RESEARCH PROPOSAL

JICHIDZA HIGH SCHOOL

MARCH 1, 2019

NGBS

NEXT GENERATION OF BIOMEDICAL SCIENTISTS

Research Proposal

Title: Combating cattle rustling in the Zaka district through the establishment of a cattle DNA profiles Database for animal tracing and identification

Study Team: The Jichidza High School Science Team composed of the following members.

Students

Name	Surname	Class	Sex
Takudzwa T	Mutote	Lower 6	Male
Munyaradzi	Masvata	Lower 6	Male
Nyashadzashe	Chisanhu	Lower 6	Male
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Pride	Nyoka	Form 3	Male
Primrose	Simbi	Lower 6	Female
Prejudice	Wapomba	Lower 6	Female
Persistence P	Bangamuseve	Form 3	Female
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Charmaine T	Balani	From 3	Female

Teachers and Community Members

Name	Role	Contact
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Nyamande T	Female Teacher	0772461389
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Subject matter Specialists & Team Mentors

Name	Institution	Role	Contact
Hlekiwe Mawere	Veterinary Service	Veterinarian	0777307150
Dr. Roslyn Thelingwani	AiBST	Forensic Scientist	0773817401
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1.0 RESEARCH QUESTION

Can cattle DNA databases be useful in cabbing the challenge of cattle rustling in the Zaka district.

The null hypothesis being H_0: Cattle DNA Databases will not be helpful in reducing cattle theft in the Zaka district.

The alternative hypothesis being H_1: Cattle DNA Databases will significantly reduce cattle theft in the Zaka district

2.0 RATIONALE FOR RESEARCH

Cattle play a pivotal role in the health and economics of people at individual and national levels. They are a major source of meat for the provision of protein in our diet. Their skins are then used to produce numerous products including shoes and jackets. In Zimbabwe and other developing societies, they are also a society of labour in ploughing fields and carrying heavy loads. Cattle are also a symbol of cultural wealth as they are used as part of lobola in marriage. For some rural communities, their sale provides the money required for family sustenance including paying for children's school fees. Cattle are therefore central to the social and economic status of individuals, families, communities and nations. This makes cattle theft a serious offence associated with various measures of deterrent legal consequences. In Zimbabwe, theft of one beast can attract up to 9 years imprisonment .The Herald Masvingo Correspondent reported that a 19 year old man from Nyajena appeared before Masvingo Provincial Magistrate Mr Langton Ndokera, who jailed him for jailed 25 years for stock theft (Herald Newspaper, 2019).

Despite such severe legal consequence, cattle theft is still rampant in Zimbabwe. It is reported that approximately 140 cattle are stolen every year and about 23 perpetrators were apprehended and imprisoned (according to statistics courtesy of ZRP, Zaka district). In Zaka District, 133 cattle were stolen in the year 2018 (ZRP Zaka) making it a serious challenge that requires a solution as shown in Table 1 below.

Table 1: Stock Theft Report for 2018 (courtesy of ZRP, Zaka district)

January	February	March	April	May	June	July	August	September	October
11	2	0	1	6	19	25	27	24	17

Zaka District has an estimated 105 000 number of cattle owned by an estimated 31 562 households. The map of Zaka District shown in Figure 1 shows the regions which will be used for the study.

