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ORIGINAL ARTICLE

Evaluation of vaccine candidates purified from the adult ticks of *Ornithodoros savignyi* (Acari: Argasidae) and *Hyalomma dromedarii* (Acari: Ixodidae) against tick infestations

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Abstract Ticks cause anemia, toxicosis, growth delay, and transmit infectious diseases in animals and humans. The current study aimed to evaluate the immunoprophylactic properties of two vaccine candidates to develop vaccine against tick infestations. These two vaccine candidates were specific fraction from the adults of the soft tick *Ornithodoros savignyi* and cross-reactive fraction from the adults of the hard tick *Hyalomma dromedarii*. Both specific and cross-reactive fractions were isolated by Cyanogen Bromide-activated Sepharose-4B affinity column chromatography. Both candidates proved their cross-reactivity by enzyme linked immunosorbent assay and Western blot. Characterization of the two vaccines by SDS-PAGE showed that the *O. savignyi* specific fraction consists of four bands; 97, 85, 66 and 11.5 kDa compared with nine bands associated with its crude antigen (196–11.5 kDa). The *H. dromedarii* cross-reactive vaccine candidate consists of three bands; 97, 66 and 45 kDa compared to eight bands of its crude antigen (196–21 kDa). Two common bands of 97 and 66 kDa between two candidates showed immunogenic cross-reactivity with the developed antisera of both infestations by Western blot. Immunization of rabbits intramuscularly with two doses of the fractions separately (40 µg/kg) led to immunological and parasitological changes. Immunologically; the level of immunoglobulins in vaccinated rabbits increased significantly compared with control infested non-vaccinated rabbits. These immunoglobulins are probably responsible for the protective effect of both candidates. Parasitologically, immunized rabbits showed protection against infestation by adult ticks as proved by significant feeding rejection percentage and significant reduction in egg and engorgement weights of *H. dromedarii*. While insignificant protection was observed against *O. savignyi* ticks infestation in feeding rejection and reduction in engorgement weight. In conclusion, this study suggests promising immunoprophylactic potentials of the purified fractions against tick infestations in rabbits through induction of IgG responses. The protective effect of both vaccine candidates deserves further evaluation in other hosts and against other tick infestations.

Keywords Cross reactive antigen · *Hyalomma dromedarii* · Purified antigen · *Ornithodoros savignyi* · Vaccination

Introduction

Ticks are obligate ectoparasites on mammals, birds and reptiles. They include two main families, the Argasidae and the Ixodidae (Latif et al. 2012; Manzano-Román et al. 2012). Ticks are of a great medical and veterinary importance. They cause anemia, toxicosis, growth delay, and transmit infectious diseases that affect animals and humans (De la Fuente et al. 2008; Manzano-Román et al. 2012).

The soft tick *Ornithodoros savignyi* is a vector of pathogens affecting cattle and camels worldwide (Peter et al. 2005; Vial 2009). During the feeding, the tick secretes toxins that might kill animals, especially young calves and lambs (Manzano-Román et al. 2012). The hard

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tick *Hyalomma dromedarii* is the most arthropod vector of pathogens such as viruses, bacteria (including rickettsiae and Q fever) and protozoa (Montasser 2005; Gunes 2006; Loftis et al. 2006; Abdel-Shafy et al. 2012; Hassan et al. 2017; Abdullah et al. 2018), some of which are zoonotic. In tropical and subtropical countries, tick infestations led to losses in the animal production (Jongejan and Uilenberg 2004; Rajput et al. 2006). Billions of dollars are annually lost due to tick infestations and their pathogens (Jongejan and Uilenberg 2004).

The extensive acaricide applications in the controlling of ticks led to the development of resistance in ticks against these acaricides and increase the pollution in the environment (Rodriguez-Vivas et al. 2011). Therefore, search for eco-friendly alternatives to acaricides becomes an important issue. Vaccination is one of the most attractive alternatives for control of ticks and tick-borne diseases (De la Fuente et al. 2007a, b, 2011). The immune responses to different tick antigens may increase weight loss or mortality in eggs and adults of ticks, prolong feeding period, and inhibit molting (Trimnell et al. 2002). The TickGARD© and GAVAC© are commercial vaccines against ixodid ticks in Australia and Southern America, respectively (Willadsen 2008). The Bm86 derived from a midgut of *Rhipicephalus* (*Boophilus*) *microplus* is also available as a commercial vaccine (De la Fuente et al. 2009). Globally, there were many trials for vaccine preparations from a number of new antigens. Some of them failed (Antunes et al. 2015) and others succeeded to obtain attractive candidates those need further evaluations (Rodríguez-Mallon et al. 2012; Toaleb et al. 2013). There are limited vaccine candidate antigens prepared from ixodid ticks (Guerrero et al. 2012; Parizi et al. 2012; Merino et al. 2013). Otherwise, the evaluated candidate vaccine antigens for argasid ticks are lower than for ixodid ticks (Vidarsson et al. 2014).

