Molecular Detection and Characterization of Bm86 Gene Homologues from *Hyalomma truncatum*, *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) decoloratus* for the Development of an Anti-Tick Vaccine in Nigeria

Dogo, Goni Abraham*, Kwaga, K.P. Jacob[1]; Umoh U. Jarlath[1]; Agbede S. Rowland[2] and Jogenjan Frans[3]

Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Jos, Jos Plateau State Nigeria

[1]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria
[2]Department of Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria
[3]Utrecht Centre for Ticks and Tick-borne Diseases, FAO Reference Centre for Ticks and Tick-borne Diseases Faculty of Veterinary Medicine Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands and Department of Tropical Veterinary Medicine, University Of Pretoria, South Africa

***Corresponding author:** gonidogo@gmail.com

Rec. Date:	Mar 27, 2015 07:11
Accept Date:	Apr 10, 2015 03:11
Published Online:	April 20, 2015
DOI	10.5455/ijlr.20150410031140

Abstract

The current study was conducted using prevalent Ixodid ticks from the Jos Plateau, were largely the use of acaricides has a number of drawbacks which include the development of resistant ticks, environmental pollution and toxicity. The molecular detection and characterization of Bm86 gene homologues in Hyalomma truncutum, Rhipicephalus (Boophilus) decoloratus and Rhipicephalus (Boophilus) annulatus for the first time in Nigeria was achieved and significantly important in the quest for alternative tick control strategies. GenBank Accession numbers has been assigned to the homologues as KF670599, KF670600 and KF670601 respectively and deposited as well. These results might assist in the development of a recombinant Bm86 gene based anti-tick vaccine which is an important component of an integrated control measure against hard ticks infesting livestock in the sub-Saharan Africa.

Key words: Molecular, Detection, Characterization, Bm86 gene, homologues, *Hyalomma* and *Boophilus* Nigeria

Introduction

The ixodid ticks from *Boophilus* and *Hyalomma* genus are known to be important pests of livestock, having major medical and veterinary significance in Northern and Sub-Saharan Africa BenSaid, et al (2012). Generally, ticks transmit a greater variety of pathogenic microorganisms than any other arthropod

vector group. Integrated tick management strategies include the adaptation of different control methods that have been used to control ticks in a particular geographical area. The use of acaricides has had limited efficacy and has a number of drawbacks which include the development of resistant ticks, environmental pollution, etc (Graf et al 2004). Control of ticks by vaccination has a number of advantages of which the most important are their cost effectiveness, reduction of environmental contamination and the prevention of drug resistant ticks caused as a result of repeated acaricide application Jongejan and Uilenberg (2004). Due to the incorrect dilution of acaricides, by under-dosing them, resistance can quickly develop in certain tick species, including *B. microplus*. Over-dosing on the other hand can cause severe damage to the skin of the animals and the person applying the acaricides Minjauw, et al (2002). It has been established that acaricides are toxic; their use also leads to the problem of possible residues in food products and contamination of the environment, especially with an increasing public concern about environmental and health issues which are of public health importance Tellam, (1992); Jongejan and Uilenberg, (2004). Apparently, tick control is encountering a number of different problems. A concealed antigen from the midgut of engorged female *Rhipicephalus (Boophilus) microplus* and called it Bm86 was identified Willadsen and Kemp (1989). Cattle immunized with Bm86 showed significant rejection of adult ticks, reduction in engorgement and egg mass and more than 80% protection against challenge infestations. Bm86 is a 89kDa glycoprotein that is coded by an open reading frame (ORF) of 1982 base pairs and sequence variation in the tick midgut surface protein Bm86 is one hypothesis for the variability in efficacy of Bm86 based vaccines against cattle fever ticks Freeman et al (2010). An inverse correlation between vaccine efficacy and sequence variation in Bm86, with variations of greater than 2.8% being the most likely to produce lower efficacy has been established (Garcia et al 1999). The efficacy of *Rhipicephalus (B.) microplus* recombinant Bm86 for the control of *H. dromedarii* infestation in Cattle and Camels was demonstrated Rodriguez-Valle et al (2012). The aim of this study was to detect and characterize the Bm86 gene homologues in *Hyalomma, Rhipicephalus* (*Boophilus*) *annulatus* and *Rhipicephalus* (*Boophilus*) *decoloratus* tick species in Nigeria.

