Research paper

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

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A B S T R A C T

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.

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 h 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 AQPs 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 microbiome, and even during tick colonisation by pathogenic microbes (Adamson et al., 2013; Kumar et al., 2016a; Budachetri et al., 2017, 2018). Because of these functions, tick selenoproteins might be suitable targets for vaccines aimed at controlling ticks and tick-transmitted pathogens, but as far as we know, no studies on their protective efficacy against tick infestations have been undertaken.

The aim of the current work was to select representative members of the AQPs, ABC transporter and selenoprotein families from *O. erraticus*, and to test their efficacy as protective antigens in animal immunisation trials, followed by tick infestations with 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 AQPs 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 h photoperiod.

Midguts from both *O. erraticus* and *O. moubata* unfed females and fed females 48 h 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 h 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 NCBInr 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) (Krogh 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 oxidisation 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.

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	

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 3 N 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 h. 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 ± 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 x [1- (S x F x N x 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 A0A293 N0K9) and ci|000090257 (Uniprot A0A293 N7C6). 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/NCBInr databases for orthologues of either A0A293 N0K9 or A0A293 N7C6 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 A0A293 N0K9 and A0A293 N7C6 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

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 h 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	4.39×10^{-01}	0.6103
ci|000016152	A0A293LYK5_ORNER	VM23	321	34.83	25.18	49.01	1.81	3.12×10^{-01}	0.6568
ci|000078002	A0A293MYA1_ORNER	Aquaporin AQPAe.a	271	28.50	416.34	729.27	1.74	4.33×10^{-04}	0.4611
ci|000079254*	A0A293N0K9_ORNER	Water-specific aquaporin	285	30.84	399.68	507.86	1.28	9.51×10^{-01}	0.5891
ci|000090257*	A0A293N7C6_ORNER	Aquaporin	303	32.84	849.94	1,343.66	1.58	4.59×10^{-05}	0.6365
ABC transporters									
ci|000079929	A0A293MZ42_ORNER	ABC transporter, putative	520	57.78	717.67	6,652.10	9.23	5.83×10^{-222}	0.4074
ci|000038204	A0A293MIC1_ORNER	ABC transporter, putative	334	36.50	5.77	24.49	3.51	3.50×10^{-02}	0.5159
ci|000048641	A0A293MM56_ORNER	ABC transporter, putative	175	20.33	11.01	33.94	2.76	5.60×10^{-02}	0.5371
ci|000082016	A0A293MJG3_ORNER	ABC transporter, putative (fragment)	464	52.04	2,163.16	5,905.55	2.72	2.67×10^{-38}	0.5435
ci|000077665*	No name	ABC transporter, putative (fragment)	1,518	168.63	21,594.58	44,470.94	2.06	1.27×10^{-25}	0.5941
Selenoproteins									
ci|000017237*	A0A293LXQ0_ORNER	Selenoprotein T, putative	111	12.30	69.19	452.54	6.29	4.74×10^{-15}	0.7096
ci|000016549	No name	Selenoprotein M	39	4.19	860.80	3,531.50	4.09	1.07×10^{-84}	0.3573
ci|000079224	No name	Selenoprotein P precursor, putative	338	38.12	48,126.37	76,721.99	1.59	3.59×10^{-06}	0.5623

