University of Zambia Journal of Agriculture and Biomedical Sciences *JABS 2021:5 (1)*

Identification of MHC Alleles Associated with Disease Resistance/ Susceptibility In Smallholder Cattle In Zambia

Isaac Kombe Silwamba[1*], Martin Simuunza[2], King Nalubamba[4], Joseph Ndebe[2], Edgar Simulundu[2], Geoffrey Mainda[3], John Bwalya Muma[2]

[1*] Livestock Laboratory, Department of Technical Services, Livestock Services Cooperative Society,
Pamodzi Highway, Showgrounds, Lusaka, Zambia
[2]Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia
[3]Department of Veterinary Services, Ministry of Fisheries and Livestock, Lusaka, Zambia
[4]Department of Clinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia

***Corresponding author**
Isaac Kombe Silwamba, Mailing address: Livestock Laboratory, Department of Technical Services, Livestock Services Cooperative Society| Stand No. 2375, Pamodzi Highway, Off Nangwenya Road| Show Grounds| P.O. Box 32025| Lusaka, Zambia| Mobile: +260 977 628318 |Email: isaacsilwambak@gmail.com; Alternative Email: isaacs@livestock.co.zm

***DOI**: https://doi.org/10.53974/unza.jabs.5.1.494*

Abstract

Background: The occurrence of Major Histocompatibility Complex (MHC) alleles associated with resistance to Mastitis, Bovine Leukaemia Virus (BLV), Theileriosis, Foot and Mouth Disease (FMD) and susceptibility Dermatophilosis in African cattle is ill defined.

Methods: The researchers used manual annotation to screen for five MHC alleles previously known to be associated with resistance/susceptibility to these diseases from a database of alleles sequenced from 846 cattle in Zambia.

Results: Overall, the researchers found twenty-eight (3.3%), twenty-one (2.6%), fifty-five (6.5%), and fifteen (1.8%) animals with resistance alleles to Mastitis, BLV, Theileriosis, FMD; and thirty-nine (4.6%) animals with susceptibility alleles to Dermatophilosis, respectively.

Conclusion: This study provides the first evidence of resistance/susceptibility alleles in smallholder cattle in Zambia and the data could aid strategies for breeding cattle with enhanced resistance to disease in endemic countries.

Keywords: *major histocompatibility complex; bovine leukaemia virus; foot and mouth disease; mastitis; theileriosis*

Introduction

Smallholder livestock production centres, on small to medium family farming systems are key to poor rural people's livelihoods, food security, incomes and poverty alleviation. In Zambia, smallholder livestock production accounts for approximately 80 per cent of the total livestock production and contributes about 45 per cent to smallholder household income [1]. This sector involves farmers who mainly rear traditional cattle breeds (Angoni, Barotse, Tonga) and some crosses with exotic breeds under extensive production management systems. As of the year 2019, Zambia's cattle population was estimated at 3,714,667 with Southern, Eastern, Central, and Western provinces having the highest cattle population [2]. According to the Food and Agriculture Organisation, high prevalence of infectious diseases, high mortality rates and lack of adequate capacity to control diseases are among the major challenges faced by livestock farmers in low- and middle-income countries (LMICs)

[3]. Constrained in this way, livestock producers are thrown into a vicious cycle of low productivity and low incomes.

The Major Histocompatibility Complex (MHC), also known as Bovine Leukocyte Antigen (BoLA) found on chromosome 23 of cattle, is known to contain alleles that are associated with resistance/susceptibility to a number of cattle diseases that cause serious detrimental effects. Therefore, utilisation of natural strategies such as breeding for disease resistance using animals with inherent disease resistance genes is crucial in efforts aimed at reducing animal disease burden, associated mortality rates, and farmer over reliance on government support as well as other costly disease control measures. Evidence from other studies, has shown that genetic information on alleles can be transferred from chromosomes of parent animals to offspring when bred [4]. Thus, exploitation of alleles that have potential to confer resistance to diseases may assist in efforts to design strategies for disease control and development of animal breeding plans, aimed at producing cattle that are more disease resistant. Further, information on genetic determinants such as MHC that have potential to be used as markers for disease resistance/susceptibility in specific animal populations is crucial for speeding up efforts to include genetic resistance in breeding programmes. The aim of this study was to identify MHC class II alleles, previously known to be associated with resistance/susceptibility to diseases in populations of cattle reared by rural smallholder farmers in Zambia.

