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Effect of recombinant glutathione S-transferase as vaccine antigen against *Rhipicephalus appendiculatus* and *Rhipicephalus sanguineus* infestation

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ABSTRACT

The ticks *Rhipicephalus appendiculatus* and *Rhipicephalus sanguineus* are the main vectors of *Theileria parva* and *Babesia* spp. in cattle and dogs, respectively. Due to their impact in veterinary care and industry, improved methods against *R. appendiculatus* and *R. sanguineus* parasitism are under development, including vaccines. We have previously demonstrated the induction of a cross-protective humoral response against *Rhipicephalus microplus* following vaccination with recombinant glutathione S-transferase from *Haemaphysalis longicornis* tick (rGST-Hl), suggesting that this protein could control tick infestations. In the present work, we investigated the effect of rGST-Hl vaccine against *R. appendiculatus* and *R. sanguineus* infestation in rabbits. *In silico* analysis revealed that GST from *H. longicornis, R. appendiculatus* and *R. sanguineus* have >80% protein sequence similarity, and multiple conserved antigenic sites. After the second vaccine dose, rGST-Hl-immunized rabbits showed elevated antibody levels which persisted until the end of experiment (75 and 60 days for *R. appendiculatus* and *R. sanguineus*, respectively). Western blot assays demonstrated cross-reactivity between anti-rGST-Hl antibodies and native *R. appendiculatus* and *R. sanguineus* GST extracts from ticks at different life stages. Vaccination with rGST-Hl decreased the number, weight, and fertility of engorged *R. appendiculatus* adults, leading to an overall vaccine efficacy of 67%. Interestingly, histological analysis of organ morphology showed damage to salivary glands and ovaries of *R. appendiculatus* adult females fed on vaccinated animals. In contrast, rGST-Hl vaccination did not affect *R. appendiculatus* nymphs, and it was ineffective against *R. sanguineus* across the stages of nymph and adult. Taken together, our results show the potential application of rGST-Hl as an antigen in anti-tick vaccine development, however indicating a broad difference in efficacy among tick species.

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1. Introduction

Ticks are hematophagous ectoparasites that, in addition to the direct harm caused to their hosts, are often responsible for pathogen transmission [1]. Among these species, *Rhipicephalus appendiculatus* and *Rhipicephalus sanguineus* cause great impact on animal health, resulting mainly from disease transmission [2,3]. *R. appendiculatus* occurs in eastern, central and southern Africa region [4], while *R. sanguineus* has a worldwide distribution; however, the taxonomical status of *R. sanguineus* is under revision since the name *R. sanguineus* has been applied for different *Rhipicephalus* spp. tick populations [3,5].

Tick feeding induces host immune responses that build resistance against future tick infestations [6], and this observation led to the hypothesis that a strategy to modulate host immunity could be used to control tick infestation. There is currently a broad

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consensus that effective anti-tick vaccines could constitute a sustainable approach for the control of ticks and tick borne diseases [7], avoiding the deleterious effects associated with acaricide application. Due to the presence, under field conditions, of multiple tick species, and the need to simplify commercial production of an effective vaccine, there is a pressing demand to identify and evaluate antigens offering heterologous protection [8–10]. For some candidate antigens, however, very low or absent effectiveness against other tick species were observed [11,12].

Glutathione S-transferases (GSTs) are proteins found in all eukaryotes; they are responsible for elimination of toxic substances by catalyzing a nucleophilic conjugation reaction of reduced glutathione with the toxic compound [13]. In the ticks *R. sanguineus* and *Rhipicephalus microplus,* GSTs are involved in permethrin detoxification [14] and resistance to synthetic pyrethroid [15], respectively. Furthermore, *R. microplus* GST plays a role in egg antioxidant defenses, as indicated by its effects on redox balance of these cells, and by a positive correlation with lipid peroxidation [16]. Recombinant *Haemaphysalis longicornis* GST (GST-Hl), used in vaccination trials as single- [17] or multi-antigenic vaccines [18], induced a partial protection against *R. microplus* infestation. The protective capacity of GST-Hl was also tested against *R. microplus* using GST-Hl-expressing *Babesia bovis* as a live vector vaccine [19]. Moreover, a multi-antigenic vaccine composed of rGST-Hl, recombinant *R. microplus* VTDCE (vitellin degrading cysteine endopeptidase), and recombinant BYC (*Boophilus* yolk cathepsin), reduced *R. microplus* survival in 35–60%, and improved cattle body weight gain in 14% [18]. These data support the use of rGST-Hl in cross-protection vaccination trials against other tick species. In this work, we evaluated the potential of rGST-Hl as a vaccine antigen against *R. appendiculatus* and *R. sanguineus* infestations.