The selected vaccine candidate has to be active against different tick species and their multiple stages. In the present study, two isolated protective common antigens between the soft tick *O. savignyi* and the hard tick *H. dromedarii* were evaluated as vaccine candidates against experimental challenge with *H. dromedarii* and *O. savignyi* in rabbits.

Materials and methods

This study was carried out in the laboratories of the Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Centre and the Animal Acarines Research Center, Department of Zoology and Agricultral Nematology, Faculty of Agriculture, Cairo University, Egypt during the summer of 2017.

Rabbits

Twenty-six healthy black German male rabbits (weight 2.5 kg) were used in the current study, from which 2 rabbits were used for preparation of anti-*O. savignyi* antibodies (RAOsA) and 24 rabbits were used in vaccination protocol. These rabbits were purchased from a private farm in Cairo. They were kept 1 week before injection with the vaccine candidates. Rabbits were maintained in the animal house of the Animal Acarines Research Center, Department of Zoology and Agricultral Nematology, Faculty of Agriculture, Cairo University.

Camels

Blood samples of heavily infested camels by *H. dromedarii* were collected from camels intended for slaughtering at the main abattoir of Nahia, Giza. Serum samples were separated and considered as positive camel sera.

Ticks

Two tick species were used in the experiment. The first one is the sand tampan tick *O. savignyi* (Audouin, 1827) (Acari: Argasidae) as an argasid tick species. The second one is the camel tick *H. dromedarii* Koch, 1844 (Acari: Ixodidae) as an ixodid tick species. *O. savignyi* was collected as nymphs and adults from the ground of camel market in Shalateen, Egypt. It was identified by using taxonomic key of Walker et al. (2003).Whereas, *H. dromedarii* ticks was collected as engorged nymphs from camel market, Burkash village, Giza Egypt. The engorged nymphs were incubated at 27 ± 2 °C, 75 ± 5% RH and a permanent darkness until they moullt to unfed adults (males and females). The unfed adults of *H. dromedarii* were identified based on the taxonomic keys (Walker et al. 2003).

Antigens preparation

The two crude extracts of ticks were prepared by the following procedures of Ghosh and Khan (1999). The two unfed adult ticks were collected and washed several times and homogenized separately in 0.15 M PBS, PH 7.2. The homogenates were then sonicated in ice, then centrifuged at 15,000 rpm for 60 min at 4 °C. The supernatants were used as antigens and their protein contents were estimated by Lowry et al. (1951).

Preparation of rabbit anti-*O. savignyi* antibodies

Two rabbits were infested by two hundreds of adults *O. savignyi* ticks (one hundred adults for each rabbit). After 6 days of ticks' attachment, blood samples were collected

from slaughtered rabbits and centrifuged at 2000 rpm for 15 min. RAOsA-sera were collected and stored at − 20 °C till use.

Affinity purification of adult *O. savignyi* and *H. dromedarii* antigens

The prepared rabbit anti-*O. savignyi* antibodies (RAOsA) was dialyzed against 100 mM NaHCO buffer, pH 8.3. Then, it coupled to CNBr-activated Sepharose-4B by strictly following the manufacture instructions. Then crude *O. savignyi* and crude *H. dromedarii* antigens were separately placed in a column as well as bound fractions were eluted using 50 mM glycine that contained 500 mM Nacl. The protein contents of specific *O. savignyi* fraction and the cross-reactive *H. dromedarii* were estimated by the method of Lowry et al. (1951).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

The two crude extracts; crude *O. savignyi*, crude *H. dromedarii* and two their fractions; specific fraction, cross reactive fraction were separately electrophoresed on 10% SDS-PAGE according to Laemmli (1970). The slab gel was colored by silver stain after separation as procedures of Wray et al. (1981). On the same gel, molecular weight standards (Sigma) were electrophoresed to compute the relative molecular weights of the antigens. Gel was analysis by Molecular Imager Gel Doc^{TM} XR+ with Image Lab Software. Atomic weight benchmarks (Sigma) were electrophoresed on a similar gel to compute the relative sub-atomic weights of the antigens.

Vaccination protocol in rabbits

Twenty-four healthy rabbits were divided into four main groups (Table 1). The first group (six rabbits) was immunized with saline (negative control). The second group (six rabbits) was infested but not vaccinated (positive control); three rabbits were infested by *O. savignyi* (subgroup A) and the other three rabbits were infested by *H. dromedarii* (subgroup B). The third group (six rabbits) is divided into two equal subgroups (C and D). The fourth group (six rabbits) is also divided into two equal subgroups (E and F). Rabbits in the third group were immunized with *O. savignyi* specific fraction and rabbits in the fourth group were immunized with *H. dromedarii* cross-reactive fraction. Rabbits in subgroups C and E were infested by *O. savignyi* while rabbits in subgroups D and F were infested by *H. dromedarii*. Rabbits' immunization was intramuscularly injected twice at 2 week intervals, with 40 µg/kg of the two fractions independently blended with equivalent volume of complete Freund adjuvant (Sigma, USA) in the first dose and with incomplete Freund adjuvant in the second dose. Twenty adult ticks of *H. dromedarii* (10 females and 10 males per rabbit) also twenty of *O. savignye* (20 adults per rabbit). They placed inside two capsules on each rabbit as previously described by Szabó and Bechara (1997). The tick feeding was monitored daily to observe the development of ticks. Rabbits were bled before immunization and at 1 week intervals post immunization from the marginal ear vein to collect blood samples and all rabbits were necropsied 2 weeks post challenge to determine IgG level.

