
V5HHU7_IXORI	VVTAYIRKYQVRLMKQDKRRITKLMNEILGGIKVKLKLYAWEKSFQARVQEIRDQEMSSLKV	522
V5HBA0_IXORI	VVTAYIRKYQVRLMKQDKRRITKLMNEILGGIKVKLKLYAWEKSFQARVQEIRDQEMSSLKV	484
A0A1E1X7G1_AMBAU	VVTAYIRKYQVQLMKHKDRRLKLMNEILGGIKVKLKLYAWEKSFQERVQSIRSDQMSSLKV	519
A0A023GP66_AMBTR	VVTAYIRKYQVQLMKHKDRRLKLMNEILGGIKVKLKLYAWEKSFQGRVQSIRRDELKSLV	130
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OeABC	QAYLSAAVIFAFTAAPFLVALSAFAVFLVDPNSVLDANKAFVSLSLFNILRVPMAFLPM	577
A0A1Z5KXC6_ORNMO	QAYLSASIIFAFTAAPFLVALSAFAVFLVDPNSVLDANKAFVSLSLFNILRVPMAFLPM	351
B7PU16_IXO5C	QAYLSAAVIFAFTSAPFLVALSAFAVFLVMDPANILDANKAFVSLSLFNILRVPMAFLPM	593
V5HHU7_IXORI	QAYLSAAVIFAFTSAPFLVALSAFAVFLVMDPANILDANKAFVSLSLFNILRVPMAFLPM	582
V5HBA0_IXORI	QAYLSAAVIFAFTSAPFLVALSAFAVFLVMDPANILDANKAFVSLSLFNILRVPMAFLPM	544
A0A1E1X7G1_AMBAU	QAYLSAAVIFAFTCAPFLVALSAFAVFTAIDPANVLNDANKAFVSLSLFNILRVPMAFLPM	579
A0A023GP66_AMBTR	QAYLSAAVIFAFTCAPFLVALSAFAVFTIIDPNVLDANKAFVSLSLFNILRVPMAFLPM	190
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OeABC	LITFTTAMFFVSLARINKYLRCDELDPDAVHHSKAEKEEPVVMSEGTFAWSKDSAPTLHDIT	637
A0A1Z5KXC6_ORNMO	LITFTTAMFFVSLARINKYLRCDELDPDAVHHPQKEEPPVVMSEGTFAWSKEASPTLHDIN	411
B7PU16_IXO5C	LITFTTAMFLVSLGRINKYLRSELDLPNAVEHSTKEEDPLVIKDSAFWSKDSNAEEDLN	653
V5HHU7_IXORI	LITFTTAMFLVSLGRINKYLRSELDLPNAVEHNTKEEDPLVIKDSAFWSKDSNAEEDLN	642
V5HBA0_IXORI	LITFTTAMFLVSLGRINKYLRSELDLPNAVEHNTKEEDPLVIKDSAFWSKDSNAEEDLN	604
A0A1E1X7G1_AMBAU	LITFTTAMFVSLGRINKYLRSELDLPNAVERNTGEGDPLVMKDSAFWSKDSQVSLDDL	639
A0A023GP66_AMBTR	LITFTTAMFVSLGRINKYLRSELDLPNAVQNTGEGDPLVMKDSAFWAKDSQPALDDL	250
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OeABC	LRVPKGSLLAVGVSGSGKSSILSAFLGDMVRLKGRVNVNGSVAYCPQQAWIQNASVKSN	697
A0A1Z5KXC6_ORNMO	LRVPKGSLLAIVGVSGSGKSSVLSAFLGDMVRLKGRVNVNGSVAYCPQQAWIQNASVKSN	471
B7PU16_IXO5C	IRIPKGSAAVGVAVGTGKSSMLSAFLGDMVKLGKTVNINGSIAYCPQQAWILNASVKSN	713
V5HHU7_IXORI	IRIPKGSAAVGVAVGTGKSSMLSAFLGDMVKLGKTVNINGSIAYCPQQAWILNASVKSN	702
V5HBA0_IXORI	IRIPKGSAAVGVAVGTGKSSMLSAFLGDMVKLGKTVNINGSIAYCPQQAWILNASVKSN	664
A0A1E1X7G1_AMBAU	ISVPKGALTAIVGVSGSGKSSMLSAFLGDMVKLGKTVNINGSVAYCPQQAWIQNASVKNN	699
A0A023GP66_AMBTR	ISVPKGALTAIVGTGCGKSSMLSAFLGDMVKLGKSVSINGSVAYCPQQAWIQNASVKSN	310
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OeABC	ILFGQRCLDGRYDQVLDACALRPDLAILPGGDETEVGEKGINLSGGQKQRIALARAVYSG	757
A0A1Z5KXC6_ORNMO	ILFGNSFDQGRYDQVLEACALKPDLAILPGGDETEVGEKGINLSGGQKQRISLARAVYSG	531
B7PU16_IXO5C	ILFGQPYDSERYEQVIEACALKPDLAILPGGDDTEVGEKGINLSGGQKQRIALARAVYSG	773
V5HHU7_IXORI	ILFGQPYDSERYEQVIEACALKPDLAILPGGDDTEVGEKGINLSGGQKQRIALARAVYSG	762
V5HBA0_IXORI	ILFGQPYDSERYEQVIEACALKPDLAILPGGDDTEVGEKGINLSGGQKQRISLARAVYSG	724
A0A1E1X7G1_AMBAU	ITFGQPFDRERYEQVIEACALKQDLIDLPGGDDTEVGEKGINLSGGQKQRISLARAVYSG	759