2. Methods

2.1. Ticks and rabbits

The standard *R. appendiculatus* Muguga stocks were maintained at the tick unit of the International Livestock Research Institute (ILRI) for at least forty years [20]. *R. sanguineus* tropical lineage were collected in Rio de Janeiro, Brazil [21], and kept at the Universidade Federal do Rio Grande do Sul (UFRGS). Ticks were fed by experimental infestation on 4-months old New Zealand rabbits (2 kg) in isolated cages, and kept at 28 °C and 85% relative humidity. The experiments were approved and conducted following the guidelines of the Ethics Committee on Animal Experimentation of UFRGS and ILRI.

2.2. In silico analyses

Amino acid sequence predictions, alignment using ClustalW algorithm, and the identity matrix of GSTs amino acid sequences (*R. appendiculatus* AY298732, *R. sanguineus* AGK29895, and *H. longicornis* AAQ74441), were performed using BioEdit software version 7.2.5 [22]. Immunogenic regions of tick GSTs were predicted by Jameson Wolf algorithm [23], using the software Lasergene version 7.0.0.

2.3. Expression and purification of rGST-Hl

rGST-Hl was expressed and purified as previously described [24] in *Escherichia coli* strain D494 (DE). Purification of the soluble fraction containing rGST-Hl was performed by affinity chromatography using GSTtrap (GE Healthcare). Protein concentration was determined by the Bradford method [25].

2.4. Tick tissues extraction

Ovaries, salivary glands and gut of partially and fully engorged *R. appendiculatus* and *R. sanguineus* female ticks were dissected using a stereomicroscope (Stemi DRC; Zeiss) as previously described [17] and lysed by sonication (Qsonica-model Q125) or by maceration, for *R. sanguineus* or *R. appendiculatus* respectively. Ten-days old eggs, whole larvae, and nymphs of both tick species were also macerated in PBS. Tissue homogenates were centrifuged at 16,000*g* for 15 min at 4 °C. For purification of native GST from tissues, 60 μl of glutathione-Sepharose 4B resin were mixed with tissue extracts aliquots (300 μg) and incubated for 10 min at room temperature. Fractions were separated by centrifugation at 10,000*g* for 10 min at 4 °C. The soluble fractions were collected and the resin containing bound native GST was washed 10 times with PBS.

2.5. In vitro cross-reactivity evaluation

The cross-reactivity of anti-rGST-Hl antibodies with native GSTs from *R. appendiculatus* and *R. sanguineus* was evaluated by western blotting. The total amount of native GST obtained by purification from each of the different tissues of *R. appendiculatus* or *R. sanguineus* (see Section 2.4) was resolved by 14% SDS-PAGE and transferred to nitrocellulose membranes [26]. Membranes were blocked with 5% non-fat dry milk in PBS for 1 h at room temperature and incubated with anti-rGST-Hl rabbit sera (1:100) for 16 h at 4 °C, followed by incubation with anti-rabbit IgG alkaline phosphatase conjugate (1:5000) for 2 h at room temperature. Detection was performed with NBT (nitro blue tetrazolium, Thermo Scientific; 0.3 mg/mL) and BCIP (5-bromo-4-chloro-3-indolyl phosphate, Fermentas; 0.15 mg/mL) in alkaline phosphatase buffer (tris-HCl 100 mM, NaCl 100 mM, and $MgCl_2$ 5 mM).

2.6. Vaccination trial

Fourteen New Zealand rabbits were divided in 4 groups for immunization experiments and tick infestation challenge as follows: *R. appendiculatus* vaccine group (rabbits GST-1Ra, GST-2Ra, and GST-3Ra) and control group (rabbits PBS-1Ra, PBS-2Ra, and PBS-3Ra); and *R. sanguineus* vaccine group (rabbits GST-4Rs, GST-5Rs, GST-6Rs and GST-7Rs) and control group (rabbits PBS-4Rs, PBS-5Rs, PBS-6Rs, and PBS-7Rs). Rabbits in vaccine groups were subcutaneously immunized (needle length of 2.5 cm) in the back with 200 μg of rGST-Hl (0.5 mL) emulsioned with Marcol/Montanide (0.5 mL) in 3 doses at 14-day intervals, a protocol similar to bovine vaccination experiment reported by Parizi et al. [17]. Control groups were injected with PBS (0.5 mL) plus Marcol/Montanide (0.5 mL). For *R. appendiculatus* challenge, each rabbit was infested with 190 nymphs placed in a chamber on one ear, and 60 adults (30 males and 30 females) in another chamber on the other ear. For *R. sanguineus* challenge, two control and two immunized rabbits were infested with 40 adults (20 male and 20 female) each, while the other two control and two immunized rabbits were infested with 80 nymphs each. Ticks of each instar were placed in separate chambers fixed on the back of the rabbits. Blood samples were collected at each inoculation and post-infestation days. Serum was separated by centrifugation at 5000*g* for 10 min and stored at −20 °C for further analysis. Tick number, weight, molt, egg laying, and hatch rates were analyzed.