Materials and Methods

Study area

This study was conducted in Jos Plateau State which is located in north central of Nigeria, with an area of 26,899 square kilometers and an estimated population of about three million people. It is located between latitude 9° 0′ to 9° 40′ North and longitude 8° 30′ to 10° 30′ East of the equator. The altitude ranges from around 1,200 meters (about 4000 feet) to a peak of 1,829 metres above sea level in the Shere Hills range near Jos. Years of tin mining have also left the area strewn with deep gorges and lakes. Though situated in the tropical zone, a higher altitude means that Plateau State has a near temperate climate with an average

DOI 10.5455/ijlr.20150410031140

temperature of between 18 and 22°C. Harmattan winds cause the coldest weather between December and February. The warmest temperatures usually occur in the dry season months of March and April. The mean annual rainfall varies from 131.75 cm (52 in) in the southern part to 146 cm (57 in) on the Plateau. The highest rainfall is recorded during the wet season months of July and August Odumodu (1983).

Ticks source

The *Hyalomma truncatum, Rhipicephalus (B.) annulatus and Rhipicephalus (B.) decoloratus* used in this study, originated from the pure tick colony reared in a Memmert low temperature incubator (Germany) at 26.5 ºC with 85% relative humidity and maintained at National Veterinary Research Institute, Vom Tick Laboratories, south of Jos in 2009 which were collected from White Fulani cattle.

PCR amplification and Sequencing of Bm86 homologues

To amplify the Bm86 gene orthologs, tick guts were dissected from 100 adult female *B. annulatus, B. decoloratus* and *H. truncatum* each using a sterile scalpel blade, and total RNA extracted using TRIzol reagents (Invitrogen, Breda, The Netherlands). The RNA from the aqueous phase was precipitated by mixing with isopropanol (isopropanol alcohol), using 0.5 ml isopropanol per 1 ml of TRIZOL reagent used for the initial homogenization. First strands cDNA was synthesized according to the manufacturer's instruction from Supercript III Kit using a 3´-RACE anchor primer with a poly-T sequence (Invitrogen, Breda The Netherlands) and used as template for reverse transcription PCR. A mixture was prepared (2 µl 3´RACE anchor primer (10pmol/µl), 1 µl 10 mM dNTP mix, 1 µg of total RNA, distilled water), heated to 65°C for 5 minutes, incubated on ice for at least 1 minute and briefly centrifuged at 1200 rpm for 10 minute. To this mixture was added: 4 µl of 5x First-strand buffer, 1 µl 0.1 M DTT, 1 µl RNAse inhibitor and 1 µl Superscript III Reverse Transcriptase. It was mixed by gentle pipetting, incubated at 55°C for 60 minutes and heated at 70°C for 15 minutes. The resulting cDNA was treated with 1 µl RNAse H (2U/ µl, Invitrogen), incubated at 37°C for 20 minutes and stored at -20°C. PCR rapid amplification of Bm86 gene from *B. annulatus*, *B. decoloratus* and *H. truncatum* was carried out using the Go Taq polymerase Kit (Invitrogen). A 25µl master mixed reaction mixture was used for gene amplification; containing 5x colorless Go Taq flexi buffer, 2.5mM Mgcl2, 10mM each deoxynucleotide triphosphate (dNTP), 10mol of each primer and 0.2 ul Go Taq polymerase in accordance with the manufacturer's instructions. DNA amplification was carried out in a thermocycler (GeneAmp® PCR System 9700; Applied Biosystems, Singapore) with a pre-cycle at 95°C for 2 minutes and final extension at 72°C for 2min were used for PCR programme.

