Ticks cause approximately $17–19 billion economic losses to the livestock industry globally. Development of recombinant antitick vaccine is greatly hindered by insufficient knowledge and understanding of proteins expressed by ticks. Ticks secrete immunosuppressant proteins that modulate the host's immune system during blood feeding; these molecules could be a target for antivector vaccine development. Recombinant p36, a 36 kDa immunosuppressor from the saliva of female *Dermacentor andersoni*, suppresses T-lymphocytes proliferation *in vitro*. To identify potential unique structural and dynamic properties responsible for the immunosuppressive function of p36 proteins, this study utilized bioinformatic tool to characterize and model structure of *D. andersoni* p36 protein. Evaluation of p36 protein family as suitable vaccine antigens predicted a p36 homolog in *Rhipicephalus appendiculatus*, the tick vector of East Coast fever, with an antigenicity score of 0.7701 that compares well with that of Bm86 (0.7681), the protein antigen that constitutes commercial tick vaccine *Tickgard®*. Ab initio modeling of the *D. andersoni* p36 protein yielded a 3D structure that predicted conserved antigenic region, which has potential of binding immunomodulating ligands including glycerol and lactose, found located within exposed loop, suggesting a likely role in immunosuppressive function of tick p36 proteins. Laboratory confirmation of these preliminary results is necessary in future studies.

1. Introduction

Ticks are considered among the most important vectors of livestock diseases worldwide as well as major vectors of pet diseases [1]. In tropical Africa, ticks and the tick-transmissible diseases constitute a major obstacle to livestock development [2]. Like elsewhere in the world, chemical acaricides have been the mainstay of tick control in this region; however, increasing resistance to this group of insecticides threatens livestock production systems, especially small-holding sectors that rely on rearing of exotic cattle breeds that are more susceptible to tick infestation and tick-borne diseases [TBDs] [3]. Integrated tick control incorporating reduced acaricide use, breeding cattle for tick resistance, rotational grazing, and use of vaccines presents a sustainable and long-term strategy to the control of ticks and TBDs in the tropics [4].

Numerous studies have shown the potential of immunological methods to control tick infestation by targeting critical tick physiological processes. Existing antitick vaccines work by eliciting humoral and cellular responses against tick cell membrane antigens [5, 6]. Vaccines capable of quelling both the arthropod vector and disease-causing pathogens are also under development [7]. Despite clear advantages of controlling ticks through vaccination, this strategy is presently hampered by antigenic sequence variations between geographically isolated tick populations and species causing
vaccine resistance in some regions [8] and lack of efficacy in others [9]. These limitations necessitate search of alternative antigens for inclusion in the next-generation tick vaccines.

Proteins found in tick saliva play critical roles during blood meal acquisition [10]. The pharmacologically active components secreted in their saliva help ticks circumvent host defenses such as haemostatic and immune responses of the host, thereby enabling blood feeding in hematophagous arthropods [11]. One such class of biological compounds is immunosuppressant proteins, which modulate the host’s immune system during tick’s blood feeding [12], making them suitable target in the search of novel vaccines against arthropod-transmitted diseases [13]. Low molecular weight proteins 5–36 kDa from tick saliva proteins have been shown to inhibit T-lymphocytes proliferation in vitro [14]. Active immunization of mice with Salp15, a 15 kDa secreted salivary gland protein from I. scapularis, showed substantial protection (60%) from tick-borne Borrelia [15]. Tick subolesin (SUB), the ortholog of insect and vertebrate akirins (AKR), was discovered as a tick protective antigen in Ixodes scapularis [16]. Vaccines containing conserved SUB/AKR protective epitopes have been shown to protect against tick, mosquito, and sandfly infestations, thus suggesting the possibility of developing universal vaccines for the control of arthropod vector infestations [17].

Protein antigens conserved across vector species could be used in developing cross-protective vaccines against multiple arthropod vectors and their associated pathogens [17, 18]. Alarcon-Chaidez et al. [19] cloned and characterized a 36 kDa immunosuppressive protein p36 from the salivary glands of partially engorged, female D. andersoni, that suppressed Con-A induced in vitro proliferation of normal murine T-lymphocytes by more than 90% [20]. Genes related to D. andersoni-derived p36 gene, such as Ra-p36, Av-p36, Hl-p36, and Rhp36, have been reported in A. variegatum [21], R. appendiculatus [22], H. longicornis [23], and R. haemaphysaloides [24]. Most proteins are, however, not sufficiently protective on their own suggesting the need for a multiantigen/chimeric vaccine that incorporates critical tick and pathogen antigenic epitopes [16, 25] to elicit synergistic antipathogen and antitick immune responses. Computational characterization and 3D structure modeling of D. andersoni p36 protein undertaken by this study is an initial step in understanding molecular basis of immune recognition which is a challenge in vaccine development [26]. The p36 conserved antigenic region predicted by this study has binding residues for ligands like glycerol and lactose which are associated with an immunomodulatory role suggesting this site may have a role in suppression of select T-cell receptor induced signaling events of D. andersoni p36 and its related proteins.