Table 1 The distribution of rabbits through main groups and subgroups in the experiment of vaccination

Item	Rabbit number	Details
Group 1	6	Immunized with saline (negative control)
Group 2		Infested by ticks but not vaccinated (positive control)
Sub-group A	3	infested by *O. savignyi*
Sub-group B	3	infested by *H. dromedarii*
Group 3		Immunized with *O. savignyi* specific fraction
Sub-group C	3	Infested by *O. savignyi*
Sub-group D	3	Infested by *H. dromedarii*
Group 4		Immunized with *H. dromedarii* cross-reactive
Sub-group E	3	Infested by *O. savignyi*
Sub-group F	3	infested by *H. dromedarii*
Total rabbits	24	

Enzyme linked immunosorbent assay

This assay was carried out to define the activity of the two eluted specific bound fractions compared with their crude extracts and unbound fractions. The assay also evaluated the IgG level raised in rabbits vaccinated with the two eluted specific bound fractions at different intervals of vaccination and challenge with two species of ticks according to Engvall and Perlmann (1971). Enzyme linked immunosorbent assay (ELISA) plates were coated, independently, by fractions in carbonate buffer. Unvaccinated infested rabbit serum samples (control positive group), vaccinated infested rabbit serum samples and non-infested non-vaccinated rabbit serum samples (control negative group) were added to the coated plates independently. Protein A horse radish peroxidase labeled-conjugates and anti-rabbit IgG horse radish peroxidase labeled-conjugate were used based on the host serum species. Ortho-

phenylenediamine substrate buffer (Sigma) was added and the plates were read spectrophotometrically at 450 nm.

Immunoblot analysis

After electrophoresis, two slab gels (each one contains the eluted fractions and crude extracts together) were blotted onto two nitrocellulose membranes according to Towbin et al. (1979). After washing and blocking, the first membrane was incubated with antiserum of experimentally infested rabbit by *O. savignyi* diluted 1:200. The 1:1000 dilutions of horse radish peroxidase-conjugated anti-rabbit IgG were used. The second membrane was incubated with antiserum of naturally infested camel by *H. dromedarii* diluted 1:200. Protein A was used at 1:2000 dilution. Membranes were revealed by adding 4-chloro-1-napthol solution. Membranes were photographed by Molecular Imager Gel Doc^{TM} XR+ with Image Lab Software.

Statistical analysis

The means and their errors of biological parameters for the *H. dromedarii* and *O. savignyi* ticks fed on the experimental rabbits were calculated. Statistical analyses between the groups of the challenge experiment were estimated by one-way ANOVA using SPSS (version 20; IBM, USA).

Results

Identification of purified fractions

The purification process of specific and cross reactive fractions was typically summarized in Tables 2 and 3. The initial antigenic activities in *O. savignyi* were 90.91% recovered in the specific fraction. Although it contains 0.91% of total protein in its crude extract (*O. savignyi*) when applied to the column, giving 27,500 purification fold which increases in the specific activities compared to its crude extract (Table 2). While, 93.97% of cross-reactivities were bound and eluted in cross reactive fraction which represents 0.83% of total protein in crude *H. dromedarii* giving 34,401.32 purification folds and increases in activities compared to its crude extract (Table 3).

Electrophoretic profile of the two vaccine candidates

The electrophoretic profile of isolated two fractions (vaccine candidates) in comparison with their crude antigens showed that the first vaccine candidate specific fraction of *O. savignyi* was resolved in only four bands of molecular weights 97, 85, 66 and 11.5 kDa (Fig. 1, Lane 3) compared to nine bands in its crude extract that located between 196–11.5 kDa (Fig. 1, Lane 2). The second vaccine candidate; cross reactive fraction of *H.*

Table 2 Quantitative summary of purification *O. savignyi* antigen

Fraction	Total protein[a] ($\mu g \times 10^{-4}$)	Activity unit[b] ($Au \times 10^{-6}$)	Specific activity[c] ($Au/\mu g \times 10^{-2}$)	Purification fold	Yield (%)
Purification of species specific fraction from O. savignyi					
Crude Os	45.1	9.02	0.2	1.0	100
Unbound to RAOsA-column	24.2	0.537	0.022	0.205	5.95
Bound and eluted specific fraction	0.410	8.2	20	27,500	90.91

[a]Protein was measured as described by Lowry et al. (1951)

[b]A unit of activity defined as the amount of protein required to give one well of agglutination

[c]Specific activity which is the number of activity per μg of protein and is related to the starting crude Os and the starting crude Hd