DOI 10.5455/ijlr.20150410031140

Agarose gel electrophoresis

Electrophoresis was carried out firstly by preparing a 1.5 % agarose gel using electrophoresis buffer 1X TAE diluted from 10x TAE stock (Invitrogen).The 1.5g agarose (Hispan Agar) was weighed into 100ml of 1x TAE to give the final 1.5 % agarose concentration.

DNA Sequencing with the Big Dye V3.1 Kit

The positive clones of *Rhipicephalus (B.) annulatus*, *Rhipicephalus (B.) decoloratus* and *H. truncatum* were sequenced using the Big Dye V3.1 kit and ran on the ABI3500XL genetic analyzer according to manufacturer's guidelines at Inqaba Biotechnical Industries (Pty) Limited, Pretoria South Africa.

Editing and Aligning of Sequence Data

The sequences were assembled and checked for accuracy using the Finch TV 4.1 version software **www.geospiza.com**. The peaks against the base call were checked and 5' – 3' ends limits were determined and all sequences were assembled to generate a contig file and saved. Sequences were exported to editor (BLAST search) and the tick species which corresponded with each sequence was determined saved as fasta files. Sequences were aligned using CLC – bio workbench 6 version software were both reference and queried sequences from text files were ran and saved as fasta files.

Results and Discussion

Sequence Analysis

All sequences were subjected to a Basic Local Alignment Search Tool (BLAST) search to determine their identities and assess their homologies and similarities to those in the GenBank.

Analysis of DNA Sequences of Ba86, Bd86 and Ht86 gene homologues

All sequences were subjected to a Basic Local Alignment Search Tool (BLAST) search to determine their identities and assess their homologies. GenBank Accession numbers has been assigned to the homologues as KF670599, KF670600 and KF670601 respectively. The result of the multiple sequence alignment (MSA) using the CLC-Bio workstation version 6.0 revealed areas of identities between the tick strains and the reference sequence of *Rhipicephalus (B.) annulatus* with the GenBank Accession number HQ014401 which are depicted by dots. Where sequences are similar, the same nucleotides are seen. Where sequences are not similar, there is either a deletion (Dashes) depicted by – or substitution of one nucleotide for another, (Fig. 1).

Phylogenetic analysis of Bm86 sequenced nucleotides

To further support the grouping of tick strains from different geographical regions, a neighbor – joining tree was generated using the Molecular Engineering Genetic Analysis (MEGA version 4) software Tamara (2007) and the CLC-Bio Workbench 6.0 version software which was based on the 1000

DOI 10.5455/ijlr.20150410031140

boothstrapping iterations. The Bm86 queried sequences tree rooted with the Bm86 sequence of referenced *Boophilus annulatus* accession number HQ014404.1 and others.

Phylogenetic analysis

The results of phylogenetic and molecular evolutionary analyses using *MEGA* version 4 Tamura *et al* (2007) and CLC Bio workstation 6.0 version are presented in Fig. 2. In this study, the phylogenic tree analysis comprises the Bm86 orthologs from *Rhipicephalus (B.) annulatus, Rhipicephalus (B.) decoloratus* and *H. truncatum* strains showing identity to 11 GenBank accession numbers.

```
                                  20                        40                        60
Hyalomma truncatum-Jos  GCATGGCTCGAGTTTTTCAGCAATGACTCCCTCGTAGATTAAGCACTTACTTTCCAGGAT 60
Boophilus annulatus-Jos .............................T....AGT..CT..ATTAAG.AC.AACTTTC 60
Boophilus decoloratus-Jos .GGAC.....................T....G.T..CT..ATTAAG.AC.AACTTTC 60
HQ014401  ....C.........C.GTC....A..A.TT..GCGGCG.CT.TATGAAT.GACAAGCTGC 60
Consensus GCATNGCTCGAGTTTTTCAGCAATGACTCTCTCGGNTATCTAGATTAAGCACTAACTTTC

                                  80                        100                       120
Hyalomma truncatum-Jos  CTGCACCGAAGTCGCAAATGGATGACTCTCTTTTCTCGACTTGAAGGATCTGAACCGAAG 120
Boophilus annulatus-Jos .A.G.T.ATCAC..A.GTCA..GC.AATGGA.GA...TCT..TCTCT.CT....GGATCT 120
Boophilus decoloratus-Jos .A.GC..TC.AC..GTCTATACAT.AATGGA.GC...TCT..TTTCT.CT.C...GATCT 120
HQ014401  TATTGT.TC.CC.AA.GTCAACATCAATGAA.GC...TCT.GATTCT.CTCC.GAATGGT 120
Consensus CAGGANCTCAACCGAAGTCANNATAAATGGATGCCTCTCTTTTTTCTACTTNAACGATCT

                                  140                       160
Hyalomma truncatum-Jos  TCAGCGAGAAAAGAGAGTCAT---------------------------------- 141
Boophilus annulatus-Jos GTGC...AGTC....CAAATGGATAACTCTCTTTCTAGCAGCTGCCATGGAAAATCAA 178
Boophilus decoloratus-Jos G.GC...AGTC....CAAATGGATAACTCTCTTTCTAGACAATATTCTCAGCTGCCAT 178
HQ014401  G..T...AGTTGCT..TCA.GAAAAACTCTGCATCCAGCAGCTGCGATGGAAAGTCAA 178
Consensus GCNCCGAAGTCAGAGNAAANGGATAACTCTCTTTCTAGCAGCTGCNATGGAAAGTCAA
```