2.7. Serological assays

Rabbit humoral immune responses were evaluated using an ELISA protocol previously published by Ali et al. [27], with slightly modified washing and blocking steps, which in our study were

performed in 0.05% PBS-tween. Sera from experimental and control groups were used as primary antibodies. Anti-rabbit IgG alkaline peroxidase conjugate (1:5000) was used as secondary antibody. Absorbance at 492 nm was measured in a microplate spectrophotometer (Spectramax Microplate Reader, Molecular Devices Corporation). Serum titration of vaccinated rabbits was evaluated by the same ELISA protocol. The titer was defined as the dilution of post-immunization serum that showed an optical density (OD) more than two times the OD of pre-immune serum.

2.8. Histology

Tissues of fully engorged *R. appendiculatus* females fed on rabbits during vaccination trial were collected for histology. A total of 9 ticks per group (3 ticks from each rabbit of vaccine and control groups) were dissected and tissues fixed in 4% paraformaldehyde/10% formalin and dehydrated in ethanol series at 15 min intervals. The resin blocks were sectioned at 3 μm on Leica RM2255 microtome, followed by staining with haematoxylin and eosin (HE), according to [28]. Slides were air-dried, mounted in Canada balsam, and photodocumented.

2.9. Statistical analysis

Vaccine efficacy was estimated based on the difference in number and weight of fully engorged females, egg laying capacity, egg fertility, and molting, between ticks fed on vaccinated or non-vaccinated rabbits. The overall protection was calculated as previously described, taking into account female numbers [29], or alternatively using female weight as parameter, since engorgement was affected by vaccination. Significant differences were identified using *t*-test, both for physiological parameters of ticks fed in vaccinated versus control groups, and for ELISA assays.

3. Results

3.1. Identity, antigenicity and cross-recognition of GSTs

The predicted amino acid sequences of *R. appendiculatus* and *R. sanguineus* GSTs were found to be 82.5% and 86.9% identical to *H. longicornis* GST, respectively (Suppl. Fig. 1). Protein alignment showed that the three GST sequences possess conserved regions within the primary structures, and *in silico* epitope mapping analysis revealed that most of the conserved regions are also antigenic (Fig. 1). Sera raised against rGST-Hl was able to cross-react with native GSTs purified from *R. appendiculatus* and *R. sanguineus* tissues (Fig. 2); no recognition was observed with pre-immune sera (data not shown). The western blot also revealed that *R. appendiculatus* and *R. sanguineus* GST are expressed in adults in different organs (ovary, salivary glands and gut), and across all different developmental stages: embryonic (egg), larvae, nymph, and adult ticks (Fig. 2).

3.2. Vaccination and challenge trials

IgG levels in rabbit sera from rGST-Hl immunized and control groups were evaluated by ELISA (Fig. 3). After two inoculations, sera from immunized rabbits were able to recognize rGST-Hl,

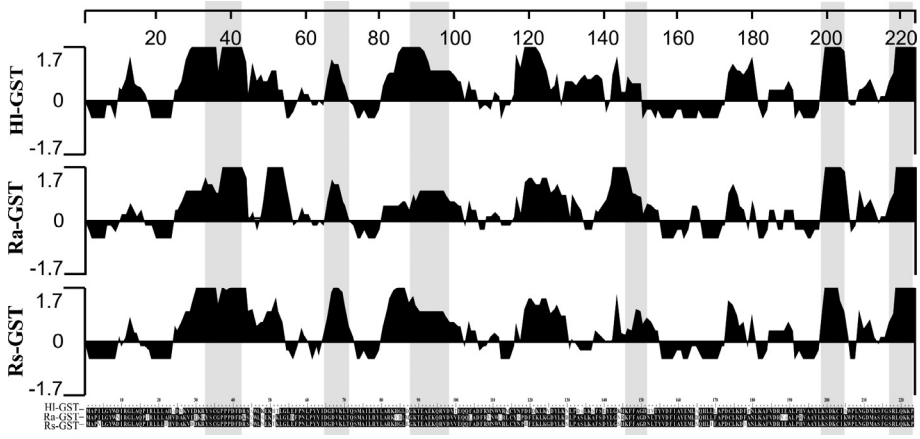


Fig. 1. Comparative analysis of conserved and antigenic regions of *H. longicornis*, *R. sanguineus* and *R. appendiculatus* GSTs. Antigenic index plots for tick GSTs were predicted using the Jameson–Wolf algorithm. Graphic increased positivity shows predicted antigenic sites. The alignment shows conserved regions among GST-Hl, *R. sanguineus* and *R. appendiculatus* GST. Regions with high antigenicity and identity are highlighted in gray boxes.