Table 3 Quantitative summary of purification *H. dromedarii* antigen

Fraction	Total protein[a] ($\mu g \times 10^{-4}$)	Activity unit[b] ($Au \times 10^{-6}$)	Specific activity[c] ($Au/\mu g \times 10^{-2}$)	Purification fold	Yield (%)
Purification of cross-reactive fraction from H. dromedarii					
Crude Hd	37.3	8.3	0.22	1.0	100
Unbound to RAOsA-column	26.4	0.71	0.027	0.173	8.6
Bound and eluted cross reactive fraction	0.31	7.8	25.16	34,401.32	93.97

[a]Protein was measured as described by Lowry et al. (1951)

[b]A unit of activity defined as the amount of protein required to give one well of agglutination

[c]Specific activity which is the number of activity per μg of protein and is related to the starting crude Os and the starting crude Hd

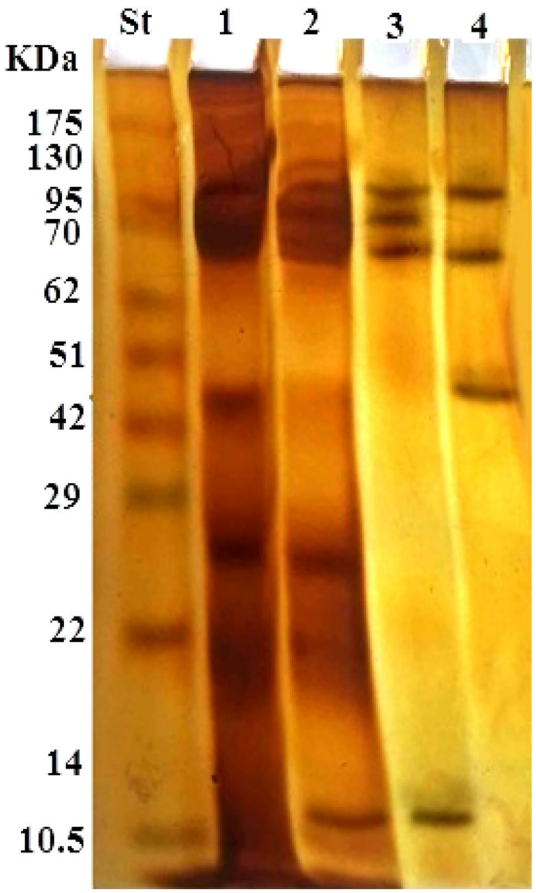

Fig. 1 Electrophoretic profile of the vaccine candidate specific fraction *O. savignyi* (lane 3), the second vaccine candidate cross-reactive fraction *H. dromedarii* (lane 4), crude *H. dromedarii* antigen (lane 1), crude *O. savignyi* antigen (lane 2) and molecular weight standards (lane St)

dromedarii was resolved into three bands of molecular weights 97, 66 and 45 kDa (Fig. 1, Lane 4) compared to its crude extract which resolved in eight bands ranged from 196 to 21 kDa (Fig. 1 Lane 1). There are two common bands between the two fractions which are 97 and 66 kDa. While there are six common bands between their crude extracts with molecular weights 196, 175, 97, 66, 25 and 21 kDa (Fig. 1).

Immunogenic activities

The specific and cross-reactive fractions showed higher potency than their crude antigens in the diagnosis of soft tick infestation in two fold serially diluted rabbit sera infested by *O. savignyi* (Fig. 2) and two fold serially diluted naturally infested camels sera by *H. dromedarii* (Fig. 3) by using ELISA. The diagnostic potency of the fractions was still valid at high dilution of serum samples reached 1:32,768 (Figs. 2, 3).

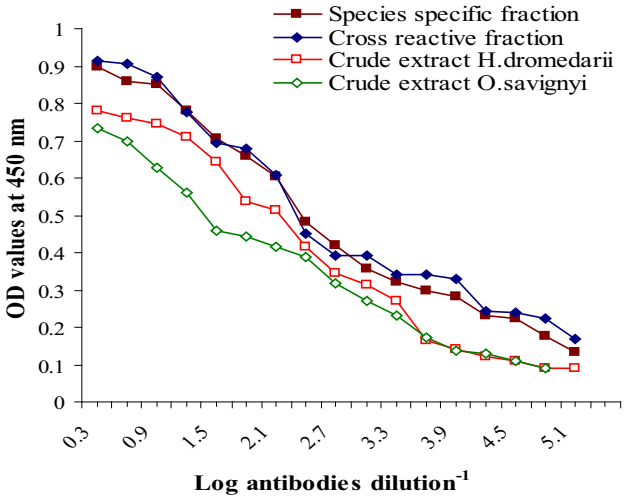

Fig. 2 Levels of IgG response measured by ELISA against both vaccine candidates of specific fraction *O. savignyi* (filled square) and cross reactive fraction *H. dromedarii* (filled diamond) and their crude antigens, crude *O. savignyi* (open square), crude *H. dromedarii* (open diamond) in detection of IgG in experimentally infested rabbit sera by *O. savignyi*