A0A023GP66_AMBTR	ITFGQPFDRDRYEQVIEACALKQDLIDLPGGDDTEVGEKGINLSGGQKQRISLARAVYSG	370
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OeABC	ADIYFLDDSLSAVDSHVGHKHFIDKVIKPGKMLRKTRILVTHRLSVLPQVGTIIVVLKDR	817
A0A1Z5KXC6_ORNMO	AEIYFLDDSLSAVDSHVGHKHFIDKVIKPGKMLRKQTRILVTHRLSVLPEVASIIVVLKDR	591
B7PU16_IXO5C	SDIYFFDDPLSAVDSHVGHKHFIDKVIKPGKLLRKTRILVTHRLSVLPQVDSVLVLIGGK	833
V5HHU7_IXORI	SEIYFFDDPLSAVDSHVGHKHFIDKVIKPGKLLRKTRILVTHRLSVLPQVDSVLVLIGGK	822
V5HBA0_IXORI	SEIYFFDDPLSAVDSHVGHKHFIDKVIKPGKLLRKTRILVTHRLSVLPQVDSVLVLIGGK	784
A0A1E1X7G1_AMBAU	SDIYFFDDPLSAVDSHVGHKHFIDKVIKPGKLLRKTRILVTHRLAVLPQVDMVLVLGDKG	819
A0A023GP66_AMBTR	SDIYFFDDPLSAVDSHVGHKHFIDKVIKPGKLLRKTRILVTHRLSVLPQVDMVLVMDGK	430
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OeABC	VSDMGTYDELVSRRGAFSEFLVQFLQVVEELPDVASDEDLQLVGEIVAQAQAGAPPELLKQY	877
A0A1Z5KXC6_ORNMO	VSDVGTYEELLVSRRGAFSEFLIQLQHVVEETPEGASDEDLQLVGEIVAQSRAPPELLIKQY	651
B7PU16_IXO5C	ISDVGTYEELLARGGAFSDFLVQFLREGEE-TEGVSDDELQLLGEIVAQAQAGAPSELLRQY	892
V5HHU7_IXORI	ISDVGTYEELLARGGAFSDFLVQFLREGEE-TEGVSDDELQLLGEIVAQAQAGAPSELLRQY	881
V5HBA0_IXORI	ISDVGTYEELLARGGAFSDFLVQFLREGEE-TEGVSDDELQLLGEIVAQAQAGAPSELLRQY	843
A0A1E1X7G1_AMBAU	ISDVGTYEELLTRGGAFSDFLVQFLQEGEE-TEGVSDDELQLLGEIVSQVGASTELARQY	878
A0A023GP66_AMBTR	INDVGTYEELLTRGGAFSDFLVQFLQEGEE-TEGVSDDELQLLGEIISQVGASTELARQY	489
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OeABC	TRLSANESDGS-DVERRGRSKSRVTSGSAEPPLSPKEKGV---PKKAAPVAGSKLTEE	933
A0A1Z5KXC6_ORNMO	SRLSGNESDSAS-DVERRGRT--HTISSGRTSLTPIKEKGV---KKKAEPAGSKLTEE	705
B7PU16_IXO5C	SRLSTNESDSTSDSERRARR-RTSSGRSLA-ERTSQKGTAEQ-VKPFAPAGAKLTEE	949
V5HHU7_IXORI	SRLSTNESDSCASDSERRARR-RTSSGRSLA-ERTSQKGTAEQ-VKPFAPAGARLTEE	938
V5HBA0_IXORI	SRLSTNESDSCASDSERRARR-RTSSGRSLA-ERTSQKGTAEQ-VKPFAPAGARLTEE	900
A0A1E1X7G1_AMBAU	SRLSANESDST-SDTERRARR-RSSARS-RTSSGRSLA-ERTSQKGPERSVKAPLAAGAKLTEE	935
A0A023GP66_AMBTR	SRLSANESDST-SDTERRARR-RTSSRSRTS-RTSSGRSLA-ERTSQKGPKEKTEKALAAGAKLTEE	546
	:*** ***** * ***:***:***:***:***:***:***:***:***:***:***:***:***	
OeABC	EVAEVSQVKKWVYLAYIKAMGWSWCTVLTFLFAYVLSHAFNIMGSLWSAWSNDALDPSLTG	993
A0A1Z5KXC6_ORNMO	EVAEVSQVKKWVYLEYVKAMGTWCTITLTVMYVLSHAFNIMGSLWSAWSNDALDAALV	765
B7PU16_IXO5C	ESAQVGSQVKKWVYIAYIKAMGLWMTAITLAAYIVSHIFNIMGSLWSAWSNDALDPSLAV	1009
V5HHU7_IXORI	ESAQVGSQVKKWVYIAYIKAMGLWMTGITLAAYIVSHIFNIMGSLWSAWSNDALDPSLAV	998
V5HBA0_IXORI	ESAQVGSQVKKWVYIAYIKAMGLWMTGITLAAYIVSHIFNIMGSLWSAWSNDALDPSLAV	960
A0A1E1X7G1_AMBAU	ESAQVGSQVKKWVYLAYIKAMGKMTVITLFSYIFSHAFNIMGSLWSAWSNDALDPSLAT	995
A0A023GP66_AMBTR	ESAQVGSQVKKWVYLAYIKAMGKMTVITLFSYIFSHAFNIMGSLWSAWSNDALDPSLAT	606
	* *****:***:***:***:***:***:***:***:***:***:***:***:***	