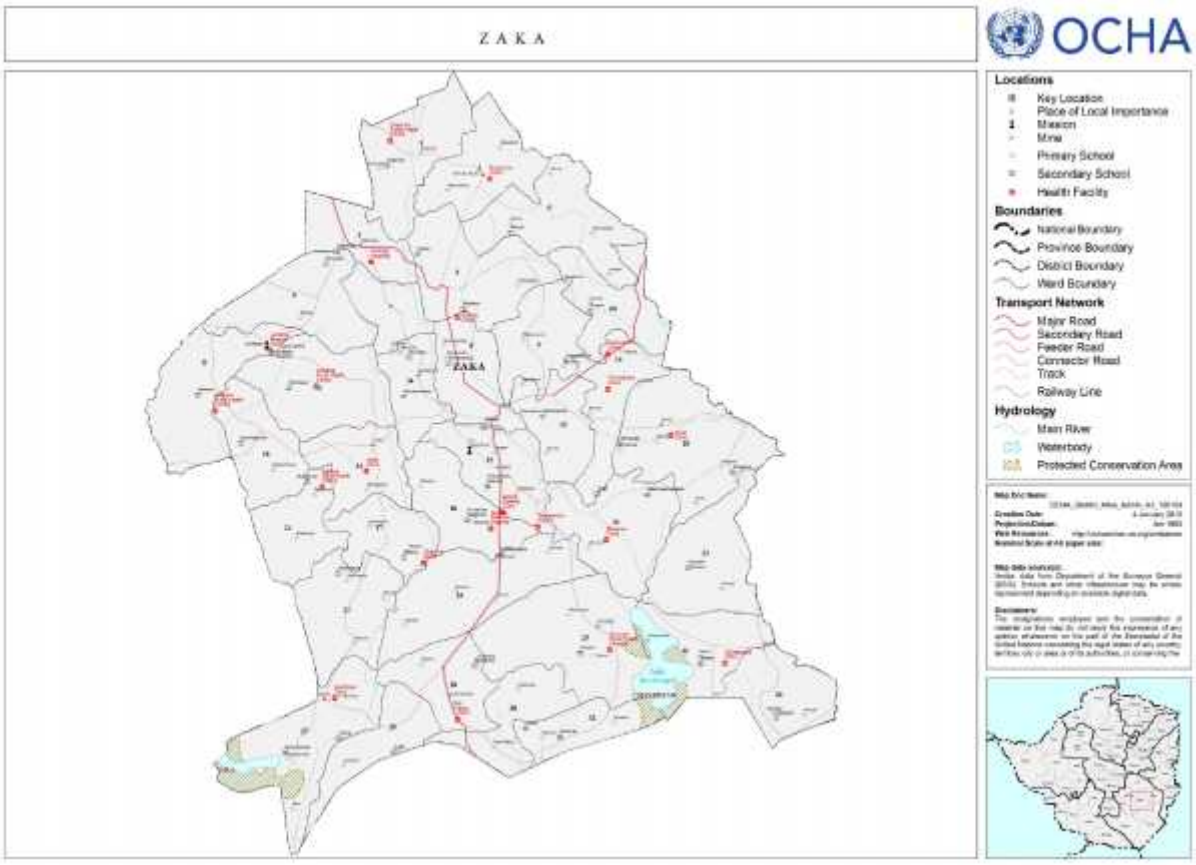

Figure 1: Map showing Zaka District.

Current methods to control cattle theft include cattle clearing processes for anyone wishing to move cattle from one district to the other. Given the porous nature of the inter-district boarders and the fact that most thefts happen at night, this process is not very effective. With respect to tracing reported missing cattle, cattle branding (use of distinct cattle marks burnt into the cattle skin) is highly recommended and used to recover stolen animals. Despite its usefulness, many people have not taken heed of Veterinary and Police's advice to do so (In Chipato village of Zaka , people are refusing to get their cattle branded due to lack of knowledge on the importance of cattle branding). Cattle branding has also proved ineffective in identifying cattle which are stolen and slaughtered as such marks can be removed.

Some technology driven solutions such as "effective stock theft control and monitoring system" are in use in other countries. Agri-Alert system used in South Africa involves Activity alarm Global Positioning System (GPS) alarm - Water-level monitoring - Panic alarm; and Temperature alarm. The available systems provide for livestock farmers to monitor their animals' movements and any disturbances, using their cell phones. GPS coordinates are also available, for the farmers in question to receive Short Message Service (SMS) in this regard (Maluleke, 2017). Such infrastructure is however still under developed in countries like Zimbabwe. There is therefore need for additional tools to help individuals, communities and the nation to reduce cattle theft and increase capacity to identify and recover those which will have been stolen and bring to book the cattle rustlers.

Deoxyribonucleic acid (DNA) is a molecule which is the instruction manual on the identity and function of living organisms hence it`s being viewed as the blue print of life. This information is passed from one generation to the next such that one can identify the ancestry of living things. This is made easy by the simple composition of the chemical that carry this information which is composed of four bases, guanine (**G**), thymine (**T**), cytosine (**C**) and Adenine (**A**), where the order and number of these letters in different units of the DNA molecule determine the difference between two individual living things. This has results in the development of distinct genetic markers called short tandem repeats (STR) markers used in human identification, applied in the field of forensic science (This was discovered by Friederich Miescher in 1869). Similar genetic markers have been developed in veterinary science to govern cattle breeding and to establish the identify of cattle. The markers for cattle identification are being used for combating cattle theft. One of the most promising system for combating cattle theft , especially in the rural resource-limited settings, which combines cattle branding and DNA profiling is called Livestock Identification Catalogue (LidCat) developed by *The Agricultural Research Council (ARC) in South Africa.* The system is based on collecting a DNA sample from an animal, allocating a unique barcoded (biobanking) the sample under controlled conditions in a laboratory (https:// www.farmersweekly.co.za/ archive/let-genes-decide-who-an-animal-belongs-to/). When cattle go missing, and suspected remains or suspected stolen cattle with defaced branding or missing tags are found, or suspected cattle thieves captured, DNA samples from the found cattle, remains on clothing of suspected thieves is compared to the DNA of the missing cattle which will be in the DNA Biobank and DNA Database.