Materials and Methods

The dataset used in this study was obtained under permission from a study conducted between 2017 and 2020 on 846 smallholder cattle in Zambia. Ethical clearance was sought from and granted by Excellence in Research Ethics and Science (ERES) Ref. No 2018-Jan-009. Clearance on the Nagoya protocol was sought and granted through the ministry responsible for natural resources in Zambia. The researchers applied manual annotation to that dataset to screen for five MHC alleles previously known to be associated with disease resistance/susceptibility. The sample was composed of indigenous cattle breeds reared by rural smallholder farmers in respective provinces namely: Tonga, Barotse and Angoni of Southern, Western and Eastern provinces respectively. The indigenous cattle breeds were identified using individual phenotypic traits and the herds within each province were formed by pooling together small 'single-owner' cattle groups, managed under traditional farming system conditions. Laboratory methods and bioinformatic analyses, that were applied to generate the dataset for the present study, were essentially published by Vasoya and co-workers [5] with few modifications. Briefly, cattle blood was collected by jugular venipuncture into EDTA tubes and peripheral blood mononuclear cells (PBMCs) extracted using ammonium chloride lysis method in which erythrocytes were lysed by incubation in 5× volume of erythrocyte lysis buffer (0.144 M ammonium chloride/0.0175 M Tris pH 7.4) for 5 min at room temperature. The white blood cell (WBC) pellet was washed three times in Phosphate Buffered Saline (PBS). Total RNA was extracted using Tri-reagent(Sigma, Gillingham, UK) while cDNA was generated using the Go script Reverse Transcription Kit (Promega, Madison, WI, USA). In both protocols, the researchers followed the manufacturers' instructions. The cDNA from individual animals was subjected to PCR amplification for the DRB3, DQA and DQB MHC class II genes using a series of gene specific 3′ (for) and 5′ (rev) pan-MHC class II primers (Data not shown), designed to specifically amplify the highly polymorphic regions of DRB3, DQA, and DQB genes within the BoLA class II locus. Following amplification, the PCR products were purified and submitted to Edinburgh Genomics (University of Edinburgh) for next generation sequencing on the Illumina MiSeq v3 platform. Analysis of sequence data was performed with bioinformatic scripting/ tools (FastQC, Flash, Fastx, BLAST, and Perl) essentially as published by Vasoya and coworkers [5] and the resulting data stored in excel spreadsheets.

Table 1: Sample structure of the dataset analysed in this study (N = 846)

Variable	**n (%)**
Sex	
Male	210 (24.8)
Female	636 (75.2)
Age group (yrs)	
< 1	146 (17.3)
1 – 5	420 (49.6)
> 5	254 (30.0)
Unknown	26 (3.1)
Herd status	
Born within herd	543 (64.2)
Brought in	303 (35.8)
Twin	
Yes	0 (0.0)
No	846 (100)
Sampling sites: Province (District)	
Southern (Mazabuka and Namwala)	347 (41.0)
Western (Senanga)	269 (31.8)
Eastern (Lundazi)	230 (27.2)

Table 2: Prevalence and distribution of MHC class II alleles associated with disease resistance/ susceptibility in small holder cattle in Zambia

Disease	Allele	Type of association	Overall allele Prevalence (N = 846)	Eastern province (Angoni cattle) (n = 230)	Western province (Barotse cattle) (n = 269)	Southern province (Tonga cattle) (n = 347)
Mastitis	BoLA-DRB3*1201 [6]	Resistance (Natural)	28 (3.3%)	16 (7.0%)	3 (1.1%)	9 (2.6%)
BLV	BoLA-DRB3*0902 [7]	Resistance (Natural)	21 (2.5%)	14 (6.1%)	3 (1.1%)	4 (1.2%)
FMD	BoLA-DRB3*1001[8]	Resistance (High responses to vaccination)	15 (1.8%)	0 (0.0%)	12 (4.5%)	3 (0.6%)
Theileriosis	BoLA-DRB3*1501[9]	Resistance (High responses to vaccination)	55 (6.5%)	24 (10.4%)	27 (10.0%)	4 (1.2%)
Dermatophilosis	BoLA-DQB*1802 [10]	Susceptibility	39 (4.6%)	3 (1.3%)	17 (6.3%)	19 (5.5%)