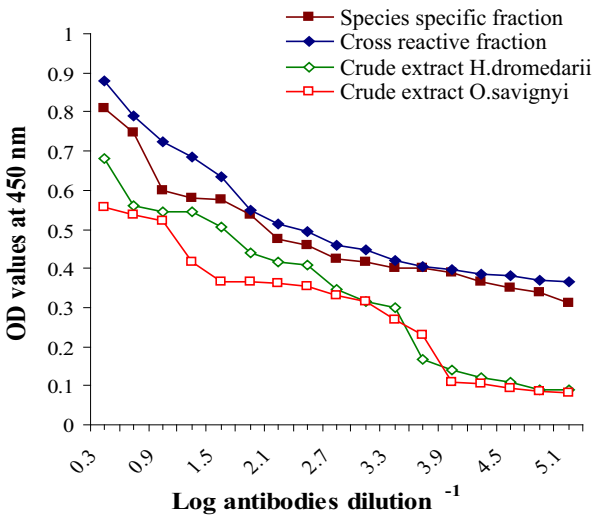

Fig. 3 Levels of IgG response measured by ELISA against both vaccine candidates of specific fraction *O. savignyi* (filled square) and cross reactive fraction *H. dromedarii* (filled diamond) and their crude antigens, crude *O. savignyi* (open square), crude *H. dromedarii* (open diamond) in detection of IgG in naturally infested camels sera by *H. dromedarii*

Protective humoral immune response

The IgG levels in rabbits of the vaccinated groups were higher than that of the control group (Figs. 4, 5). The level of IgG in rabbits vaccinated with specific fraction and challenged with *H. dromedarii* is higher than IgG level in rabbits vaccinated with cross-reactive fraction and

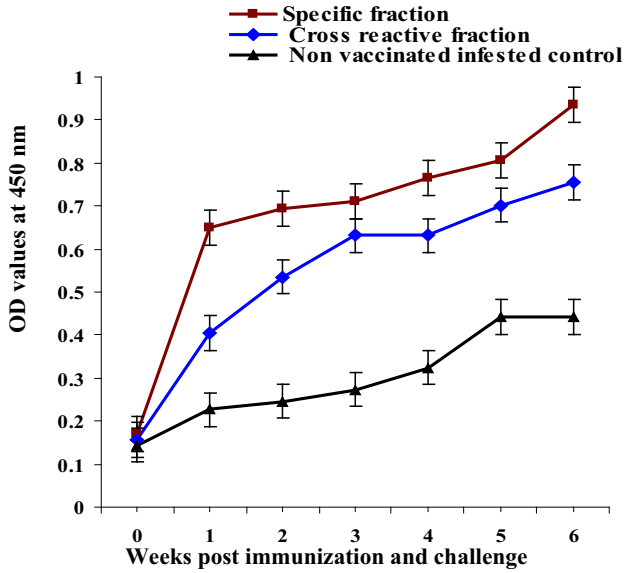

Fig. 4 Antibodies response in rabbits immunized with two vaccine candidates separately, before and after challenge with *H. dromedarii* ticks. Bars in the figure represent mean ± standard error of the optical densities

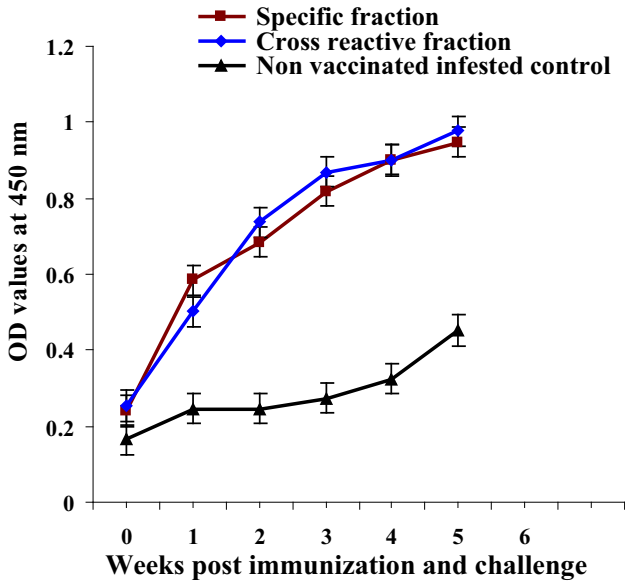

Fig. 5 Antibodies response in rabbits immunized with two vaccine candidates separately, before and after challenge with adult *O. savigenyi* ticks. Bars in the figure represent mean ± standard error of the optical densities

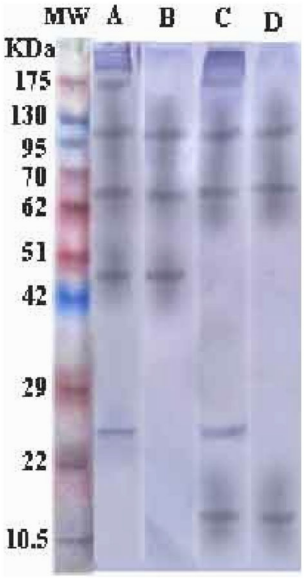

Fig. 6 Immunoreactive bands identified by sera from naturally infested camel by *H. dromedarii* ticks by using immunoblot assay; lane A: crude Hd, lane B: cross-reactive fraction, lane C: crude Os, lane D: specific fraction and MW: Molecular weight standards in KDa

