Journal of Agriculture, Food Security and Sustainable Environment. *ISSN: 3635-3547*
Volume 2: December 2018
I **2018**

COMPARATIVE HOMOLOGY OF *HSP* 70 GENE IN FOUR NIGERIAN *BOS INDICUS* BREEDS REVEALED COMMON EVOLUTION AND ANCESTRAL LINEAGE

***[124]Onasanya, G. O., [4]Thiruvenkadan, A. K., [2]Sreekumar, C., [5]Tirumurugaan, K. G., [3]Sanni, M. T., [3]Olowofeso, O., [3]Fafiolu, A. O., [6]Msalya G. M., [7]Decampos J. S., [3]Ikeobi, C. O. N.**

[1] Department of Animal Science, Federal University Dutse, Dutse, Nigeria
[2] Biotechnology Center, Post Graduate School in Animal Sci. Research Kattupakkum, Tamil Nadu Veterinary and Animal Sci University, Chennai, India
[3] Department of Animal Breeding and Genetics, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria
[4] Mecheri Sheep Research Station Pottaneri, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
[5] Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India
[6]Department of Animal, Aquaculture and Range Sciences, Sokoin University of Agriculture, Morogoro, Tanzania
[7]Department of Animal Production, University of Ilorin, Ilorin, Nigeria
***Correspondence author:**: onasanya.gbolabo@gmail.com; onasanya.g@fud.edu.ng
Phone Number: +2347069209207

ABSTRACT

Heat shock protein (HSP) 70 gene is a member of HSPs sub-family that act as molecular chaperons whenever animals come under thermal assault , they fulfill essential roles of providing cellular protection, immune response, protein synthesis, protein folding and unfolding, protection proteins from cellular stress, inhibitory apoptosis and adaptation during thermal assault. A total of ninety (90) bulls from across four extant breeds of Nigerian Zebu cattle comprising of White Fulani (25), Sokoto Gudali (21), Red Bororo (21) and Ambala (23) sampled from northern parts of Nigeria. We report from the findings that the rooted evolutionary study based on Neighbour-joining dendrogram of HSP70 sequences of White Fulani, Ambala, Sokoto Gudali and Red Bororo Nigerian Zebu breeds of cattle revealed that HSP 70 sequences of four Nigerian cattle breed showed shared homology which is suggestive of common ancestral lineage. Similarly, nucleotide sequences of HSP70 in four Nigerian B. Indicus and those of goat, sheep, yak, buffalo, camel, horse and taurine demonstrated common clade architecture, therefore suggesting evolution from common ancestor. Phylogenetic analyses of four breeds of Nigerian cattle and comparative homology between four Nigerian breeds of cattle and those of goat, sheep, yak, buffalo, camel, horse and taurine at HSP 70 gene locus suggested that these animals had shared ancestral lineage and common evolution. Therefore, the degree of relatedness of HSP 70 gene established within four Nigerian breeds of cattle and those of selected mammalian species suggested that HSP 70 gene is conserved among wide range of animals and as such it can be used as bio-marker for marker assisted selection of thermo-tolerance in wide range of livestock animals under thermal assault.

Keywords: *HSP* 70 gene thermal stress, phylogenetic analyses, bio-marker, cattle

INTRODUCTION

The concern about the influence of environmental heat stress has increased in recent years with the realisation of global warming's influence on the environment and subsequently on animal production (West, 2003). Heat stress phenomenon has become a major issue in the era of climate change as its affect adaptability and survivability of livestock to thermal assault which is the consequent effect of global warming. Animals can succumb to hyperthermia if they fail to abate the impact of heat stress load. Heat shock protein 70 gene is a member of molecular chaperone families that are known to be highly expressed under stressful environmental and physiological conditions. This *HSP 70* gene is a member of *HSPs* sub-family which fulfill essential roles of providing cellular protection / cyto-protection, immune response, protein synthesis, cyto-skeletal protection, protein folding and unfolding, protein translocation and regulation of steroid hormone receptors, transportation, re-folding of protein, protection proteins from cellular stress, inhibitory apoptosis and adaptation during and after thermal assault (Hart *et al*., 2011; Kapila *et al*., 2013; Sodhi *et al*., 2013). This *HSP* 70 gene also act as molecular chaperons in regulating cellular homeostasis and folding-unfolding of damaged proteins during thermal assault or any other physiological stress, thereby conferring on stressed animals the adaptive capacity to cope under stressful environmental and physiological conditions (Kapila *et al*., 2013). Previous studies in Zebu cattle breeds suggested superiority of in adapting to the tropical climatic conditions compared to their European counterparts (Hansen, 2004). Several Zebu cattle are naturally adapted to different agro-climatic conditions of India (Kapila *et al*., 2013; Sodhi *et al*., 2013). The impact of heat stress must be ameliorated to maintain animal health status, adaptability, survivability and performance. Several

managemental strategies have helped to lower the stress in dairy and beef animals but to a limited extent (Kapila *et al*., 2013).

Therefore, this study intends to determine degree of relatedness and evolutionary relationship between four Nigerian breeds of cattle and some selected mammalian species at *HSP* 70 gene locus with a view to providing baseline genomic information for thermo-tolerance selection of tropical animals under thermal assaults.