Results and Discussion

The sample structure of the dataset used in this study comprised of 846 animals (of which 75.2 per cent were females) from Southern (n=347), Western (n=269) and Eastern (n=230) Provinces of Zambia (Table 1). The majority (49.6%) of animals were within their productive age group of approximately one to five years and large proportions (64.2%) of these were born within the herd potentially increasing the likelihood of gene/allele localisation. The study provides the first genetic evidence for the presence of five MHC class II alleles previously known to be associated with disease resistance/ susceptibility in Zambian cattle herds. Distribution of theses alleles was variable and the overall prevalence Table 2 of alleles associated with resistance to Mastitis (BoLA-DRB3*1201), Bovine Leukaemia Virus (BoLA-DRB3*0902), FMD (BoLA-DRB3*1001), Theileriosis (BoLA-DRB3*1501) and susceptibility to Dermatophilosis (BoLA-DQB*1802) was 3.3 per cent (28/846), 2.5 per cent (21/846), 1.8 per cent (15/846), 6.5 per cent (55/846) and 4.6 per cent (39/846), respectively. In Eastern province, alleles associated with resistance to Mastitis, Bovine Leukaemia Virus

(BLV), Theileriosis and FMD were found in 7.0 per cent (16/230), 6.1 per cent (14/230), 10.4 per cent (24/230) and 0 (0.0%) of animals, respectively. In Western province, alleles associated with resistance to Mastitis, FMD, BLV and Theileriosis were detected in 1.1 per cent (3/269), 4.5 per cent (12/269), 1.1 per cent (3/269) and 10.0 per cent (27/269) of animals, respectively. In Southern province, the resistance alleles for these conditions were identified in 2.6 per cent (9/347), 0.6 per cent (3/347), 1.2 per cent (3/347) and 1.2 per cent (4/347) of animals, respectively. The alleles associated with susceptibility to Dermatophilosis were found in 6.3 per cent (17/269), 5.5 per cent (19/347), and 1.3 per cent (3/230) of animals in Western, Southern and Eastern provinces, respectively.

Of the three provinces studied, Eastern province had the highest number of animals with resistance alleles to Mastitis and BLV. Western province had the highest number of animals with resistance alleles to FMD and Theileriosis. Southern province had the highest number of animals with resistance alleles to Dermatophilosis. The lowest number of resistance alleles to Mastitis and BLV were found in Western province and the lowest numbers of resistance alleles to Theileriosis were found in Southern Province. Eastern province had the lowest number of susceptibility alleles to Dermatophilosis and no resistance alleles to FMD.

The geographical distribution for these alleles presented a pattern like relationship to known prevalence of diseases. For instance, the prevalence of Dermatophilosis disease is known to be high in Southern Province [11] where we found a large number of alleles associated with susceptibility to Dermatophilosis. Similarly, the prevalence of BLV is known to be low in Eastern province [12] and we found a large number of resistant alleles to BLV in this region. However, this relationship of disease prevalence and allelic distribution exclusively based on the findings of this study should be interpreted with caution because our sampling depth was low and we still cannot explain why cattle from Eastern and Western provinces, despite having significantly high numbers of resistance alleles to Theileriosis; are still known to record large numbers of Theileriosis cases in Zambia [13] .

A striking feature observed in this study was the repeated co-occurrence of alleles associated with disease resistance/ susceptibility to BLV (DRB3*0902) and Theileriosis (BoLA-DRB3*1501) with other unknown alleles (i.e. BoLA-DRB3*0902 with BoLA-DQA*0301 and BoLA-DRB3*1501 with BoLA-DQB*1301). Unfortunately, with this data alone, the researchers could not clearly tell whether such allele co-occurrences had a functional implication on disease resistance or susceptibility. Therefore, it would be of interest to determine the extent, if any, to which these co-existing alleles at MHC loci contributed to variations in disease resistance or susceptibility. Despite the huge possibility of breeding cattle for resistance to diseases, using marker alleles in molecular marker-assisted selective breeding programmes, there is still lack of information on application of genetic methods involving MHC alleles to breed cattle for disease resistance. An example of a study that demonstrated possibility of producing livestock that is genetically resistant to disease was done by Whitworth and colleagues [14] who reported to have successfully produced a litter of pigs that are genetically resistant to a deadly porcine virus. Since Mastitis, BLV, FMD, Theileriosis and Dermatophilosis are still among the most common disease problems affecting efficiency and viability of cattle production in many countries, more studies in the context of MHC are still needed in order to speed up efforts to include genetic resistance in breeding programmes [15,16,17]. Although our study reports the first evidence of the presence of MHC class II alleles, associated with disease resistance/ susceptibility in rural smallholder cattle in Zambia, the data generated in this present study has some limitations. These include low or biased sampling and lack of genetic characterisation of the indigenous cattle breed studied. Therefore, the data generated cannot translate into meaningful breeding programmes to genetically breed for disease resistance. Nonetheless, our data provides a useful resource of information to aid breeding strategies in the future. Other future studies aiming to generate data for breeding programmes should make an effort to genetically characterise the breeds for proper identification.