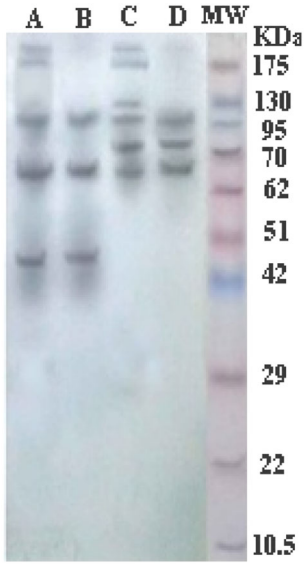

Fig. 7 Immunoreactive bands identified by sera from experimentally infested rabbit by *O. savignyi* ticks by using immunoblot assay; lane A: crude Hd, lane B: cross-reactive fraction; lane C: Crude Os; lane D: Specific fraction and MW: Molecular weight standards in KDa

challenged with the same infestation (Fig. 4). Despite the level of IgG against two fractions increased gradually after the first booster until the date of challenge (on the fourth week) with adult *O. savigenyi*. The cross-reactive fraction is of higher potency than specific fraction starting from the third week post immunization and still enhanced immune response reached the maximum on the fifth week post challenge with *O. savigenyi* ticks in immunized rabbit (Fig. 5).

Immunogenic reactive bands

The recognition of immunogenic bands in two fractions and their crude extracts by sera from natural infested

camels by *H.dromedarii* ticks and sera from experimented infested rabbit by *O. savignyi* adopted by immunoblot assay (Figs. 6, 7). The camel sera reacted strongly with all antigens. Crude extract *H. dromedarii* (Fig. 6; Lane A) revealed many bands at high and low molecular weights 196,175, 97, 66, 45 and 25 kDa. Cross-reactive fraction revealed three reactive bands at molecular weights 97, 66 and 45 kDa (Fig. 6; Lane B). Whereas, crude extract *O. savignyi* reacted with camel sera in five immunogenic bands at molecular weights 175, 97, 66, 25 and 11.5 kDa (Fig. 6; Lane C). While the specific fraction from *O. savignyi* revealed three immunogenic bands at molecular weights 97, 66 and 11.5 kDa (Fig. 6; Lane D). Figure 7 shows immunoreactive bands of crude extract *H. dromedarii* reacted with sera from experimented infested rabbit by *O. savignyi* at molecular weights 196,175, 97, 66 and 45 kDa (Fig. 7; Lane A). Its cross-reactive fraction exhibited three bands at 97, 66 and 45 kDa (Fig. 7; Lane B). While, the *O. savignyi* crude antigen reacted at six immunogenic bands at molecular weights 196,175, 129, 97, 85 and 66 kDa (Fig. 7; Lane C). The specific fraction revealed three immunogenic bands at 97, 85 and 66 kDa (Fig. 7; Lane D).

Comparative performance of ticks after feeding on vaccinated rabbits

Two tick species, one soft tick *O. savignyi* and another hard tick *H. dromedarii* were fed on vaccinated rabbits with the two fractions, specific fraction (group 3) and cross-reactive fraction (group 4) compared with those fed on unvaccinated rabbits (group 2). Low insignificant rejection (6.67%) was recorded in *O. savignyi* fed on rabbits vaccinated with cross-reactive fraction (group 4), while no rejection was observed on *O. savignyi* fed on the rabbits vaccinated with specific fraction (group 3) or unvaccinated control group (group 2). However, both specific fraction (group 3) and cross-reactive fraction (group 4) achieved significant rejection (20%) in feeding of *H. dromedarii* comparing with 3.33% in unvaccinated group (group 2). Feeding period was very short (2–3 h) in *O. savignyi* as all species of soft tick, therefore it is not listed in the Table 4. Moreover, the tested fractions had not any significant effect on feeding period of *H. dromedarii* (subgroups B, D and F). In general, the average of feeding period was ranged between 8.08 and 8.80 days. The percentage in increasing weight of twenty *O. savignyi* adults was insignificantly lower in those fed on vaccinated groups in comparing those fed on unvaccinated group (subgroups A, C and E). Whereas, *H. dromedarii* females fed on vaccinated rabbits with the specific fraction in subgroup D ingested blood significantly lower (0.94 g) than those in control in subgroup B (1.13 g), on the other hand, females fed on vaccinated rabbits with the cross-reactive fraction in subgroup F ingested blood insignificant lower (0.97 g) than those in control group (in subgroup B). Both fractions in subgroups D and F produced significant lower in egg weight of *H. dromedarii* (0.43 g for the specific fraction and 0.50 g for the cross-reactive fraction) comparing with control group (0.58 g) in subgroup B. Reproductive index indicated that the specific fraction in subgroup D is significantly better (RI: 0.45) than the cross-reactive fraction in subgroup F (RI: 0.51), while the latter fraction recorded insignificant RI lower than that of control group in subgroup B (RI: 0.52). In general, the species specific fraction is better than the cross-reactive fraction against the hard tick *H.*