2. Methods

2.1. Sequence Characterization of Tick p36 Proteins. All tick proteins deposited in National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) protein database were retrieved and deposited in a standalone MySQL based database (https://www.mysql.com). D. andersoni p36 protein was used as reference sequence in conducting homology searches, Blastp [27] and OrthoMCL [28, 29], of tick proteins in the database.

The identified tick p36-related proteins were subjected to MEME tool search (http://meme-suite.org/tools/meme) to predict conserved motifs characteristic of p36 proteins. Motif search tool (http://www.genome.jp/tools/motif) then searched for function of identified common motifs in the database of known motifs. Tick p36 protein sequences were then aligned by a multiple sequence alignment tool, Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo). Phylogenetic tree construction was by maximum likelihood method [30] and evolutionary distance computed using Poisson correction method [31]. Bootstrap resampling (1000 replicates) assessed robustness of the groupings.

2.2. Identification of Antigenic Determinants in the p36 Proteins. SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP), TMHMM (http://www.cbs.dtu.dk/services/TMHMM), and PredGPI (http://gpcr.biocomp.unibo.it/predgpi) servers were used to determine if tick p36 proteins are preferably secretory, transmembrane, or have a glycosylphosphatidylinositol (GPI) sites, respectively. Antigenic potentials of tick p36 proteins against reference Bm86, a known antitick vaccine antigen, were evaluated by vaxijen tool (http://www.ddg-pharmfac.net/vaxijen/Vaxijen/Vaxijen.html); the model selected was parasite whose standard threshold is 0.5000. The antigenic regions of D. andersoni p36 and other p36 proteins predicted with an antigenic score above 0.7000 were mapped by online tools that predict antigenic peptides (Immunomedicine) (http://imed.med.ucm.es/Tools/antigenic.pl) and SVMTrip [32]. Immunogenic segments/residues of the predicted antigenic region were identified by an online Epitopia tool (http://epitopia.tau.ac.il). Sprint-Pep tool (http://sparks-lab.org/server/SPRINT) was then used to predict protein-peptide binding sites while Coach tool (https://zhanglab.ccmb.med.umich.edu/COACH) predicted ligands likely to bind these sites found within the region predicted as a potential p36 protein conserved site.

2.3. Structural Modeling of D. andersoni p36 Protein. Physico-chemical properties of D. andersoni p36 protein were analyzed by ExPASyProtParam (https://www.expasy.org) server while its secondary structure was characterized by online tool Spider2 (http://sparks-lab.org/yueyang/server/SPIDER2). The crystal or NMR structure of tick p36 protein is currently not available in the protein data bank (PDB) (https://www.rcsb.org/pdb/). The 3D structure of D. andersoni p36 protein was developed by QUARK ab initio modeling [33] that builds 3D structure from “Scratch,” based on physical principles rather than previously solved structures. 10 models, designated as 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10, were generated and validated by analyzing Verify 3D scores [34] of Ramachandran plots for each model. Based on the scores, models 2 and 9 were selected as likely 3D structures of D. andersoni p36 protein because they scored 81.41% and 88.44%, respectively, meeting Verify 3D
validation tool limit of 80% of the amino acids residues scoring \( \geq 0.2 \) in the 3D/1D profile.

The two selected models had their atomic structures refined by ModRefiner [35] after which their respective generated Ramachandran plots were validated by RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) and ProQ (https://proq.bioinfo.se/ProQ/ProQ.html). The validation scores guided selection of model 2 as the best 3D structure of *D. andersoni* p36 protein. PDBsum (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=index.html) was used to check location of predicted conserved antigenic region in the 3D structure of *D. andersoni* p36 protein.