MATERIALS AND METHODS

Sampling regions and experimental animals

A total of ninety (90) bulls from across four extant breeds of Nigerian Zebu cattle comprising of White Fulani (25), Sokoto Gudali (21), Red Bororo (21) and Ambala (23) sampled from northern parts of Nigeria (Figure 1) were examined in this present study. The animals originated from different herds (Plates 1-4) and were reared under the traditional extensive system where they grazed during the day on natural pasture containing forages such as Stylo (*Stylosanthes gracilis*), Leucaena (*Leucaena leucocephala*) and Guinea grass (*Panicum maximum*), crop residues and scavenged on kitchen wastes whenever available.

Skin tissue sample collection for genomic DNA isolation

From each of the 90 samples, 200 g of skin tissue sample was excised from each the animals prior to bleeding in the abattoir / slaughter house and same were sliced into less than 0.5 cm (1 mg weight) and were quickly submerged into 0.5 ml Eppendorf tubes containing RNA*later* reagent. Subsequently, they were transported for the duration of 3 to 10 hours with iced pack to laboratory and stored at -20^{o}C until further analyses. Genomic analyses including polymerase chain reactions (PCRs) were conducted in the Biotechnology Center Laboratory of Post Graduate Research Institute in Animal Science, Tamil Nadu University of Veterinary and Animal Sciences, Chennai, India