Table 4 Estimation of different biological aspects of the soft tick *O. savignyi* adults and the hard tick *H. dromedarii* females fed on vaccinated rabbits with specific and cross reactive fractions

Tick species	Parameter	Unvaccinated control (group 2)	Vaccinated groups		*F*	*p*
			Specific (group 3)	Cross reactive (group 4)		
		Subgroup A	Subgroup C	Subgroup E		
O. savignyi	Rejection (%)	0.00	0.00	6.67 ± 4.41 (0.00–15.00)	2.286	0.183
	Increase in weight (%)	181.32 ± 13.87 (153.59–195.19)	143.15 ± 11.50 (129.60–166.02)	143.77 ± 9.08 (125.65–153.88)	3.524	0.097
		Subgroup B	Subgroup D	Subgroup F		
H. dromedarii	Rejection (%)	3.33 ± 3.33a (0.00–10.00)	20.00 ± 0.00b (20.00–20.00)	20.00 ± 0.00b (20.00–20.00)	25.000	0.001
	Feeding period (day)	8.80 ± 0.30 (6.00–12.00)	8.67 ± 0.27 (6.00–11.00)	8.08 ± 0.18 (6.00–9.00)	2.046	0.136
	Engorgement weight (g)	1.13 ± 0.05a (0.67–1.44)	0.94 ± 0.06b (0.51–1.49)	0.97 ± 0.04ab (0.59–1.21)	4.335	0.017
	Egg weight (g)	0.58 ± 0.02a (0.30–0.74)	0.43 ± 0.03b (0.20–0.68)	0.50 ± 0.02b (0.29–0.63)	8.803	< 0.001
	Reproductive index (RI)	0.52 ± 0.01a (0.45–0.60)	0.45 ± 0.01b (0.34–0.56)	0.51 ± 0.01a (0.41–0.61)	12.176	< 0.001

Data were presented as Mean ± SE (minimum–maximum)

The different letters (a and b) at the same row indicate the significant difference at $p < 0.05$

dromedarii, while, the cross-reactive fraction is better than the specific fraction against *O. savignyi* (Table 4).

Discussion

It is possible to develop vaccines against infestations by many tick species using highly conserve tick-protective or induced immune cross-reactivity antigens. The active antigens used in vaccination should to be easy to use, cheap to produce and its costs are less than acaricides (Willadsen 2004). The main target of the present study was to evaluate two vaccine candidates isolated from the crude extract of two different types of adult ticks; specific fraction (first vaccine candidate) isolated from *O. savignyi* and cross-reactive fraction (second vaccine candidate) isolated from *H. dromedarii*. The two fractions (vaccine candidates) isolated by immunoaffinity column chromatography with 27,500 and 34,401.32 purification folds than two crude extracts respectively. The success of the purification process was supported by the most antigenic activity was associated with the two pure bound fractions 90.91% and 93.97% specific fraction and cross-reactive fraction respectively.

Electrophoretic profile of the crude antigens (*O. savignyi* and *H. dromedarii*) showed that crude antigens were shared in six common bands at molecular weights 196, 175, 97, 66, 25 and 21 kDa. While their purified specific fractions consist of four bands at molecular weights 97, 85, 66 and 11.5 kDa and cross-reactive fraction was resolved into three bands of molecular weights 97, 66 and 45 kDa. The two purified fractions have two common bands of 97 and 66 kDa. These two common bands of 97 and 66 kDa may be representing the most antigenic activities (90.91% and 93.97%) in the two fractions. In the current study, the immunogenic activities showed that the two purified fractions (vaccines candidates) have high potency to detected anti-ticks (IgG) level of infested animals at high diluted antibodies (1: 32,768).

Immunoblot assay showed that; three major immunogenic reactive bands in cross-reactive fraction were identified by two sera (rabbit sera experimentally infested by *O. savignyi* and camel sera naturally infested by *H. dromedarii*) with molecular weights 97, 66, and 45 kDa. While, specific fraction revealed reactive bands at 97, 85 and 66 kDa against *O. savignyi* infested rabbit serum and 97, 66 and 11.5 kDa reactive bands against *H. dromedarii* infested camel serum. The two common bands of 97 and 66 kDa in the two purified fractions were immunogenic reactive bands against different infested sera and may be they responsible for immunogenic activity.