* Transcripts selected as vaccine antigenic candidates.
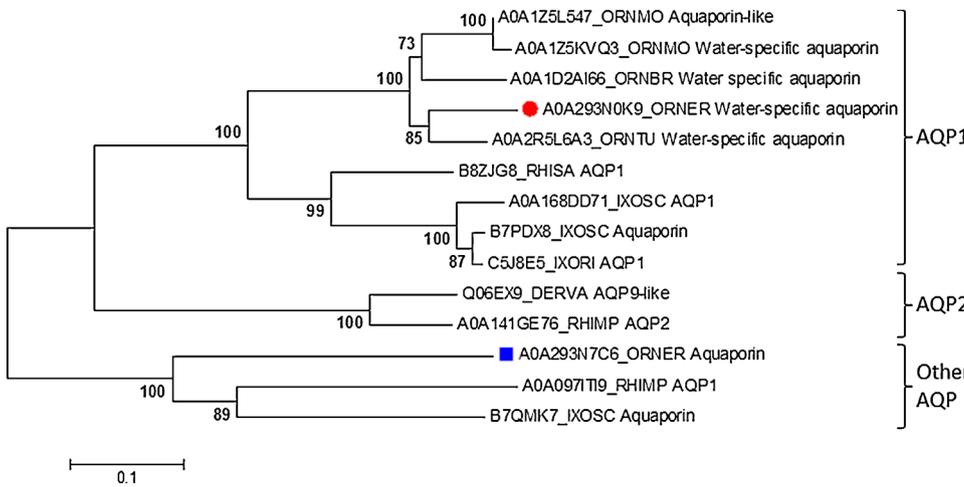
Fig. 1. Neighbour-joining analysis of the phylogenetic relationship of tick AQPs. Uniprot accession numbers followed by the species acronym are shown for each sequence. Q06EX9_DERVA from *Dermacentor variabilis*; C5J8E5_IXORI from *Ixodes ricinus*; A0A168DD71_IXOSC, B7PDX8_IXOSC and B7QMK7_IXOSC from *Ixodes scapularis*; A0A1D2AI66_ORNBR from *Ornithodoros brasiliensis*; A0A293 N7C6_ORNER and A0A293 N0K9_ORNER from *Ornithodoros erraticus*; A0A1Z5L547_ORNMO and A0A1Z5KVQ3_ORNMO from *Ornithodoros moubata*; A0A2R5L6A3_ORNTU from *Ornithodoros turicata*; A0A097ITI9_RHIMP and A0A141-GE76_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 (A0A293 N7C6) and OeAQP1 (A0A293 N0K9) are marked by a blue square and red dot, respectively.

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* A0A293 N0K9 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* A0A293 N7C6 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 1518 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 NCBInr databases for orthologues of OeABC retrieved a range of highly conserved sequences, from
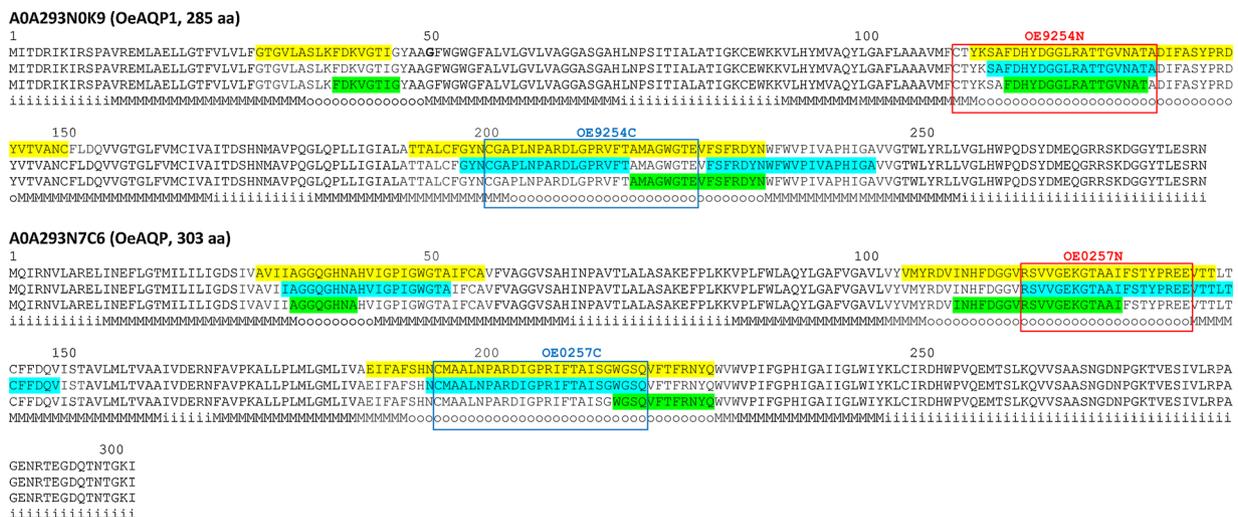
Fig. 2. Linear B-cell epitope predictions for the extracellular domains of OeAQP1 and OeAQP. The sequence of each protein is represented in triplicate, showing the ABCpred (yellow), BCEpred (blue) and BepiPred-2.0 (green) predictions. Amino acids with antigenicity predictions made by at least two of 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.