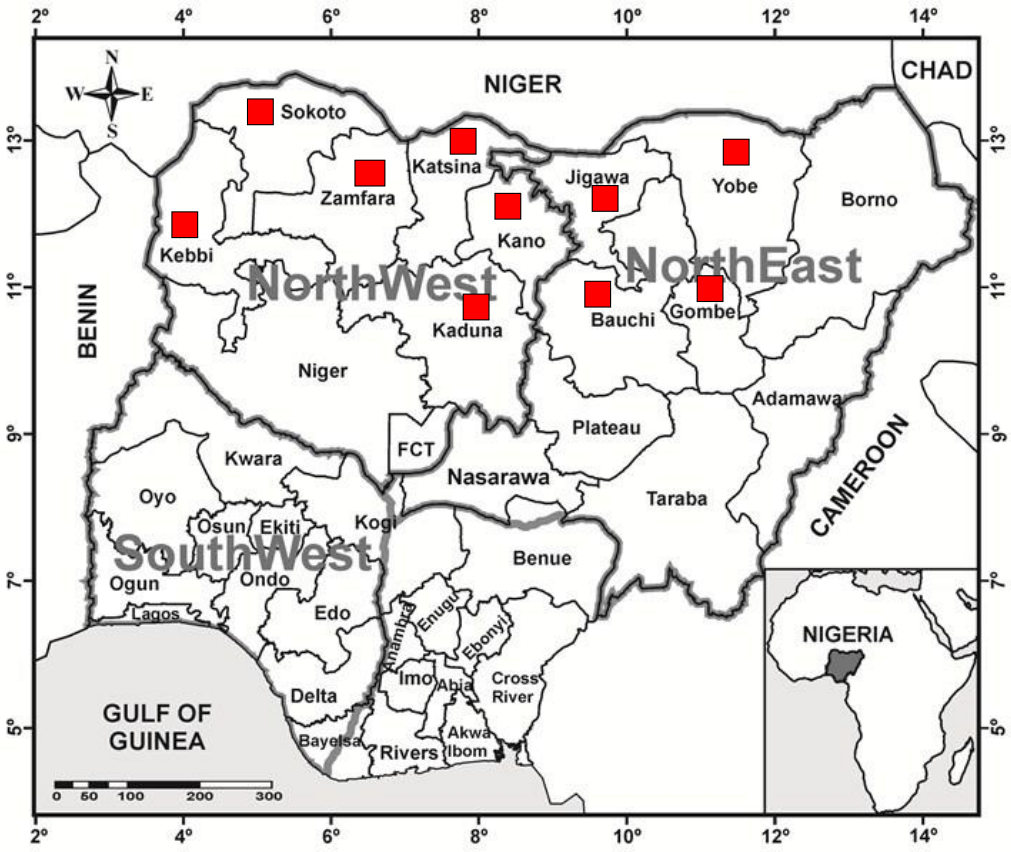

Figure 1. Map of Nigeria showing the regions of sampling

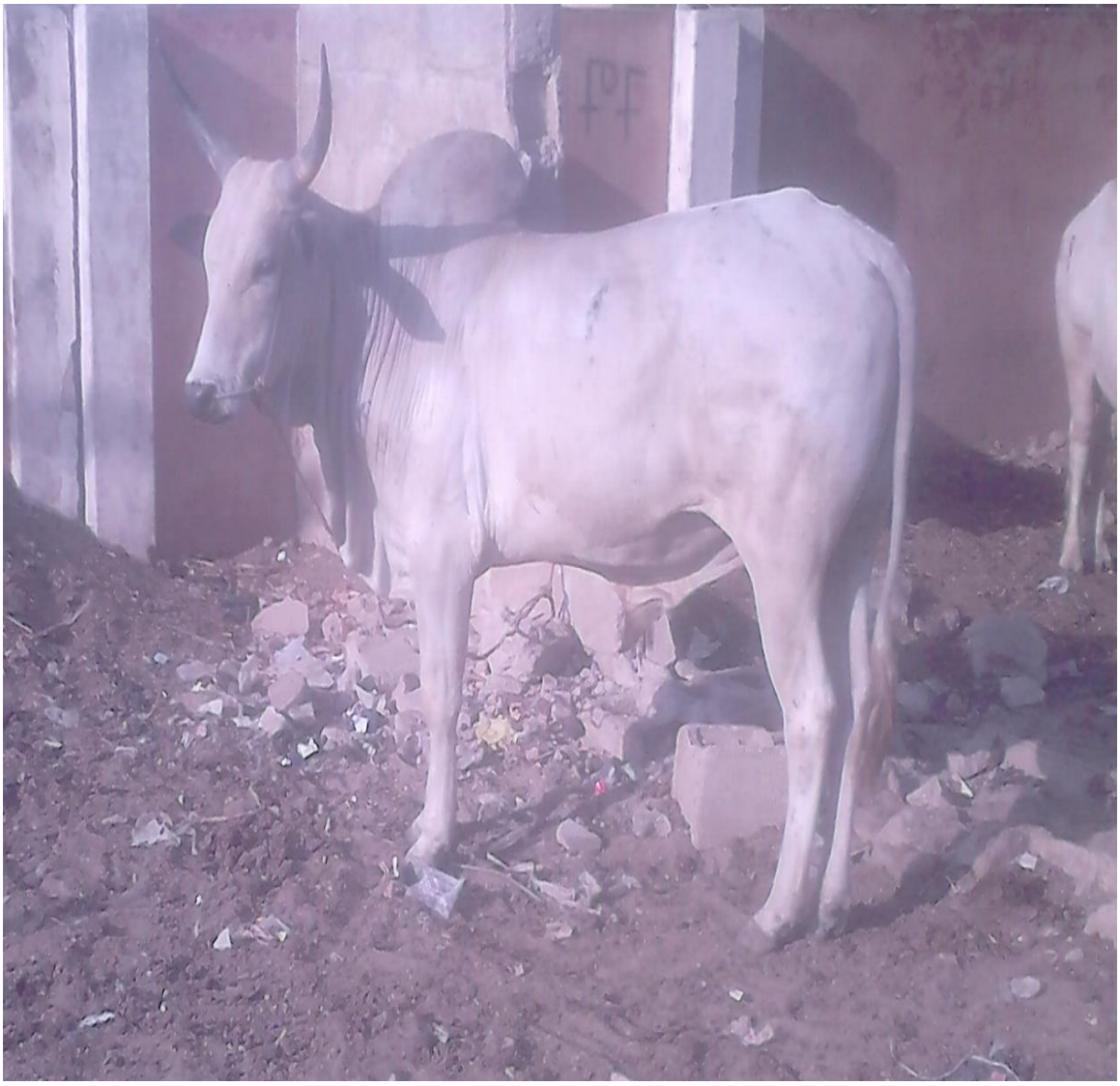

Plate 1 White Fulani cattle breed

Plate 2 Ambala cattle breed

Plate 3 Sokoto Gudali cattle breed

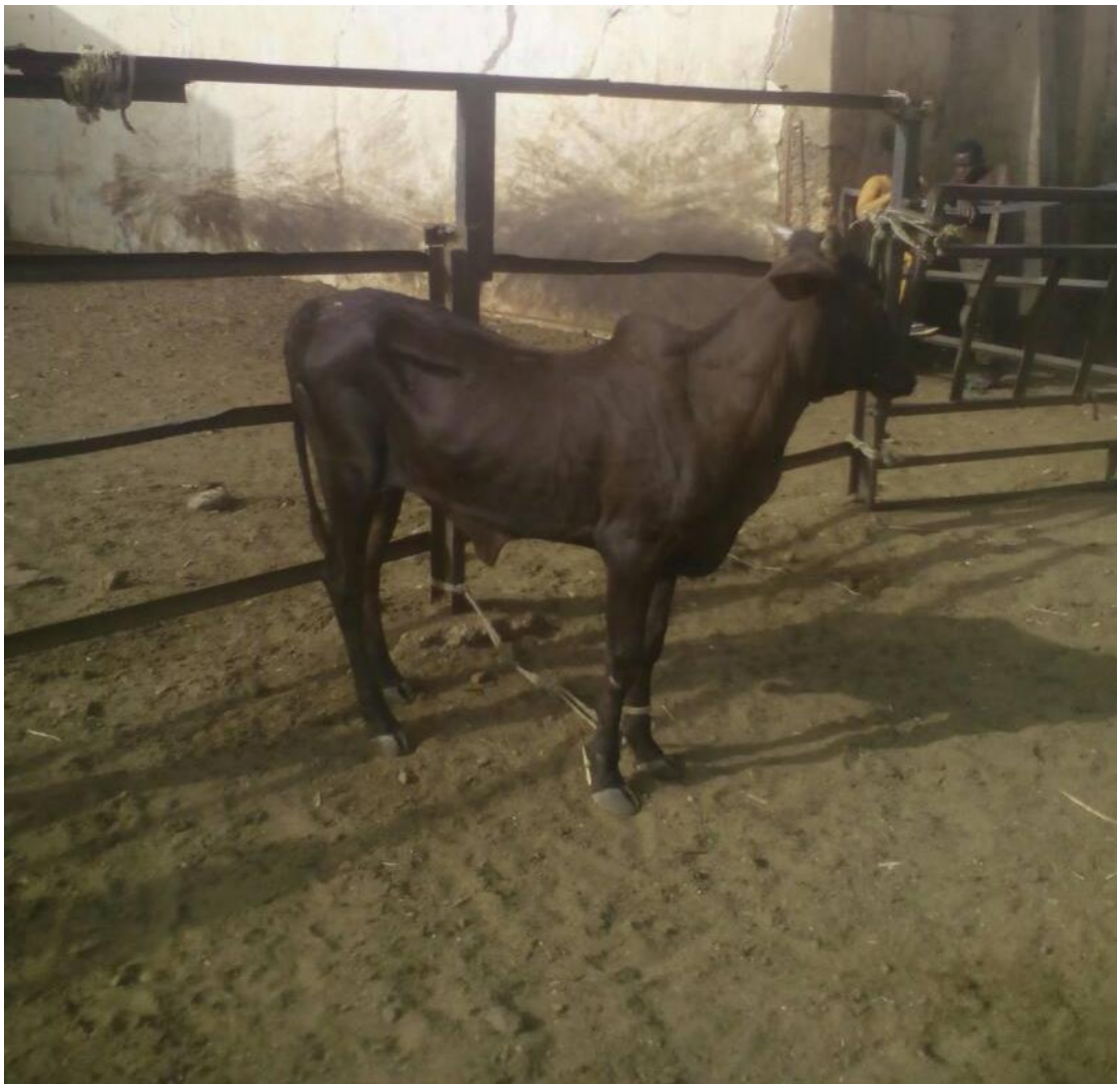

Plate 4 Red Bororo cattle breed

DNA extraction of *HSP* 70 gene from collected skin tissue samples

The protocol for DNA extraction employed for this study was according to HiPurA™ Multi-Sample DNA Purification procedure, MolBio™Himedia®, Mumbai, India. 25 mg of skin tissue was fetched from each of the 90 samples of skin tissue preserved in RNA*later,* the skin samples were weighed using sensitive scale and excised into smaller pieces and transferred into 2 ml collection tube. 180 ul of resuspension buffer was added into the 2 ml collection tube containing the excised skin tissue. Thereafter, 20 ul of proteinase K solution was added to the skin tissue in the 2 ml collection tube and was thoroughly mixed by vortexing for proper tissue digestion. Incubation of the samples was done using ACCUBLOCK™ digital dry bath at 55 ^{o}C for 2-4 hours until the tissue was completely digested with no residues. During incubation, the samples were mixed occasionally by vortexing. After digestion, the samples were vortexed briefly for 30 seconds.

The preparation of lysate (lysation) was done by measuring 200 ul of lysis solution and adding into the 2 ml collection tube containing the digested tissue. This was mixed by vortexing thoroughly for 15 seconds and subsequently incubated using ACCUBLOCK™ digital dry bath at 70 ^{o}C for 10 minute to generate lysate. The lysate was subsequently prepared for binding to the spin

column, 200 ul of ethanol (100%) was added to the lysate obtained and subsequently mixed thoroughly by gentle pipetting.