3. Results and Discussion

3.1. Identification and Phylogenetic Analysis of p36 Proteins from Ixodid Ticks. The study identified 32 homologs of *D. andersoni* p36 protein among 6 ixodid (hard) tick species (Table 1). These included p36 genes reported in earlier studies from *R. appendiculatus*, *A. variegatum*, *H. longicornis*, and *Rhipicephalus haemaphysalooides* tick species as well as those found by this study in *Amblyomma scultum* and *Amblyomma aureolatum*. Among these homologs, 4 co-orthologs which are potential orthologs of *D. andersoni* p36 protein were identified in *R. appendiculatus* species (Table 1). Occurrence of p36 protein across a range of tick species may be related to a biological function for this protein in tick feeding [36]. Several p36 immunosuppressant protein sequences were found in a single tick species suggesting functional and structural redundancy in which a tick expresses multiple similar proteins in minute quantities during feeding [37]. Such redundancy may render saliva proteins less immunogenic, as reported with cystatins [38].

The tick p36 proteins have 3 potential common motifs designated as 1, 2, and 3 with motif 2 being the only one conserved among p36 proteins (Figure 1). Motif 2 is located between amino acid positions “107–127” in the reference *D. andersoni* p36 protein. This motif 2 may be associated with a functional domain, possibly a role in immunomodulatory activity of tick p36 proteins [39]. The 3 common motifs were not found in the motif database and could be representing an orphan protein family [40].

Alignment of tick p36 proteins revealed a conserved region occurring between amino acid positions “107–115” (“IDKGMMLSPF”) in the reference *D. andersoni* p36 protein (Figure 2). This region that coincides with location of conserved motif 2 has polar amino acid residue serine (S) and charged residues lysine (K) and aspartate (D), which are associated with potential active sites [41]. Phylogenetic tree (Figure 3) showed that, among homologs with higher amino acid percentage similarity and E-scores, *D. andersoni* was closely related to homologs from *R. haemaphysalooides* and *R. appendiculatus* as compared to homolog from *A. variegatum* indicating more recent ancestry between Dermacentor and *Rhipicephalus* than with *Amblyomma* genera as inferred by phylogeny [42].

3.2. Identification and Characterization of Antigenic Regions in the p36 Proteins. Most tick p36 proteins were predicted as secretory with signal peptide cleavage site at position 21-22 (Supplementary Table S1 and Figure S1). Secretory proteins are favoured candidates for vaccine development as they are easily accessible microbial antigens to the immune system [43]. *D. andersoni* p36 protein and most p36 variants were predicted as antigenic with several homologs having antigenicity score above 0.7000 (Table 2, Supplementary Table S1), surpassing the vaxijen tool threshold of 0.5000. JAP81944.1, a homolog in *R. appendiculatus* had antigenicity score of 0.7701, comparably higher than that of Bm86 (0.7681), the constituent antigen of Tickgard and Gavac™ commercial tick vaccines. Whether this theoretically predicted immunogenicity can confer protection against tick infestation there is need to be evaluated empirically through an immunization/tick challenge set up.

The potentially conserved motif 2 in p36 protein was predicted as a likely epitope-rich antigenic region with binding residues for glycerol and lactose ligands which are associated with an immunomodulatory role [44, 45]. To facilitate tick feeding a single tick saliva protein ligand may bind receptors on several immune cell types in the vertebrate host; alternatively, multiple tick saliva proteins may bind to a common receptor [37].

3.3. 3D Structure of *D. andersoni* p36 Protein. *D. andersoni* p36 protein has an instability index of 35.53 and GRAVY score of −0.324 classifying it as a stable, globular protein [46]. The protein’s high aliphatic index of 86.41 is associated with increase in thermostability of globular protein [47]. The stable secondary structures alpha-helix (α) and beta-sheets (β) comprised approximately 55% of *D. andersoni* p36 protein amino acid sequence (Supplementary Figure S2). The predicted conserved immunogenic region “74–107” in processed secretory *D. andersoni* p36 protein had several segments within loop regions where epitopes are generally found [48]. The combination of α-helices and β-structures through loops with specific geometric arrangements with respect to each is responsible in forming conserved structural motifs [49, 50].

Based on Verify 3D [34] scores of the 10 models designated as 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 generated for *D. andersoni* p36 protein; models 2 and 9 were selected for further validation as they passed tool limit of 80% of the amino acids residues scoring \( \geq 0.2 \) in the 3D/1D profile (Supplementary Table S2). Comparison of validation scores of the selected models 2 and 9 (Table 3, Supplementary Figures S3 and S4) identified model 2 as the best 3D structure of *D. andersoni* p36 protein.