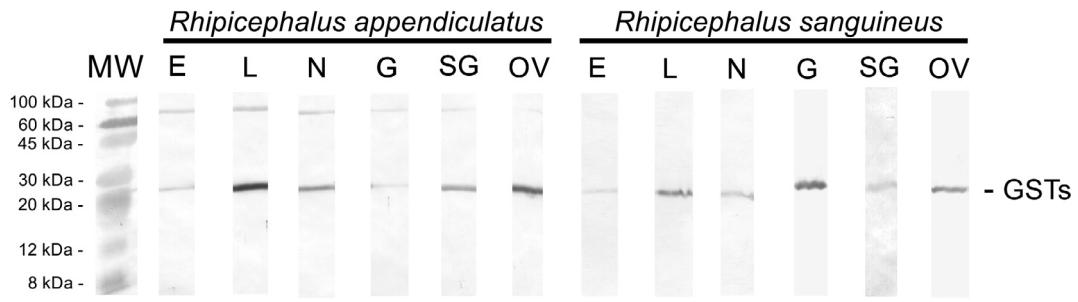


Fig. 2. *In vitro* cross-reactivity between *H. longicornis* and *R. sanguineus* GSTs. Immune sera from rabbit vaccinated against rGST-Hl cross-react with native GST from *R. appendiculatus* and *R. sanguineus* tissues: egg (E), larvae (L), nymph (N), midgut (G), salivary glands (SG) and ovary (OV). The recognition of native GST is indicated by the protein band of molecular weight (MW) 25 kDa.

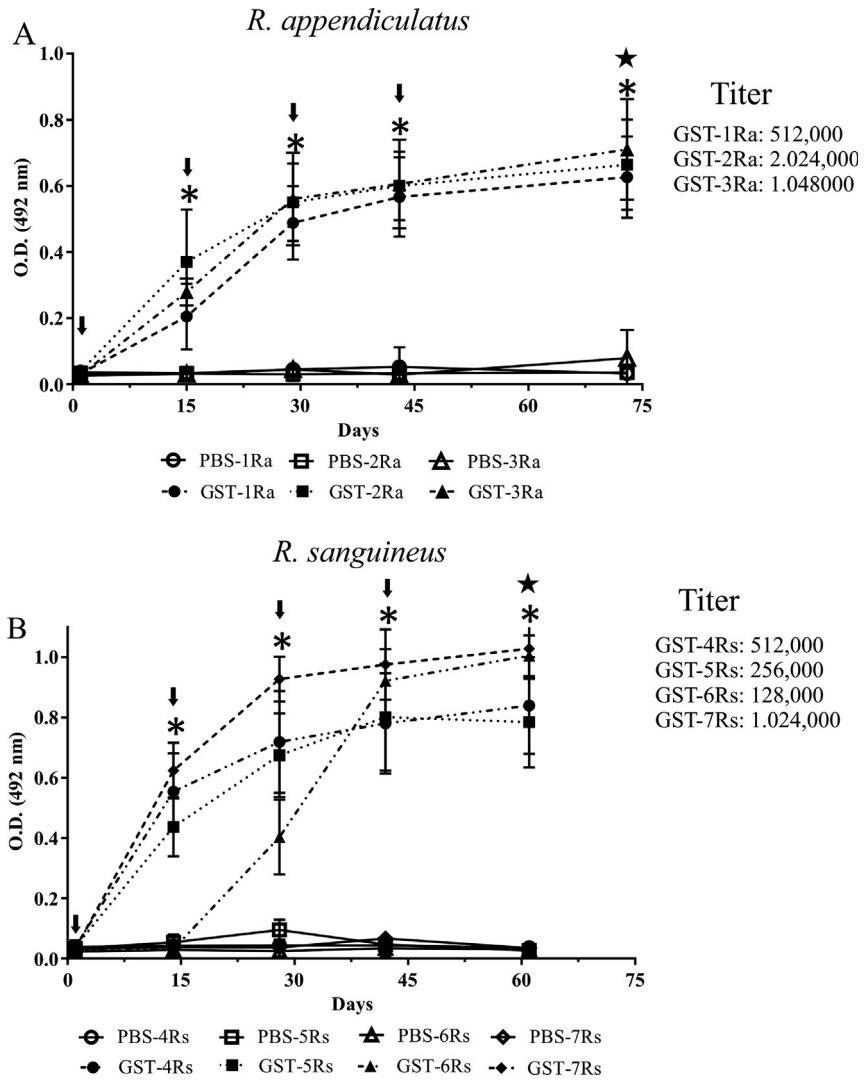


Fig. 3. Humoral immune response of rabbits from vaccinated (GST) and control (PBS) groups analyzed by ELISA. Sera from rabbits infested with (A) *R. appendiculatus* (Ra) and (B) *R. sanguineus* (Rs). The titers from immunized animals are shown on the right. Arrows indicate immunization days. Star indicates the first day of infestation. Asterisks (*) show significant differences between immunized and control groups ($p < .005$).

maintaining stable antibody levels until the end of vaccination experiment. At the time of tick infestation, sera titer to rGST-Hl ranged from 128,000 to 2,048,000 (Fig. 3), showing that rGST-Hl is immunogenic in rabbits.