B

	outside	1	30
1	TMhelix	31	53
	inside	54	64
02	TMhelix	65	87
	outside	88	99
03	TMhelix	100	122
	inside	123	128
04	TMhelix	129	151
	outside	152	165
05	TMhelix	166	188
	inside	189	298
06	TMhelix	299	321
	outside	322	335
07	TMhelix	336	358
	inside	359	416
08	TMhelix	417	436
	outside	437	440
09	TMhelix	441	463
	inside	464	524
10	TMhelix	525	547
	outside	548	566
11	TMhelix	567	589
	inside	590	958
12	TMhelix	959	981
	outside	982	1005
13	TMhelix	1006	1028
	inside	1029	1087
14	TMhelix	1088	1110
	outside	1111	1183
15	TMhelix	1184	1206
	inside	1207	1210
16	TMhelix	1211	1233
	outside	1234	1518

Supplementary Fig. 3. (A) Alignment of the amino acid sequences of tick selenoproteins T. Uniprot accession numbers are shown for each sequence (AMBAU, *Amblyomma aureolatum*; AMBMA, *Amblyomma maculatum*; AMBPA, *Amblyomma parvum*; AMBTR; *Amblyomma triste*; HYAEX, *Hyalomma excavatum*; IXORI, *Ixodes ricinus*; ORNER, *Ornithodoros erraticus*; ORNTU, *Ornithodoros turicata*; RHIAP, *Rhipicephalus appendiculatus*; RHIPU, *Rhipicephalus pulchellus*; RHIZA, *Rhipicephalus zambiensis*). The amino acids conserved in all the sequences are labelled with asterisks (*); the conservative and semi-conservative substitutions are labelled with colons (:) and periods (.), respectively. The immunogenic peptide designed from OeSEL (A0A293LXQ0_ORNER) is bolded and underlined. The percentage of sequence identity showed by OeSEL to the other tick selenoproteins is indicated in the column on the lower right corner. **(B)** Topology prediction for OeSEL using the TMHMM server. The values indicate the amino acids forming part of each region and the blue box indicates the location of the designed immunogenic peptide.