The predicted 3D structure of *D. andersoni* p36 protein (Figures 4(a) and 4(b)) is a ball-like structure comprised of 1 alpha-helix and several antiparallel beta-strands. The region predicted as a likely conserved antigenic region “74–107” in *D. andersoni* p36 protein is not only located in between the alpha-helix and beta-strands but also occurs within the potentially groove region of the predicted 3D structure and further has its loop exposed on the protein surface.
**Table 1:** Tick proteins related to *D. andersoni* p36 protein.

| Tick species            | NCBI accession number | Protein description | Sequencesimilarity % identity | Length | Alignment | Value | Bit score | OrthoMCL Search | Reference          |
|-------------------------|-----------------------|---------------------|-------------------------------|--------|-----------|-------|-----------|-------------------|--------------------|
| *D. andersoni*          | AAF03683.1            | p36*               | 100                           | 220    | 1.00E−165 | 459   |           | Reference         | Bergman et al., 2000 |
| *R. appendiculatus*     | JAP82151.1            | Da-p36 family member | 37.72                         | 228    | 2.00E−034 | 124   |           | Co-ortholog       | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP85101.1            | Da-p36 family member | 37.8                          | 209    | 3.00E−034 | 123   |           | Co-ortholog       | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP87284.1            | Da-p36 family member | 38.2                          | 201    | 3.00E−033 | 120   |           | Co-ortholog       | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP86350.1            | Da-p36 family member | 35.82                         | 201    | 6.00E−032 | 116   |           | Co-ortholog       | Decastro et al., 2016 |
| *A. variegatum*         | BAD11807.1            | Da-p36              | 35.04                         | 234    | 1.00E−029 | 111   |           |                  |                    |
| *R. h. haemaphysaloides*| ABB90890.1            | Rhh-ISP partial     | 36.71                         | 158    | 2.00E−027 | 103   |           | In-paralog        | Xiang et al., 2005  |
| *A. sculptum*           | JAU03129.1            | Hypothetical protein partial | 35.06                         | 231    | 1.00E−026 | 103   |           | In-paralog        | Eliane et al., 2016 |
| *R. appendiculatus*     | JAP89144.1            | Da-p36 family member | 32.3                          | 226    | 2.00E−024 | 97.4  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP88013.1            | Da-p36 family member partial | 31.58                         | 228    | 2.00E−024 | 97.4  |           | In-paralog        | Decastro et al., 2016 |
| *A. sculptum*           | JAU02613.1            | Hypothetical protein | 27.65                         | 217    | 2.00E−016 | 74.3  |           | In-paralog        | Eliane et al., 2016 |
| *R. appendiculatus*     | JAP85022.1            | Hypothetical protein | 25.11                         | 231    | 7.00E−016 | 72.8  |           | In-paralog        | Decastro et al., 2016 |
| *A. sculptum*           | JAU02539.1            | partial Da-p36 family member | 32                            | 175    | 2.00E−015 | 70.9  |           | In-paralog        | Eliane et al., 2016 |
| *A. variegatum*         | DAA34959.1            | Da-p36 like         | 27.95                         | 229    | 3.00E−015 | 70.9  |           | In-paralog        | Ribeiro et al., 2011 |
| *A. aureolatum*         | JAT98922.1            | Hypothetical protein partial | 31.65                         | 218    | 4.00E−015 | 70.5  |           | In-paralog        | Martins et al., 2016 |
| *A. aureolatum*         | JAT98921.1            | Hypothetical protein | 30.19                         | 212    | 3.00E−013 | 65.5  |           | In-paralog        | Martins et al., 2016 |
| *R. appendiculatus*     | JAP85729.1            | Da-p36 family member | 26.24                         | 221    | 4.00E−013 | 65.1  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP89564.1            | Da-p36 family member | 25.47                         | 212    | 2.00E−012 | 63.5  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP82843.1            | Da-p36 family member | 23.31                         | 236    | 2.00E−011 | 60.1  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP86306.1            | Da-p36 family member | 25.74                         | 237    | 3.00E−011 | 60.1  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP81863.1            | Da-p36 family member | 27.14                         | 210    | 6.00E−011 | 59.3  |           | In-paralog        | Decastro et al., 2016 |
| *A. variegatum*         | DAA34748.1            | Da-p36 like         | 29.24                         | 171    | 1.00E−010 | 57.8  |           | In-paralog        | Ribeiro et al., 2011 |
| *R. appendiculatus*     | JAP70801.1            | Da-p36 family member | 26.23                         | 244    | 3.00E−009 | 54.3  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP86680.1            | Da-p36 family member | 27.72                         | 202    | 7.00E−009 | 53.5  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP88255.1            | Da-p36 family member | 26.73                         | 202    | 2.00E−008 | 52    |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP85730.1            | Da-p36 family member | 24.82                         | 141    | 2.00E−008 | 51.2  |           | In-paralog        | Decastro et al., 2016 |
| *H. longicornis*        | BAG11660.1            | Isp-p36             | 31.78                         | 107    | 1.00E−007 | 50.1  |           | In-paralog        | Nakajima et al., 2008 |
| *R. appendiculatus*     | JAP81735.1            | Da-p36 family member | 24.58                         | 240    | 6.00E−004 | 38.9  |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP86324.1            | Da-p36 family member | 24.24                         | 198    | 0.00      | 38.1  |           | In-paralog        | Decastro et al., 2016 |
| *A. sculptum*           | JAU02519.1            | Hypothetical protein partial | 33.7                          | 92    | 0.00      | 36.6  |           | In-paralog        | Eliane et al., 2016 |
| *A. variegatum*         | DAA34145.1            | ISP-p36 partial     | 28.95                         | 76    | 0.002     | 36.6  |           | In-paralog        | Ribeiro et al., 2011 |
| *R. appendiculatus*     | JAP81446.1            | Da-p36 family member | 25.74                         | 237    | 0.01      | 35    |           | In-paralog        | Decastro et al., 2016 |
| *R. appendiculatus*     | JAP86032.1            | Da-p36 family member | 25.18                         | 139    | 0.089     | 34.3  |           | In-paralog        | Decastro et al., 2016 |