To evaluate whether the observed humoral response against rGST-Hl was protective, rabbits were infested with *R. sanguineus* or *R. appendiculatus* nymphs and adults. Tick physiological parameters evaluated were: number and weight of replete ticks recovered, fecundity, and molting success of nymph (Table 1: *R. appendiculatus* and Table 2: *R. sanguineus*). Vaccinated rabbits challenged with *R. appendiculatus* showed a statistically significant ($p < .05$) reduction in the number of detaching replete ticks (11.5%), and the engorgement weight (22.7%) in females. Moreover, a decrease of 35% in the amount of eggs, and 32.3% in egg hatching rate was observed. The overall protection achieved with rGST-Hl against adult *R. appendiculatus* infestation was 62.7% (based on number of engorged ticks) or 67.1% (based on the weight of engorged ticks). Fig. 4 shows the differences in phenotype and egg production between engorged adult females fed on vaccinated versus control groups, showing that host immunization with rGST-Hl partially disrupted normal blood feeding and egg laying by adult *R. appendiculatus*. Interestingly, no protection against *R. appendiculatus* nymphs was observed in any of the analyzed parameters. Despite the anti-rGST-Hl antibody response produced in immunized rabbits, and the cross-reactivity between heterologous GSTs demonstrated by western blotting, no significant differences in biological parameters were observed for *R. sanguineus* fed on immunized or control rabbits (Table 2).

3.3. Histological analysis

To investigate the effects of rGST-Hl antibodies on histophysiological aspects of *R. appendiculatus* ticks fed on immunized rabbits, tissue specimens were examined. Salivary glands from the control group did not show pathological alterations (Fig. 5a), with cells from salivary gland acini types I, II and III showing normal morphology as described previously [30]. The acini retained their normal rounded shape while the lumen diameter of glandular ducts was equally preserved. In contrast, salivary glands from ticks fed on host immunized with rGST-Hl exhibited severe morphological damage characterized mainly by irregular-shaped acini (Fig. 5b). In addition, extensive cytoplasmic vacuolation of acinar cells, loss of cell limits, and nuclear damage were evident. Besides losing its typically rounded morphology, nuclei exhibited pyknosis. The granular acini also presented disruption in secretory activity,

Table 1
Biological parameters of *Rhipicephalus appendiculatus* fed in rGST-Hl immunized and control rabbits.

Group	*Nymph*					
	Engorged[a]					Weight[b]
Control	95.26% ± 7.31					10.40 ± 0.66
Vaccine	97.72% ± 1.99					9.54 ± 0.52
Reduction	−3.16%					8.27%
	Adult female					
	Engorged females[a]	Female weight[b]	Egg laying[c]	Egg fertility[d]	Vaccine efficacy	
					Number[e]	Weight[f]
Control	97.78% ± 3.85	477 ± 16.68	0.53 ± 0.04	0.29 ± 0.04		
Vaccine	86.67% ± 3.33	373 ± 66.31	0.34 ± 0.08	0.20 ± 0.04		
Reduction	11.36%*	21.68%*	35.03%*	32.67%*	62.75%	67.13%

[a] Percentage of engorged ticks recovered on rabbits (average values ± S.D.).
[b] Average weight (mg) of engorged ticks (average values ± S.D.).
[c] Total weight of laid eggs per total weight of females.
[d] Total weight of larvae per total weight of laid eggs.
[e] Vaccine efficacy = 100 × [1 − (NFE × WE × WL)], where NFE, WE and WL are the number of fully engorged females, egg laying capacity and egg fertility from vaccinated cattle/control cattle, respectively.
[f] Vaccine efficacy = 100 × [1 − (WFE × WE × WL)], where WFE, WE and WL are the weight of fully engorged females, egg laying capacity and egg fertility from vaccinated cattle/control cattle, respectively.
* $p < .05$ (Student's *t*-test).

Table 2
Biological parameters of *Rhipicephalus sanguineus* fed in rGST-Hl immunized and control group rabbits.

	Nymph		
	Engorged [a]	Weight[b]	Moulting[e]
Control	43.12% ± 34.47	3.72 ± 0.01	65.55% ± 17.28
Vaccine	51.25% ± 10.61	3.78 ± 0.13	86.50% ± 11.00
Reduction	−18.60%	−1.61%	−31.96%
	Adult female		
	Engorged females[a]	Female weight[b]	Egg laying[c]	Egg fertility[d]
Control	95.00% ± 7.07	127 ± 0.98	0.44 ± 0.01	0.52 ± 0.03
Vaccine	95.00% ± 7.07	126 ± 3.00	0.46 ± 0.02	0.50 ± 0.07
Reduction	0.00%	0.79%	−5.54%	3.85%

[a] Percentage of engorged ticks recovered on rabbits (average values ± S.D.).
[b] Average weight (mg) of engorged ticks (average values ± S.D.).
[c] Total weight of laid eggs per total weight of females.
[d] Total weight of larvae per total weight of laid eggs.
[e] Rate of molting success.

displaying irregularities in distribution and constitution, which were deduced from cytoplasmic granules strongly stained by eosin.