A

A0A293LXQ0_ORNER	-----MQLAHALGLLKLGVIALLVLLGVDPFH	27
A0A2R5LKM1_ORNTU	GYRRAYEQYAGLLQEKYPALALEGAFFPPPPRMQLAHALGLLKLGVIALLVLLSVDPFHS	60
A0A147BVB9_IXORI	GYRRAFEQYAGLLQEKYPSLSVEGDTYPPPTPRMQMAHALSLLKLAVIALVLLGVDPFHY	60
A0A023HQI3_AMBMA	-----MEKYPSLLVEGDADFPPPPRMQLAHALSLLKLALIALVLLGLDPFAR	47
A0A023GF10_AMBTR	-----MEKYPSLLVEGDADFPPPPRMQLAHALSLLKLALIALVLLGLDPFAR	47
A0A023FUX9_AMBPA	-----MEKYPSLLVEGDAYPPPPRMQLAHALSLLKLALIALVLLGLDPFTR	47
A0A1E1WZU0_AMBAU	GYRRAFEQYAGLLMEKYPSLLVEGDAYPPPPRMQLAHALSLLKLALIALVLLGLDPFTR	60
A0A131X9Z0_HYAEX	GYRRAFEQYAGLLMEKYPSLLVEGDAYPPPPRMQLAHALSMLKLALIALVLLGINPFAW	60
A0A224Z6C8_RHIZA	-----MEKYPSLLVEGDAYPPPPRMQLAHALSLLKLALIALVLLGFNPFTW	47
A0A131Z2T8_RHIAP	-----MEKYPSLLVEGDAYPPPPRMQLAHALSLLKLALIALVLLGFNPFTW	47
L7MED7_RHIPU	GYRRAFEQYAGLLMEKYPSLLVEGDAYPPPPRMQLAHALSLLKLALIALVLLGFNPFTW	60
	:**.:**.:*****.:**	
A0A293LXQ0_ORNER	LG-RPTPSFVAVMLNNKLYSCLMVFFVCGAIESKLISTGAFEIYFNDVRVWSKIETGRIP	86
A0A2R5LKM1_ORNTU	LG-RPTPSFVGWMLTNKLYSCLMVFFVCGAIESKLISTGAFEIYFNDVRVWSKIETGRIP	119
A0A147BVB9_IXORI	LG-THTPAPFVWMLNNKLYSCLMLFFVCGAIESRLVSTGAFEIYFNDVRVWSKIETGRIP	119
A0A023HQI3_AMBMA	FGWGPTPAFFTWMMLTNKLYSCLMVFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	107
A0A023GF10_AMBTR	FGWGPTPAFFTWMMLTNKLYSCLMVFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	107
A0A023FUX9_AMBPA	LGWGQTPAFFTWMMLTNKLYSCLMVFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	107
A0A1E1WZU0_AMBAU	LGWGQTPAFFTWMMLTNKLYSCLMVFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	120
A0A131X9Z0_HYAEX	MG-TPTPAFFSWMVGKLYSCLMLFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	119
A0A224Z6C8_RHIZA	MG-YPTPAFYTWVMVGKLYSCLMLFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	106
A0A131Z2T8_RHIAP	MG-YPTPAFYTWVMVGKLYSCLMLFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	106
L7MED7_RHIPU	MG-YPTPAFYTWVMVGKLYSCLMLFFVCGAIESKLVSTGAFEIYFNDVRVWSKIETGRIP	119
	:* **:* **:* *****:*****.*:*****:*****:*****	
A0A293LXQ0_ORNER	SPPELFQIIDNQVFLKSPVLPLADT	111
A0A2R5LKM1_ORNTU	SPPELFQIIDNQVFLKSPVLPLADT	144
A0A147BVB9_IXORI	SPPELFQIIDNQVFLKSSVLSLADR	144
A0A023HQI3_AMBMA	SPPELFQIIDNQVFLKSSVLGLADS	132
A0A023GF10_AMBTR	SPPELFQIIDNQVFLKSSVLGLADS	132
A0A023FUX9_AMBPA	SPPELFQIIDNQVFLKSSVLGLADS	132
A0A1E1WZU0_AMBAU	SPPELFQIIDNQVFLKSSVLGLADS	145
A0A131X9Z0_HYAEX	SPPELFQIIDNQVFLKSSTLDLANS	144
A0A224Z6C8_RHIZA	SPPELFQIIDNQVFLKSSTLGLADS	131
A0A131Z2T8_RHIAP	SPPELFQIIDNQVFLKSSTLGLADS	131
L7MED7_RHIPU	SPPELFQIIDNQVFLKSSTLGLADS	144
	*****.* **:	
		Identity
		-
		97.3%
		85.5%
		84.8%
		84.8%
		84.8%
		84.8%
		80.2%
		82.0%
		82.0%
		82.0%

B

Figure

Supplementary Fig. 4. ELISA. IgG antibody response in rabbits vaccinated with synthetic peptides derived from antigens OeABC, OeAQPs (AQP+AQP1), and OeSEL against midgut protein extracts from *Ornithodoros erraticus* **(A)** and *O. moubata* **(B)** female ticks taken either before blood feeding (Unfed) or 48 hours post-feeding (Fed). Protein extracts were fractionated into soluble proteins (Sol) and membrane-associated proteins (Mem). Values are the average OD $\pm$ SD at 492 nm ($OD_{492\text{ nm}}$ immune sera – $OD_{492\text{ nm}}$ preimmune sera) from each rabbit group. Sera were taken before immunisation (preimmune), 14 days post-immunisation (14 d.p.i.), immediately before the infestation and 14 days post-infestation (28 d.p.i.) and were used at 1/300 dilution.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author contributions

RPS and AO conceived the study. RPS, POM, RPM and AO performed the experiments. RPS, RPM and AO performed data analyses. RPS wrote the paper, and other co-authors made additional suggestions and approved the manuscript.