Acknowledgments

The Centre for Tropical Livestock Genetics and Health (CTLGH) financially supported the laboratory work for the dataset used in this work.

Conflict of Interest Statement

The authors declare no conflict of interest.

References

1. Lubungu M, and Mofya R, The status of the smallholder livestock Sector in Zambia. 2012; Technical Report.
2. Ministry of Fisheries and Livestock, Central Statistical Office; the 2017/2018 Livestock and aquaculture census. Summary Report 2019; Lusaka Zambia.
3. Food and Agriculture Organization of the United Nations, Economic analysis of animal diseases. Animal Production and Health Guidelines. No. 18. 2016; Rome Italy.
4. Oldenbroek K, and Liesbeth van der W, Textbook animal breeding Animal breeding and genetics for BSc students. Centre for Genetic Resources and Animal Breeding and Genomics Group. Wageningen University and Research Centre, 2014; the Netherlands. Groen Kennisnet. p. 46.
5. Vasoya D, Law A, Motta P, Yu M, Muwonge A, Cook E, et al. Rapid identification of bovine MHCI haplotypes in genetically divergent cattle populations using next-generation sequencing, Immunogenetics. 2016; 68:765–781.
6. Kulberg S, Heringstad B, Guttersrud O, Olsaker I, Study on the association of BoLA-DRB3.2 alleles with clinical mastitis in Norwegian Red cows. *Journal of Animal Breeding and Genetics* 2007; 124 [4] 201–207.
7. Xu A, van Eijk MJ, Park C, and Lewin HA, Polymorphism in BoLA-DRB3 exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukaemia virus. *Journal of Immunology* 1993; 6977-85.
8. Baxter R, Craigmile SC, Haley C, Douglas AJ, Williams JL, and Glass EJ, BoLA-DR peptide binding pockets are fundamental for foot-and-mouth

disease virus vaccine design in cattle. Vaccine 2009; 28, 28–37.

9. Ballingall K, Luyai A, Rowlands S J, Musoke A, Morzaria S, and Mckeever D, Bovine Leukocyte Antigen Major Histocompatibility Complex Class II DRB3*2703 and DRB3*1501 Alleles Are Associated with Variation in Levels of Protection against Theileria parva Challenge following Immunization with the Sporozoite p67 Antigen. Infection and Immunity. 2004; 72. 2738-41.
10. Maillard JC, Chantal I, Berthier D, Stachurski F, and Elsen JM, Molecular markers of genetic resistance and susceptibility to bovine dermatophilosis. Archives of Animal Breeding 1999; 42 93–96.
11. Samui KL and Hugh-Jones ME, The prevalence of bovine dermatophilosis in zambia proceedings of the 5th International Symposium on Veterinary Epidemiology and Economics. Acta Veterinaria Scandinavia, 1988; Supplementum 84.
12. Phiri MM, Kaimoyo E, Changula K, Silwamba I, Chambaro HM, Kapila P, et al., Molecular detection and characterization of genotype 1 bovine leukemia virus from beef cattle in the traditional sector in Zambia. Archives of virology 2019; 04350-6
13. Nambota A, Samui K, Sugimoto C, Kakuta T, and Onuma M, Theileriosis in Zambia. Aetiology, epidemiology and control measures. *Japanese Journal of veterinary research* 1994; 42.1.1
14. Whitworth KM, Rowland RR, Petrovan V, Resistance to coronavirus infection in amino peptidase N-deficient pigs. Transgenic Research; 2018; DOI: 10.1007/s11248-018-0100-3
15. World organisation for animal health, listed diseases, infections and infestations in force. 2019; https://www.oie.int/animal-health-in-the-world/oie-listed-diseases.
16. Food and Agriculture Organization of the United Nations, Impact of mastitis in small scale dairy production systems. Animal Production and Health Working Paper. 2014; No. 13. Rome Italy.
17. Samui KL and Hugh-Jones ME, The financial and production impacts of bovine dermatophilosis in Zambia. Veterinary Research Communications. 1990; [14] 357.