The oocytes at different stages of development (I-V oocytes) from control group all showed regular morphological characteristics as described previously [31] (Fig. 5c). For ticks fed on rGST-Hl immunized rabbits, alterations in oocytes at early stages of development (types I, II and III) were found, with loss of the characteristic rounded shape and the structure of germinal vesicle (Fig. 5d). Surrounding the entire extent of oocytes IV and V periphery, small vacuoles below the cytoplasmic membrane were observed; they were mostly evident in the area adjacent to the pedicel, which means that this structure allows the absorption of hemolymph components into the oocytes. On the other hand, yolk granulation was preserved in shape and color in oocytes of all stages.

4. Discussion

A number of proteins have been proposed for inclusion as antigens in vaccines against ticks [32]. Some studies went further and have evaluated tick antigens that potentially protect against heterologous tick infestation [8–10], aiming to reduce the number of antigens required in a vaccine to control multiple tick species. Along these lines, the development of a cross-protective vaccine against *Rhipicephalus* spp. could control *R. decoloratus* and *R. appendiculatus* cattle infestation in east Africa, where both species are prevalent [33]. An important step in the selection of antigens with high level of cross-species reactivity is the degree of sequence identity among target species. The presence of conserved antigenic regions is desirable to elicit broad-spectrum protective immune responses [10,34,35]. Based on these premises, we have searched for conserved epitopes between *H. longicornis* GST and its homologues in *R. appendiculatus* and *R. sanguineus*. We found conserved GST sequences in surface-exposed regions, indicating that rGST-Hl and native GSTs from *R. appendiculatus* and *R. sanguineus* contain epitopes able to be bind anti-rGST-Hl antibodies. *In silico* deduced cross-binding was corroborated by western blot analysis showing cross-reactivity between anti-rGST-Hl rabbit sera and native GSTs from *R. appendiculatus* and *R. sanguineus* tissues. Previously, Parizi et al. [17] demonstrated through *in silico* and *in vitro* analysis that antibodies against rGST-Hl are able to bind *R. microplus* GSTs; furthermore, rGST-Hl experimental vaccination cross-protected cattle against *R. microplus* infestation [17,18]. Here, *R. appendiculatus* feeding on rabbits immunized with rGST-Hl had deleterious effects on the ticks blood ingestion, oviposition and egg hatching. Further histological analysis on these ticks showed unusual lesions in the

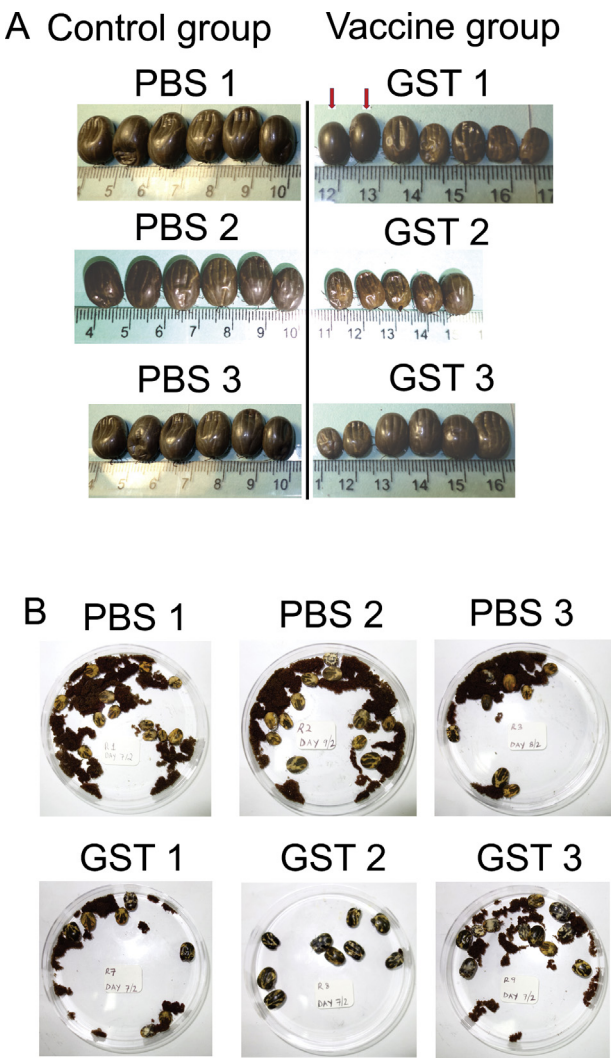


Fig. 4. Phenotypes and egg laying rates in *R. appendiculatus* females affected by rGST-Hl vaccination. Representative examples of female phenotypes (A) and egg mass recovery (B) of engorged adult *R. appendiculatus* from control (PBS) and vaccinated groups (GST). Red arrows indicate dead ticks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ovaries and salivary glands. These effects could result from anti-GST antibodies inhibition of GSTs functions, such as repairing molecules damaged by reactive oxygen species (ROS). The presence of high levels of ROS induces oxidative damage at protein and DNA level, which may lead to cellular apoptosis [36].