The thoroughly-mixed lysate obtained was transferred into the HiElute Miniprep spin column in the collection tube and centrifuged at 10000 rpm for 1 minute using Thermo Scientific Nanofuge (MCROCL 21/21R) micro-centrifuge. The flow–through liquid was discarded and the column was placed into a new 2 ml collection tube. 500 ul of dilute pre-wash solution (12 ml pre-wash + 18 ml ethanol) was added to the column containing the lysate and was centrifuged at 10,000 rpm for 1 minute using Thermo Scientific Nanofuge (MCROCL 21/21R) micro-centrifuge. The flow – through liquid was subsequently discarded. 500 ul of diluted wash solution (8 ml of wash solution + 24 ml of ethanol) was added to the column containing the lysate and was centrifuged at 13, 000 rpm for 3 minutes to dry the column using Thermo Scientific Nanofuge (MCROCL 21/21R) micro-centrifuge and the flow through liquid was discarded.

The empty column was again spinned at 13, 000 rpm for 1 minute using Thermo Scientific Nanofuge (MCROCL 21/21R) micro-centrifuge and the collection tube containing flow–through liquid was discarded. The column was then placed into a new 2 ml collection tube. After which 100 ul of elution buffer was pipetted directly onto the column obtained above without spilling to the sides or walls of the collection tube and was incubated for 5 minute at room temperature (15-25 ^{o}C). The above was then centrifuged at 10,000 rpm for 1 minute to elute the DNA into the collection tube using Thermo Scientific Nanofuge (MCROCL 21/21R) micro-centrifuge and the column was discarded. The eluted DNA was incubated at 90 ^{o}C to free the DNA of any contamination and was subsequently stored at -20 ^{o}C for further analyses. The quality and quantity of DNA was also estimated by Thermo Scientific-nano drop 2000 spectrophotometer (Shimadzu co-operation, Japan). The absorbance ratio between OD_{260} and OD_{280} ($OD_{260/280}$ (DNA purity) was observed for each sample. DNA sample with absorbance ratio of 1.9 was considered good and taken for further analysis.