Figure 1: Aligned sequences from Nigeria showing conserved regions of the fragment length from 1 to 178bp regions of the Bm86 gene in *Rhipicephalus (B.) annulatus, Rhipicephalus (B.) decoloratus* and *H. truncatum* sequences compared with the reference sequence HQ14401

We present the first report of molecular detection and characterization of *Bm86* gene homologues from *Rhipicephalus (B.) annulatus, Rhipicephalus (B.) annulatus* and *Hyalomma truncatum* in Nigeria. It also corroborates with work reported in The Netherlands, probably because of the fact that similar primers and protocols were employed in amplifying the Bm86 gene homologues (18). The study is not in agreement with the work done by some authors in Iran who reported that Ixodid ticks had higher amplicon sizes and there was a 100% homology between fragments of *Bm86* gene amongst the Iranian *Hyalomma anatolicum anatolicum* Rezaei and Akhshabi (2011). Furthermore, diversity was expected because the primers used in this study were completely different as they were designed using local ticks indigenous to Iran and could be probably due to species differences amongst tick genera also.

DOI 10.5455/ijlr.20150410031140

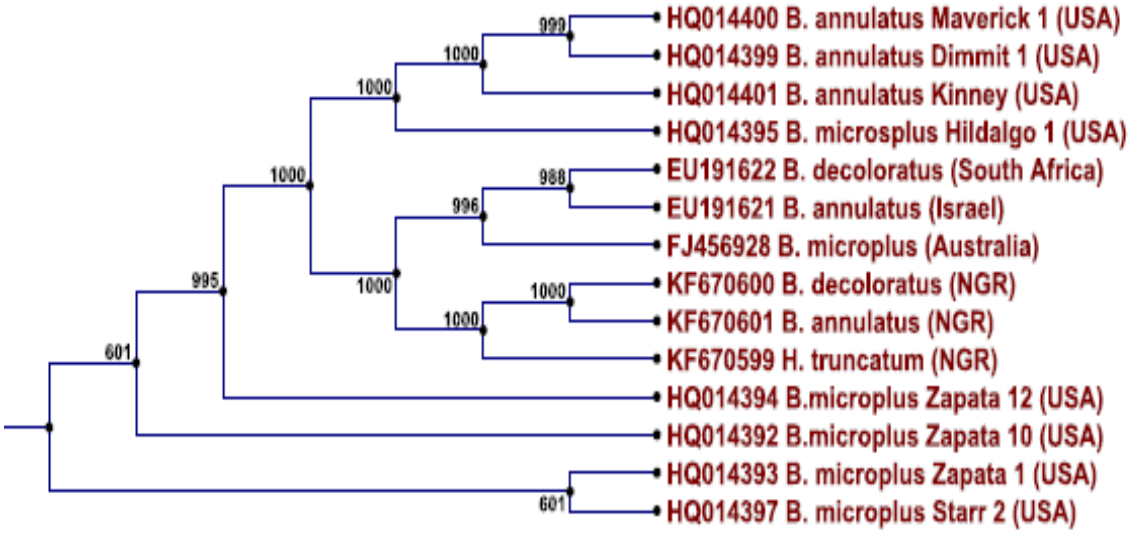

Figure 2: The presence of *Bm86* gene orthologs in Ixodid ticks as revealed by a neighbor-joining tree analysis of the DNA sequences with GenBank Accession numbers as KF670599, KF670600 and KF670601. The numbers on branch represent 1000 replications (Bootstrapping support) for which the same branching patterns were obtained.

The areas of non-identities noticed in *Hyalomma truncatum* strains of 39 nucleotide position (np) in numbers could likely be as a result of mutation or other unexplainable factors. This particular feature is possibly related to natural allele variations in the Bm86 gene orthologs de la Fuente and Kocan (2003). The phylogenetic analysis revealed that there was 3-8% sequence variation with the consensus sequence between the host and the following nucleotide sequences: HQ014394, FJ456928, EU191621, EU191622, HQ14399 and HQ14400 which was in agreement with work reported by other authors Garcia et al. (1999). It have been postulated that sequence variations in the tick midgut surface protein Bm86 is one hypothesis for the variability in efficacy of Bm86 based vaccines against cattle fever ticks Freeman et al. (2010). It has been shown also, that there was an inverse correlation between