Recombinant GST-Hl was found to be immunogenic in rabbits, with individual variation in serum titers ranging from 128,000 to 2,024,000. The immunogenicity of rGST-Hl reported here in rabbits was also observed in cattle during two previous experiments [17,18]. For *R. appendiculatus,* the vaccination caused reduction of all parameters evaluated in adult female ticks: number, weight, egg laying, and fertility, but no significant difference in number and weight of nymphs. Adult female ticks feed larger amounts of blood compared with nymphs, leading to larger amounts of heme being processed and demanding detoxification in this phase, which could explain the differential vaccine effect. GSTs play an essential role in heme detoxification, therefore anti-GST antibodies could be blocking enzyme activity, causing cell and tissue injury and leading to the observed phenotypical damage. In addition, a number of studies have shown that tick immature stages (larvae and nymph) have lower host specificity compared with adult stages [37–39]. It is possible that the loss of phenotypical plasticity in adults impairs the tick responses against antibodies, exacerbating vaccination effects in this phase. The total protection was assessed using a formula that considers the number of fully engorged females [28], as well as an adapted formula, which uses the female weight. This parameter was considered because the average weight was different between ticks in the vaccinated and control groups, thus allowing a more comprehensive assessment of the protection rate achieved. It is important to observe that the adult phase is the major stage involved in pathogen transmission, therefore vaccination with rGST-Hl could potentially affect the tick-host-pathogen interface [40]. Interestingly, rGST-Hl cattle vaccination showed similar final protection against *R. microplus* [17,18], another tick species adapted to cattle. In cattle trials, *R. microplus* was affected during larva, nymph, and/or initial fed adult stages, decreasing the total number of fully engorged females recovered. For *R. appendiculatus*, in contrast, rGST-Hl vaccination was effective during female engorgement and egg development, indicating a distinct protection mechanism for this tick. The diversity of GST isoforms observed in genomes and transcriptomes of arthropods [41–43], and GSTs transcripts processed though alternative splicing, result in a potentially large number of GST isoforms, which could explain different susceptibilities along *R. appendiculattus* and *R. microplus* life cycle.

Interestingly, vaccination with rGST-Hl against *R. sanguineus* failed to induce significant differences in all tick physiologic parameters analyzed. Despite both *R. appendiculatus* and *R. sanguineus* having a three-host life cycle, *R. appendiculatus* is more adapted to feed in bovidae, while *R. sanguineus* can parasite a higher number and diversity of host species [44]. Generalist ticks like *R. sanguineus* are better adapted to environmental variations [45], being able to more efficiently change salivary protein profile to evade distinct host responses against tick feeding [46,47]. This phenomenon could account for a lower vaccine effectiveness against *R. sanguineus*. Also, the presence of different GST isoforms, which have functional redundancies in different sub-cellular compartments, cell conditions or requirements, can be a reason for the reduced effectivity of rGST-Hl vaccine. Moreover, the efficacy of the host natural immune response developed against ticks depends on the host species and immune background, affecting tick protein expression [48]. For example, *R. sanguineus* repeated infestations trigger low immune protection levels for dogs, its natural host [49–51]. In contrast, high immune protection is developed after repeated infestations in experimental hosts, such as guinea pig and rabbits [49,51,52]. A direct comparison between these apparently discrepant observations is not possible, however, since experimental infestation are performed in naive animals inoculated with one or a few antigens, as opposed to the repeated exposure to several saliva antigens in successive natural infestations. Despite the distinct levels of host susceptibility, there are reports of cross-protective antigens reducing *R. sanguineus* fitness in dogs (Bm86 antigen) [53], guinea pigs (64TRPs antigen) [34], and rabbits (ATAQ antigen) [10]. Taken together, these results show that even susceptible hosts can be protected by vaccination against *R. sanguineus* infestation, although in the current protocol rGST-Hl was not able to develop protective immunity.