Primer sequence and target regions

The *HSP70* gene primers set (Table 1) used for this study was obtained from the earlier works of Bhat *et al.* (2016) and was optimised for primer specificity. The fragment size for *HSP*70 gene was 295 bp covering coding region in exon 1.

Polymerase chain reaction and amplification condition for *HSP* 70 gene

The PCR reactions were carried out in a total volume of 15 µl containing template DNA of 1.0 µl, 1.0 µl of each of forward and reverse primers, PCR Master Mix (2x) (GeNei™ Red Dye PCR Master Mix) of 7.5 µl, and 4.5 µl of nuclease free water. PCR amplification was performed in a TaKaRa Thermal Cycler Dice™ version III (Takara Bio Inc., Japan). The amplification condition involved initial denaturation at 94 °C for 5 minutes, followed by 45 cycles of denaturation at 94 °C for 60 seconds, annealing temperature of 65°C for 45 seconds, extension at 72 °C for 1 minutes followed by final extension at 72 °C for 7 minutes. PCR products were evaluated using 2% agarose gel electrophoresis after staining with 1 ug/ml of ethidium bromide and the fragments were visualised under Bio-RAD Gel Doc™ XR+ Imaging System version 5.1 (Gel Documentation Molecular Imager, Bio-Rad Laboratories, Inc., U.S.A.). Subsequently the PCR products for *HSP* 70 gene were sequenced using an automated ABI DNA Sequencer (Eurofins Genomics Pvt. Ltd., Bangalore, India).

Table 1 Heat Shock protein 70 gene primer sequence and target region (181...475)

HSP 70 Sequence (5'-3')	No. of bases	Targeted region	Amplicon size
F-AAACATGGCTATCGGCATCGACCT	24	Exon 1	295bp
R-AGGCTTGTCTCCGTCGTTGATGA	23		

Source: Bhat *et al*. (2016)

DNA extraction and isolation of *HSP70* gene in four Nigerian Zebu cattle as revealed polymerase chain reaction and gel electrophoresis analyses

Plate 5 shows amplified region of *HSP70* gene of 295 bp at coding region of exon 1 in four Nigerian Zebu cattle breeds