* Amino acid; * reference p36 protein in *D. andersoni*. 
Table 2: Conserved motif 2 of tick p36 proteins mapped as a potential antigenic/binding site region.

| Tick species | NCBI accession number | Antigenic score | Conserved Motif 2 location | Mapped immunogenic segments of Motif 2 | Mapped Binding sites of Motif 2 | Predicted ligands for Motif 2 |
|--------------|-----------------------|-----------------|-----------------|----------------------------------------|---------------------------------|-----------------------------|
| D. andersoni | AAF03683.1             | 0.5880          | 107–127         | IDKMLSPFNLSATVKFPLIP                  | IDKGMSPFNLSATVKFPLIP            | Lactose, Glycerol, NAG-(4-1)GAL, Sucrose |
| R. appendiculatus | JAP81944.1             | 0.7701          | 117–137         | IDNGIKTPFHLKAVFSFPTTG                 | IDNGIKTPFHLKAVFSFPTTG           | -                           |
| R. appendiculatus | JAP88013.1             | 0.7379          | 113–133         | IDNGIKTPFHLKAVFSFPTTG                 | IDNGIKTPFHLKAVFSFPTTG           | -                           |
| R. appendiculatus | JAP81510.1             | 0.7258          | 102–122         | IDDSMYSPFNNMTTVAFPLIG                 | IDDSMYSPFNNMTTVAFPLIG           | B-Octylglucoside            |
| R. appendiculatus | JAP86350.1             | 0.7072          | 78–98           | IDYGIYSPFNNLTVQPQFPLMG                | IDYGIYSPFNNLTVQPQFPLMG          | Alpha-D-Mannose, Alpha-D-Lactose, B-Octylglucoside |

Bold sections: tick p36 conserved region residues mapped as potentially antigenic/binding sites.
Table 3: Validation of 3D structures for models 2 and 9 of *D. andersoni* p36 protein.

| Number | Validation tool | Parameter monitored | Limit | Results |
|--------|-----------------|----------------------|-------|---------|
| (1)    | ProQ PsiPred    | LGscore              | LG score > 1.5 fairly good model | Model 2: LGscore 2.797, Model 9: LGscore 2.366 |
|        |                 |                      | LG score > 2.5 very good model   |                     |
|        |                 |                      | LG score > 4 extremely good model|                     |
|        |                 | MaxSub               | MaxSub > 0.1 fairly good model   | Model 2: MaxSub 0.208, Model 9: MaxSub 0.055 |
|        |                 |                      | MaxSub > 0.5 very good model     |                     |
|        |                 |                      | MaxSub > 0.8 extremely good model|                     |
| (2)    | ProQ J Pred     | LGscore              | LG score > 1.5 fairly good model | Model 2: LGscore 2.94, Model 9: LGscore 2.231 |
|        |                 |                      | LG score > 2.5 very good model   |                     |
|        |                 |                      | LG score > 4 extremely good model|                     |
|        |                 | MaxSub               | MaxSub > 0.1 fairly good model   | Model 2: MaxSub 0.219, Model 9: MaxSub 0.046 |
|        |                 |                      | MaxSub > 0.5 very good model     |                     |
|        |                 |                      | MaxSub > 0.8 extremely good model|                     |
| (3)    | Rampage tool    | Favoured region residues | 156 (79.2%) | 152 (77.2%) |
|        |                 | Allowed region residues | 26 (13.2%) | 24 (12.2%) |
|        |                 | Outlier region residues | 15 (7.6%) | 21 (10.7%) |
Figure 1: (a) Occurrence of tandem motifs among p36 proteins. Motif 2 is conserved across all homologs; (b) motifs sequence logo analysis.