5. Conclusions

The results presented here demonstrate rGST-Hl as a potential vaccine antigen against different tick species. Vaccinating animals with this antigen raised protective immunity against *R. appendiculatus* in rabbits, similarly to what was previously observed for *R. microplus* in cattle [17,18]. *R. appendiculatus* parasitism on rGST-Hl immunized rabbits led to salivary glands and ovary damage in the ticks, decreasing adult female fitness and resulting in a protec-

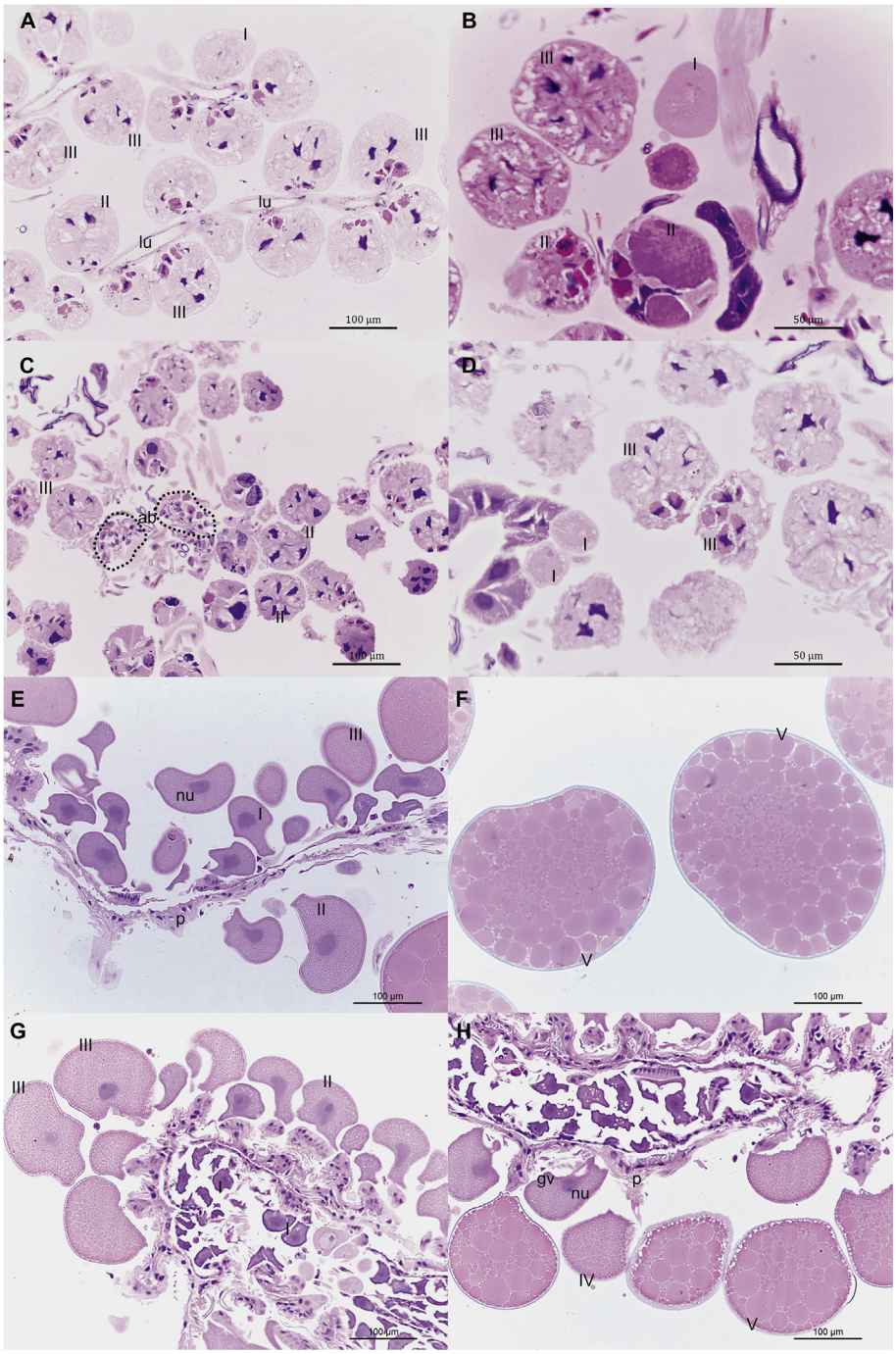


Fig. 5. Histologic sections of *R. appendiculatus* tissues stained with hematoxylin and eosin (HE). (A and B) Salivary glands of females fed on host from control group. (C and D) Salivary glands from females fed on host immunized with rGST-Hl. I, II and III: acini types; ab: apoptotic body; lu: lumen. (E and F) Ovaries of females fed on host from control group. (G and H) Ovaries from females fed on host immunized with rGST-Hl. I, II, III, IV and V: ovaries types; p: pedicel; nu: nucleus; gv: germinal vesicle.

tion level of about 65%. Despite the high similarity among tick GSTs, *R. sanguineus* life cycle was not affected by vaccination, showing the need to investigate protection levels developed for rGST-Hl against specific target tick species. Field vaccination trials against *R. appendiculatus* and *R. microplus* infestations performed in regions where these two ticks occur could lead to a new generation of anti-vector vaccines for these notorious ectoparasites.

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Conflict of interest

The authors certify that they have no affiliations with, or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2017.10.026.

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