There are several methods for DNA analysis that can be applied on the cattle DNA identification, there are simple PCR-RFLP (polymerase chain reaction- restriction length polymorphism), Sanger sequencing and next generation sequencing (NGS). Genotyping 30 SNP loci that exhibit variability across all common beef breeds would be sufficient to uniquely identify 900,000 cattle.5 The odds that two individuals coincidentally possess identical 30-SNP loci genotypes is less than one in a trillion! And 45 highly-informative SNP loci are estimated to be sufficient to identify all of the cattle in the world (estimated to be approximately 1 billion) (Heaton, *et al.*, 2002). The use of short tandem repeats (STR) in animal identification has however shown to be both rapid and cost effective. As with human DNA, genetic material obtained from cows contains short tandem repeat (STR) markers that are highly variable among individuals and thus useful in differentiating between animals. Below is a list of some of the cattle STR loci described in the literature and used by the International Society of Animal Genetics (ISAG) (https://strbase.nist.gov/cattleSTRs.htm)

A widely used genotyping test is provided by Thermo Scientific, the Bovine Genotypes Panel 1.2 which encompasses all the 12 STR loci recommended by the International Society for Animal Genetics (ISAG) for routine use in bovine parentage testing and identification, including:TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, BM1818, ETH3, ETH225, BM1824. (https:// www.thermofisher.com/nl/en/home/ brands/thermo-scientific/molecular-biology/thermo-scientific-specialized-molecular-biology-applications/genotyping-thermo-scientific/bovine-genotyping-thermo-scientific.html)

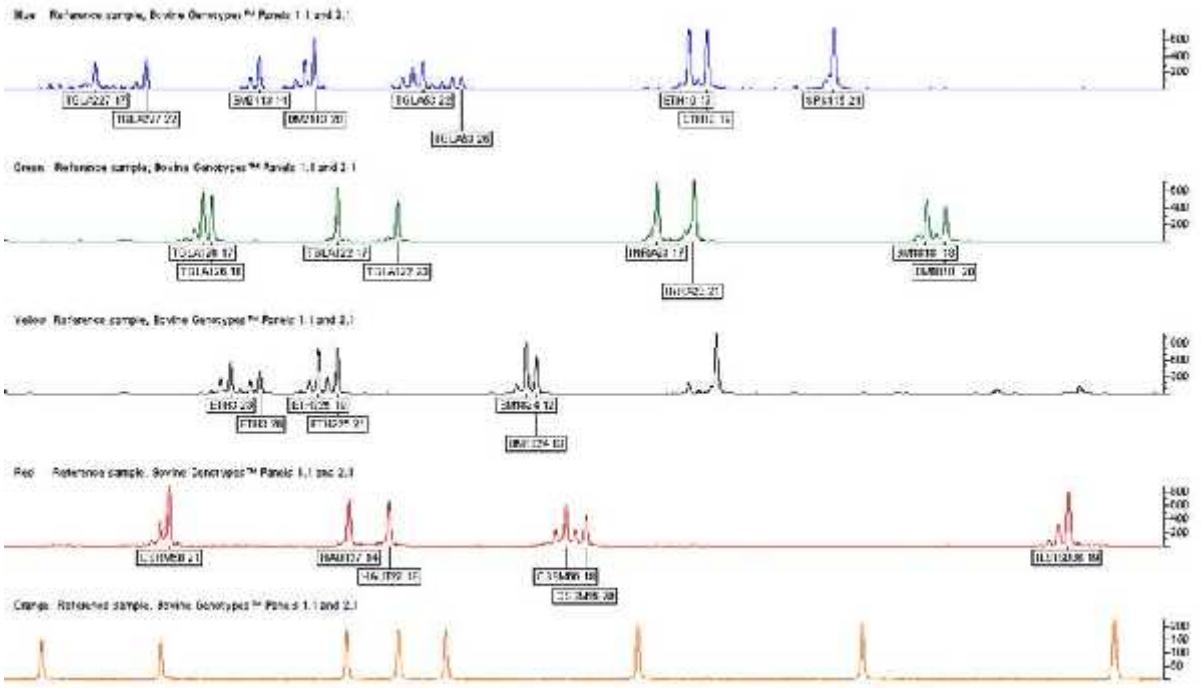

Fig. 1: Typical Electropherogram from the reference sample. The genotypes are reported in the proposed nomenclature by Van de Goor et al. (2009) based on the number of repeat units and adopted from the recommendations of the ISFG for the nomenclature in human STRs.