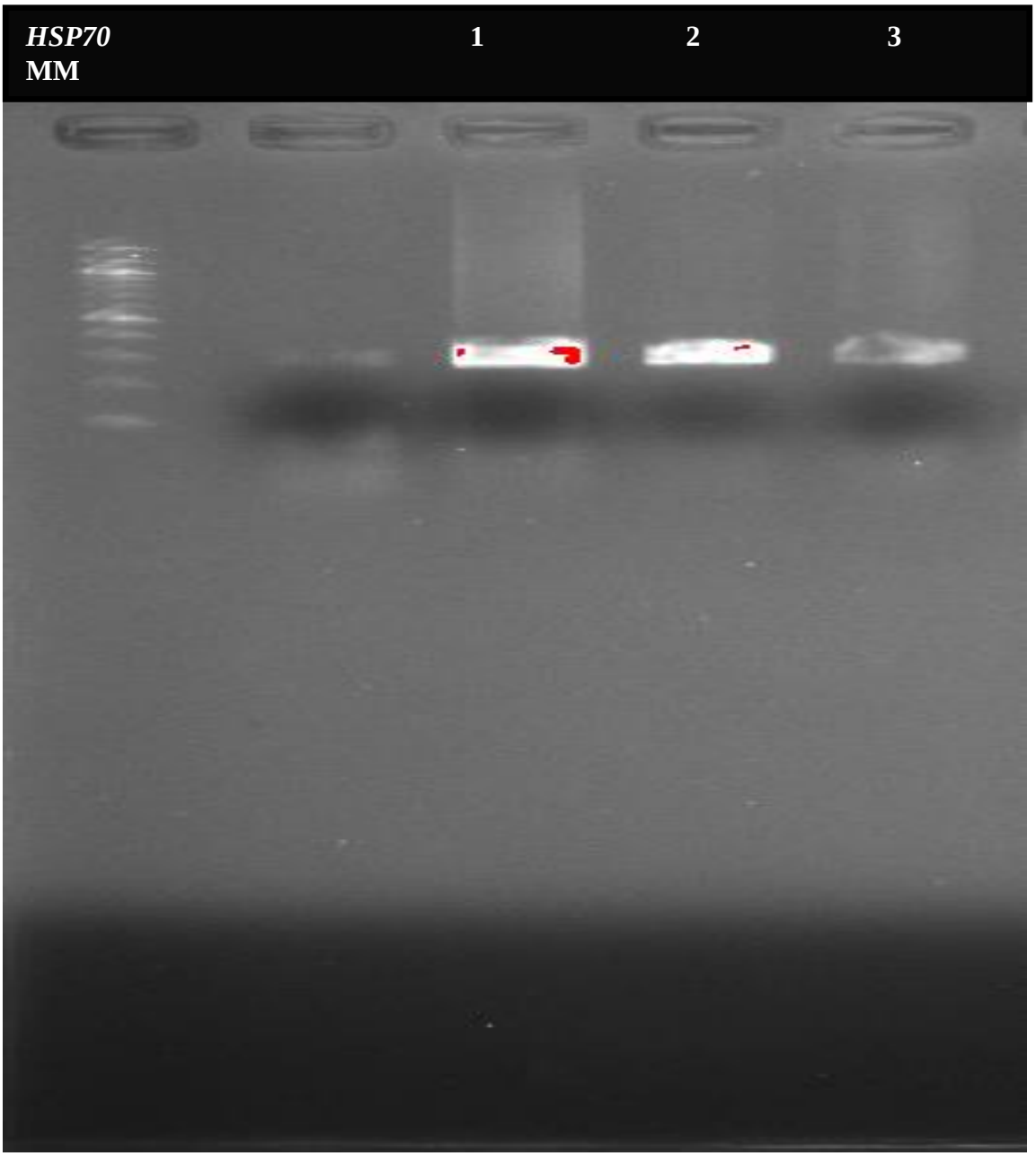

Plate 5 Gel bands of amplified fragment of *HSP70* gene (295 bp)

Lane 1= MM= Molecular Marker
Lanes 1-3: Amplified fragment of *HSP70* gene 295 bp

Phylogenetic analysis of *HSP* 70 gene of four Nigerian Zebu cattle breeds and some selected mammalian species

Rooted phylogenetic tree was constructed from nucleotide sequences of *HSP* 70 gene of four Nigerian Zebu breeds of cattle (White Fulani (WF), Ambala (AM), Sokoto Gudali (SG) and Red Bororo (RB) and GenBank-downloaded sequences of some selected mammalian species using MEGA 5.2 software to evaluate the degree of relatedness and ancestral evolution of *HSP* 90 nucleotide sequences of four Nigerian *B. indicus* and that of selected mammalian species (Tamura *et al.,* 2011).

Phylogenetic analysis of *HSP* 70 gene in four Nigerian Zebu breeds of cattle

Neighbour-joining dendrogram of the nucleotide sequences of *HSP70* gene of four Nigerian Zebu *Bos indicus* (White Fulani cattle breed, Ambala cattle breed, Sokoto Gudali cattle breed and Red Bororo cattle breed showed shared clade (Figure 2).

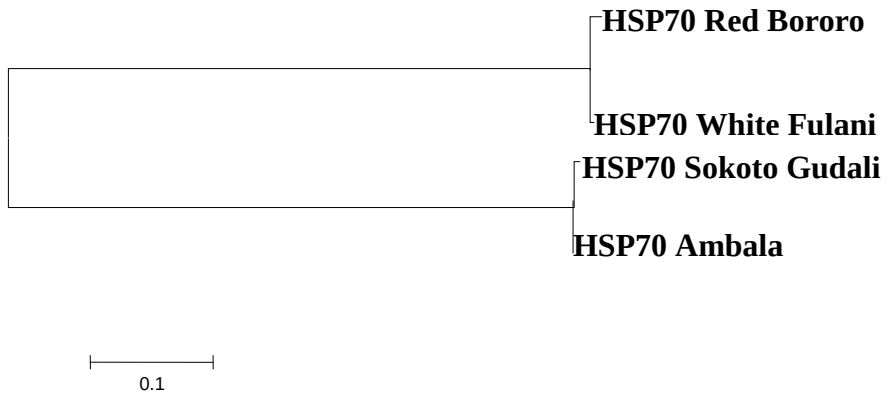

Figure 2: Neighbour-joining dendrogram of *HSP70* sequences of White Fulani. Ambala, Sokoto Gudali and Red Bororo Nigerian Zebu breeds of cattle.

Phylogenetic analysis of *HSP70* gene in four Nigerian Zebu breeds of cattle and selected mammalian species

A rooted phylogenetic tree of *HSP70* nucleotide sequences of Nigerian Zebu breeds of cattle (White Fulani. Ambala, Sokoto Gudali and Red Bororo) and selected mammalian species showed shared cluster and homology (Figure 3)