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	CHDVTEGGDNISVGQRQLVC	2,130
OeSEL	OE7237/1	19	<u>C</u>RPTPSFVAWMLNNKLYS<u>C</u>	2,230

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 (Supplementary Fig. 2A) showed a short, variable aminoterminal region (residues 1–390) and a long, highly conserved carboxiterminal region (residues 399–1518), which included the two ABC membrane domains and nucleotide binding domains that are characteristic of the full ABC transporters (Dermauw and Van Leeuwen, 2014).

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

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

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

3.3. Selenoproteins: selected candidate and designed peptide

Five transcripts annotated as selenoproteins were found in the *O. erraticus* midgut transcriptome, and three of them were upregulated upon feeding (Table 2). Among them, the transcript with the highest fold-change (6.285) was also the most antigenic (Vaxijen score, 0.7096). This transcript, A0A293LXQ0_ORNER, encoded a selenoprotein T that was 111 amino acids long, which was selected as a candidate and termed OeSEL.
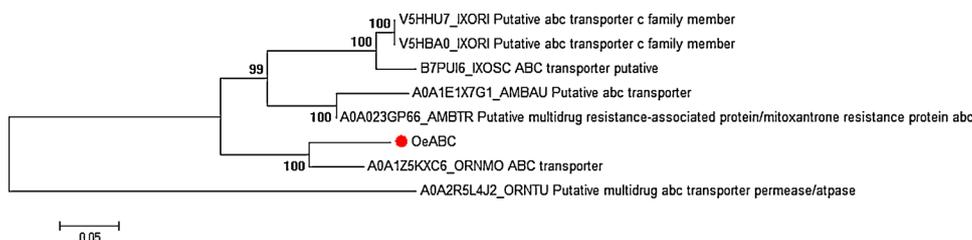
The top 10 tick orthologues for OeSEL, with E values $< 10^{-60}$ and sequence identities > 80%, were obtained from the Uniprot and NCBInr databases. Multiple alignments of all these sequences and OeSEL showed very high conservation throughout the whole OeSEL sequence, including the fragment where the designed 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, D). 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, C, E). 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–I). Lastly, the sera from rabbits immunised with peptide OE7237/1 (group 3, anti-OeSEL)

Fig. 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. A0A1E1 × 7G1_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.

Fig. 4. Partial amino acid sequence of the OeABC protein, showing its carboxi 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.

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, L). 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–J).

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 non-specific 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, D).

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

A

B

Fig. 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*; A0A131 × 9Z0_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.

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 multispanning 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–J), 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, L). Accordingly, the tick bites did not boost the vaccine-induced humoral responses either (Fig. 6A–J), indicating that natural tick-host contacts would not serve as boosting antigen doses in immunised hosts.

Fig. 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 ± 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.