Objectives

The objectives of the research are therefore to:

1. To map define a study site representative of the Zaka District cattle types, ownership, density and movement patterns
2. To collect samples for DNA isolation from selected cattle in the delineated area in the Zaka District
3. To conduct DNA profiling using standard cattle STR marker tests
4. To create a DNA database of profiles that Veterinary and Zimbabwe Police Services can use in investigating cattle theft.

3. METHODS

To answer the research question and test our hypothesis, we will conduct a set of experiments which include defining the sampling sites in the Zaka district, obtaining permission form relevant authorities (Veterinary, Zimbabwe Service Police, District authorities, Chief and village heads), collection of samples for DNA extraction lead by a professional trained to do, maintain proper chain of custody to ship samples to the AiBST laboratory, and to conduct DNA analysis at the AiBST laboratory.

Selection of sample collection sites and Sample size

In consultation with the community leadership and Veterinary authorities, selection of villages and cattle kraal from which sampling will be done will be agreed on. The sites must include elements important in combating cattle rustling such as villages at the borders of the district, well distributed across the district to be representative of the future utility of the cattle DNA database, and other factors deemed important in combating cattle theft.

In this preliminary effort, convenient sampling will be done instead of powering the study. A target of 100 cattle will have their DNA collected for analysis.

Sample collection

The cattle from which samples will be branded in a special way to enable their recognition. Veterinary officials will assist in branding of the 100 cattle including placing ear tags with barcodes. A blood sample will be collected in EDTA tubes and drops of the blood put on filter paper to have dried blood spots. Collection will be done by a trained professional from Veterinary. After drying the 5 blood spot cards, they will be put in zip lockable plastic containers and shipped to AiBST. A unique barcoded reference number, similar to a human ID number, accompanies each card, while a third replica of the number is retained by the farmer. A comprehensive information form (see attached appendix 1) about the cattle and the farmer will be completed of which a copy will remain with the cattle owner and the other accompany the sample to the laboratory.

AiBST will provide the research team with a complete kit for sample collection and shipment which will include the following:

1. Information form with printed barcode and an EDTA tube with a similar barcode for blood collection.
2. Guthrie filter cards for collection of dried blood spots
3. Ziplock plastic bags for the packaging and shipment of the Guthrie cards
4. Samples to be Biobanked at AiBST waiting for analysis by the school team.

STR (Short Tandem Repeat) DNA analysis and Establishment of a Database

The School Research team will spend one week at AiBST analysing the DNA samples for their STR marker profiles. This will be done suing the 16 STR Marker Bovie Genotypes Panel 1.2. This will be done according to the manufacturer's guide (https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2FMAN0012412_Bovine_Genotypes_Panel_1.2_UG.pdf

&title=VXNlciBHdWlkZTogQm92aW5lIEdlbm90eXBlcyBQYW5lbCAxLjI=, Appendix 2).

The kit will be able to generate profiles of 100 cattle for the markers indicated in Table 1.

Table 1: Cattle STR information on Locus name, chromosomal location, repeat structure and repeat sequence, original reference, primer sequences, and the true size ranges of the amplicons.