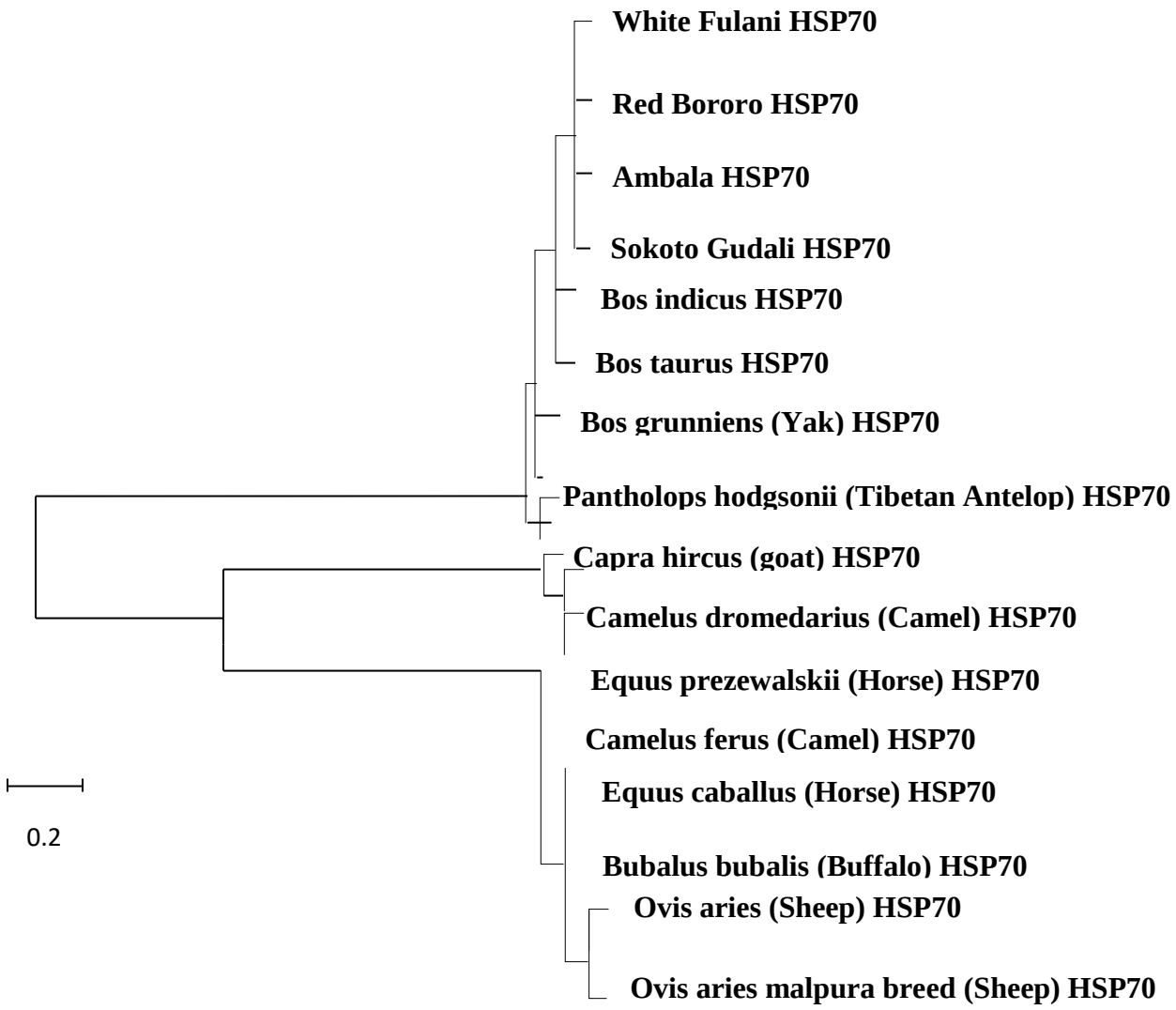

Figure 3: Neighbour-joining dendrogram of *HSP70* sequences of four Nigerian Zebu breeds of cattle (White Fulani, Ambala, Sokoto Gudali and Red Bororo) and selected mammalian species

DISCUSSION

The rooted evolutionary study based on Neighbour-joining dendrogram of *HSP70* sequences of White Fulani, Ambala, Sokoto Gudali and Red Bororo Nigerian Zebu breeds of cattle revealed *HSP* 70 sequences of four Nigerian cattle breed showed shared cluster. Similarly, sequences of *HSP70* in four Nigerian *B. Indicus* and those of goat, sheep, yak, buffalo, camel, horse and taurine demonstrated common clade architecture, therefore suggesting evolution from a common ancestor (Gade *et al*., 2010). This confirms the earlier works of Gade *et al*. (2010) who reported that *HSP70* gene of mammalian species showed high degree of relatedness. Pelham (1982) reported that *HSP* 70 proteins were highly conserved both in protein-coding sequence and in regulatory sequence with common homology. Gutierrez and Guerriero (1995) found that amino acid sequences of *HSP70* gene were highly conserved among *HSPs* sub-family. The degree of relatedness of nucleotide sequences of *HSP* 70 gene established within four Nigerian breeds of cattle and those of selected mammalian species suggested that *HSP* 70 gene is conserved among wide range of animals (Pelham, 1982; Sodhi *et al*., 2012; Kapila *et al*., 2013; Wang *et al*., 2015) and such this candidate gene can be used as bio-marker for thermo-tolerance selection of animal under thermal assault during.