The immune sera to OeABC and to OeAQPs recognised protein bands compatible with their respective native protein targets on the membrane proteins of *O. erraticus* ticks (Fig. 7B). Although the identity of these bands as OeABC and OeAQPs was not confirmed here by mass spectrometry, they have already been identified in the midgut proteome of *O. erraticus* by LC-MS/MS from gel fragments containing these re-active bands (Oleaga et al., 2015). Accordingly, the coincidence in molecular weight, the high specificity of the immune sera (Fig. 6), and the previously demonstrated presence of these proteins in the midgut proteome of *O. erraticus* led us to assume those 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), an-ticipating the possibility of some cross-protection between these spe-cies.

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 home-ostasis 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 chal-lenging 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 can-didate. Although this hypothesis needs to be demonstrated, the high number of ABC transporter family members that have been found in the
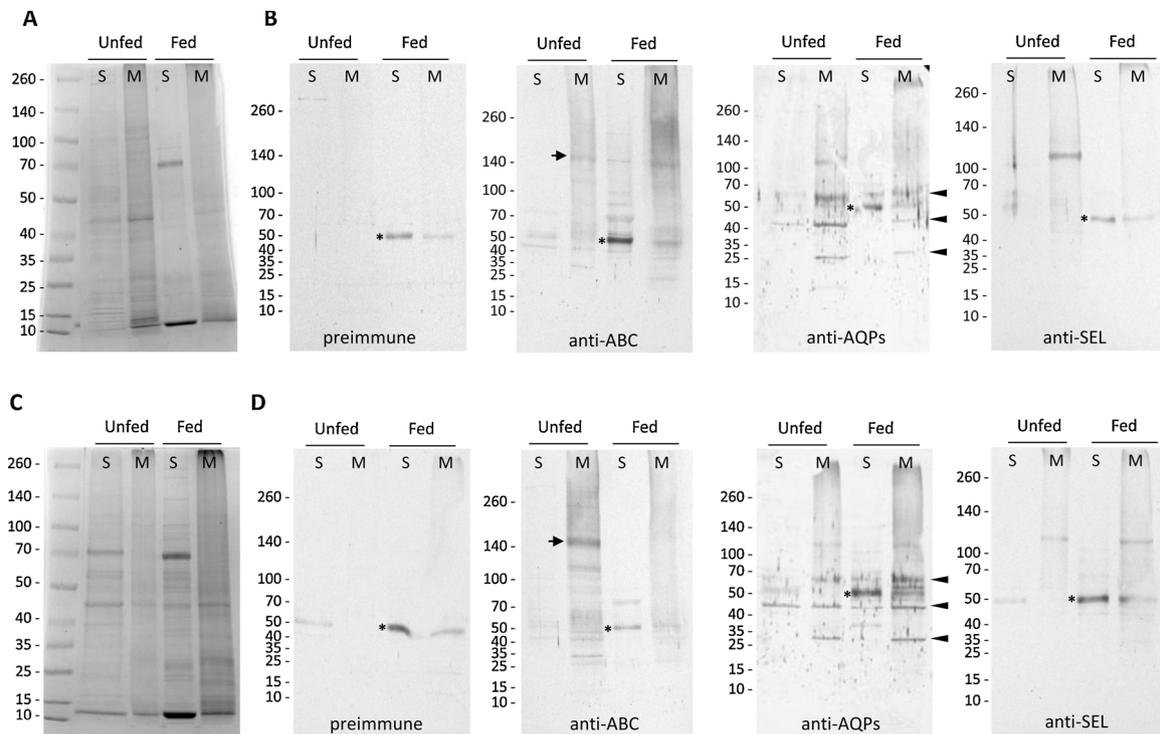
Fig. 7. **(A, C)** Coomassie Blue-stained 5–20% SDS-PAGE gels showing the soluble (S) and membrane (M) proteins of the midgut from *Ornithodoros erraticus* (**A**) and *Ornithodoros moubata* (**C**) female ticks taken before feeding (Unfed) and 48 h after engorgement (Fed). **(B, D)** Western blots: 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.

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

[*]p < 0.05; [**]p < 0.01.

O. erraticus mialome 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, D). 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 el 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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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2019.06.016.

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