Locus	Chromosomal Location	Repeat structure	Repeat sequence	Original Reference	Primer sequences (Forward and Reverse)	Amplicon length (bp)
BM1818	D23S21	simple	$(TG)_n$	Bishop et al. (1994)	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	253-277
BM1824	D1S34	simple	$(GT)_n$	Barendse et al. (1994)	F: GAGCAAGGTGTTTTTCCAATC R: CATTCTCCAACTGCTTCCTTG	176-188
BM2113	D2S26	simple	$(CA)_n$	Sunden et al. (1993)	F: GCTGCCTTCTACCAAATACCC R: CTTCCTGAGAGAAGCAACACC	124-146
CSRM60	D10S5	simple	$(AC)_n$	Baylor College of Medicine Human Genome Sequencing Center (2006)	F: AAGATGTGATCCAAGAGAGAGGCA R: AGGACCAGATCGTGAAAGGCATAG	91-117
CSSM66	D14S31	simple	$(AC)_n$	Barendse et al. (1994)	F: AATTTAATGCACTGAGGAGCTTGG R: ACACAAATCCTTTCTGCCAGCTGA	177-203
ETH3	D19S2	compound	$(GT)_nAC(GT)_6$	Solinas-Toldo et al. (1993)	F: GAACCTGCCTCTCCTGCATTGG R: ACTCTGCCTGTGGCCAAGTAGG	100-128
ETH10	D5S3	simple	$(AC)_n$	Solinas-Toldo et al. (1993)	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTCTCTTCTC	206-222
ETH225	D9S2	compound	$(TG)_4CG(TG)(CA)_n$	Steffen et al. (1993)	F: GATCACCTTGCCACT	139-157

					ATTTCCT R: ACATGACAGCCAGCTGCTACT	
HAUT27	D26S21	simple	$(AC)_n$	Thieven et al. (1997)	F: TTTTATGTTCATTTTTTGACTGG R: AACTGCTGAAATCTCCATCTTA	137-155
ILSTS006	D7S8	simple	$(GT)_n$	Brezinsky et al. (1993)	F: TGTCTGTATTTCTGCTGTGG R: ACACGGAAGCGATCTAAACG	279-297
INRA023	D3S10	simple	$(AC)_n$	Vaiman et al. (1994)	F: GAGTAGAGCTACAAGATAAACTTC R: TAACTACAGGGTGTTAGATGAACTC	201-225
SPS115	D15	compound	$(CA)_nTA(CA)_6$	Baylor College of Medicine Human Genome Sequencing Center (2006)	F: AAAGTGACACAACAGCTTCACCAG R: AACCGAGTGTCCTAGTTTGGCTGTG	247-261
TGLA53	D16S3	compound	$(TG)_6CG(TG)_4(TA)_n$	Georges & Massey (1992)	F: GCTTTCAGAAATAGTTTGCATTCA R: ATCTTCACATGATATTACAGCAGA	151-187
TGLA122	D21S6	compound	$(AC)_n(AT)_n$	Georges & Massey (1992)	F: AATCACATGGCAAATAAGTACATAC R: CCCTCCTCCAGGTAAATCAGC	136-182
TGLA126	D20S1	simple	$(TG)_n$	Georges & Massey (1992)	F: CTAATTTAGAATGAGAGAGGCTTCT R: TTGGTCCTCTATTCTCTGAATATTCC	111-127
TGLA227	D18S1	simple	$(TG)_n$	Georges & Massey (1992)	F: GGAATTCCAAATCTGTTAATTTGCT R: ACAGACAGAAACTCAATGAAAGCA	76-104

RISKS / BENEFITS TO PARTICIPANTS

There is no social, economic or legal risk to the volunteer cattle owners nor to the cattle themselves in this study. Instead, there is benefit in them being the first to have this novel solution applied towards reducing the risk of cattle theft or increasing chances of their recovery upon theft.

COSTS, COMPENSATION AND REIMBURSEMENTS

The volunteer cattle owners will not receive at money for having their cattle involved in the study.

CONFIDENTIALITY ASSURANCES

The cattle DNA samples will be kept at the AiBST Biobank and DNA database. On behalf of the Zaka District Veterinary office. Access and use of the samples and database will be controlled. By Zaka District Veterinary Office and other approved authorities in the fight against cattle theft. AiBST, all Biobank samples and DNA databases are kept under strict security and confidentiality according to international guidelines for doing so.

CONFLICT OF INTEREST (real or apparent)

There is no conflict of interest in the parties involved in the conduct of this study

COLLABORATIVE AGREEMENTS

A letter of collaboration indicating the parties involved such as the Research team representative, the School, the Veterinary Officials, Zimbabwe Service Policy, the Ministry of `Higher and tertiary Education, Science and Technology Development and the African Institute of Biomedical Science and technology.

INTENDED USE OF RESULTS

The results of this study will be communicated to the communities of Zaka District, presented at national symposia and meetings. Based on the significance of the results. Intellectual property rights might be sought for by AiBST as the sponsor of the study with indicated benefits for the other participants to the study. The IP might also be translated to commercial products and services to which involved parties will be beneficiaries.

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