CONCLUSION

The shared homology of *HSP70* gene in four Nigerian cattle breeds is an implication of high nucleotide sequences similarity which is indicative of common ancestral lineage. Similarly, shared cluster architecture between *HSP* 70 gene of four Nigerian *Bos indicus* and some selected mammalian species e.g. goat, sheep, camel, horse, yak, buffalo etc. is suggestive of shared evolution and ancestry. Therefore the degree of relatedness of nucleotide sequences of *HSP* 70 gene established within four Nigerian breeds of cattle and those of selected mammalian species suggested that *HSP* 70 gene is conserved among wide range of animals and as such it can be used as bio-marker for marker assisted selection for thermo-tolerance in wide range of livestock animals under thermal assault.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to the government of India who funded this research work through the Research Fellowship for Developing Countries Scientist (RFD-CS) offered to me. I am grateful to Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, India, my host University for giving me unfettered access to Central Biotechnology laboratory, Post Graduate Research Institute in Animal Science Kaattupakkum, Chennai, Tamil Nadu, India; Department of Veterinary Parasitology laboratory, Madras Veterinary College, Chennai, Tamil Nadu, India; Central University Laboratory, TANUVAS, Madhavaram Milk Colony, Chennai, Tamil Nadu, India, Mecheri Sheep Research Station, Pottaneri District Salem and Veterinary Science and Research Institute, Namakkal, Chennai, Tamil Nadu, India. I am sincerely grateful to Prof. A. K. Thiruvekandar, Prof. C. Sreekumar, Prof. K. G. Tirumurugan of TANUVAS, Chennai, India for their immense contribution towards the success of this research work.

REFERENCE

Bhat S., Kumar P., Kashyap, N., Deshmukh B, Dige, M. S., Bhushan, B., Chauhan, A., Kumar A. and Singh, G. (2016) Effect of heat shock protein 70 polymorphism on thermotolerance in Tharparkar cattle, *Veterinary World.* 9(2): 113-117. doi: 10.14202/vetworld.2016.

Gade, N., Mahapatra, R. K., Sonawane, A., Singh, V. K., Doreswamy, R. and. Saini, M. (2010). Molecular Characterization of Heat Shock Protein 70-1 Gene of Goat (*Capra hircus*). *Molecular Biology International*. 7: 1-7 doi:10.4061/2010/108429.

Gutierrez, J. A. and Guerriero, V. Jr. (1995). Chemical modifications of a recombinant bovine stress-inducible 70 kDa heat-shock protein (Hsp70) mimics Hsp70 isoforms from tissues. *Biochemical Journal* 305 (1): 197–203.

Hansen, P. J. (2004). Physiological and cellular adaptations of Zebu cattle to thermal stress.

Animal Reproductive Science. 82–83: 349–360.

Kapila, N., Kishore, A., Sodhi, M., Sharma, A., Kumar P., Mohanty, A. K., Jerath, T. and Mukesh, M. (2012). Identification of appropriate reference genes for qRT-PCR analysis of heat stressed mammary epithelial cells in riverine buffaloes (*Bubalus bubalis*). *International Journal of Animal Biotechnology*. 3: 10-19. http://dx.doi.org/10.5402/2013/735053

Kapila, N., Kishore, A., Sodhi, M., Sharma, A., Mohanty, B., Kumar P., and Mukesh, M. (2013). Temporal changes in mRNA expression of heat shock protein genes in mammary epithelial cells of riverine buffalo in response to heat stress in vitro. *International Journal of Animal Biotechnology*. 3: 5-9.

Kishore, A., Sodhi, M., Khate, K., Kapila, N., Kumari, P., Mukesh, M. (2013). Selection of stable reference genes in heat stressed peripheral blood mononuclear cells of tropically adapted Indian .

Pelham, H. R. B. (1982). A regulatory upstream promoter element in the Drosophila *HSP 70* heat-shock gene. *Cell*. 30 (2): 517–528.

Sodhi, M., Kishore, M., Khate, K., Kapila, N., Mishra, B. P., Kataria, R.S., Mohanty, A. K., Varshney, N. and M. Mukesh, M. (2012). Evaluating suitable internal control genes for transcriptional studies in heat-stressed mammary explants of buffaloes. *Journal of Animal Breeding and Genetics.* 931-2668

Sodhi, M, Mukesh, M., Kishore, A., Mishra, B. P. and Joshi, B. K. (2013). Novel polymorphisms in UTR and coding region of inducible heat shock protein 70.1 gene in tropically adapted Indian Zebu cattle (*Bos indicus*) and riverine buffalo (*Bubalus bubalis*). *Gene.* 527: 606–615

St. Louis, USA ABI Prism® Big Dye™Terminator Cycle sequencing kit (Applied Biosystem, Foster City, CA).

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*. 28: 2731–2739.

Wang, W. and Shi, C. (2009). Molecular cloning, distribution and ontogenetic expression of the oligopeptide transporter PepT1 mRNA in Tibetan suckling piglets. *Amino Acids* 37 (4): 593–601.

Wang, L., Yang, S., Han, L., Zhao, K. and Ye, L. (2015). Expression Profile of Two HSP70 Chaperone Proteins in Response to Extreme Thermal Acclimation in *Xestia c-nigrum* (Lepidoptera: Noctuidae). *Florida Entomologist*, 98(2): 506-515.doi.org/10.1653/024.098.0218

West, J. W. (2003). Effects of heat stress in dairy cattle. *Journal of Dairy Science*. 86: 2131–2144.