vaccine efficacy and sequence variation in Bm86, with variations of greater than 2.8% being the most likely to produce lower efficacy Garcia et al. (1999). It is worthy of note that when designing anti-tick vaccine in the future, these findings are to be taken into serious consideration because there was a 100% homology amongst the *Rhipicephalus (B.) annulatus* and *Rhipichephalus (B.) decoloratus.* An alignment of the DNA sequences from the three tick species in this study agrees with work done somewhere else (Fragoso et al.,1998, Pipano et al., 2003, Ard et al., 2009). This might likely be unconnected to environmental and or global warming as reported in a model simulation developed against *Rhipicephalus (B.) decoloratus* and *Rhipicephalus (B.) microplus* in a multi-tick population, Dogo et al. (2010a). In this study, a 100%

DOI 10.5455/ijlr.20150410031140

homology was observed within some strains from the phylogenetic tree constructed using Bootstrapping at 1000 iteration. The diversity of Bm86 homologues between *Hyalomma* and *Rhipicephalus (Boophilus)* tick species using degenerate and anchor primers was based on available sequence information from the GenBank which was supported by phylogenetic tree analysis Nijhof, (2010). The phylogenetic tree analysis has confirmed that tick species from Nigeria clustered with those of USA, South Africa, Israel and the Australian Yeerongpilly strains suggestive of common ancestral origin. This might probably assist in adopting similar control strategies in a multi-species population and to sustain the efforts in the development of suitable control measures against cattle ticks infesting Livestock in Nigeria.

Conclusion

The study provides new information on the presence of Bm86 gene homologues from Nigerian Ixodid tick species and probably gives an insight on the ancestral origin of the ticks linked with other ticks strains from different countries as revealed by the Phylogenetic analysis.

Acknowledgment

The authors are grateful to the Internal Management Committee (IMC) of the National Veterinary Research Institute, Vom for sponsorship of the research project and permission to publish. The research was partly funded by Trifolio – M, Lanau GbmH Germany, under the contract agreement no. 200901 with National Veterinary Research Institute, Vom. Authors wish to thank staff of Parasitology Division, National Veterinary Research Institute, Vom for their technical support.