(Figure 4(c)). Ligands bind in the largest cleft in over 83% of the proteins [51]; thus presence of the predicted conserved antigenic region within this potential groove may be associated with immunosuppressive function of *D. andersoni* p36 protein, as internal cavities in proteins are important structural elements that may produce functional motions such as ligand binding [52].

Potentially exposed loop region “87⋯94” (Figure 4(d)) in predicted 3D structure of *D. andersoni* p36 protein coincides with its likely conserved alignment region “107⋯115”
after cleavage of signal peptide at amino acid position 21-22. This suggested loop region might be associated with binding site of *D. andersoni* p36 protein. The ligands predicted with potential to bind on this site include fatty acid glycerol and sugars like lactose. The hydroxyl group of polar amino acid residue serine (S), hydrophobic amino acid residue leucine (L), and charged amino acids lysine (K) and aspartic acid (D) found in this region could, respectively, have a role in binding of these ligands [53]. Immunomodulator ligands predicted with potential of binding at this site include fatty acid glycerol and sugars like lactose. There is need for future studies to evaluate whether immunomodulator ligands such as glycerol and lactose. A further study is necessary on suitability of this potentially conserved region in development of a multi/chimeric antitick vaccine that incorporates critical antigenic regions. The predicted 3D model of *D. andersoni* p36 protein may be used as a template to model structures of other orphan proteins related to p36. This work is a step towards developing cross-protective next-generation antitick vaccines, as the results expand our knowledge of p36 tick saliva protein and lay ground for future studies to determine their exact role in tick feeding process, which is useful in designing blockade approaches targeting these proteins.

4. Conclusion

The p36 immunosuppressive proteins from ticks exhibit antigen traits worth evaluating in future experimental *in vitro* and *in vivo* trials. This includes potential conservation across several tick species and presence of a likely conserved antigenic region that may be bound by immunomodulator ligands such as glycerol and lactose. A further study is necessary on suitability of this potentially conserved region in development of a multi/chimeric antitick vaccine that incorporates critical antigenic regions. The predicted 3D model of *D. andersoni* p36 protein may be used as a template to model structures of other orphan proteins related to p36. This work is a step towards developing cross-protective next-generation antitick vaccines, as the results expand our knowledge of p36 tick saliva protein and lay ground for future studies to determine their exact role in tick feeding process, which is useful in designing blockade approaches targeting these proteins.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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Supplementary Materials

Supplementary Table S1: protein targeting pathway and antigenic potential of tick p36 proteins. Supplementary Table S2: Verify 3D validation scores of models generated for *D. andersoni* p36 protein. Supplementary Figure S1: *D. andersoni* p36 protein signal peptide cleavage site location. Supplementary Figure S2: Spider2 tool secondary structure characterization of *D. andersoni* p36 protein.
Figure 4: (a, b) *D. andersoni* p36 protein predicted 3D structure ribbon and space field model; (c) predicted antigenic region "74–107," in 3D structure of *D. andersoni* p36 protein. (d) Topology of *D. andersoni* p36 protein showing the likely predicted conserved exposed loop. Yellow: predicted conserved antigenic region "74–107"; red: α-helix secondary structure; purple: β-strands secondary structure. ↑: predicted exposed loop region "87–94" ("DKGMLSPF") in *D. andersoni* p36, showing conservation in alignment of tick p36 proteins.

Figure S3: rampage tool assessment of Ramachandran plot for model 2 of *D. andersoni* p36 protein. Supplementary Figure S4: rampage tool assessment of Ramachandran plot for model 9 of *D. andersoni* p36 protein. (Supplementary Materials)

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