The vaccinated rabbits with the two purified fractions emulsified in friend's adjuvant separately resulted significant rejection (20%) in feeding of *H. dromedarii* adults comparing with control group. Whereas, low insignificant rejection was recorded in *O. savignyi* adults fed on rabbits vaccinated with cross-reactive fraction, while no rejection was observed on *O. savignyi* fed on the rabbits vaccinated with specific fraction or control group. Both fractions produced significant lower egg weight of *H. dromedarii* comparing with control group. The Engorgement weight percentage of *O. savignyi* adults was insignificantly lower in those fed on vaccinated groups than those fed on control group. *H. dromedarii* females fed on vaccinated rabbits with the specific fraction ingested blood significantly lower. Females fed on vaccinated rabbits with the cross-reactive fraction ingested blood insignificant lower than those in control group. In general, the specific fraction is better than the cross-reactive fraction against the hard tick *H. dromedarii*, while, the cross-reactive fraction is better than the specific fraction against *O. savignyi*. We can say the two bands 97 and 66 kDa in the two purified fractions may be responsible for protection of animals from the infestation of different species of ticks. Our current study was confirmed by Trimnell et al. (2005) who indicated that the potential of 64TRPs was a broad-spectrum anti-tick vaccine. It provided cross-protection against *Rhipicephalus sanguineus* and *Ixodes ricinus*. Additionally, The 94 kDa prepared from the cement of the tick *R. appendiculatus* cross-reacted with the two *Rhipicephalus* tick species *R. pulchellus* and *R. evertsi* (Shapiro et al. 1989). Hence, Trimnell et al. (2002) reported that the cross-reactivity studies can facilitate identification of anti-tick vaccine candidates, including potential effective against both immature and adult tick stages. Also Contrerasa and de la Fuentea (2016) confirmed that Q38 is a universal candidate against more than one tick species those infest a single host. Furthermore, *Boophilus microplus* Bm86-based vaccine appeared cross-protection against the other *Boophilus* spp. and partial-protection against *Hyalomma* spp. and *Rhipicephalus* spp. (Fragoso et al. 1998; De la Fuente et al. 2009). In the present study, probably the band 45 kDa in the cross-reactive fraction may be induced the protective effect on rabbits infested by *O. savignyi*. Where this band a 45-kDa previously, was detected as protective antigen, and partly purified from the midgut membranes of *Ornithodoros erraticus* (Oe45), which show antigenic cross-reactivity (Manzano-Román et al. 2007). Furthermore, may be similar the molecule *Ornithodoros moubata* (Om44) provides protective responses, and suitable targets for anti-tick vaccines (Garcîa-Varas et al. 2010). In the same time, the specific fraction has the band 85 KDa which may be play a role in protection against hard ticks; whereas, the TickGARD© and GAVAC© are commercial vaccines against ixodid tick *Rhipicephalus microplus* based on the antigen Bm86 in

Australia and Southern America, respectively (Willadsen 2008). The protective effect of the isolated fraction (two vaccine candidates) is resulting from immune response of the animals towards these vaccine candidates. So in the current study, we found that the humoral immune response due to the two vaccines candidates in immunized rabbits was higher than that in non-vaccinated infested rabbits. The level of IgG antibodies increased in immunized rabbits until 6 weeks post challenge. The cross-reactive fraction induced higher humoral response than specific fraction after vaccination and challenge with the soft tick *O. savigenyi*. Our results agreed with Trimnell et al. (2002, 2005) who demonstrated that a potent humoral immune response, enhanced by tick challenge, using 64TRPs. Overall, these results confirm that specific fraction and cross-reactive fraction able to induce protective immune responses and cross-reactivity between two types of ticks (soft and hard ticks). Trimnell et al. (2005) showed that the ixodid ticks attaching to the host skin by the cement for feeding. Therefore, it can be used as a potential anti-tick vaccine candidate against different species/stages of ticks. In subsequent the recombinant SUB/AKR also revealed antigen-specific antibodies on ixodid ticks, argasid ticks, mosquitoes, sand flies, poultry red mites and sea lice (Carpio et al. 2011; Moreno-Cid et al. 2013).

Conclusion

The vaccine candidates of the argasid ticks are lower than that of ixodid ticks. Thus, it is essential to recognize novel tick protective-antigens to develop successful anti-tick vaccines. The current study showed that immunization with the two purified fractions successfully utilized in eliciting the humoral responses and significant protection against *O. savignyi* and *H. dromedarii* infestation, resulted in the partial inhibition of blood feeding, rejection (%), engorgement weight (g), egg weight (g) and reproductive index (RI). Finally the cross reactive fraction has effect as antigen specific antibodies on hard and soft ticks. This study introduces new antigens as vaccine candidates for different tick species. The two candidates deserve further investigations to evaluate different other vaccination protocols, with other adjuvant and their protective effect in other hosts.

Author contributions All authors participated in the study design. NIT and SA collected ticks and blood samples. NIT and EHA performed affinity chromatography, ELIZA, SDS-PAGE and western blot assays. NIT, HGS and SA shared in the rabbit-vaccine experiment. NIT and SA analyzed, figured and tabulated the data. NIT, SA and EHA participated in writing the manuscript. All authors revised and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was approved to the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals by Medical Research Ethics Committee (No, 17132) at National Research Centre in Egypt.

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