References

1. Ard MN, Jesper AB, Milagros P and Frans J (2009). Selection of reference genes for quantitative RT-PCR studies in Rhipicephalus (Boophilus) microplus and Rhipicephalus appendiculatus ticks and determination of the expression profile of Bm86. BMC Molecular Biology. 10: 112
2. BenSaid M, Galai Y, Mhadhbi M, Jedidi M, de la Fuente J and Darghouth MA (2012). Molecular characterization of Bm86 gene orthologs from Hyalomma excavatum, Hyalomma dromedarii and Hyalomma marginatum marginatum and comparison with a vaccine candidate from Hyalomma scupense. Veterinary Parasitology. 190 (1-2): 230-240
3. de la Fuente J and Kocan KM (2003). Advances in the identification and characterization of protective antigens for for development of recombinant vaccines against tick infestations. Expert Review of Vaccines. 2: 583-593
4. Dogo G I, Lombin L H, Asad SS, Jongejan F and Heesterbeek JAP (2010a). Modelling the field efficacy of recombinant rDNA (Bm86) anti-tick vaccine TickGARD against Boophilus microplus and Boophilus decoloratus ticks. Nigerian Veterinary Journal. 13(2): 154-163.
5. Fragoso H, Hoshmand Rad P, Ortiz M, Rodriguez M, Redo M, Hettera L and de la Fuente J (1998). Protection against Boophilus annulatus infestation in cattle vaccinated with the B. microplus Bm86 containing vaccine Gavac. Vaccine 16: 1990-1992
6. Freeman MJ, Ronald BD, Lowell SK, Diane MK and Pia UO (2010). Bm86 midgut protein sequence variation in South Texas cattle fever ticks. *Parasites and Vectors.* 3:101
7. Garcia GJC, Montero C, Redondo M, Vargas M, Canales M, Boue O, Rodriguez M, Joglar M, Garcia-Garcia H, Gonzalez JC, Gonzalez IL, Valdes DM, Mendez M and Lamberti L (1999). Sequence

DOI 10.5455/ijlr.20150410031140

variations in the Boophilus microplus Bm86 locus and implications for immuno-protection in cattle vaccinated with this antigen. Experimental and Applied Acarology. 23: 883–895
8. Graf JF, Gogolewski R, Leach-Bing N, Sabatini GA, Molento MB, Bordin EL and Arantes, G J (2004). Tick control: an industry point of view. Parasitology. 129: S427-S442
9. Jongejan F and Uilenberg G (2004). The global importance of ticks. Parasitology. 129: S3-14
10. Minjauw, B and McLeod A (2002). Tick-borne diseases and poverty: The impact of ticks and tick-borne diseases on the livelihood of small-scale and marginal owners in India and eastern and Southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.116
11. Nijhof, AM (2010). A contribution to the development of anti-tick vaccines PhD Thesis, Utrecht University, The Netherlands.
12. Odumodu, LO (1983). Rainfall distribution, variability and probability in Plateau State. Journal of Climate. 3: 385-393
13. Pipano E, Alekceey E, Galker F, Fish L, Samish M and Shkap V (2003). Immunity against Boophilus annulatus induced by the Bm86 (Tick-GARD) vaccine, Experimental and Applied Acarology. 29: 141–149
14. Rezaei A and Akhshabi S 2011. Evidence for the Identity Homologue Bm86 of Gene in Natural Strains Hyalomma anatolicum anatolicum Cattle Tick for Sequencing. Advance Studies in Biology. 3(2): 89 – 101
15. Rodriguez-Valle M, Amar T, Valdes M, Montero C, Ibrahin H, Hassan SM, Jongejan F and de la Fuente J (2012). Efficacy of Rhipicephalus (Boophilus) microplus Bm86 against Hyalomma dromedarii and Amblyomma cajennese tick infestation in camels and cattle. Vaccine 30: 3453-3458
16. Tamura K, Dudley J, Nei, M and Kumar S 2007: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596-1599
17. Tellam RL, Smith D, Kemp DH and Willadsen P (1992). Vaccination against ticks. In: Animal Parasite Control Utilizing Biotechnology. Edited by Yong WK. Boca Raton: CRC Press: 303-331
18. Willadsen P, Kemp DH (1989).Novel vaccination for control of the Babesia vector, Boophilus microplus. Trans boundary Royal Society of Tropical Medicine and Hygiene 83: 107

DOI 10.5455/ijlr.20150410031140